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THE SYNTHESIS OF NOVEL MAST CELL STABILISERS FROM BENZOCYCLOALKANONES

BY

TAO ZHANG B.SC.



A thesis presented to The University of Dublin for the degree of Doctor of Philosophy

Based on research carried out under the supervision and direction of

Dr. John Walsh

The School of Pharmacy and Pharmaceutical Sciences,
Trinity College Dublin.

October 2009

DECLARATION

This thesis is submitted by the undersigned to the University of Dublin, Trinity College, for examination for the degree of Doctor of Philosophy.

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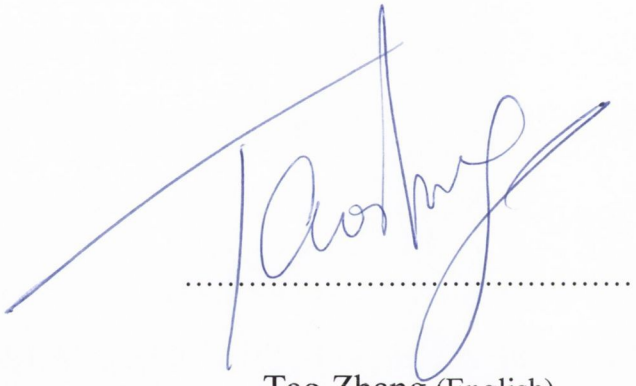

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Tao Zhang (English)



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张涛 (Chinese)

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ABSTRACT

The purpose of this project was to design and synthesise novel classes of mast cell stabilisers from benzocycloalkanones. This thesis is introduced with an overview of the increasing prevalence of allergic conditions throughout the world, and the fundamental role mast cells and their associated mediators play in allergic reactions. The mechanism of the interaction between the antibody, immunoglobulin E (IgE), and its cell surface high affinity IgE-Fc receptors (FcεRI) is outlined. There then follows a review of numerous preformed and newly synthesized inflammatory mediators which are released by the activated mast cells following allergen subsequent exposure. This leads on to a discussion of the consequent pathological effects of these biological mediators in; allergic diseases, autoimmune disorders and inflammatory conditions. Chapter 1 concludes with an assessment of both currently available and investigational therapeutic options in the treatment of these mast cell-mediated diseases.

Chapter 2 opens with a discussion on the design of a new series of compounds which were expected to overcome the design faults associated with a tetralone series of mast cell stabilisers. Having identified an appropriate solution to the problem, the remaining part of this chapter is concerned with a study on the synthesis of compounds within the new design, where modifications to the electronic properties of the benzylic ring are investigated. The chapter closes with a discussion on the bioactivity of the compounds synthesised.

The novel mast cell stabilisers described in Chapter 2 also contain a cyclohexenyl amino group as part of their design. Replacing this group with a π-rich dihydronaphtyl amine is central to the discussion outlined in the first section of Chapter 3. A method is described for its synthesis while experimental data is provided to demonstrate the route chosen to acquire this intermediate was successful. Following on from this study, details are provided on a nine step synthesis of a potent mast cell stabiliser, namely compound **(3.30)**, which is a regio-isomer of compound **(2.10)**, a novel compound whose synthesis is described in Chapter 2.

The primary objective of the work described in Chapter 4 was to investigate the effect on mast cell stabilisation of incorporation of a 3-dimethylaminopropyl group onto previously synthesised indanol and tetralol-based dimer compounds. A series of compounds were successfully synthesised containing this group. One of the compounds synthesised namely, compound **(4.10)**, exhibited good activity in an *in vivo* model of passive cutaneous anaphylaxis.

The aim of the work outlined in Chapter 5 was to generate highly aromatic mast cell stabilising compounds analogous to those described in the preceding Chapter, but which would contain; (i) fewer steps to synthesise and (ii) reduced number of asymmetric centres. From this work, several compounds were synthesised which displayed good activity *in vivo*. The Chapter closes with a description of an elegant synthetic route devised to incorporate into the most active compound, an acetic acid residue. The concept underlying this strategy was to promote through hydrogen bonding the formation of a single geometric isomer of this compound while at the same time increasing its solubility profile in physiological media.

The Experimental section contains details on the synthetic methods used synthesise the novel mast cell stabilising compounds discovered during the course of this work, together with a complete set of their spectral data.

SYMBOLS AND ABBREVIATIONS

α	alpha
Ac	acetate
AHNMD	a hematologic non-mast cell lineage disorder
AIA	adjuvant induced arthritis
AKC	keratoconjunctivitis
APCs	antigen presenting cells
aq.	aqueous
AR	allergic rhinitis
ASM	aggressive systemic mastocytosis
β	beta
BBB	blood brain barrier
Bn	benzyl
BOC	*tert*-butoxy carbonyl
br s	broad singlet
cAMP	cyclic adenosine monophosphate
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane condutance regulator
CIA	collagen-induced arthritis
conc	concentration
cm^{-1}	wavenumber
CRF	corticotropin-releasing factor
^{13}C-NMR	carbon-13 nuclear magnetic resonance
CM	cutaneous mastocytosis
CNS	central nervous system
COSY	correlated spectroscopy
C=O	carbonyl bond
δ	chemical shift in ppm downfield from TMS
°C	degrees Celsius
eq	equatorial
equiv.	equivalents
Et	ethyl
^{19}F-	fluorine-19 nuclear magnetic resonance
FTIR	fourier transform infrared
g	gram(s)
h, min	hour(s), minute(s)
Hz	hertz
IBS	irritable bowel syndrome
IgE	immunoglobulin E
IL	interleukin
IP_3	inositol triphosphate
IR	infrared
ISM	indolent systemic mastocytosis
J	coupling constant (nmr), in Hz

L	litre
LDA	lithium diisopropylamide
M	molar
M^+	mass of the molecular ion (mass spectrometry)
MAO-B	monoamine oxidase-B
MCL	mast cell leukaemia
Me	methyl
mg	milligram
MHz	megahertz
ml	milliliter
mol, mmol	mole, millimole
μM, mM	micromolar, millimolar
MMPs	matrix metalloproteinases
Mp	melting point
MPM	methoxyphenylmethyl
MS	multiple sclerosis
NGF	nerve growth factor
NOE	nuclear overhauser effect
NT	neurotensin
PAC	perennial allergic conjunctivitis
Ph	phenyl
ppm	parts per million (nmr)
RA	rheumatoid arthritis
RANTES	regulated on activation normal T cells, expressed and secreted
R_f	retention factor
RT	room temperature
SAC	seasonal allergic conjunctivitis
SCF	stem cell factor
SM	systemic mastocytosis
SP	substance P
SRS-A	slow-reacting substance of anaphylaxis
t	*tert*
TBS	*tert*-butyldimethylsilyl
TGF-β	transforming growth factor-β
TLC	thin layer chromatography
TMS	trimethylsilane
TNF-α	tumour necrosis factor-α
wk	weak
UV	ultraviolet
VKC	vernal keratoconjunctivitis

SOLVENTS AND REAGENTS

ACN/CH_3CN	acetonitrile
AcOH	acetic acid
$AlCl_3$	alluminium trichloride
BOP-Cl	Bis(2-oxo-3-oxazolidinyl)phosphinic chloride
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethylsulfoxide
CD_3OD	deuterated methanol
EtOAc	ethyl acetate
EtOH	ethanol
Et_2O	diethylether
H_2	hydrogen gas
HCl	hydrochloric acid
H_2O	water
H_2SO_4	sulphuric acid
K_2CO_3	potassium carbonate
KBr	potassium bromide
MeI	iodomethane/methyl iodide
MeOH	methanol
$MgSO_4$	magnesium sulfate
$NaBH_4$	sodium borohydride
NaCl	sodium chloride
Na_2CO_3	sodium carbonate
$NaHCO_3$	sodium hydrogen carbonate
NaI	sodium iodide
NaN_3	sodium azide
NaOH	sodium hydroxide
NH_4Cl	ammonium chloride
Pd/C	palladium on charcoal
PGs	prostaglandin
Py	pyridine
$SnCl_4$	tin (IV) chloride
TBDMS	t*ert*-Butlydimethylsilyl chloride
TEA	triethylamine
THF	tetrahydrofuran
TFA	trifluoroacetic acid
TsOH	*p*-toluene sulfonic acid

TABLE OF CONTENTS

TITLE PAGE
DECLARATION
ACKNOWLEDGEMENTS
ABSTRACT
SYMBOLS AND ABBREVIATIONS
TABLE OF CONTENTS
TABLE OF FIGURES
TABLE OF TABLES

CHAPTER 1

1.1 Introduction....1

1.2 Mast cells in allergic inflammation....2
1.2.1 Basic mast cell biology....2
1.2.2 Mast cell activation....3
1.2.2.1 Allergens....4
1.2.2.2 IgE binding receptors (FcεRI and FcεRII)....6
1.2.2.3 Mechanism of IgE mediated degranulation-the classic pathway....7

1.3 Mast cell inflammatory mediators....8
1.3.1 Histamine and its receptors....9
1.3.1.1 Histamine H1 receptor....11
1.3.1.2 Histamine H2 receptor....14
1.3.1.3 Histamine H3 receptor....15
1.3.1.4 Histamine H4 receptor....17
1.3.2 Mast cell-specific serine proteases....18
1.3.2.1 Tryptase....18

1.3.2.2 Chymase 19
1.3.3 Serotonin 20
1.3.4 Lipid mediators 20
1.3.4.1 Leukotrienes and slow-reacting substance of anaphylaxis 20
1.3.4.2 Prostaglandins (PGs) and Thromboxanes 21
1.3.4.3 Platelet-activating factor 22
1.3.5 Immunoregulatory cytokines 22
1.3.6 Epidermal growth factor and vascular endothelial cell grow factor 24

1.4 Mast cells associated disorders 25
1.4.1 Allergic diseases 25
1.4.1.1 Asthma 25
1.4.1.2 Allergic rhinitis 26
1.4.1.3 Allergic conjunctivitis 27
1.4.2 Autoimmune Disorders 28
1.4.2.1 Rheumatoid arthritis 28
1.4.2.2 Multiple sclerosis 30
1.4.3 Disorders Involving Mast Cell 31
1.4.3.1 Angiogenesis 31
1.4.3.2 Irritable bowel syndrome 33
1.4.3.3 Mastocytosis 34
1.4.3.4 Cystic fibrosis 36
1.4.3.5 Hepatic fibrosis 37

1.5 Clinical therapy with mast-cell stabilising activity 38
1.5.1 Chromones 39
1.5.2 Ketotifen 41
1.5.3 Lodoxamide 42
1.5.4 Olopatadine, Azelastine and Epinastine 43

1.5.5 Dihydropyridines 46
1.5.6 Mediator inhibitors 47
1.5.6.1 Leukotriene antagonists 47
1.5.6.2 Spleen tyrosine kinase inhibitor 48
1.5.6.3 Phosphodiesterase inhibitors 51
1.5.6.4 Serine proteinase inhibitors 52
1.5.6.5 Corticotropin-releasing factor (CRF) antagonists 54
1.5.6.6 Interleukin-10 55
1.5.7 Anti-Immunoglobulin E (anti-IgE) therapy 56
1.5.8 Natural products with mast cell-stabilising properties 57

CHAPTER 2

2.1 Introduction 60

2.2 Synthetic targets and strategy 63

2.3 Synthesis of analogues of structural type **(A)** 65
2.3.1 Synthesis of the target secondary amine **(2.3)** 65
2.3.2 Structure determination of the secondary amine **(2.3)** 65
2.3.3 *N*-alkylation of the secondary amine **(2.3)** with electron rich benzyl groups 68
2.3.3.1 Synthesis of *N*-alkyl derivatives **(2.8)** and **(2.9)** 69
2.3.3.2 Synthesis of *N*-alkyl derivatives **(2.10-2.20)** containing either electron withdrawing or water-solubilising substituents 70
2.3.3.3 Structural determinations of compounds **(2.8)**, **(2.9)** and **(2.10)** 72
2.3.3.4 Structural determinations of the fluorinated derivatives **(2.11)**, **(2.12)** and **(2.13)** 79

2.3.3.5 Structural determinations of compounds **(2.14)** and **(2.16)**..... 87
2.3.3.6 Structural determinations of compounds **(2.17)** and **(2.18)**..... 91
2.3.4 Attempted synthesis of phenylamine analogues 92
2.3.5 Preparation of a merged hybrid and π-rich mast cell stabilizer 93
2.3.6 Attempted synthesis of pyridine analogue 102

2.4 Biological data on compounds synthesised 102

2.5 Conclusion 103

CHAPTER 3

3.1 Introduction 104

3.2 Synthetic targets and strategy 104

3.3 Synthesis of compounds of structural type (B) 105
3.3.1 Synthesis of the intermediate amine (3.11) 105
3.3.1.1 Synthesis of primary amine (3.4) 106
3.3.1.2 Modified primary amine **(3.4)** synthesis-route A 108
3.3.1.3 Modified primary amine **(3.4)** synthesis-route B 109
3.3.1.4 Attempted synthesis of ketone **(3.6)** 110
3.3.1.5 Formation of compound **(3.11)** 115
3.3.2 Coupling reactions 117
3.3.2.1 Attempted synthesis of model compound **(3.12)** 118
3.3.2.2 Attempted synthesis of structural type **(B)** 119

3.4 Synthesis region-isomer structural type **(C)** 120
3.4.1 Synthesis of enone **(3.17)** 120
3.4.1.1 Synthesis of the desired molecule **(3.17)** (Method A) 121
3.4.1.2 Synthesis of the desired molecule **(3.17)** (Method B) 121

3.4.1.3 IBX mediated synthesis of molecule **(3.17)** (Method C)..... 122

3.5 Synthesis of compound **(3.30)**..... 124

3.6 Conclusion..... 137

CHAPTER 4

4.1 Introduction..... 138

4.2 Synthetic targets and strategy..... 138

4.3 Synthesis of the target compound **(4.5)**..... 140

4.3.1 Synthesis of the benzyl-alkylated dimer **(4.3)**..... 142

4.3.1.1 Synthesis of enol trimethylsilyl ether **(4.0)**..... 142

4.3.1.2 Synthesis of cyclic acetal **(4.1)**..... 143

4.3.1.3 Crossed Aldol addition..... 145

4.3.1.4 Synthesis of the intermediate ketone **(4.3)**..... 147

4.3.2 Synthesis of the benzyl-alkylated dimeric alcohol **(4.4)**..... 148

4.3.3 Formation of the amino alkene **(4.5)**..... 158

4.4 Synthesis of the amino alcohol **(4.10)** and amino alkene **(4.11)**..... 160

4.4.1 Synthesis of benzyl-alkylated dimer **(4.9)**..... 160

4.4.1.1 Synthesis of compound **(4.6)**..... 161

4.4.1.2 Synthesis of compound **(4.7)**..... 161

4.4.1.3 Synthesis of compound **(4.8)**..... 162

4.4.1.4 Synthesis of intermediate ketone **(4.9)**..... 163

4.4.2 Synthesis of the amino alcohol **(4.10)**..... 176

4.4.3 Formation of the alkene **(4.11)**..... 182

4.5 Modification to the *para*-position of the benzyl ring..... 186

4.5.1 Synthesis of trifluoromethoxy benzyl derivative **(4.13)**..... 175

4.5.2 Synthesis of amino ketone **(4.15)** 183

4.6 Conclusion 195

CHAPTER 5

5.1 Introduction 196

5.2 Synthetic targets and strategy 196

5.3 Synthesis of dibenzyl amino alcohol **(5.1)** 198

5.4 Formation of dichloro and di-*tert*-butyl derivatives of compounds **(5.1)** and **(5.2)** 208

5.4.1 Synthesis of the 4-chloro analogue 208

5.4.2 4-*tert*-butyl analogue 212

5.4.3 Synthesis of trifluoromethoxy analogues of compound **(5.3)** 215

5.4.3.1 Structure elucidation studies on compound **(5.10)** and its regioisomer **(5.13)** 216

5.4.3.2 Structure elucidation studies on compound (5.11) and its regioisomer **(5.14)** 218

5.4.3.3 Structure elucidation studies on compound (5.12) and its regioisomer **(5.15)** 219

5.4.4 Synthesis of (pyridin-2-yl)methyl analogues of **(5.1** and **5.2)** 226

5.5 Synthesis of compound **(5.2)** derivatives with either a seven- or a five-membered hydroaromatic ring 231

5.5.1 Attempted synthesis of benzosuberyl analogues of **(5.2)** and **(5.6)** 232

5.5.2 Five membered hydroaromatic ring analogues 234

5.6 Synthesis of amino acid analogues 245

5.6.1 Synthesis of tetralone acetic acid **(5.34)** 246

5.6.2 Benzylation of compound **(5.34)** 253

5.7 Biological data on compounds synthesised 265

5.8 Conclusion 267

TABLE OF FIGURES

1.0 Image of a resting mast cell (A)

1.1 Image of a activated and degranulated mast cell (B)

1.2 Image of single mast cell molecule bound by IgE antibodies

1.3 Biosynthesis and metabolism of histamine

1.4 Histamine H1-receptor antagonists

1.5 Chemical structure of the series of 1-substituted-4-(2-methylphenyl)-4H-[1,2,4]trizolo[4,3-a] quinazolin-5-ones

1.6 Chemical structure of Chlorpheniramine maleate **(2.2)**

1.7 Histamine H2-receptor antagonists

1.8 Methylation of histamine

1.9 Histamine H3-receptor antagonists

1.10 Histamine H4-receptor antagonists

1.11 Proposed targets of mast cell stabilisers and antihistamines

1.12 Chemical structures of Khellin and K18

1.13 Chemical structures of disodium cromoglycate and sodium nedocromil

1.14 Chemical structures of FPL55757 and FPL55787

1.15 Chemical structures of Ketotifen

1.16 Chemical structure of Lodoxamide

1.17 Chemical structure of Lodoxamide tromthamine

1.18 Chemical structure of Olopatadine hydrochloride

1.19 Chemical structures of Azelastine

1.20 Chemical structures of Epinastine

1.21 Chemical structures of dihydropyridine

1.22 Chemical structures of Nifedipine and Verapamil

1.23 Chemical structures of DS-4574 and LY171883

1.24 Chemical structures of BAY61-3606 and R112

1.25 Chemical structures of Syk kinase inhibitor R406

1.26	Chemical structure of 2-(2-aminoethylamino)-4-{3-(trifluoromethyl) phenylamino}pyrimidine-5-carboxamide
1.27	Chemical structure of YM193306
1.28	Chemical structures of Theophylline and BRL 22321
1.29	Chemical structure of APC366
1.30	Chemical structure of human chymase inhibitor SPF32629A
1.31	Chemical structure of Luteolin-7-*O*-rutinoside
1.32	Chemical structures of thunberginols A, B and F
1.33	Chemical structure of FK 506 (or tacrolimus)
1.34	Chemical structure of cyclosporine
4.1	Indanone-2-aminotetralin dimmer
2.1	Tetralone-2-aminotetralin dimer
2.2	Cyclohexenyl-substituted tertiary amine
2.3	Alkylating agents used to generate analogues of structural **(A)**
2.4	Secondary amine **(2.3)** and molecular design of structural type **(A)**
2.5	^{1}H NMR spectrum of compound **(2.3)**
2.6-2.7	^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(2.3)**
2.8	IR spectrum of compound **(2.3)**
2.9	^{1}H NMR spectrum of compound **(2.9)**
2.10-2.12	^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(2.9)**
2.13	H-H COSY NMR spectrum of compound **(2.9)**
2.14-2.15	C-H COSY NMR spectrum of compound **(2.9)**
2.16	HMBC NMR spectrum of compound **(2.9)**
2.17	^{1}H NMR spectrum of compound **(2.11)**
2.18-2.20	^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(2.11)**
2.21	^{19}F NMR spectrum of compound **(2.12)**
2.22	^{1}H NMR spectrum of compound **(2.13)**
2.23-2.25	^{13}C and DEPT 135 NMR spectra of compound **(2.13)**
2.26-2.27	C-H COSY NMR spectrum of compound **(2.13)**
2.28	^{19}F NMR spectrum of compound **(2.13)**
2.29	^{1}H NMR spectrum of compound **(2.14)**

2.30-2.32 ^{13}C and DEPT 135 NMR spectra of compound **(2.14)**

2.33 ^{1}H NMR spectrum of compound **(2.20)**

2.34-2.36 ^{13}C and DEPT 135 NMR spectra of compound **(2.20)**

2.37 ^{1}H NMR spectrum of compound **(2.21)**

2.38-2.40 ^{13}C and DEPT 135 NMR spectra of compound **(2.21)**

2.41-2.42 H-H COSY NMR spectrum of compound **(2.21)**

2.43-2.44 C-H COSY NMR spectrum of compound **(2.21)**

2.45 HMBC NMR spectrum of compound **(2.21)**

3.0 Molecular designs of structural types **(B)**, **(C)** and compound **(3.30)**

3.1 Chemical structure of primary amine **(3.4)**

3.2 ^{1}H NMR spectrum of compound **(3.8)**

3.3-3.4 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(3.8)**

3.5 ^{1}H NMR spectrum of compound **(3.11)**

3.6 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(3.11)**

3.7 Molecular design of structural type **(C)**

3.8 Chemical structural of enone **(3.17)**

3.9 Chemical structures of compounds **(3.28)** and **(3.30)**

3.10 ^{1}H NMR spectrum of compound **(3.29)**

3.11-3.13 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(3.29)**

3.14 H-H COSY NMR spectrum of compound **(3.29)**

3.15-3.16 C-H COSY NMR spectrum of compound **(3.29)**

3.17 ^{1}H NMR spectrum of compound **(3.30)**

3.18-3.20 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(3.30)**

3.21-3.23 C-H COSY NMR spectrum of compound**(3.30)**

4.0 Examples of dimeric compounds

4.1 Chemical structures of Olopatadine and Impramine

4.2 Chemical structures of compounds **(4.3)** and **(4.9)**

4.3 ^{1}H NMR spectrum of compound **(4.4)a**

4.4-4.5 ^{13}C, DEPT 135and DEPT 90 NMR spectra of compound **(4.4)a**

4.6-4.8 H-H COSY NMR spectrum of compound **(4.4)a**

4.9-4.10 C-H COSY NMR spectrum of compound **(4.4)a**

4.11	^{1}H NMR spectrum of compound **(4.4)b**
4.12-4.14	^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(4.4)b**
4.15	^{1}H NMR spectrum of compound **(4.5)b**
4.16-4.17	^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(4.5)b**
4.18	Chemical structure of intermediate dimeric ketone **(4.9)**
4.19	^{1}H NMR spectrum of compound **(4.10)**
4.20-4.23	^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(4.10)**
4.24-4.25	H-H COSY NMR spectrum of compound **(4.10)**
4.26-4.27	C-H COSY NMR spectrum of compound **(4.10)**
4.28	^{1}H NMR spectrum of compound **(4.11)**
4.29-4.30	^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(4.11)**
4.31-4.32	C-H COSY NMR spectrum of compound **(4.11)**
4.33	Chemical structures of target compounds **(4.13)** and **(4.15)**
4.34	^{1}H NMR spectrum of compound **(4.13)**
4.35-4.36	^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(4.13)**
4.37	H-H COSY NMR spectrum of compound **(4.13)**
4.38-4.41	C-H COSY NMR spectrum of compound **(4.13)**
4.42	HMBC NMR spectrum of compound **(4.13)**
4.43	^{19}F NMR spectrum of compound **(4.13)**
4.44	Chemical structure of target compound **(4.15)**
4.45	^{1}H NMR spectrum of compound **(4.15)a**
4.46-4.48	^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(4.15)a**
4.49	H-H COSY NMR spectrum of compound **(4.15)a**
4.50-4.51	C-H COSY NMR spectrum of compound **(4.15)a**
4.52-4.53	HMBC NMR spectrum of compound **(4.15)a**
4.54-4.55	^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(4.15)b**
4.56	C-H COSY NMR spectrum of compound **(4.15)b**
4.57-4.58	HMBC NMR spectrum of compound **(4.15)b**
5.0	Chemical structures of the alcohols (DFs)
5.1	Target compounds of structural types of **(H)**, **(I)**, **(5.41)** and **(5.43)**
5.2	Chemical structure of compound **(5.34)**

5.3 ^{1}H NMR spectrum of compound **(5.1)**

5.4-5.5 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(5.1)**

5.6 ^{1}H NMR spectrum of compound **(5.2)**

5.7-5.8 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(5.2)**

5.9 H-H COSY NMR spectrum of compound **(5.2)**

5.10-5.11 C-H COSY NMR spectrum of compound **(5.2)**

5.12-5.13 HMBC NMR spectrum of compound **(5.2)**

5.14 NOE NMR spectrum of compound **(5.2)**

5.15 ^{1}H NMR spectrum of compound **(5.6)**

5.16 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(5.6)**

5.17 ^{1}H NMR spectrum of compound **(5.15)**

5.18 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(5.15)**

5.19 H-H COSY NMR spectrum of compound **(5.15)**

5.20 C-H COSY NMR spectrum of compound **(5.15)**

5.21-5.22 HMBC NMR spectrum of compound **(5.15)**

5.23 ^{19}F NMR spectrum of compound **(5.15)**

5.24 Chemical structure of (pyridin-2-yl) methyl analogues

5.25 ^{1}H NMR spectrum of compound **(5.20)**

5.26-5.27 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(5.20)**

5.28 Benzosuberyl and indane analogues of **(5.2)** and **(5.6)**

5.29 ^{1}H NMR spectrum of compound **(5.25)**

5.30-5.31 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(5.25)**

5.32-5.34 C-H COSY NMR spectrum of compound **(5.25)**

5.35 ^{1}H NMR spectrum of compound **(5.28)**

5.36-5.37 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound **(5.28)**

5.38-5.39 C-H COSY NMR spectrum of compound **(5.28)**

5.40 Amino acid analogues **(5.41)** and **(5.43)**

5.41 Chemical structure of compound **(5.34)**

5.42 ^{1}H NMR spectrum of compound **(5.43)**

5.43-5.44 ^{13}C and DEPT 135 NMR spectra of compound **(5.43)**

5.45 1D H-H COSY NMR spectrum of compound **(5.43)**

5.46-5.47 C-H COSY NMR spectrum of compound **(5.43)**

5.48 HMBC NMR spectrum of compound **(5.43)**

TABLE OF TABLES

2.0 Tertiary amine derivatives **(2.10)-(2.20)**

2.1 Summary statistics for compounds **(2.8-2.14)**, **(2.16-2.18)** and **(2.20-2.21)**

3.0 List of methods for BOC protection of the amine

3.1 *In vitro* mast cell stabilisation data on compound **(3.30)**

5.0 ^{1}H NMR characterisation of compounds **(5.12) and (5.15)**

5.1 ^{13}C NMR characterisation of compounds **(5.12) and (5.15)**

5.2 Summary statistics for compounds synthesised

To my family,

For all their love and support!!!

1.1 Introduction

The immune and the lymphatic systems represent remarkably versatile defence systems which have developed to protect animals and human beings from invaders such as bacteria, parasites, and other pathogenic microorganisms. Lymphocytes and antigen presenting cells (APCs) are the two major cell types involved in the generation of effective immune responses (Rao, 2002a). Abnormal immune response against antigens, trigger various allergic diseases. The prevalence of allergic conditions has increased at an incredible rate over the past few decades throughout the world, especially in industrialised countries, where an estimated 20% of the population are affected (Nauta et al., 2008). Allergic disorders have become a major cause of substantial morbidity and mortality worldwide. An overview of chronic respiratory diseases was presented by World Health Organization (WHO) (2009) and revealed that approximately 300 million people around the world currently suffer from asthma and 255,000 asthma related deaths occurred in 2005. The statistics also show that Ireland has the fourth highest incidences of asthma in the world (McGowan et al., 2009), and nearly one in every three school-aged children in the UK are affected by asthma (Sohi & Warner, 2008).

Even though allergy-related diseases may in many cases be benign, prolonged therapies may still be required. Much research focusing on the molecular level of allergic disorders has been performed in the last two decades. As a result, the fundamental role of mast cells in inflammatory processes, the acute and delayed-phase allergic reactions has been well studied and documented (Theoharides & Cochrane, 2004). A resident cell of mucosal and connective tissue, the mast cell is a central player in allergic reactions as well as innate and acquired immunity, wound healing, fibrosis, tumours and autoimmune diseases (Puxeddu et al., 2003). The involvement of mast cells in cardiac diseases has been reported, including atherosclerosis (Laine et al., 2000), myocardial ischemia (Singh & Saini, 2003), myocardial infarction (Jin et al., 2001). A very recent study showed that an increase in density of heart mast cells was associated with hyperhomocysteinemia-induced cardiac hypertrophy (Singh et al., 2008).

1.2 Mast cells in allergic inflammation

1.2.1 Basic mast cell biology

Mast cells were first described by Paul Ehrlich in 1878, who named them "mastzellen", meaning "feeding cells" because of their appearance (Ehrlich, 1878; Galli et al., 1999). The precursor to mast cells expressing $CD34^+$ molecule are formed in the bone marrow during haematopoiesis and circulate into the blood stream (Robbie & Brown, 2002). The haematopoietic progenitor cells remain undifferentiated in the blood and become differentiated only upon entering the tissue, where they become mature mast cells under the influence of local factors. Mast cells can be very long-lived, ranging from weeks to months (Wdemeyer et al., 2000). (Fig. 1.0 and Fig. 1.1)

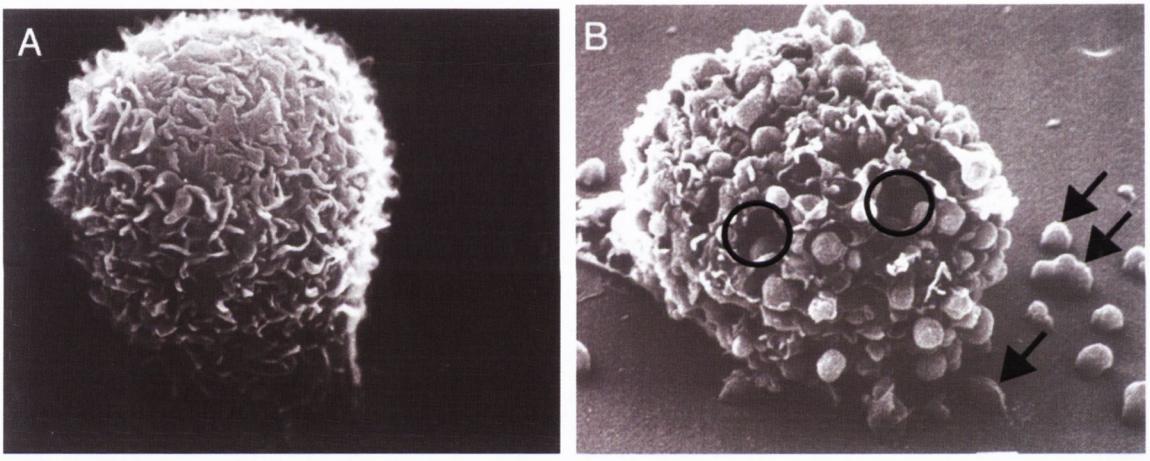


Fig. 1.0 Image of a resting mast cell (A) (Lindstedt & Kovanen, 2006)

Fig.1.1 Image of a activated and degranulated mast cell (B) arrows point to expelled granule remnants; two open degranulation channels showing intracellular granule remnants are encircled. (Lindstedt & Kovanen, 2006)

Mast cells are found in almost all parts of the body along with the endothelial cells of the blood vessel wall as well as the mucosal epithelial tissue (Nauta et al., 2008). It has been established by many studies that mast cell proliferation, differentiation and survival are strictly regulated by stem cell factor (SCF), which act through its receptor expressed on the mast cell surface (Sundström et al., 2001).

Under normal conditions, mast cell numbers in tissue are considered to be relatively constant, whereas mast cell hyperplasia is established in different pathologies such as chronic inflammatory processes, fibrotic disorders and wound healing (Bischoff & Sellge, 2002).

Two distinct phenotypes of mast cells are distinguishable based on the types of proteases contained in their exocytotic granules. Mucosal mast cells contain only tryptase (namely M_T), which are mainly found in the mucosa of the gastrointestinal system and in the lamina of the respiratory tract, whereas those found in connective tissue mast cells contain tryptase, chymase, cathepsin G and carboxypeptidase (namely M_{TC}), which are localized in the sub-mucosa of the gastrointestinal tract, skin and peritoneum (Rao, 2002a; Puxeddu et al., 2003).

1.2.2 Mast cell activation

Mast cells express a vast array of stimulatory and inhibitory receptors. Mast cell activation can be induced by both immunologic (Immunoglobulin E (IgE) related or non-IgE related) and non-immunologic substances. Crosslinking of IgE, Immunoglobulin G_1(IgG_1), Immunoglobulin G_{2a} (IgG_{2a}) and Immunoglobulin G_{2b} (IgG_{2b}) antibodies on the high affinity IgE receptor (FcεRI), the FcγRI (human), FcγRIIa (mouse and human) or FcγRIIIa (mouse) receptor by allergen incites antigen-specific mast cell activation. In addition, mast cell activation and degranulation can also occur by crosslinking of immunoglobulin free light chains (Nauta et al., 2008).

It has been demonstrated that mast cells can also be activated by neuropeptides released by neuronal stimulation during stress, which include corticotropin-releasing factor (CRF), urocortin (Ucn), nerve growth factor (NGF), substance P (SP), neurotensin (NT) (Suzuki et al., 1999; Theoharides et al., 2004). Activated mast cells secrete vasoactive, pro-inflammatory and neurosensitizating molecules which interact with keratinocytes, endothelial cells or nerve endings, resulting in chronic inflammation and neuropathic

hypersensitivity or pain. It has been shown that such mast cell activations can either be mediated through specific receptors (i.e. NK_1 receptors), or by activating G protein directly, which is receptor-independent (Theoharides & Cochrane, 2004).

1.2.2.1 Allergens

In atopic patients, an allergic reaction normally begins with an immediate wheal, followed by a flare response, and ends up with a chronic late phase reaction by the interaction of allergens with mast cells or basophil-bound IgE molecules (Maurer et al., 1994). (Fig. 1.2)

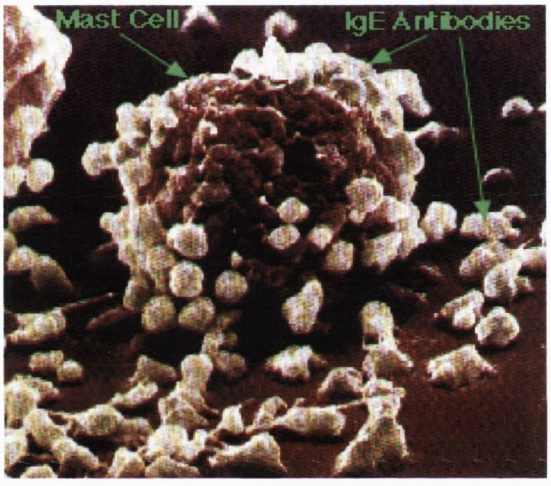


Fig. 1.2 Image of single mast cell molecule bound by IgE antibodies (Chandler & Stadtmauer, 2009)

Allergens are substances which enter the body and subsequently induce allergic reactions (Rao, 2002b). Research studies on allergens at a molecular level were first performed in the late 1980s (Radauer et al., 2008). Hundreds of allergens have been identified, cloned and sequenced from many sources, for example: pollen, mites, molds, animal dander, insect venoms, natural rubber latex, drugs, and foods (Rao, 2002b). Different allergens encounter different organs and tissues resulting in various clinical forms of allergy such as asthma, urticaria, hay fever, etc (Rao, 2002c). Most of the allergens are proteins, glycoproteins or protein bound substances (Rao, 2002c; Pomes, 2008).

Almost 60% of plant food allergens can be grouped into 4 protein superfamilies (the prolamin, the cupin, the Bet v 1 superfamilies and the profilins). In addition, pollen allergens were recently classified into 29 protein families within 3 major families (expansins, profilins, and calcium-binding proteins) (Breiteneder, 2008). It has been demonstrated that some of the allergens are haptens capable of reacting with body proteins to form a stable complex, which can incite an immune response. IgE, IgG and IgM antibodies are believed to be involved in this mechanism (Rao, 2002b).

There is no a common molecular structure to all allergens; some only have α-helices, some only have β-sheets, and most possess both secondary structural elements. The relationships between structure and function of allergens have been investigated and some conclusions have been made. Molecular shape, flexibility and specific amino acids are the key factors in determining allergen functions (Breiteneder, 2008).

Furthermore, Pfam (Protein Family) Database have demonstrated that most members of a given protein family seem to be non-allergic, only around 2% of the 9318 protein families possess a limited range of biologic function to act as allergens (Breiteneder, 2008). This significant finding was also confirmed by AllFam (allergome Family) Database (Radauer et al., 2008). The most interesting finding is that the closer a potential animal allergen is to a human protein, the less likely it is to be an allergen (Breiteneder, 2008). However, the factors that contribute to allergenicity are currently still under investigation.

In order to try to explain and reduce the globally increasing incidence of allergic diseases, many epidemiological studies have been performed examining various factors (i.e. immune responses of the host, characteristics of the invading microorganisms, feto-maternal events, lifestyles, diet and genetic factors) in the past 20 years (Miescher & Vogel, 2002; Schaub et al., 2006; Bloomfield et al., 2006). Strachan first proposed in 1989, which stated that the rapid increase in atopic disorders could be attributed to a cleaner environment and lower levels of childhood infections, known as "hygiene

hypothesis" (Boomfield et al., 2006). This inverse relationship between atopy and repeated infections was supported by studies using serology for hepatitis A, *Helicobacter pylori, Toxoplasma gondii,* childhood measles and *Mycobacterium tuberculosis* (Miescher & Vogel, 2002). However, the hygiene hypothesis suffers from many anomalies. For example, no correlation was found between the increase in allergic disorders and the decrease in infection with pathogenic organisms (Boomfield et al., 2006).

1.2.2.2 IgE binding receptors (FcεRI and FcεRII)

IgE-Fc receptor I and IgE-Fc receptor II (FcεRI and FcεRII) are the two types of Fc receptors expressed by different cell types (Rao, 2002d). Allergen mediated cross linkage of the bound IgE leads to aggregation of the FcεRI receptors and rapid tyrosine phosphorylation, from where mast cell degranulation process initiates to release preformed mediators from granules, synthesis of lipid mediators, and activation of genes for many cytokines and chemokines (Puxeddu et al., 2003). FcεRI expression has been demonstrated to have direct correlation with IgE concentration (Owen, 2002). The α chain is believed to be responsible for IgE binding through a prominent extracellular loop. The β chain and two γ chains are responsible for signalling after receptor cross link. In atopic patients, cellular binding of monomeric IgE to monocytes was also generated by high affinity FcεRI receptors (Maurer et al., 1994).

FcεRII receptor (also named CD23) has low affinity for IgE receptors. Allergen cross-linking of IgE expressed on FcεRII receptors results in activation of B cells, macrophages and eosinophils. In atopic individuals, higher levels of CD23 on their lymphocytes and macrophages are detected (Owen, 2002; Rao, 2002d).

1.2.2.3 Mechanism of IgE mediated degranulation-the classic pathway

IgE-dependent activation of mast cells, basophils, monocytes, and macrophages are believed to play a vital role in the pathogenesis of allergic reactions (Maurer et al., 1994;

Sutton et al., 2000; Owen, 2002). IgE is the major antibody in allergic diseases. The main physiological role of IgE is believed to be to protect the external mucosal surface of the body by the local recruitment of plasma factors and effector cells, by inducing an acute inflammatory reaction (Rao, 2002e). IgE is largely cell bound, especially to mast cells. Mast cell degranulation results from antigen cross-linking of IgE on cell surface receptors. However, several agents such as opioids and physical stimuli also activate mast cells and they do this independently of IgE (Machado et al., 1996).

The bridging of the high affinity FcεRI, by cross linking of IgE molecules on the membrane of mast cells, results in a sequence of downstream intracellular signalling, which leads to the recruitment and activation of several tyrosine kinases and associated adaptor molecules (Blank et al., 2001). This is rapidly followed by the following: breakdown of phosphatidyl inositol to inositol triphosphate (IP_3), the formation of diacyl glycerol and elevation of intracytoplasmic free calcium. As in the case of B cell activation, both IP_3 which mobilizes intracellular Ca^{2+} and diacyl glycerol are produced by phospholipase C activation. Moreover, diacyl glycerol results in activation and translocation of protein kinase C to the plasma membrane and phosphorylation of many different proteins. The biochemical activation of membrane generates membrane active *fusogens* such as lysophosphotidic acid, which are thought to facilitate granular membrane fusion, degranulation and production of arachidonic acid metabolites formed by the cyclo-oxygenase and lipoxygenase pathways (Rao, 2002d).

However, the classical pathway doesn't explain all allergic responses. For example, active systemic anaphylaxis developed in mice that were deficient in mast cells, IgE, or FcεRIα chain (Strait et al., 2002). Various studies have demonstrated the existence of other major distinct pathways resulting in allergen-triggered systemic anaphylaxis, which are mediated by basophils, IgG, IgG receptor, and plate-activating factor (PAF) (Tsujimura et al., 2008).

Moreover, Der p 1 (a major house dust mite allergen) and bee venom phospholipase A2 (major allergen in bee sting allergy), have been demonstrated to induce mast cell activation independently of the classical pathway (Machado et al., 1996). Interestingly, research studies showed that certain stimulators could induce biological mediators' release (i.e. histamine, IL-4, TNF) from unsensitized mast cells in the absence of detectable changes in intracellular Ca^{++} such as TSL-1 antigens from *Trichinella spiralis* muscle larvae (Arizmendi-Puga et al., 2006).

1.3 Mast cell inflammatory mediators

Cross linking of IgE and activation of mast cells by subsequent exposure to the specific allergen induce the release of a vast array of preformed mediators (i.e. histamine, serotonin, proteases) localised in specialised granules increasing blood vessel permeability and leukocyte extravasation. Activated mast cells also discharge many newly synthesised lipid mediators (prostaglandin D2, and leukotriene C4, platelet-activating factor) are released into adjacent tissues, which evokes the development of inflammation. These mediators have been suggested to be mainly involved in the acute-phase of inflammation. As the third class of mediators, the synthesis of cytokines and chemokines has been believed to be responsible for the delayed-phase reactions (Bingham & Austen, 2000; Williams & Galli, 2000).

Moreover, activation of non-IgE-dependent mast cells releases neurotransmitters, bacterial toxins, and substances of the complement system, which contribute to the tissue inflammation (Untersmayr & Jarolim, 2006).

1.3.1 Histamine and its receptors

Histamine, 2-(4-imidazolyl)ethylamine, is one of the smallest biomolecules synthesised and released by many cell types such as human basophils, mast cells, lymphocytes and neurons in response to both immunological and nonimmunological stimulation. It is believed to be the most predominant pharmacological mediator in allergy, asthma, autoimmunity, and malignancies (Dale & Laidlaw, 1910; Dale & Laidlaw, 1911; Akdis

& Blaser, 2003). Compelling evidence has shown that histamine can induce and/or modulate cytokine synthesis during allergic inflammation process (Marone et al., 2003). Increased secretion of pro-inflammatory cytokines (IL-1α, IL-1β, IL-6), chemokines (regulated on activation normal T cells, expressed and secreted (RANTES), IL-8) by histamine may facilitate the progression of many of the symptoms of allergy and inflammatory diseases (Vannier & Dinarello, 1993; Jeannin et al., 1994). Mast cell-derived histamine exits as its precursor histidine, along with heparin. It is formed by decarboxylation of the amino acid histidine, which is the major component of mast cell granules and constitutes about 10% of the granule weight (Dale & Laidlaw, 1910; Rao, 2002c; Akids & Blaser, 2003). The reaction is catalysed by the enzyme L-histidine decarboxylase (HDC). Nearly 97% of the histamine can be broken down by histamine-*N*-methyltransferase and diamine oxidase before excretion (Abe et al., 1993). The mechanism of biosynthesis and biotransformation of histamine was depicted in Fig. 1.3.

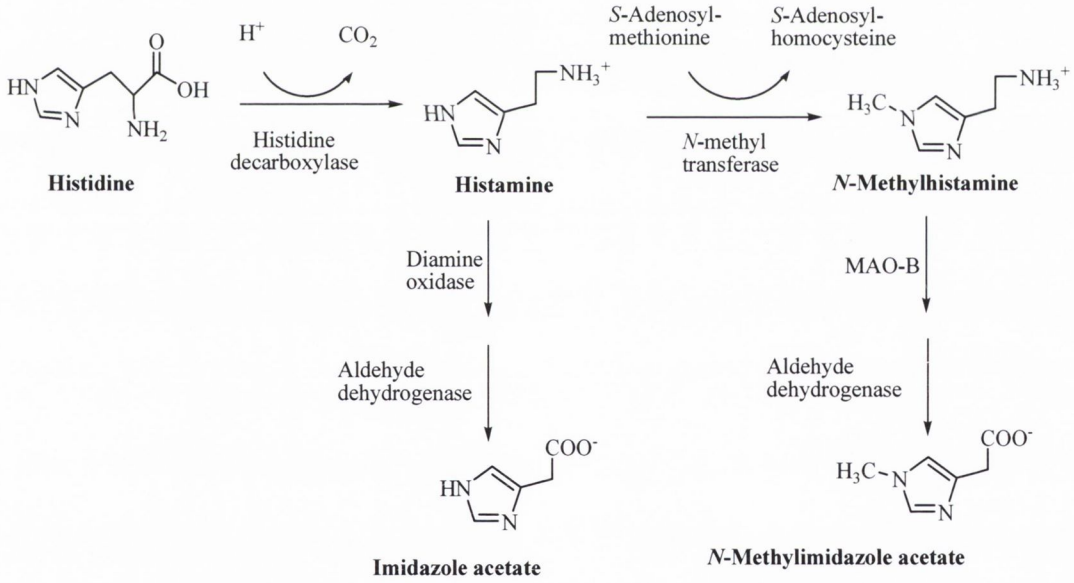


Fig. 1.3 Biosynthesis and metabolism of histamine (Falus et al., 2006)

In the brain, histamine-*N*-methyltransferase metabolises the majority of histamine to *N*-methylhistamine, which is further metabolised by monoamine oxidase-B (MAO-B) to form the derivative of imidazole acetic acid (*N*-methylimidazole acetate), while 15%-30% of histamine in the periphery is broken down by diamine oxidase to imidazole

acetate (Akdis & Blaser, 2003; Maintz & Novak, 2007). Histamine has been shown to be stored in brain and the cardiac sympathetic nerve system as a neurotransmitter, as well as in several tissue types, where it is regarded as non-mast cell derived histamine (Broide, 2001; Li et al., 2006). Its effects are observed immediately after activation and degranulation of mast cells.

It has been well established that the effects of histamine are mediated through 4 types of G-protein-coupled receptors: histamine H1, H2, H3, H4 receptors (Rao, 2002c; Akdis & Blaser, 2003). G-protein-coupled receptors possess the following structural features: 7 transmembrane domains, amino terminal glycosylation sites, and protein kinase A and protein kinase C phosphorylation sites (Gelfand, 2002). Histamine receptors exist in equilibrium between their inactive and active conformations (Akdis & Simons, 2006). Dimers or oligomers of histamine receptors form have been observed, resulting in cooperation between histamine receptors and other G protein-coupled receptors (Akdis & Simons, 2006). H1 and H2 receptor antagonists have been successfully used therapeutically. The existence of a fifth receptor subtype, namely an intracellular receptor H_{IC}, has been suggested due to the observation of low amounts of histamine within cells which not conventionally thought to be associated with histamine (MacGlashan, 2003).

1.3.1.1 Histamine H1 receptor

The H1 receptor is the most important histamine receptor subtype associated with acute inflammatory and allergic disorders (Hill et al., 1997). It has been detected in a wide range of tissues and organs, including mammalian brain, airway smooth muscle, gastrointestinal tract, genitourinary system, cardiovascular system, adrenal medulla, endothelia and various immune cells. Its predominant activities include intestinal and bronchial smooth muscle contraction, interaction with vascular endothelium resulting in vasodilatation, increased vascular permeability, increased mucous secretion by goblet cells as well as itchy wheals and flares in urticaria (Hill et al., 1997; Rao, 2002c; Akdis & Blaser, 2003). It has been reported that increased cyclic adenosine monophosphate

(cAMP) is also associated with stimulation of H1 receptors, by means of elevation in intracellular calcium level, even though most cAMP was upregulated by H2 receptor (Togias, 2003).

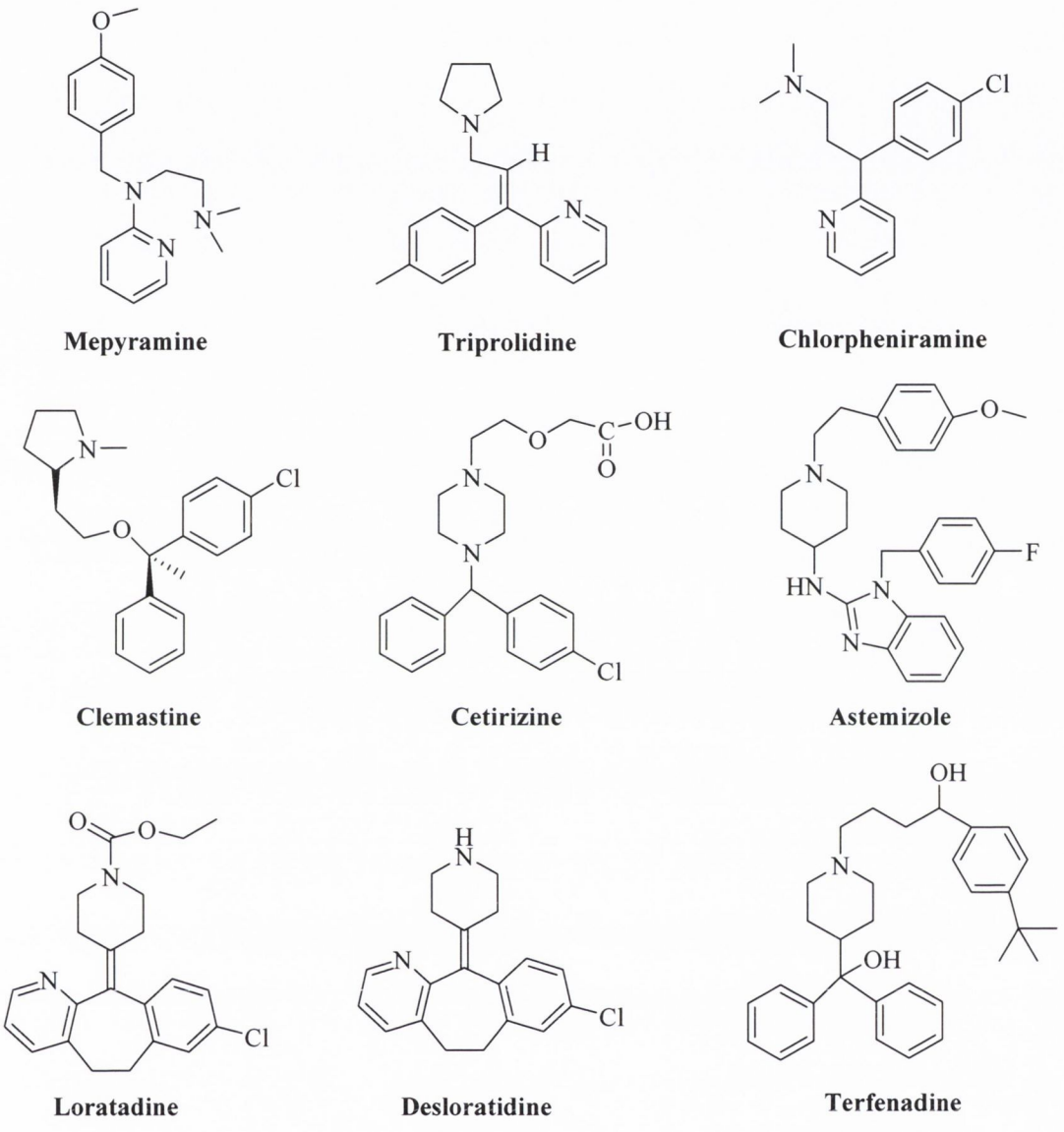
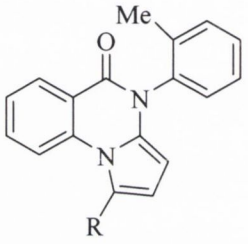


Fig. 1.4 Histamine H1-receptor antagonists

A number of H1 receptor antagonists, so-named as classical antihistamines, have been developed and investigated over the past years. Mepyramine, Triprolidine, Chlorpheniramine and Clemastine are examples of traditional antihistamines, which easily penetrate the brain and interact with central H1 receptors to produce bronchodilation. However, these drugs cause sedation as a common side effect (Hill et al., 1997; Gelfand, 2002). New generation antihistamines have been developed, with no

blood-brain-barrier passage and sedating side effects. Studies showed that these drugs, including Cetirizine, Astemizole, Loratadine, Desloratidine and Terfenadine provided highly variable effects on allergen-induced airway inflammation (Hill et al., 1997; Gelfand, 2002).



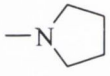
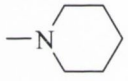
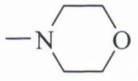
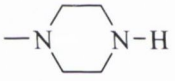
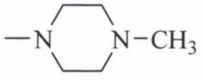
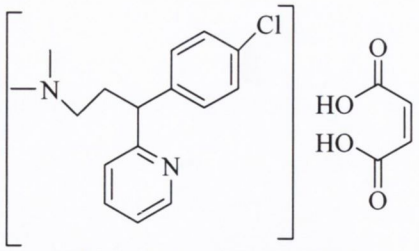
Compounds	R
1.0	—H
1.1	—CH_3
1.2	—CH_2CH_3
1.3	—$CH_2CH_2CH_3$
1.4	—CH_2-Cl
1.5	
1.6	
1.7	
1.8	
1.9	

Fig.1.5 Chemical structures of the series of 1-substituted-4-(2-methylphenyl)-4H-[1,2,4]triazolo[4,3-a]quinazolin-5-ones



Chlorpheniramine maleate

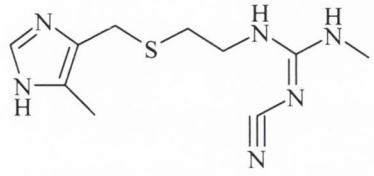
Fig. 1.6 Chemical structure of Chlorpheniramine maleate

A class of novel 1-substituted-4-(2-methylphenyl)-4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5

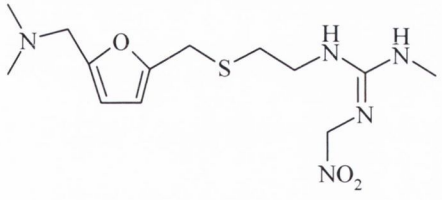
-ones have been developed possessing H1 antihistamine properties. An *in vivo* study indicated that all the compounds in this class (Fig. 1.5) showed great inhibitory effects on histamine-induced bronchospasm. Compound **(1.1)** {1-methyl-4-(2-methylphenyl) -4*H*-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one} was the most efficacious with 72.45% inhibition, in comparison with 71% for Chlorpheniramine maleate (Fig. 1.6). Negligible sedation as a side effect (11%) was seen compared to Chlorpheniramine maleate (30%) (Alagarsamy et al., 2008).

1.3.1.2 Histamine H2 receptor

Histamine H2 receptors are expressed on gastric mucosa, in uterus, and in brain, as well as on lymphoid and inflammatory cells (Gelfand, 2002). The H2 receptor is involved in a series of physiological histamine actions, such as regulation of right atrial and ventricular muscles of the heart and inhibition of basophil chemotactic responsiveness. It is believed that the principal role of the histamine H2 receptor is stimulating gastric acid secretion (Hill et al., 1997). Many compounds with H2 receptor antagonistic properties have been developed, including Cimetidine, Ranitidine, Tiotidine, Famotidine. The newly synthesised brain-penetrating H2 antagonist Zolantidine has been widely used as a powerful agent in central nervous system (CNS) research studies (Hill et al., 1997) (Fig. 1.7).



Cimetidine



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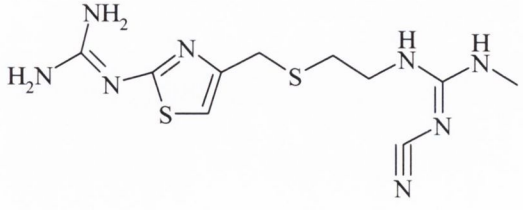
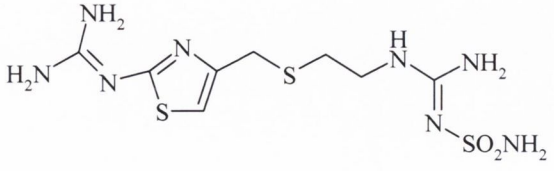
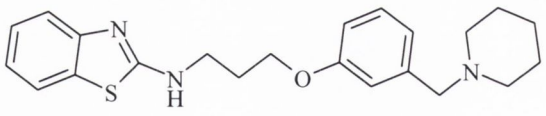


Tiotidine



Famotidine



Zolantidine

Fig. 1.7 Histamine H2-receptor antagonists

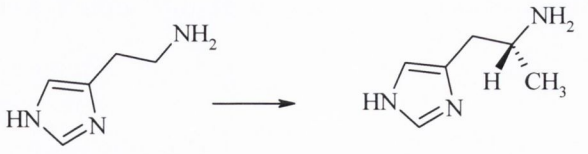
Increasing evidence shows that the H2 receptor antagonists such as Cimetidine have significant beneficial adjunctive effects in patients with immune suppression secondary to surgical trauma, burns, blood transfusion, sepsis, renal transplants, acquired immunodeficiency disease due to HIV, as well as in patients after surgical excision of colorectal, gastric, breast, ovarian cancers and melanoma (Akdis & Blaser, 2006; Akdis & Simons2006).

1.3.1.3 Histamine H3 receptor

Histamine H3 receptor was first identified in 1983 (Arrang et al., 1983). The association of H3 receptor with inflammatory processes has been well established. The H3 receptor is widely located in the CNS, and in bronchial smooth muscle. It regulates the release and synthesis of neuronal histamine in the nervous system, as well as the release of other neurotransmitters. In addition, peripheral histamine function in peripheral neurotransmission is also regulated by the H3 receptor, for example, in the cardiovascular system, gastrointestinal tract and airways (Hill et al., 1997; Leurs et al., 1998). Many studies show that the H3 receptor is involved in many CNS functions, including sleep/wakefulness, cognition, memory processes, and in conditions such as attention-deficit hyperactive disorder and epilepsy (Leurs et al., 1998; Passani, 2004; Leurs et al., 2005).

The very selective H3 receptor agonist, R-(α)-methylhistamine (Fig. 1.8), was successfully made through methylation of the α–carbon atom of the ethylamine side chain of histamine. Together with its less active S-isomer, this compound provides a novel approach for the pharmacological characterisation studies of H3 receptor-mediated

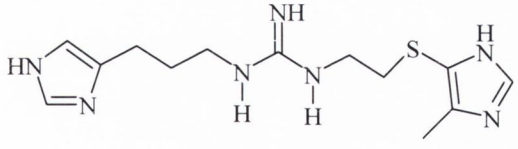
effects. The potential use of H3 receptor agonists has been suggested to treat myocardial ischaemic arrhythmias, inflammation, asthma, migraine (Leurs et al., 2005).



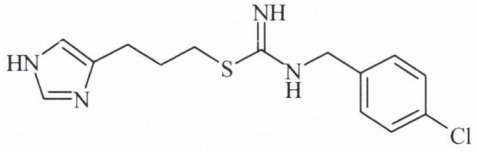
Histamine **R-(α)-methylhistamine**

Fig. 1.8 Methylation of histamine

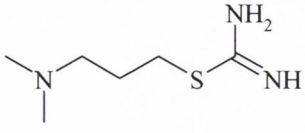
Interestingly, some H2 agonists can act as H3 receptor antagonists; including Impromidine and Dimaprit. Thioperamide, the most effective H3 receptor antagonist with excellent brain penetration, has been widely used. However, visceral discomfort has been reported as the major side effect of Thioperamide. Furthermore, the H3 receptor antagonist, Clobenpropit has been reported to have reduced CNS passage (Hill et al., 1997). Many other H3 receptor antagonists have been demonstrated to be potentially useful as anti-obesity drug. Thioperamide and Ciproxifan resulted in weight loss in rodents (Leurs et al., 2005; Hancock et al., 2005). In combination with H1 receptor antagonists, the H3 receptor antagonist is believed to be a novel approach for nasal decongestion (Leurs et al., 2005).



Impromidine



Clobenpropit



Dimaprit

Fig. 1.9 Histamine H3-receptor antagonists

1.3.1.4 Histamine H4 receptor

The existence of the histamine H4 receptor was first announced in 2002, after the sequencing of the human genome and cloning of the human H4 receptor cDNA from fetus (De Esch et al., 2005). The H4 receptor shows 40% homology with the H3 receptor. It is believed that the H4 receptor is located predominantly on haematopoietic and immunocompetent cells, with high level expression in mast cells, peripheral blood mononuclear cells, eosinophils, neutrophils and bone marrow. The H4 receptor activation induces the accumulation of inflammatory cells (particularly mast cells) at sites of allergic inflammation. This suggests that the H4 receptor has a potential role in mediating immune or inflammatory responses (De Esch et al., 2005; Akdis & Simons, 2006).

Histamine-induced chemotaxis and migration of mast cells have been demonstrated to be mediated by the H4 receptor (De Esch et al., 2005; Akdis & Simons, 2006). In combination with the H2 receptor, the H4 receptor has been suggested to be involved in the release of IL-16 from human lymphocytes, and a selective H4 antagonist was thought to be a novel approach in treating asthma (De Esch et al., 2005; Parsons & Ganellin 2006). Recently, many studies showed the H4 receptor was involved in histamine-induced pruritus, thus H4 receptor antagonists might also be a new therapy in treating such disease (De Esch et al., 2005). Moreover, immunohistochemistry has confirmed the presence of H4 receptor in nerves from the human nasal mucosa, which indicated that the H4 receptor might play a role in CNS functions (De Esch et al., 2005).

Many of the known H3 receptor agonists and antagonists exhibit significant binding affinity to the H4 receptor (Jablonowski et al., 2003). OUP16 and 4-methylhistamine are the most potent, selective H4 receptor agonists. JNJ7777120 and its analogue VUF6002 are identified as the most effective, selective H4 receptor antagonist, and the former has thus been widely used as a radioligand for H4 receptors, as well as a pharmacological tool in many research studies (Parsons & Ganellin 2006) (Fig. 1.10).

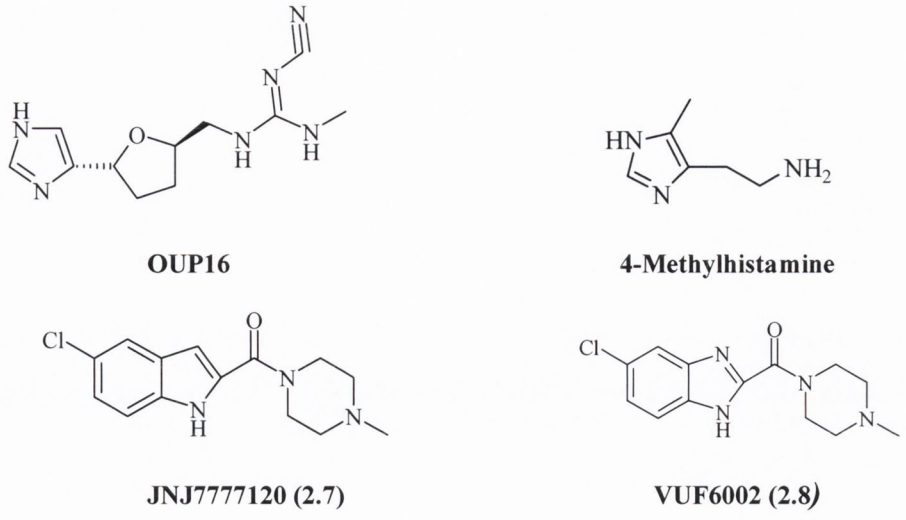


Fig. 1.10 Histamine H4-receptor antagonists

1.3.2 Mast cell-specific serine proteases

It is an undisputed fact that serine proteases, including tryptic enzyme tryptase, the chymotryptic enzyme chymase, cathepsin G, and carboxypeptidase, are associated with inflammation processes, tissue remodelling, bronchoconstriction, and mucus secretion. (Walls, 1998; He et al., 1999). The released proteases may generate additional mediators from precursor proteins, which are suggested to stimulate further mast cell activation.

1.3.2.1 Tryptase

Human mast cell tryptase is a neutral tetrameric serine protease, which constitutes about 20% of the total protein within human mast cells (Pallaoro et al., 1999; Levi-Schaffer & Piliponsky, 2003). Tryptase has been divided into two main types, α-tryptase and β-tryptase. Each subunit has one active site (Schwartz, 2001). Evidence has emerged that the release of tryptase is a chief feature of mast cell degranulation as it has been found to have a role in airway homeostasis, psoriatic skin, angiogenesis, vascular relaxation and contraction, gastrointestinal smooth muscle activity and intestinal transport, mediated by activation of protease activated receptor type 2 (PAR2) (Blair et al., 1997; Reed et al., 2003). Inhibitors of tryptase were discovered to possess the ability to inhibit human skin, tonsil, synovial and colon mast cells (Blair et al., 1997). Many studies have shown that

the level of released tryptase is useful to investigate possible anaphylactic reaction during anaesthesia (Fisher & Baldo, 1998). Increased mast cell tryptase levels are highly suggestive of an immune reaction but may reflect direct histamine release. In addition to mast cells, tryptase can also induce the activation and growth of eosinophils and neutrophils causing alterations in cell behaviour (Walls et al., 1995; He et al., 1997), such as triggering the release of inflammatory cytokines (as growth factor) from epithelia and endothelial cells (Cairns & Walls, 1996; Compton et al., 1998), and the release of collagen and collagenase from fibroblasts (Cairns & Walls, 1997).

1.3.2.2 Chymase

Recent discoveries have implicated chymase as a key mediator in cardiovascular and allergic inflammation, and fibrotic disorders (Vegi et al., 2008). Chymase is a 137 amino acid, cathepsin G-like, an inactive enzyme found primarily in secretory granules of mast cells (McEuen et al., 1995; Muramatsu et al., 2000). Mast cell chymase is particularly abundant in the dermis of human skin.

Chymase is believed to play a crucial role in cardiovascular diseases, adhesion formation and fibrosis via generating angiotensin (II) from angiotensin (I) through hydrolysis, which contributes to vascular proliferation and apoptosis of vascular smooth muscle cells (Muramatsu et al., 2000; Okamoto et al., 2002; Takai et al., 2004). Over expression of vascular chymase was found to result in the occurrence of hypertension in transgenic mice (Ju et al., 2001). Chymase is also able to activate matrix metalloproteinase 9 (MMP-9), which is suggested to be important for the development of aneurysms and angiogenesis (Takai et al., 2004).

1.3.3 Serotonin

Serotonin is another mediator of immediate hypersensitivity reactions released during mast cell degranulation. Serotonin is derived from tryptophan and stored in the CNS of all species. Its chief function is acting as a neurotransmitter. Serotonin is also stored in

platelet granules and is released during blood clotting. In addition, serotonin can be found in the enterochromaffin cells of the gastrointestinal tract mucosa. Like histamine, serotonin has receptor-mediated vasoactive activities causing contraction of smooth muscle, increase in the respiratory rate, pain production, depression of CNS activity and further stimulation of histamine release. Recently, it has been found that human mast cells are able to synthesis and release serotonin (Mossner & Lesch, 1998; Kushnir-Sukhov et al., 2007; Ringvall et al., 2008).

1.3.4 Lipid mediators

Lipid mediators, a complex group of chemical mediators, include leukotrienes, prostaglandins (PGs) and thromboxanes, as well as platelet-activating factor (PAF) (Rosenberg & Gallin, 2003). These lipid mediators participate in both the acute and the chronic inflammatory responses. In particular, they act rapidly to induce smooth muscle contraction, elevated vascular permeability, mucus release, and cause the influx and activation of leukocytes which result in the late phase of the allergic response (Murphy et al., 2008).

1.3.4.1 Leukotrienes and slow-reacting substance of anaphylaxis

Leukotrienes, leukocyte-derived group of secondary mediators, are the oxidation products of arachidonate (Rosenberg & Gallin, 2003) and are only synthesised following mast cell degranulation (Sjöström et al., 2002). It is believed that leukotrienes contribute to a series of proinflammatory activities such as vasoconstriction, increased vascular permeability, elevated endothelial adhesiveness, as well as neutrophil chemotaxis and activation (Bulger & Maier, 2000; Dahlen, 2000). Regulating the function of leukotrienes in the control of asthma has been well established (Bisgaard, 2001).

Slow-reacting substance of anaphylaxis (SRS-A) is a mixture of three-leukotrienes (i.e. LTE_4, LTC_4, LTD_4), which are mainly produced by macrophages, eosinophils, basophils as well as mast cells (Hayes et al., 1983; Lotti et al., 2000). Ishizaka et al., (1972)

demonstrated the existence of SRS-A as mast cell mediator by the observation of the histamine and SRS-A release through IgE-anti-IgE reactions on monkey mast cells. SRS-A can cause prolonged smooth muscle contraction, induce edema by capillary dilation, and stimulate mucus secretion. (Lotti et al., 2000; Gao & Raj, 2005). It has been reported that SRS-A has a particularly marked bronchial constricting effect in man and is probably the predominant pharmacological mediator in human asthma (Koshino et al., 1985).

1.3.4.2 Prostaglandins (PGs) and Thromboxanes

Mast cells also release prostaglandins and thromboxanes following degranulation. Prostaglandin D2 is the major prostaglandin produced by mast cell, eosinophils, and basophils. When allergen binds to IgE on the mast cell, the cell membrane is perturbed, resulting in enzymatic breakdown of phospholipids, and release of arachidonic acid, the precursor of PGs and thromboxanes. These mediators show pronounced and long lasting effects, which act as vasodilators, bronchoconstrictiors, and regulators of blood coagulation (Schulman et al., 1981; Murphy et al., 2008).

1.3.4.3 Platelet-activating factor

Platelet-activating factor (PAF) is a substituted derivative of glycerol phosphate which exists in both circulating and cellular forms (Rosenberg & Gallin, 2003). The production of PAF is mainly caused by the remodelling pathway in a wide array of cell types (i.e. platelets, neutrophils, eosinophils, macrophages, vascular endothelial cells and mast cells) in response to cell-specific stimulators (Dent et al., 1989). It has been widely reported that IgE-dependent mast cell activation induces the production and release of PAF, which is involved in many allergic inflammation processes, particularly affecting the respiratory, vascular, digestive and reproductive system (Nakajima et al., 1997). Biosynthesis of PAF in cultured mast cells has been suggested to occur via a mechanism in which the CoA-independent transacylase is involved (Colard et al., 1993). Moreover,

Mustafa & Pearce, (2005) have demonstrated that PAF has the ability to generate a dose-dependent histamine release from rat peritoneal mast cells.

1.3.5 Immunoregulatory cytokines

Cytokines, proteins and glycoproteins with low molecular weight, and acting as chemical messengers in the immune system, are released by a variety of cell types in the body. Cytokines such as interleukin IL-3, IL-4, IL-9, IL-10 and RANTES can control mast cell differentiation (Wedemeyer et al., 2000; Puxeddu et al., 2003; Theoharides et. al., 2004). IL-3, IL-4, IL-5, IL-6 also maintain the survival of mast cells (Bradding & Holgate, 1999). Nerve growth factor (NGF) released from nerve endings has been found to be involved in mast cell differentiation, survival and growth (Puxeddu et al., 2003).

Mast cell-derived cytokines, for instance IL-1, IL-6, IL-8, tumour necrosis factor-α (TNF-α), are believed to play both a physiological and a pathological role in innate and acquired immunity, inflammation, wound healing as well as tumor growth. In addition, mast cells produce cytokines such as IL-4 and IL-13, and increased levels of IL-4 and IL-13 have been found in mast cell related atopic dermatitis (AD) (Obara et al., 2002). Temann et al., (2002) further confirmed that IL-9 incites the mast cell proliferation in mucosal tissues, which is significantly associated with allergic airway disease (Hauber et al., 2004), such as bronchial hyper-responsiveness (McLane et al., 1998), IgE production (Dugas et al., 1993) and mucus hypersecretion (Louahed et al., 2000).

In contrast with mast cell survival factor (SCF), transforming growth factor (TGF) β1 (a member of TGFβ superfamily of structure related cytokines) showed inhibitory effects on mast cell development (Kashyap et al., 2005), expression and function of IgE receptors (Gomez et al., 2005), and even provoked mast cell apoptosis (Norozian et al., 2006).

The tumour necrosis factor family contains tumour necrosis factor-α (TNF-α), lymphotoxin-α (LT-α, also referred to as TNF-β) and lymphotoxin-β (LT-β). TNF-α is a protein that can remain attached to the membrane by its long signal sequence until it is processed by a matrix metalloproteinase. TNF-α is produced by monocytes and macrophages, as well as T and B lymphocytes and fibroblasts. Mast cells are suggested to be the largest source of pre-formed TNF-α in the body (Theoharides & Conti, 2004). TNF-α alters the growth and morphology of endothelial cells that line the blood vessels supplying the tumour, increases the synthesis of factors favouring blood clotting and enhances endothelial cell adhesiveness for inflammatory cells. However, TNF-α also has cytotoxic or cytostatic effects on tumour cells, probably mediated by prostaglandins, proteases or free radicals. TNF-α exists as trimers, all three forms being biologically active. TNF-α may have antiviral effects, although it is possible that these are actually mediated by IL-6, whose production it stimulates. It has been demonstrated that TNF-α expression is increased markedly in severe asthma, as shown by increased protein in bronchoalveolar lavage (Berczi et al., 2003; Berry et al., 2006).

1.3.6 Epidermal growth factor and vascular endothelial cell grow factor

Epidermal growth factor (EGF) is a polypeptide containing 53 amino acids (Kang et al., 1998). EGF and TGF-α are the two members of this family, which are only peripherally involved in immunity. EGF and TGF-α express similar biological effects by binding to the same receptor, epidermal growth factor receptor (EGFR) (Kramer et al., 1994). EGF has been proven to be the mitogenic molecule that plays an important part in the control of normal cell growth (Boonstra et al., 1995), and in wound healing (Mian et al., 1992) by accelerating epithelisation, increasing tensile strength and decreasing histamine levels in mast cells (Babul et al., 2004). Erkasap et al., (2004) have elucidated that the protective effect of EGF on gastric mucosa to ethanol-induced gastric injury might be due to its mast cell stabilising and antioxidant properties.

Mast cells also generate vascular endothelial cell growth factor (VEGF) which induces angiogenesis (the development of new blood vessels) and vascular permeability (Hiromatsu & Toda, 2003; Croll et al., 2004). It has been believed that VEGF is vital for developmental, physiologic and pathologic angiogenesis. In increased levels of VEGF can be observed before or during the anagiogenic process in many diseases such as, rheumatoid arthritis, endometriosis and tumors (Maharaj & D'Amore, 2007). In brain, receptors for VEGF have been localised to vascular endothelium, neurons, and glia (Levi-Schaffer & Pe'er, 2001). VEGF is upregulated after hypoxic injury to the brain, which can occur during cerebral ischemia or high-altitude edema, and has been implicated in the blood-brain barrier breakdown associated with these conditions (Croll et al., 2004). Given its recently-described role as a potent inflammatory mediator, VEGF can also contribute directly or indirectly to the inflammatory responses observed in cerebral ischemia, trauma, tumor ascites, etc. (Croll et al., 2004; Maharaj & D'Amore, 2007). Study suggested that neuroinflammatory conditions might be exacerbated by the over-expression of VEGF (Kirk & Karlik, 2003). Lower level of extremity of edema and hypotension was found in clinical trails when using VEGF as a clinical therapy (Baumgartner et al., 2000).

1.4 Mast cells associated disorders

Since these biological mediators exert a broad range of biological effects, mast cells contribute to a broad spectrum of allergic diseases and inflammatory diseases, including those of the pulmonary system, the central nervous system and the gastrointestinal tract (Esposito, 2001; Puxeddu et al., 2005; Theoharides & Cochrane, 2004; Chavarria & Varela, 2004).

1.4.1 Allergic diseases

1.4.1.1 Asthma

WHO claimed that over 300 million people suffered from asthma, and 255,000 died of asthma in 2005 (Bloemen et al., 2007). It is the most common chronic complex inflammatory disorder and is characterised by smooth muscle contraction and narrowing of the airway lumen, eventually causing airflow obstruction, dyspnoea, mucus secretion, and mucosal edema (Peachell, 2005; Bradding et al., 2006). Various cells types are involved in pathophysiology of asthma, including T cells, basophils, eosinophils, neutrophils, macrophages, dendritic cells, endothelial cell, etc. However, most crucial mediators in the early-phase asthmatic reaction, such as histamine, proteases, proteoglycans, prostaglandins, leukotrienes, cytokines, chemokines, are synthesised and secreted by mast cells (Gelfand, 2002; Bloemen et al., 2007). The observation of mast cell microlocalisation in asthmatic airway smooth muscle bundles shows that airway smooth muscle infiltration by mast cells can be used as indicator for asthmatic phenotype (Bradding et al., 2006). The location of mast cells within airway epithelium and submucosal glands indicates that mast cells are also important in regulating mucous gland secretion (Bradding et al., 2006). Strong evidence shows that many mast cell-derived mediators are involved in wall thickening, subepithelial fibrosis, hyperplasia of airway cells, cartilage break down, angiogenesis known as airway remodelling (Bradding et al., 2006; Okayama et al., 2007).

1.4.1.2 Allergic rhinitis

Allergic rhinitis (AR), another common atopic disease, is a nasal inflammatory state characterized by sneezing, rhinorrhea, nasal congestion and nasal itching or postnasal drainage (Rosenwasser, 2002). More than 600 million patients suffer from this disease worldwide (Mullol et al., 2008). The very close relationship between AR and asthma has been well documented, such as having a common immunopathology and pathophysiology. Statistics show that over 80% of patients with allergic asthma tend to develop AR (Gelfand, 2004).

It is well known that nasal epithelial mast cells are responsible for the outset of the AR response, as well as being key to maintaining the disease process in a chronic phase. Exposure of the nasal mucosa to allergens (i.e. pollens, dust mites, moulds, etc) leads to the accumulation and degranulation of IgE-sensitised mast cell, which triggers the rapid release of preformed and newly synthesized mediators (Salib et al., 2004). Histamine, tryptase, cytokines (such as, TNF-α, IL-4, IL-5, IL-6, IL-13), prostaglandins and leukotrienes are involved during the initial phase of AR (White & Kaliner, 1992). During the late phase of the AR response, infiltration of nasal mucosa with mast cells, basophils, eosinophils, neutrophils, and mononuclear cells develops (Gelfand, 2004). In addition, the involvement of an influx of basophils at the late phase is suggested to be due to the increase of histamine release in spite of unchanged levels of tryptase (Baraniuk, 1997).

The accumulation of mast cells within the epithelium of patients with naturally occurring AR might be attributed to epithelial expression of mast cell chemoattractants, transforming growth factor-β (TGF-β). Moreover, the correlations between epithelial immunoreactivity of TGF-β1, TGF-β2, TGF-β receptor I, TGF-β receptor II and the number of mast cells have also been established (Salib et al., 2004).

Furthermore, an increased expression of IL-9 leading to the seasonal development of c-Kit$^+$ mast cell growth in the nasal mucosa has been observed, which suggests IL-9 might be a new therapeutic target for the treatment of chronic mast cell related allergic inflammation (Nouri-Aria et al., 2005).

1.4.1.3 Allergic conjunctivitis

A recent study indicated that about 35% of families surveyed have suffered from allergic reactions, with more than 50% of this group reporting allergies associated with eye symptoms (Stahl & Barney, 2004). Allergic conjunctivitis is a group of inflammatory diseases affecting the ocular surface. Type 1 anaphylactic hypersensitivity mechanism, also named as IgE mediated mechanism, is believed to be a vital role in the pathogenesis

of seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC), which are the most common forms of ocular allergy (Friedlaender, 1995). Atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC) are chronic allergic diseases, affecting just about 2% of all ocular allergies. AKC and VKC can lead to remodelling of the ocular surface tissues, through more complex pathogenesis (Ono & Abelson, 2006).

Conjunctival mast cells are connective tissue type and are located beneath the epithelium, adjacent to small venules (Bundoc & Keane-Myers, 2007). It is well known that mast cell degranulation can be detected in most biopsy samples obtained from SAC patients. Conjunctival mast cell activation and release of mediators, histamine as primary contributor, result in the development of early-phase response in conjunctivitis with clinical symptoms: itching, tearing, redness, swelling of the conjunctiva (Ono & Abelson, 2006). In addition, mast cell activation causes the synthesis of arachidonic acid from membrane phospholipids. Arachidonic acid further generates prostaglandins and thromboxanes, causing redness, itching, eosinophil infiltration and mucous discharge (Bisca, 1997).

A recent *in vivo* study indicated that IL-10, a regulatory cytokine, can stabilise mast cells and prevent compound 48/80 (a well known mast cell secretagogue) stimulated mast cell degranulation in a mouse model of allergic conjunctivitis (Bundoc & Keane-Myers, 2007). Increasing attention has focussed on IL-10 as one of the targets in allergic conjunctivitis treatment.

1.4.2 Autoimmune Disorders

Autoimmunity occurs when the immune system fails to recognise the self-components of the body, thus evokes an immune response and the production of auto-antibodies and auto-reactive T cells (Roitt, 2006; Rao, 2002f). Autoimmune mechanisms are associated with many diseases: organ-specific (i.e. Hashimoto's thyroiditis, Addison's disease, myocardial infarction, etc), and systemic autoimmune diseases (i.e. Rheumatoid arthritis,

multiple sclerosis, Sjogren's syndrome, etc) (Konttinen et al., 2000; Rao, 2002d). The potential role for mast cells in the complex, long, convoluted molecular and cellular chains which result in autoimmune disease has been well discussed over many years (Benoist & Mathis, 2002). Interestingly, most autoimmune disorders are more common in females than in males. The reason for this gender difference is still under investigation (Theoharides & Cochrane, 2004).

1.4.2.1 Rheumatoid arthritis

Rheumatoid arthritis (RA), first named by Dr. Garrod in 1858 (Kimpel, 2005), is a chronic, synovial inflammatory disease marked by cartilage destruction, bone erosion, reduction in bone integrity and severe joint deformity (Kobayashi & Okunishi, 2002). In addition to dendritic cells, B lymphocytes, plasma cells, osteoclasts and mast cells, T lymphocytes, macrophages and fibroblast-like synoviocytes are believed to be major contributors to the pathology of RA (Maruotti et al., 2007). RA is considered as the product of interaction between cell populations in RA synovium.

Mast cells (MC_{TC} subtype) mainly constitute up to 3% of the cells in normal synovial membrane (Crisp, 1984; Maruotti et al., 2007). Involvement of mast cells in adjuvant induced arthritis (AIA), collagen-induced arthritis (CIA) as well as streptococcal cell wall-induced arthritis has been confirmed (Bakharevski & Ryan, 1999). Evidence shows that mast cell numbers are increased in the periphery of the lymphoid infiltrates, sites of cartilage erosions, joint fluids and the areas of active fibrosis in RA (Bromley & Woolley, 1984; Tetlow & Woolley, 1995a; Gotis-Graham & McNeil, 1997; Bakharevski & Ryan, 1999). Gotis-Graham & McNeil (1997) have demonstrated a positive correlation between the density of mast cells and RA severity. In the early phase of RA, MC_T was found to be present in the lymphoid infiltrate and hyperplastic lining layer (Gotis-Graham et al., 1998). In contrast, MC_{TC} was found in the areas of cellular fibrosis in the late phase of RA (Gotis-Graham & McNeil, 1997).

Elevated levels of mast cell mediators incite edema, destroy connective tissue, and are also responsible for lymphocyte chemotaxis, infiltration and pathological fibrosis of RA joints (Maruotti et al., 2007). The examples of those mediators are histamine, heparin, chymase, tryptase and cytokines (i.e. IL-6, IL-8, TNF-α, etc), mast cell chemo-attractants (i.e. transforming growth factor-β, platelet activating factor, etc), RANTES, prostaglandins and leukotrienes (Bromley & Woolley, 1984; Malone et al., 1986; Matsushima & Oppenheim, 1989; Schwartz, 1994; Conti et al., 1997). In addition, Tetlow & Woolley (1995b) showed mast cell mediators were associated with the production of matrix metalloproteinases (MMPs) which were thought to be the contributors to cartilage destruction and bone erosions in RA. Moreover, macrophages cause further IL-1 release by activated mast cells (Yoffe et al., 1984). Furthermore, the involvement of mast cells in angiogenesis during RA causing cartilage destruction and bone remodeling, has also been confirmed (Hiromatsu & Toda, 2003).

1.4.2.2 Multiple sclerosis

Multiple sclerosis (MS) is a major demyelinating, chronic inflammatory disease affecting the CNS (Behi et al., 2005). MS is characterised by relapsing-remitting episodes which cause progressive disability, clinical symptoms include visual and sensory impairment, bowel and bladder dysfunction, severe fatigue and paralysis (Stinissen et al., 1997; Ewing & Bernaed, 1998).

Mast cells have long been known to be involved in MS. Documented evidence can be dated from as early as 1890 (Zappula et al., 2002). Activation and proliferation of mast cells were identified both inside and around demyelinated plaques in the CNS of MS patients (Ibrahim et al., 1996). Moreover, elevated levels of mast cell-derived mediators, such as tryptase, histamine, TNF-α, IL-1β and IL-6, were also observed in MS lesions (Bebo et al., 1996; Malamud et al., 2003; Theoharides & Cochrane, 2004). Interestingly, a decreased number of mast cells around plaques in MS patients with acute lesions was shown, compared to those with chronic active plaques (Behi et al., 2005).

It has been well documented that blood brain barrier (BBB) disruption is associated with many pathological or clinical symptoms of MS. A breakdown of the BBB was thought to be the early and key stage in the development of new MS lesions (Kermode et al., 1990). Malamud et al., (2003) showed tryptase could alter the permeability of the BBB of MS patients by secreting bradykinin. Growing evidence indicated that acute stress could also activate mast cells resulting in increased permeability of BBB by the activation of H2 receptor, which precipitated or exacerbated symptoms of MS (Esposito et al., 2001; Theoharides & Cochrane, 2004). Likewise, mast cell mediators sustained such alteration of BBB permeability (Mayhan, 2001).

Mast cells act as antigen-presenting cells to mediate destruction of the myelin sheath and the neuronal cells in MS, which results in the demyelineation and the secretion of several myelin antigens (Johnson et al., 1988; Behi et al., 2005). Such antigens subsequently interact with T cells by means of mast cells, promoting inflammation and epitope spreading(Zappula et al., 2002). Several studies indicated that sex hormones, environmental factors and genetic factors could modulate the pathophysiology of MS. (Zappula et al., 2002). However, the pathogenesis and the nature of the autoantigen(s) that induce this autoimmune disease are still under investigation.

1.4.3 Disorders Involving Mast Cell

1.4.3.1 Angiogenesis

Angiogenesis is defined as the development of new blood vessels from pre-existing microcirculation (Risau et. al., 1997), and is vital for the regulation of physiological functions, for example wound healing, embryonic development and formation of collateral circulation in ischaemic tissue, etc (Hiromatsu & Toda, 2003). It is also related to many other pathological conditions such as growth, maintenance and metastasis of solid tumours (Folkman, 1995), chronic inflammatory disorders (Muramatsu et al., 2000) as well as diabetic retinopathy (Alon et al., 1995). Strong evidence demonstrates the formation and growth of angiogenesis is associated with mast cells (Hiromatsu & Toda,

2003). In addition, mast cell accumulation has been observed within and around the tumor angiogenesis in colorectal cancer (Lachter et al., 1995; Acikalin et al., 2005), cervical cancer (Benitez-Bribiesca et al., 2001), breast carcinoma (Kankkunen et al., 1997), pulmonary adenocarcinoma (Imada et al., 2000), basal cell carcinoma (Yamamoto et al., 1997), soft tissue tumors (Donhuijsen et al., 1992), and melanoma (Doncan et al., 1998), gastric cancer (Kondo et al., 2006), oral squamous cell carcinoma (Iamaroon et al., 2002) and human endometrial carcinoma (Ribatti et al., 2005).
The codependent relationship between angiogenesis and chronic inflammation has been established (Puxeddu et al., 2005). It is notable that many mast cell mediators are angiogenic and contribute to tube formation, function and proliferation of endothelial cells (Hiromatsu & Toda, 2003). These include heparin, histamine, IL-8 (known as an angiogenesis factor or tumor cell chemotactic factor), TNF-α, proteases (Blair et al., 1997; Theoharides & Conti, 2004).

It has been observed that higher levels of mast cell-derived tryptase present in malignant breast lesions than in benign lesions, with similar finding in the mast cell-mediated human renal tissue tumour (Ribatti et al., 2005). Furthermore, chymaste, acting as a pro-angiogenic factor, is associated with basic fibroblast growth factor (FGF)-induced angiogenesis as well as mast cell-dependent angiogenesis in hamster sponge granulomas (Muramatsu et al., 2000), lung cancer (Ibaraki et al., 2005) and gastric cancer (Kondo et al., 2006).

Interestingly, considerable findings demonstrate that certain mast cell mediators can also induce apoptosis of tumor cells, for instance, IL-1, IL-4, IL-6 and TNF-α (Gordon & Galli, 1990; Gooch et al., 1998; Theoharides & Conti, 2004). As the most vital growth factor, TNF-α can stimulate angiogenesis as well as cause tumor regression which might result in coagulation, haemorrhage and necrosis of the tumor by bloodstream administration into the tumor vessels. By contrast, if TNF-α is delivered at the exterior of the blood vessel as a result of the synthesis by macrophages, mast cells or tumor cells, migration and proliferation of endothelial cells may occur (Fajardo et al., 1992; Lichtenbeld et al., 1996).

Therefore, angiogenesis has been suggested to be a novel target for anticancer therapy, as well as for the treatment of pathological neovascularisation, for example hypoxic retina (Aiello et al., 1995; Folkman et al., 1996; Levi-Schaffer & Pe'er, 2001).

1.4.3.2 Irritable bowel syndrome

Irritable bowel syndrome (IBS), one of the most common chronic gastrointestinal tract disorders (Santos et al., 2005), is characterised by the presence of abdominal pain, altered bowel motility causing either diarrhea or constipation, increase in visceral hypersensitivity (Thompson et al., 1999; Rijnierse et al., 2007) and an overactivation of the hypothalamic-pituitary-adrenal axis (Dinan et al., 2006). Both central nervous system and peripheral mechanisms (i.e. gastrointestinal tract) are widely accepted to be related to the origin of abdominal pain in IBS (Drossman et al., 2002).

The presence of mast cells in IBS was first documented in 1890, when increased numbers of mast cells were discovered in colonic biopsies from spastic colon patients (Rijnierse et al., 2007). Activation of mast cells located in close vicinity (<5μm) to enteric nerve fibers in the colonic mucosa (Barbara et al., 2004a), caecum (O'Sullivan et al., 2000; Park et al., 2003) and rectum, was observed in IBS patients (Park et al., 2003). Notably, Barbara et al., (2004b) indicated that the number of activated mast cells around nerve endings in the gut wall correlated with the frequency and severity of abdominal pain in IBS patients. Increased levels of intestinal and colonic mucosa mast cell mediators (i.e. histamine, tryptase, IL-6, IL-8), and low–grade infiltration of inflammatory cells have been described in IBS patients (Barbara et al., 2004a, 2004b; Dinan et al., 2006).

Mast cell-nerve interactions have been proposed to account for the pathophysiology and symptom development of IBS, and the exacerbation of the inflammation in IBS (Rijnierse et al., 2007). Indeed, bidirectional interactions between mast cells and nerves have been well established. Mast cell activation is triggered by neuronal mechanisms;

conversely, mast cell mediators can act as principle transducers of information between local inflammatory events and peripheral nerves (Rijnierse et al., 2007). Excitation of nociceptive visceral sensory nerves caused by mucosa mast cell mediators in IBS has also been reported (Barbara et al., 2007). However, direct mast cell and nerve interaction is still not well established. Psychological factors (i.e. stress, emotions), bacterial toxins, food hypersensitivity and gastrointestinal infection are suggested to be important in the pathophysiology of IBS (Barbara et al., 2002; Santos et al., 2005).

Post-infective irritable bowel syndrome (PI-IBS) refers to patients who develop IBS symptoms after an acute enteric infection (Collins et al., 1999). Clinically, Dunlop et al., (2003) demonstrated that PI-IBS patients are a distinct subgroup marked by diarrhea symptoms, less psychiatric illness, and increased serotonin-containing enterochromaffin (EC) cells compared to patients with non-PI-IBS.

1.4.3.3 Mastocytosis

Mastocytosis is an unusual disorder characterised by significantly abnormal growth and proliferation in mast cell density (Tharp, 1997; Metcalfe et al., 2001) and associated pathologic changes in numerous organ systems, including skin, bone marrow, liver, spleen, lymph nodes, and GI tract (Marone et al., 2001). With a better understanding of the clinical presentation of mastocytosis, a categorization scheme was developed by the WHO (Pardanani et al., 2006):

- Systemic mastocytosis (SM) usually develops in adults (Valent et al., 2001) with gastrointestinal symptoms: abdominal pain, diarrhea, nausea, and vomiting (Jensen, 2000).
    1. Indolent systemic mastocytosis (ISM) is usually associated with hemodynamic instability, maculopapular skin lesions and good prognosis (Valent et al., 2001).
    2. SM associated with a hematologic non-mast cell lineage disorder (AHNMD). Increased numbers of mast cells in one or more organs are

observed in patients with AHNMD. Clinically, bone marrow abnormality is commonly (Valent et al., 2001).

3. Aggressive systemic mastocytosis (ASM) which is a rapid, progressive condition without skin involvement. ASM is usually associated with destruction of organ function resulting from the infiltration of the bone marrow first, and then gastrointestinal tract, liver, spleen and lymph nodes via pathologic mast cells (Valent et al., 2001; Wolff et al., 2001).
4. Mast cell leukaemia (MCL), a primary leukemic process, is characterized by increased numbers of mast cell in bone marrow smears and peripheral blood, absence of skin lesions, poor prognosis. However, the incidence of MCL is rare (Valent et al., 2001).

- Non-systemic mastocytosis encompasses cuaneous mastocytosis and mast cell sarcoma (MCS

The most commonly involved organ affected by mastocytosis is the skin (Wolff et al., 2001). Cutaneous mastocytosis (CM) tends to develop in infancy or childhood and has a good prognosis with low incidence of systemic involvement. Spontaneous remissions are often observed in puberty (Valent et al., 2001).

CM in most patients is the indolent subgroup with clinical features, which encompasses flushing, pruritus due to an increase numbers of dermal mast cells; nausea, vomiting, diarrhea, ulcers of the stomach and the duodenum associated with amplified gastric acid secretion; malabsorption resulting from mast cell infiltration of the intestine; skeletal disease resulting from the activation of mast cells on bony surfaces as well as vascular instability, headache and neuropsychiatric problems (Marone et al., 2001; Wolff et al., 2001). Maculopapular CM (Urticaria pigmentosa), where lesions develop in a generalised and random distribution, is the most frequent form of CM (Valent et al., 2001); others include: diffuse CM with diffuse involvement of approximately the entire skin and nodular CM with solitary or multiple lesions (Wolff et al., 2001). Moreover, mast cell sarcoma (MCS), a very rare type of non-systemic mastocytosis, is marked by a

local destructive (sarcoma-like) tumor growth consisting of highly atypical mast cells (Valent et al., 2001).

Proelss et al., (2007) have indicated that the measurement of tryptase in bone-marrow blood would be a novel, sensitive maker of the mast cell burden in bone marrow of SM patients. Treatment with IFN-α has been suggested to be the first-line cytoreductive therapy of mastocytosis. However, a significant incidence of dose-limiting toxicities has been observed (Pardanani et al., 2006).

1.4.3.4 Cystic fibrosis

Cystic fibrosis (CF) is a hereditary, fatal, autosomal recessive disorder. CF is a disease of exocrine glands, which results in altered ion composition and increased viscosity of epithelial secretions, such as sweat duct and salivary glands, small intestine, pancreatic exocrine gland ducts, biliary tract, vas deferens and respiratory tract (Voynow et al., 2008).

Chronic obstructive pulmonary disease and pancreatic insufficiency are the main clinical symptoms of CF. Pulmonary disease causes 95% of both morbidity and mortality in CF patients (Voynow et al., 2008). Infection of CF airways at early age was caused by *Staphylococcus aureus* and *H. influenza*, then later by *Pseudomonas aeruginosa* and other opportunistic gram negative organisms (Ramsey, 1996).

CF disease results from loss of expression and function mutations in the cystic fibrosis transmembrane condutance regulator (CFTR), primarily a membrane protein, expressed in epithelial cells. Airway epithelium has long been believed to be the key player in progression of CF lung disease by producing various inflammatory mediators (cytokines, chemokines, enzymes, adhesion molecules, etc.) (Jacquot et al., 2008). There are significantly increased numbers of mast cell in CF airways compared with non-CF airways during fetal development (Huneau et al., 2001b).

Kulka et al., (2002) showed that nasal polyps and chronic inflammation of sinuses and nasal mucosa are strongly associated with CF. In addition, increased levels of mast cells were found in nasal polyps from CF patients. Rhinosinusitis was identified in about 92.9% of patients surfing from CF (Babinski & Trawinska-Bartnicka, 2008). Infectious chronic non-specific rhinosinusitis was found to be frequently presented by patients with CF, whereas nasal polyps were seen in 6-44% of CF patients depending on the type of mutations (Kulka et al., 2005).

1.4.3.5 Hepatic fibrosis

It is widely accepted that mast cells contribute to tissue fibrosis, for instance hepatic fibrosis, putmonary fibrosis, tubulointerstitial fibrosis, scleroderma, and keloid (Shen, 2008). Hepatic fibrosis, characterised by the net accumulation of ECM including collagen, glycoproteins and proteoglycans, is a very common pathological process associated with all the chronic hepatic diseases, such as: cirrhosis, alcoholic hepatic fibrosis, primary biliary cirrhosis, portal hypertension, liver failure, and hepatocellular carcinoma (Shen, 2008). The increased number of mast cells in chronic liver disease has been demonstrated to correlate with the increasing severity of hepatic fibrosis (Sugihara et al., 1999; Matsunaga et al., 1999; Matsunaga & Terada, 2000). The complex interactions among mast cells, myofibroblasts, macrophages, and nerve fibers were suggested to play a significant role in the development of hepatic fibrosis process (Akiyoshi & Terada, 1998; Jeong et al., 2002). Moreover, mast cell-derived histamine, basic fibroblast growth factor (bFGF) and tryptase promote fibroblast growth by producing collagen, which results in fibrosis (Yamashiro et al., 1998). In the late stages of hepatic fibrosis, ECM degradation by matrix metallic proteinase (MMP) (Arthur, 1997) is inhibited by mast cells, presumably by displaying protease inhibitors, such as tissue inhibitors of matrix metallic proteinase (TIMP) (Benyon et al., 1996; Armbrust et al., 1997). Hence, the extent of TIMP expression is often applied as a biochemical marker to assess the degree of fibrosis in many research studies (Iredale et al., 1996; Christina et al., 2006).

Much evidence has already shown that herbal medicines have preventive and inhibitory effects on hepatic fibrosis, such as Rhei rhizome (Jin et al., 2005), *Piper longum* Linn (Christina et al., 2006), *Hibiscus sabdariffa* L. (Liu et al., 2006), *Paeonia lactiflora*, *Astragalus membranaceus* (Sun et al., 2007), Inchin-ko-to (TJ-135) (Inao et al., 2004), Sho-saiko-to (TJ-9) (Sakaida et al., 2004).

1.5 Clinical therapy with mast-cell stabilising activity

With the better understanding of the roles of mast cells in physiologic, immunologic and pathologic conditions, mast cell stabilisers have been developed and used clinically to prevent or treat allergic disorders and inflammation. It has long been accepted that such medication stabilises mast cells by blocking calcium influx across the membranes, by this means inhibiting mast cell degranulation and mediator release. However, other mechanisms have also been suggested and investigated. Due to the involvement of various mast cell mediators, a combination of mast cell stabilisation and antihistamine activity has proven to be a very desirable therapeutic approach. Recently, stabilising mast cells was found to significantly improve cardiac contractile function following hemorrhagic shock and resuscitation (Santone et al., 2008).

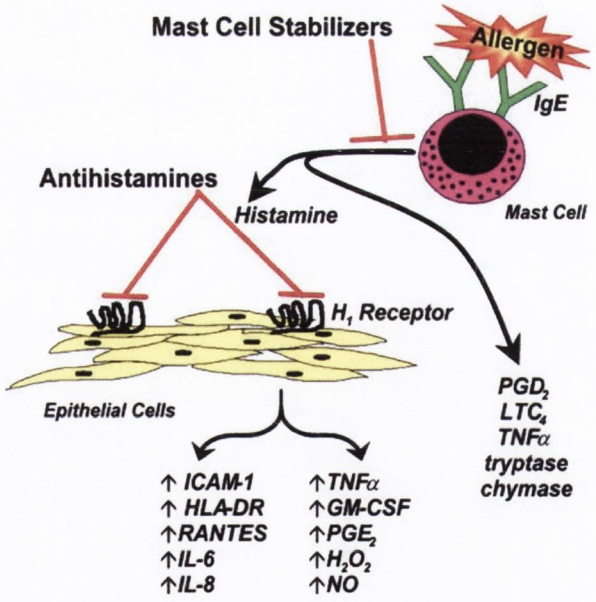
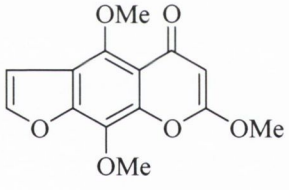


Fig. 1.11 Proposed targets of mast cell stabilizers and antihistamines (Cook at el., 2004)

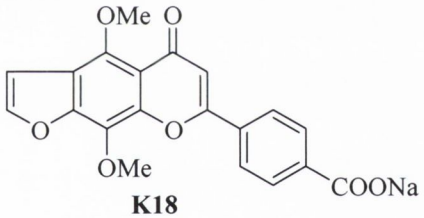
1.5.1 Chromones

Chromones are the one of the most common mast cell-stabilising drugs used to treat various types of inflammatory diseases, which are caused by immunological as well as non-immunological responses. Clinically used as prophylactic treatments, chromones have demonstrated an effect in conjunctivitis, early and mild allergic rhinitis and mild asthma have been shown (Parikh & Singh, 1998; Chang & Shiung, 2006).

Khellin was the first chromone to be used clinically (Fig. 1.12). Traditionally, it was used as a diuretic as well as a smooth muscle relaxant. Khellin was originally extracted from the seeds of the *Ammi visnaga* plant (Edwards & Howell, 2000). It was first chemically synthesised in 1947 (Baxter et al., 1949); and soon was providing complete and prolonged relief in bronchial asthma. K18 (Fig. 1.12), the first of the soluble chromones, showed inhibition of bronchoconstriction activity in 1954 (Edwards & Howell, 2000).



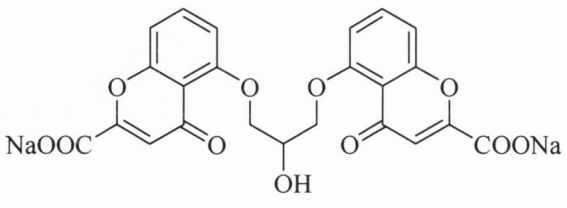
Khellin



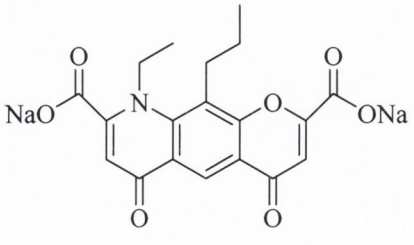
K18

Fig. 1.12 Chemical structures of Khellin and K18

Inhaled disodium cromoglycate (DSCG) and sodium nedocromil have been considered as two of the most successful drugs to provide a balance between efficacy and safety of drugs in the treatment of asthma (Edwards & Howell, 2000) (Fig. 1.13).



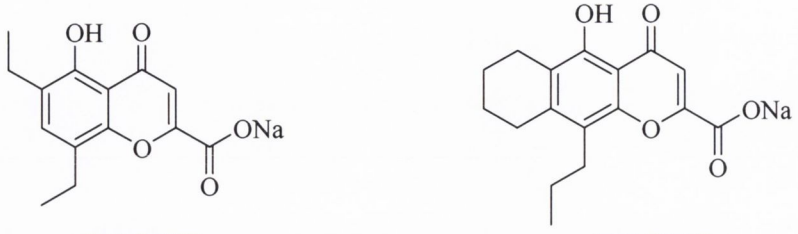
disodium cromoglycate



sodium nedocromil

Fig. 1.13 Chemical structures of disodium cromoglycate and sodium nedocromil

During the 1970s, orally active chromones, FPL55757 and FPL55787, were designed and synthesised after the development of DSCG. However, the clinical trials of FPL55757 and FPL55787 failed due to increased liver enzymes in patients, and the potential carcinogenicity in rats, respectively (Edwards & Howell, 2000) (Fig. 1.14).



FPL55757 **FPL55787**

Fig. 1.14 Chemical structures of FPL55757 and FPL55787

The mast cell-desensitising effect of chromones have been well known to act through the inhibition of Ca^{++} mobilisation, transport of Cl^- ions, and the exocytotic process, resulting in the inhibition of mast cell degranulation, mediator release and synthesis of lipid mediators. Moreover, chromones can inhibit the activation of many cell types, including mast cells, neutrophils, eosinophils, monocytes, alveolar macrophages, and lymphocytes. (Kay et al., 1987; Tsicopoulos et al., 1988; Chang & Shiung, 2006)

1.5.2 Ketotifen

Ketotifen {4-(1-Methyl-4-piperidylidene)-*4H*-benzo[4,5]cyclohepta[1,2-b]thiophen-10 (9*H*)-one Fumarate} (Fig. 1.15), an H1 receptor antagonist, is a synthesised cycloheptathiophene with both antihistamine and anti-allergic properties (Serna et al., 2006; Sánchez-Patán et al., 2008). It works to stabilise the membrane of mast cells, and inhibits the release of inflammatory mediators (Lau & Pearce, 1990). Ketotifen is extensively used in asthma management (not acute attacks), and in the treatment of allergic conditions including rhinitis and conjunctivitis (Crampton, 2003) and edema, as a result of reducing vascular leakage (Sánchez-Patán et al., 2008).

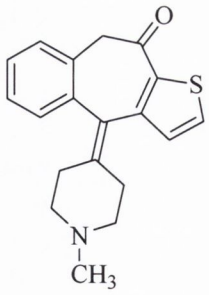


Fig. 1.15 Chemical structure of Ketotifen

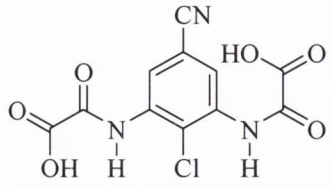
Administration of ketotifen showed significant protection in the development of dextran sulfate sodium (DSS)-induced colitis in rats, which suggested that ketotifen might play a therapeutic role in inflammatory bowel diseases (Oda et al., 1995). In addition, mast cell activity is also directly associated with the development of intestinal hypermotility caused by infection and inflammation. Serna et al., (2006) described ketotifen as a potential treatment for motor disorders in IBS and inflammatory bowl disease (IBD).

Recently, compelling evidence showed that ketotifen could reduce the release of mast cell mediators involved in the development of prehepatic portal hypertension, by means of preventing mast cell migration and activation in the mesenteric lymph nodes. This suggested that ketotifen might have therapeutic implication in reducing early splanchnic inflammatory response with prehepatic portal hypertension, such as an increased infiltration of the intestinal mucosa and mesenteric lymph nodes by mast cells (Sánchez-Patán et al., 2008).

1.5.3 Lodoxamide

Lodoxamide [*N',N'*-(2-choloro-5-cyano-m-phenylene)dioxamic acid] (Fig. 1.16) is another newly developed effective antiallergy medication acting as a mast cell stabiliser. It showed significantly greater inhibition of mediator release than DSCG by several routes of administration (i.e. inhalation, oral, intraperitoneal injection, intravenous injection) in animal models of asthma (i.e. rats, primates), especially, 2,500 times more active than DSCG (ID50 = 0.001 mg/kg) shown in rat studies. However, pilot evaluation

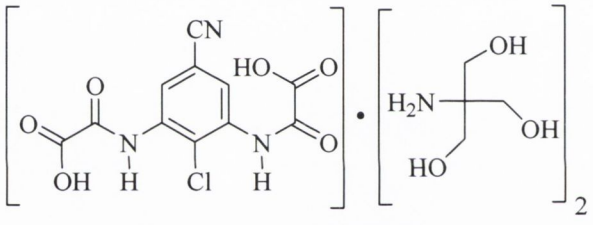
studies observed less activity in comparison to placebo as treatment for asthma patients (Johnson & Sheridan, 1986).



Lodoxamide

Fig. 1.16 Chemical structures of Lodoxamide

Lodoxamide tromethamine {*N',N'*-(2-chloro-5-cyano-m-phenylene) dioxamic acid tromethamine salt} (Fig. 1.17), a Lodoxamide derivative, has been successfully developed. Lodoxamide tromethamine ophthalmic solution 0.1% (LOS), topical Lodoxamide therapy, was shown to be safe and effective for treatment in all ocular allergic diseases (Avunduk et al., 2000).



Lodoxamide tromethamine

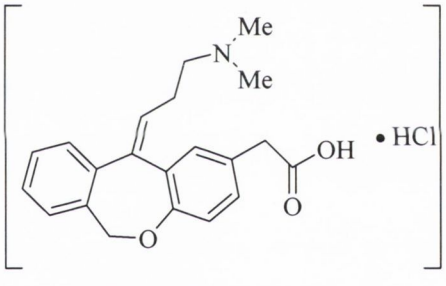
Fig. 1.17 Chemical structure of Lodoxamide tromethamine

A comparative study demonstrated that Lodoxamide tromethamine was more efficacious than DSCG in rabbits with passive allergic conjunctivitis (Özturk et al., 2002). Furthermore, in the treatment of VKC, the greater effect of LOS in resolving the signs and symptoms was demonstrated to be associated with dramatically decreasing tear eosinophil cationic protein levels (a marker in monitoring the treatment of VKC) (Leonardi et al., 1997), inflammatory cells in the tear fluid (Oguz et al., 1999), and $CD4^+$ cell numbers (Avunduk et al., 2000). A recent study showed that mast cell stabilisation of Lodoxamide only accelerated colonic transit in comparison to ketotifen increasing postoperative small intestinal and colonic transit (Glatzle et al., 2003).

1.5.4 Olopatadine, Azelastine and Epinastine

Olopatadine, Azelastine and Epinastine belong to a class of anti-allergy drugs which possess antihistamine as well as mast cell stabilising properties.

Karaman et al., (2008) indicated that Olopatadine hydrochloride {(*z*)-11-[3-(dimethylam ino)propylidene]-6,11-dihydrodibenz[b,e]oxepin-2-acetic acid hydrochloride} (Fig. 1.18) was more effective in patients with acute than in those with chronic allergic disorders, without demonstrating major detected side effects. Olopatadine hydrochloride ophthalmic solution 0.1% (Opatanol) is widely used in clinical treatment of ocular allergic diseases. It shows a rapid onset of action in the treatment of allergic conjunctivitis (Abelson & Spitalny, 1998). Recently, a 0.2% Olopatadine hydrochloride ophthalmic solution (Pataday) has been proven to be the first effective ophthalmic antiallergy drug with once-daily dosing (Abelson & Gomes, 2008).



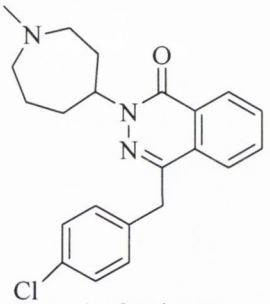
Olopatadine hydrochloride

Fig. 1.18 Chemical structure of Olopatadine hydrochloride

Several comparative studies showed that different concentrations of Olopatadine hydrochloride nasal spray (0.1%, 0.2%, 0.4%, 0.6%) were efficacious in suppressing allergic reactions by reducing total allergen-induced nasal symptoms in comparison to placebo nasal spray. Olopatadine at 0.2% concentration inhibited mast cell degranulation and at 0.6% resulted in remarkably fast onset as well as long lasting action (Sanico et al., 2004; Patel et al., 2007; Pipkorn et al., 2008). An intranasal formulation of Olopatadine hydrochloride (Patanase, Olopatadine hydrochloride nasal spray 0.6%) has been developed to treat allergic rhinitis (Saltoun & Avila, 2008).

Suppression of increased levels of mast cell derived NGF and VEGF, as well as histamine H1 receptor antagonism account for Olopatadine's therapeutic effects in the treatment of allergic inflammations (Tamura & Komai, 2008).

Azelastine hydrochloride {4-[(4-chlorophenyl)methyl]-2-(1-methyl-azepan- 4-yl)-phthal azin-1-one hydrochloride} (Fig. 1.19), has been extensively studied and has been shown to possess anti-allergic and anti-inflammatory activities *in vivo* as well as *in vitro*. Many observations are reported upon administration of Azelastine, including the inhibition of IL-6, IL-8, TNF-α secretion from mast cells (Kempuraj et al., 2003), blockage of eosinophils, infiltration of T lymphocytes into the bronchial mucosa, and a dose-dependent inhibition of intra and extracelluar platelet-activating factor-like activity from asthmatic and non-asthmatic eosinophils, neutrophils, and macrophages (Hanashiro et al., 2006).



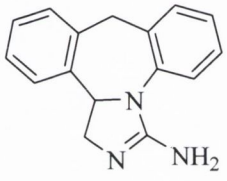
Azelastine

Fig. 1.19 Chemical structures of Azelastine

Comparative studies show that in allergic rhinitis, treatment with Olopatadine 0.6% and Azelastine 0.1% both present good therapeutic effects, however, the sensory attributes of Olopatadine were greater than azelastine with regard to immediate taste post-dosing, overall aftertaste, overall patient preference, and likelihood adherence (Meltzer et al., 2008). The combination of Azelastine and nasal corticosteroids delivered by nasal spray was demonstrated to be more effective in both allergic rhinitis and vasomotor rhinitis (Kaliner, 2007) than either agent alone. However, in contrast, no therapeutic advantage was recorded by using oral administration of Azelastine in combination with intranasal corticosteroid (Ratner et al., 2008). A predominantly toxic effect on conjunctival cells

was observed when Ketotifine and Azelastine were topically administrated in the treatment of allergic conjunctivitis (Lee et al., 2008).

Epinastine hydrochloride [3-amino-9,13b-dihydro-1*H*-dibenz(c,f)imidazo(1,5-a)azepine chloride] (Fig. 1.20), was first approved for the treatment of rhinitis in 1981 (Finegold et al., 2006). In a randomised, double-masked, crossover study, Torkildsen et al., (2008) revealed that epinastine was more comfortable for patients with allergic conjunctivitis following a single drop administration compared to azelastine and ketotifen. More importantly, none of these anti-allergic agents generated significant acute ocular drying effects.



Epinastine

Fig. 1.20 Chemical structures of Epinastine

1.5.5 Dihydropyridines

Dihydropyridines (DHPs) (Fig. 1.21), a class of molecules based on the structure of pyridine, are also well known as L-type Ca^{++} channel blockers (Chen et al., 2009).

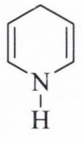
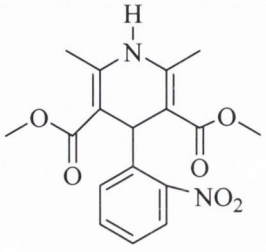


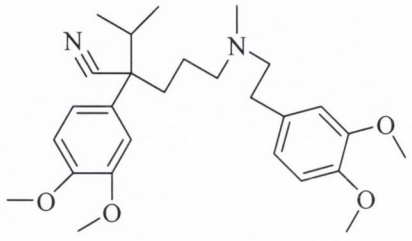
Fig. 1.21 Chemical structure of dihydropyridine

Clinically, DHPs have been exclusively used to treat mild to moderate hypertension (Kawabe et al., 2008), angina, atrial arrhythmia, and myocardial ischaemia by inhibiting transmembrane influx of Ca^{++} through L-type Ca^{++} channel, and as such inciting vascular smooth muscle relaxation, and the repression of cardiac contractility (Struyker-Boudier et al., 1990).

Some DHP derivatives have been developed and found to possess anti-allergic activity acting as mast cell stabilisers, including inhibitory effects on histamine release, the synthesis of SRS-A and the formation of thromboxane A2. The examples of these derivatives are Nifedipine [3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyri dine-3,5-dicarboxylate} and Verapamil {2-(3,4-dimethoxyphenyl)-5-[2-(3,4-dimethoxyp henyl)ethyl-methyl-amino]-2-(1-methylethyl)pentanenitrile} (Fig. 1.22) (Chand et al., 1986).



Nifedipine



Verapamil

Fig. 1.22 Chemical structures of Nifedipine and Verapamil

Nifedipine showed positive therapeutic effects in bronchial asthma, including diminishing asthmatic symptoms, bronchomotor tone and airway hyperresponsiveness (Dhar, 2001), whereas, Nifedipine failed as maintenance therapy at doses which generate cardiovascular effects (Ann Twiss et al., 2001). As a result, Nifedipine was suggested to be used to treat angina, hypertension or migraine headache in patients with persistent asthma, where β–adrenoceptor inhibitors are relatively contraindicated (Ann Twiss et al., 2001). Moreover, a response to Nifedipine treatment in a patient with symptomatic urticaria pigmentosa was also observed (Fairley et al., 1984).

1.5.6 Mediator inhibitors

1.5.6.1 Leukotriene antagonists

When pharmacotherapy can not apply to certain patients who are unresponsive to the treatment or unable to receive medications, immunotherapy becomes more clinically favourable.

Leukotriene antagonists have been suggested and demonstrated to be as effective in allergic inflammations (i.e. asthma, allergic rhinitis) as non-steroid anti-inflammatory treatment (Robinson et al., 2001; Lai et al., 2005). DS-4574 {6-(2-cyclohexylethyl)-[1,3,4]thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(3*H*)-one} (Fig. 1.23), is a peptidoleukotriene D4 receptor antagonist with mast cell stabilising activity and low toxicity (Aibara et al., 1991; Yokohama et al., 1992). It has generally been believed that DS-4574 has a role in the treatment of bronchial asthma, allergy, edema, gastric ulcer, peptic ulcers (Tabuchi et al., 1992; Aibara et al., 1993; Tabuchi et al., 1993; Tabuchi et al., 1994). Tabuchi et al., (1995) have shown the mast-cell stabilising activity of DS-4574, leading to the significant inhibition of histamine release and hyperhistaminemia from rat peritoneal mast cells induced by compound 48/80, and the remarkable suppression of gastric lesion formation triggered by compound 48/80, in comparison to LY171883 (another peptidoleukotriene D4 receptor antagonist) (Fig. 1.23), which protected mucosal edema formation, but did not inhibit mucosal mast cells degranulation (Tabuchi et al., 1993).

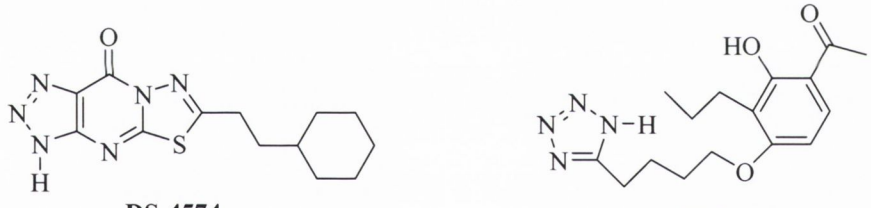


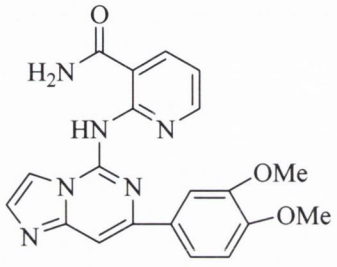
Fig. 1.23 Chemical structures of DS-4574 and LY171883

1.5.6.2 Spleen tyrosine kinase inhibitor

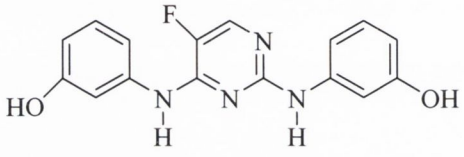
Spleen tyrosine kinase (Syk) is a soluble, cytosolic, non-receptor protein tyrosine kinase expressed mainly in most hematopoietic cells, including mast cells, neutrophils, macrophages, B cells, dendritic cells, and epithelial cells (Beaven & Baumgartner, 1996; Miller et al., 2009). It has been proven that Syk is involved in the activation and degranulation of mast cells, lipid mediator synthesis and cytokine production through FcεRI-mediated signaling (Costello et al., 1996). In addition, Syk plays a critical role in signal transduction from immunoreceptors for IgG (FcγR). Thereby, it has been believed that Syk is a vital enzyme strongly associated with both a variety of allergic disorders,

antibody-mediated autoimmune diseases as well as certain cancers (Li et al., 2009). Accordingly, Syk kinase inhibitor becomes an attractive therapeutic strategy for novel anti allergic treatment.

Newly developed BAY61-3606 {2-[7-(3,4-dimethoxyphenyl)-imidazo[1,2-c] pyrimidin-5-ylamino]-nicotinamide dihydrochloride} (Fig. 1.24), a potent and selective Syk kinase inhibitor, showed significant suppression of antigen-induced passive cutaneous anaphylactic reaction, bronchoconstriction, and bronchial edema, in addition to the reduction of antigen-induced airway inflammation in the rat (Yamamoto et al., 2003).



BAY 61-3606



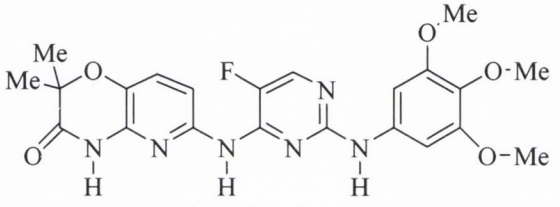
R112

Fig. 1.24 Chemical structures of BAY 61-3606 and R112

A double-blind, placebo-controlled clinical trial revealed Syk kinase inhibitor R112 {3-[2-(3-hydroxyphenylamino)-5-fluoropyrimidin-4-ylamino]phenol} (Fig. 1.24), showed its remarkable, powerful, complete and fast onset action to diminish all mast cell activation cascades induced by IgE receptor cross-linking. Consequently, significant alleviation in a broad range of clinical symptoms of allergic rhinitis was observed with R112. However, topical therapy (nasal spray) as the only possible administration route has limited its clinical use (Rossi et al., 2006).

With the advance in molecular and cell biology technologies, there are numerous tyrosine kinases which have been identified. Many of these tyrosine kinases are believed to be involved in the physiopathology of RA (Tristano, 2009). Syk activation is strongly linked to RA as its essential role in the production of cytokine and matrix metalloproteinase by TNF-α in RA fibroblast-like synoviocytes (Cha et al., 2006). R406 {*N*4-(2,2-dimethyl-3-oxo-4*H*-pyrid[1,4]oxazin-6-yl)-5-fluoro-*N*2-(3,4,5-trimethoxyphenyl)-2,4-pyrimidinediamine} (Fig. 1.25), a novel orally active Syk kinase inhibitor with a

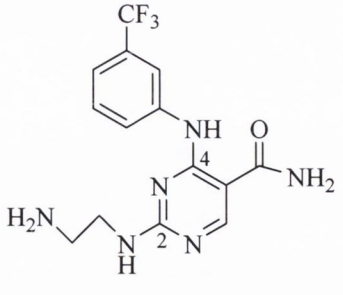
potential broad spectrum of anti inflammatory properties, has also been developed. Many significant observations have been made after administration of R406, which include assuage of airway hyperresponsiveness and pulmonary eosinophilia (Matsubara et al., 2006a, 2006b), reduction of allergic airway inflammation, amelioration of a rodent collagen-induced arthritis model, inhibition of inflammation in rat models of RA (Pine et al., 2007). All these findings are proved without interfering remarkably with the innate immune response or hemostasis (Braselmann et al., 2006). Immunotoxicity assessment study found that R406 had minimal functional immunotoxicity without causing unacceptable mechanism-based adverse effects, which offered R406 as a potential candidate for modulating Syk activity in human diseases (Zhu et al., 2007).



R406

Fig. 1.25 Chemical structure of Syk kinase inhibitor R406

A series of pyrimidine-5-carboxamide derivatives, as novel Syk kinase inhibitors have been synthesised and biologically evaluated. The class of 4-anilionpyrimidine-5-carbox-amides was shown to possess good selectivity for Syk.



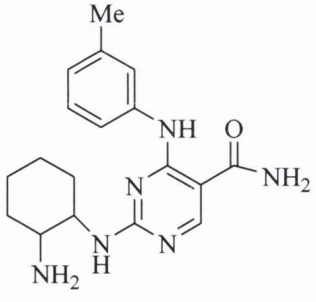


Fig. 1.26 Chemical structure of 2-(2-aminoethylamino)-4-{3-(trifluoromethyl) phenylamino}pyrimidine-5-carboxamide (left)

Fig. 1.27 Chemical structure of 2-(2-aminocyclohexylamino)-4-(m-tolylamino) pyrimidine-5-carboxamide (YM193306) (Right)

One of the derivatives, 2-(2-aminoethylamino)-4-{3-(trifluoromethyl)phenylamino}pyri midine-5-carboxamide (Fig. 1.26), exhibited significant inhibition of the passive cutaneous anaphylaxis reaction in mice by subcutaneous administration (Hisamichi et al., 2005).

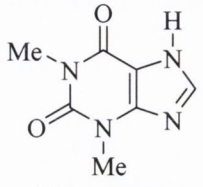
They also found that an aminoethylamino moiety at the 2-position of the pyrimidine ring, and an anilino function group at the 4-position was markedly associated with Syk kinase inhibitory activity. Based on those significant findings, Syk kinase inhibitor YM193306 (Fig. 1.27) was successfully developed with extraordinary bioactivity (Li et al., 2009).

1.5.6.3 Phosphodiesterase inhibitors

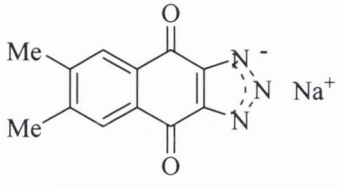
Phosphodiesterases (PDEs) are a class of enzyme hydrolysing cyclic nucleotide, which is central to their role in modulating cell functions by regulating intracellular levels of cAMP and cGMP. As PDE activity is observed in almost every cell in the body, PDE has been suggested to be a novel target for treatment (Boswell-Smith et al., 2006). Many selective PDE inhibitors have been designed, developed and studied for the clinical anti-allergy and anti-inflammation properties in a variety of diseases, for example the utilize of PDE2 inhibitors in sepsis; PDE4 inhibitors in asthma, allergic rhinitis, multiple sclerosis, psoriasis and PDE5 inhibitors in sexual dysfunction in females, cardiovascular disease, and pulmonary hypertension (Boswell-Smith et al., 2006). Some PDE inhibitors have been found to possess mast cell stabilising activity (Peachell et al., 1988).

Theophylline (1,3-dimethyl-7*H*-purine-2,6-dione) (Fig. 1.28) has been long used to treat asthma because of its anti-inflammatory and bronchodilator activities, as well as its ability to increase diaphragm contractility. In comparison with Nedocromil sodium, Theophylline showed significant inhibition of histamine release induced by anti-IgE, compound 48/80 or substance P from human basophils, lung and skin fragments (Louis & Radermecker, 1990). However, a series of side effects in the gastrointestinal, central nervous, and cardiovascular systems limited its clinical use (Torphy & Undem, 1991).

Research study indicated that BRL 22321 (4,9-dihydro-6,7-dimethyl-4,9-dioxo-1H-naph tho[2,3-d]-v-triazole sodium salt) (Fig. 1.28) had greater mast cell stabilising activity than Theophylline and Nedocromil sodium, in studies including inhibition of rat passive cutaneous and peritoneal anaphylaxis, blockage of cAMP and cGMP, inhibition of histamine release by antigen from passively sensitised rat peritoneal cells *in vitro*, suppression of histamine secretion by antigen from chopped lung taken from hyperimmune guinea pigs, as well as anti-IgE-induced histamine release from buffy coat-derived mast cells. Smooth muscle relaxing effect of BRL 22321 was also detected (Spicer et al., 1983; Wang et al., 2007).



Theophylline



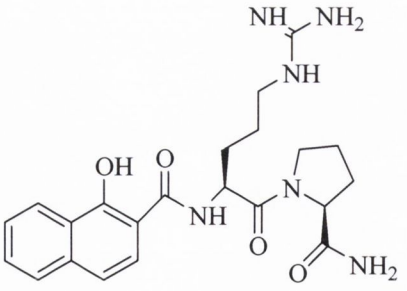
BRL 22321

Fig. 1.28 Chemical structures of Theophylline and BRL 22321

1.5.6.4 Serine proteinase inhibitors

Serine proteases are one of the most important groups of therapeutic targets for allergic inflammations. Indeed, as the major secretory mediator of mast cells, proteinase tryptase inhibitors are believed to be ideal candidates as mast cell-stabilising agents.

Tryptase inhibitors are able to reduce the action of both tryptase and trypsin, and inhibit both IgE- and non-IgE-dependent histamine release from mast cells (He et al., 2001; He et al., 2004). APC366 [*N*-(1-hydroxy-2-naphthoyl)-$_L$-arginyl-$_L$-prolinamide hydrochloride] (Fig. 1.29), a tryptase inhibitor, has been reported to be able to significantly reduce the late phase responses of mild to moderate, allergen-induced asthma (Krishna et al., 2001). In a study with APC366, remarkable inhibition of histamine release elicited by anti-IgE antibody or calcium ionophore from enzymatically dispersed human lung cells was also observed (He et al., 2004). Heparin was also thought to contribute to the stabilisation of tryptase activity.



APC366

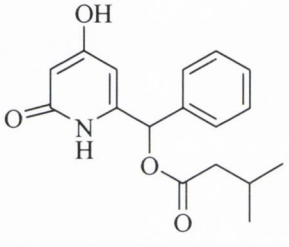
Fig. 1.29 Chemical structure of APC366

Lactoferrin is a 78kDa protein of the transferrin class with anti-bacterial (bacteriostatic, bactericidal), anti-viral, anti-fungal activities (Azuma et al., 1999; Samaranayake et al., 1997; Ikeda et al., 2000; He et al., 2003; Kolm et al., 2007). Lactoferrin can be found in a range of secretions (i.e. milk, tears, saliva, etc), tissues (i.e. epidermis, hair follicles, etc) and epithelial (Cumberbatch et al., 2000). Being a part of the innate immune system, lactoferrin also has immunomodulatory properties by means of down-regulating certain cytokines, for example TNF-α, IL-6 (Machnicki et al., 1993; Haversen et al., 2002). In addition to keratinocytes, cutaneous lactoferrin is also associated with mast cells, lymphocytes, neutrophils (Kolm et al., 2007). As an endogenous mast cell stabiliser, an increased level of cutaneous lactoferrin was found during IgE-mediated late phase reactions in human skin allergen challenged sites (Zweimann & von Allmen, 2000). In addition, lactoferrin was thought to have potential greater inhibitory effects of IgE-dependent histamine release from skin mast cells than most anti-allergic drugs (He et al., 2003), which was suggested to be mediated by interaction with mast cell specific tryptase (Elrod et al., 1997; He et al., 2003). Furthermore, He et al., (2003) demonstrated that lactoferrin can counteract the activity of cathepsin G, a product of both neutrophils and mast cells.

The mast cell stabilising properties of chymase inhibitors have been extensively discussed. Many research studies showed that the degranulation of rodent mast cells could be inhibited by inhibitors of chymotryptic proteinases (chymase), such as chymostatin and certain peptide boronic acid inhibitors. In addition, experimental

evidence has shown that chymotryptic proteases inhibitor (i.e. chymostatin) can effectively suppress IgE-dependent activation of certain populations of human mast cells, particularly those in skin tissue. More interestingly, chymase induced by mast cell degranulation could also mediate a feedback mechanism, suppressing further degranulation (He et al., 1999).

Chymase inhibitors have also been demonstrated to be useful in preventing cardiovascular diseases, postopervative adhesion formation and fibrosis, probably by the inhibition of angiotensin II formation and transforming growth factor-β activation (Okamoto et al., 2002; Takai et al., 2004). Recently, the synthesis of novel human chymase inhibitor (SPF32629A) (Fig. 1.30), originally isolated from the cultured broth of Penicillium sp., SPF32629, has been successfully established (Vegi et al., 2008).



SPF32629A

Fig. 1.30 Chemical structure of human chymase inhibitor SPF32629A

1.5.6.5 Corticotropin-releasing factor (CRF) antagonists

Corticotropin-releasing factor (CRF, a 41 amino acid pepteide) and structurally CRF-related peptide Urocortin (Ucn) and their receptors were found in the CNS and in many peripheral tissues, such as the immune and cardiovascular systems (Grammatopoulos & Chrousos, 2002). The pro-inflammatory characteristics of CRF and Ucn have been confirmed through mast cell activation, degranulation and mast cell dependent vascular permeability (Kempuraj et al., 2004). The synthesised and released of CRF and Ucn by mast cells in response to IgE receptor (FcεRI) cross linking have also been clearly documented (Kempuraj et al., 2004). An array of CRF receptor antagonists has been developed to treat brain disorders, for example anxiety and Alzheimer's disease (Grammatopoulos & Chrousos, 2002). CRF receptor antagonists,

together with natural plants, such as retinol and plant-derived flavonoids with mast cell stabilising activity, have been believed to play a beneficial role in the treatment of skin diseases (Middleton et al., 2000; Theoharides et al., 2004).

1.5.6.6 Interleukin-10

Interleukin-10 (IL-10), known as cytokine synthesis inhibitor factor (Moore et al., 2001), is another anti-inflammatory cytokine with immunoregulatory properties. In addition to suppressing proinflammatory cytokine formation (i.e. IFN-γ, TNF-α, IL-2, IL-6, IL-8) which results in inhibition of allergic and inflammatory responses, IL-10 also regulates mast cell function, development, proliferation, apoptosis (combined stimulation with IL-4), as well as protease gene expression (Thompson-Snipes et al., 1991; Ghildyal et al., 1993; Bundoc & Keane-Myers, 2007). Moreover, *in vivo* studies showed that IL-10 could also inhibit c-Kit expression and FcεRI expression in mast cells (Mirmonsef et al., 1999; Gillespie et al., 2004). So far, IL-10 has also been proven to inhibit compound 48/80-induced mast cell activation in an *in vivo* rodent model of allergic conjunctivitis (Bundoc & Keane-Myers, 2007), as well as IgE-dependent activation of human mast cells (Royer et al., 2001). Thus, IL-10 has been proposed as a novel mast cell stabiliser for ocular allergic disease. Furthermore, psoriasis, a common chronic cutaneous inflammation, is characterized by tryptase- and chymase-positive mast cell (MC_{TC}) proliferation (Ichaelsson et al., 1997; Harvima et al., 2008). As relative IL-10 deficiency is another key feature in psoriasis. Asadullah et al., (1998) detected an increased level of IL-10 expression of peripheral blood mononuclear cells in patients with psoriasis compared with patients before antipsoriatic therapy. There is rising evidence that IL-10 administration could improve the intracutaneous IL-10 mRNA expression (Asadullah et al., 1998). Hence, therapeutic administration or induction of endogenous IL-10 is believed to be a useful novel treatment for cutaneous disorders.

1.5.7 Anti-Immunoglobulin E (anti-IgE) therapy

Anti-IgE therapy was first introduced as a potential mast cell stabilising agent in 1987

(Chang et al., 1990; Davis et al., 1993; Presta et al., 1993; Chang & Shiung, 2006). As the first anti-IgE agent, Omalizumab (marketed under the name Xolair) has been evaluated and proved to be a safe and effective treatment for moderate to severe allergic asthma (Busse et al., 2001; Lanier et al., 2003; Berger et al., 2003; Milgrom, 2004; Holgate et al., 2004), seasonal and perennial allergic rhinitis (Adelroth et al., 2000; Kuehr et al., 2002; Nayak et al., 2003), allergic and anaphylactic reactions to peanuts (Leung et al., 2003; Leung et al., 2004), allergic reactions to latex products (Leynadier et al., 2004), etc.

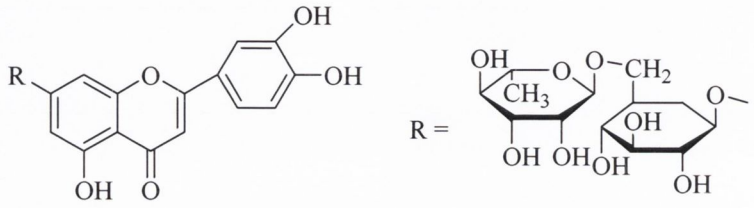
Omalizumab is a recombinant humanised monoclonal anti-IgE antibody (Ruffin & Busch, 2004). Omalizumab demonstrates mast cells stabilisation action by selectively binding to the IgE molecules, then forming an Omalizumab-IgE complex in order to prevent IgE from binding to FcεRI receptors on mast cells. Consequently, the IgE free FcεRI receptors are unstable, and are routed back inside the cells for decomposition (Chang & Shiung, 2006).

It is believed that in the initial few weeks of anti-IgE treatment, the anti-IgE-IgE complex binds to incoming allergen molecules to prevent them binding to IgE-FcεRI on mast cells (Chang & Shiung, 2006). This novel medication is recommended for asthma patients aged $\geq$ 12 years to improve asthma control and reduce requirement for oral and inhaled corticosteroids. However, the high cost limits the availability of this therapy.

1.5.8 Natural products with mast cell-stabilising properties

Clinical treatments using natural products have been broadly employed in the treatment of various aspects of allergies. Structure identification, exact mechanisms at the molecular levels, and the relationship between structure and bioactivity have drawn much attention from many research groups in order to provide efficacious anti-allergy drugs with minimum side effects.

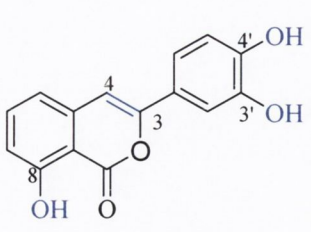
Luteolin-7-*O*-rutinoside (Fig. 1.31), one of six flavonoid glycosides isolated from the aerial part of *Mentha piperita* L., expressed a remarkable inhibition of histamine release triggered by compound 48/80 and antigen-antibody mediated allergic reaction (Inoue et al., 2002). A notable dose-related inhibition of the antigen-induced nasal response such as sneezing and nasal rubbing was also observed, which indicated luteolin-7-*O*-rutinoside could be of benefit to allergic rhinitis patients to attenuate their nasal symptoms (Inoue et al., 2002).



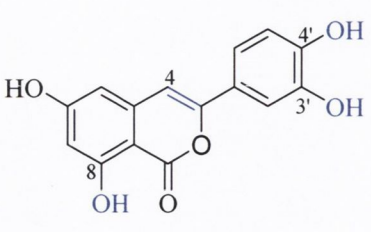
Luteolin-7-*O*-rutinoside

Fig. 1.31 Chemical structure of Luteolin-7-O-rutinoside

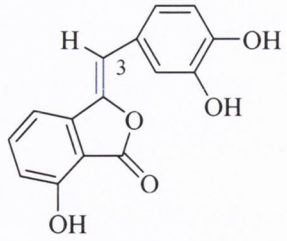
The methanol extract of Hydrangeae Dulcis Folium obtained from the leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO (Saxifragaceae), has been identified as possessing compelling anti-allergic, anti-bacterial, anti-oxidative, anti-ulcer, and cholagoic activities (Matsuda et al., 1999). A structure-bioactivity relationship study revealed that two isocoumarins (thunberginols A and B) and a benzylidenephthalide (thunberginol F) isolated from Hydrangeae Dulcis Folium, exhibited excellent inhibitory effects on histamine release from rat peritoneal mast cells. The existence of the double bond at the 3-position and the hydroxyl groups at the 8-, 3'-, 4'- positions of the two isocoumarins was believed to be important for their potent mast cell stabilising activity (Matsuda et al., 1999).



Thunberginol A



Thunberginol B



Thunberginol F

Fig. 1.32 Chemical structures of thunberginols A, B and F

Matsuda et al., (1999) also demonstrated that the plane conformation structure of thunberginol F (Fig. 1.32) owing to presence of the double bond at the 3-position could account for its increased bioactivity.

Mosla dianthera (Maxim.) was traditionally used to treat colds, headaches, intestinal and skin diseases in oriental countries (Lee et al., 2006). The aqueous extract of *Mosla dianthera* (Maxim.) has recently been proven to diminish histamine release and secretion of inflammatory cytokines (i.e. TNF-α, IL-8, IL-6) *in vitro* as well as *in vivo*, by inhibiting mast cell activation and mast cell-mediated immediate-type allergic diseases (Lee et al., 2006).

Albizzia lebbeck Benth. (Mimosaceae), a large, erect, unarmed, deciduous spreading tree, contains many components including alkaloids, flavanoids, tannins, proteins and saponins (Kumar et al., 2007). It has widely been accepted that *Albizzia lebbeck* Benth. has numerous therapeutic effects in many medicinal conditions. Its bark was demonstrated to have immunomodulatory function (Chintawar et al., 2002), anti-microbial properties (Barua et al., 2000), and it is utilised as an astringent, expectorant, anti-inflammatory, anti-allergic and anti-asthmatic (Kumar et al., 2007). In the same manner, the leaf part has been claimed to possess anticonvulsant (Kasture et al., 1996) and nootropic activities (Kumar et al., 2007), and it is used for the treatment of night blindness (Shashidhara et al., 2008). In a recent *in vitro* study, methanol and water extracts of bark together with the methanol extract of leaf showed its maximum mast cell stabilising activity in comparison to DSCG (Shashidhara et al., 2008). Moreover, a selective inhibitory ability of the water extract of *Rehmannia glutinosa* steamed root on IgE-mediated allergic reaction *in vivo* and *in vitro* was significantly observed (Kim et al., 1998). Similar results were also obtained from the aqueous extract of Salviae radix root, which implied that such plant extract may become a future therapeutic intervention for the treatment of allergic skin reaction (Kim et al., 1999).

Furthermore, many natural products from microbial sources have been claimed to demonstrate effective inhibition of IgE-dependent mast cell activation, therefore preventing histamine release and production of certain cytokines, such as TNF-α and IL-4 by mast cells. These include FK506 (also called tacrolimus) (Fig. 1.33) (Sengoku et al., 1999) and cyclosporine (Fig. 1.34) (Harrison et al., 2007), both of which are immunosuppressants which are commonly used to treat autoimmune diseases and prevention of organ rejection following transplantation. It has been reported that the mast cell-stabilising abilities of FK506 and cyclosporine are modulated by different mechanisms with unnecessary calcineurin involvement (Harrison et al., 2007).

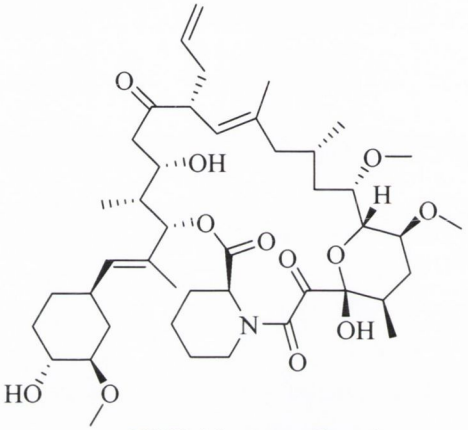


Fig. 1.33 Chemical structure of FK506 (or tacrolimus)

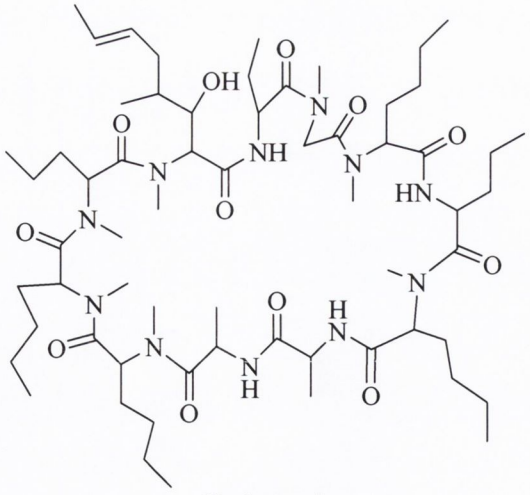


Fig. 1.34 Chemical structure of cyclosporine

2.1 Introduction

The design and synthesis of novel, potent mast cell stabilising compounds of the type depicted in Fig. 2.0 by Barlow (2002), has opened up the possibility of identifying a new class of mast cell stabilising drugs, which possess a basic indanone skeleton coupled to an *N*-alkylated-2-aminotetralin. These compounds demonstrated potent mast cell-stabilising activity in a series of *in vitro* and *in vivo* assays. However, stability studies indicated that their true potential maybe somewhat compromised by their tendency to fragment to form the more stable conjugated indenone derivatives under physiological conditions (37°C, pH 7.4) (Scheme 2.0).

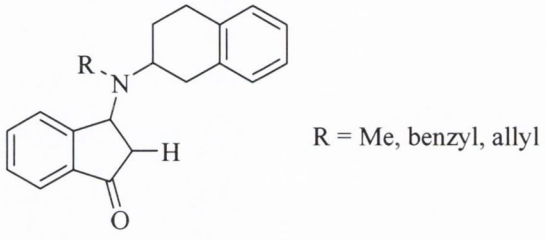
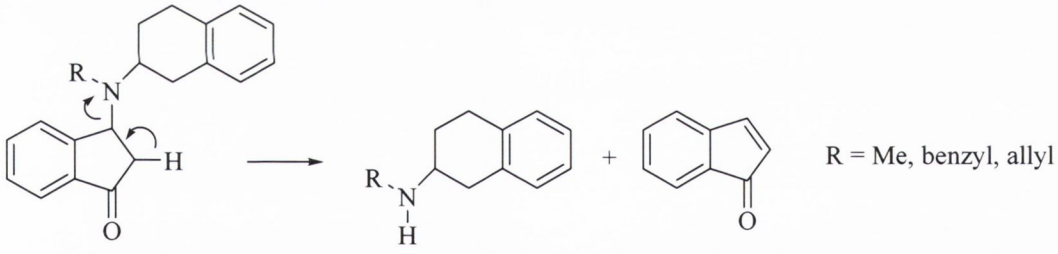


Fig.2.0 Indanone-2-aminotetralin dimer



Scheme 2.0 Formation of conjugated indenone derivatives

Substituting the indanone component with a tetralone moiety (Fig. 2.1) also resulted in fragmentation of the compounds in this series (Scheme 2.1). In this case, the driving force for the breakdown is the formation of naphthol akin to 1-indenone from the series depicted in Scheme 2.0.

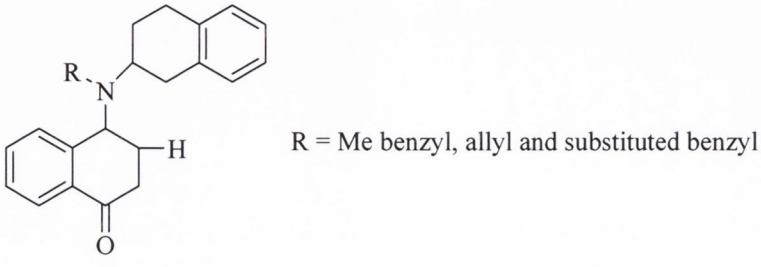
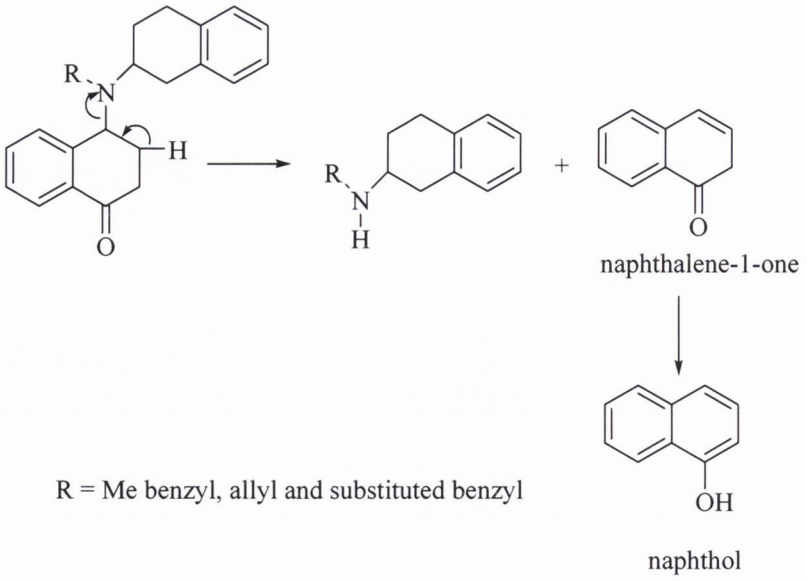


Fig.2.1 Tetralone-2-aminotetralin dimer



Scheme 2.1 Formation of naphthol from tetrahydronaphthalene based tertiary amine

In addition to the synthesis of a series of compounds based on Figs. 2.0 and 2.1, previous studies by Barlow (2002) also recognised that substitution of the 2-aminotetralin moiety with a cyclohexenyl group resulted in the generation of a series of compounds with good mast cell stabilisation activity, when compound 48/80 was used to induce histamine release from rat peritoneal mast cells (Fig. 2.2).

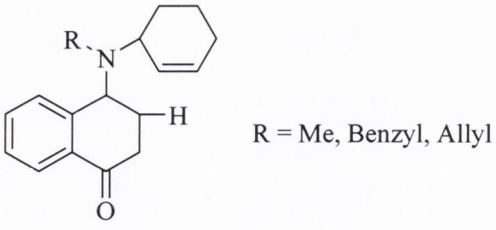
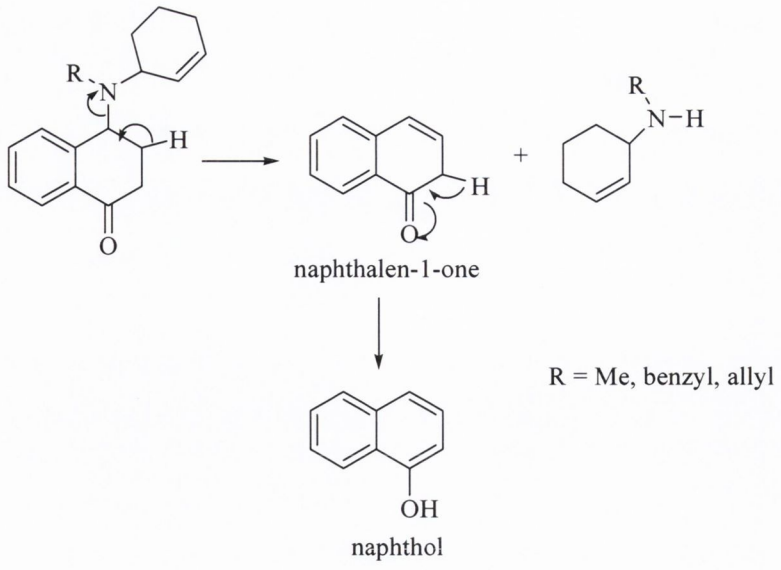


Fig.2.2 Cyclohexenyl-substituted tertiary amine

Whilst active compounds within this series were synthesised, their potential as true mast cell stabilising compounds was still hampered by their tendency to fragment and form the more stable naphthol derivative (Scheme 2.2).



Scheme 2.2 Formation of naphthol from cyclohexenyl-substituted tertiary amine

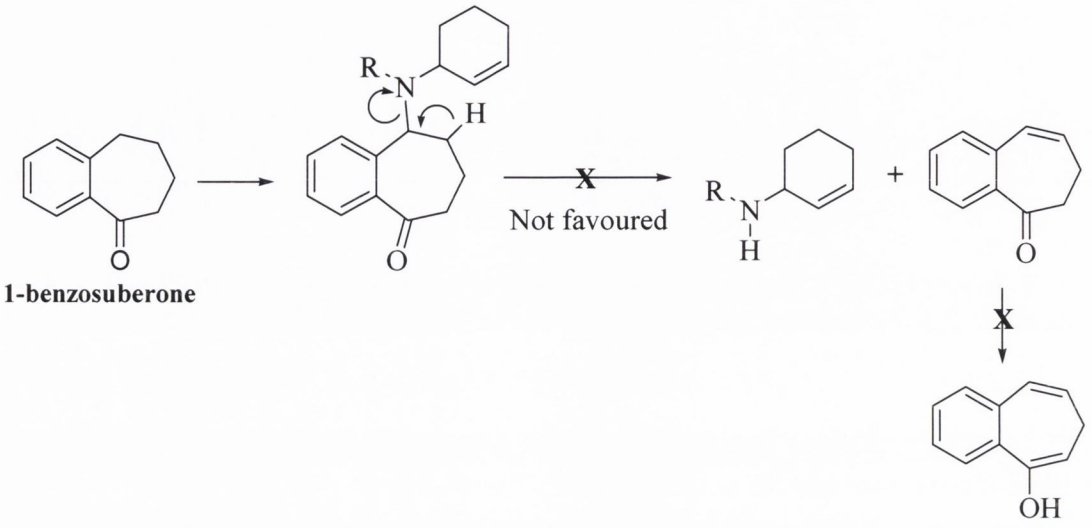
Although the mast cell data generated on the above compounds demonstrated that they had significant potential as novel mast cell stabilising compounds, it was nevertheless felt their shortcomings in respect of their stability needed to be addressed when the design of new compounds was being considered (Scheme 2.2). The data generated by Barlow yielded a number of interesting facts regarding the requirement of compounds of this type to demonstrate potent mast cell stabilisation activity; namely a 1-indanone or 1-tetralone unit can be used while the *N*-alkylated secondary amine could be derived from a variety of secondary amines, those tested to date included *N*-alkylamino; tetralin, indane, cyclohexenyl and cyclopentyl groups.

2.2 Synthetic targets and strategy

The aim of the work described in this Chapter was fourfold; (i) To identify a series of mast cell stabilising compounds that addressed the stability issue associated with the Barlow series of compounds. (ii) To investigate the effect of varying the electronic properties of substituents on the benzylic ring. (iii) To incorporate ligands with potential to bind to the histamine H_1 receptor and thus act as dual mast cell stabilisers and antihistamines. (iv) To enhance the pharmaceutical properties of lead molecules through the placement of water-solubilising groups onto the benzylic ring.

The strategy was based on the premise that if novel mast cell stabilisers could be built from 1-indanone or 1-tetralone then it should be possible to build a similar series from 1-

benzosuberone. The obvious advantage with this building block is that the resulting compounds are unlikely to be cleaved, as the additional methylene unit eliminates the opportunity to form stable conjugated derivatives. Hence it was expected that within this series of compounds it should be possible to synthesise candidate molecules with the requisite molecular properties and appropriate stability profile.



Scheme 2.3 Cleavage resulting in non-conjugated fragments

A series of benzylic groups (Fig. 2.3) were chosen containing electron-withdrawing and/or electron-donating substituents. Moreover, an additional group of substituents were selected that continued highly "aromatised" functionality in the form of a 3-phenylbenzyl group and a biphenylmethyl group.

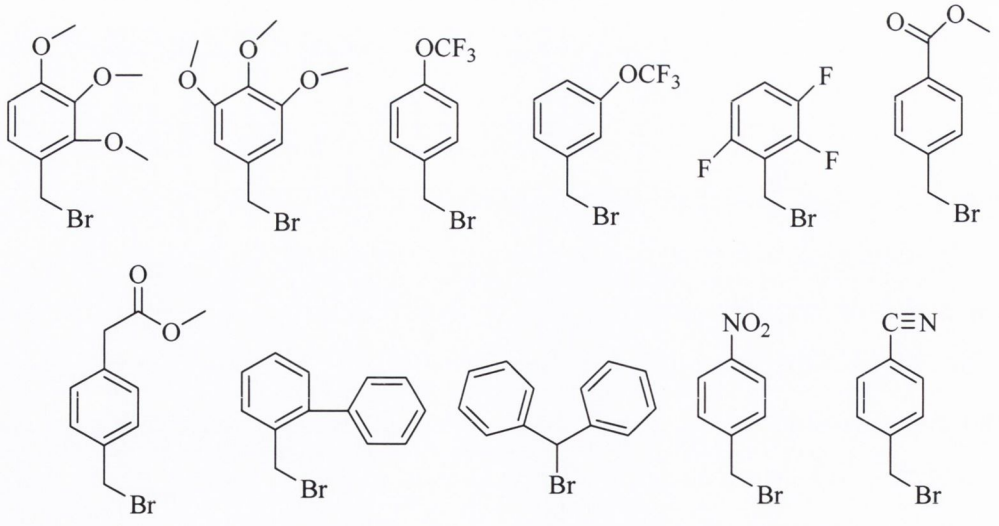
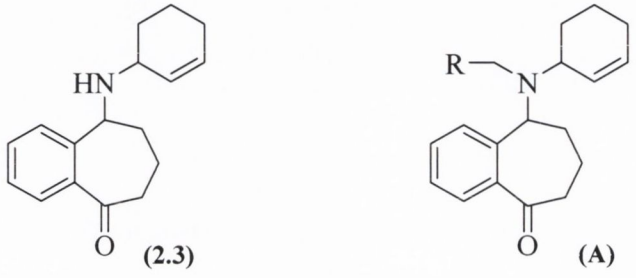


Fig. 2.3 Alkylating agents used to generate analogues of structural (A)

2.3 Synthesis of analogues of structural type (A)

In order to successfully generate the first series of target compounds of structural type **(A)**, an effective synthetic approach was designed to produce the key secondary amine intermediate **(2.3)** (Fig. 2.4). The logical starting point for the synthesis of compound **(2.3)** was benzosuberone. To introduce the amino substituent at the benzylic position, initial efforts were made around benzylic bromination of benzosuberone, followed by nucleophilic displacement of the bromine atom with an azide anion, and then subsequent reduction of the azide product.

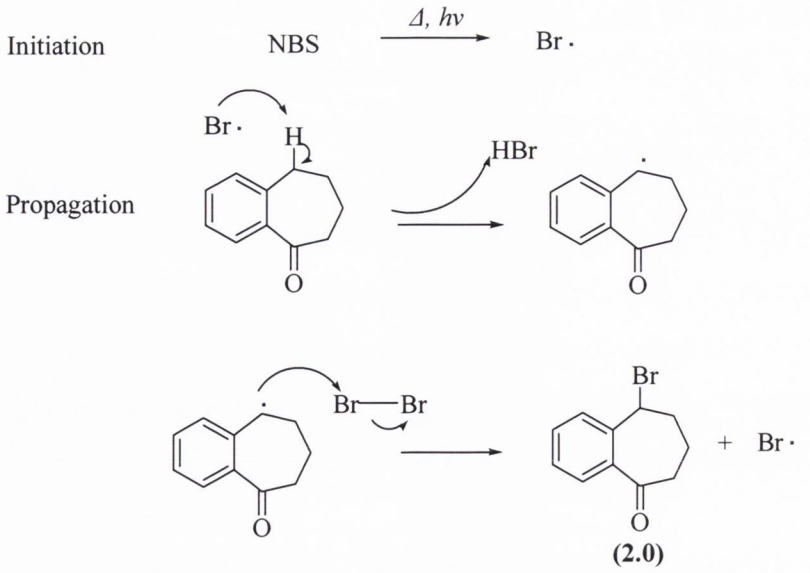


R groups designed to investigate the effect on mast cell stabilisation of changing the electronic properties of this ring and introduce H1 binding ligands.

Fig. 2.4 Secondary amine (2.3) and molecular design of structural type (A)

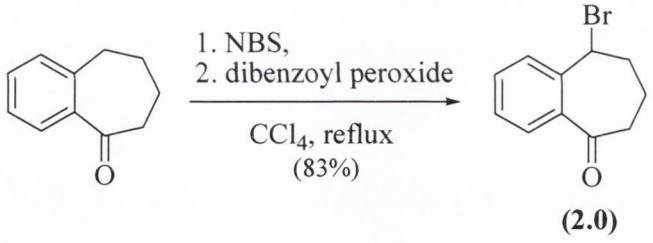
2.3.1 Synthesis of the target secondary amine (2.3)

To effect benzylic bromination of 1-benzosuberenone, *N*-bromosuccinimide (NBS) was employed with the addition of a catalytic amount of the radical initiator, dibenzoyl peroxide. Under such conditions the preferred solvent to use is carbon tetrachloride (CCl_4). NBS is the most common reagent to employ for benzylic or allylic bromination reactions. This transformation is known as the Wohl-Ziegler reaction and was first introduced in the 1940s (Djerassi, 1948). The reaction is believed to proceed through a free radical mechanism as shown in Scheme 2.4, with initial dissociation of NBS, allowing the release of a bromine free radical, which then either selectively abstracts a benzylic hydrogen to give a benzylic radical, or combines with another bromine radical to form bromine. The resultant reactive benzylic radical intermediate then reacts with the bromine molecule thus formed. This leads to its homolytic cleavage and attachment of one of the released bromine radicals onto the benzylic position to produce the desired benzylic bromide **(2.0)**.



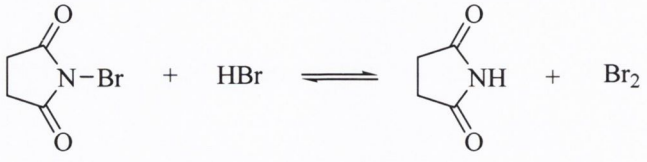
Scheme 2.4 Mechanism of benzylic radical bromination of 1-benzosuberone

Free radical bromination of 1-benzosuberone with NBS in the presence of CCl_4 proceeded under reflux conditions. Progress of the reaction was monitored by TLC as well as the observation of a colour change to the reaction mixture from bright yellow to orange (Scheme 2.5).



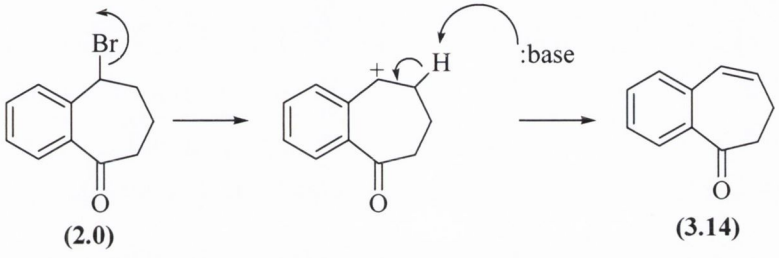
Scheme 2.5 Formation of compound (2.0)

The concentration of bromine plays a key role in this reaction. The advantage of using NBS rather than bromine itself is that NBS acts like a turnstile, allowing only one molecule of bromine to form for every molecule of HBr produced by the reaction. The liberated HBr produced by the reaction reacts with NBS to maintain low concentrations of bromine (Scheme 2.6).



Scheme 2.6 Reaction of HBr with NBS

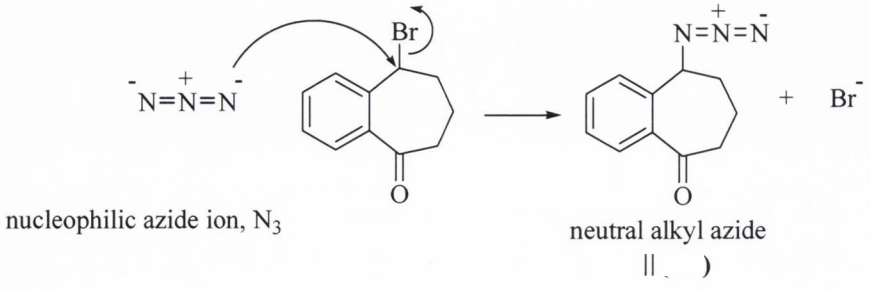
Although this reaction produced good yields of the desired product (**2.1**, 83%), optimisation of the reaction proved problematic especially when it was carried out on a large scale (>5.0g). In particular, the reaction suffered from competing side-reactions, including loss of HBr from the product resulting in considerable quantities of the alkene **(3.14)** being formed, especially when the reaction time was prolonged (>2.5 hours). It is presumed that loss of HBr may be occurring by an E1 mechanism with the rate-determining step being loss of the leaving group (Br^-), resulting in the formation of a carbocation. Subsequent loss of a hydrogen ion from the neighbouring carbon resulted in formation of the alkene **(3.14)** (Scheme 2.7).



Scheme 2.7 Formation of by-product (3.14) of radical bromination

The characteristic feature of the ^1H NMR spectrum of compound **(2.0)** was the double doublet (J_1=6.02Hz, J_2=2.02Hz) for the benzylic methine proton at 5.58ppm, and its corresponding benzylic carbon was located at 54.1ppm in the ^{13}C NMR spectrum. The key signal in the infrared spectrum was that of $C{=}O_{str}$ at 1686.5cm^{-1}.

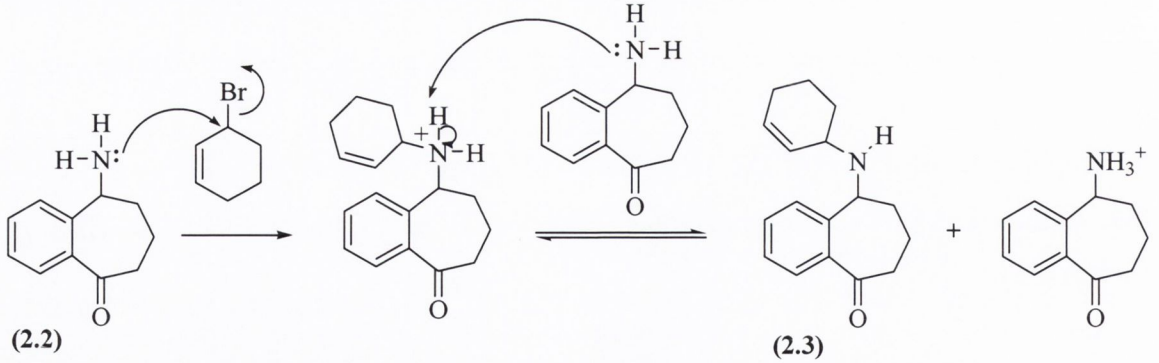
Once the desired benzylic bromide **(2.0)** was obtained, a suitable amino precursor could then be readily introduced by displacement of the bromine substituent at the benzylic position. This was achieved following treatment of the bromide **(2.0)** with sodium azide (NaN_3) in *N,N*-dimethylformamide (DMF) at 60°C, conditions that favour the nucleophilic substitution of the bromine by an azide ion over elimination reactions (McMurray, 1984). This reaction may be accounted for by the following mechanism (Scheme 2.8):



Scheme 2.8 Mechanism of azide nucleophilic substitution (S_N2)

The azide **(2.1)** was isolated in good yield (81%) as a dark brown oil. Upon azide substitution of the benzylic bromide **(2.0)**, the benzylic methine proton was shifted downfield to 4.91ppm, appearing as a triplet (J=5.52Hz) in the ^{1}H NMR spectrum of compound **(2.1)**. Conversely, the benzylic methine carbon moved upfield to 63.7ppm in the ^{13}C NMR spectrum. The infrared spectrum was characterised by the presence of azide ($-N_3$) at 2104.1cm^{-1}, in addition to the $C{=}O_{str}$ of the ketone at 1680.6cm^{-1}. The azide **(2.1)** was analysed by high resolution mass spectrometry (HRMS). The calculated mass was 202.0980g/mol, which correlates well to the actual mass of 202.0977g/mol. The following elemental composition for that peak, ($C_{11}H_{12}N_3O$), corresponds to the structure of compound **(2.1)** ($+H^+$).

The azide **(2.1)** was conveniently reduced to the primary amine **(2.2)** after initial treatment of the azide **(2.1)** with zinc powder in acetic acid at 0°C and then allowing the temperature to slowly rise to room temperature. Attachment of the cyclohexenyl group onto the amino functionality of the amine **(2.2)** was accomplished, following coupling of the amine **(2.2)** to 3-bromocyclohexene in DMF at room temperature, using caesium carbonate (Cs_2CO_3) as base. After ten days, the reaction yielded a stereoisomeric mixture of the desired secondary amine **(2.3)** in low yield (39%), as a pale yellow oil. When the coupling conditions were changed to using potassium carbonate (K_2CO_3) as base in acetone under reflux conditions for twelve hours, the desired secondary amine **(2.3)** was obtained in a relatively good yield of 62%. The reaction is presumed to proceed *via* an S_N2 mechanism, as depicted in Scheme 2.9.

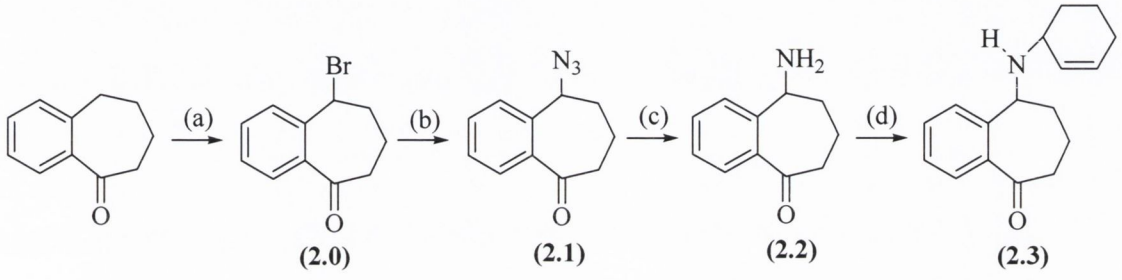


Scheme 2.9 Alkylation of the primary amine (2.2)

The yield of the target secondary amine **(2.3)** was substantially increased from 10% to 62% yield when the solvent was changed to acetone; the base used was postassium carbonate and when the reaction was carried out under reflux. The potential reason for

the increased yield may be accounted for on the basis that in this nucleophilic substitution reaction (S_N2), the lone pair of electrons on the amine were solvated by the polar solvent, DMF, and consequently decelerated the reaction considerably. On the contrary the relatively nonpolar solvent, acetone, would not be expected to solvate the primary amine to the same degree or the transition state and thus accelerated the reaction considerably.

In summary, Scheme 2.10 illustrates the entire sequence of events towards the secondary amine (**2.3**) from 1-benzosuberone.



(a) NBS, dibenzoyl peroxide, CCl_4, reflux, 2hrs, 83%; (b) NaN_3, DMF, 40-60°C, 2.5hrs, 81%
(c) Zn, CH_3COOH, RT, 7.5hrs, 56%; (d) 3-bromocyclohexene, acetone, K_2CO_3, 12hrs, 62%

Scheme 2.10 The overall synthetic pathway of the secondary amine (2.3)

2.3.2 Structure determination of the secondary amine (2.3)

The identity of compound **(2.3)** was confirmed through the use of ^1H, ^{13}C nuclear magnetic resonance (NMR), infrared (IR) and high resolution mass spectroscopy (HRMS). In the ^1H NMR spectrum of compound **(2.3)**, strong aliphatic resonances with complex coupling were evident in the 1.10-2.10ppm range of the spectrum. The complexity of the coupling can be further attributed to the fact that compound **(2.3)** exists as a mixture of stereoisomers. Ten of the twelve methylene protons resonated from 1.21-2.07ppm. A breakdown of this region revealed that a single methylene system resonated between 1.21-1.30ppm, there was one between 1.31-1.50ppm, one between 1.56-1.65ppm, four between 1.67-1.81ppm, two between 1.88-1.95ppm and one between 2.00-2.07ppm. The remaining two methylene protons within the aliphatic region of the spectrum, those α to the carbonyl group (C-2) were visible as two separate pairs of signals. These signals appeared to take the form of a pair of multiplets, with resonance values between 2.48-2.57ppm for the first, and values between 2.89-2.98ppm for the second. While in between these two protons the cyclohexenyl methine (C-10) resided at

2.82ppm as a broad multiplet. The second methine proton (C-5), that of the benzosuberyl unit, was found as two overlapping triplets signals between 4.22-4.26ppm. The protons of the alkene moiety (C-11 and C-12) form a complex multiple signal, ranging from 5.54-5.77ppm. In the aromatic region, two of the four proton system resonated between 7.28-7.38ppm, one at 7.42ppm while the remaining unassigned proton that of C-9 resonated at 7.47ppm. The ^{13}C NMR spectrum of compound **(2.3)** was along expected lines with most signals doubling up as expected for a stereoisomeric mixture (Fig. 2.6 and Fig. 2.7).

However, the carbonyl signal was absent even though the dominant resonance characteristic of the carbonyl group, at 1681.3cm^{-1}, was evident in the infrared spectrum. Another notable feature of the infrared spectrum was broad NH stretch at 3322.4cm^{-1}. The identity of compound **(2.3)** was also confirmed through HRMS analysis with the most significant peak representing the ($+H^+$) ion at 256.1691 that calculated being 256.1701 with molecular formula $C_{17}H_{22}NO$.

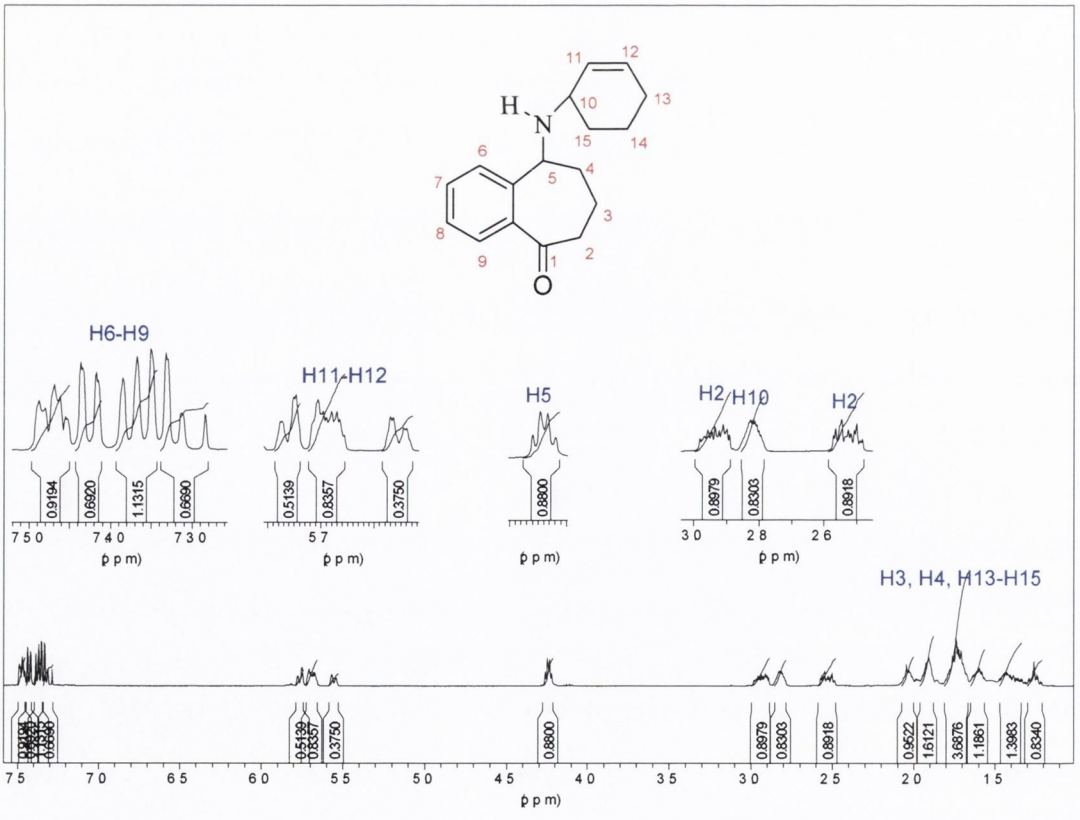


Fig. 2.5 ^1H NMR spectrum of compound (2.3)

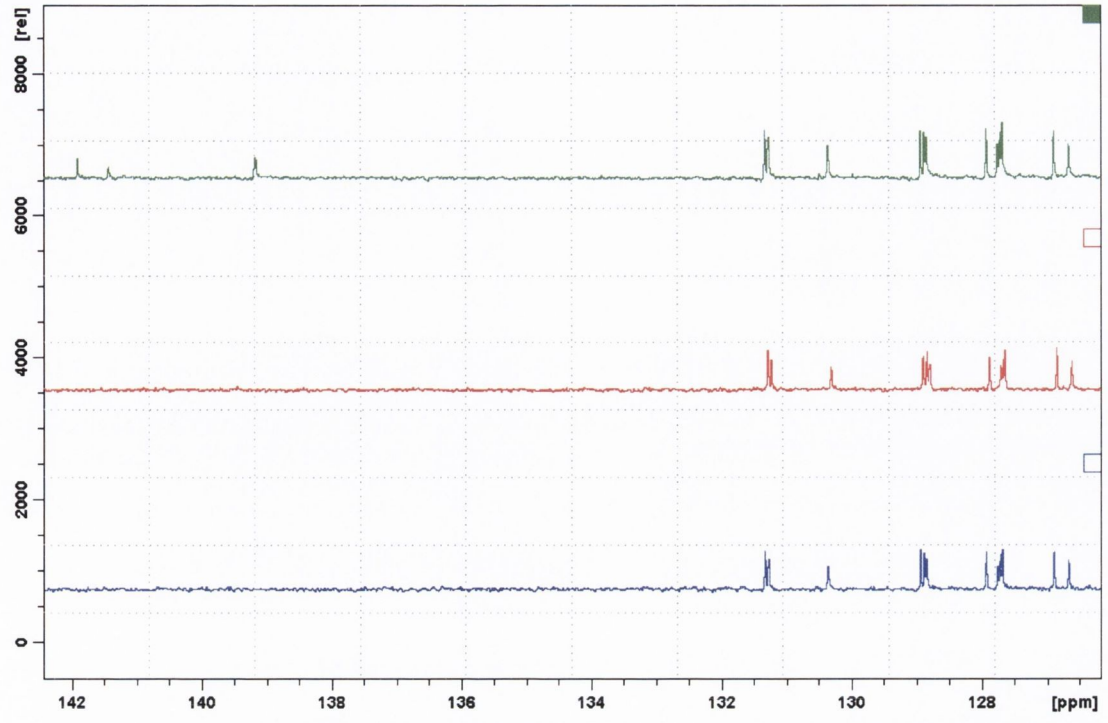


Fig. 2.6 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (2.3) from 126.8-142.5ppm

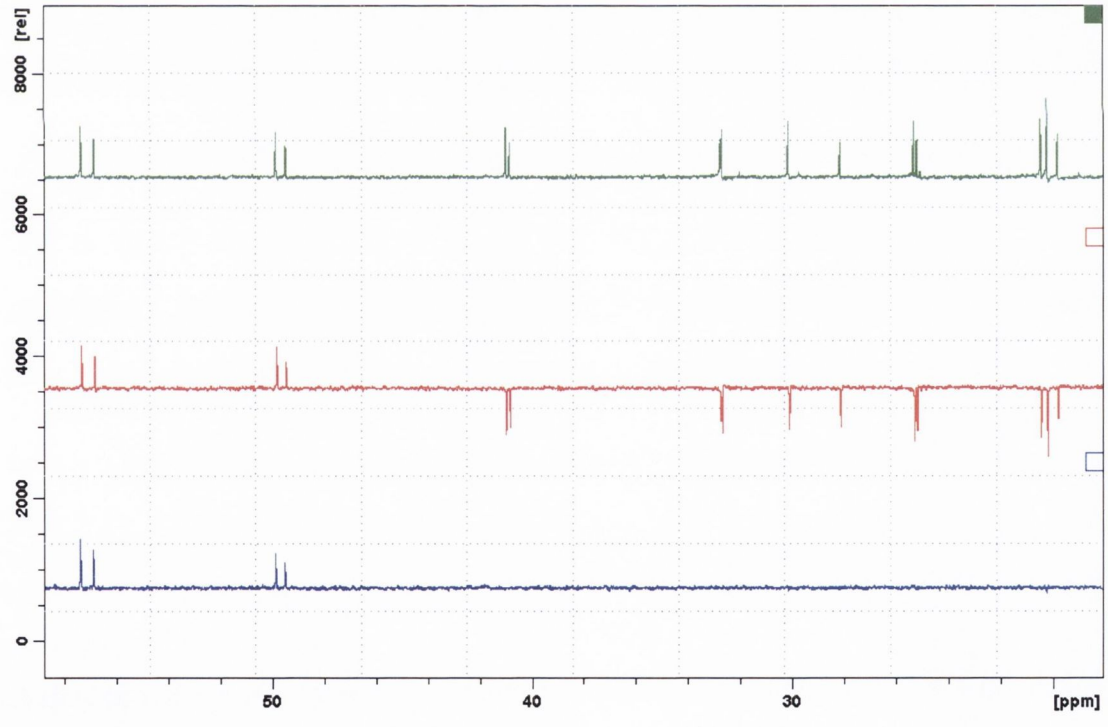


Fig. 2.7 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (2.3) from 22.0-59.0ppm

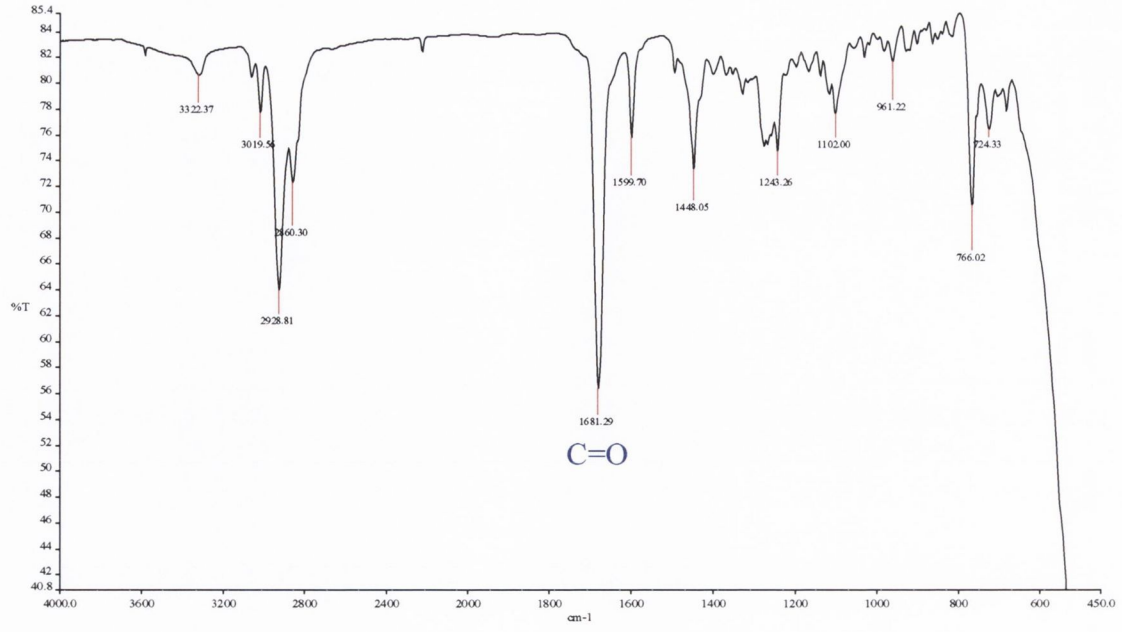
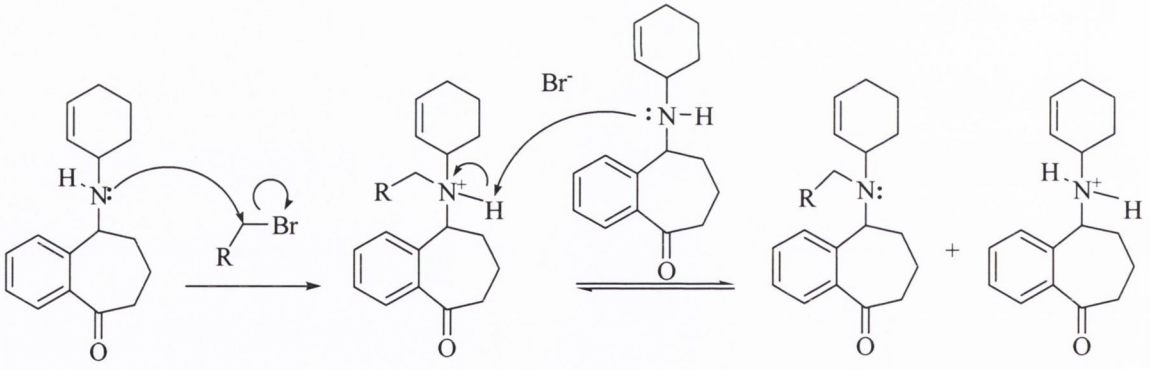


Fig. 2.8 IR spectrum of compound (2.3)

2.3.3 *N*-alkylation of the secondary amine (2.3) with electron rich benzyl groups



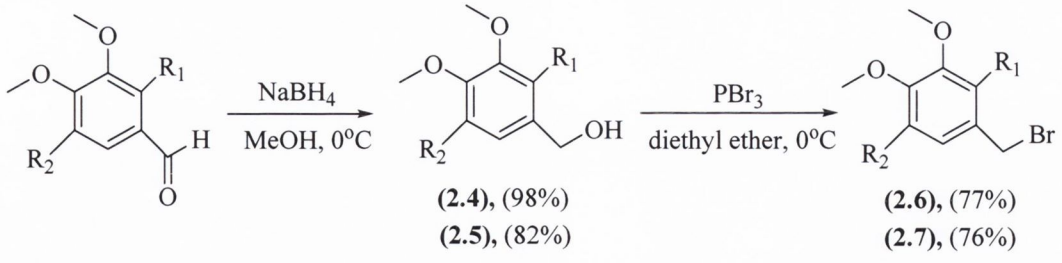
Scheme 2.11 Synthesis of N-alkyl derivatives of compound (2.3)

Having identified an appropriate synthetic method to allow for the ready attainment of satisfactory quantities of the amine **(2.3)**, the next target was to develop the conditions necessary for *N*-benzylation of this secondary amine. As might have been anticipated formation of the desired benzylic derivatives of compound **(2.3)** proved to be quite problematic. This may be accounted for on the basis that the two alkyl substituents (benzosuberyl and cyclohexenyl rings) attached to the amino functionality of compound **(2.3)** are quite bulky and thus steric factors may also be an additional important parameter when determining the rate at which this secondary amine could be alkylated.

The mechanism of the *N*-alkylation reaction of the secondary amine **(2.3)** is shown in Scheme 2.11.

2.3.3.1 Synthesis of *N*-alkyl derivatives (2.8) and (2.9)

Benzyl halides containing methoxy substituents were chosen as examples of electron rich substituents for the initial studies with, 2,3,4-trimethoxybenzyl bromide **(2.6)** and its 3,4,5-trimethoxy-isomer **(2.7)**, the preferred candidates to use based on observations by Barlow (2002) with the tetralin series of mast cell stabilisers. The strategic plan was also to directly compare their activities to a simple non-substituted benzyl analogue. Although the methoxy benzyl halides are not commercially available, they can be readily synthesised from their respective aldeydes. Scheme 2.12 depicts the sequence of steps involved in their preparation. The first step involved reduction of the parent aldehyde with sodium borohydride in the presence of methanol at 0°C. This transformation occurred smoothly in an hour to form the expected products in good yield **(2.4,** 94%) or **(2.5,** 89%). The conversion of the resulting alcohols to their corresponding trimethoxybenzyl bromides was achieved by using phosphorous tribromide in the presence of diethyl ether at -20°C for ninety minutes. This substitution step proved to be slightly problematic as it tended to be slightly exothermic upon work-up. Moreover, purification of the resulting trimethoxybenzyl bromides was difficult through traditional silica gel based chromatographic methods, but nevertheless yielded the desired halides **(2.6)** and **(2.7)** in 76% and 77% yields, respectively. As the stability of both benzyl halides on storage proved problematic they were used immediately for the subsequent alkylation step (Scheme 2.12).



(2.4), (98%)
(2.5), (82%)

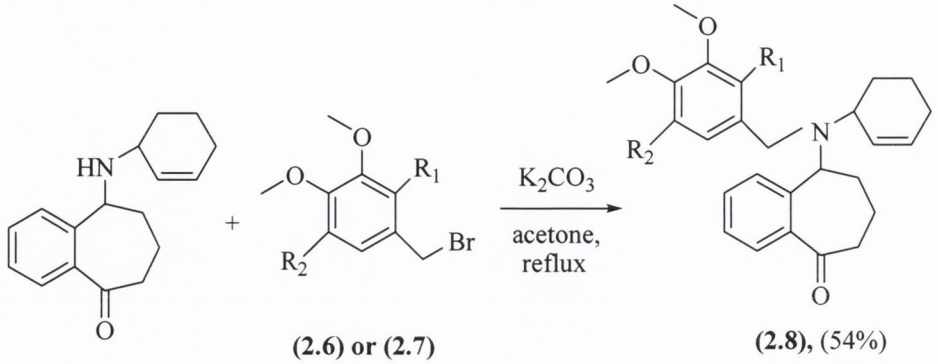
(2.6), (77%)
(2.7), (76%)

compounds (2.4) and (2.6): R_1 = OMe, R_2 = H compounds (2.5) and (2.7): R_1 = H, R_2 = OMe

Scheme 2.12 Synthesis of compounds (2.4)-(2.7)

The final step towards the tertiary amine derivatives **(2.8)** and **(2.9)** was alkylation of compound **(2.3)** with bromides **(2.6)** and **(2.7)**. As with the formation of the secondary amine **(2.3)**, the preferred condition to use was to employ K_2CO_3 as base in acetone

under reflux conditions (Scheme 2.13). This modification was found to proceed much quicker, with improved overall yields **(2.8**, 54%**)** and **(2.9**, 59%**)** in comparison to the initial reaction condition, Cs_2CO_3 in DMF at room temperature.



(2.6) or (2.7)

(2.8), (54%)
(2.9), (59%)

compounds **(2.6)** and **(2.8)**: R_1 = OMe, R_2 = H compounds **(2.7)** and **(2.9)**: R_1 = H, R_2 = OMe

Scheme 2.13 Synthesis of compounds (2.8) and (2.9)

2.3.3.2 Synthesis of *N*-alkyl derivatives (2.10-2.20) containing either electron withdrawing or water-solubilising substituents

Although relatively good yields of compounds **(2.8)** and **(2.9)** were achieved when the same conditions were employed for this series of compounds, the benzylation step could at best be described as inconsistent. The reactions tended to be sluggish, even when allowed to proceed for more than one week. As a result, very little of the desired tertiary amines were formed even when different benzylic halides were utilised. When we reverted back to using DMF as solvent and Cs_2CO_3 as base at higher temperatures this also resulted in poor yields of the desired product with numerous side products forming in the synthesis of compounds **(2.13)**, **(2.17)**, **(2.20)** and **(2.21)** especially.

Previous studies by Morre et al, (2005) indicated that tertiary amines can be synthesized efficiently by direct *N*-alkylation of secondary amines with alkyl halides in the presence of Hunig's base (*N,N*-diisopropylethylamine) using acetonitrile as solvent at room temperature. To test this observation, these conditions were utilized to prepare compound **(2.18)**. Thus compound (**2.3**) in the presence of acetonitrile at room temperature was treated with 4-(bromomethyl) benzonitrile and *N,N*-diisopropylethylamine. Under these conditions, a slow rate of reaction was observed. In an effort to improve the yield of product, the temperature of the reaction was raised to a gentle reflux. Under these conditions the yield of desired product was increased to 90%

with no evidence of side products forming when analysed by TLC. After slight modifications to the method, the best set of conditions to employ was to use the following ratio of secondary amine, alkyl halide, and Hunig's base, 1.0, 1.1 to 1.5 respectively. These alkylation reaction conditions were repeated to furnish the targeted tertiary amines **(2.10)-(2.21).** Details on their reaction times and overall yield obtained for each compound is shown in Table 2.0.

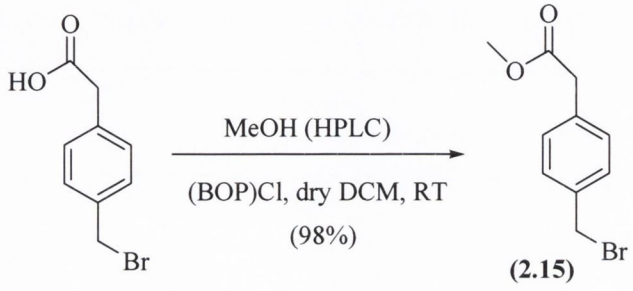
The exact role of Hunig's base in this alkylation reaction has not been established. However, Klok et al., (2006) suggested that Hunig's base enhances the alkylation step probably due to its non-nucleophilic properties as a sterically hindered base. The reaction itself is also believed to be reversible under mild kinetically controlled conditions. It is thought that salt formation of this non-nucleophilic strong base with the released hydrogen halide, allows alkylation of the secondary amine to proceed under mild kinetically controlled conditions (Moore et al., 2005).

R	Compound	Reaction time	% Yield
Benzyl	**2.10**	7 days	63
4-(trifluoromethoxy)benzyl	**2.11**	12 days	81
3-(trifluoromethoxy)benzyl	**2.12**	12 days	73
2,3,6-trifluorobenzyl	**2.13**	12 days	70
Methyl 4-benzoate	**2.14**	13 days	54
Methyl 4-phenylacetate	**2.16**	24 hours	12
4-nitrobenzyl	**2.17**	10 days	64
Methyl 4-benzonitrile	**2.18**	12 days	85
2-phenylbenzyl	**2.20**	12 days	41
Diphenylmethanyl	**2.21**	4 days	32

Table 2.0 Tertiary amine derivatives (2.10)-(2.20)

Aside from methyl 2-[4-(bromomethyl)phenyl]acetate, all of the benzylic halides used to synthesise compounds **(2.10)-(2.14)** and **(2.16)-(2.21)** were commercially available. However, its synthesis was easily accomplished through methylation of 2-[4-(bromomethyl)phenyl]acetic acid. The reaction involved simply treating a solution of the acid in DCM with 2 equivalents of methanol and employing bis(2-oxo-3-oxazolidinyl)phosphonic chloride as coupling reagent. After allowing the solution to stir

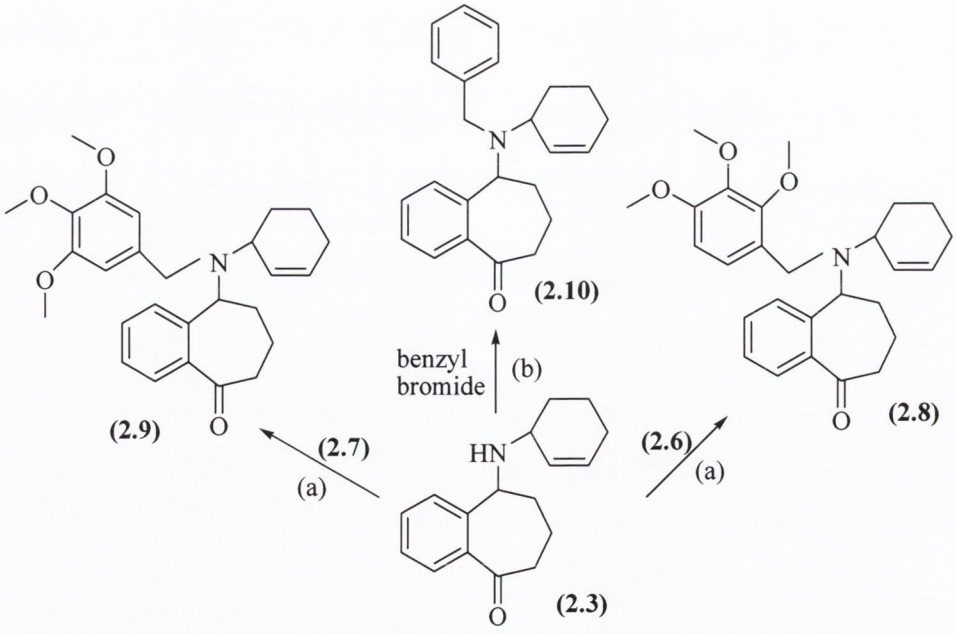
at room temperature for twenty-four hours, the desired ester **(2.15)** was obtained in near quantitative yield, following work-up and purification by flash column chromatography (Scheme 2.14). Its identity was confirmed through analysis of its ^{1}H NMR spectrum where a sharp singlet at 3.72ppm integrating for three protons represented the methyl group. The DEPT 135 NMR spectrum also confirmed the existence of the methyl carbon at 52.2ppm while in the infrared spectrum a strong ester carbonyl absorption band was evident at 1736.3 cm^{-1}.



Scheme 2.14 Synthesis of compound (2.15)

2.3.3.3 Structural determinations of compounds (2.8), (2.9) and (2.10)

In each case, the benzyl amines **(2.8)**, **(2.9)** and **(2.10)** were isolated as a mixture of stereoisomers. Their NMR spectra were somewhat different from that of the parent amine **(2.3)**.



(a) acetone, K_2CO_3, reflux, 16days (b) acetonitrile, *N,N*-diisopropylethylamine, reflux, N_2, 7days

Scheme 2.15 N-alkyl derivatives (2.8-2.10)

Additional complexity was introduced into the spectra when compared to the aliphatic region aside from the signal for the benzyl methylene, in comparison with compound **(2.3)**. The main difference in the spectrum occurred around 3.90ppm for compounds **(2.8)** and **(2.9)**. As the simplest N-benzyl derivative, namely compound **(2.10)**, mainly affected the complexity of the aromatic region, with the exception of the distinctive aminomethylene resonance at 3.76-4.02ppm in the ^{1}H NMR spectrum. A particularly noteworthy characteristic of the ^{13}C NMR spectrum for this molecule was the resonance for the carbonyl carbon, 205.6 and 206.5ppm, one for each diastereomer which was surprisingly absent in the ^{13}C NMR spectrum of compound **(2.3)**.

The main additional feature of the compound **(2.8)** to the general pattern seen in the ^{1}H NMR spectrum of compound **(2.3)**, was the presence of the strongly overlapping singlets for the nine methyl protons at 3.88ppm; their corresponding ^{13}C signals being found at 55.5, 60.3 and 60.4ppm. The aromatic region integrated for a total of six protons: two doublets (J=7.76Hz) at 7.82 and 7.89ppm indicating a diastereomeric mixture of products, which was attributed to the proton on C-9. A sharp doublet (J=9.04Hz) integrating for one proton, at 6.72ppm was considered to be the aromatic proton next to the methoxy group. A multiplet, integrating for two protons, in the region of 3.68 and 3.86ppm was indicative of the two protons of the methylene (C-16). The three quaternary carbons of the methoxy substituents were also observed as three additional quaternary carbons at 126.6 and 127.3, 150.8 and 151.1, 151.6 and 151.7ppm in the ^{13}C NMR spectrum, one for each diastereomer pair. The DEPT 135 NMR spectrum confirmed the presence of the benzylic methylene (C-16) at 44.3 and 44.9ppm. A total of sixteen methine peaks were observed in the DEPT 90 NMR spectrum between 106.8-131.3ppm, including the two carbons for the alkene moiety (C-11and C-12) being present at 129.8, 129.9, 130.1, 130.3ppm. These signals were identified following inspection of the C-H COSY NMR spectrum of compound **(2.8)**.

Rotation of the trimethoxy-substituent from the more hindered 2,3,4-position to the less hindered 3,4,5-position resulted in the formation of compound **(2.9)**, generated through alkylation of compound **(2.3)** with 3,4,5-trimethoxybenzyl bromide. As might have been predicted ^{1}H signals in the NMR spectra of compounds **(2.8)** and **(2.9)** were found to have many identical resonance values. The main features of the ^{1}H NMR spectrum of compound **(2.9)** were the two separated singlet signals, which integrated for a total of

nine protons, representing the trimethoxy protons, at 3.85 and 3.89ppm. Their corresponding ^{13}C signals resonated at 56.0ppm as an overlapping signal, perhaps due to the slightly symmetrical arrangement of the methoxy substituents on the ring. In the aromatic region of the ^{1}H NMR spectrum, two singlets integrating for two protons, at 6.63 and 6.66ppm, was assigned to the two protons (C-17 and C-18) adjacent to the methoxy groups. Two overlapping doublets (J=7.78Hz) at 7.77ppm were assigned to the aromatic methine proton on C-9. It's resonance position being influenced by the adjacent carbonyl group. A total of six quaternary carbons were identified in the region between 135.8 and 152.6ppm in the ^{13}C NMR spectrum.

For clarity, the H-H COSY, C-H COSY and HMBC NMR spectra of compound **(2.9)** are shown in the Figures below. Assignments derived from these Figures are included in the experimental section.

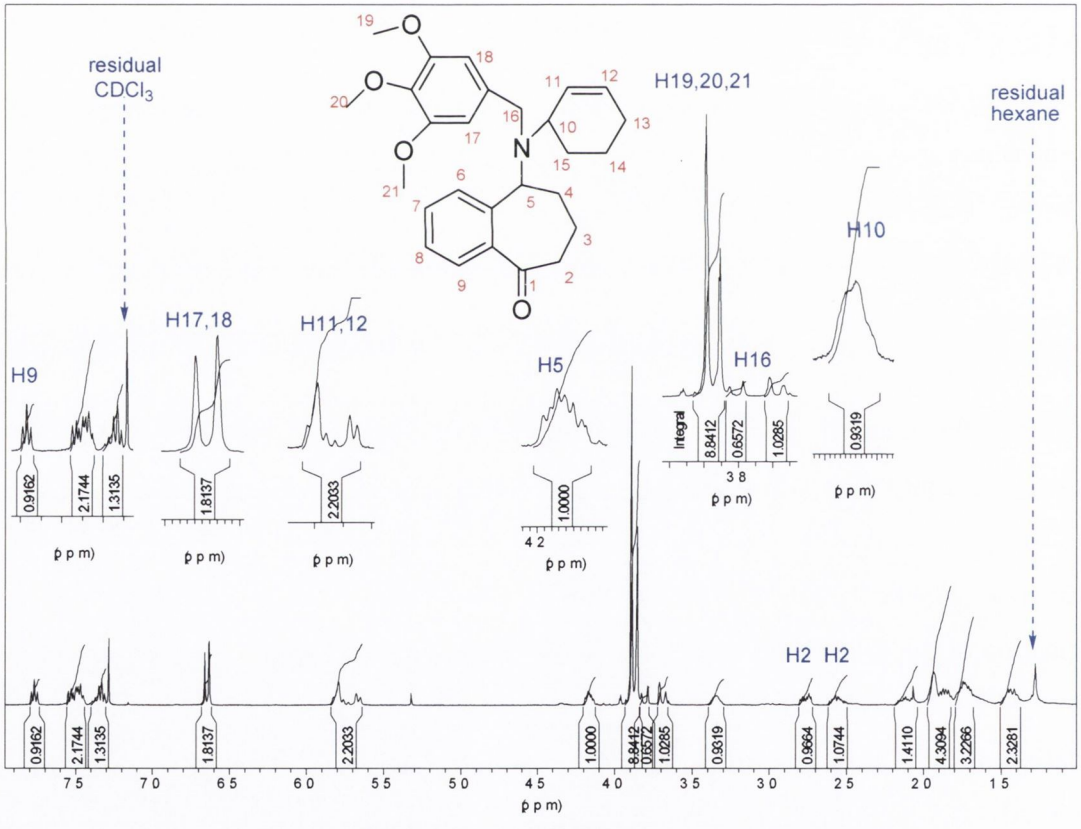


Fig. 2.9 ^{1}H NMR spectrum of compound (2.9)

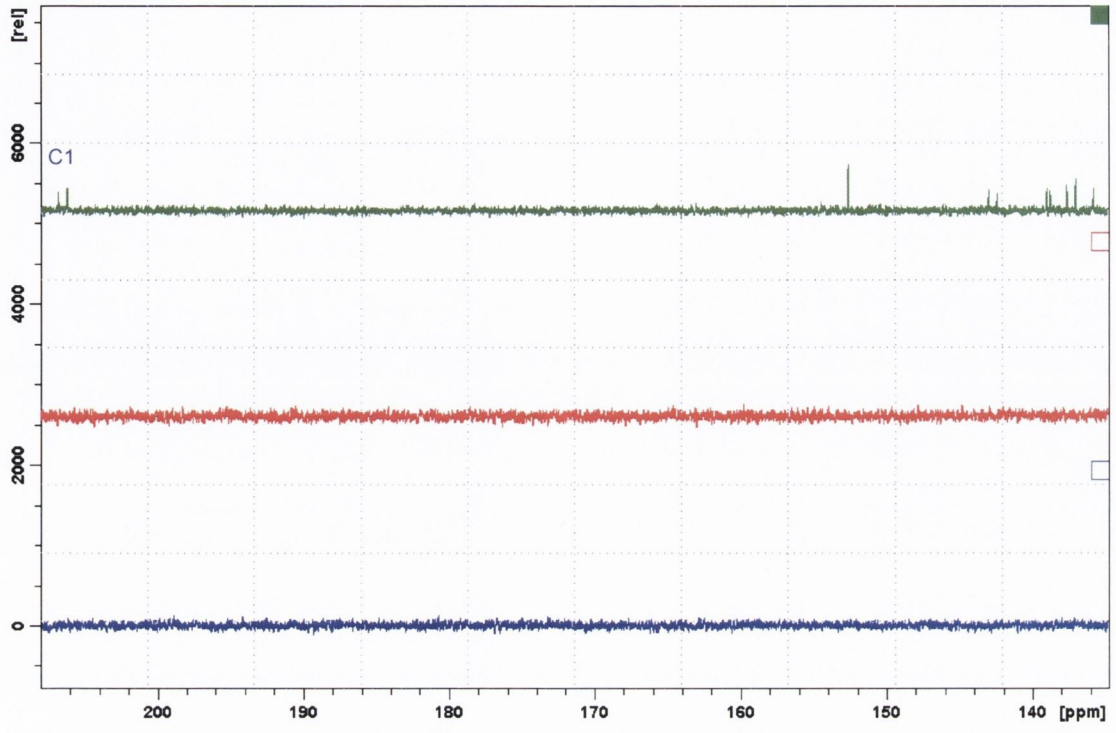


Fig. 2.10 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (2.9) from 135.2-208.0ppm

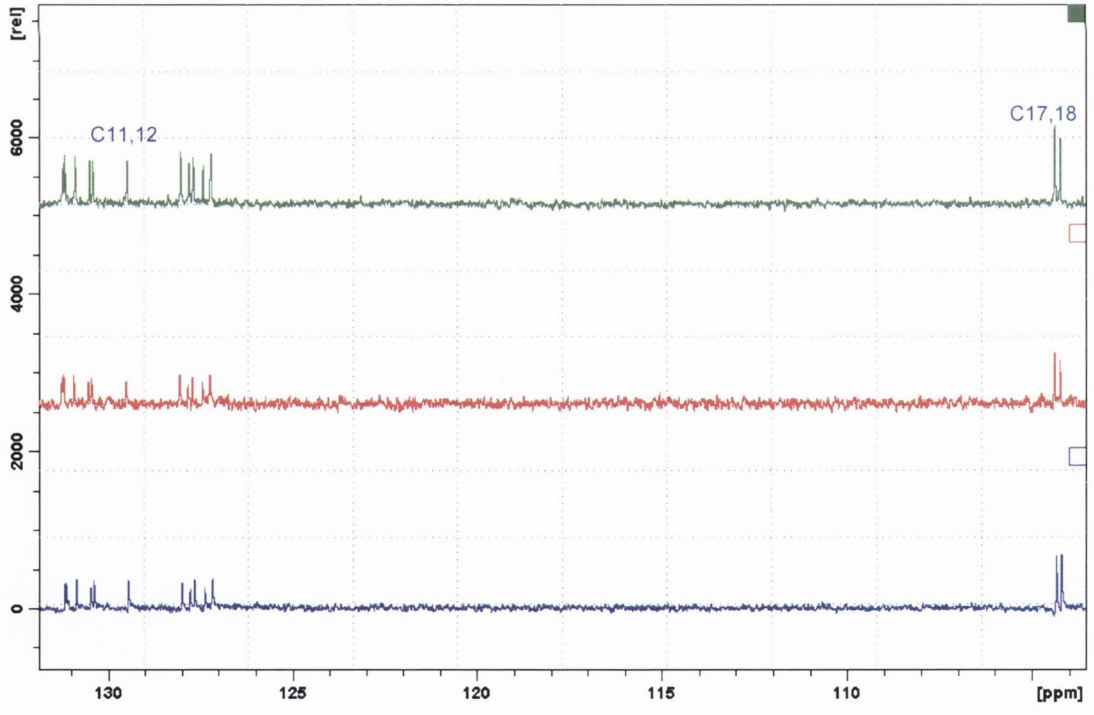


Fig. 2.11 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (2.9) from 103.5-132.2ppm

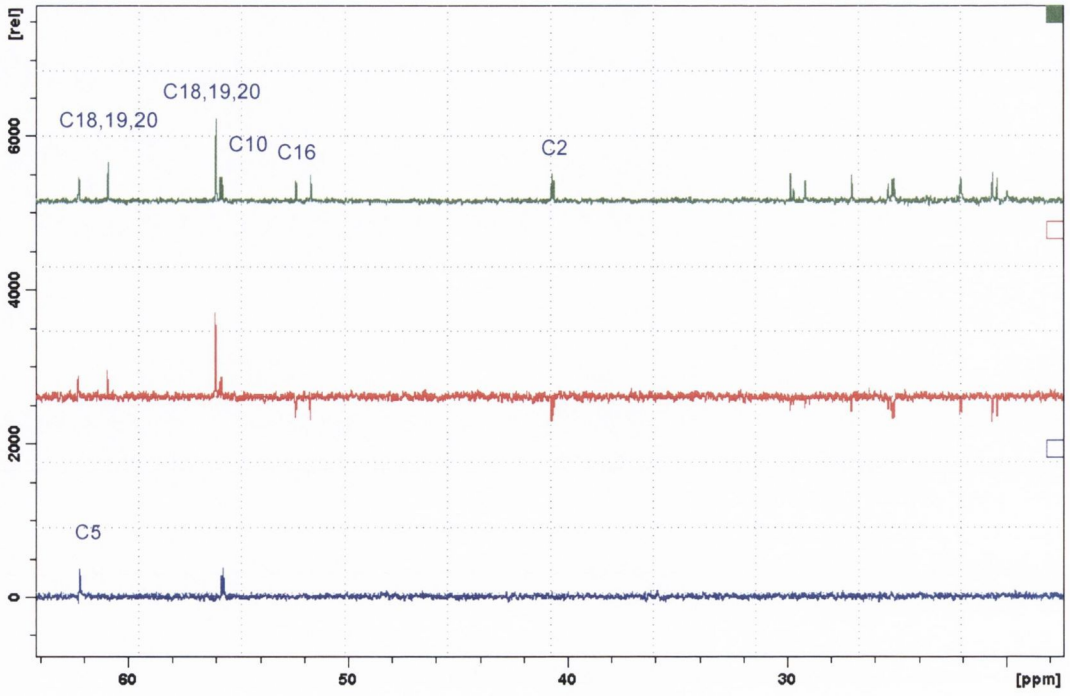


Fig. 2.12 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (2.9) from 17.6-64.1ppm

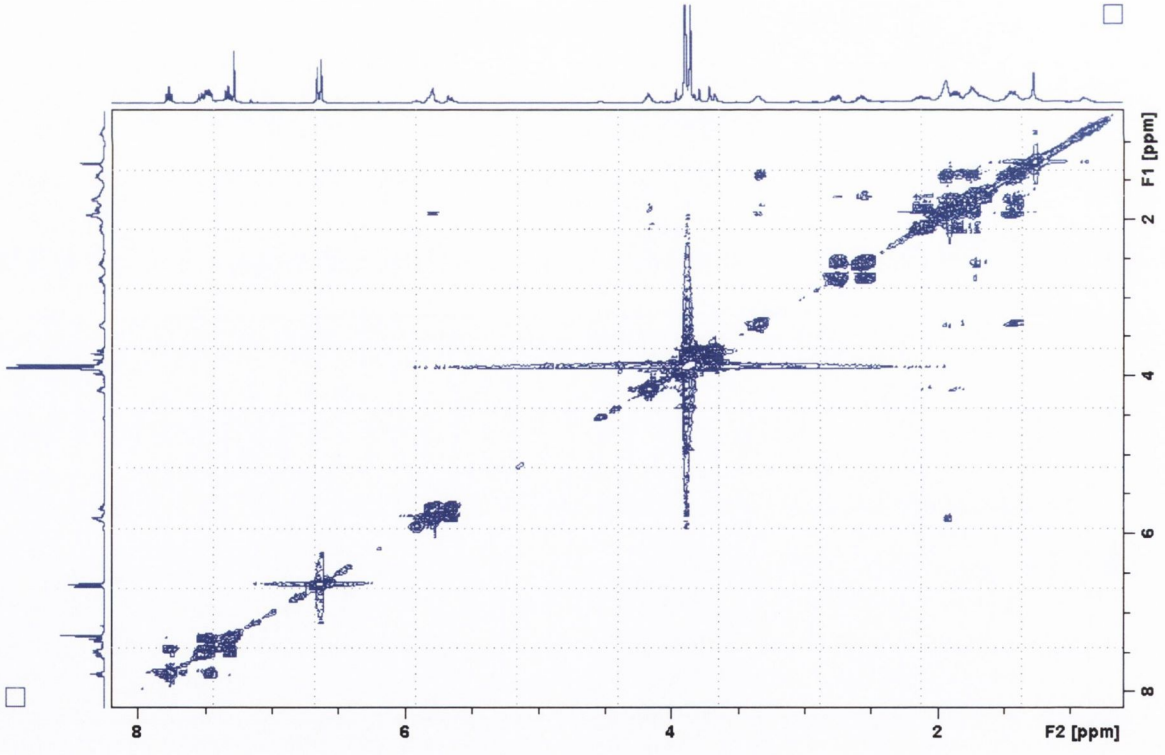


Fig. 2.13 H-H COSY NMR spectrum of compound (2.9)

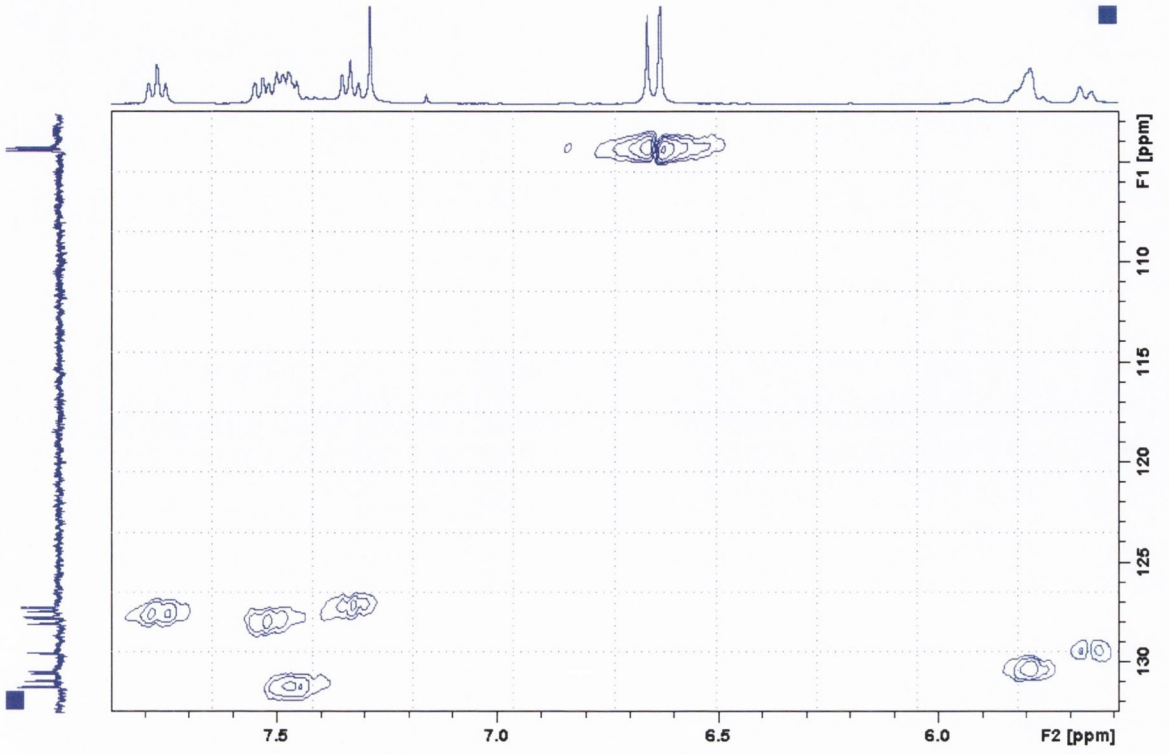


Fig. 2.14 C-H COSY NMR spectrum of compound (2.9) from 5.59-7.87ppm

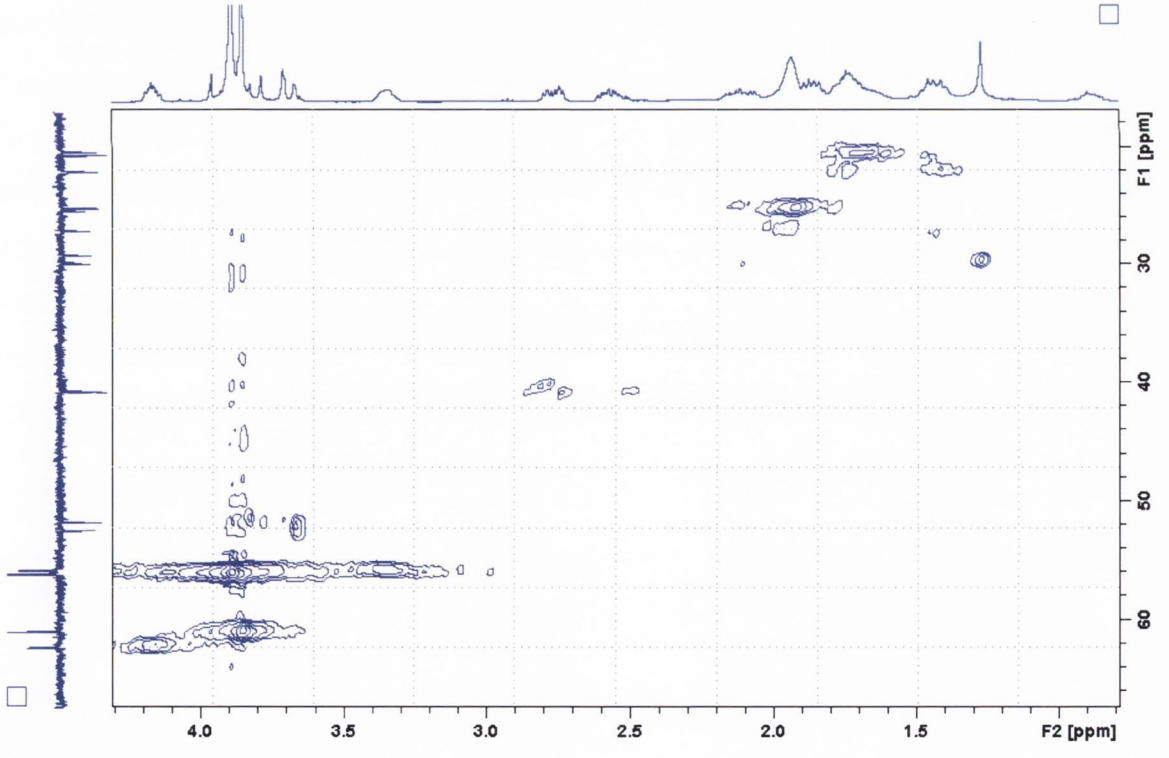


Fig. 2.15 C-H COSY NMR spectrum of compound (2.9) from 0.78-4.31ppm

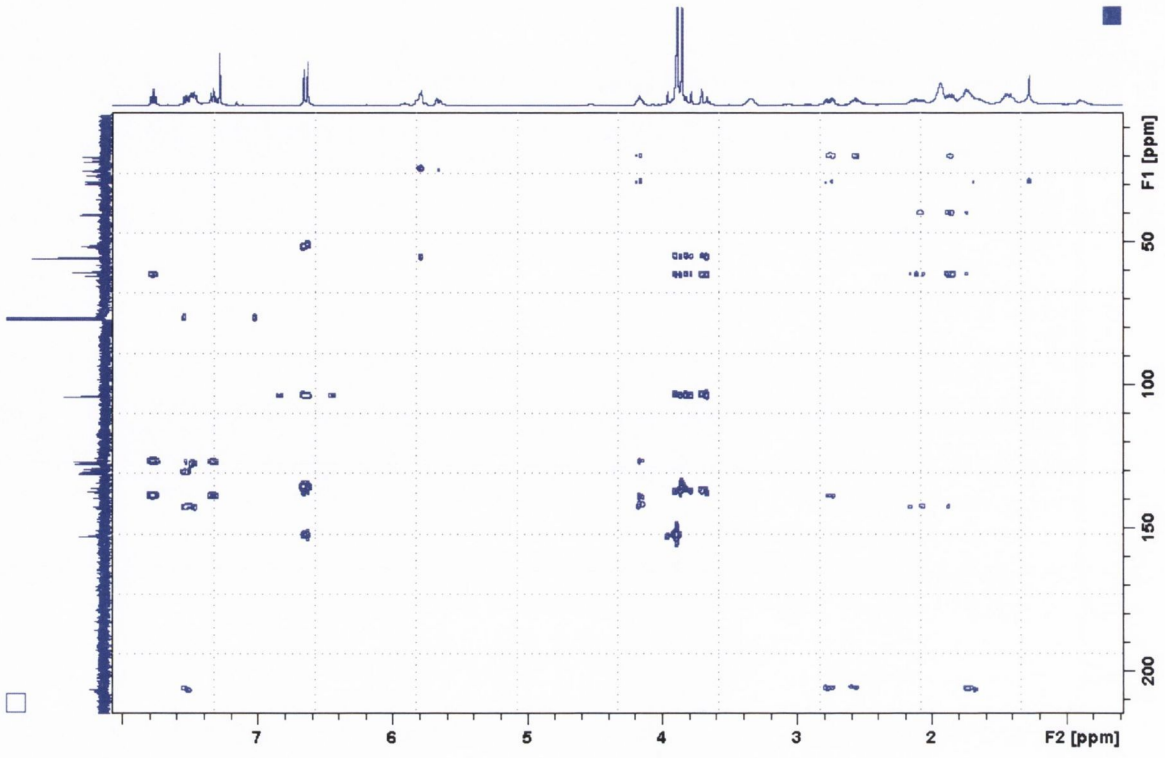
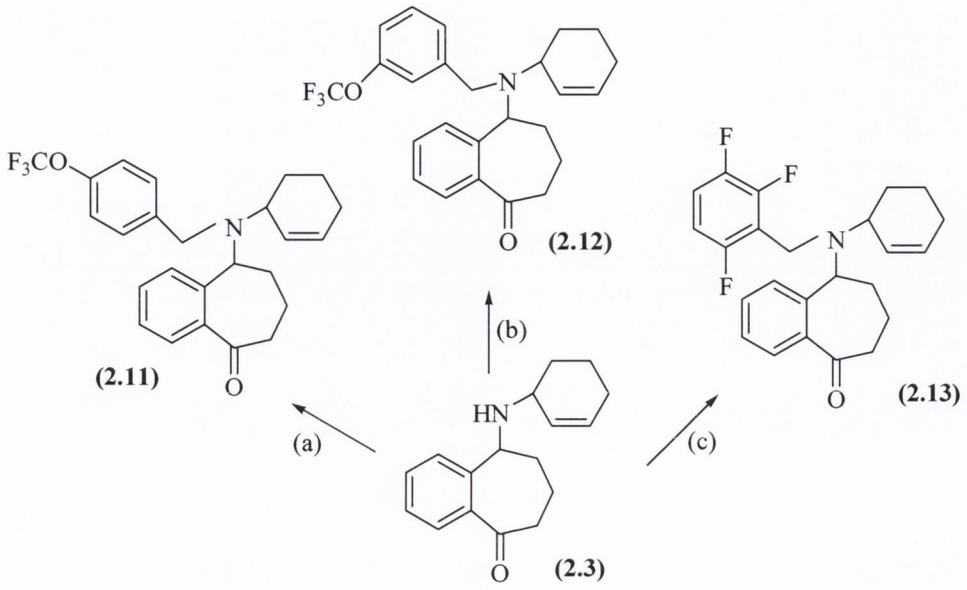


Fig. 2.16 HMBC NMR spectrum of compound (2.9)

When substituted by a trimethoxy benzyl group, the infrared spectra of compounds **(2.8)** and **(2.9)** were characterised by the presence of ethereal C-O_{str} at 1101.0 and 1287.2cm^{-1}, 1125.8 and 1253.9cm^{-1} respectively, in addition to the C=O_{str} of the ketone at 1683cm^{-1} for both compounds **(2.8)** and **(2.9)**. In all cases **(2.8, 2.9** and **2.10)**, HRMS spectral analysis gave acceptably accurate molecular masses: for compound **(2.8)**, 436.2480 (+H^+), calculated 436.2488 with molecular formula $C_{27}H_{34}NO_4$, for compound **(2.9)**, 458.2322 (+H^+), calculated 458.2307 with molecular formula $C_{27}H_{33}NO_4Na$, and for compound **(2.10)**, 346.2186 (+H^+), calculated 346.2171 with molecular formula $C_{24}H_{27}NO$.

2.3.3.4 Structural determinations of the fluorinated derivatives (2.11), (2.12) and (2.13)



(a) 4-(trifluoromethoxy)benzyl bromide, acetonitrile, *N,N*-diisopropylethylamine, reflux, N_2, 12days
(b) 3-(trifluoromethoxy)benzyl bromide, acetonitrile, *N,N*-diisopropylethylamine, reflux, N_2, 12days
(c) 2,3,6-trifluorobenzylbromide acetonitrile, *N,N*-diisopropylethylamine, reflux, N_2, 12days

Scheme 2.16 N-alkyl derivatives (2.11-2.13)

Upon *N*-benzylation of the secondary amine by a trifluoromethoxy group, which was believed to be more electron withdrawing than their methoxy analogues. The ^{1}H NMR spectra of compounds **(2.11)** and **(2.12)** were extremely complex in the aromatic region, due to the formation of diastereomeric mixtures of products.

In the ^{1}H NMR spectrum of compound **(2.11)** (Fig. 2.17), two doublets (J=8.04Hz) at 7.76 and 7.82ppm integrating as one proton, represented the proton at C-9 position. Three protons resonated within a narrow region between 7.42-7.53ppm as a multiplet, one proton at 7.38ppm as a doublet (J=8.52Hz) and one proton at 7.30ppm appearing as a double doublet (J_1=13.56Hz, J_2=6.52Hz). The final two protons in the aromatic region were present at 7.16ppm being considered as two overlapping triplets (J=7.04Hz). The alkenic protons (C-11 and C-12) resonated as a multiplet between 5.64-5.79ppm. The methine proton (C-5) of the benzosuberone ring was accounted for by a multiplet in the region of 4.14-4.17ppm. The protons of the methylene (C-16) attached to the amino moiety form a complex multiple signal, ranging from 3.72-3.93ppm. The methine proton (C-10) of the cyclohexenylamino ring resonated as a broad multiplet at 3.37ppm. The two separate multiplets located between 2.55-2.63ppm and 2.67-2.75ppm, represented

the α-keto methylene group of the bezosuberone ring at C-2 position. Complex coupling patterns gave rise to four multiplets for the aliphatic protons, resonating in the region of 1.39-2.18ppm.

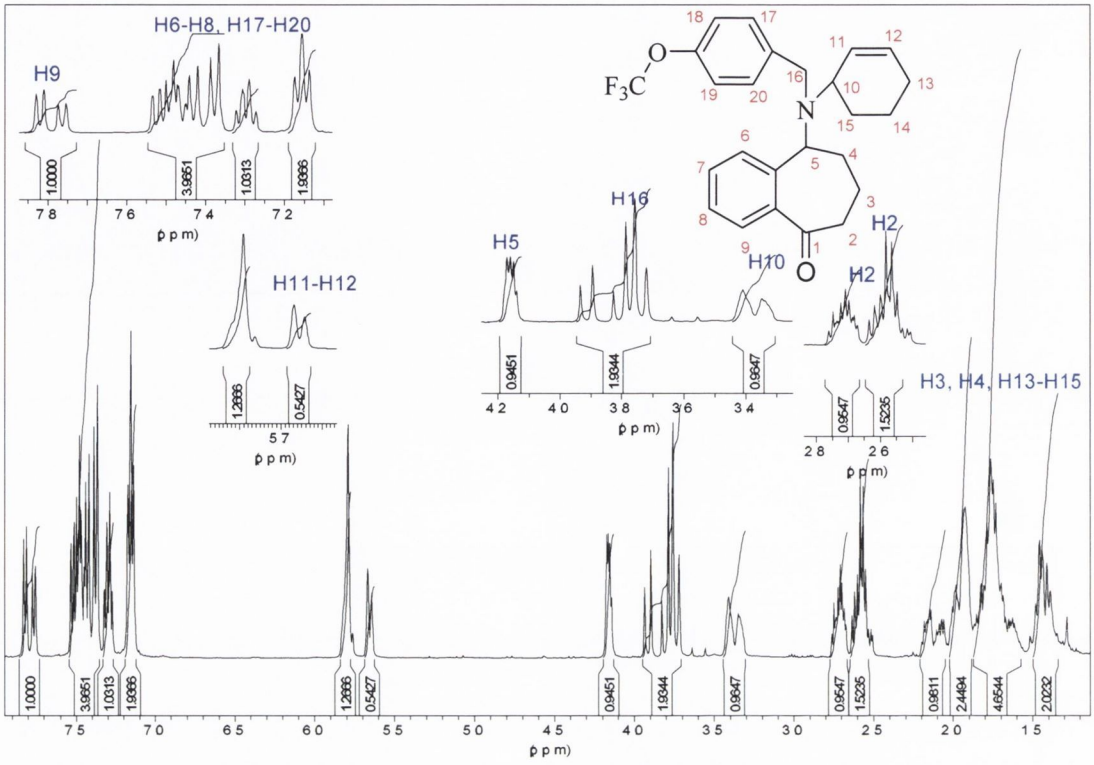


Fig. 2.17 ^{1}H NMR spectrum of compound (2.11)

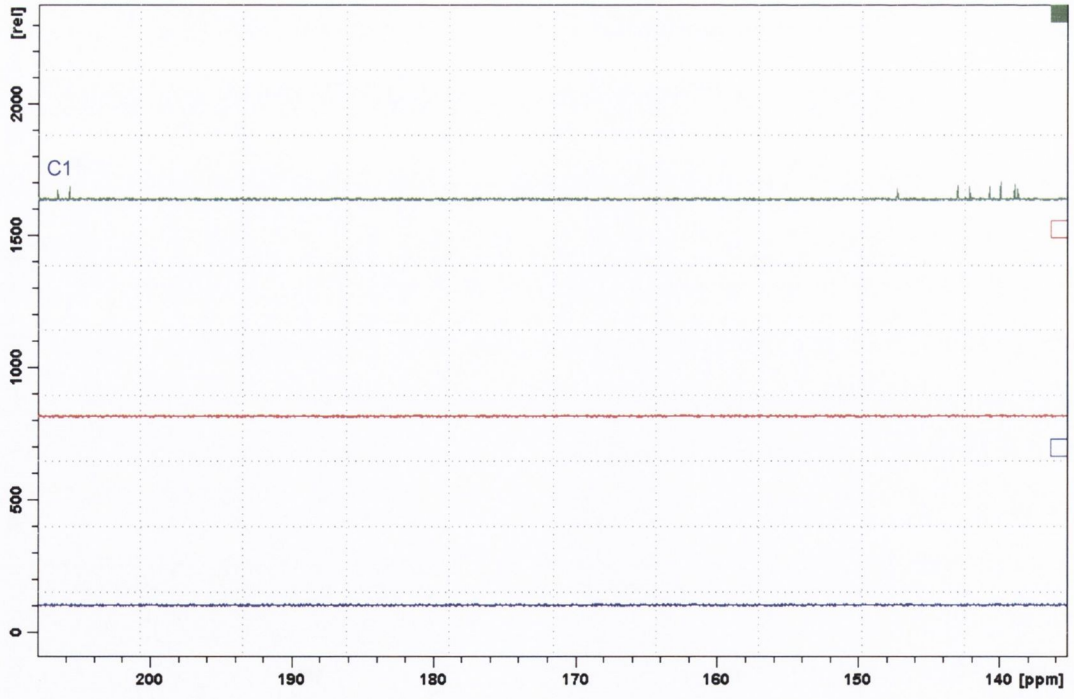


Fig. 2.18 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (2.11) from 135.7-208.2ppm

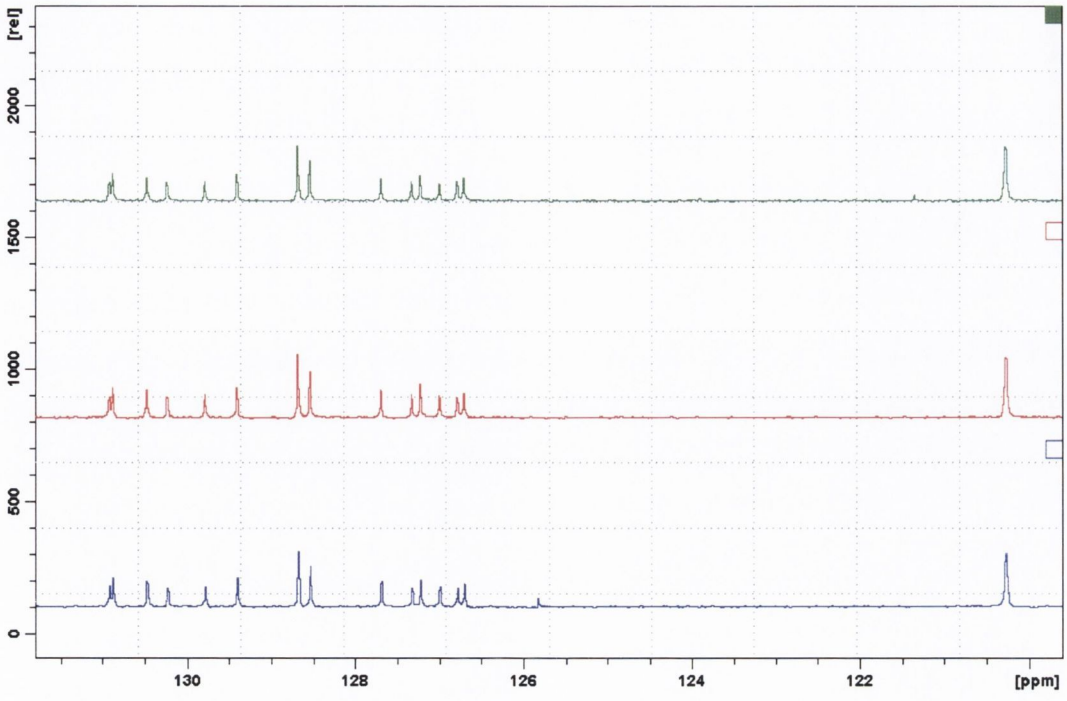


Fig. 2.19 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (2.11) from 119.5-131.8ppm

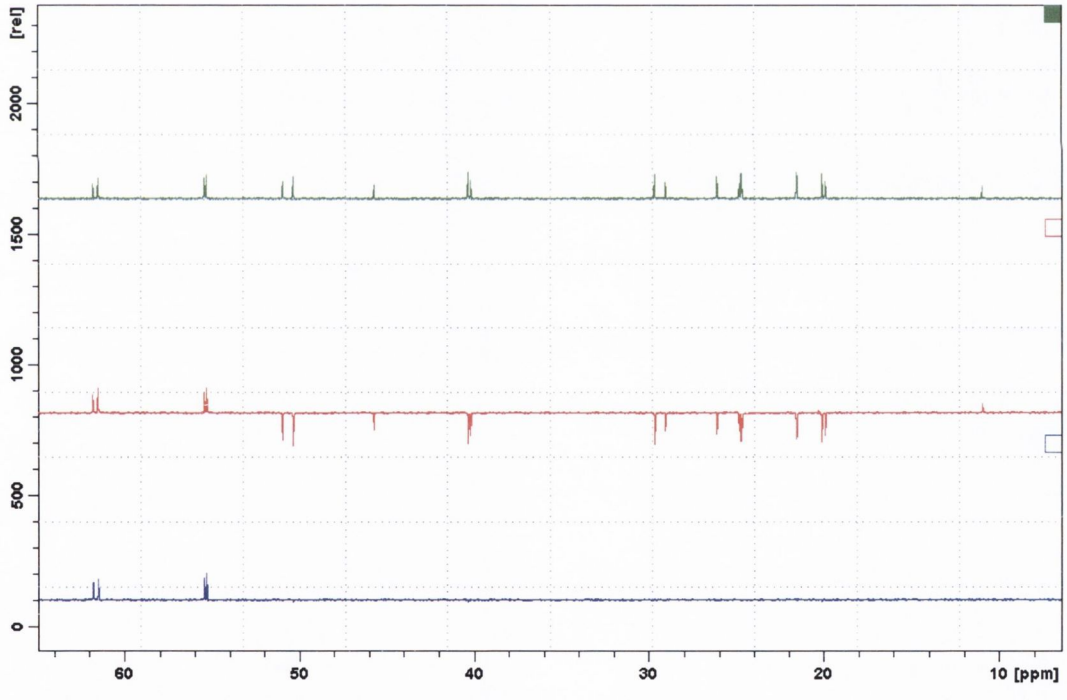


Fig. 2.20 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (2.11) from 6.2-64.4ppm

The main features in the ^{13}C NMR spectrum (Fig. 2.18-2.20) were the four separated signals for the fluorinated carbon attached to the oxygen atom, which resonated at 116.2, 118.8, 121.3 and 123.9ppm, and the presence of the carbonyl at 205.8 and 206.6ppm.

Three diagnostic signals for the four unsubstituted postitions on the trifluoromethoxybenzyl ring were found at 120.3, 128.5 and 128.3ppm in the DEPT 90 NMR spectrum.

Rotation of the trifluoromethoxy-substituent from the *para*-position of compound **(2.11)** to the *meta*-position compound **(2.12)** still gave many identical resonance values in the NMR spectra. Key signals in the ^{1}H NMR spectrum of compound **(2.12)** were again those of the aromatic region when additional complexity was observed over that of compound **(2.3)**. Two separate doublets (J=7.76Hz) resonated at 7.79 and 7.85ppm, three multiplets resonated at 7.08, 7.33 and 7.52ppm respectively, represented eight protons in the aromatic region. The fluorinated carbon attached to the oxygen atom located at 119.3, 119.5, 120.8 and 121.8ppm in the ^{13}C NMR spectrum. A total of twenty methine signals were seen in the DEPT 90 NMR spectrum; four peaks for the two alkenic carbons, sixteen peaks for the eight aromatic methine carbons. To further aid the structural elucidation of these two molecules, ^{19}F NMR experiment was also performed on the compound **(2.12)** as a representative of the series, and confirmed the presence of the OCF_3 group (Fig. 2.21).

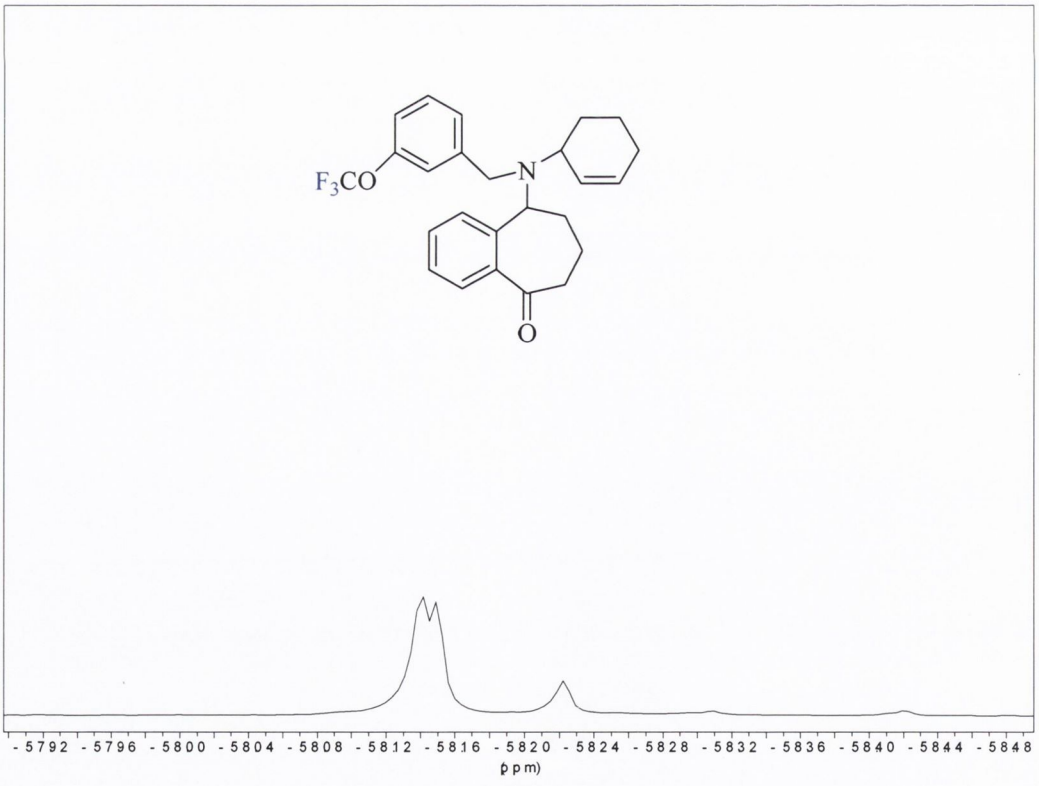


Fig.2.21 ^{19}F NMR spectrum of compound (2.12)

The infrared spectrum displayed the $C=O_{str}$ at 1682.4cm^{-1} compound **(2.11)** and 1687.6cm^{-1} compound **(2.12)**, ethereal $C\text{-}O_{str}$, $C\text{-}F_{str}$ at 1161.2 and 1259.5cm^{-1} compound **(2.11)**, 1163.6 and 1260.8cm^{-1} compound **(2.12)**. HRMS analysis in both cases gave acceptably accurate ($+H^+$) molecule masses: for both compounds **(2.11)** and **(2.12)**, 452.1813 ($+H^+$), calculated 452.1813 with molecular formula $C_{25}H_{26}NO_2F_3$.

Relative to compound **(2.3)**, the ^1H NMR (Fig. 2.22) of the N-trifluorobenzyl derivative **(2.13)** showed the aminomethylene protons at C-16 position, overlapping with the aliphatic signals of the methine proton (C-5) of the benzosuberone ring at 3.81-4.06ppm. Two separate multiplets at 6.72-6.81ppm and 6.97-7.07ppm respectively, which integrated for one proton for each, were considered to be for the two unsubstituted positions on the trifluorobenzyl ring. In the remaining part of the aromatic region, one proton appeared as a multiplet between 7.23-7.29ppm, which was followed by a tall, sharp triplet (J=7.34Hz), integrating for one proton, at 7.42ppm. A very intense double doublet (J_1=2.51Hz, J_2=5.02Hz) located at 7.52ppm for one proton, while the aromatic proton adjacent to the carbonyl resonated as two discrete doublets (J=7.56Hz) at 7.83 and 7.88ppm. The ^{13}C NMR spectrum of compound **(2.13)** (Fig. 2.23-Fig. 2.24), showed the presence of the six quaternary carbons: two carbons (four peaks) on the benzosuberone ring located at 139.1 and 139.3ppm, 143.7 and 144.1ppm; four carbons (three pairs of quartet signals and one pair singlet signals) of the triflurobenzyl group at 46.1 and 147.7ppm, 148.3 and 149.2ppm, 156.2 and 157.8ppm respectively. The carbonyl resonance was situated at 206.3 and 207.1ppm. The DEPT 90 NMR spectrum confirmed the presence of six tertiary carbon and two alkenic carbons in the aromatic region; two multiplet signals at 110.2 and 115.4ppm were attributed to the two tertiary carbons on the trifluorobenzyl ring. The ^{19}F NMR spectrum of compound **(2.13)** clearly demonstrated the presence of three fluorine moieties (Fig. 2.28).

The infrared spectrum of compound **(2.13)** contained as main features, a carbonyl stretching frequency at 1684.2cm^{-1} and the C-F bond stretching frequency at 1495.7cm^{-1}. HRMS ($+H^+$) data yielded actual molecular mass as 400.1877, calculated 400.1697 with molecular formula $C_{25}H_{25}NOF_3$.

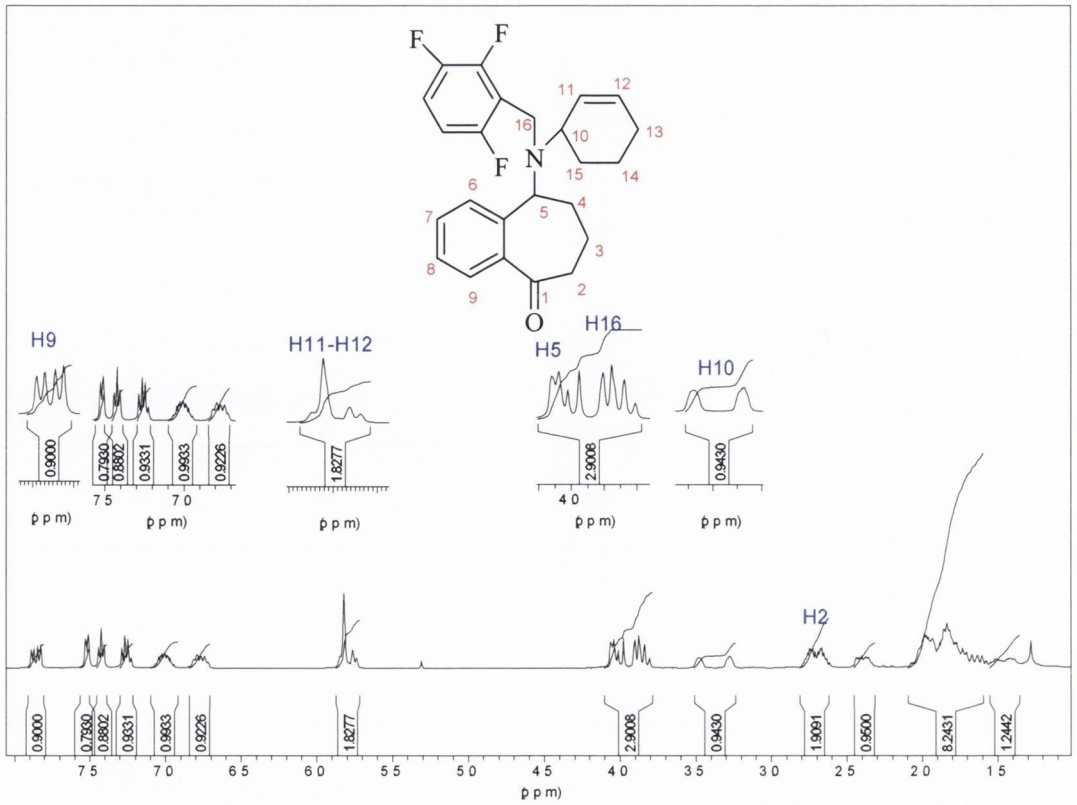


Fig.2.22 ^{1}H NMR spectrum of compound (2.13)

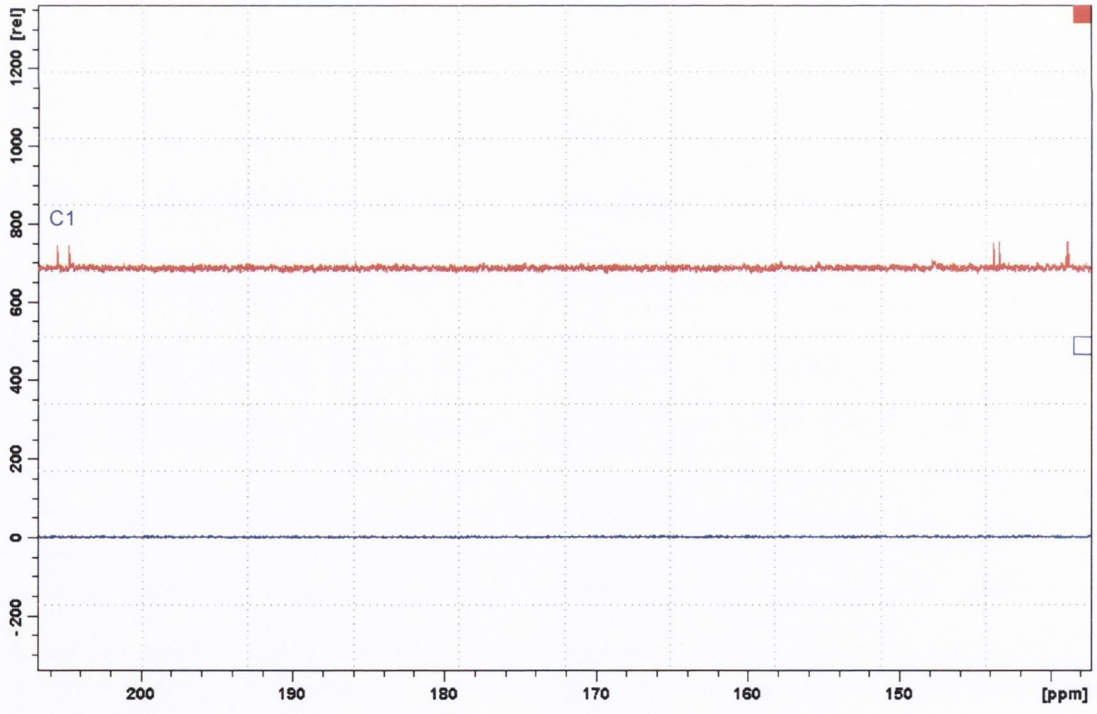


Fig. 2.23 ^{13}C and DEPT 135 NMR spectra of compound (2.13) from 137.8-206.7ppm

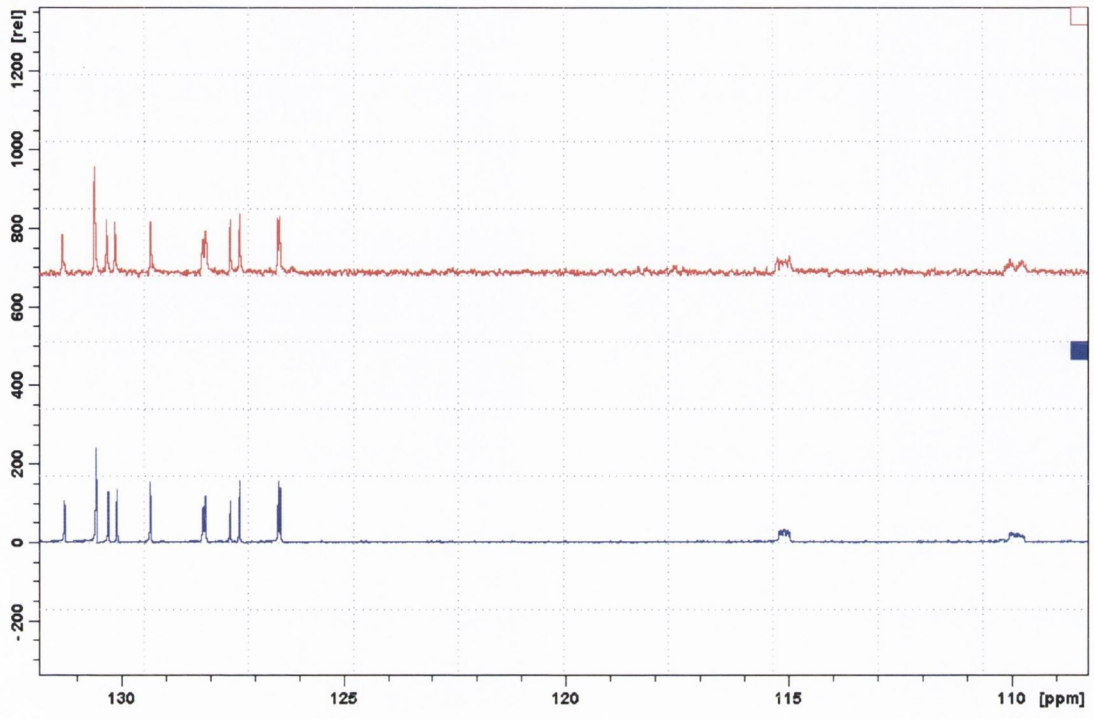


Fig. 2.24 ^{13}C and DEPT 135 NMR spectra of compound (2.13) from 108.2-132.0ppm

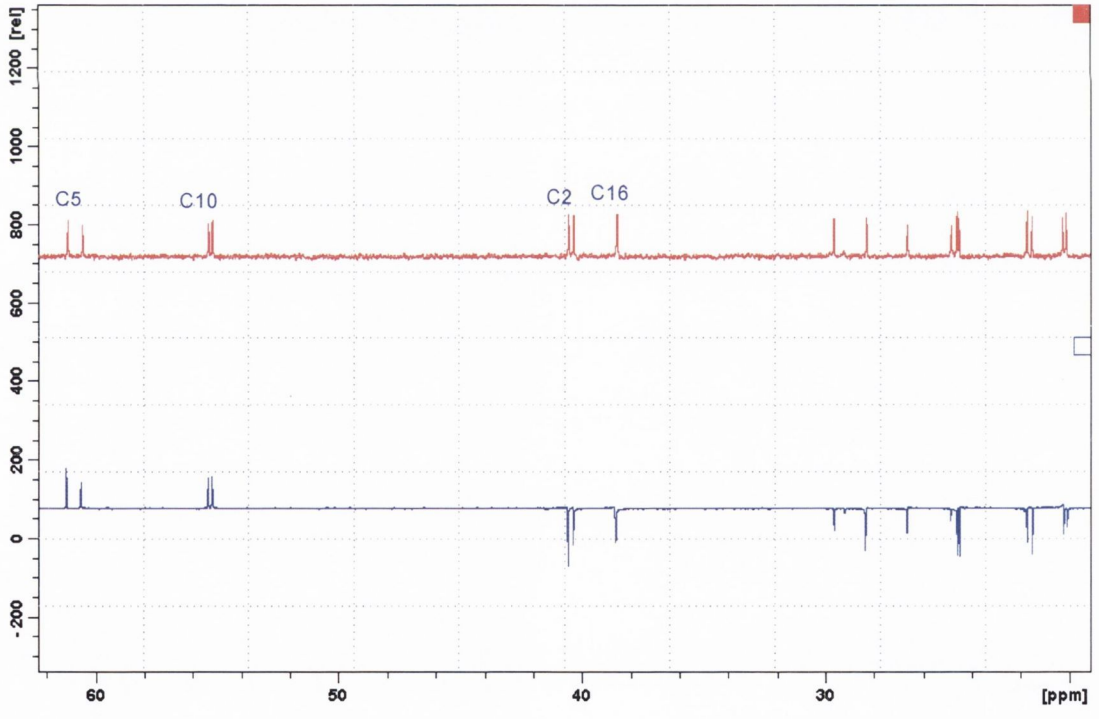


Fig. 2.25 ^{13}C and DEPT 135 NMR spectra of compound (2.13) from 18.2-62.2ppm

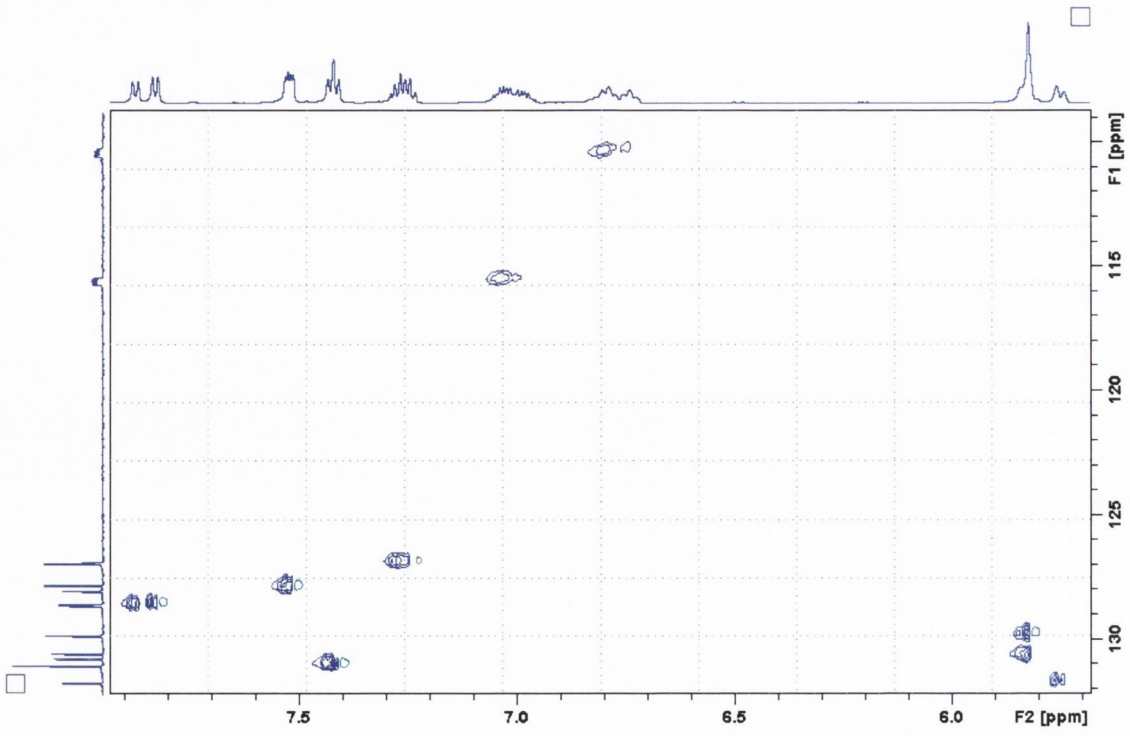


Fig.2.26 C-H COSY NMR spectrum of compound (2.13) from 5.69-7.93ppm

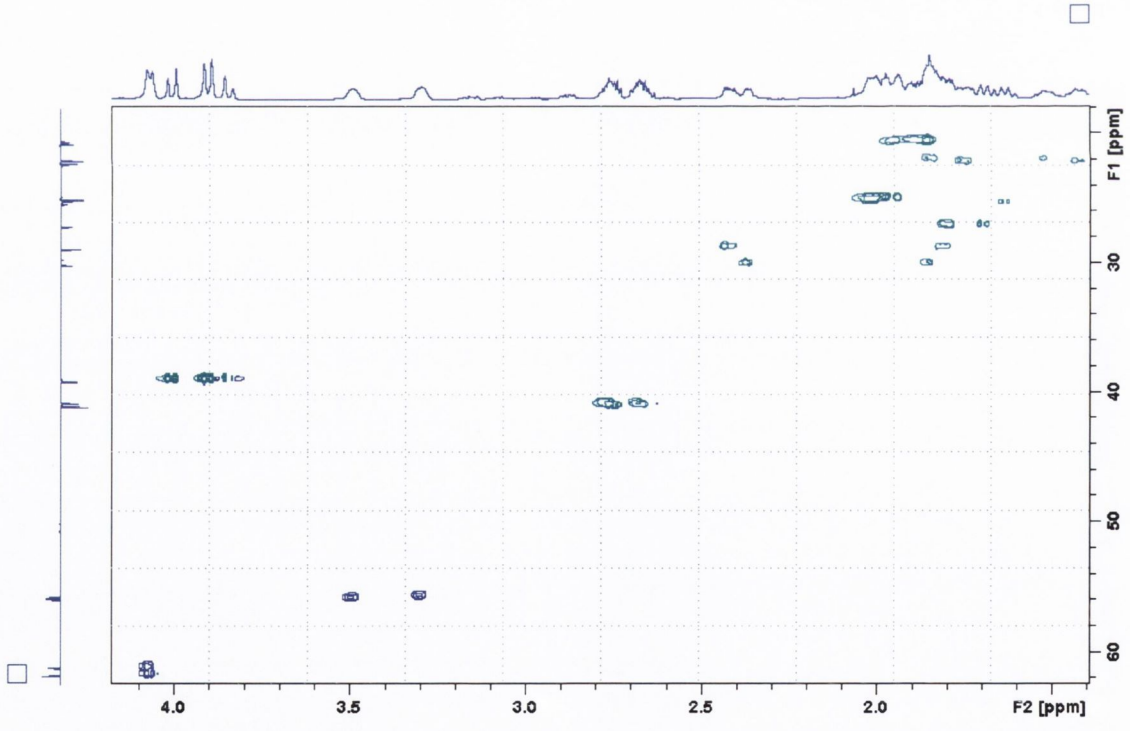


Fig. 2.27 C-H COSY NMR spectrum of compound (2.13) from 1.40-4.18ppm

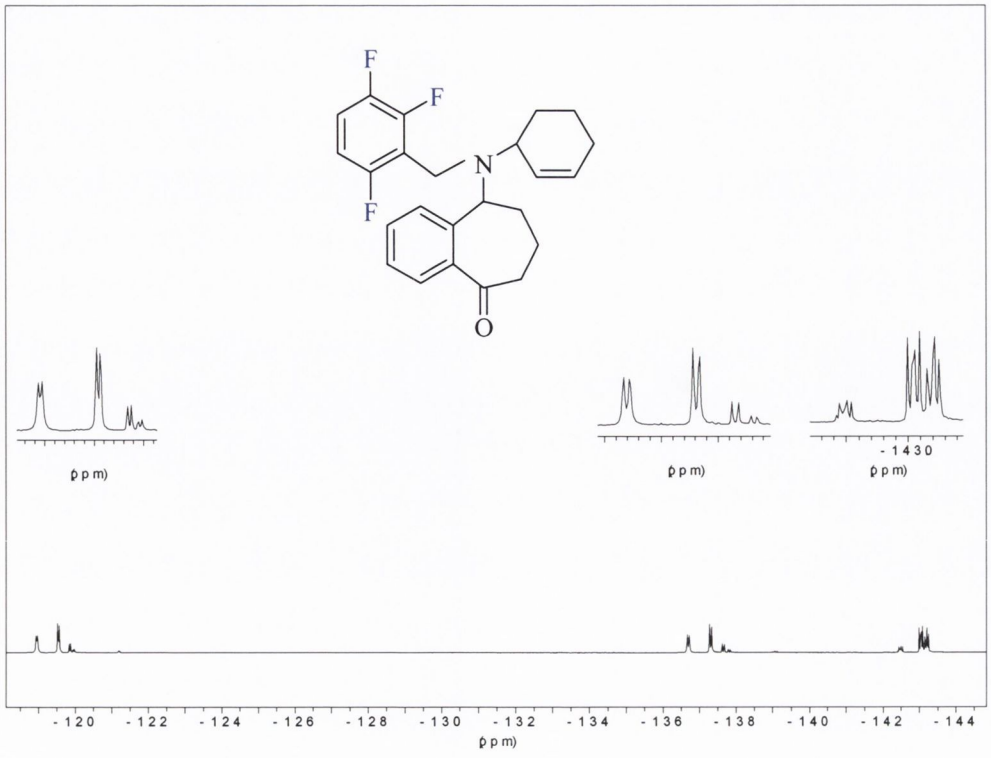
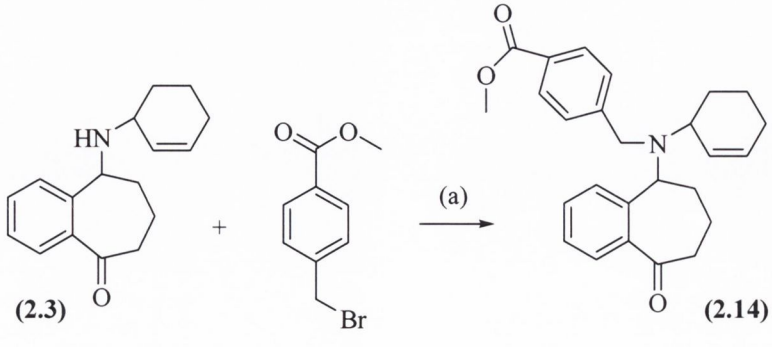


Fig.2.28 ^{19}F NMR spectrum of compound (2.13)

2.3.3.5 Structural determinations of compounds (2.14) and (2.16)

As the synthesis of a representative series of derivatives of **(2.3)**, containing fluorine-based electron withdrawing substituents on the benzyl ring was accomplished, the focus of the project now changed to the inclusion onto the benzyl ring of carboxyl, cyano and nitro-based electron withdrawing groups. Their conversion to potential water-soluble derivatives also served as a secondary impetus for their preparations. As shown in Table 2.0, the reaction time for each was approximately eleven days on average. Difficulties arose in the preparation of **(2.16)** as the starting benzyl halide tended to be prone to decomposition under the reaction conditions employed.



(a) acetonitrile, *N,N*-diisopropylethylamine, reflux, N_2, 13days

Scheme 2.17 N-alkyl derivatives (2.14)

The key features of the ^{1}H NMR spectrum of compound **(2.14)** (Fig. 2.29) were signals for the α-ketone protons on the benzosuberone ring at C-2 position were split, resonating as two multiplets (one for each diastereomer), at 2.54 and 2.56ppm. The methine proton of the cyclohexene ring at C-10 position took the form of a broad multiplet at 3.32ppm. As expected the methyl protons resonated as a tall singlet at 3.87ppm, which overlapped with a multiplet for the amino methylene proton occupying the range 3.83-3.97ppm. Considerable signal overlap was observed in the aromatic region of the spectrum; two multiplets at 7.25-7.29ppm and 7.42-7.50ppm respectively accounted for five protons, followed by two separate doublets (J=7.52Hz), representing each diasteromeric pair for the proton (C-9), at 7.74 and 7.80ppm. Those protons adjacent to the benzoate carbonyl resonated as two intense overlapping doublets (J=8.04Hz) at 7.96 and 7.97ppm, respectively. In the ^{13}C NMR spectrum of compound **(2.14)**, the two carbonyl groups resonated at 205.7 and 206.6ppm (ketone), 165.5ppm (ester) respectively while the methyl carbon was found at 51.5ppm.

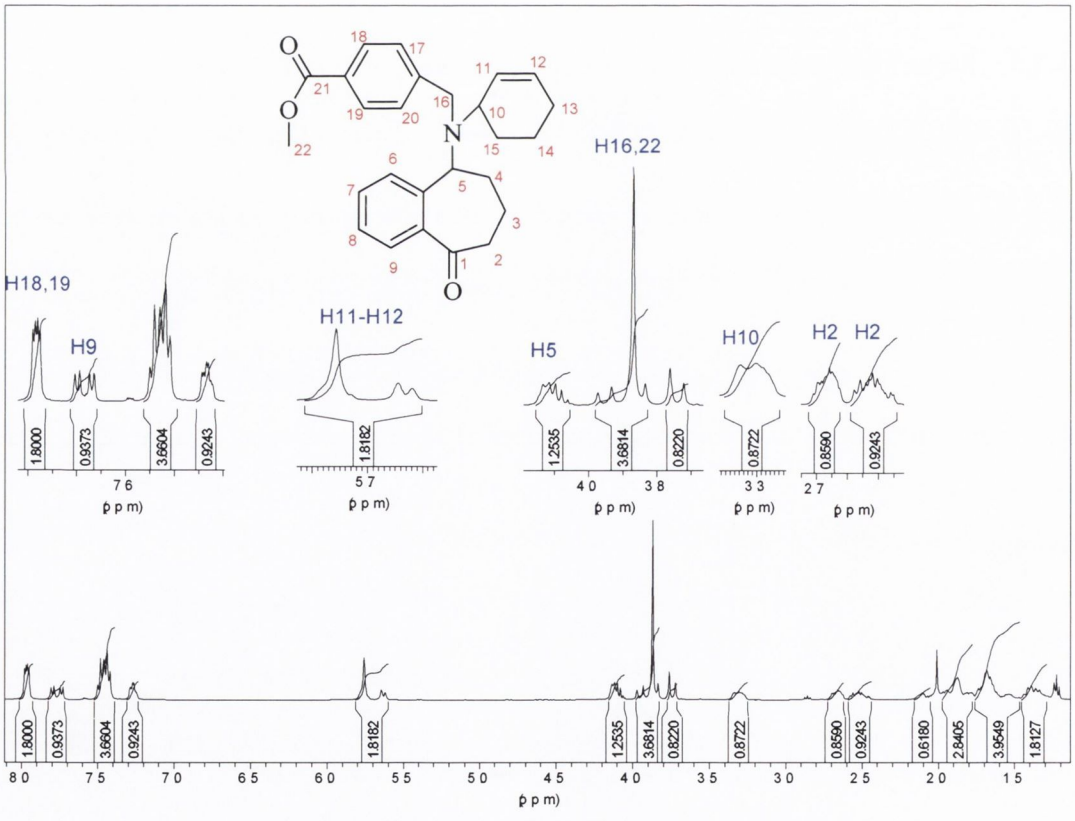


Fig.2.29 ^{1}H NMR spectrum of compound (2.14)

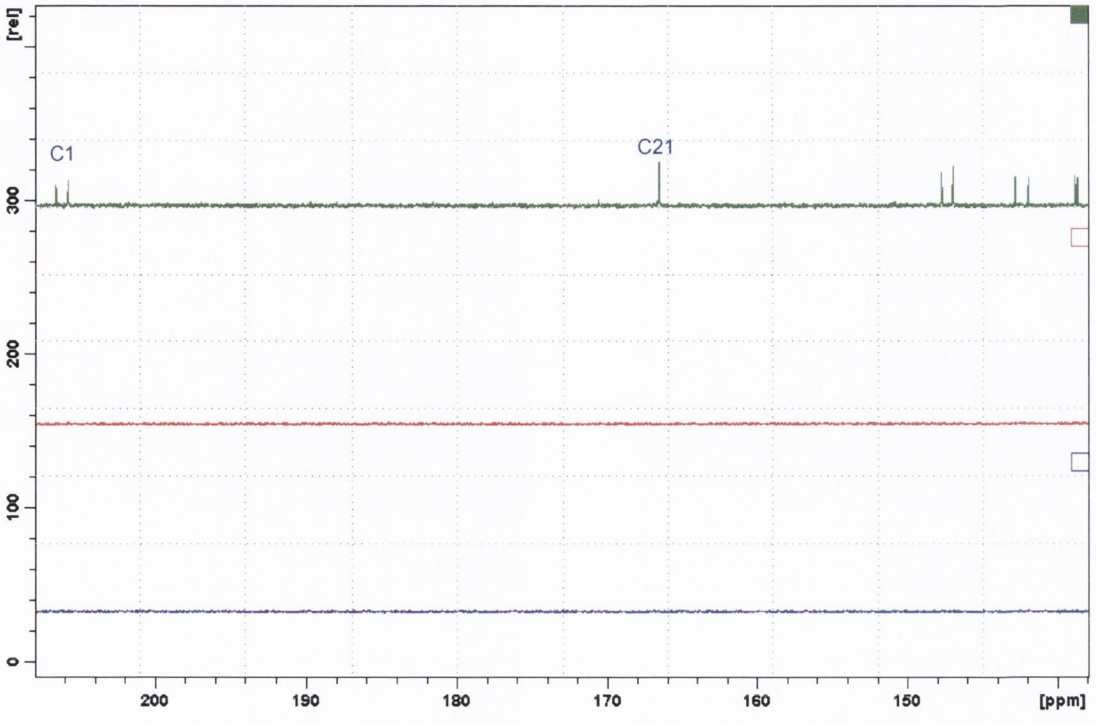


Fig. 2.30 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (2.14) from 138.0-208.0ppm

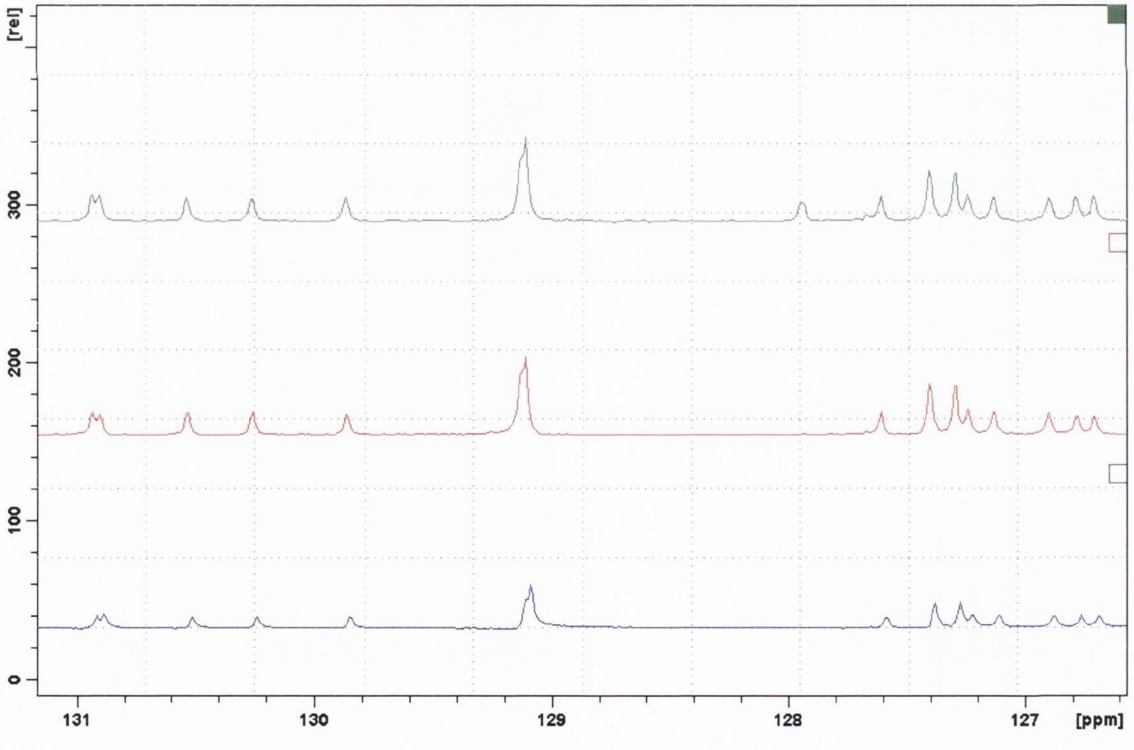


Fig. 2.31 ^{13}C and DEPT 135 NMR spectra of compound (2.14) from 125.9-131.2ppm

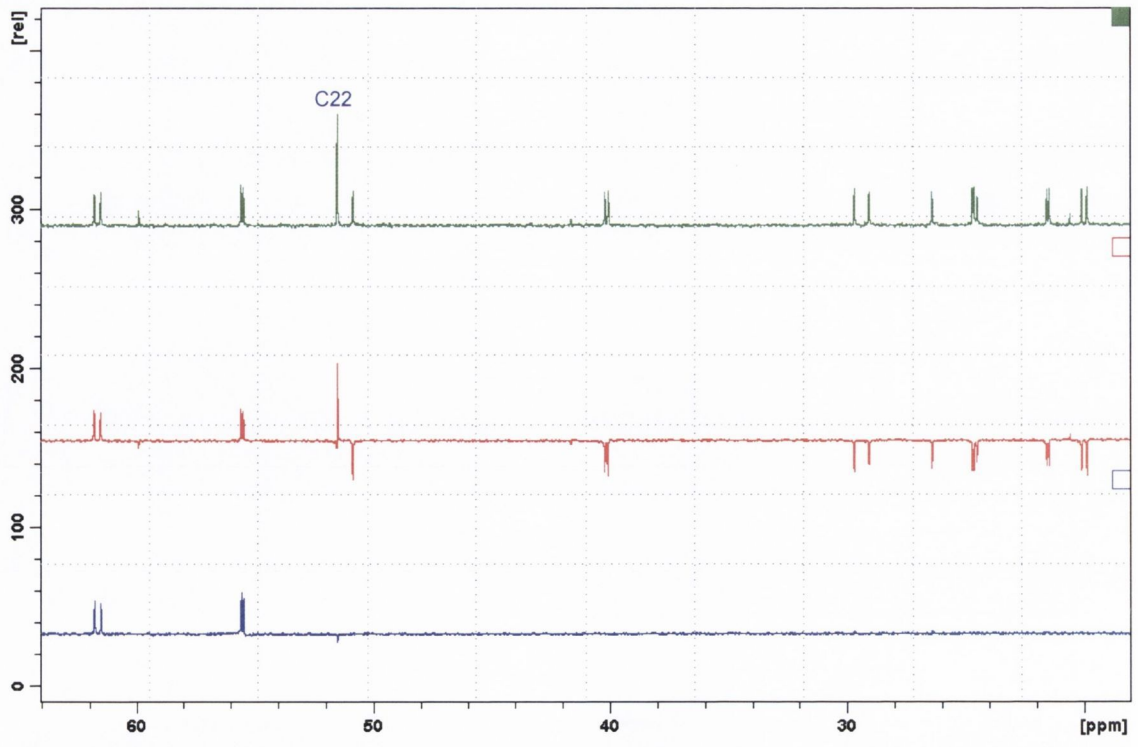
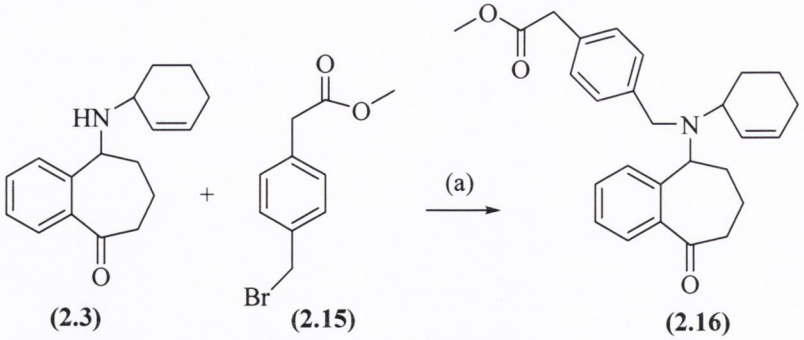


Fig. 2.32 ^{13}***C and DEPT 135 NMR spectra of compound (2.14) from 18.0-64.0ppm***

Infrared analysis displayed C=O_{str} (ketone) at 1683.0cm^{-1} and C=O_{str} (ester) at 1721.2cm^{-1}. HRMS analysis gave the accurate molecular mass (+Na^+) as actual 426.2053, calculated 426.2045 with molecular formula $C_{26}H_{29}NO_3Na$.

As stated earlier, the most significant problem with the synthesis of the ester **(2.16)** was the instability of the benzyl bromide **(2.15)** under the reaction conditions employed. Nevertheless, the ester was synthesised in low yield, 12%.



(2.3) **(2.15)** **(2.16)**

(a) acetonitrile, *N,N*-diisopropylethylamine, reflux, N_2, 12days

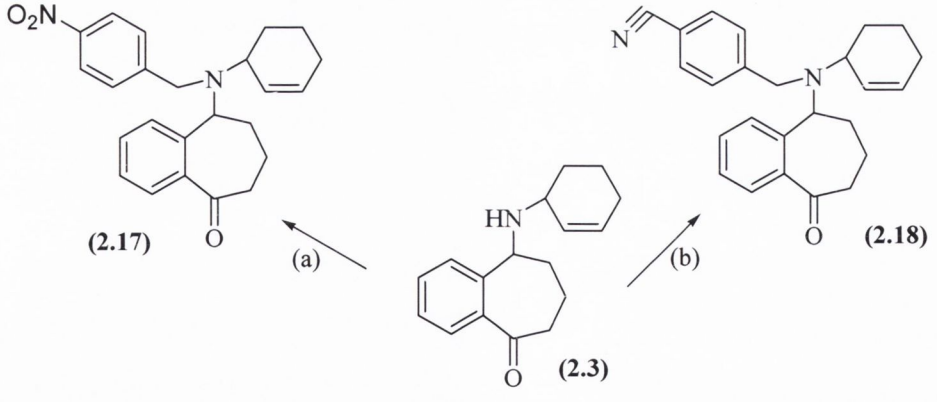
Scheme 2.18 N-alkyl derivatives (2.16)

As expected, considerable similarities existed in the NMR spectra of the esters **(2.14)** and **(2.16)**. The main features of the ^{1}H NMR spectrum of compound **(2.16)**, was the tall singlet at 3.64ppm for the benzylic methylene protons next to the ester group. The

signals for the amino methylene group were split, appearing at 3.73 and 3.88ppm, the former signal was found to overlap slightly with the tall singlet for the methyl protons. The aromatic region integrated for eight protons. Again considerable overlap of signals was observed with two overlapping doublets (J=8.02Hz) at 7.25ppm which integrated for two protons. This was followed by a triplet (J=7.52Hz) at 7.32ppm for one proton and a further two overlapping doublets at 7.39ppm (J=9.56Hz) and 7.41ppm (J=8.00Hz). The signal between 7.48-7.63ppm representing two protons appeared as a broad multiplet. The remaining proton signal appearing in the aromatic region resonated as two split doublets, at 7.84 and 7.94ppm, which was considered to be the proton adjacent to the carbonyl on the benzosuberone ring. The two carbonyl groups were evident at 171.8 (ester), 205.8 and 206.8ppm (ketone) in the ^{13}C NMR spectrum of compound **(2.16)**. The DEPT 135 NMR spectrum indicated the presence of the additional methylene carbon at 40.8ppm.

The ester **(2.16)** displayed similar infrared behaviour to the ester **(2.14)**, with the two carbonyl stretching frequencies at 1682.4cm^{-1} (ketone) and 1738.8cm^{-1} (ester). Compound **(2.16)** also provided satisfactory molecular mass (+Na^+) on HRMS analysis as actual 440.2211, calculated 440.2202 with molecular formula $C_{27}H_{31}NO_3Na$

2.3.3.6 Structural determinations of compounds (2.17) and (2.18)



(a) 4-nitrobenzyl bromide, acetonitrile, *N,N*-diisopropylethylamine, reflux, N_2, 10days
(b) 4-(bromomethyl)benzonitrile, acetonitrile, *N,N*-diisopropylethylamine, reflux, N_2, 12days

Scheme 2.19 N-alkyl derivatives (2.17) and (2.18)

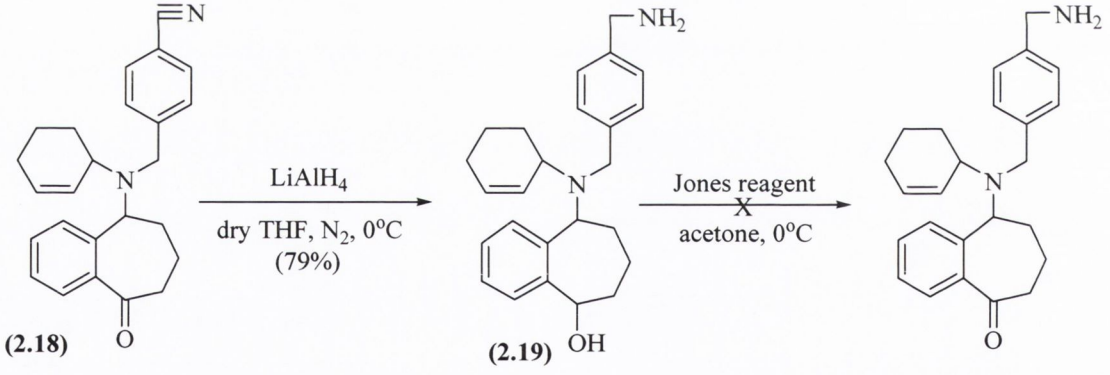
In brief ^1H NMR analysis of the benzylated analogues **(2.17, 2.18)** showed important common aliphatic signals as the other substituted benzylated molecules. The

corresponding signals for these protons in both compounds also showed similar splitting patterns. Once again, the main additional ^{1}H NMR features present in the spectra of the two molecules were found in the aromatic region, with considerable signal overlap. Diagnostic signals in the ^{13}C NMR spectrum included the quaternary nitrile carbon at 118.7ppm in the spectrum of compound **(2.18)**.

The infrared spectra of compounds **(2.17)** and **(2.18)** were dominated by the carbonyl signal at 1684.2 and 1681.1cm^{-1} respectively. Important infrared characteristics of both compounds included the intense and strong N-O_{str} at 1343.6 and 1518cm^{-1} for the aromatic nitro compound **(2.17)** and C≡N_{str} at 2226.4cm^{-1} for the nitrile compound **(2.18)**.

2.3.4 Attempted synthesis of phenylamine analogues

Apart from the tertiary amines outlined above, it was of interest to synthesise some *N*-alkyl derivatives with electron-donating amino substituent (i.e. phenylamine, aniline), as it was felt that these molecules might possess better water solubility properties. The nitrile **(2.18)** was employed to reduce to its corresponding alcohol **(2.19)**, as the first step in the synthesis of phenylamine analogue.



Scheme 2.20 N-alkyl derivatives (2.19)

Reduction of *N*-alkyl derivative **(2.18)** occurred easily within five-six hours by the slow addition of a solution of compound **(2.18)** in anhydrous tetrahydrofuran (THF) to a solution of lithium aluminium hydride ($LiAlH_4$) in anhydrous THF at 0°C under anhydrous conditions. The temperature of the reaction was slowly allowed to increase to room temperature. On completion confirmation that the amine had formed was evidenced by the positive reaction of the baseline spot with ninhydrin (developing reagent) on TLC. After completion, the reaction was quenched by the slow addition of

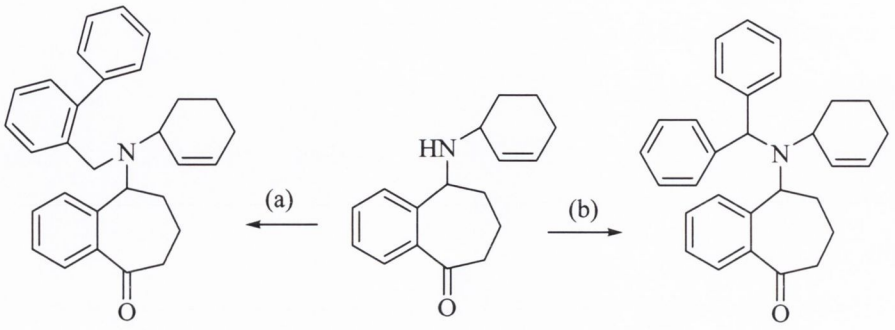
ice water. Following acid (2M aq. HCl) and base (2M aq. NaOH) work-up, the product was extracted with diethyl ether. The resulting alcohol **(2.19)** was furnished in relative good yield (79%) without further purification by flash column chromatography.
The ^{1}H NMR spectrum of compound **(2.19)** indicated the presence of the alcohol methine proton resonating in the region of 4.64-4.67ppm. The signals of the additional methylene group were split into two multiplets located in the regions of 3.74-3.78ppm and 3.92-3.94ppm respectively, integrating as two protons. The amino methylene protons resonated as a singlet, at 3.86ppm. ^{13}C NMR analysis revealed the absence of the carbonyl group. The diagnostic signal of alcohol methine carbon observed at 75.8 and 75.9ppm in DEPT 90 NMR spectrum.

The next step towards the designed aminoketone was oxidation of the alcohol **(2.19)**. This transformation was performed through the slow addition of Jones's reagent (Rangarajan & Eisenbraun, 1985), a well known oxidizing agent for the selective oxidation of hydroxyl groups under acidic conditions, into a solution of **(2.19)** in acetone at 0°C over a period of thirty minutes. Reaction was maintained at 0°C and monitored by TLC with mobile phase (MeOH:EtOAc, 3:1). The time scale involved in order to allow the reaction to go to completion (six and a half hours) resulted in the formation of numerous by-products, which were observed on TLC with mobile phase (hexane:EtOAc, 2:1). After many attempts, it was found that the optimum reaction time to use was between four and five hours, which still only yielded crude product in very little percentage following acid-base work-up. Unfortunately, NMR analysis indicated that a mixture of compounds were obtained.

2.3.5 Preparation of a merged hybrid and π-rich mast cell stabilizer

Within the last series of compounds that are the subject of this chapter, there are two design concepts in mind; namely to explore the creation of a π-rich region centered on the amino group, and secondly to incorporate a substituent with the potential to bind to the histamine H1 receptor. In doing so, the objective is to create a merged hybrid/designed multiple ligand, with mast cell stabilization and histamine H1 antagonist activity. In respect of the latter objective it was expected that compound **(2.21)** with the diphenylmethyl substituent would serve as an ideal candidate molecule to test this hypothesis.

Alkylation of the secondary amine **(2.3)** with the bulky benzyl moieties resulted in the formation of the desired compounds in reasonable yields **(2.20**, 41%**)** and **(2.21**, 32%**)**. Both molecules, upon isolation as colourless oils, showed strong carbonyl absorption bands at 1684.2 and 1681.1cm^{-1}, respectively in their infrared spectra.



(2.20) **(2.3)** **(2.21)**

(a) 2-phenylbenzyl bromide, acetonitrile, *N,N*-diisopropylethylamine, reflux, N_2, 12days
(b) bromodiphenylmethane, acetonitrile, *N,N*-diisopropylethylamine, reflux, N_2, 4days

Scheme 2.21 N-alkyl derivatives (2.20) and (2.21)

The ^{1}H NMR spectrum of compound **(2.20)** (Fig. 2.33) contained a complex multiplet ranging from 1.26-1.95ppm integrating for ten protons, the α-ketone methylene proton signals at 2.44-2.65ppm, resonated as two mulitplets, immediately followed by a very broad multiplet signal for the proton of the cyclohexenyl methine. The methine proton of the benzosuberone ring appeared as a multiplet and occupied the region from 3.62-3.69ppm. The next multiplet locating from 3.83-3.98ppm was thought to be the amino methylene protons. As expected the two protons for the alkene formed a multiple signal residing from 5.48-5.73ppm. As might have been anticipated the addition of the second aryl ring added great complexity to the aromatic region, with many proton signals overlapping. Two doublets were found at 7.18ppm and 7.27ppm, one integrated for one proton, and the other one integrated for four protons, respectively. This was followed by a multiplet for six protons from 7.35-7.55ppm. Two separate doublets (J=7.28Hz) resonated at 7.75 and 7.92ppm and integrated as one proton, in between which was another two doublets at 7.82 and 7.85ppm (J_1=7.32Hz, J_2=8.04Hz), again overlapping, and represented one proton. In the ^{13}C NMR spectrum of compound **(2.20)** (Fig. 2.34-Fig. 2.36), the carbonyl carbon resonated at 206.0 and 206.9ppm one for each diastereoisomer. Five quaternary carbons were present in the region between 138.1-143.7ppm. The DEPT 135 NMR spectrum clearly demonstrated the presence of seven

methylene carbons. A total of fifteen tertiary carbons (thirteen for the aromatic carbons and two for alkenic carbons) were identified in the aromatic region between 125.6-130.9ppm in the DEPT 90 NMR spectrum.

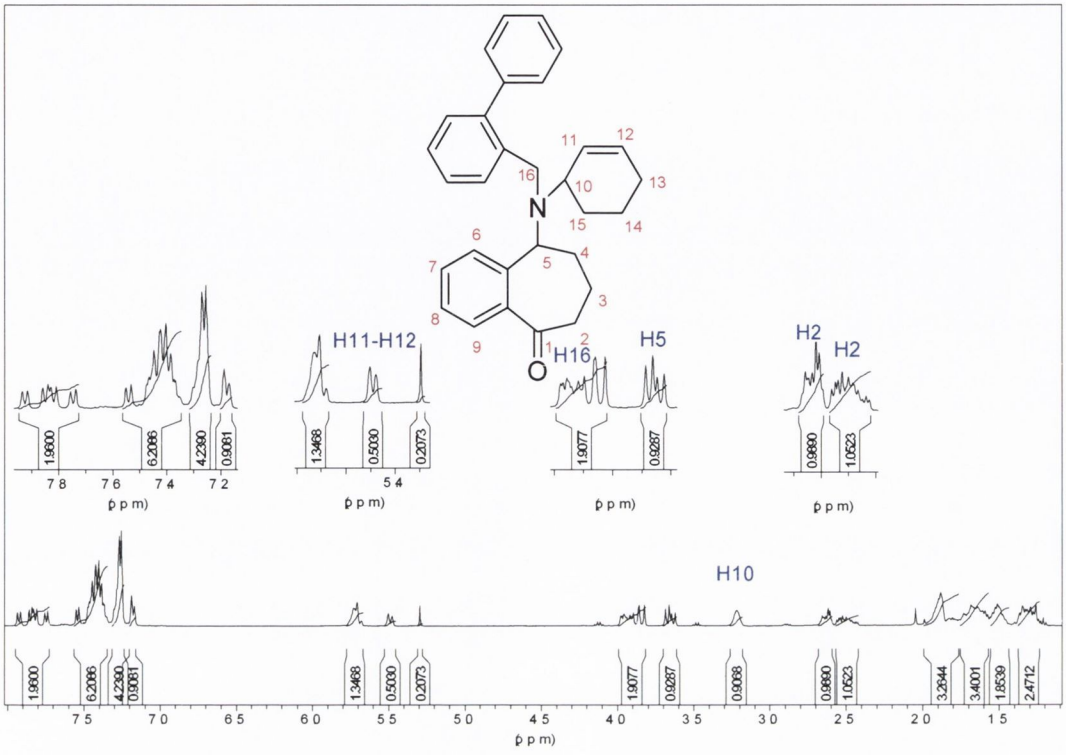


Fig.2.33 ^{1}H NMR spectrum of compound (2.20)

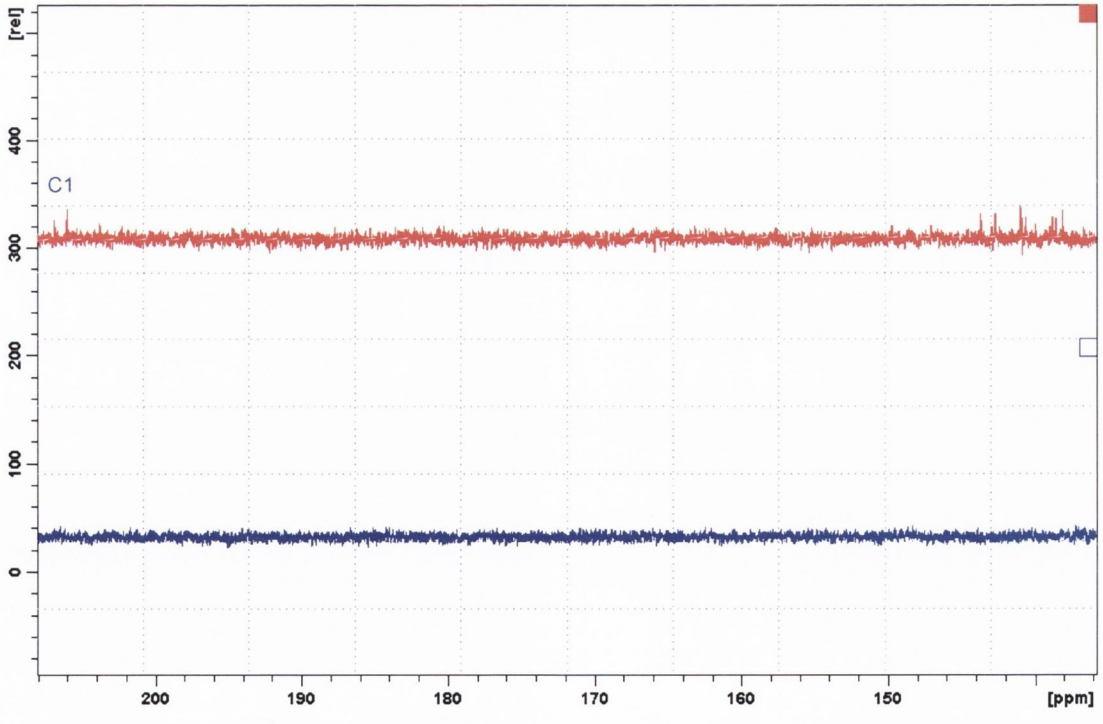


Fig.2.34 ^{13}C and DEPT 135 NMR spectra of compound (2.20) from 136.0-208.1ppm

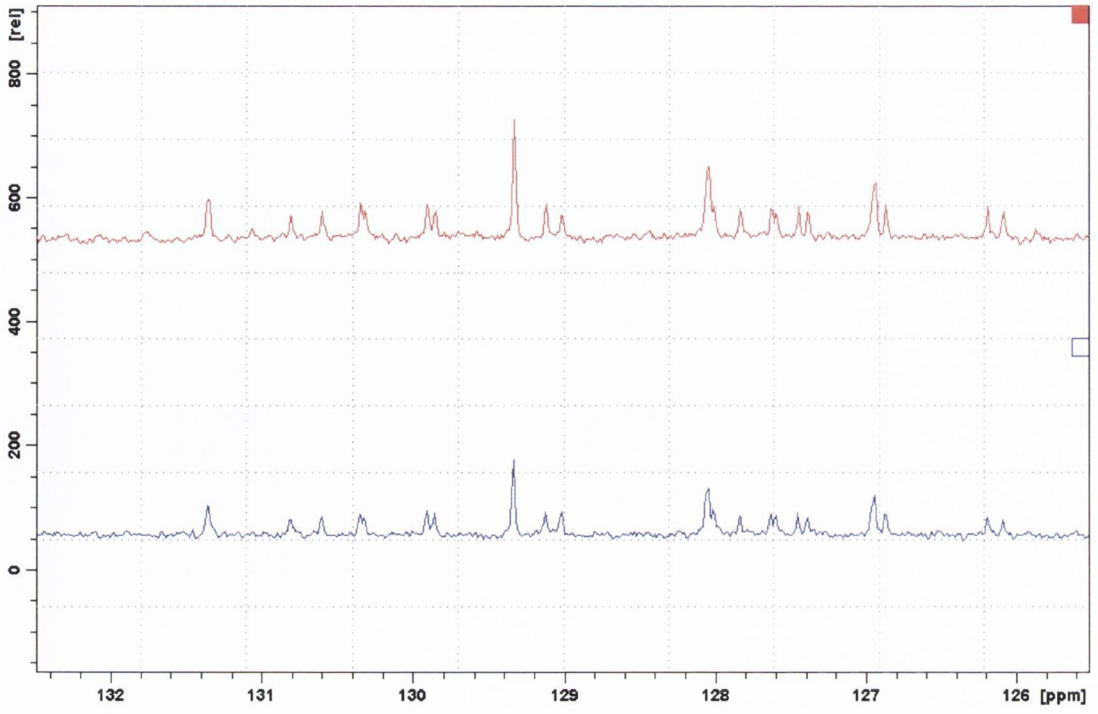


Fig.2.35 ^{13}C and DEPT 135 NMR spectra of compound (2.20) from 126.5-132.5ppm

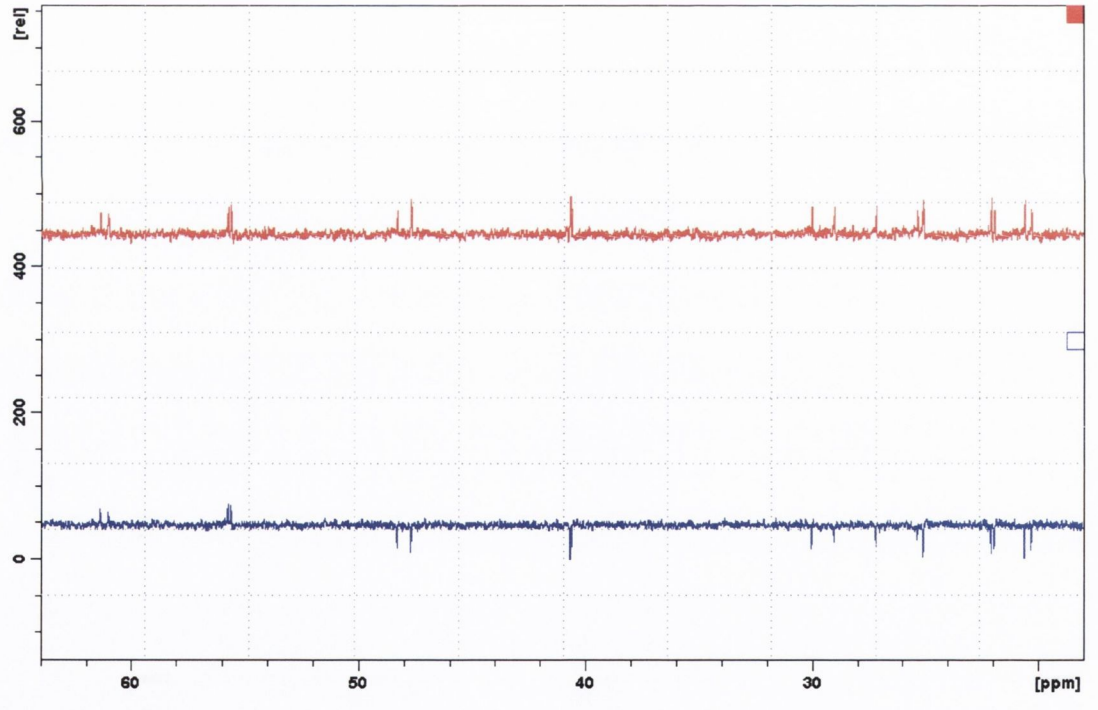


Fig.2.36 ^{13}C and DEPT 135 NMR spectra of compound (2.20) from 18.0-64.0ppm

The HRMS spectrum of compound **(2.20)** gave an accurate mass ($+H^+$) as 422.2478 with that calculated being 422.2484, molecular mass $C_{30}H_{32}NO$.

Very similar NMR spectra were obtained for compound **(2.21)** compared with those for **(2.20)**, but with one obvious exception, the two singlets of the methine proton on C-16 position at 5.41 and 5.50ppm. The ^{1}H NMR spectrum of compound **(2.21)** showed aromatic region to be quite complex. Two split doublets (J=7.92Hz) at 7.78 and 7.95ppm were assigned to the proton of the benzosuberone ring close to the ketone group, in between which lay a tall doublet signal (J=7.14Hz), at 7.84ppm. Two triplets at 7.53ppm (J=7.70Hz), 7.63ppm (J=7.40Hz) and a multiplet at 7.57ppm accounted for three protons. The broad multiplet in the region from 7.14-7.42ppm was assigned to the remaining nine protons. The alkenic proton signals appeared as repeated pair of mirror imaged broad multiplets, resonating from 5.79-5.99ppm. The methine proton linked to the two phenyl groups showed two sharp singlets for the diastereomers at 5.41 and 5.50ppm. The ^{13}C NMR spectrum showed the presence of the carbonyl group at 206.3 and 206.7ppm. Other important peaks in the spectrum included those for the methine carbon (C-16) bridging the two phenyl rings at 65.7 and 65.9ppm while the other two methine carbons (C-10 and C-5) in this region resonated at 55.5 and 55.7ppm, 58.7 and 59.0ppm respectively. The six methylenes were also prominent each showing two peaks representative of the fact that compound **(2.21)** is presented as a mixture of stereoisomers.

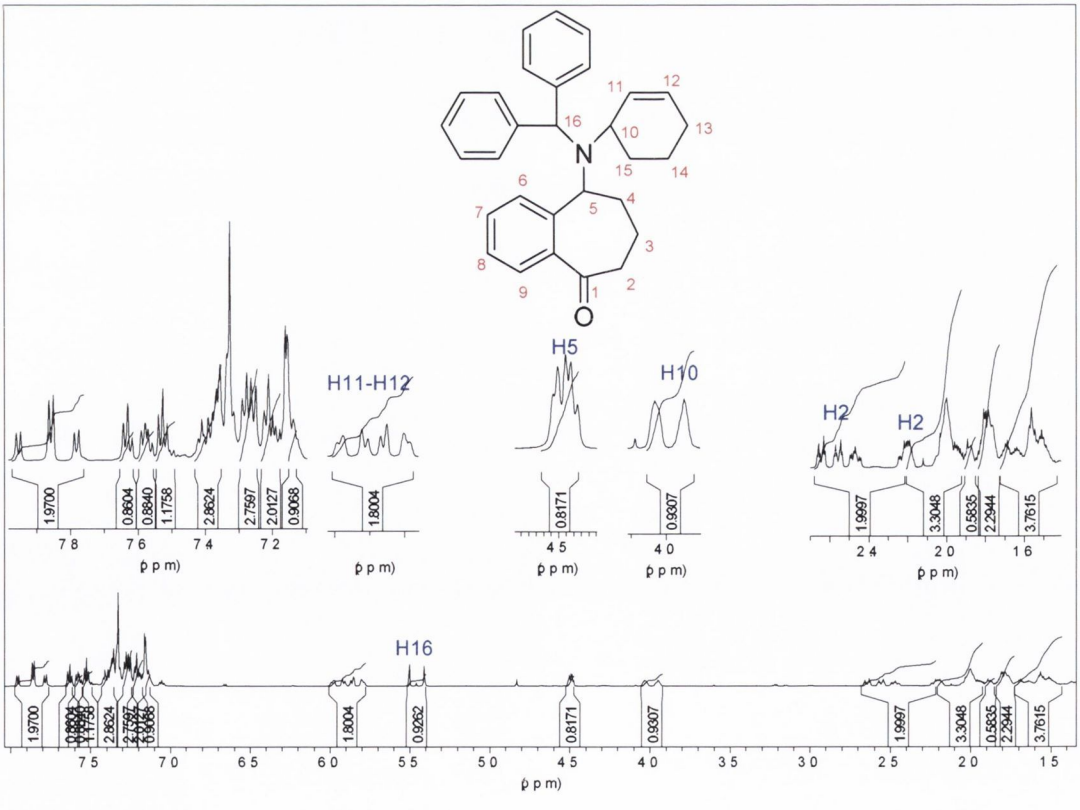


Fig.2.37 ^{1}H NMR spectrum of compound (2.21)

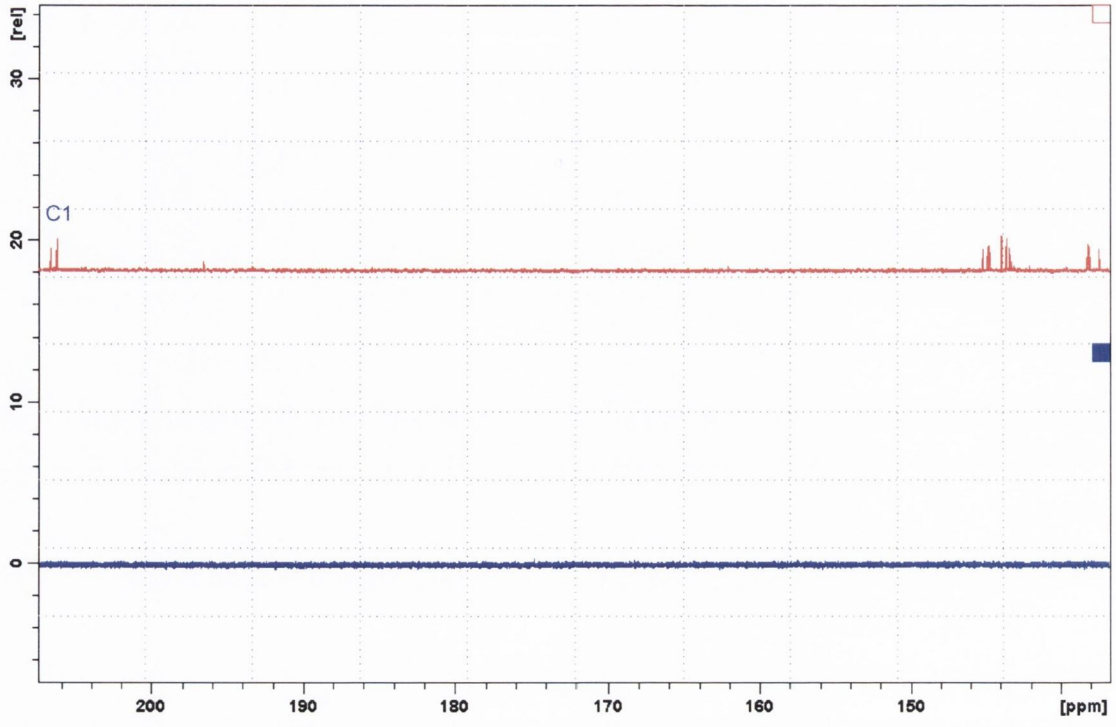


Fig.2.38 ^{13}C and DEPT 135 NMR spectra of compound (2.21) from 137.0-207.8ppm

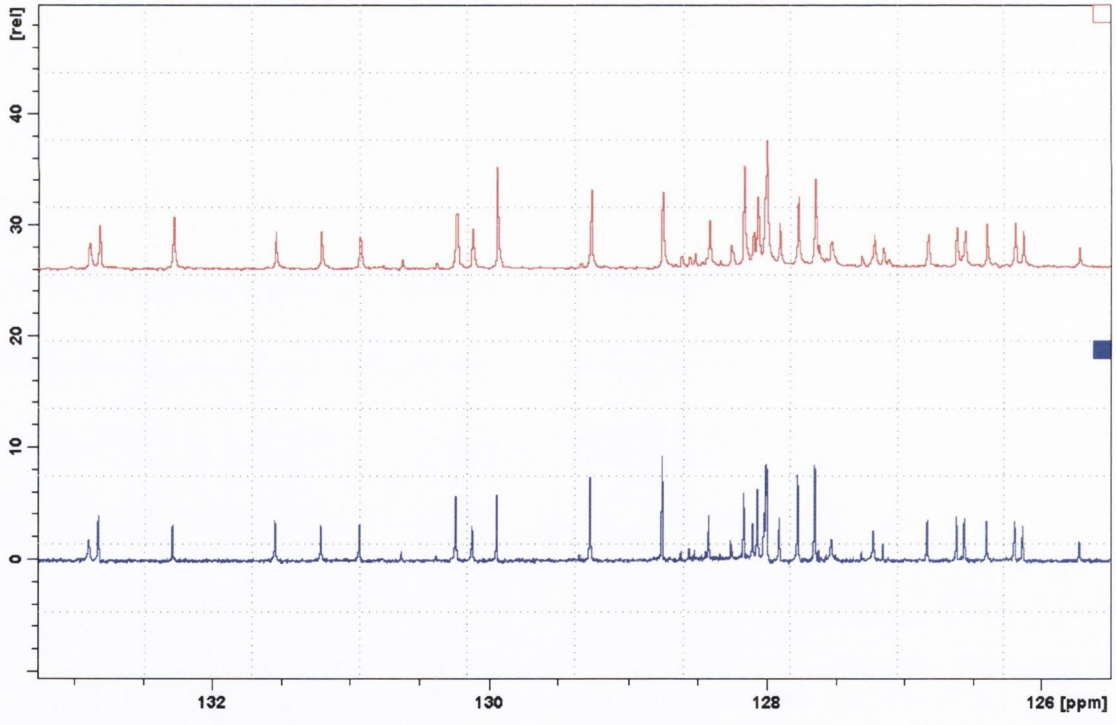


Fig.2.39 ^{13}C and DEPT 135 NMR spectra of compound (2.21) from 125.5-133.5ppm

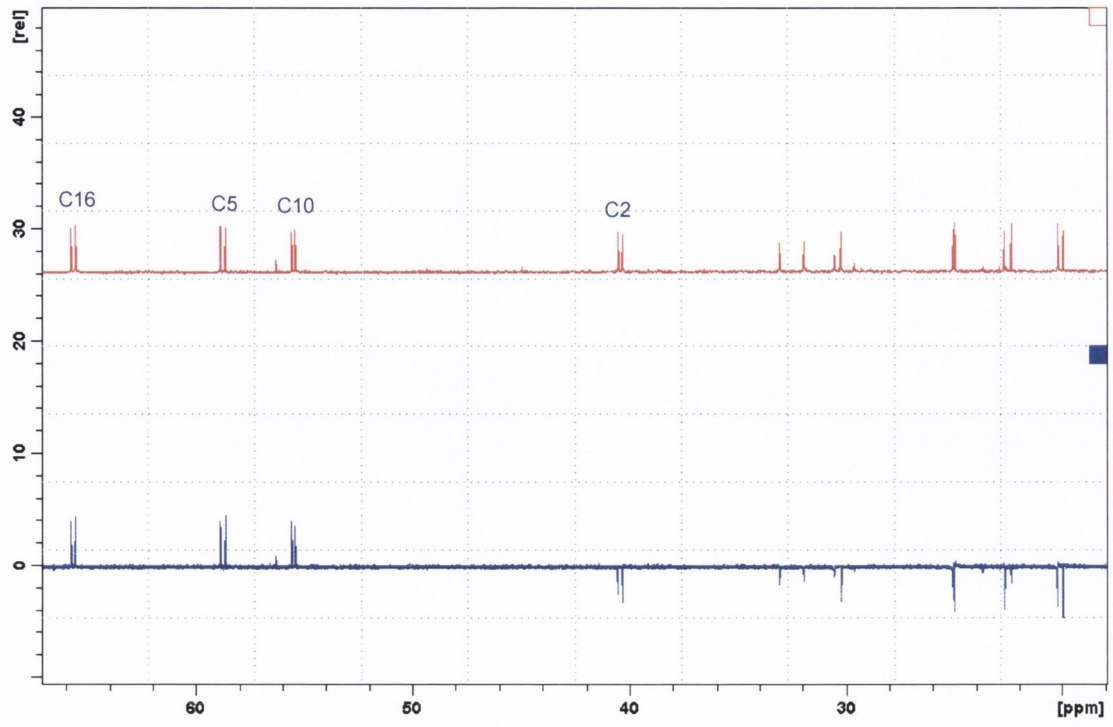


Fig.2.40 ^{13}C and DEPT 135 NMR spectra of compound (2.21) from 18.0-66.5ppm

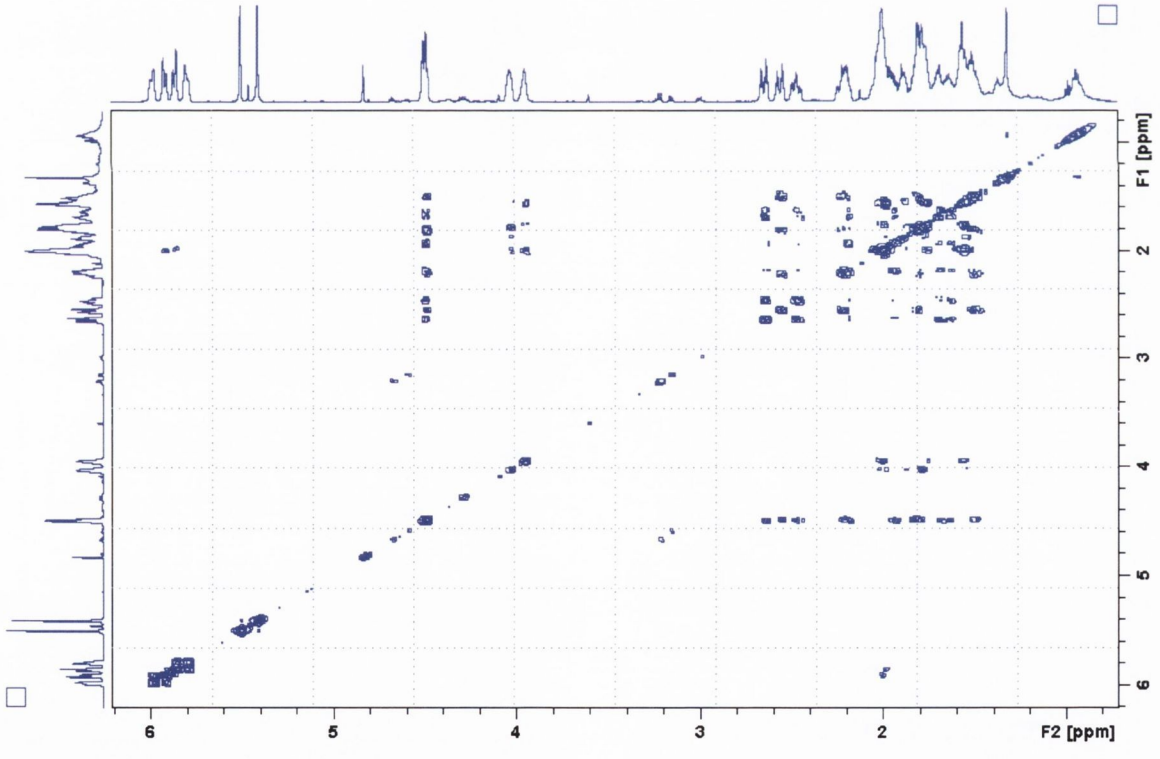


Fig.2.41 H-H COSY NMR spectrum of compound (2.21) from 0.8-6.2ppm

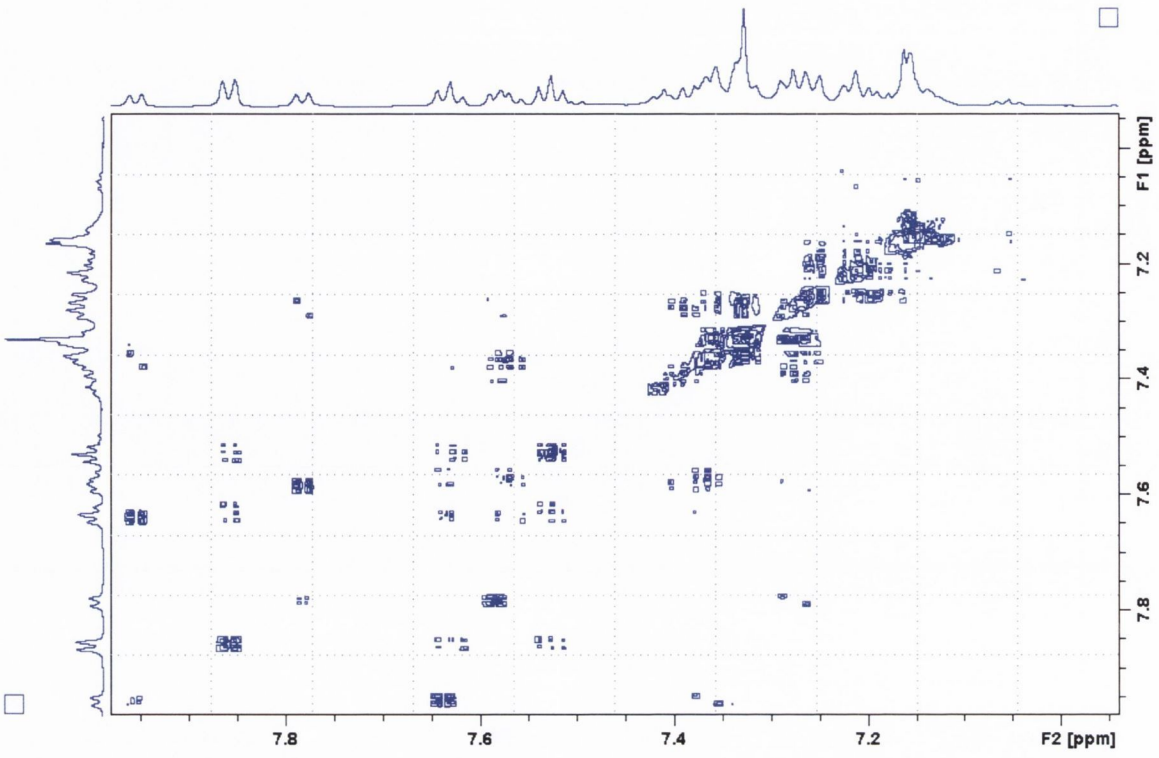


Fig.2.42 H-H COSY NMR spectrum of compound (2.21) from 7.02-7.95ppm

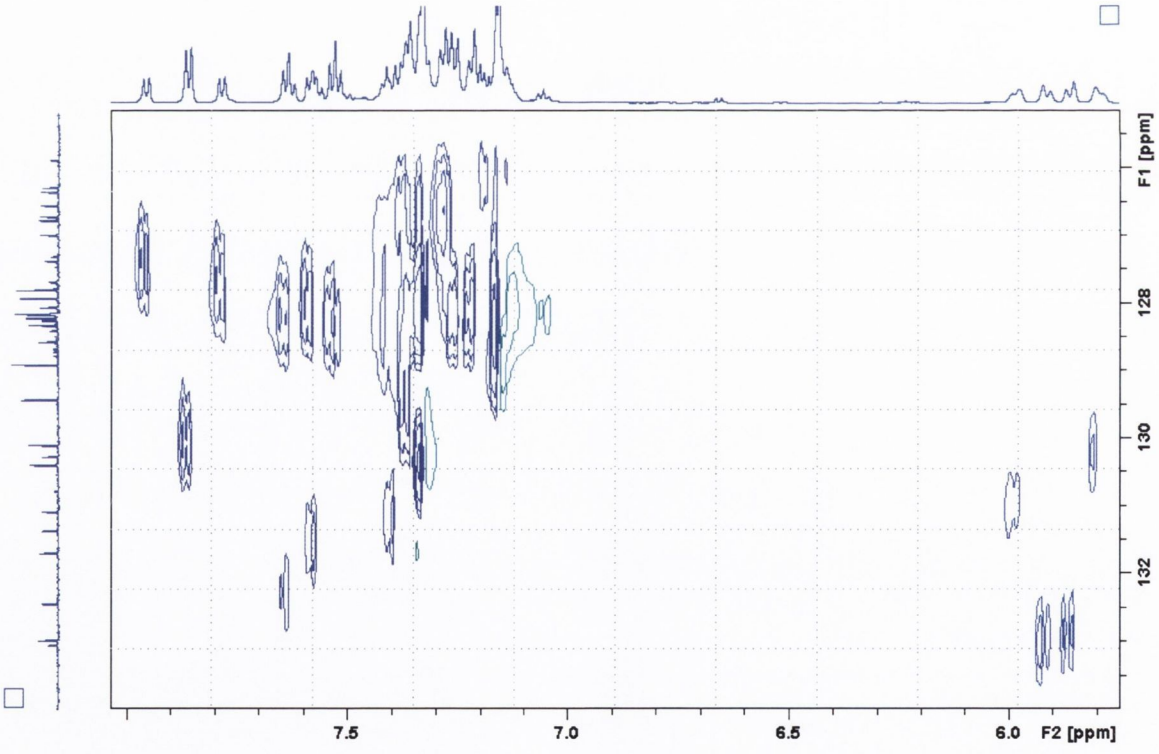


Fig.2.43 C-H COSY NMR spectrum of compound (2.21) from 5.75-8.05ppm

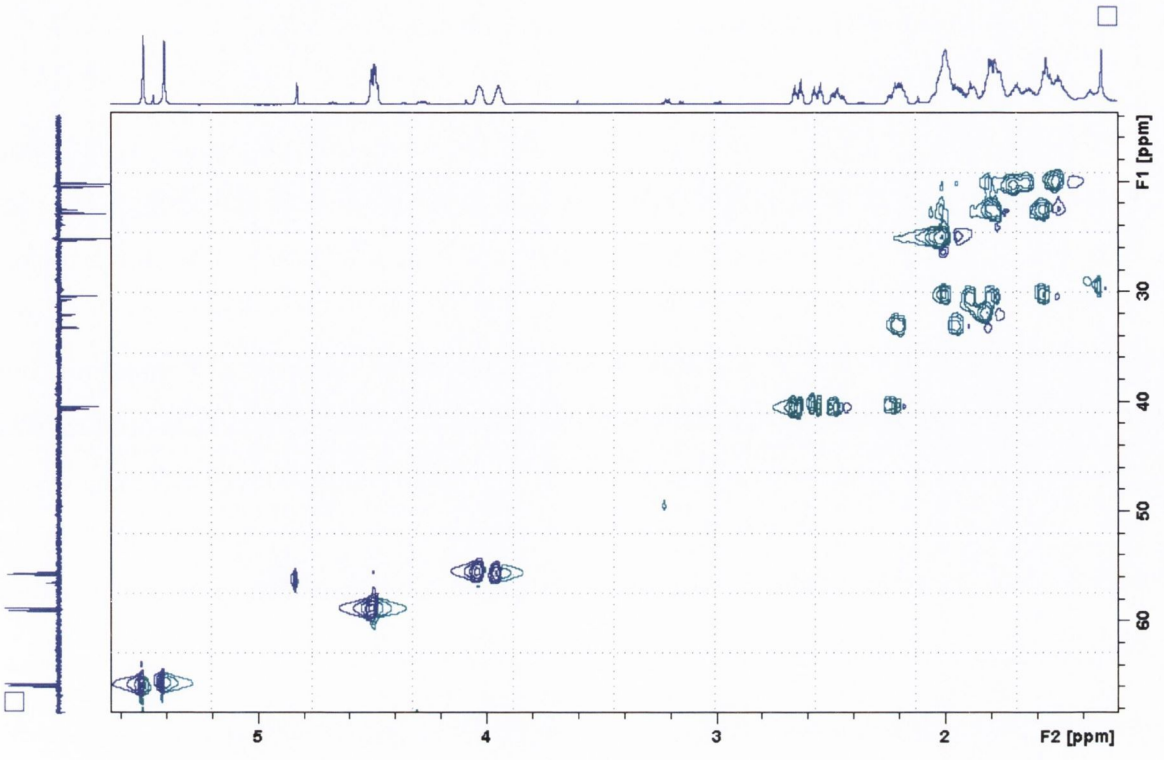


Fig.2.44 C-H COSY NMR spectrum of compound (2.21) from 1.32-5.62ppm

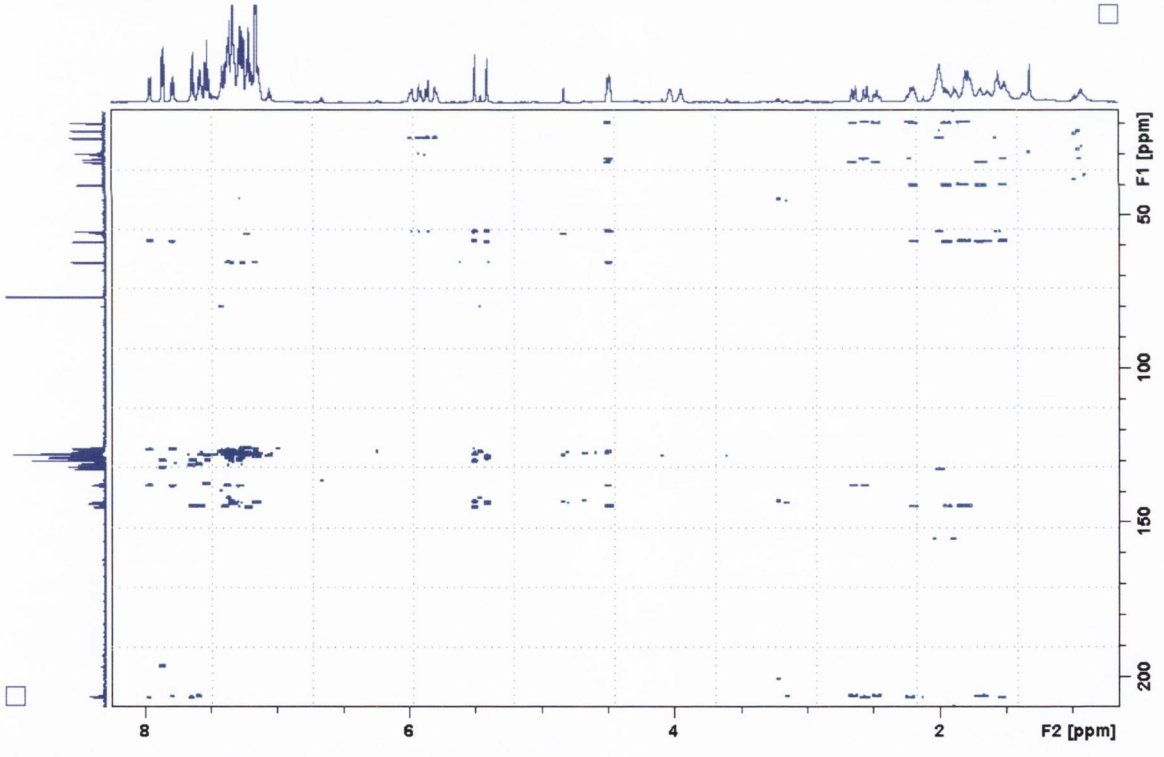
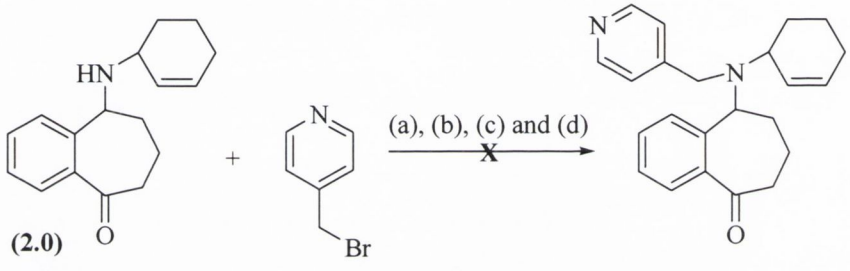


Fig.2.45 HMBC NMR spectrum of compound (2.21)

For clarity, the H-H COSY, C-H COSY, HMBC NMR spectra are shown in Fig. 2.41-Fig. 2.45. HRMS analysis revealed the calculated mass ($+H^+$) as 421.2303, which correlated well to the actual mass as 421.2318 with molecular formula $C_{30}H_{31}NONa$.

2.3.6 Attempted synthesis of pyridine analogue

Numerous reports in the literature had cited that the relationship between pyridine and mast cells. Many pyridine compounds have showed mast cell stabilising activities (Haining, 1963; Obata et al., 1996; Venkatachalam et al., 2003). Therefore, it was decided to produce pyridine-substituted analogues. Several approaches made to couple the secondary amine **(2.3)** to 4-(bromomethyl)pyridine were unsuccessful under all four modifications of reaction conditions listed in Table 2.1. In particular, crude product was formed from the reaction under Cs_2CO_3 in DMF at room temperature. Unfortunately, it was found that degradation of the crude product occurred while it was being concentrated *in vacuo* during work-up (Scheme 2.22). This strategy was hence not pursued any further.



(a) DMF, Cs_2CO_3, RT (b) acetone, K_2CO_3, reflux
(c) acetonitrile, *N,N*-diisopropylethylamine, RT, N_2
(d) acetonitrile, *N,N*-diisopropylethylamine, reflux, N_2

Scheme 2.22 Attempted synthesis of pyridine analogue

2.4 Biological data on compounds synthesised

Test compounds were evaluated for inhibition of compound 48/80-induced degranulation of rat peritoneal mast cells (RPMC), which performed within Department by Woods (2009). Mast cell stabilising activity of novel compounds and the reference compound DSCG in RPMC stimulated by compound 48/80. Values are mean of at least n=5, standard error in parentheses. All test compounds and DSCG were at 2 x 10^{-5}M, and challenged with compound 48/80 at 0.2g/ml, 5min exposure. All of the compounds synthesised displayed greater activity over DSCG when compound 48/80 was used to induce histamine release from rat peritoneal mast cells. However, at the concentration examined, the 4-trifluoromethoxy analogue **(2.11)** and the highly "aromatised"

diphenylmethyl analogue **(2.21)** were much less active (24% and 11%, respectively). It was evident from the results attained by Woods, that the compound with the unsubstituted benzyl moiety, namely compound **(2.10)** showed almost complete inhibition (99%) of histamine release, again reinforcing the importance of the presence of the benzyl and cyclohexenyl groups to mast cell-stabilisation activity. Rotation of the 2,3,4-trimethoxy substituents to the 3,4,5-position on the benzyl ring increased activity from 58%-101%, again suggesting that the positioning of these groups on the ring is critical for effective mast cell stabilisation activity.

Compounds	% Inhibition	Compounds	% Inhibition
2.8	58 (6)	**2.14**	89 (7)
2.9	101 (10)	**2.16**	95 (11)
2.10	99 (1)	**2.17**	91 (13)
2.11	24 (8)	**2.18**	90 (14)
2.12	Not tested	**2.20**	87 (5)
2.13	90 (5)	**2.21**	11 (4)
DSCG	10 (3)		

Table 2.1 Summary statistics for compounds (2.8-2.14), (2.16-2.18) and (2.20-2.21)

2.5 Conclusion

The primary objective of the work described in this Chapter was to synthesise a series of compounds where the indanone (or tetralone) portion of the compounds synthesised by Barlow (2002), was replaced by 1-benzosuberone. The initial focus of the work was to establish an effective synthetic approach to generate the targeted secondary amine intermediate **(2.3)**. Having identified an appropriate method, the intermediate secondary amine **(2.3)** was then *N*-benzylated with a range of benzyl groups containing electron withdrawing, donating substituents or π-rich aryl moiety. Problems were encountered with the *N*-benzylation step but were addressed when the method was changed from Cs_2CO_3/DMF, as base and solvent, to *N,N*-diisopropylethylamine and acetonitrile. The mast cell stabilisation data obtained on the compounds synthesised indicate that they are more active than DSCG, with a significant number capable of complete inhibition of histamine release from mast cells, when compound 48/80 is used to elicit release.

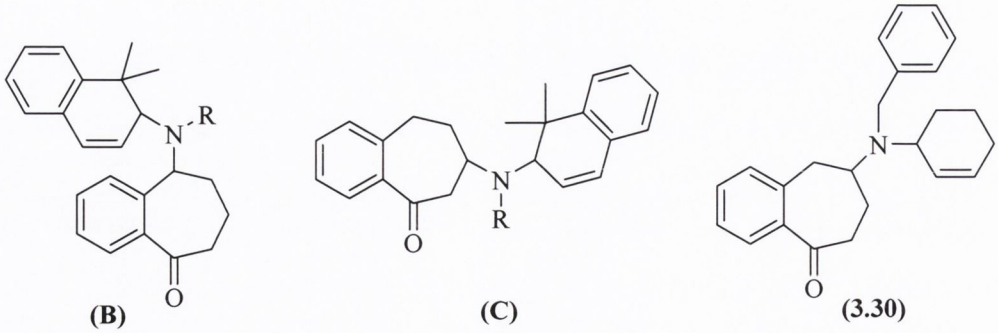
3.1 Introduction

In Chapter 2, a range of compounds were synthesised, containing both electron withdrawing and donating substituents on the benzylic ring and identified in agreement with Barlow (2002), that the cyclohexenyl unit is an important moiety for mast cell stabilisation activity. Interestingly, previous work by Barlow has shown that reduction of the cyclohexenyl double bond in a tetralone series of compounds abolished their activities *in vivo*. It would therefore seem plausible that perhaps the π-electron system may be important for molecular interaction at the cellular level.

3.2 Synthetic targets and strategy

This Chapter now explores the synthesis of compounds which increased the π-character at this site through substitution of the cyclohexenyl group by a π-rich dihydronaphthalene moiety, namely structural type **(B)** and its regio-isomer structural type **(C)**.

In addition, this Chapter will outline the development of a synthetic method for the synthesis of compound **(3.30)**, of the type shown in Fig 3.0, where the amine moiety is now attached to position-4 of benzosuberone ring. In doing so, it was expected to determine the degree of structural diversity that can be tolerated within the series of mast cell stabilisers, described in the preceding Chapter.



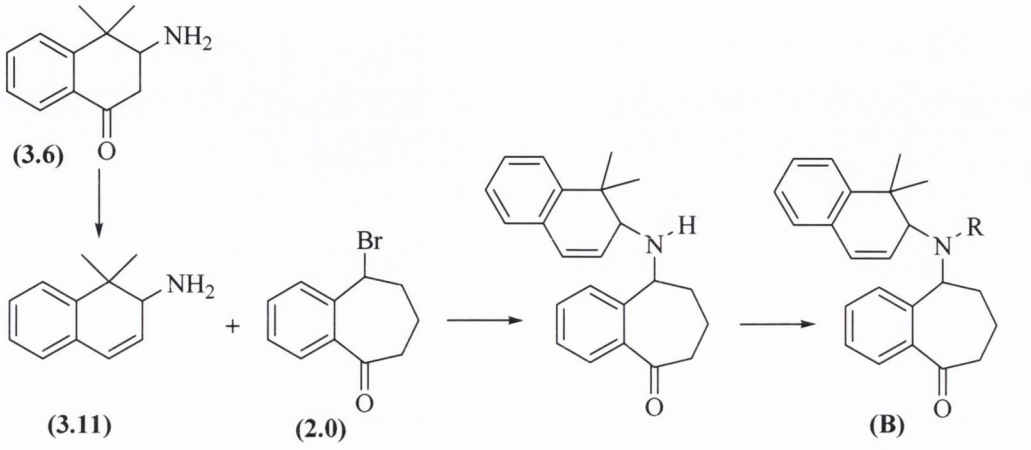
(B) **(C)** **(3.30)**

R = Me, benzyl, allyl

Fig. 3.0 Molecular designs of structural types (B), (C) and compound (3.30)

3.3 Synthesis of compounds of structural type (B)

In order to generate compounds of structural type **(B)**, the first objective was to establish a synthetic route toward the primary amine **(3.11)** that furnished the ketone **(3.6)** as one of its intermediate (Scheme 3.0).



Scheme 3.0 Synthetic approach to structural type (B)

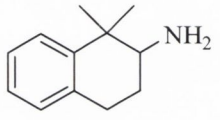
The inclusion of the dimethyl substituents on the dihydronaphthalene unit of structural type **(B)** was decided upon in order to circumvent the breakdown of the resulting compound to form naphthene, in a similar manner to that used for the indanone and tetralone mast cell stabilisers designed by Barlow (2002). Clearly, an alternative option was to attach the methyl substituents adjacent to the carbonyl group, but in our hands this inevitably resulted in inactive mast cell stabilisers, perhaps suggesting that the acidic hydrogens adjacent to the carbonyl are important for activity.

3.3.1 Synthesis of the intermediate amine (3.11)

The synthesis of the naphthalene fragment **(3.11)** dictated that in order to secure it as a thermodynamically stable intermediate that could then be coupled to the benzosuberone ring, it was essential that any opportunity for it to conjugate into a more stable derivative should be negated. Consequently, as stated above, inclusion of the dimethyl group adjacent to the amino substituent was deemed essential. Therefore, the proposed synthetic strategy involved introduction of two methyl groups at the benzylic position of

β-tetralone, conversion of dimethyltetralone to a primary amine **(3.4)** (Fig. 3.1) and benzylic oxidation to give compound **(3.6)**. The two critical challenges presented in this synthetic approach were to convert the dimethylated tetralone to the desired primary amine **(3.4)**, and secondly, insert the carbonyl functionality β to the amino moiety by oxidation of the primary amine **(3.4)**. If this strategy proved successful, the next objective was to insert the double bond to give the alkene **(3.11)**, followed by the coupling step between allylic amine **(3.11)** and benzylic bromide **(2.0)**. The final step was the *N*-alkylation of the resulting secondary amine (Scheme 3.14).

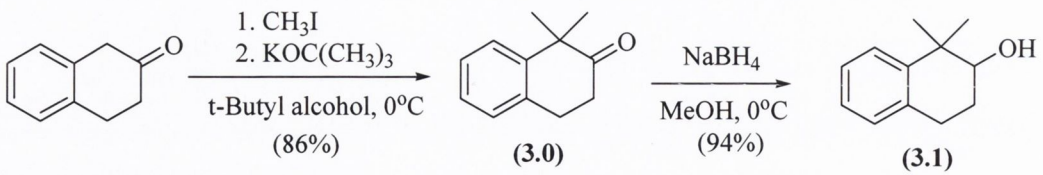
3.3.1.1 Synthesis of primary amine (3.4)



(3.4)

Fig. 3.1 Chemical structure of primary amine (3.4)

The dimethylation step involved treatment of β–tetralone with excess methyl iodide using potassium t-butoxide as base in the presence of t-butyl alcohol (Hill et al., 1965). Although the reaction was conducted at 0°C, it still tended to be slightly exothermic. Nevertheless after work-up and purification by flash column chromatography, compound **(3.0)** was isolated in good yield (86%) (Scheme 3.1). The identity of this compound was confirmed through analysis of its ^{1}H NMR spectrum where the two methyl groups resonated as a very sharp singlet signal at 1.48ppm while their corresponding ^{13}C signals were found at 26.5ppm.

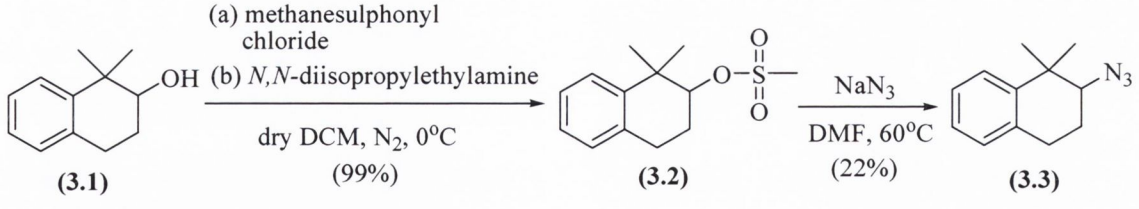


Scheme 3.1 Synthesis of compounds (3.0) and (3.1)

The dimethylated ketone **(3.0)** was then reduced with sodium borohydride to its corresponding tetralol **(3.1)**. This transformation occurred smoothly within an hour in

near quantitative yield (94%) (Scheme 3.1). Analysis of its ^{1}H NMR spectrum confirmed the presence of the methine proton at 3.80ppm, with its corresponding carbon being found at 75.1ppm.

Conversion of the hydroxyl to a suitable leaving group was necessary for the next step to enable substitution with azide. Various options in the choice of leaving group have been reported in the literature, including halide and sulphonyl groups. Indeed iodo-substituted naphthalene was first reported in 1946 (Arbit, 1946). The preferred approach was to convert the alcohol **(3.1)** into the mesylate **(3.2)**. The transformation of the alcohol moiety to the mesylate **(3.2)** occurred with ease by treatment with methane sulphonylchloride in the presence of *N,N*-diisopropylethylamine as base in DCM (Scheme 3.2). The detection of the endpoint of the reaction was somewhat complicated by the fact that both the starting material and mesylate had identical R_f values on TLC. Nevertheless, it didn't pose a significant problem as the alcohol tended to be completely converted to the mesylate. After rapid purification of the crude product by flash column chromatography on silica gel, the desired mesylate **(3.2)** was obtained in 99% yield. The most notable feature of its ^{1}H spectrum over that of the alcohol **(3.1)** was the shift of the methine proton which appeared as double doublet (J_1=3.38Hz, J_2=7.42Hz) at 4.87ppm relative to 3.80ppm for the alcohol **(3.1)**.

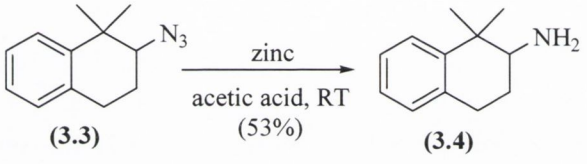


Scheme 3.2 Synthesis of mesylate (3.2) and azide (3.3)

While it was possible to isolate the mesylate **(3.2)** in pure form, it nevertheless was unstable on storage. Consequently, it was always treated immediately with the appropriate amine-based nucleophile. In our hands, this was most conveniently conducted by treatment with sodium azide in DMF at 60°C (Iida et al., 1985) (Scheme

3.2). Possibly owing to the steric effects of the adjacent methyl substituents, conversion to the azide **(3.3)** tended to be quite sluggish and required long reaction times. Typically the reaction took up to sixteen days to give the azide **(3.3)** in very modest yields, usually less than 25%. Its identity was easily confirmed through its sharp azide peak at 2098.2cm^{-1} in its infrared spectrum.

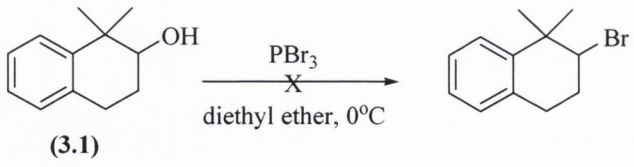
To furnish the amine **(3.4)**, the resulting azide **(3.3)** was reduced by using zinc in acetic acid at room temperature (Scheme 3.3). After the acid-base work-up, the primary amine **(3.4)** was obtained as a yellow oil. Its identity was confirmed chemically by a TLC by its strong reaction with ninhydrin, while the absence of the azide group was also confirmed by infrared analysis of the product. Although this synthetic route produced the desired amine, the yield in the substitution step with azide was especially disappointing.



Scheme 3.3 Synthesis of amine (3.4)

3.3.1.2 Modified primary amine (3.4) synthesis-route A

In an effort to improve upon the yield of the target primary amine **(3.4)**, it was decided to transform the alcohol **(3.1)** to the resulting bromide and then attempt to displace the bromide with azide ion. The alcohol **(3.1)** was brominated using phosphorous tribromide. The reaction was carried out in the presence of diethyl ether at 0°C and this substitution reaction was complete within one hour (Scheme 3.5). Unfortunately, TLC analysis showed that products produced from this bromination process had lower R_f values than the starting alcohol. Unsurprisingly, NMR data confirmed that the desired bromo-substituted naphthalene was not afforded.

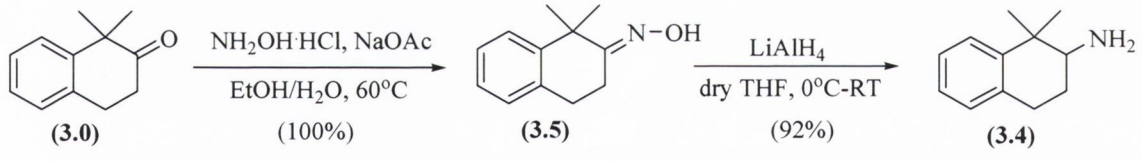


(3.1)

Scheme 3.4 Attempted bromination of compound (3.1)

3.3.1.3 Modified primary amine (3.4) synthesis-route B

An alternative strategy was simply to convert the ketone **(3.0)** into the oxime **(3.5)** and then reduce the corresponding oxime **(3.5)** with lithium aluminium hydride ($LiAlH_4$). This was easily effected following treatment of compound **(3.0)** with hydroxylamine hydrochloride and sodium acetate in a 2:1 mixture of ethanol and water at 60°C (Scheme 3.5). This step occurred readily in two hours and in excellent yield (100%). It also allowed the product to be purified and characterised before proceeding to the next step. The key feature in the characterisation of the oxime **(3.5)** was the presence of $O\text{-}H_{str}$, C=N, N-O in the infrared spectrum at 3264.5, 1655.3, 936.6cm^{-1}, respectively while the ^{13}C NMR spectrum also confirmed the absence of the carbonyl group.



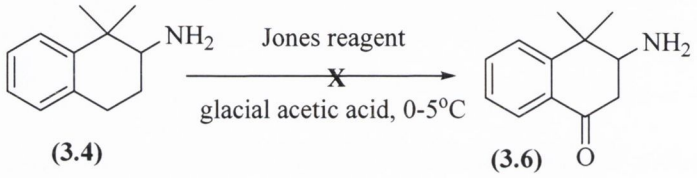
(3.0) (3.5) (3.4)

Scheme 3.5 Synthesis of amine (3.4) through the formation of oxime (3.5)

Upon isolation, the pure oxime **(3.5)** was reduced to the primary amine **(3.4)** following treatment with a large excess of $LiAlH_4$ (six mole equivalents) in anhydrous THF at 0°C. This transformation was simple once the starting compound was pure and the reaction conditions were anhydrous (Scheme 3.5). The amine **(3.4)** was isolated in good yield (92%) after acid-base work-up, without the need for further purification by flash column chromatography. Once again, the presence the amine **(3.4)** was confirmed by the positive reaction with ninhydrin on TLC and the absence of the oxime peaks in the infrared spectrum.

3.3.1.4 Attempted synthesis of ketone (3.6)

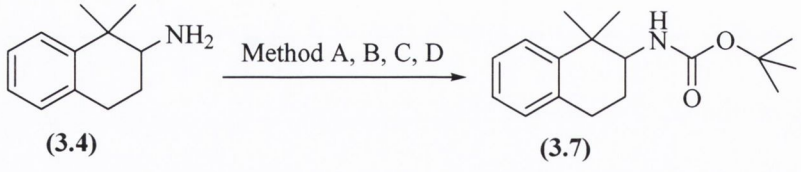
It was felt that introduction of the carbonyl moiety at the benzylic position could be effected through oxidation with Jones's reagent, as these conditions are acidic. The transformation was carried out by the slow addition of a 10% solution of chromium trioxide in glacial acetic acid at 0°C to a solution of the amine **(3.4)** in glacial acetic acid as solvent (Scheme 3.6). When the reaction was worked up through an acid-base extraction procedure, the NMR data from the isolated material proved inconclusive at best.



(3.4) **(3.6)**

Scheme 3.6 Attempted oxidation of primary amine (3.4)

An alternative two-step approach was pursued in order to introduce the carbonyl group. It was thought that it was necessary to protect the amino functionality of compound **(3.4)**, as *BOC* protected amine derivative prior to the benzylic oxidation step. This transformation was carried out by four different methods in order to optimise this process, as listed below in Table 3.0.



(3.4) **(3.7)**

Scheme 3.7 Synthesis of N-BOC protected amine (3.7)

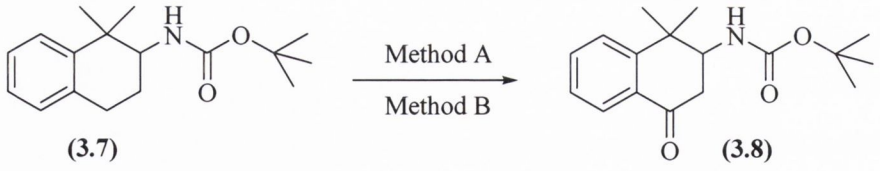
All attempts used produced the desired *N-BOC* protected amine **(3.7)** in good yields, with method D proving to be the most effective, yielding compound **(3.7)** in an excellent yield (96%).

The ^{1}H NMR spectrum of compound **(3.7)** showed three methyl groups (as a part of the *BOC* functionality) as a tall, sharp singlet at 1.49ppm. The methine proton appeared as a triplet (J=8.02Hz) at 3.88ppm and the proton attached to the amino group as a doublet (J=9.52Hz) at 4.65ppm. Important features of the ^{13}C NMR spectrum were the methyl resonances; those of the methyl carbons in the *BOC* group resonated at 28.0ppm, those of the methyl carbons on the tetralin ring at 25.4 and 29.7ppm and the diagnostic carbonyl signal of the carbamate at 155.4ppm. Infrared analysis confirmed the presence of the carbamate functionality ($C=O_{str}$) at 1701.6cm^{-1}.

	Reaction Conditions	Time	Yields (%)
Method A	di-*tert*-butyl dicarbonate, DMAP, DCM, *N,N*-diisopropylethylamine, RT	1 hr	53%
Method B	di-*tert*-butyl dicarbonate, MeOH, Cs_2CO_3, RT	4 hrs	71
Method C	di-*tert*-butyl dicarbonate, DCM, *N,N*-diisopropylethylamine, RT	overnight	95
Method D	di-*tert*-butyl dicarbonate, H_2O/*t*-butanol (*v:v*, 2:9), RT	4 hrs	96

Table 3.0 List of methods for BOC protection of the amine (3.7)

As illustrated above with Jones reagent, there are several approaches that can be adopted to affect an oxidative step at the benzylic position. Of primary interest was the use of *meta*-chloroperoxybenzoic acid (*m*-CPBA) (Ma et al., 1999), as an oxidising agent. The reaction was conducted by the dissolving *BOC*-protected amine **(3.7)** in DCM, followed by the addition of *m*-CPBA and sodium hydrogen carbonate. In order to facilitate the oxidative process a condenser was attached to the flask to allow exposure of the reaction solution to atmospheric oxygen. The reaction was allowed to proceed for seven days. After work-up and purification by flash column chromatography, the desired ketone **(3.8)** was isolated in a modest yield (24%). Nevertheless the unreacted starting material from the mixture could be achieved and continuously used in repeated oxidation processes.



(3.7) **(3.8)**

Method A: *m*-CPBA, DCM, $NaHCO_3$, 0°C, 7days, 24%
Method B: Jones reagent, acetone, 0°C, 45mins, 22%

Scheme 3.8 Synthesis of compound (3.8)

As expected the ^1H NMR spectrum of the ketone **(3.8)** was relatively straightforward to assign (Fig. 3.2). Two peaks at 1.25 and 1.30ppm, integrating for a total of fifteen protons, confirmed the presence of the five methyl groups. One proton of the tetralin methylene group at C-2 position resonated as two separate doublets (J=8.04Hz) at 2.65 and 2.69ppm, while the other methylene proton at C-2 appeared as a double doublet (J_1=4.00Hz, J_2=17.56Hz) at 2.85ppm. The methine proton at C-3 position existed as a broad triplet at 4.06ppm and the proton on the amino moiety appeared as a doublet (J=9.52Hz) at 5.09ppm. The aromatic region was well resolved, a triplet (J=7.26Hz) for one proton at 7.18ppm, followed by a doublet (J=8.04Hz) at 7.35ppm. Another triplet (J=7.28Hz) was seen at 7.43ppm. The proton nearest the carbonyl group existed as a doublet (J=7.87Hz) at 7.87ppm. In the ^{13}C NMR spectrum of compound **(3.8)** a strong signal at 27.8ppm was attributed to the three methyl carbons of the *BOC* group. Two methyl carbons of the tetralin ring at C-11 and C-12 positions resonated at 24.2 and 27.9ppm, respectively. Four quaternary carbons were found at 38.4, 78.7, 130.3 and 149.6ppm. The carbonyl groups (C-13) were present at 155.0ppm (*BOC* moiety) while the signal at 195.5ppm represented the ketone functionality (C-1). The DEPT 135 NMR spectrum confirmed the presence of the methylene carbon at the C-2 position at 41.0ppm. In the DEPT 90 NMR spectrum, a methine carbon was seen at 54.2ppm and a total of four tertiary carbons at 125.8, 126.2, 126.7 and 134.0ppm confirmed the expected aromatic signals (Fig. 3.3 and Fig. 3.4).

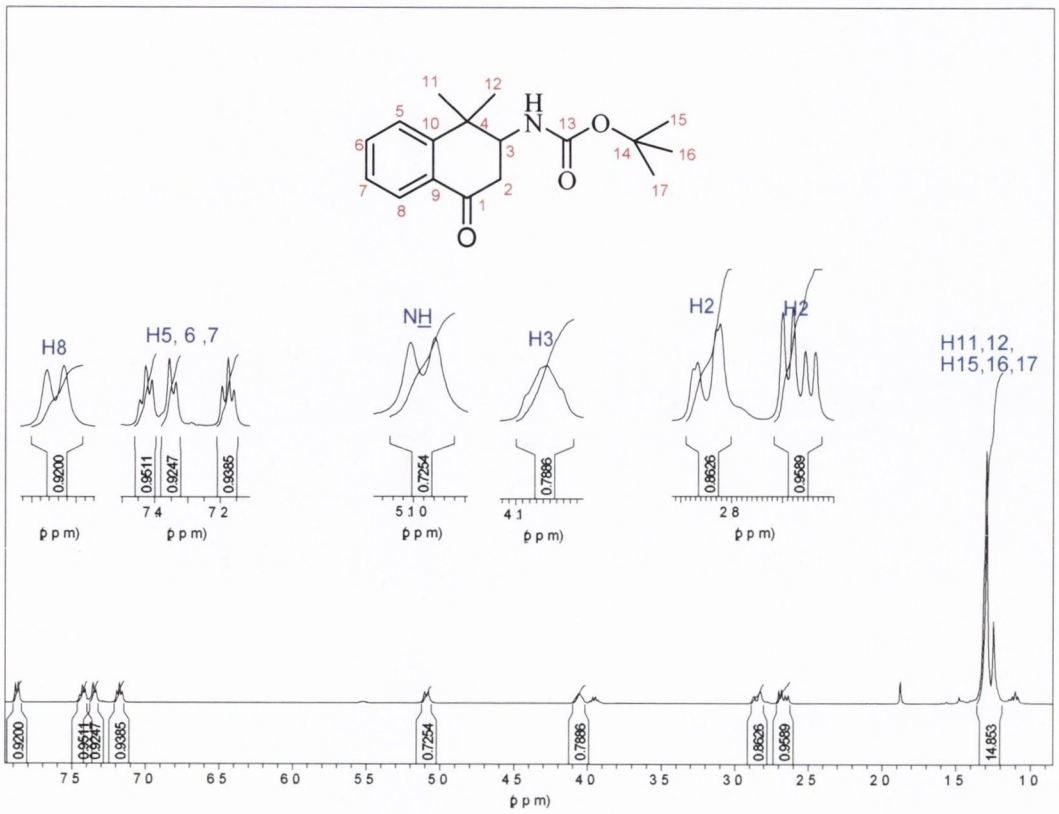


Fig. 3.2 ^{1}H NMR spectrum of compound (3.8)

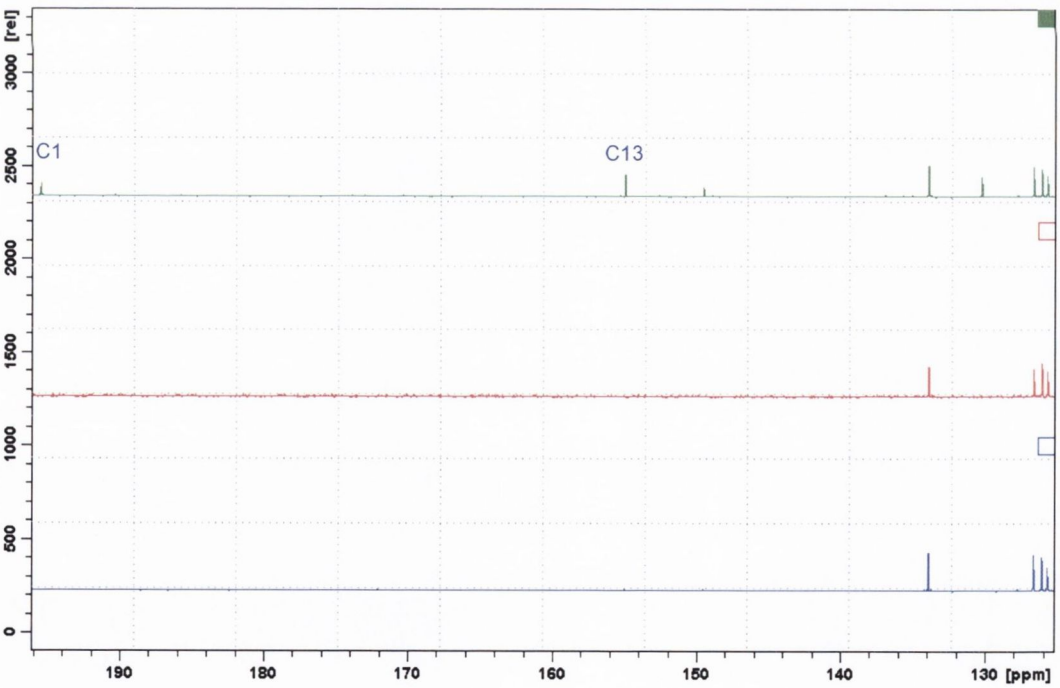


Fig. 3.3 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (3.8) from 125.8-196.0ppm

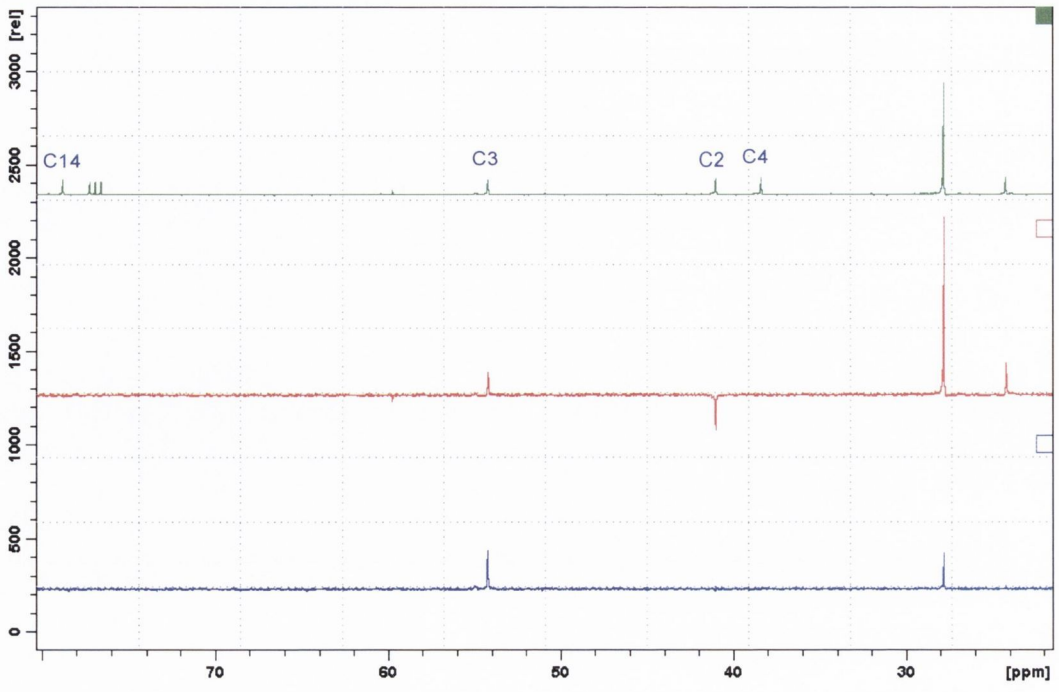


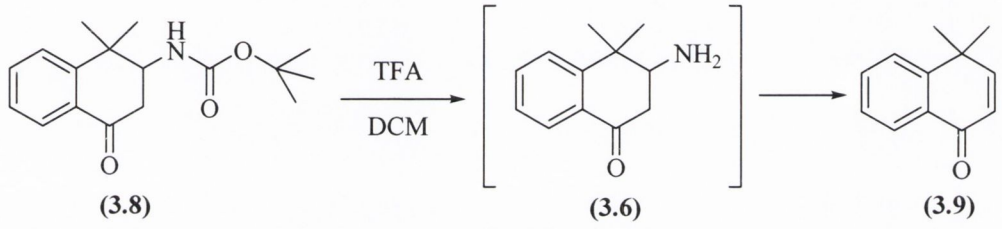
Fig. 3.4 ***^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (3.8) from 21.9-80.1ppm***

The carbamate **(3.8)** exhibited a characteristic C=O stretch at 1683.0cm^{-1} in its infrared spectrum. HRMS analysis gave the accurate molecular mass (+Na^{+}) as 312.1562, calculated 312.1576, molecular formula $C_{17}H_{23}NO_3Na$.

Although the oxidative step to produce the ketone **(3.8)** was clean, it nevertheless gave a relatively poor yield of product (24%). It was decided to revert back to using Jones reagent (Bowden et al., 1946) to affect this oxidation process. Jones reagent is commonly employed for the conversion of secondary alcohols to ketones and for the oxidation of benzylic hydrocarbons (Rangarajam & Eisenbraun, 1985). The reaction was performed by slowly adding Jones reagent to the cooled solution of compound **(3.7)** in order to avoid removal of the *N-BOC* protecting group. The reaction temperature was set at 0°C initially, and then allowed to rise to 5-10°C. After maintaining the reaction at this temperature for forty minutes, it was then quenched, worked up, and purified by flash column chromatography to furnish the ketone **(3.8)** in 22% yield. Attempts to optimise this transformation resulted in the generation of many side products especially when prolonged reaction times were employed. Unfortunately, the effect of changing the

oxidising agent did not improve the yield of the title compound. Nevertheless it did secure the title compound after a reaction time of only forty-five minutes in comparison to four days with *m*-CPBA as oxidising agent.

Removal of the *N-BOC* protecting group using a mixture of trifluoracetic acid and DCM (*v:v*, 1:1) at 0°C gave the corresponding free amine **(3.6)**. Unfortunately, on acid-base work-up and removal of solvent, the liberated amine **(3.6)** was found to decompose rapidly to a white crystalline solid at room temperature. This was believed to be the elimination product **(3.9),** which was confirmed by NMR analysis. This immediate transformation was also observed even at -4°C (Scheme 3.9).

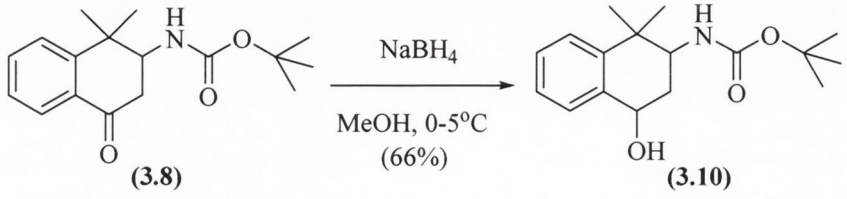


(3.8) **(3.6)** **(3.9)**

Scheme 3.9 Formation of compound (3.9)

3.3.1.5 Formation of compound (3.11)

Due to the instability of compound **(3.6)**, a different approach was examined. Insertion of the alkene group into compound **(3.8)** was achieved following reduction of the ketone **(3.8)** to the alcohol **(3.10)**, and elimination of the alcohol **(3.10)**. Reduction of compound **(3.8)** to yield the alcohol **(3.10)**, was effected by the use of sodium borohydride (Chaikin & Brown, 1949). The reaction was conducted in the presence of methanol at 0°C. Upon work-up and purification by flash column chromatography, the alcohol **(3.10)** was obtained in good yield (66%) (Scheme 3.10).

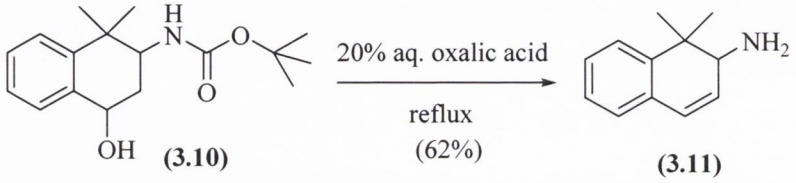


(3.8) **(3.10)**

Scheme 3.10 Synthesis of compound (3.10)

This naphthol was easily identified by ^{1}H NMR analysis, showing the diagnostic signal of the benzylic methine proton as a broad triplet at 4.91ppm. The corresponding carbon was found to resonate at 78.7ppm in the ^{13}C NMR spectrum.

The direct one-step conversion of compound **(3.10)** to the allylic amine **(3.11)** was accomplished by treating the alcohol **(3.10)** with a 20% of solution of oxalic acid in water under reflux conditions. Following an acid-base work-up, the allylic amine **(3.11)** was obtained in an acceptable yield (62%) (Scheme 3.11).



Scheme 3.11 Synthesis of compound (3.11)

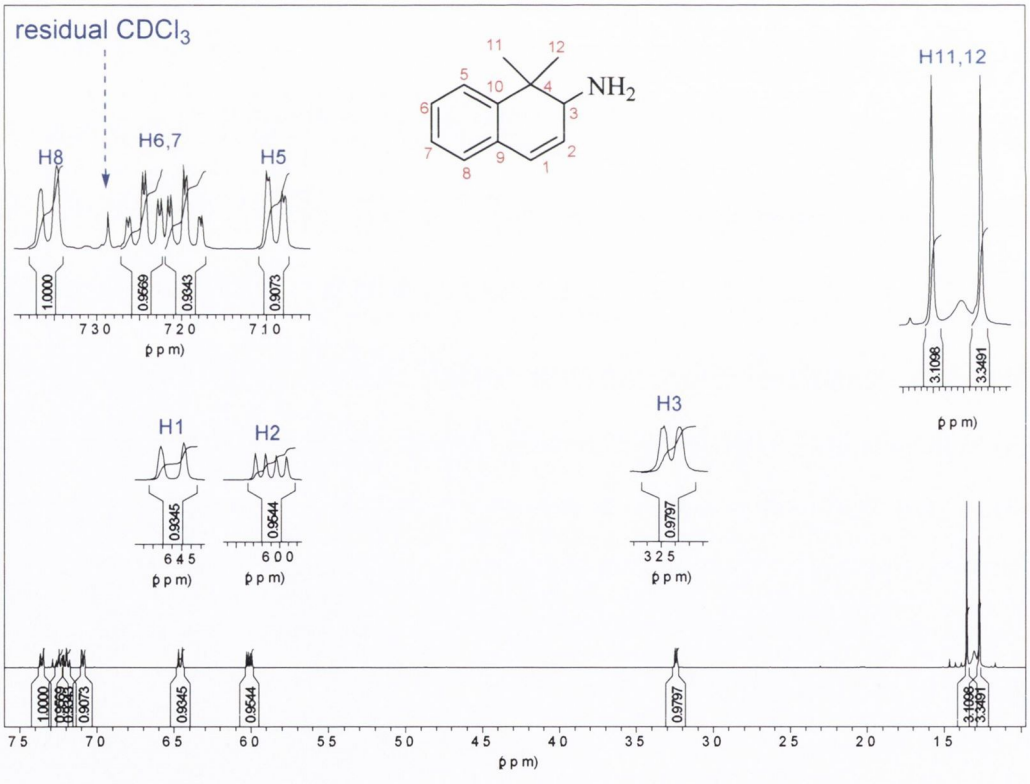


Fig. 3.5 ^{1}H NMR spectrum of compound (3.11)

The most obvious feature of its ^{1}H NMR spectrum is the absence of the methine protons and the presence of the two alkenic protons resonating as a double doublet (J_1=4.52Hz,

J_2=9.52Hz) and doublet (J=9.56Hz), at 6.01 and 6.46ppm respectively (Fig. 3.5). A total of six signals were observed in the region of 125.0-132.0ppm in the ^{13}C NMR spectrum, representing four aromatic tertiary carbons and two alkenic carbons (Fig. 3.6).

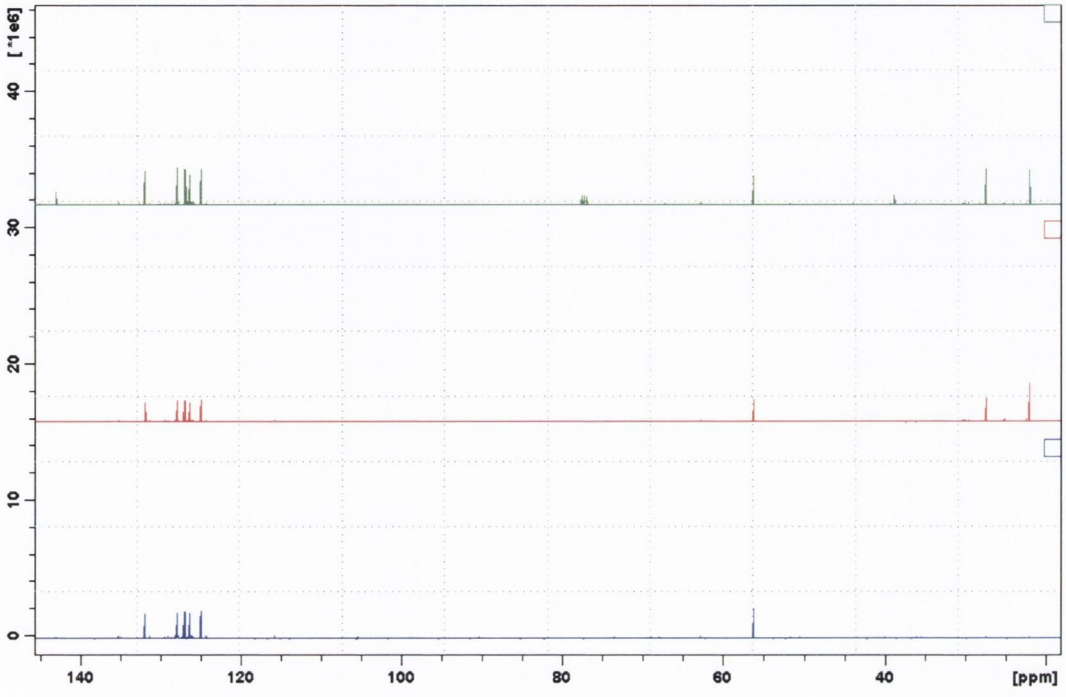


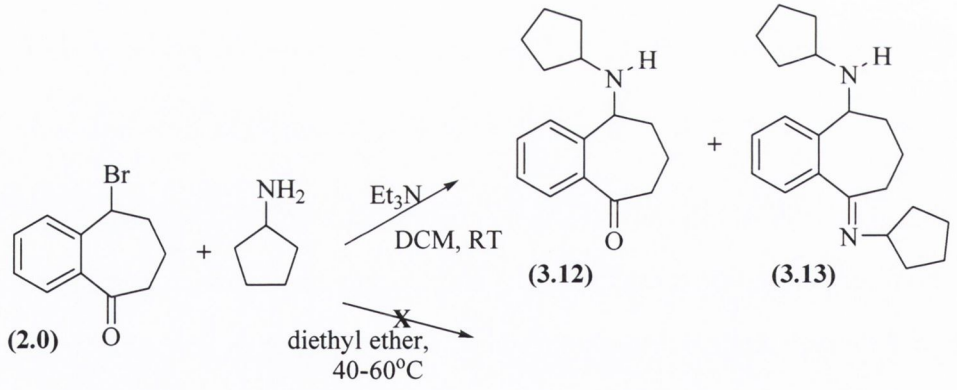
Fig. 3.6 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (3.11)

3.3.2 Coupling reactions

Having obtained the π-rich amine **(3.11)**, the next step was to complete its attachment to benzosuberone. Two points on benzosuberone for the attachment were decided upon, namely positions 3 and 5. It was felt that coupling to the 3-position could be achieved through a Michael addition step (structural type **C**) and in the case of 5- through displacement of a highly reactive benzylic halide (structural type **B**) (Fig 3.0). Displacement of benzylic halides of 3-bromoindanone with amine based nucleophiles proceeds efficiently in DCM with triethylamine as base. As most of the work to date had been on amine coupling to the benzylic position, this served as the most logical point for the first coupling step. Assuming these transformations were successful, the focus of the work would then turn towards completion of the synthesis through *N*-benzylation of the secondary amines.

3.3.2.1 Attempted synthesis of model compound (3.12)

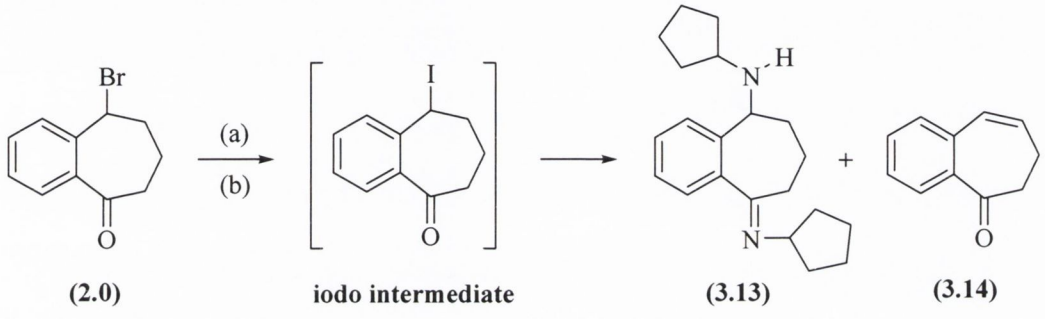
Since considerable quantities of the valuable intermediate **(3.11)** were required for the synthesis of the designed target compounds, alternative synthetic strategies were investigated. Initially, it was decided to optimise the displacement of the benzylic bromide (**2.0**) with readily available primary amines. Taking cyclopentylamine as a suitable model compound, the secondary amine **(3.12)** was prepared. Compound **(3.12)** was synthesised by direct coupling reaction of the benzylic bromide **(2.0)** with cyclopentylamine in DCM with triethylamine as base (Barlow, 2002). Although the reaction was successful in the sense that it produced the desired compound, it nevertheless suffered from the competing side reaction, namely formation of the imine **(3.13)** the most significant product (Scheme 3.12). In an attempt to avoid the formation of imine **(3.13)** as direct coupling of the bromide **(2.0)** to the cyclopentylamine was attempted in the presence of diethyl ether at 40-60°C. After a period of seven days, a mixture of products was formed. Unfortunately, there was no evidence of the formation of the desired product **(3.12)** by NMR analysis.



Scheme 3.12 Synthesis of compound (3.12) via direct coupling

Owing to the lack of success of the direct coupling method, a more sequential approach was attempted. It was felt that one could favour the formation of amine **(3.12)** over imine **(3.13)** by ultilising a better leaving group at the benzylic position. The alternative route examined was via the *in situ* displacement of the bromide with iodide using sodium iodide. This one-pot reaction was performed in the presence of acetonitrile as

solvent and Hunig's base as tertiary amine (Klok et al., 2006). After stirring the reaction at room temperature for one day, there was little evidence of the formation of amine **(3.12)** as judged by TLC. Applying heat to the reaction resulted in the formation of two significant products; namely imine **(3.13)** and the competing elimination product compound **(3.14).** Both compounds were isolated and identified by NMR analysis. When the solvent was changed to DMF from acetonitrile there was no observable sign of the formation of amine **(3.12)**, with compounds **(3.13)** and **(3.14)** being the dominant products isolated (Scheme 3.13).



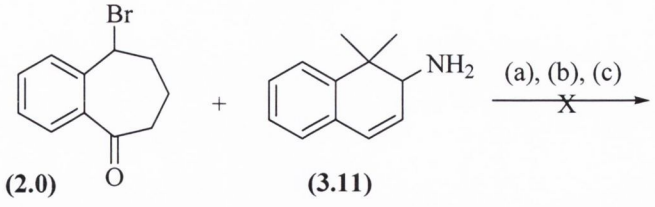
(2.0) **iodo intermediate** **(3.13)** **(3.14)**

(a) acetonitrile, Hunig's base, NaI, cyclopentylamine, reflux
(b) DMF, Hunig's base, NaI, cyclopentylamine, 60°C

Scheme 3.13 Attempted synthesis of compound (3.11) via iodo intermediate

3.3.2.2 Attempted synthesis of structural type (B)

Unsurprisingly, all attempts to couple the key intermediate amine **(3.11)** with the benzylic bromide **(2.0)** proved fruitless (Scheme 3.14). Therefore, this synthetic strategy was not pursued any further.



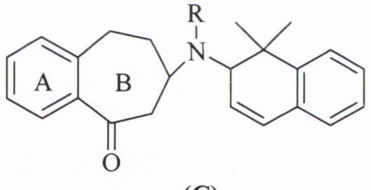
(2.0) **(3.11)**

(a) DCM, triethylamine, RT (b) acetonitrile, Hunig's base, NaI, reflux
(c) DMF, Hunig's base, NaI, 60°C

Scheme 3.14 Attempted couplings of compounds (2.0) and (3.11)

3.4 Synthesis regio-isomer structural type (C)

It was somewhat disappointing to observe that displacement of the benzylic bromide **(2.0)** with amine based nucleophiles proved difficult especially as the corresponding benzylic bromide of 1-indanone could readily be displaced with amine based nucleophiles. As the most significant product that formed was the elimination product **(3.14)**, we surmised that perhaps the coupling reaction, in the case of 3-bromoindanone with amines, proceeds through an enone intermediate rather than through direct displacement of the halide. Indeed, Finn (2008) has since demonstrated that amine based nucleophiles readily couple with indenone under identical conditions for those used for 3-bromoindanone. Within the benzosuberone series the opportunity now exists for the attachment of the amine to the 3-position of benzosuberone if one could secure the enone derivative of benzosuberone. Indeed this served as an ideal opportunity to investigate the effect of rotating the amino substituent to different positions on the B-ring and then to investigate the effect on activity of these changes (Fig. 3.7).

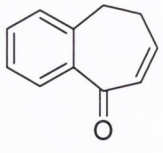


R = Me, benzyl, allyl

(C)

Fig. 3.7 Molecular design of structural type (C)

3.4.1 Synthesis of enone (3.17)

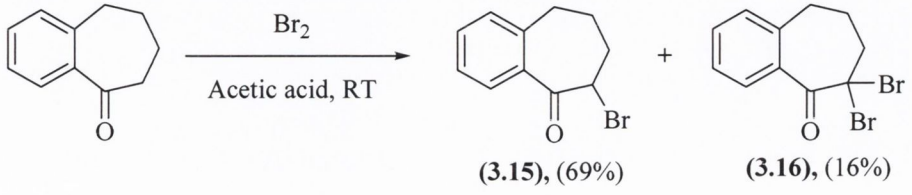


(3.17)

Fig. 3.8 Chemical structural of enone (3.17)

The approach was to start the synthesis from benzosuberone and conduct a monobromination step followed by an elimination reaction. The bromination reaction

was effected by dissolving benzosuberone in acetic acid, followed by the drop-wise addition of one equivalent of bromine at room temperature (Rueeger et al., 2004). This transformation was complete within fifty minutes and the desired monobromide **(3.15)** was obtained in satisfactory yield (69%), together with a small percentage of the dibrominated product **(3.16)** (Scheme 3.15). The structures of both compounds were confirmed through analysis of their ^{1}H NMR spectra.

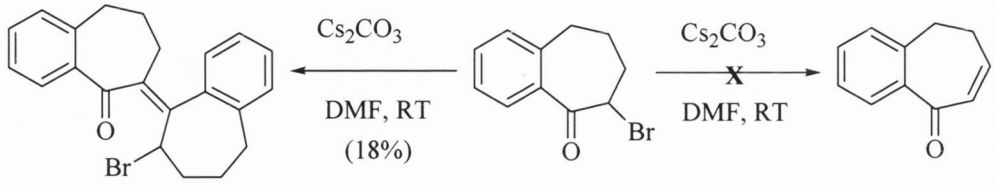


(3.15), (69%) **(3.16),** (16%)

Scheme 3.15 Synthesis of molecules (3.15) and (3.16)

3.4.1.1 Synthesis of the desired molecule (3.17) (Method A)

Having obtained compound **(3.15)**, the next step was to effect the loss of HBr from this compound. The first approach employed caesium carbonate as base in the presence of DMF at room temperature (Scheme 3.18). However, it was discovered that although **(3.15)** was consumed in the reaction, the only product isolated was the dimer **(3.18)**, presumably as a result of the reaction of compound **(3.15)** with unreacted benzosuberone from the preceding step (Scheme 3.16).



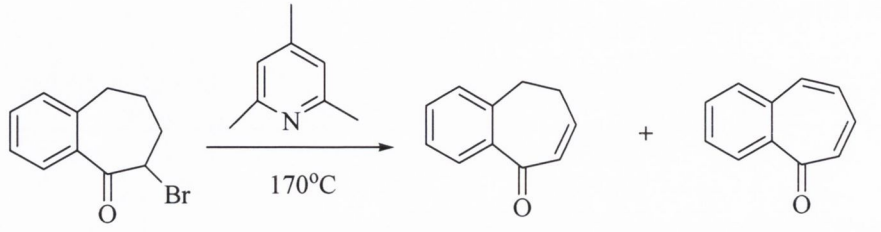
(3.18) **(3.15)** **(3.17)**

Scheme 3.16 Synthesis of molecule (2.42)

3.4.1.2 Synthesis of the desired molecule (3.17) (Method B)

Consequently, a milder set of reaction conditions were sought that would facilitate the process of unsaturation without causing dimer formation. Many alternative synthetic methods have been developed for dehydrohalogenation including the use of collidine as

base (Buchanan & Lockhart, 1959). Compound **(3.15)** was converted to the enone **(3.17)** upon exposure to 2,4,6-trimethyl pyridine (collidine) at 170°C. The reaction was complete within six hours. Following work-up, under acidic conditions and purification by flash column chromatography, two main compounds; namely compounds **(3.17)** (46%) and **(3.19)** (14%) were isolated from numerous side products (Scheme 3.17).

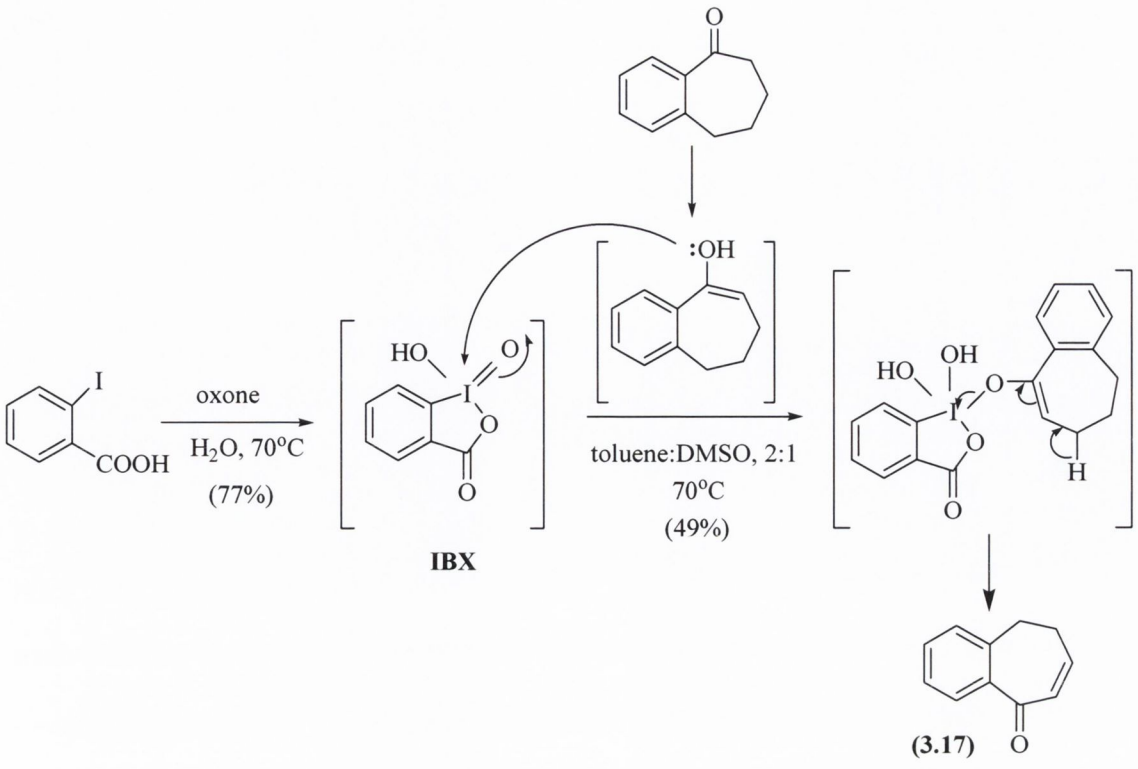


(3.15) **(3.17),** (46%) **(3.19),** (14%)

Scheme 3.17 Collidine mediated synthesis of compound (3.17)

3.4.1.3 IBX mediated synthesis of molecule (3.17) (Method C)

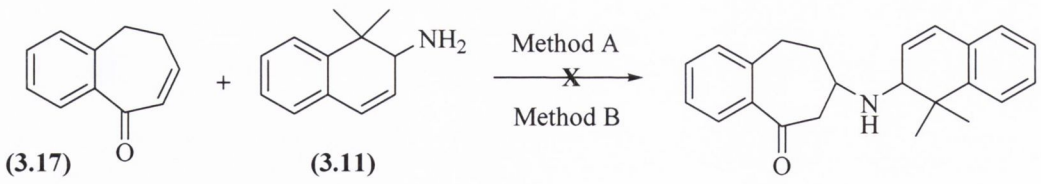
Although compound **(3.17)** was attained in a reasonable overall yield, it was nevertheless decided to investigate if it could be synthesised through a one-pot procedure, using 2-iodoxybenzoic acid (IBX). IBX was prepared according to the method reported by Frigerio et al. (1999), which involved treating 2-iodobenzoic acid with oxone ($2KHSO_5$-$KHSO_4$-K_2SO_4) in water at 70-75°C for thirty-six hours. Following completion and work-up of the reaction, IBX was obtained as a white solid, with a 77% yield. The NMR and infrared spectra of IBX were in agreement with literature data. The oxidation step itself involved treating benzosuberone with IBX in a solvent mixture consisting of toluene and DMSO (*v*:*v*, 2:1) at 70°C (Nicolaou et al., 2000). The progress of the reaction was monitored by TLC. After two days, it was worked up and purified by flash column chromatography to give the title compound **(3.17),** in reasonable yield 49%, together with a small quantity (15%) of unreacted starting material.



Scheme 3.18 Mechanism of the oxidation of benzosuberone with IBX

3.4.2 Coupling of α, β-unsaturated carbonyl compound (3.17) to compound (3.11)

It was hoped that direct coupling of the amine **(3.11)** to the enone **(3.17)** could proceed in a similar manner to that used by Finn (2008) for the coupling of amines to 1-indenone. The reaction was performed in DMF at room temperature with caesium carbonate as base. After leaving the reaction to stir for ten minutes at room temperature, an acid/base extraction step was performed to isolate the basic constituents. Analysis of the isolated material by NMR indicated that the only compound isolated was the amine **(3.11)**, even though analysis of the reaction mixture by TLC indicated that the enone **(3.17)** was consumed. When the procedure was changed to using triethylamine as base, there was still no evidence of the desired coupled product (Scheme 3.19). Having been unsuccessful trying to couple the amine **(3.11)** to benzosuberone, it was decided to direct on efforts towards the attachment of the amine, containing cyclohexenyl and benzyl substituents to position-4 of benzosuberone.



Method A: caesium carbonate, DMF, RT, 10mins
Method B: triethylamine, DMF, 10days

Scheme 3.19 Attempted coupling of amine (3.11) and enone (3.17)

3.5 Synthesis of compound (3.30)

Following the successful synthesis of a series of mast cell stabilising compounds of the types depicted in Chapter 2, our efforts were now directed towards the study of compounds where the amino substituent is appended to position-4 of benzoberone, γ-position relative to that of the carbonyl group.

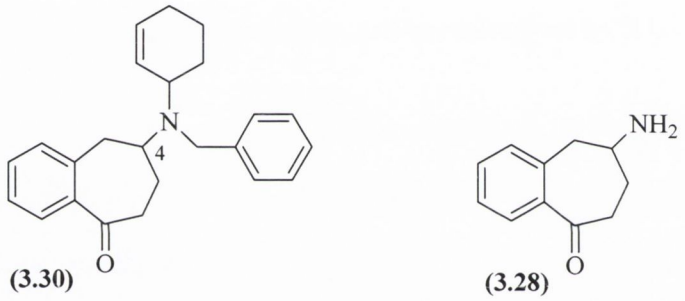
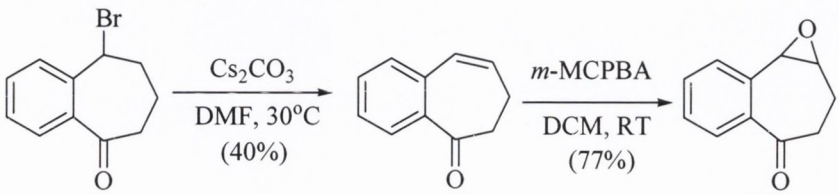


Fig. 3.9 Chemical structures of compounds (3.28) and (3.30)

In an effort to generate compound **(3.30)**, the first objective was to complete the synthesis of the primary amine **(3.28)**. The synthetic method devised dictated that we start from 1-benzosuberone. Following benzylic bromination to form bromide **(2.0)**, the alkene **(3.14)** was furnished following treatment of bromide **(2.0)** with 2.5 equivalents of caesium carbonate in the presence of DMF at 30°C for three days (van Tamelen et al., 1957; Dunkelblum et al., 1977; Smith et al., 1994). The identity of the alkene **(3.14)** was confirmed through analysis of its ^{1}H NMR spectrum, with the signals for the alkene protons resonating at 6.16-6.21ppm as a muliplet for one proton, and at 6.49ppm as a doublet (J=11.52Hz) for the other proton. Their corresponding ^{13}C NMR signals were found between 127.0-133.1ppm.

Treatment with *meta*-chloroperbenzoic acid gave the desired epoxide **(3.20)**, the spectral data from which was consistent with that reported in the literature data (Kotkowska-Machnik & Zakrzewski, 1980; Jean et al., 1981). The diagnostic signals in the ^{1}H NMR spectrum were the two methine protons which existed as one multiplet and one doublet (J=4.52Hz), at 3.63-3.66 and 3.92ppm, respectively. Again, the corresponding peaks for their respective carbons in the ^{13}C NMR spectrum were at 56.3 and 57.5ppm.



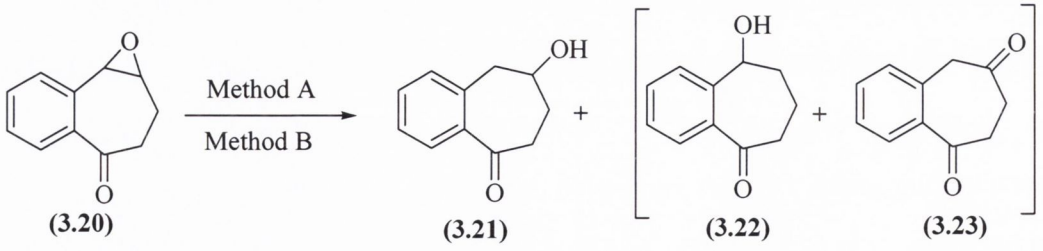
(2.0) **(3.14)** **(3.20)**

Scheme 3.20 Synthesis of compounds (3.14) and (3.20)

Opening of the epoxide ring of compound **(3.20)** was initially carried out using 10% palladium (Pd/C) in a solvent system consisting of methanol and ethyl acetate under an atmosphere of hydrogen (Method A, Scheme 3.21). The reaction was complete within four hours and the desired product **(3.21)** was obtained in good yield (75%). The main feature of the ^{1}H NMR spectrum of compound **(3.21)** was the methine proton as a multiplet resonating from 4.22-4.28ppm. The corresponding signal in the ^{13}C NMR spectrum was at 67.7ppm. However upon analysis by NMR and HRMS, a mixture of two side products (8.9%) of identical R_f values were found to have also formed. One was attributed to the benzylic alcohol **(3.22)** and the other to the dione compound **(3.23)**. HRMS analysis gave an accurate molecular mass for compound **(3.22)** ($+Na^{+}$) of 199.0729 with molecular formula $C_{11}H_{12}O_2Na$ requiring 199.0735, and compound **(3.23)** ($+H^{+}$) of 175.0438, $C_{11}H_{11}O_2$ with calculated mass of 175.0754.

In an effort to overcome this problem and further improve the yield of compound **(3.21)**, the reaction was repeated without the presence of methanol (Method B, Scheme 3.21).

The reaction occurred smoothly and the desired compound **(3.21)** was obtained in an excellent yield, 98%. Its structure was confirmed by NMR and HRMS analysis.

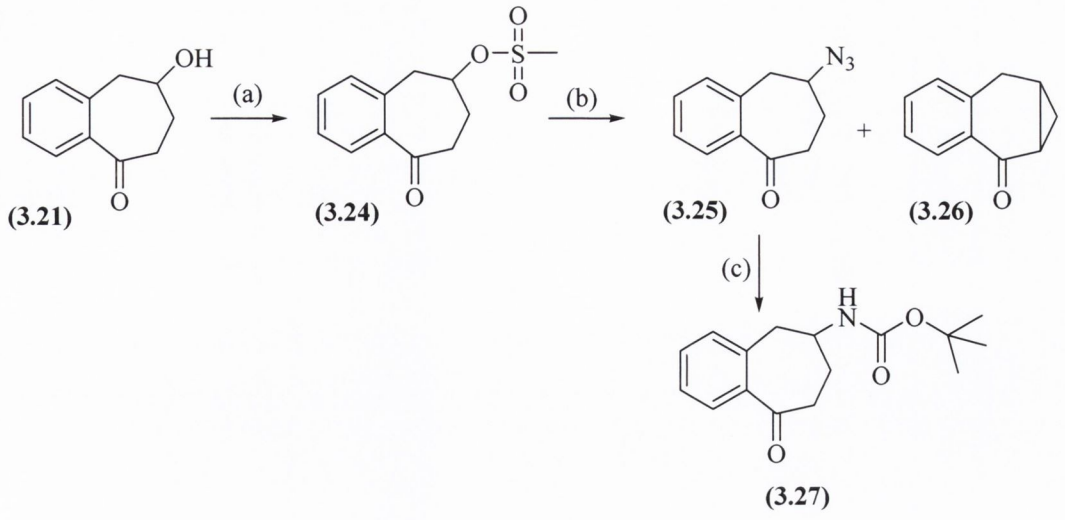


(3.20) **(3.21)** **(3.22)** **(3.23)**

Method A: EtOAc, MeOH (1mol, eq.), 10% Pd/C, H_2, **(3.21),** (75%), **{(3.22) and (3.23)}**, (8.9%)
Method B: EtOAc, 10% Pd/C, H_2, **(3.21),** (98%), **(3.22),** (1%)

Scheme 3.21 Opening of epoxide ring of compound (3.20)

Transformation of the hydroxyl group to a suitable leaving group was important for insertion of the amine group. As with the synthesis of the amine **(3.11)**, it was decided to convert the alcohol to the mesylate **(3.24)** employing identical conditions to that used to generate the mesylate **(3.2)**. After four hours, the starting alcohol **(3.21)** had been consumed as judged by TLC, and the desired mesylate **(3.24)** was isolated in excellent yield (92%). The mesylate was then treated with sodium azide in DMF at 60°C. The reaction was left on for fifteen hours and upon analysis by TLC, two very closely related spots on TLC were noted, which precluded their separation by flash column chromatography. Nevertheless, it was suggested that the desired azide **(3.25)** had formed owing to the sharp azide peak in the infrared spectrum of the mixture. As the presence of the azide functionality in the molecule was confirmed, it was decided to effect an *in situ* reduction of the azide under catalytic hydrogenation conditions (10% Pd/C, H_2) and *BOC* protection of the resulting amine in the presence of di-*tert*-butyl-dicarbonate. Following work-up and purification, the corresponding *BOC*-protected amine **(3.27)** was isolated in good yield (84%), together with the unexpected cyclopropyl derivative, compound **(3.26)** (Scheme 3.22).



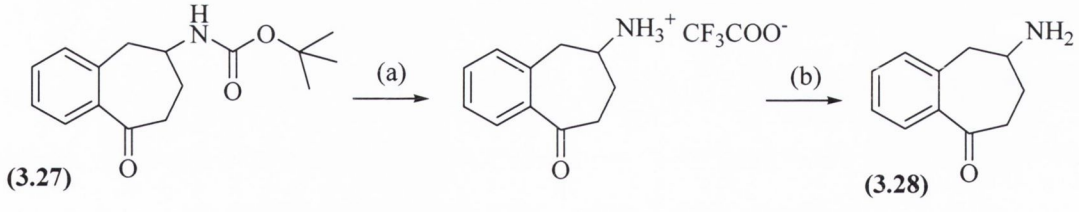
(a) methanesulphonyl chloride, DIEA, DCM, 0°C, 4hrs, (92%)

(b) NaN_3, DMF, 60°C, 15hrs, (71%)

(c) Di-*tert*-butyl dicarbonate, 10% Pd/C, EtOH/EtOAc, (*v:v*, 1:1), RT, H_2, over night, (84%)

Scheme 3.22 Synthesis of BOC-protected amine (3.27))

The main feature of the ^1H NMR spectrum of compound **(3.27)** was the sharp singlet at 1.48ppm integrating as nine protons for the three methyl groups. The corresponding peak in the ^{13}C NMR spectrum was at 28.0ppm and carbonyl moieties were at 154.6ppm (*BOC* group) and 204.8ppm (keto). Compound **(3.26)** was isolated in pure form and confirmed by NMR and HRMS analysis.



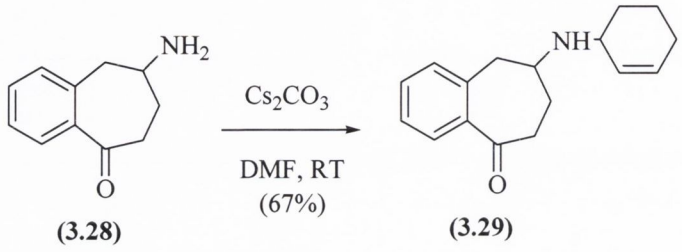
(a) TFA, dry DCM, 0°C, 1hr (b) base work up, (81%)

Scheme 3.23 Formation of primary amine (3.28)

Liberation of the free amine **(3.27)** was accomplished smoothly within one hour using trifluoroacetic acid in the presence of anhydrous DCM. The reaction was conducted at 0°C initially and then allowed to rise to room temperature. Following base work-up, the corresponding primary amine **(3.28)** was isolated in good yield (81%) (Scheme 3.23).

The identity of which was confirmed by the positive reaction of the baseline spot with ninhydrin on TLC plate.

Reaction of the primary amine **(3.28)** with 3-bromocyclohexene in DMF, using caesium carbonate as base, yielded a diastereomeric mixture of the secondary amine **(3.29)** (Scheme 3.24).



(3.28) **(3.29)**

Scheme 3.24 Synthesis of amine (3.29)

This amine was isolated as a yellow oil, which showed a similar pattern to compound **(2.3)** on the TLC. However it was much polar than expected. In the ^{1}H NMR spectrum of compound **(3.29)** (Fig. 3.10), the aliphatic region was well resolved. The amine proton was a broad singlet at 1.23-1.26ppm. There were seven multiplets in the region from 1.38-2.99ppm, integrating as twelve protons in total. The signals for the two methine protons were overlapping as a broad multiplet at 3.32ppm. The two alkenic protons appeared as two separate multiplets in the regions from 5.64-5.67ppm and 5.75-5.78ppm, respectively. The four protons in the aromatic region were also well resolved, each integrating as one proton. The first of these aromatic protons resonated as a doublet (J=7.52Hz) at 7.19ppm, followed by the second, a triplet signal (J=7.54Hz) at 7.31ppm and another triplet (J=7.28Hz) at 7.43ppm. The remaining aromatic proton existed as a doublet (J=7.52Hz) at 7.72ppm. In the DEPT 135 NMR spectrum, a total of six methylene carbons were found in the region from 19.6-38.1ppm, while two methine carbons were located at 49.2-51.2ppm (Fig. 3.13). The correlations observed in the H-H COSY and C-H COSY NMR spectra were used to aid identifications of the carbons at C-9, 11, 12 and 6 positions, which resonated at 2 x 128.1ppm, 128.5 and 128.7ppm, 129.3 and 129.4ppm, 2 x 130.1ppm, respectively (Fig. 3.14-Fig. 3.16). However, as with the amine **(2.3)**, the carbonyl signal was not detected in the ^{13}C NMR spectrum (Fig.

3.11), even though a significant signal for the carbonyl group was evident at 1675.2cm^{-1} in the infrared spectrum.

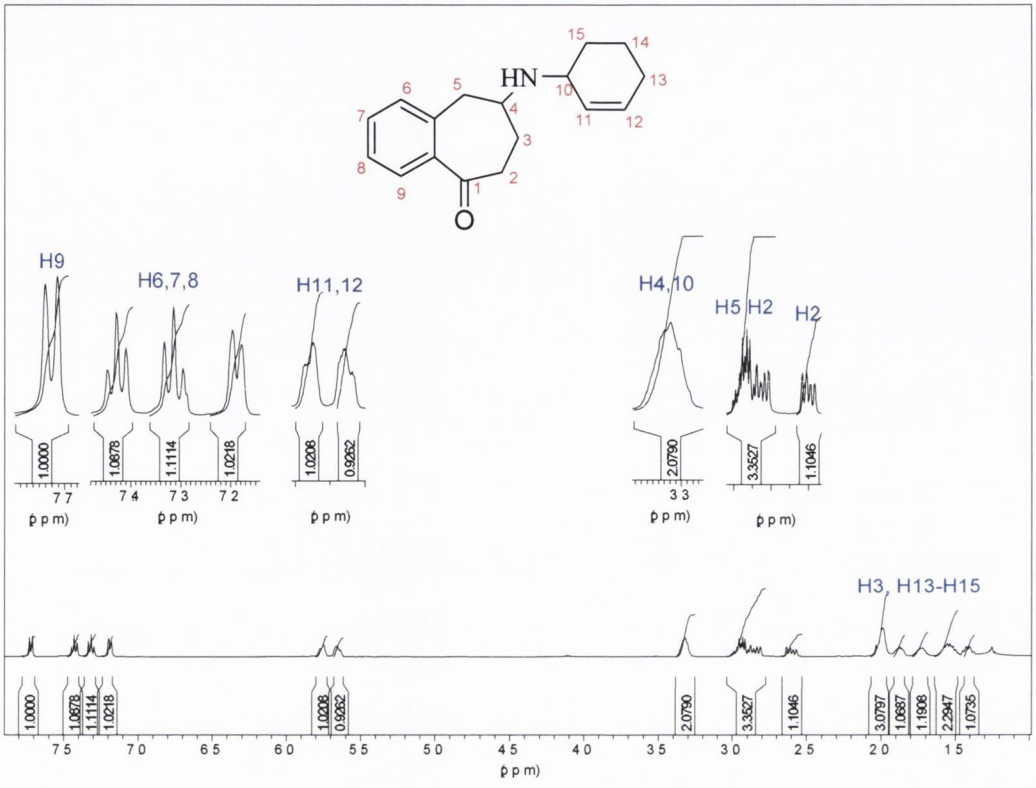


Fig. 3.10 ^{1}H NMR spectrum of Compound (3.29)

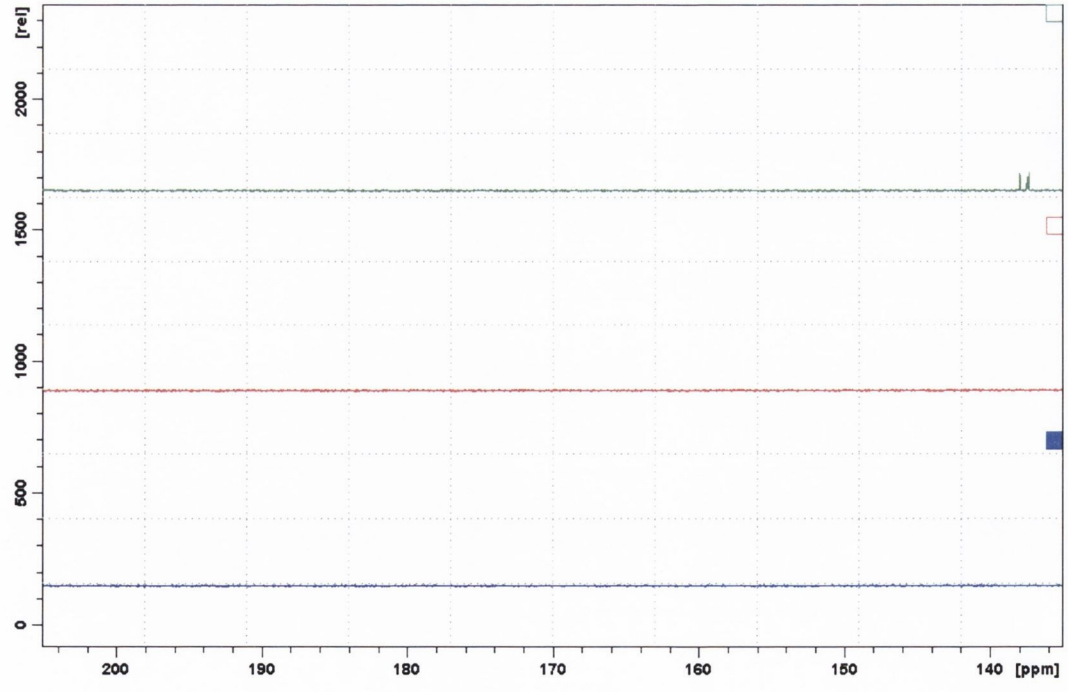


Fig. 3.11 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (3.29) from 135.5-205.2ppm

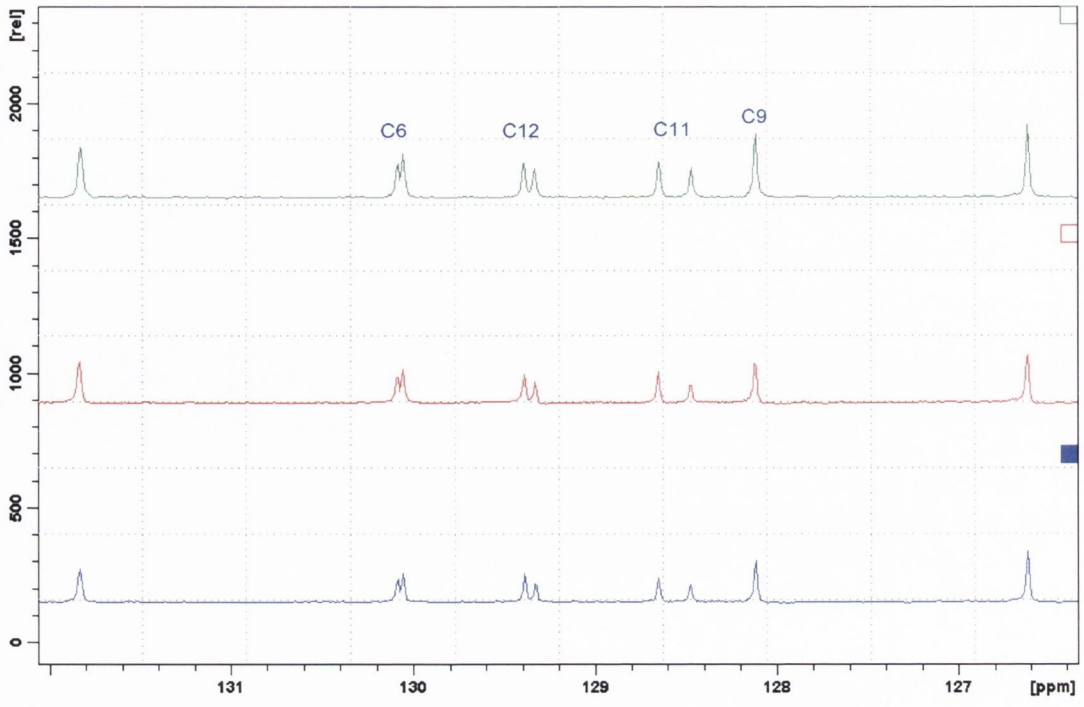


Fig. 3.12 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (3.29) from 126.3-132.2ppm

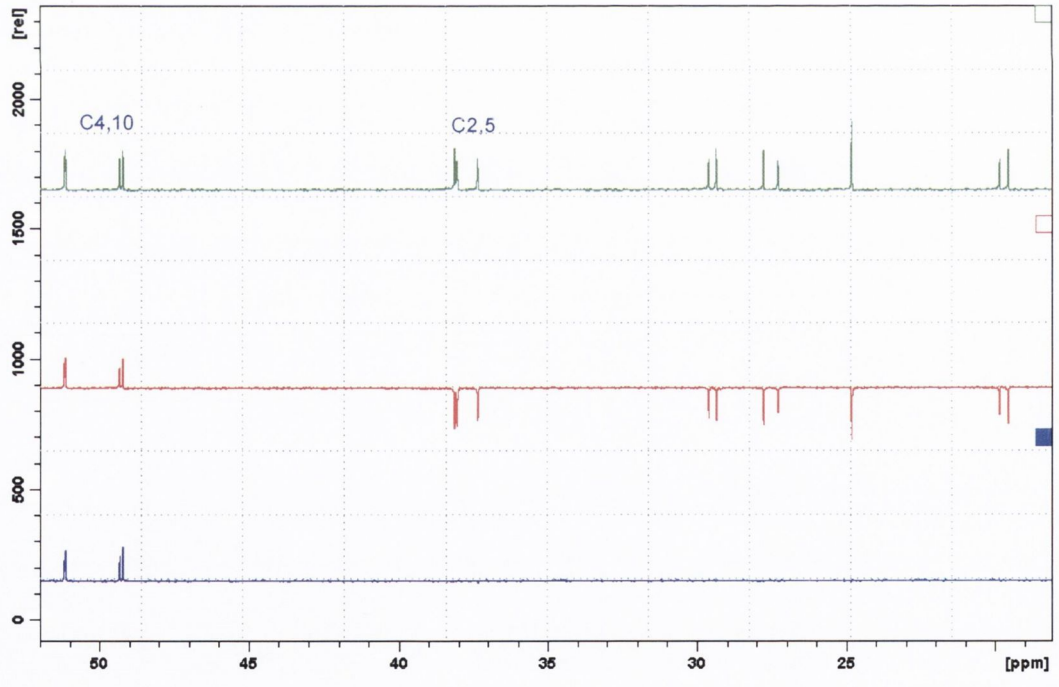


Fig. 3.13 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (3.29) from 18.0-52.0ppm

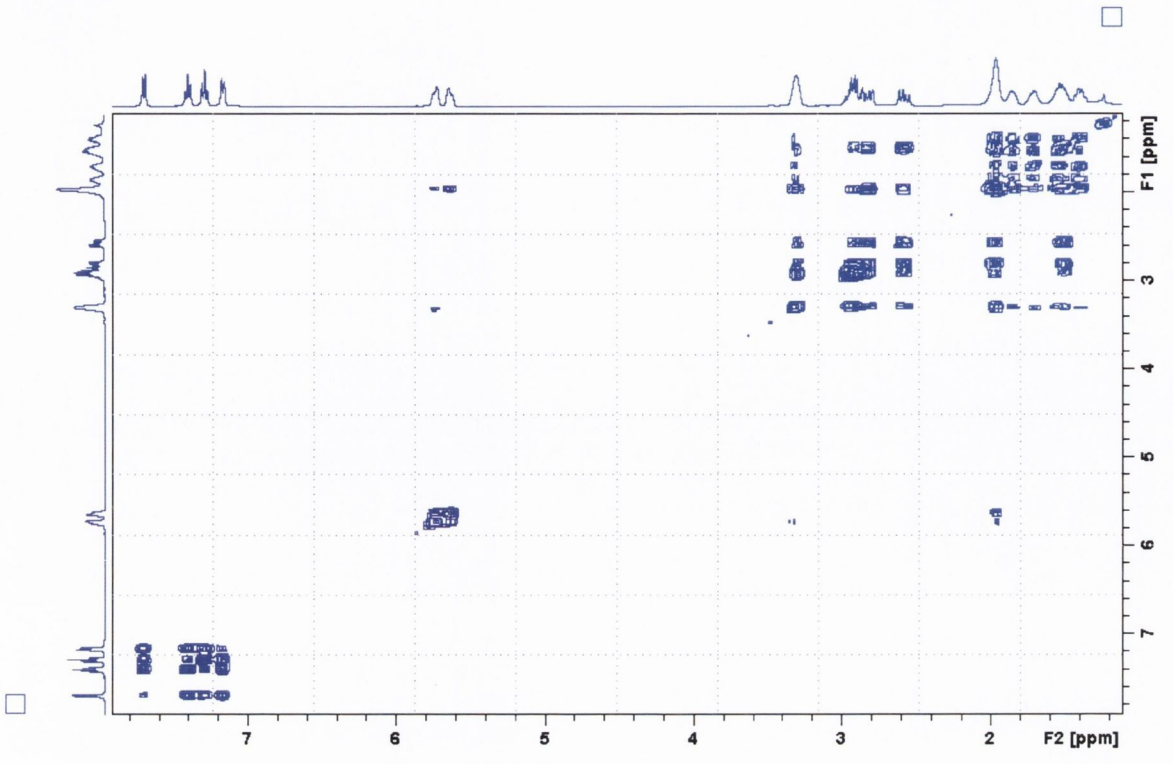


Fig. 3.14 H–H COSY NMR spectrum of compound (3.29)

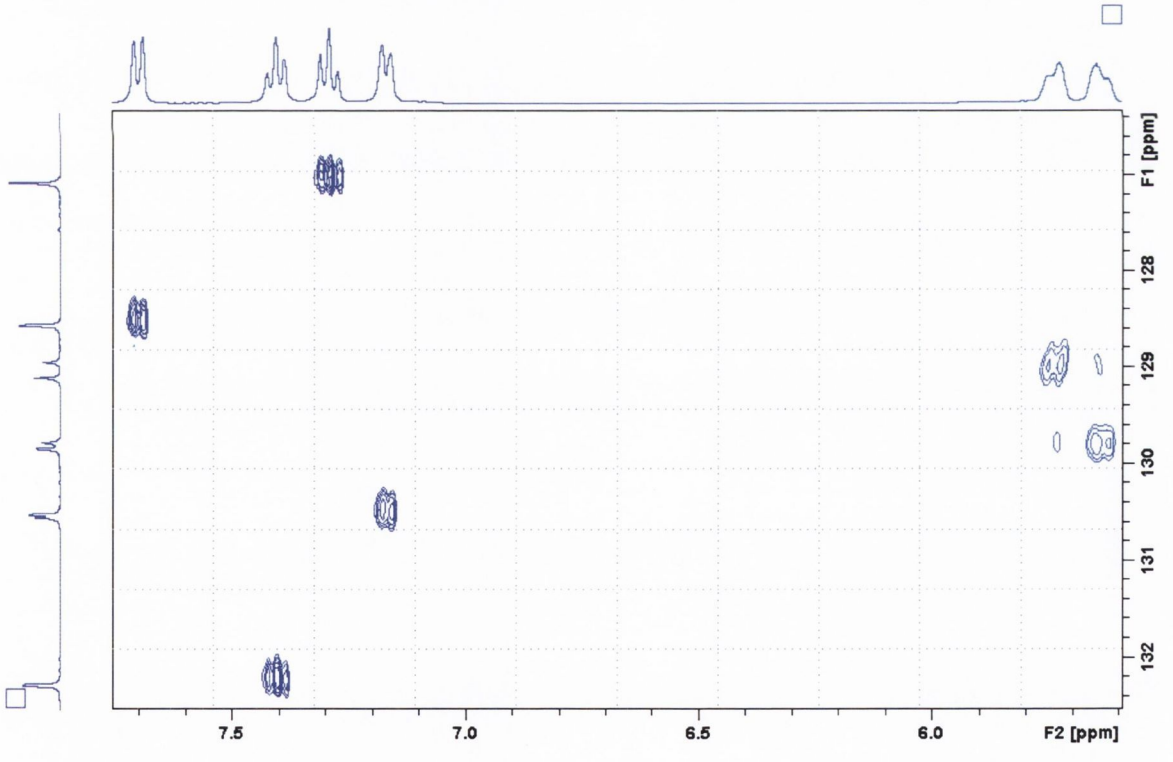


Fig.3.15 C-H COSY NMR spectrum of compound (3.29) from 5.60-7.75ppm

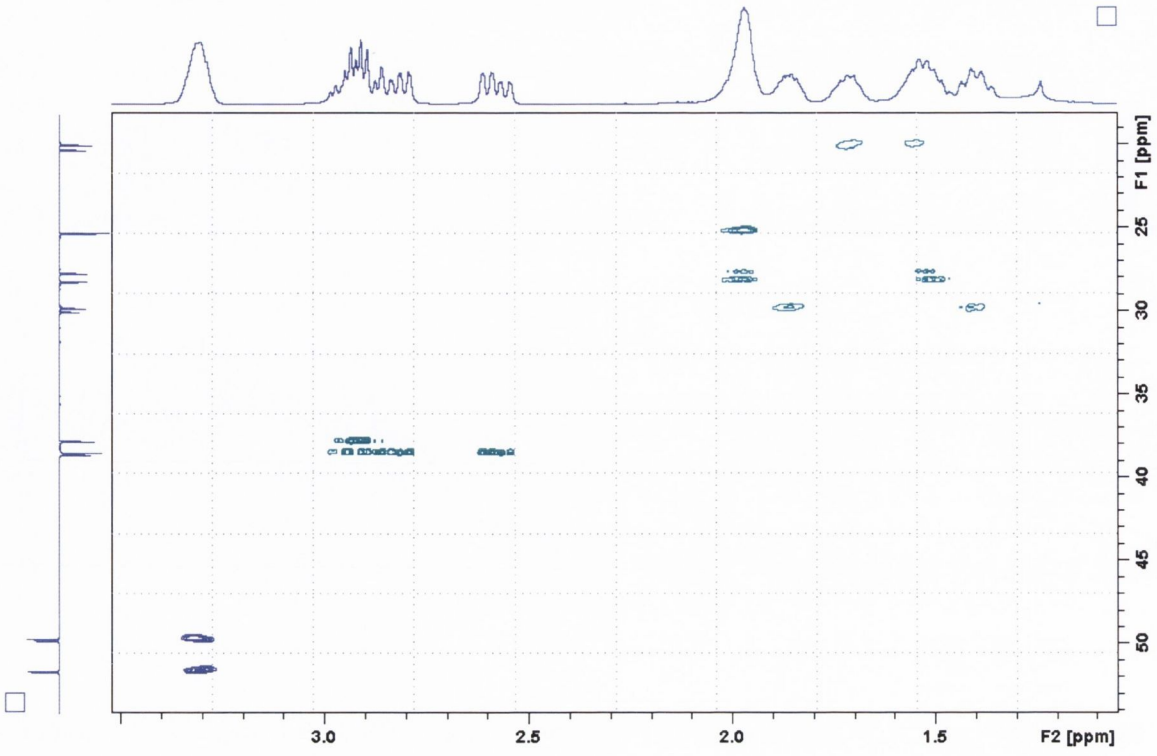
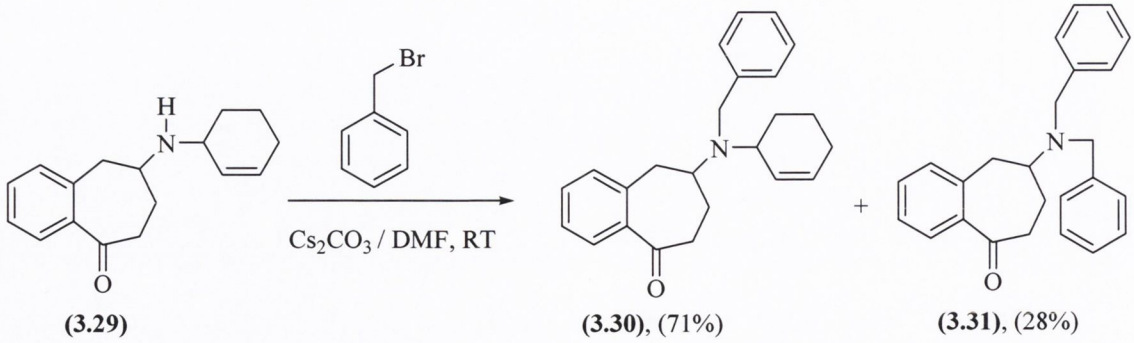


Fig. 3.16 C-H COSY NMR spectrum of compound (3.29) from 0.50-3.52ppm

HRMS also gave the accurate mass ($+H^+$) as 256.1696 with molecular formula $C_{17}H_{22}NO$, requiring 256.1701.

To effect *N*-benzylation, the amine **(3.29)** was treated with benzyl bromide using caesium carbonate as base in the presence of DMF at room temperature, over a period of three weeks. Following work-up and purification, compound **(3.30)** was isolated as the most significant product in good yield (71%). The minor product, compound **(3.31)**, was also isolated from the reaction in 28% yield (Scheme 3.25).



(3.29) **(3.30)**, (71%) **(3.31)**, (28%)

Scheme 3.25 Formation of target compound (3.30)

The main diagnostic signal in the ^{1}H NMR spectrum of compound **(3.30)** was the two additional benzyl methylene protons (C-16), resonating as a multiplet between 3.76-3.86ppm. In the aromatic region, four multiplet signals were evident, integrating for a total of seven protons in the region from 7.20-7.39ppm, followed by an apparent double triplet (J_1=1.05Hz, J_2=14.72Hz) at 7.43ppm integrating for one proton. The final proton signal at C-9 appeared as two discrete doublets (J=7.66Hz) at 7.77ppm (Fig. 3.17). The key features of the ^{13}C NMR spectrum were the signal for the carbonyl group at 204.9ppm, as well as those signals for the three quaternary carbons at 137.9 and 138.1, 140.2 and 140.4, 141.8 and 142.0ppm, respectively (Fig. 3.18). The DEPT 135 NMR spectrum showed the existence of nine tertiary carbons in the aromatic region from 126.6-132.5ppm within the molecule (Fig. 3.19). The DEPT 90 NMR spectrum clearly demonstrated the presence of the additional methylene carbon (C-16) at 50.5 and 50.9ppm (Fig. 3.20). Two alkenic carbons were identified at 130.2 and 130.3ppm, 131.1 and 131.7ppm respectively. Their resonance position was confirmed through inspection of the C-H COSY NMR spectrum (Fig. 3.21).

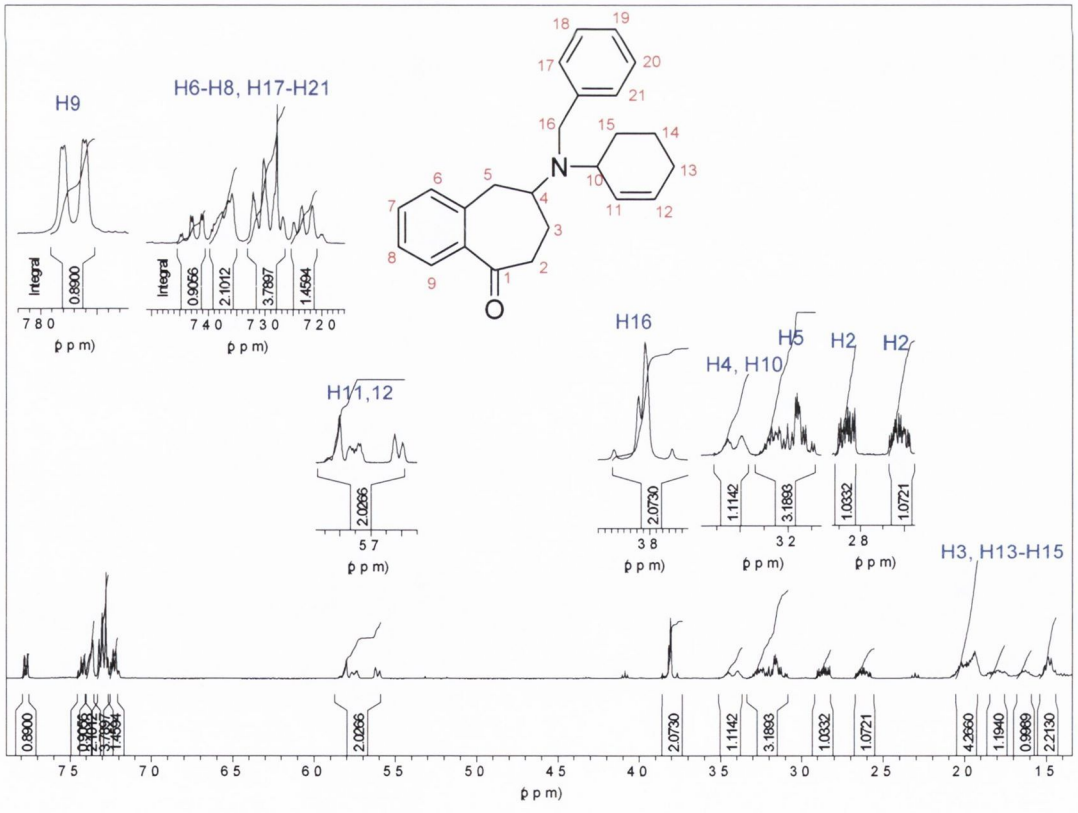


Fig. 3.17 ^{1}H NMR spectrum of compound (3.30)

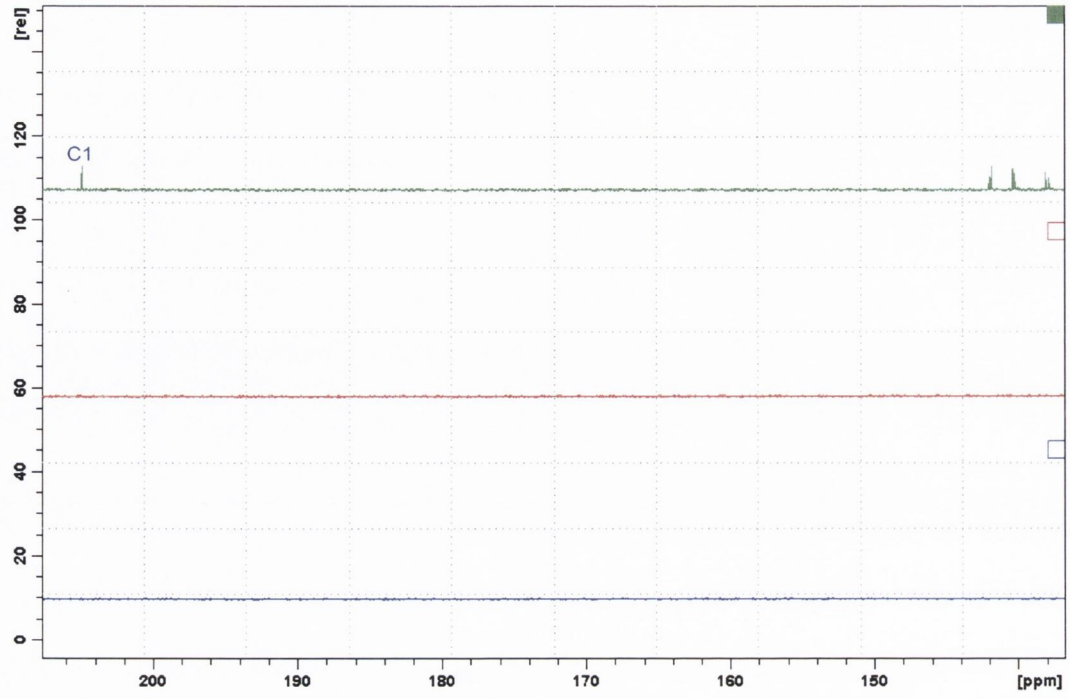


Fig. 3.18 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (3.30) from 137.0-208.0ppm

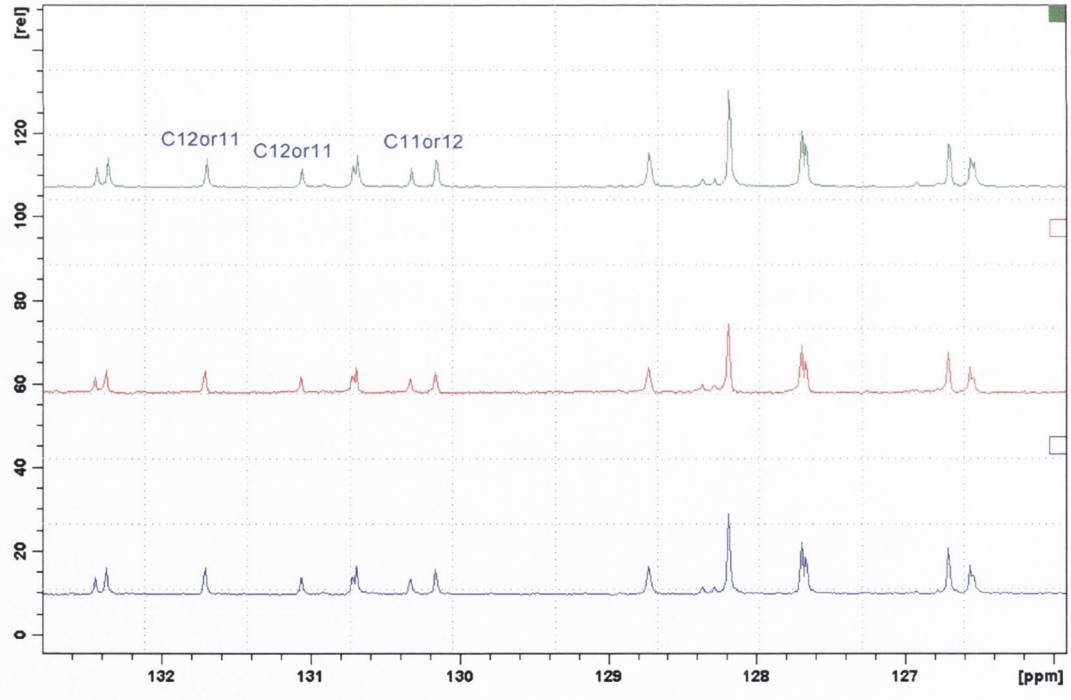


Fig. 3.19 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (3.30) from 125.6-132.8ppm

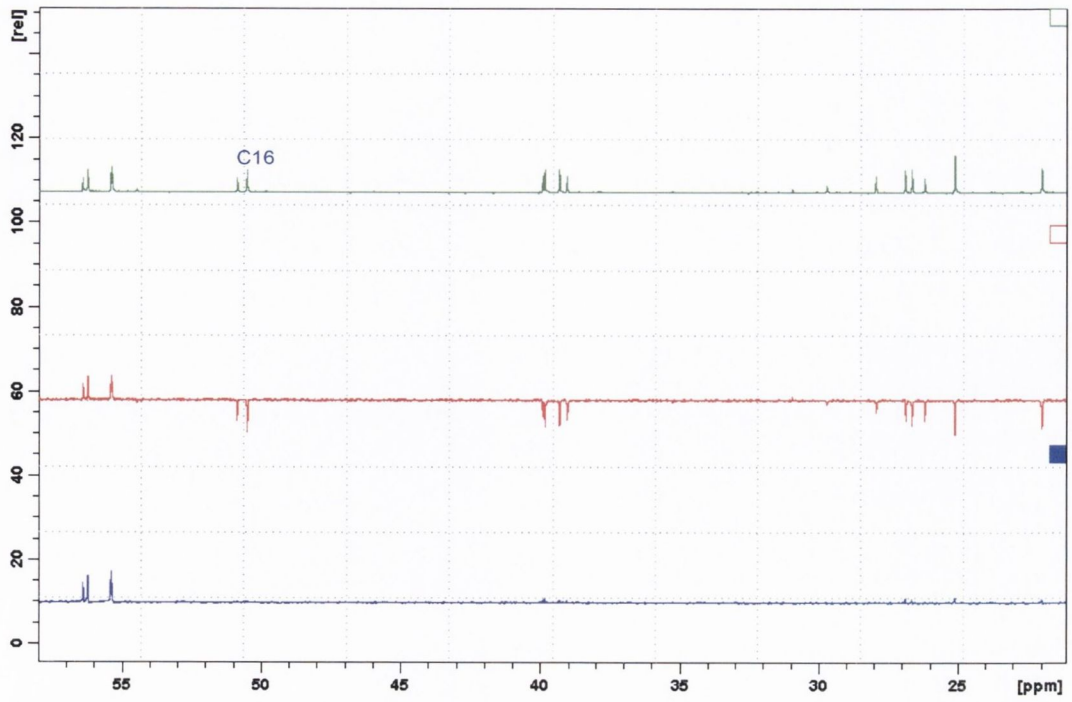


Fig. 3.20 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (3.30) from 21.0-58.0ppm

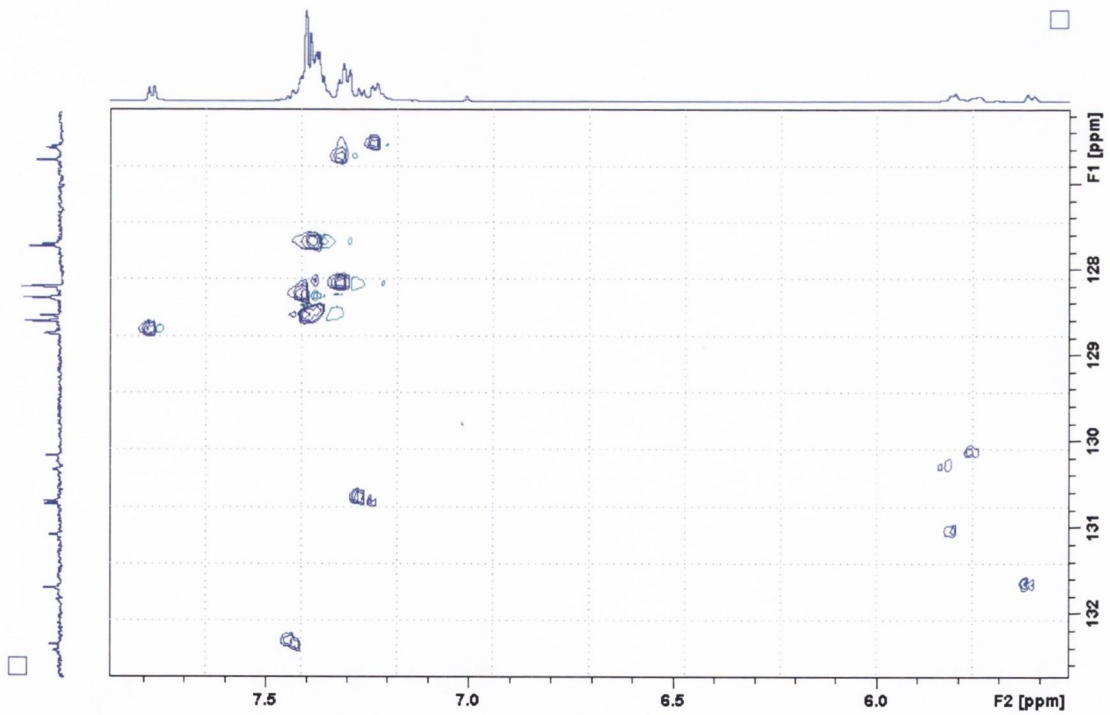


Fig. 3.21 C-H COSY NMR spectrum of compound (3.30) from 5.53-7.88ppm

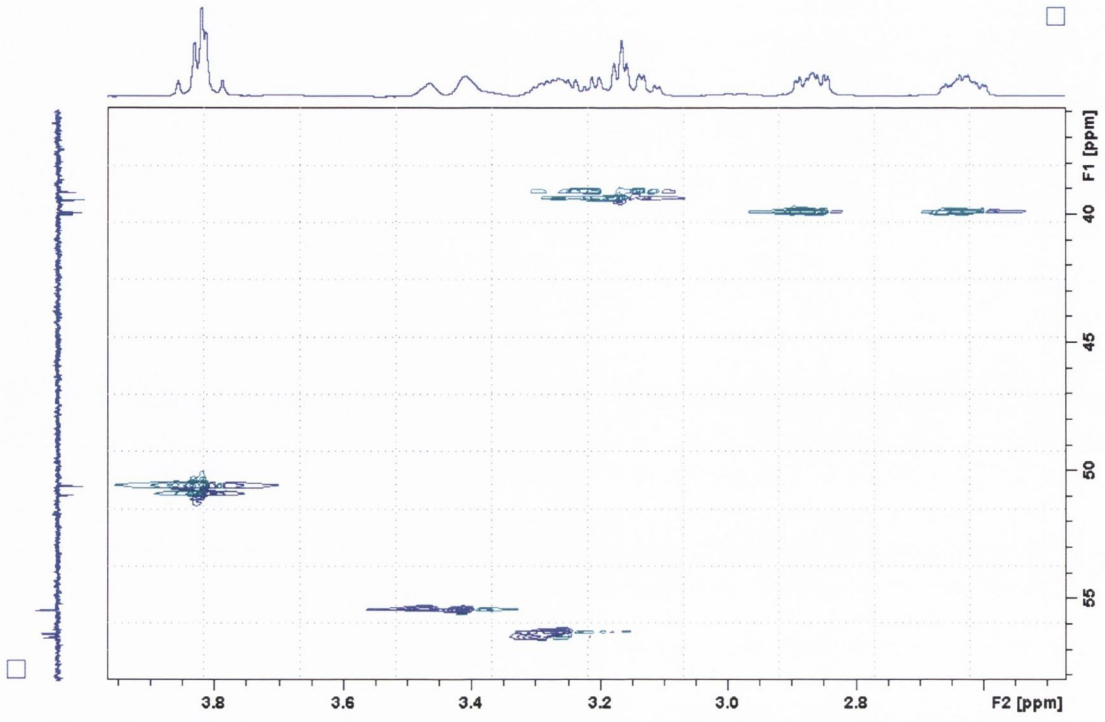


Fig. 3.22 C-H COSY NMR spectrum of compound (3.30) from 2.45-3.96ppm

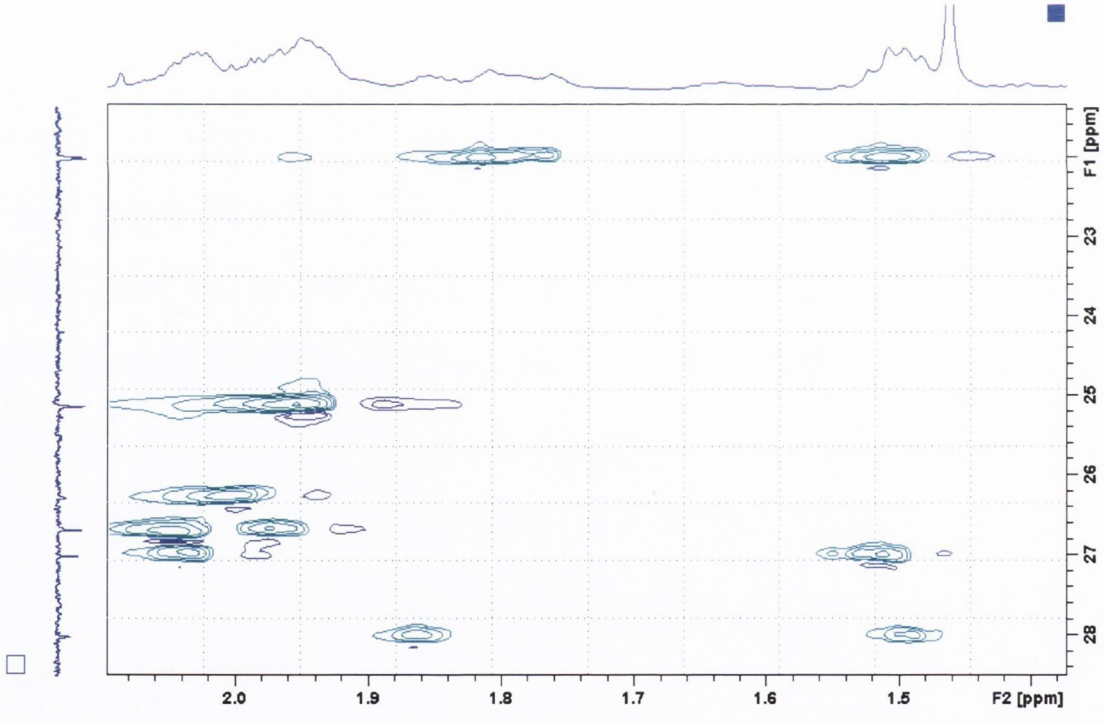


Fig. 3.23 C-H COSY NMR spectrum of compound (3.30) from 1.38-2.10ppm

The key feature of the tertiary amine **(3.30)** in the infrared spectrum was the stretching frequency for the carbonyl group at 1745.6 cm^{-1}. HRMS analysis gave a mass of 346.2161 ($+H^+$), that calculated being 346.2171 with molecular formula $C_{24}H_{27}NO$.

3.6 Conclusion

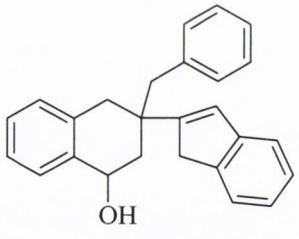
The purpose of the work described in the first section of this Chapter was to explore the synthesis of compounds of the type described in Chapter 2, where the cyclohexenyl amine group was to be replaced by a π-rich dihydronaphthyl amine moiety. This intermediate was synthesised in six steps from β-tetralone. Coupling of the amine to either bromo-benzosuberone or enone **(3.17)** was unsuccessful despite repeated attempts and slight changes to the reaction conditions. It is presumed that the dimethylsubstituents on the dihydronaphthyl amine prevent the amino group from being able to; conjugate to the enone **(3.17)** or displace the bromo moiety from benzosuberone. Having been unsuccessful with this study, the remainder of the work described in this Chapter was concerned with the synthesis of compound **(3.30)**, a regioisomer of the potent mast cell stabiliser **(2.10)**. This compound was synthesised in nine steps from benzosuberone. At a range of different concentrations tested, compound **(3.30)** demonstrated good activity *in vitro* when both compound 48/80 and calcium ionophore were used to induce histamine release from mast cells (Table 3.1) (Woods, 2009). From the data generated, it is apparent that relocation of the amino group to position-4 of benzosuberone does not adversely affect the mast cell stabilisation activity of compounds within this molecular design.

Elicitor	(3.30) Conc.	% inhibition	Elicitor	(3.30) Conc.	% inhibition
Compound 48/80	20μM	67.97±5.297	Calcium Ionophore	20μM	71.45±6.051
	10μM	61.1±14.89		10μM	62.02±5.99
	5μM	41.84±15.06		5μM	22.63±5.936
	1μM	13.14±12.85		1μM	14.01±4.234

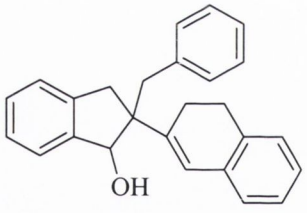
Table 3.1 In vitro mast cell stabilisation data on compound (3.30)

4.1 Introduction

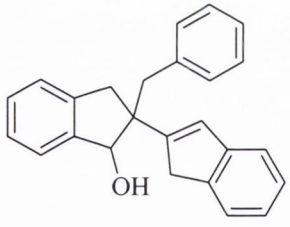
The synthesis and mast cell stabilisation activity of indanol and tetralol-based dimer compounds of the type depicted in Figure 4.0, have been the subjects of several studies (Walsh, 2001; Barlow, 2002). Several compounds within these studies displayed potent activity *in vitro* when a variety of elicitors were used to induce histamine release from rat peritoneal mast cells. Regardless of the elicitor used, from compound 48/80 to calcium ionophore, to concanvalin A, the compounds demonstrated superior activity to disodium cromoglycate, the clinically used mast cell stabiliser. However, the activities of these compounds *in vitro* did not translate into an effect *in vivo* when they were evaluated in the *in vivo* model of passive cutaneous anaphylaxis. The principal issue associated with these compounds is their inherent lack of solubility in physiological solutions, necessitating a high percentage of Tween 80 or DMSO (up to 5% in each case) to effect their complete solubilisation in for example, water for injection at 1mg/ml. The assay itself requires test compounds to be soluble in a 3mg/ml saline solution.



(JWB 5.9)



(JWB 5.29)



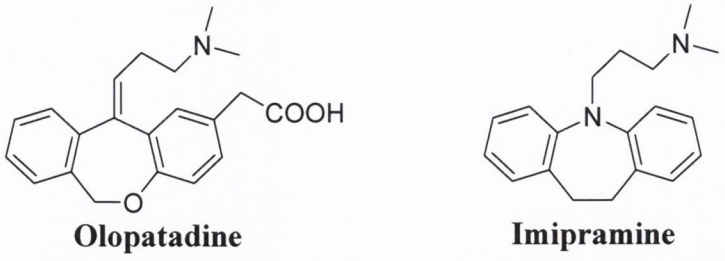
(BRA 100)

Fig. 4.0 Examples of dimeric compounds

4.2 Synthetic targets and strategy

The principle objective of the work described in this Chapter was to identify a series of compounds which retained the basic skeleton of the lead molecules shown in Fig 4.0, but which incorporated into their structures a suitable polar group to enhance their solubility in physiological solutions. In deciding upon a suitable group we were cognisant of the fact that the 3-(dimethylamino)propyl functional group has been incorporated into molecules that possess either or both mast cell stabilisation and histamine H1 receptor

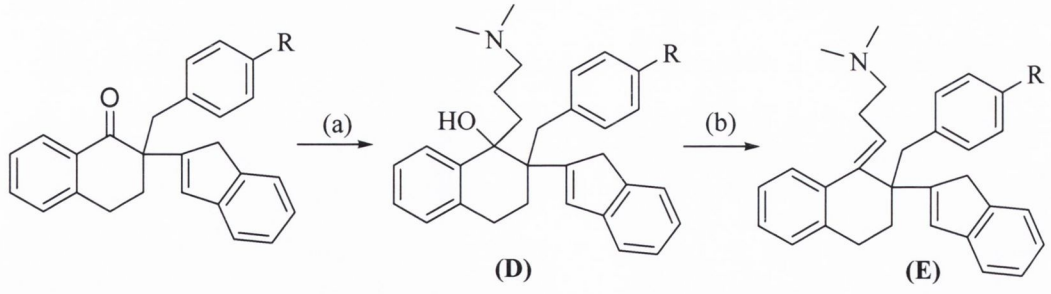
antagonist activities. Indeed there are many drugs on the market that contain this side chain. Examples include Olopatadine and Imipramine (Fig. 4.1).



Olopatadine **Imipramine**

Fig. 4.1 Chemical structures of Olopatadine and Imipramine

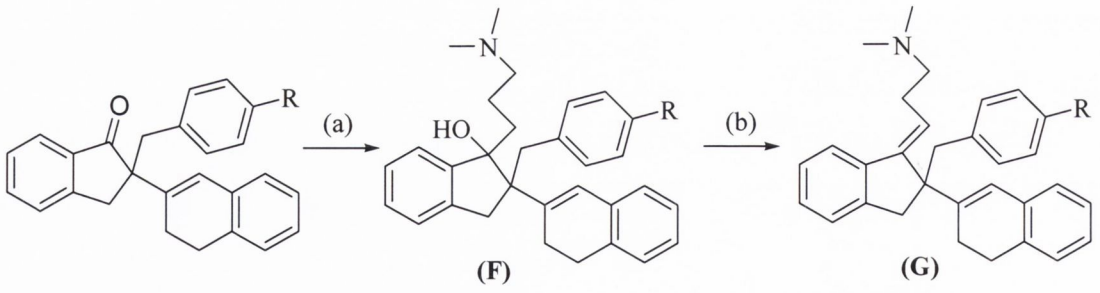
In principle, it was felt that this group could easily be introduced through a Grignard reaction on the precursor carbonyl compounds, resulting in the formation of structural of types **(D)** and **(F)** depicted in Schemes 4.0 and 4.1. Of secondary importance, it was also decided to generate derivatives where the R substituent on the benzyl ring contained the *N,N*-dimethylpropyl substituent, in effect increasing the overall polarity of the molecule in the process, with the objective of generating molecules with greater drug-like properties. A third series of compounds were to be synthesised where the amino alcohols were dehydrated to give the target dimethylpropylidene series of compounds of structural types **(E)** and **(G)** (Schemes 4.0 and 4.1). The argument for the introduction of the alkene group was two fold (i) its presence on the structure of olapatadine and (ii) that the resultant compounds would be more stable than the precursor alcohols.



(D) **(E)**

(a) Grignard reaction (b) Dehydration R: H, Alkyl, *N,N*-dimethylpropyl

Scheme 4.0 Synthetic approach to structural types (D) and (E)



(a) Grignard reaction (b) Dehydration R: H, Alkyl, *N,N*-dimethylpropyl

Scheme 4.1 Synthetic approach to structural types (F) and (G)

4.3 Synthesis of the target compound (4.5)

The basic building blocks used to synthesise the series of target compounds shown in Schemes 4.0 and 4.1, required us to start from either 1, or 2-indanone and α- or β-tetralone. Clearly the most logical way of coupling these building blocks together was through a directed aldol condensation reaction, thus eventually generating ketonic compounds **(4.3)** and **(4.9)** (Fig 4.2).

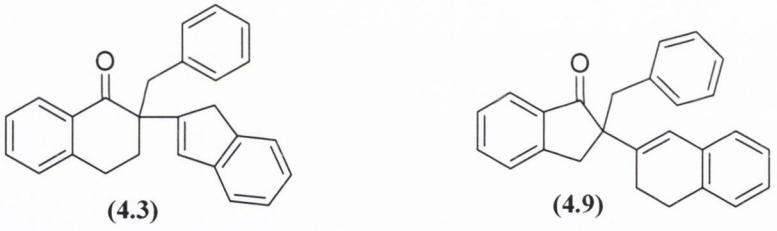


Fig. 4.2 Chemical structures of compounds (4.3) and (4.9)

The crossed-aldol addition reaction has been well documented and utilised in carbon-carbon bond forming reactions for decades (Wittig & Reiff, 1968). The electrophiles used in this type of transformation are generally ketones or aldehydes; while the corresponding nucleophiles used have been generated by formation of enols, enolates, enol ethers of the appropriate ketone or aldehyde (Murata et al., 1980). Historically, the conventional aldol-type reaction, as illustrated in Scheme 4.2, required generation of the carbon based nucleophile through the use of a strong base like lithium diisopropylamide (LDA) at -78°C. Once the enolate was formed, it was then reacted with the appropriate ketone to generate the aldol product (Clayden et al., 2001a). The mechanism of the methodology involves with three stages:

Stage 1

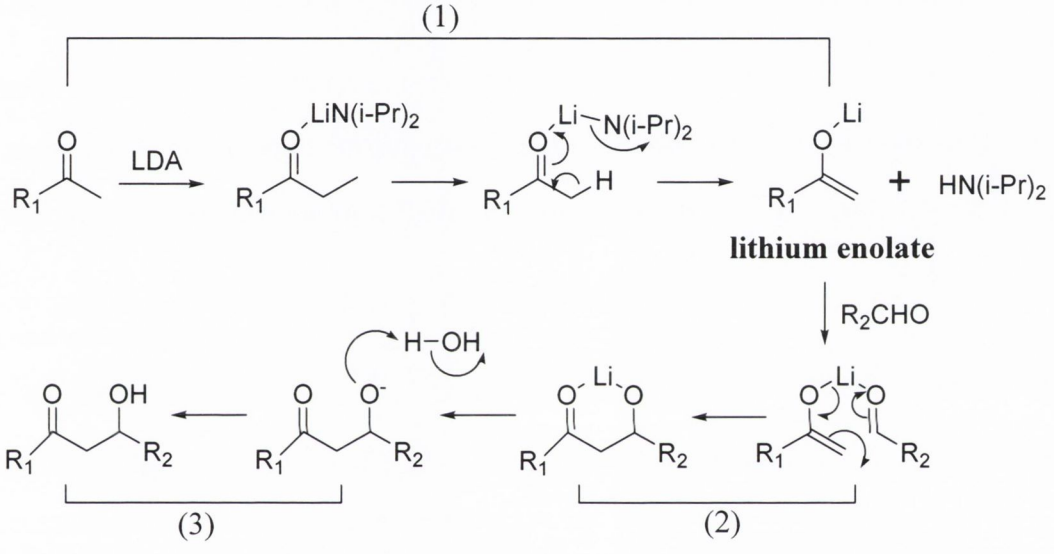
Formation of lithium enolate resulting in Li-O bond formation before removal of the proton from the α-position by the basic nitrogen atom. This transformation can be effected by LDA at low temperature.

Stage 2

Reaction of the lithium enolate with a ketone or aldehyde leads to coupling *via* a cyclic intermediate.

Stage 3

Aldol product is then developed after an aqueous work-up.



Scheme 4.2 Mechanism of aldol reaction by lithium enolates

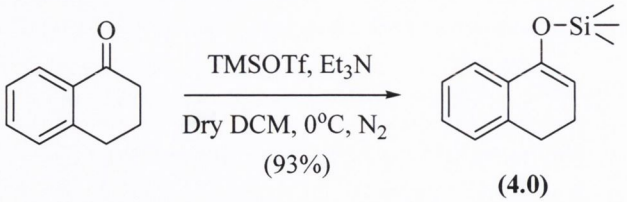
An alternative approach to complete a directed aldol reaction is to employ Lewis acid catalysts to attack the carbonyl compounds (or when in the form of an acetal or ketal) onto Lewis acid-complexed enol ether derivatives (House et al., 1973), such as bromozinc (Spencer et al., 1967), boron (Mukaiyama et al., 1973), aluminum (Maruoka et al., 1977), and tin (Yamamoto et al., 1981) enolates. Whilst many studies have demonstrated that remarkable regio- and stereoselectivity can be attained in the synthesis of aldol type compounds, by the employment of titanium tetrachloride ($TiCl_4$) (Mukaiyama et al., 1974), trimethylsilyl trifluoromethanesulfonate (TMSOTf) (Murata et al., 1980) or tin triflate (Franck-Neumann et al., 1999).

In addition, aldol reactions can also be effected by the reaction between enol ethers and unactivated carbonyl compounds when fluoride ion is employed as catalyst (Noyori et al., 1977; Nakamura et al., 1983a). Recently, Brønsted acid triflimide ($HNTf_2$)-catalysed aldehyde-crossed aldol reaction using tri(trimethylsilyl)silyl (super silyl) enol ethers have proven to be a very effective and efficient method to generate aldol products (Boxer & Yamamoto, 2006).

4.3.1 Synthesis of the benzyl-alkylated dimer (4.3)

4.3.1.1 Synthesis of enol trimethylsilyl ether (4.0)

The preferred route towards the synthesis of the aldol intermediates that are the focus of this Chapter is to use the method described by Boxer & Yamamoto (2006). Here the nucleophilic carbon is generated through formation of the enoltrimethylsilyl ether of the chosen aldehyde or ketone. Enol trimethylsilyl ethers are used in many types of aldol reactions and as protecting group for enolates, as they are stable enough to be isolated (Clayden et al., 2001a). Historically, generation of enoltrimethylsilyl ethers was accomplished by the reaction of carbonyl compounds with excess trimethylsilyl chloride in DCM or DMF using triethylamine as base (House et al., 1969). For the purpose of the project, the enol trimethylsilyl ether **(4.0)** was prepared by the reaction of α-tetralone, with the super-silylating reagent, trimethylsilyl trifluoromethanesulfonate (TMSOTf), containing the highly electron-withdrawing triflate moiety which can activate many oxygen-containing compounds (Murata et al., 1988). The reaction was performed in dry DCM at 0°C using triethylamine as base. Following rapid purification by flash column chromatography, the enol **(4.0)** was isolated as a clear oil and stored under an nitrogen atmosphere at 0°C until required for use (Scheme 4.3).



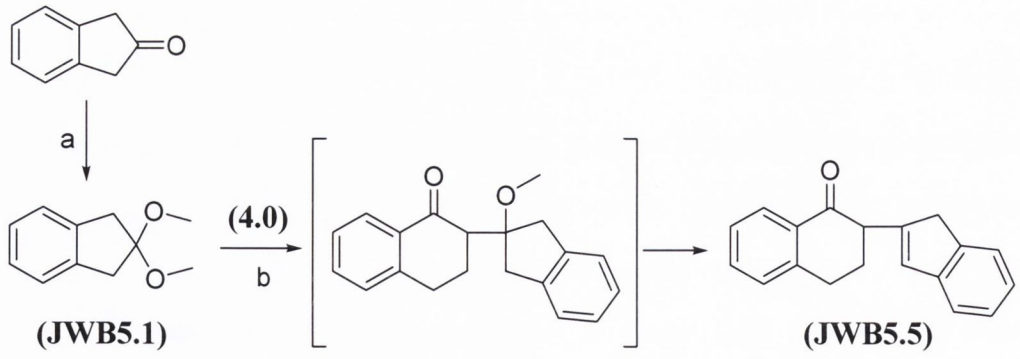
(4.0)

Scheme 4.3 Synthesis of molecule (4.0)

Analysis of the ^{1}H NMR spectrum of compound **(4.0)** indicated the presence of trimethylsilyl ether. A singlet at 0.51ppm, integrating for nine protons was considered to be the three methyl groups. The allylic and benzylic methylene protons resonated at 2.35-2.40ppm and 2.82ppm, appearing as a multiplet and a triplet (J=7.96Hz), respectively. A triplet (J=4.60Hz) at 5.25ppm was attributed to the alkene proton. The aromatic region was well resolved, a multiplet was observed in the region of 7.15-7.29ppm, integrating for three protons. The doublet (J=7.40Hz) of the aromatic proton nearest the carbonyl resonated at 7.48ppm. The existence of the trimethylsilyl ether group was evident in the ^{13}C NMR spectrum. The three methyl groups were evident at -0.18ppm. The DEPT 90 NMR spectrum indicated the presence of the two alkene carbons at 104.9ppm and 147.7ppm.

4.3.1.2 Synthesis of cyclic acetal (4.1)

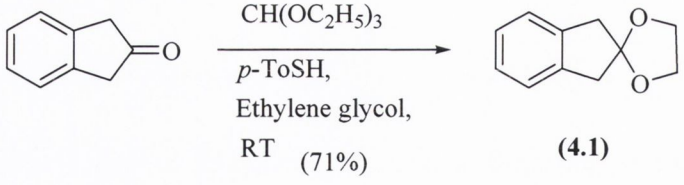
Having obtained the enoltrimethylsilyl derivative of α-tetralone, the next step prior to coupling was to activate the carbonyl group of 2-indanone towards nucleophilic attack. While, it has well been accepted that the acetal functionality is one of the most common protecting group for carbonyl compounds such as aldehydes and ketones (Tsunoda et al., 1980), it can also serve as a very suitable means to activating carbonyl groups towards nuclephilic attack. Dimethylacetals have been used successfully to perform aldol reactions using a variety of enol trimethylsilyl ethers (Murata et al., 1980). Acetalisation of the parent carbonyl compound can be accomplished either in alcoholic media containing a brønsted acid catalyst, or by employing organosilicon compounds (i.e. alkoxytrimethylsilanes) and TMSOTf functioning as acetalising reagent as well as catalyst (Tsunoda et al., 1980). However, the preparation of the dimethyl acetal of 2-indanone was proved difficult when *p*-toluenesulphonic acid (*p*-ToSH) was employed as catalyst and trimethylorthoformate as dehydrating agent in methanol. In addition, when the dimethyl acetal **(JWB5.1)** was coupled to compound **(4.0)**, the resulting product **(JWB5.5)** had the same R_f on TLC as α-tetralone, regenerated from compound **(4.0)** under the coupling conditions employed.



a. $CH(OCH_3)_3$, *p*-ToSH, MeOH, RT; b. cat. TMSOTf, dry DCM, -78°C, N_2

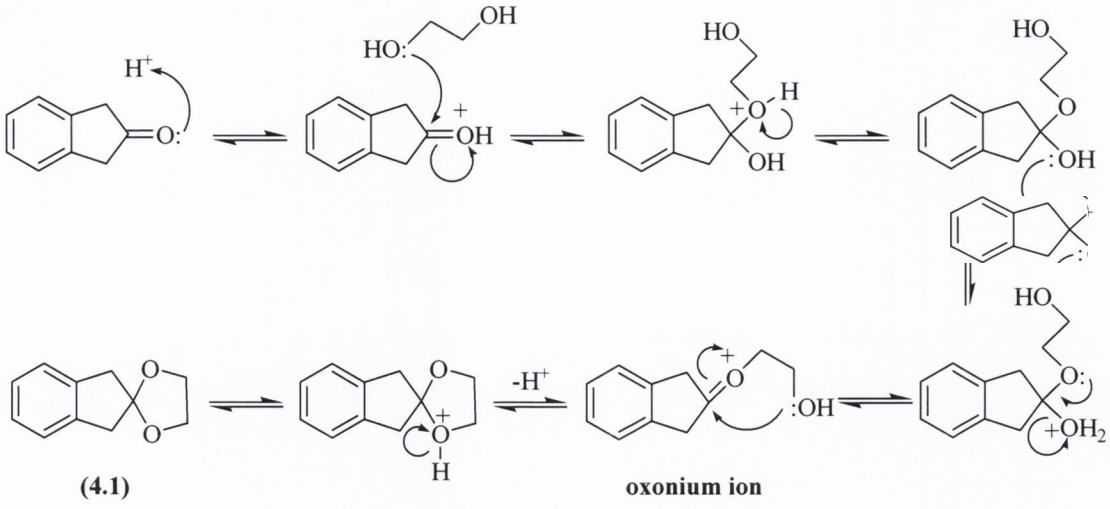
Scheme 4.4 Attempted coupling of the dimethylacetal and enol trimethylsilyl ether

The solution and indeed in agreement with that of Barlow (2002), was to synthesise the cyclic acetal **(4.1).** When coupled to the enol trimethylsilyl ether **(4.0)**, it was expected that the aldol product **(4.2)** with the extended primary alcohol functionality should easily separate from any of the less polar side products including α-tetralone and 2-indanone. Indeed cyclic acetals/dioxolanes are also more stable towards hydrolysis than acyclic acetals (Clayden et al., 2001b).The formation of the cyclic acetal **(4.1)** was easily accomplished through the reaction of 2-indanone with ethylene glycol. *p*-ToSH served as catalyst with triethyl orthoformate being employed as dehydrating reagent. The reaction was conducted at room temperature (Scheme 4.5).



Scheme 4.5 Synthesis of cyclic acetal (4.1)

The mechanism of the acid-catalysed formation of the cyclic aetal/dioxolane **(4.1)** in outlined in Scheme 4.6.



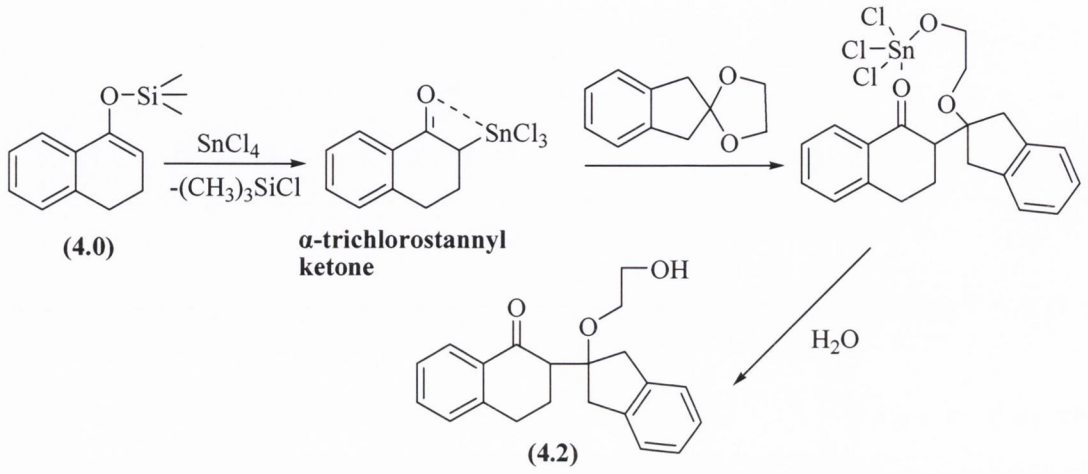
Scheme 4.6 Mechanism of formation of cyclic acetal (4.1)

To our surprise monitoring of the progress of the reaction was slightly problematic as both 2-indanone and the dioxolane **(4.1)** had identical R_f values on TLC regardless of the solvent system used. The solution to the problem was simply to leave the reaction on overnight which inevitably resulted in complete consumption of the starting material. Following work-up and purification, compound **(4.1)** was normally obtained in good yield (~70%). Its ^{1}H NMR spectrum was relatively straightforward as one might expect from a symmetrical molecule. The four benzylic methylene protons resonated as a singlet at 3.32ppm while those of the dioxolane skeleton also resonated as a singlet at 4.05ppm. As expected, the aromatic region integrated for a total of four protons in the region of 7.29-7.33ppm. In the ^{13}C NMR spectrum, the additional carbon signal for the two dioxolane methylene carbons was observed at 64.1ppm, while the quaternary signal for the dioxolane carbon resonated at 117.1ppm.

4.3.1.3 Crossed Aldol addition

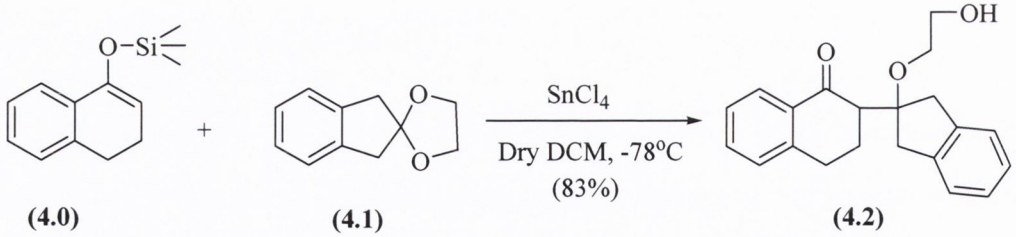
Having obtained the silylenolether **(4.0)** and the cyclic acetal **(4.1)** in good yields, the attention was now focused on the method to be utilized for preparation of the coupled product **(4.2)**. Following examined literature methods, it was decided to employ a Lewis acid catalyst for the coupling step. The Mukaiyama method (Mukaiyama, 1977) which employs tin tetrachloride ($SnCl_4$) as Lewis acid catalyst was the preferred approach to

use. The reaction is thought to proceed through formation of the intermediate α–trichlorostannyl ketone initially following reaction of the silylenol ether (**4.0**) with $SnCl_4$. The resultant stannyl ketone then reacts with the acetal (**4.1**), which following hydrolysis yields the coupled product (**4.2**) (Nakamura & Isao, 1983b,c) (Scheme 4.7).



Scheme 4.7 Mechanism of crossed Aldol addition

The optimum conditions to employ for the Mukaiyama reaction (Mukaiyama, 1977), was to add one molar equivalent of $SnCl_4$ in DCM to a solution of the enol ether (**4.0**) and cyclic acetal (**4.1**) at -78°C under anhydrous conditions. After an hour, the reaction was quenched through the addition of water. Following work-up and purification the desired aldol product (**4.2**) was obtained in good yield (83%) (Scheme 4.8).



Scheme 4.8 Synthesis of compound (4.2)

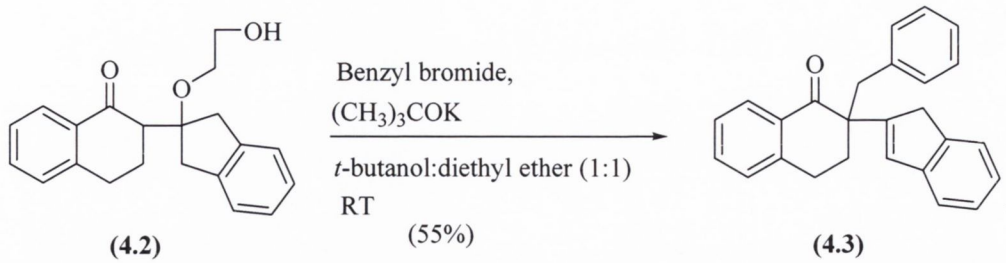
The identity of compound (**4.2**) was established through the use of a combination of spectroscopic techniques including NMR, IR and HRMS. The presence of the alcohol functionality was evident as a singlet resonating at 2.03ppm in the ^1H NMR spectrum of compound (**4.2**). Two multiplets resonating at 2.16-2.24ppm and 2.39-2.44ppm were identified as those belonging to the two methylene protons on the tetralone ring. The remaining aliphatic region of the spectrum was complex, with eleven methylene and one

methine protons resonating closely together between 2.82-2.84ppm. The aromatic region showed a similar level of complexity to that observed in the aliphatic region of the spectrum. A series of overlapping signals accounted for the six protons in the region of 7.16-7.29ppm in the spectrum. The triplet (J=7.28Hz) at 7.44ppm represented an individual proton, while the doublet (J=7.56Hz) at 8.02ppm was believed to be the proton β to the carbonyl group. Important features of the ^{13}C NMR spectrum were the methine carbon and the two ethereal methylene carbons at 54.8, 61.5 and 63.7ppm, respectively. The carbonyl signal resonated at 198.1ppm.

Many significant infrared absorptions of compound **(4.2)** were observed in the infrared spectrum, including those of $C\text{-}O_{str}$ (ether), $C\text{-}O_{str}$ and $O\text{-}H_{def}$ (alcohol) at 1093.1, 1188.6 and 1251.5 cm^{-1} respectively, while the peak at 1686.3cm^{-1} confirmed the presence of the carbonyl group. The very broad peak at 3500.2cm^{-1} repersented the hydroxyl group.

4.3.1.4 Synthesis of the intermediate ketone (4.3)

Having completed the synthesis of the hydroxy ketone **(4.2)**, its conversion into the benzylated derivative **(4.3)** was effected through the base catalysed deprotonation of compound **(4.2)** with potassium *tert*-butoxide and simultaneous alkylation with benzyl bromide. The benzylation reaction occurred smoothly at room temperature in a solution of *tert*-butanol and diethyl ether (*v*:*v*, 1:1) and yielded the desired product **(4.3)** in moderate yield (55%) (Scheme 4.9).



(4.2) **(4.3)**

Scheme 4.9 Formation of key intermediate ketone (4.3)

The most notable feature of the NMR spectrum generated on the benzylated compound **(4.3)** was its reduced complexity in comparison with the hydroxyl ketone **(4.2)**. Removal of one of the stereogenic centres leads to compound **(4.3)** being presented as a mixture

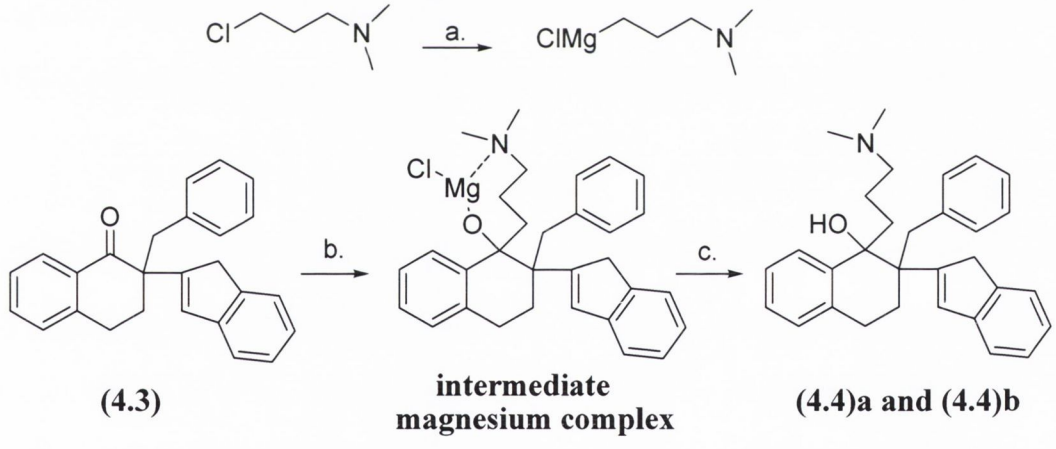
of enantiomers. As a result, insertion of the unsubstituted benzyl substituent as in **(4.3)** gave a very clean ^{1}H NMR spectrum, especially in the aliphatic region.

The four methylene protons on the tetralone ring resonated as distinct multiplets between 2.30-2.38ppm and 2.48-2.52ppm for the α–keto methylene protons while the benzylic protons resonated between 2.89-2.94ppm and 3.13-3.24ppm. Two multiplets for the remaining four methylene protons resonated in the region between 3.33-3.45ppm and 3.67-3.78ppm respectively, each integrated as two protons. The alkenic proton resonated at 6.70ppm as a singlet. As might be expected, the aromatic region was quite complex following insertion of the benzyl group. A double triplet (J_1=1.52Hz, J_2=7.04Hz) considered as overlapping signals for three protons at 7.31ppm, was followed by a multiplet from 7.50-7.57ppm. A doublet was present at 8.40ppm, which was attributed to the aromatic proton at the β–keto position on the tetralone ring. In the DEPT 135 NMR spectrum, the four methylene carbons were found to resonate in the region between 25.6-44.2ppm. As expected a total of thirteen aromatic tertiary carbons and one alkenic tertiary carbon were present in the region of 120.5-130.7ppm in the DEPT 90 NMR spectrum. The seven quaternary carbons were seen in the ^{13}C NMR spectrum which also indicated the presence of the carbonyl group at 198.1ppm. Insertion of the benzyl group had very little effect on the $C=O_{str}$ in the infrared spectrum in comparison with the keto alcohol **(4.2)**, it being present at 1678.0cm^{-1}. HRMS analysis revealed the molecular mass as 373.1573 ($+Na^{+}$), calculated 373.1568.

4.3.2 Synthesis of the benzyl-alkylated dimeric alcohol (4.4)

Following successful synthesis of the ketone **(4.3)**, insertion of the 3-(dimethylamino)propyl (DAP) side chain onto this compound was to be achieved via an organometallic reaction to furnish the amino alcohol **(4.4)**. The preferred method was to convert DAP chloride (DAPCl) into its corresponding Grignard reagent and then to use this reagent for the attachment of the side chain onto **(4.3)**. In order to furnish the Grignard reagent, DAPCl hydrochloride was first converted into its free base by simply adding DAPCl hydrochloride salt to crushed potassium hydroxide pellets. The liberated

free amine was then distilled using a micro distillation apparatus. Having furnished the free base as a clear liquid, the Ohshima's method (Ohshima et al., 1992) was adopted for the coupling reaction in order to generate compound **(4.4)**. Formation of the Grignard reagent was accomplished by injecting anhydrous DAPCl into a three-neck flask containing magnesium and 1,2-dibromoethane in dry THF under an atmosphere of nitrogen. In order to activate the magnesium and initiate reaction, the reaction mixture was heated gently (~50°C). After an initial slow period, the mixture became cloudy and exothermic as the Grignard reagent began to form (Miodownik et al., 1981). Having obtained the Grignard reagent, a solution of the ketone **(4.2)** in dry THF was injected into the Grignard reagent. The progress of the reaction was closely monitored by TLC analysis. After fifteen hours, the starting ketone was totally consumed and the reaction was quenched through the addition of sat. aq. ammonium chloride (NH_4Cl_4) solution. The crude product was extracted with diethyl ether and purified by a combination of flash column chromatography and preparative TLC. The amino-alcohol was isolated as two single isomers **(4.4)a** and **(4.4)b**, both as colourless oils.



(4.3) **intermediate magnesium complex** **(4.4)a and (4.4)b**

a. Mg, dry THF, 1,2-dibromoethane, Δ b. Grignard reagent, dry THF, RT
c. NH_4Cl/H_2O, (93%)

Scheme 4.10 Synthesis of the amino-alcohol (4.4)a and (4.4)b

In the ^1H NMR spectrum of compound **(4.4)a** (Fig. 4.3), the aliphatic region was well resolved. Two multiplets for the two methylene protons at C-10 position resonated at 1.20-1.28ppm and 1.56-1.65ppm, respectively, followed by another multiplet for one of the methylene protons on C-9 in the region of 1.66-1.72ppm. A double doublet

(J_1=8.02Hz, J_2=14.04Hz) was observed at 1.87ppm, which was considered to be one of the protons on C-3. The second methylene proton at C-9 position was located at 2.00ppm, appearing as a double doublet (J_1=8.54Hz, J_2=12.54Hz). A multiplet in the region from 2.10-2.30ppm, as signals overlapping, represented the methylene protons at C-11 position and the second methylene proton on C-3. The sharp singlet peak for the two methyl protons was found at 2.39ppm. Both benzyl protons on C-14 were clearly identifiable, as a doublet (J=14.56Hz) at 2.65 and 3.50ppm. Both methylene protons on the tetralone ring at position-4 appeared as double doublets at 2.97ppm (J_1=8.52Hz, J_2=18.04Hz), and at 3.08ppm (J_1=9.04Hz, J_2=18.56Hz), respectively. The remaining two methylene protons on C-20 were identified as a broad triplet (J~12.56Hz) at 3.85pp, via the w-coupling correlation with alkenic proton at 6.74ppm, observed in H-H COSY NMR spectrum (Fig. 4.8). In the aromatic region of the ^{1}H NMR spectrum, two multiplets were seen at 6.85-6.86ppm and 7.09-7.11ppm integrating for two and three protons, respectively. An additional five aromatic protons appeared as overlapping signals in the region of 7.20-7.34ppm, followed by two doublets at 7.38ppm (J=7.00Hz) and 7.52ppm (J=7.04Hz), respectively integrated for a total of two protons. As expected the remaining proton at position-8 was a doublet (J=7.52Hz) at 7.76ppm. The DEPT 90 NMR spectrum clearly demonstrated the absence of six quaternary carbon signals between 134.1-154.6ppm (Fig. 4.4), and a total of thirteen carbons were found in the region of 119.9-129.5ppm, while the alkenic methine carbon (C-22) was at 129.7ppm. In the DEPT 135 NMR spectrum (Fig. 4.5), six methylene carbons resonated at 20.3, 24.5, 25.2, 38.9, 39.6 and 41.3ppm, which were identified for C-10, C-4, C-3, C-14, C-9 and C-20 positions, respectively. The ^{13}C NMR spectrum confirmed the existence of the two methyl carbons (C-12 and C-13) at 45.1ppm, as well as the quaternary carbon (C-2) at 48.6ppm. The remaining methylene carbon (C-11) resonated at 60.5ppm, followed by the signal at 76.1 for the carbon at C-1 position.

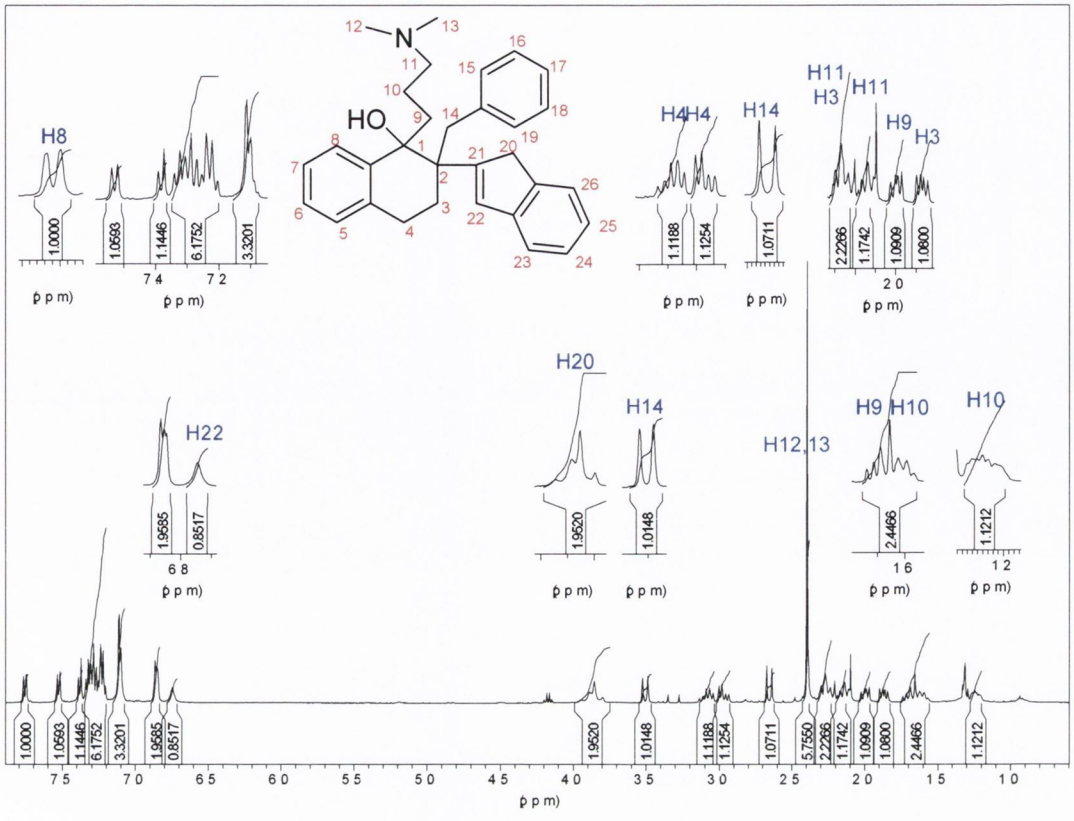


Fig. 4.3 ^{1}*H NMR spectrum of compound (4.4)a*

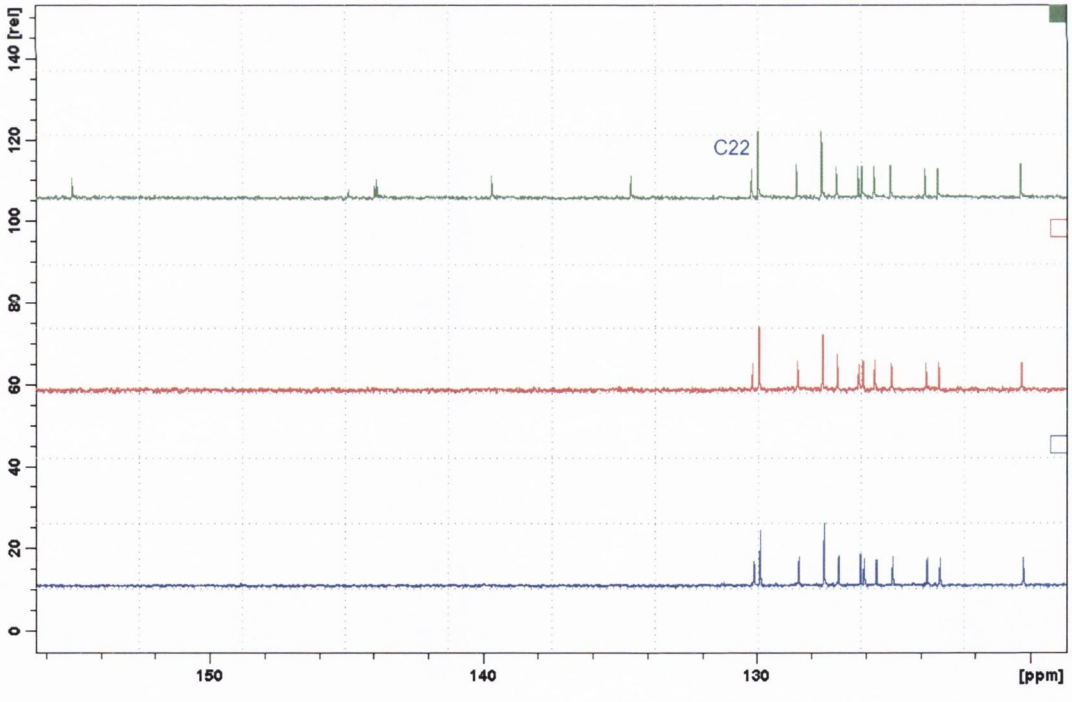


Fig. 4.4 ^{13}*C, DEPT 135 and DEPT 90 NMR spectra of compound (4.4)a from 119.5-156.2ppm*

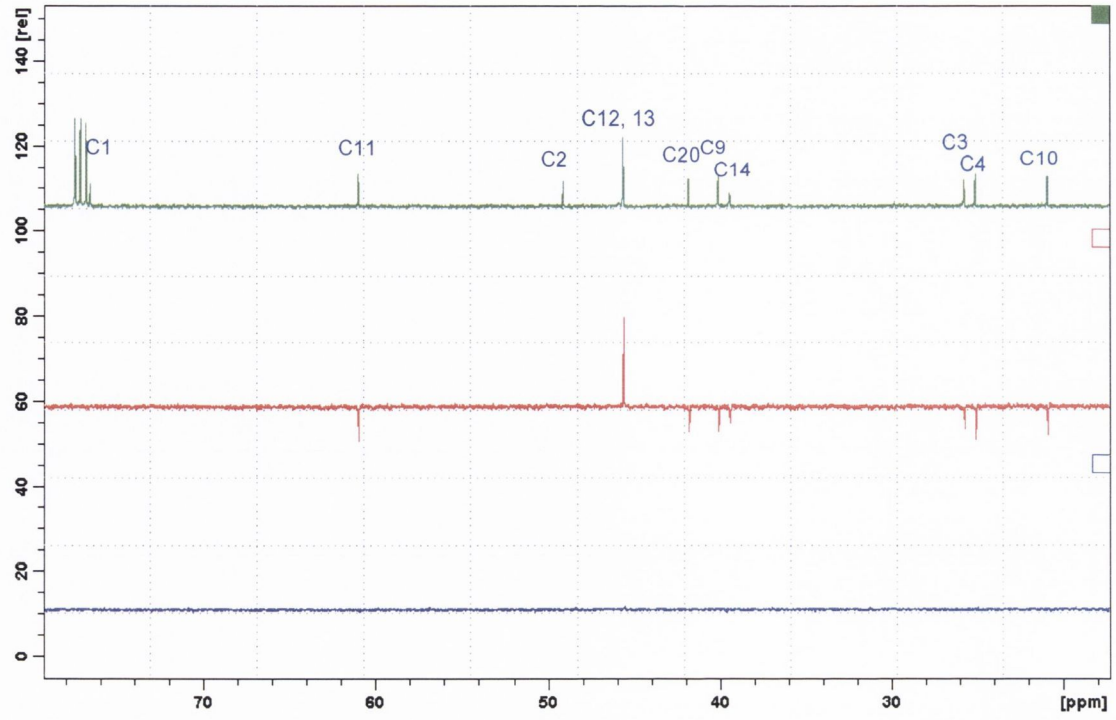


Fig. 4.5 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.4)a from 28.0-80.0ppm

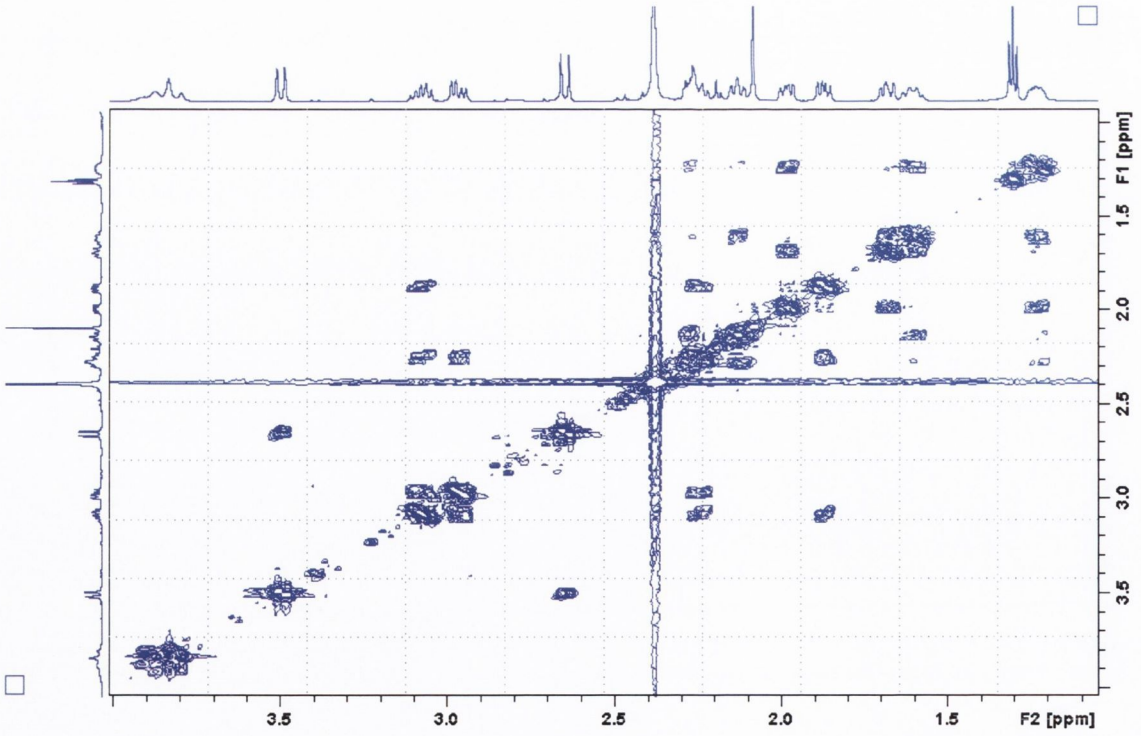


Fig. 4.6 H-H COSY NMR spectrum of compound (4.4)a from 1.00-4.00ppm

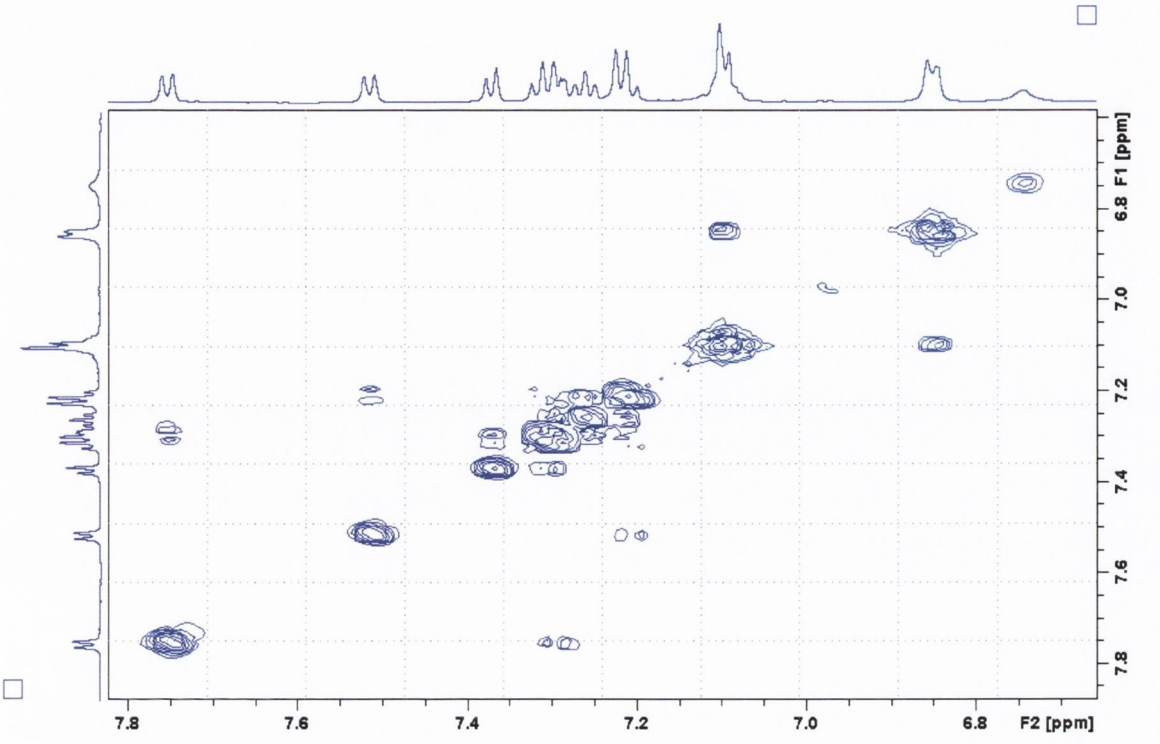


Fig. 4.7 H-H COSY NMR spectrum of compound (4.4)a from 5.82-8.00ppm

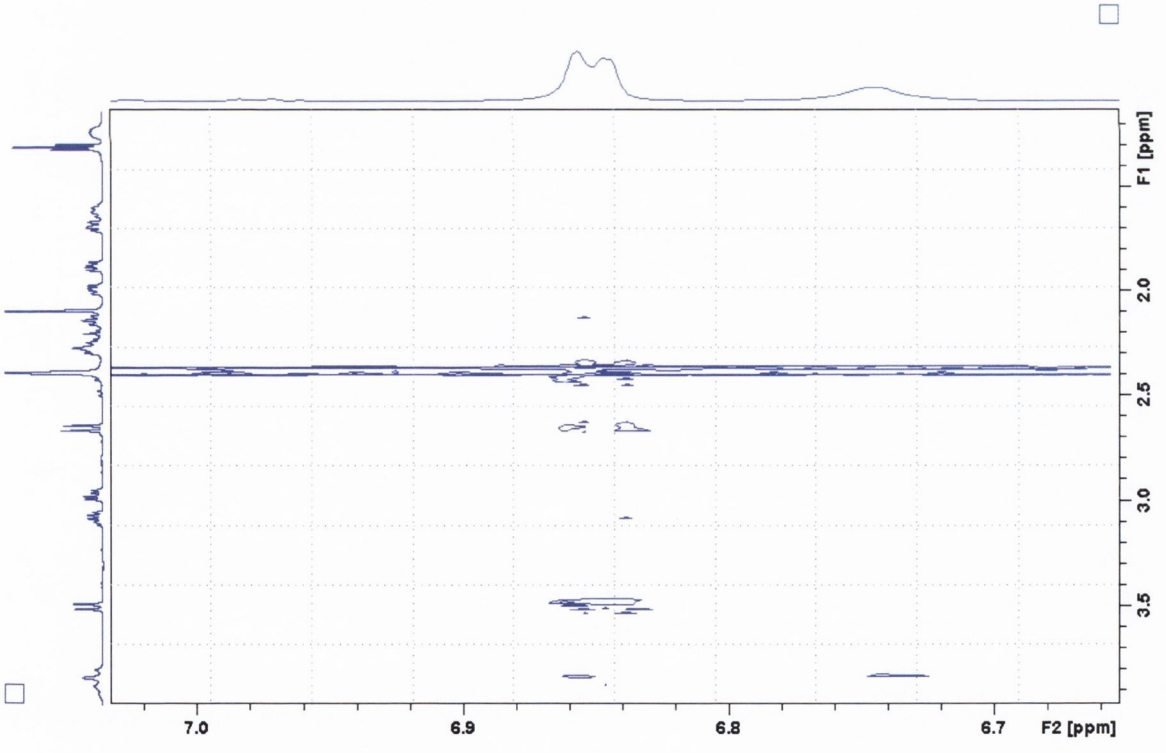


Fig. 4.8 H-H COSY NMR spectrum of compound (4.4)a from 6.65-7.04ppm

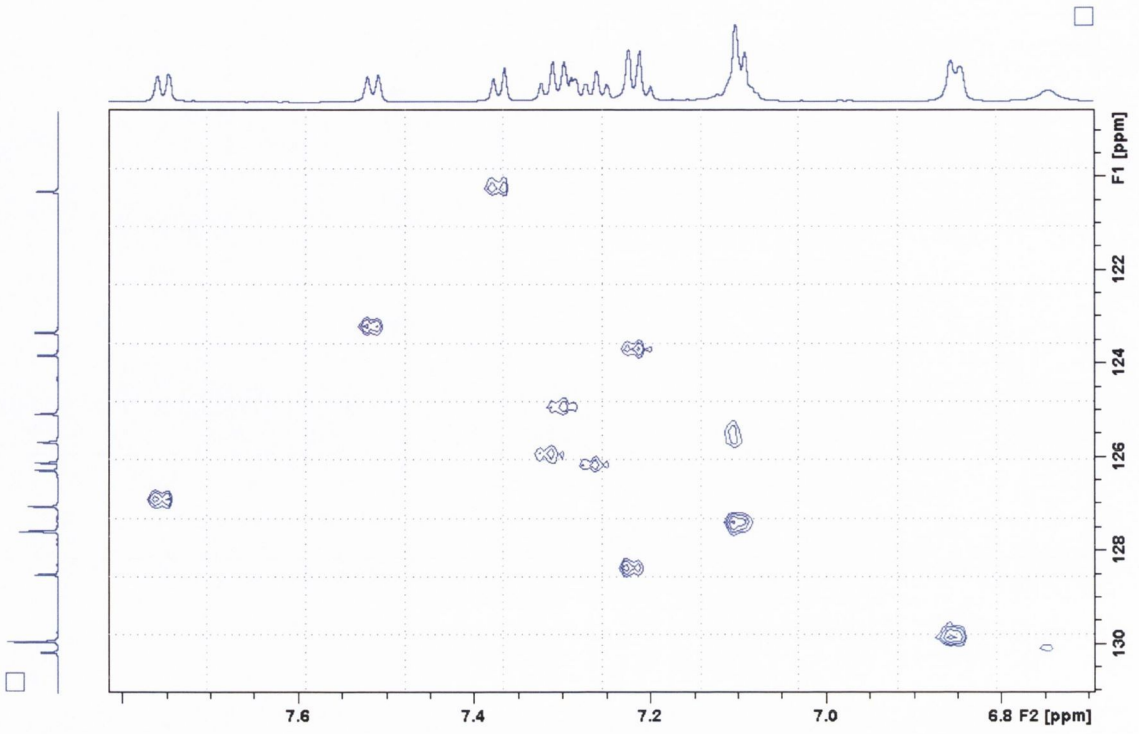


Fig. 4.9 C-H COSY NMR spectrum of compound (4.4)a from 6.72-7.82ppm

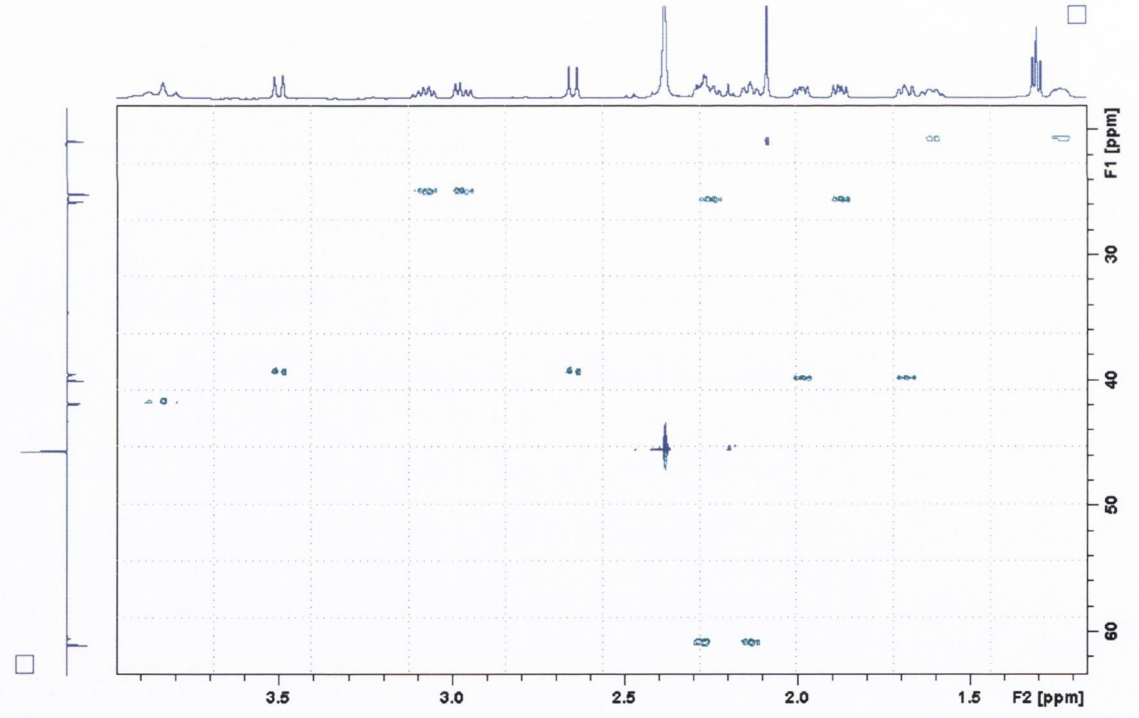


Fig. 4.10 C-H COSY NMR spectrum of compound (4.4)a from 1.25-4.08ppm

All of above assignments of the proton and carbon data were confirmed by the H-H and C-H COSY NMR spectra (Fig. 4.6-Fig. 4.10).

In the infrared spectrum of compound **(4.4)a**, two diagnostic peaks were evident, namely the $C{=}C_{wk}$ stretch at 1643.7cm^{-1} and the $O\text{-}H_{br}$ signal at 3435.8cm^{-1}, while the identity of compound **(4.4)a** was confirmed through the HRMS data with ion at 438.2782 for the ($+H^+$) ion, that calculated being 438.2797 for molecular formula $C_{31}H_{36}NO$.

^{1}H NMR analysis of isomer **(4.4)b** (Fig. 4.11) in the aliphatic region showed slight differences to isomer **(4.4)a**, with two multiplets close together at 1.41-1.45ppm and 1.55-1.62ppm respectively for the two protons at position-10, followed by a vast multiplet between 2.13-2.26ppm integrating for four methylene protons, including the two protons on C-9. The signal for the *N,N*-dimethyl protons resonated as a singlet at 2.43ppm, its sharpness being slightly obscured by two methylene protons which resonated at the same position. The signals for the two methylene protons on C-4 of the tetralin ring appeared as a multiplet at 2.93-2.97ppm. Two separate doublets, at 3.08ppm (J=13.04Hz) and 3.54ppm (J=13.04Hz) respectively, represented the two methylene protons on the benzyl moiety (C-14). The remaining two benzylic methylene protons on the indene ring split into two discrete doublets at 3.43ppm (J=23.08Hz) and 3.62ppm (J=23.08Hz). The aromatic region of the ^{1}H NMR spectrum was similar to that of its isomer **(4.4)a**. A complex splitting pattern was observed in this region possibly owing to the fact that many proton signals overlapped, with eleven protons resonating between 6.93-7.20ppm. A doublet (J=7.04Hz) at 7.36ppm represented a single proton while the aromatic proton adjacent to the hydroxyl substituent resonated as a doublet (J=7.52Hz) at 7.63ppm. The vinylic proton at C-12 resonated as a singlet at 6.22ppm, its position being considerably upfield from that of its isomer **(4.4)a**. The carbon spectra of isomer **(4.4)b** (Fig. 4.12-Fig. 14) correlated well to its proposed structure. The DEPT 135 NMR spectrum showed the presence of seven methylene carbons in the region of 20.9-41.1ppm and at 60.6ppm. The ^{13}C NMR spectrum confirmed the presence of the two methyl carbons at 45.4ppm and the quaternary carbons (C-2 and C-1) at 49.2 and 76.7ppm, respectively. The DEPT 90 NMR spectrum indicated the presence of eleven methine carbon resonances in the aromatic and alkene region. Moreover, five aromatic

quaternary carbons and one quaternary alkenic carbon resonated in the region of 135.2-152.2ppm in the ^{13}C NMR spectrum.

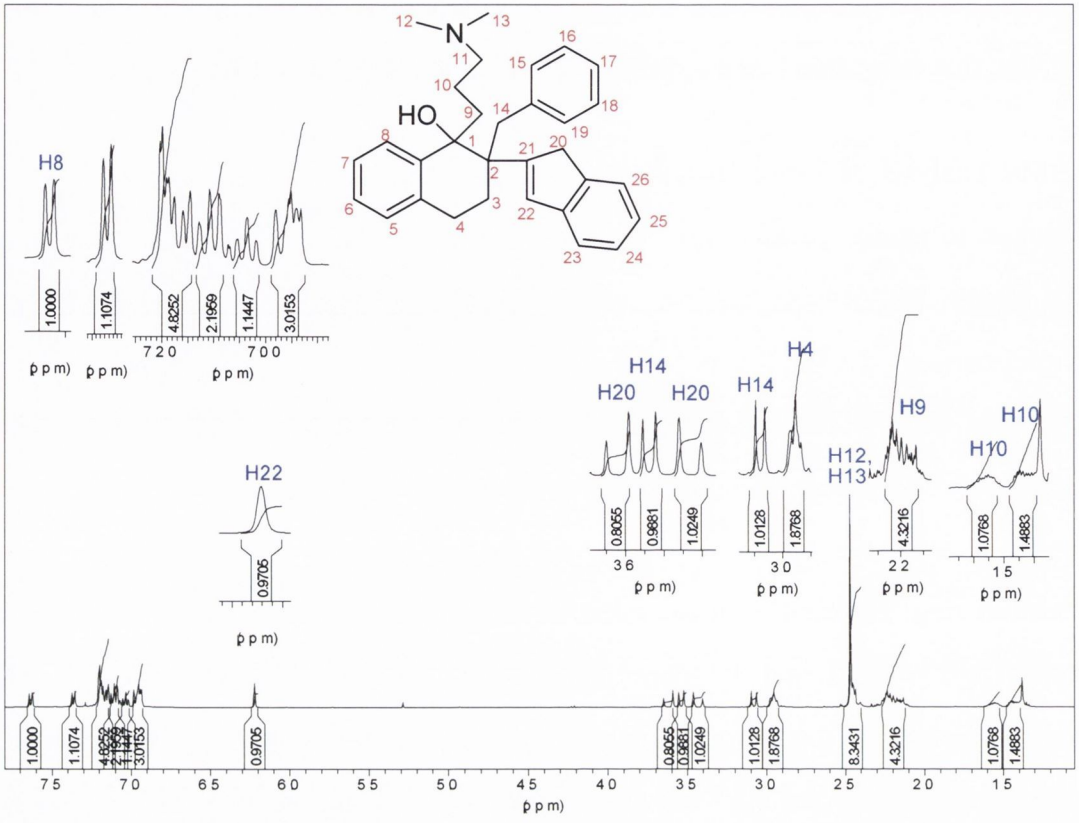


Fig. 4.11 ^{1}H NMR spectrum of compound (4.4)b

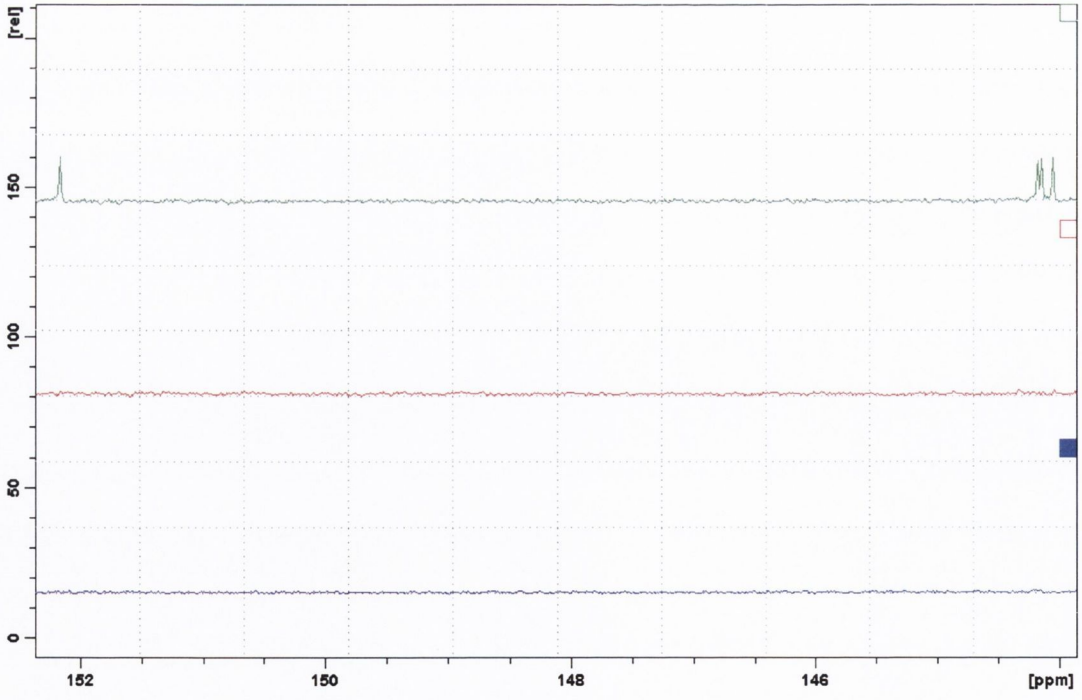


Fig. 4.12 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.4)b from 143.8-152.3ppm

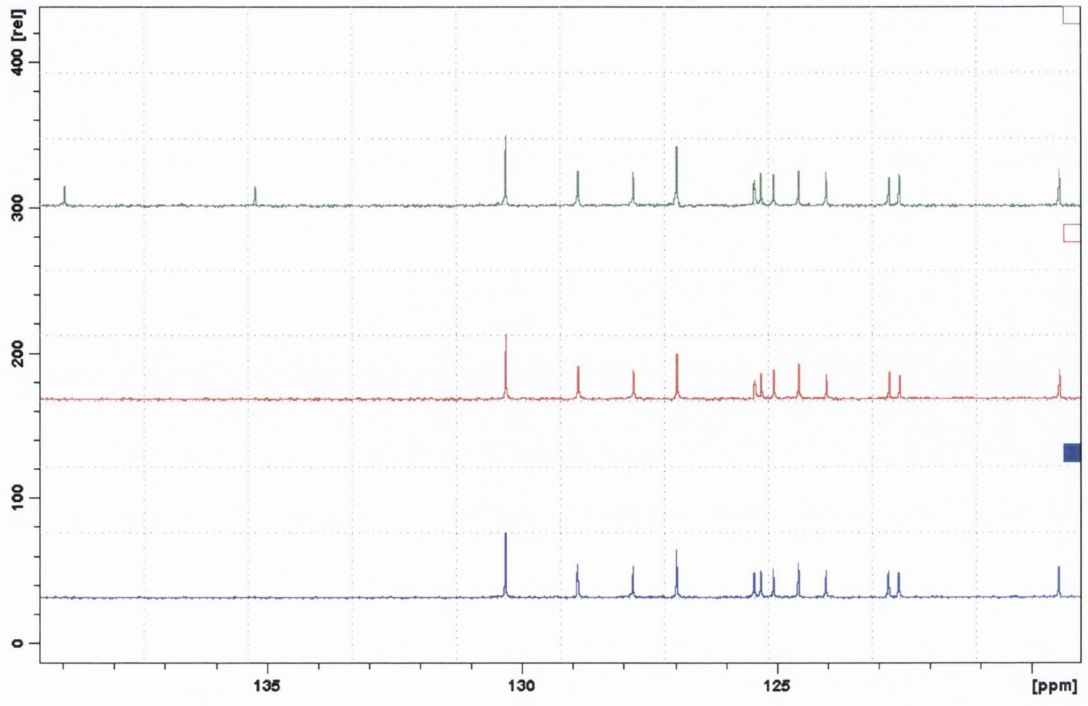


Fig. 4.13 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.4)b from 118.0-139.5ppm

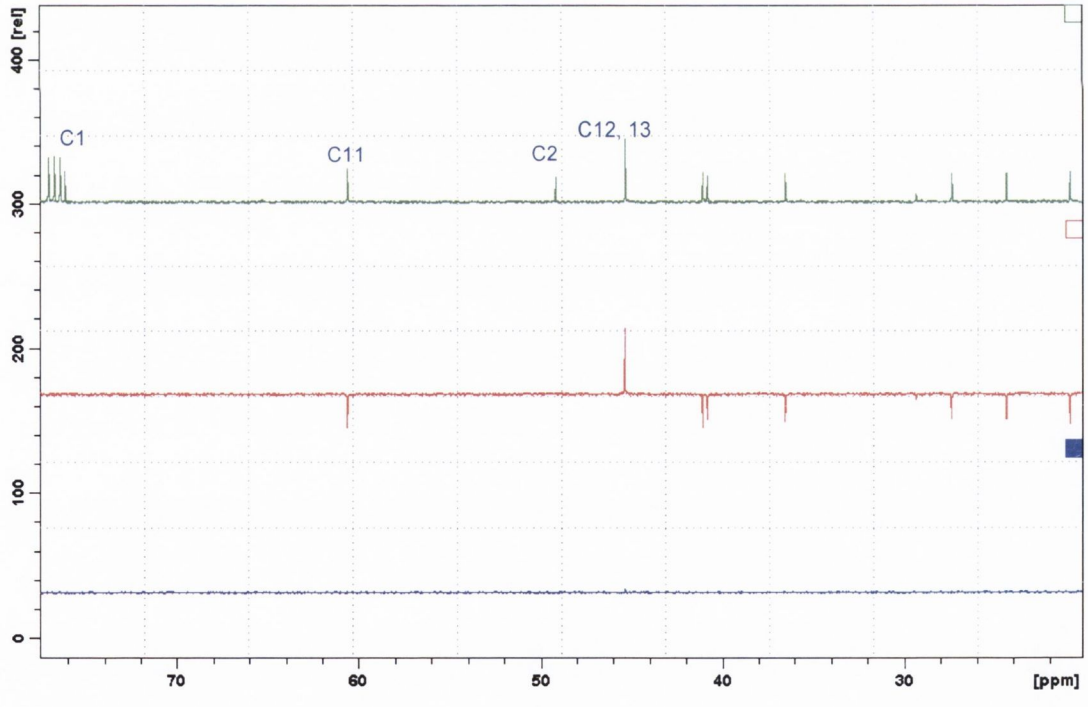
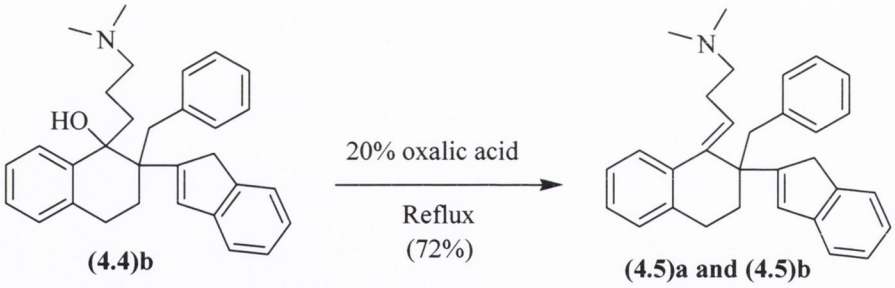


Fig. 4.14 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.4)b from 20.0-76.8ppm

In the infrared spectrum of **(4.4)b**, two diagnostic peaks were evident, namely the $C{=}C_{wk}$ stretch at 1642.5cm^{-1} and the $O\text{-}H_{br}$ signal at 3432.1cm^{-1}, while the identity of **(4.4)b** was also confirmed through the HRMS data with ion at 438.2792 for the ($+H^+$) ion, that calculated being 438.2797 for molecular formula $C_{31}H_{36}NO$.

4.3.3 Formation of the amino alkene (4.5)

Although both alcohols were perfectly stable on standing, it was nevertheless felt that derivatives containing the alkene group may exhibit enhanced activity as well as stability over the alcohols, **(4.4)a** and **(4.4)b**. The elimination reaction was carried out using isomer **(4.4)b**. This transformation process proceeded smoothly by the reaction of the alcohol **(4.4)b** in the presence of 20% aq. oxalic acid under reflux conditions. The starting material was totally consumed within two hours and following acid-base work-up and purification by flash column chromatography, and the alkene **(4.5)** was afforded, as a geometric mixture of isomers **(4.5)a and (4.5)b** in an overall yield of 72% (Scheme 4.11). Isomer **(4.5)b** was further purified by preparative TLC in a mixture of hexane:ethyl acetate:triethylamine (*v:v*, 10:10:1) as eluant.



(4.4)b **(4.5)a and (4.5)b**

Scheme 4.11 Synthesis of compound (4.5)a and (4.5)b

The ^{1}H NMR spectrum of compound **(4.5)b** revealed the triplet (J=7.06Hz) at 5.77ppm, which was believed to be for the alkenic proton at C-9 position (Fig. 4.15). Its corresponding ^{13}C resonance was in the region of 119.8-128.8ppm (Fig. 4.16). Dehydration of the alcohol **(4.4)b** to afford the alkene **(4.5)b** did not greatly affect the infrared spectral characteristics; the two important signals, $C{=}C_{wk}$, were located at 1708.3 and 1655.9cm^{-1}. HRMS analysis of compound **(4.5)b** gave accurate molecular mass ($+H^+$) as 420.2697, calculated as 420.2691 with molecular formula $C_{31}H_{34}N$.

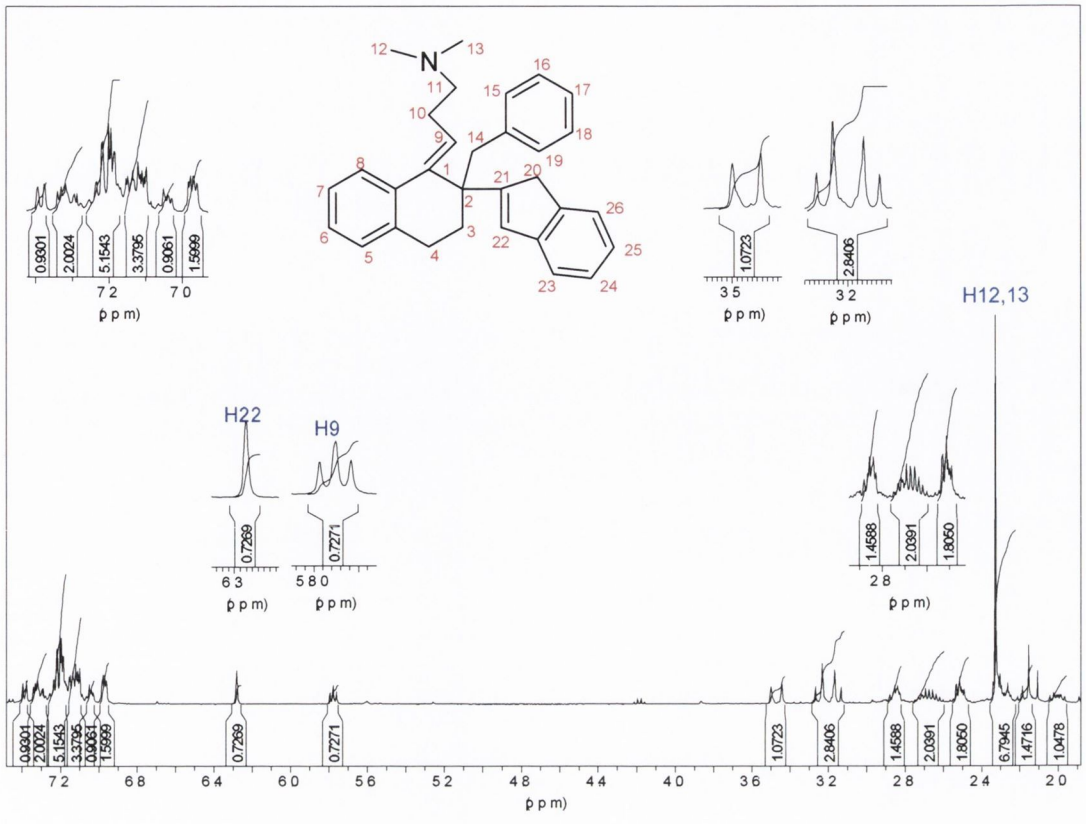


Fig. 4.15 ^{1}H NMR spectrum of compound (4.5)b

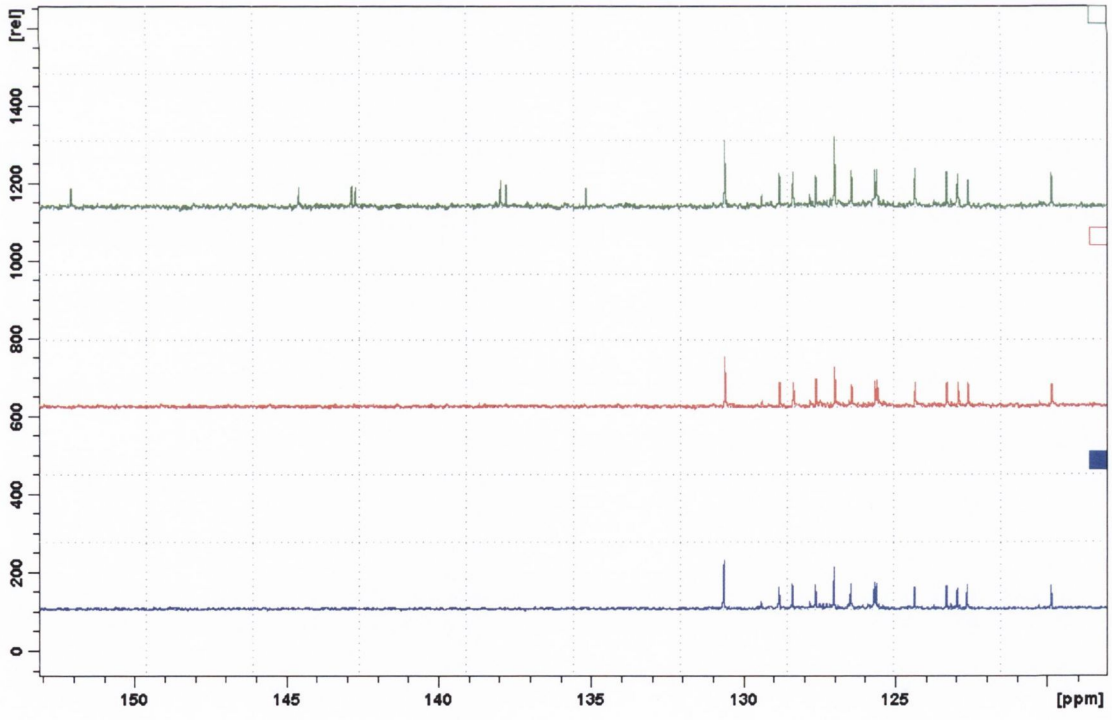


Fig. 4.16 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.5)b from 118.0-153.0ppm

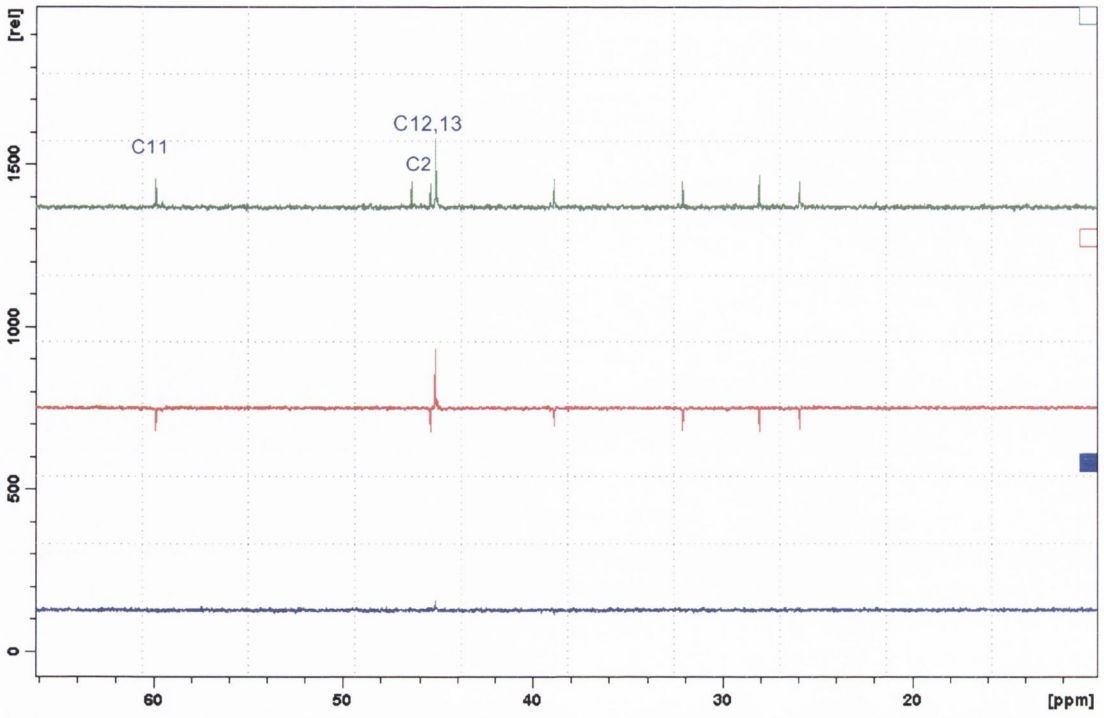
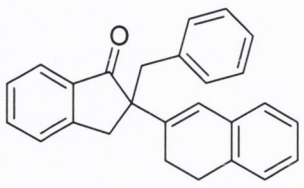


Fig. 4.17 ^{13}C*, DEPT 135 and DEPT 90 NMR spectra of compound (4.5)b from 10.0-66.0ppm*

4.4 Synthesis of the amino alcohol (4.10) and amino alkene (4.11)

The mast cell stabilising compounds generated by Barlow (2002) also contained a series where the hydroxyl group was placed on the indane unit and the alkene functional group was positioned on the tetralin moiety. It was therefore of interest to investigate the effect on mast cell stabilising activity of attaching the *N,N*-dimethylpropyl group onto ketone **(4.9)**, the analogous dimer to ketone **(4.3)**.

4.4.1 Synthesis of benzyl-alkylated dimer (4.9)

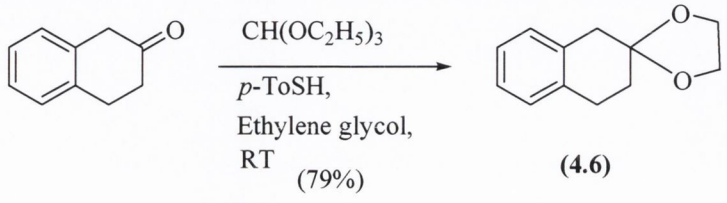


(4.9)

Fig. 4.18 Chemical structure of intermediate dimeric ketone (4.9)

4.4.1.1 Synthesis of compound (4.6)

The synthetic pathway which generated compound **(4.3)** was followed in a similar manner to furnish compound **(4.9)**. The cyclic ketal **(4.6)** was generated by the reaction of β-tetralone with ethylene glycol using p-ToSH as catalyst and triethyl orthoformate as dehydrating agent at room temperature (Scheme 4.12). The transformation was complete within three hours and gave compound **(4.6)** in a good yield (79%).



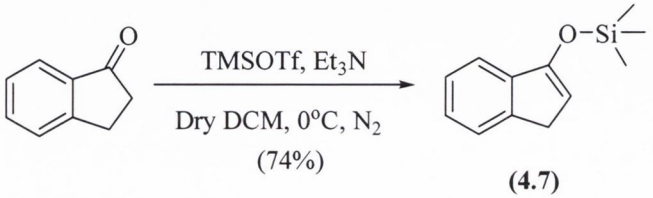
(4.6)

Scheme 4.12 Synthesis of compound (4.6)

In the ^{1}H NMR spectrum of compound **(4.6)**, a triplet (J=6.78Hz) was found at 2.05ppm, represented the two methylene protons at the non-benzylic position of the tetralin ring, followed by a multiplet at 3.07-3.09ppm for the remaining four methylene protons of the tetralin ring. The four acetal methylene protons resonated as a multiplet in the region of 4.06-4.11ppm, while the downfield region of the spectrum contained the four aromatic protons which resonated as a multiplet between 7.15-7.22ppm. The most notable feature of the ^{13}C NMR spectrum was the presence of the acetal methylene carbons resonating at 64.2ppm, and the expected absence of the carbonyl signal. The important infrared features of compound **(4.6)** were the $C\text{-}O_{str}$ at 1060.4, 1094.5 and 1124.0cm^{-1}, respectively.

4.4.1.2 Synthesis of compound (4.7)

Generation of the trimethylsilyl enol ether **(4.7)**, from 1-indanone, was readily accomplished using the same method as that employed to furnish compound **(4.0)**.



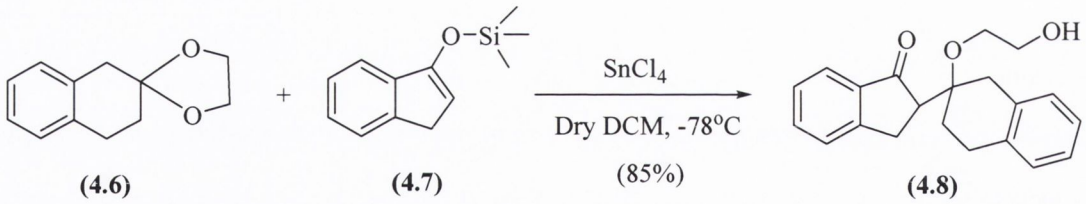
(4.7)

Scheme 4.13 Synthesis of compound (4.7)

As expected, the ^{1}H NMR spectrum showed a sharp singlet for the trimethyl protons at 0.49ppm, followed by two overlapping doublets (J=2.32Hz) at 3.42ppm for the methylene protons while the triplet (J=2.38Hz) at 5.59ppm, confirmed the presence of the methine proton. The aromatic region was very well resolved. Four signals were observed with, a double triplet (J_1=0.80Hz, J_2=7.45Hz) at 7.37ppm, a triplet (J=7.36Hz) at 7.46ppm, a doublet (J=11.44Hz) at 7.55ppm and a doublet (J=11.52Hz) at 7.56ppm. All characteristic signals were found in the ^{13}C NMR spectrum, with a sharp signal at 0.34ppm for the methyl carbons, one signal for the methylene carbon at 33.6ppm and the alkene tertiary carbon at 105.8 while the quaternary signal for the alkenic carbon was found at 153.2ppm.

4.4.1.3 Synthesis of compound (4.8)

The keto alcohol **(4.8)** was synthesised through the directed aldol reaction of cyclic acetal **(4.6)** with the silyl enol ether **(4.7)**. The reaction was performed at -78°C and employed tin tetrachloride as Lewis acid catalyst. After an hour the reaction was quenched, worked up, and purified by flash column chromatography to afford the titled compound **(4.8)** in good yield (85%) (Scheme 4.14).



(4.6) **(4.7)** **(4.8)**

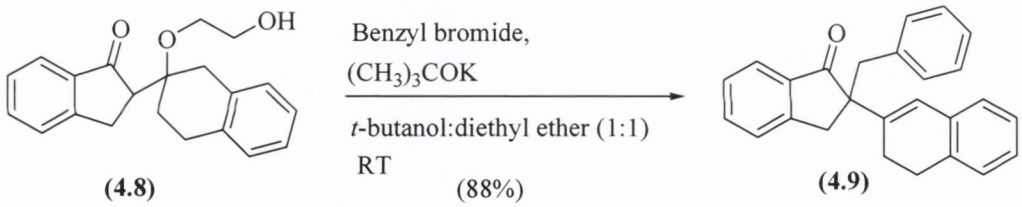
Scheme 4.14 Synthesis of compound (4.8)

The aliphatic region of the ^{1}H NMR spectrum of compound **(4.8)** showed a similar pattern to that of compound **(4.2)**, with nine discrete multiplet signals representing twelve methylene protons and one methine proton and the hydroxyl proton in the region from 1.90-3.65ppm. Signals in the aromatic region were well resolved in comparison to that of compound **(4.2)**, with two multiplets between 7.01-7.15ppm for four protons, two multiplets from 7.51-7.66ppm for two protons, followed by a triplet (J=7.40Hz) centred at 7.41ppm integrating for one proton. A broad set of doublets (J=7.66Hz) for the aromatic proton nearest the carbonyl resonated at 7.77ppm. The ^{13}C NMR spectrum

confirmed that compound **(4.8)** was formed as a mixture of stereoisomers. Fourteen signals were observed for the six methylene carbons between 25.6-63.3ppm, and one methine carbon at 51.8 and 52.5ppm, followed by two signals at 77.1 and 77.2ppm for the quaternary carbon β to the carbonyl. In the aromatic region, nine signals for the eight tertiary carbons and six signals for four quaternary carbons were found between 123.9-153.6ppm. The carbonyl signal was found at 206.6ppm. Infrared analysis of dimeric alcohol **(4.8)** showed the strong carbonyl peak at 1699.0cm^{-1} and a broad O-H_{str} at 3415.1cm^{-1}.

4.4.1.4 Synthesis of intermediate ketone (4.9)

Benzylation of the keto alcohol **(4.2)** with benzyl bromide was completed using exactly the same procedure at that used to acquire compound **(4.3)**. The desired compound **(4.9)** was isolated in good yield (88%) after work-up and purification by flash column chromatography (Scheme 4.15).



(4.8) **(4.9)**

Scheme 4.15 Formation of the key intermediate ketone (4.9)

The ^{1}H NMR spectrum of compound **(4.9)** again showed similar coupling pattern to that of compound **(4.3)**, with two multiplets in the regions of 2.31-2.39 and 2.48-2.52ppm for the two methylene protons next to the alkenic carbon. Two doublets as overlapping signals were located centred at 2.81 and 2.84ppm, followed by a multitplet for the remaining four methylene protons. The vinylic proton resonated at 6.58 as a singlet, with all thirteen of the aromatic protons between 7.07-7.79ppm. The DEPT 135 NMR spectrum indicated the four methylene carbons at 24.9, 28.5, 36.7 and 40.7ppm, respectively, and the absence of the quaternary carbon at 59.6ppm. The DEPT 90 NMR spectrum confirmed the presence of the fourteen tertiary carbons with the signals in the region of 124.1-130.2ppm. The five aromatic quaternary carbons together with the

alkene quaternary carbon and carbonyl carbon were present at 134.2, 134.8, 136.5, 137.3, 140.6, 152.7 and 207.2ppm respectively in the ^{13}C NMR spectrum. In the infrared spectrum, the principal absorption was for that of the carbonyl stretching peak at 1707.1 cm^{-1}. HRMS data was in agreement with the proposed structure with the ($+Na^+$) 373.1575, that calculated being 373.1568.

4.4.2 Synthesis of the amino alcohol (4.10)

Having successfully synthesised compound **(4.9)**, the attachment of the *N,N*-dimethylpropyl side chain was proceeded smoothly via a Grignard reaction to give the amino alcohol **(4.10)** in near quantitative yield (Scheme 4.16).



(4.9) **(4.10)**

Scheme 4.16 Synthesis of compound (4.10)

The NMR spectra of the amino alcohol **(4.10)** was somewhat complicated by the fact that it was isolated as a mixture of stereoisomers. The aliphatic region of the ^1H NMR spectrum (Fig. 4.19) revealed very complex coupling patterns with considerable overlap of signals (Fig. 4.19). In the aliphatic region, three of four methylene protons at C-9 position from both two isomers were located in the regions of 1.44-1.48ppm, 1.69-1.77ppm and 1.78-1.81ppm, as three discrete multiplets. The two methylene protons at C-8 from one single isomer occurred as a broad multiplet centred at 1.90ppm. The mulitplet from 1.99-2.04ppm integrated for two protons, considered to be the remaining one methylene proton at C-9 and one methlyene proton at C-8 from the other single isomer. The signal of the final methylene proton from C-8 overlapped with that of one methylene protons, as a multiplet in the region of 2.18-2.26ppm representing two protons. A very complex pattern of signals was evident in the region between 2.31-2.73ppm where a total of twenty-three protons resonated. Twelve of which

represented the methyl protons (from both isomers), all four methylene protons at C-10 (from both isomers), the two methylene protons at C-3 of the indane ring (one from each isomer) and five methylene protons from C-21 and C-22 positions (from both isomers). The second methylene proton at C-3 resonated as a doublet (J=10.16Hz) at 2.97ppm. A multiplet, as overlapping signals, was observed in the region of 3.02-3.09ppm, integrated for four protons, including the two protons on C-13 (one from each isomer). The signal for the remaining proton on C-3 was found at 3.30ppm, as a doublet (J=9.36Hz). The following two doublets at 3.35ppm (J=10.52Hz) and 3.49ppm (J=9.40Hz) respectively, represented the remaining two methylene protons on C-13, one from each isomer. The alkenic proton on C-19 appeared as a broad singlet and a sharp singlet at 6.12 and 6.36ppm, respectively. In the aromatic region, a broad multiplet was found at 6.90ppm for two protons, followed by a doublet (J=4.68Hz) for one proton at 6.96ppm. A broad doublet (J=4.16Hz) at 7.03ppm, a broad triplet (J~4.56Hz) at 7.08ppm, a double triplet (J_1=0.92Hz, J_2=4.81Hz) at 7.11ppm and a double triplet (J_1=0.96Hz, J_2=4.84Hz) at 7.14ppm represented four aromatic protons. In the region between 7.19-7.38ppm, a total of eighteen aromatic protons were found within three multiplets. The terminal broad doublet (J=4.92Hz) of the aromatic proton nearest the hydroxyl group (C-7) resonated at 7.49ppm. The ^{13}C NMR spectrum of compound **(4.10)** also confirmed that the amino alcohol was procured as a mixture of stereoisomers. The signals for the methylene carbon at C-9 resonated at 21.4 and 22.2ppm. The signals of the carbons at C-21 and C-22 (from both isomers) were found in the region of 26.9-28.4ppm. The signals of the carbon on C-8 were located at 34.6 (one isomer) and 36.5ppm (second isomer), while the signals of the carbon on C-3 were found at 35.7 and 36.2ppm, one for each isomer. The two signals at 38.0 and 40.1ppm represented the C-13 carbon. The methyl signals for both isomers resonated at 45.4ppm. The remaining methylene carbon at C-10 resonated at 60.6 and 60.7ppm. The two quatenary carbons at C-2 and C-1 positions were found to resonate at 61.5 and 62.7ppm, and 85.0 and 85.9ppm, respectively. Signals for a total of twenty-six aromatic tertiary carbons were observed between 123.8-130.0ppm, with the identification of alkenic tertiary carbon at 127.2ppm. The

DEPT 135 NMR spectrum confirmed the absence of the signals for the twelve quaternary carbons in the aromatic region.

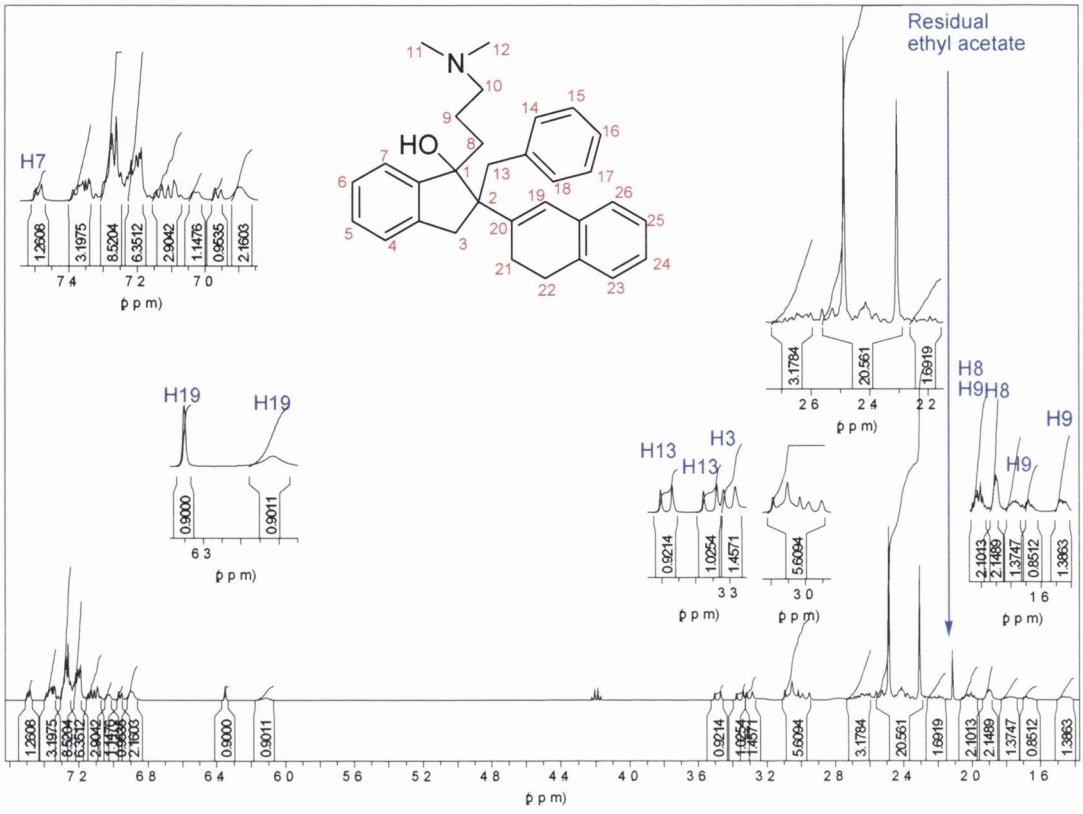


Fig. 4.19 ^{1}H NMR spectrum of compound (4.10)

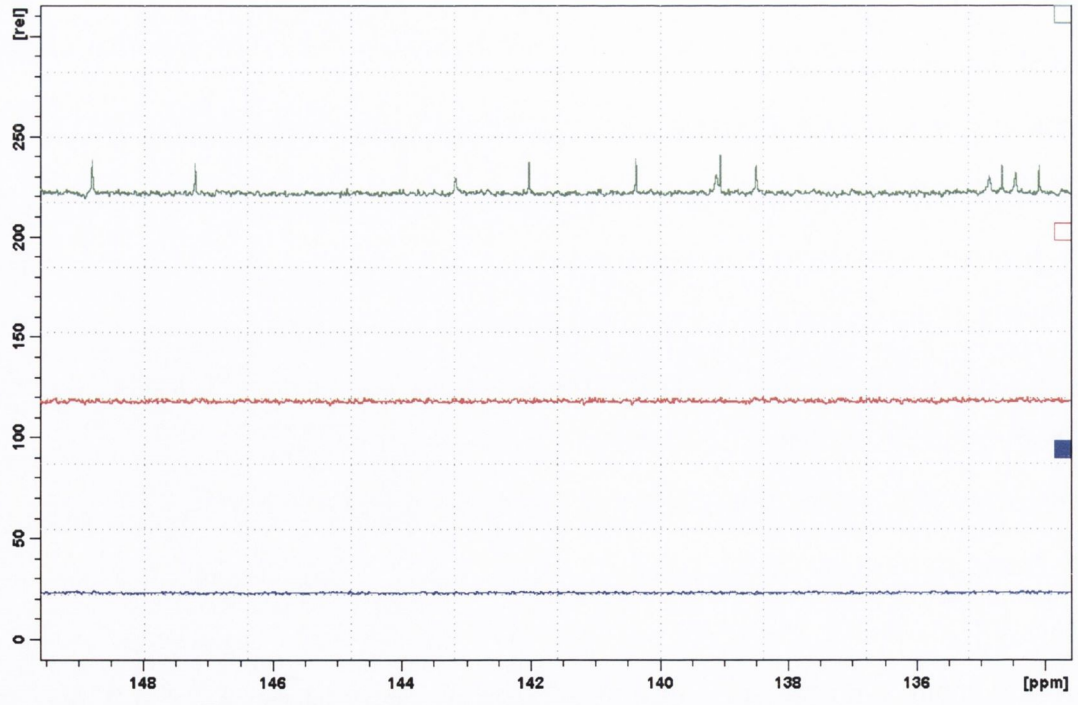


Fig. 4.20 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.10) from 134.6-149.6ppm

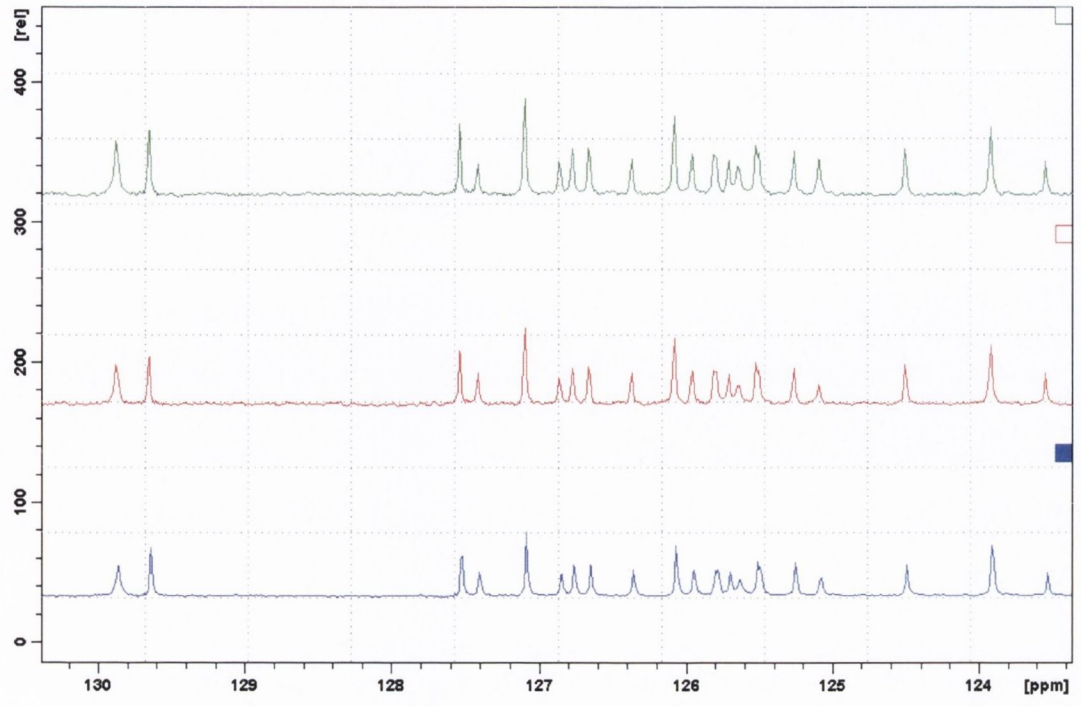


Fig. 4.21 ^{13}C, *DEPT 135 and DEPT 90 NMR spectra of compound (4.10) from 123.9-130.0ppm*

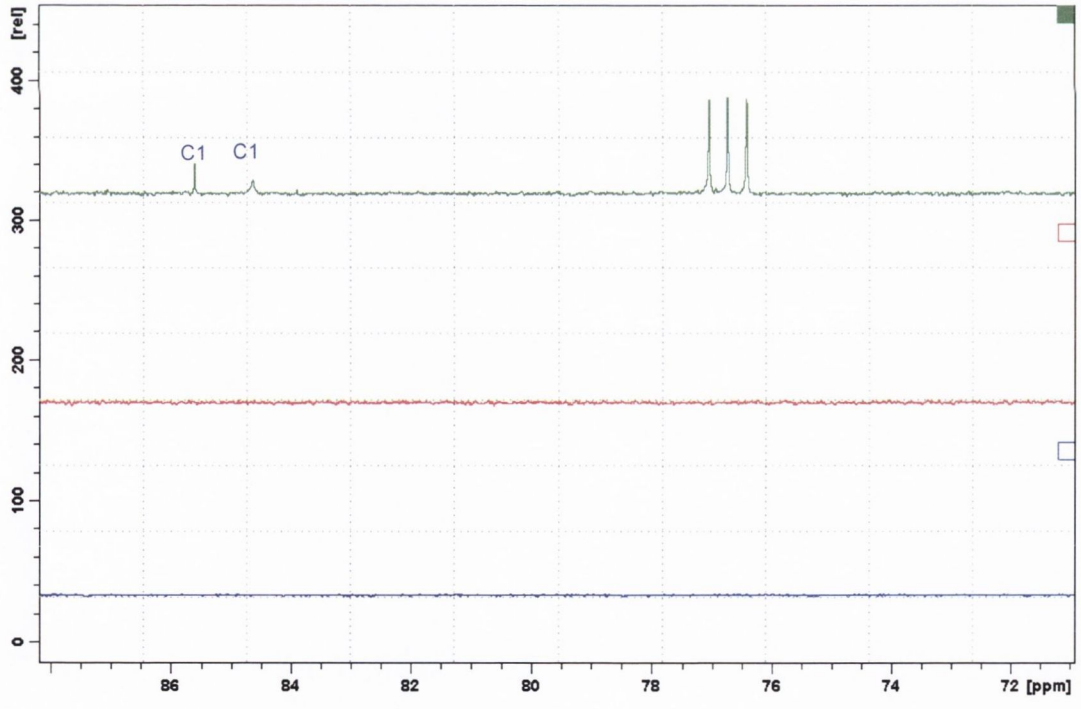


Fig. 4.22 ^{13}C, *DEPT 135 and DEPT 90 NMR spectra of compound (4.10) from 71.2-88.4ppm*

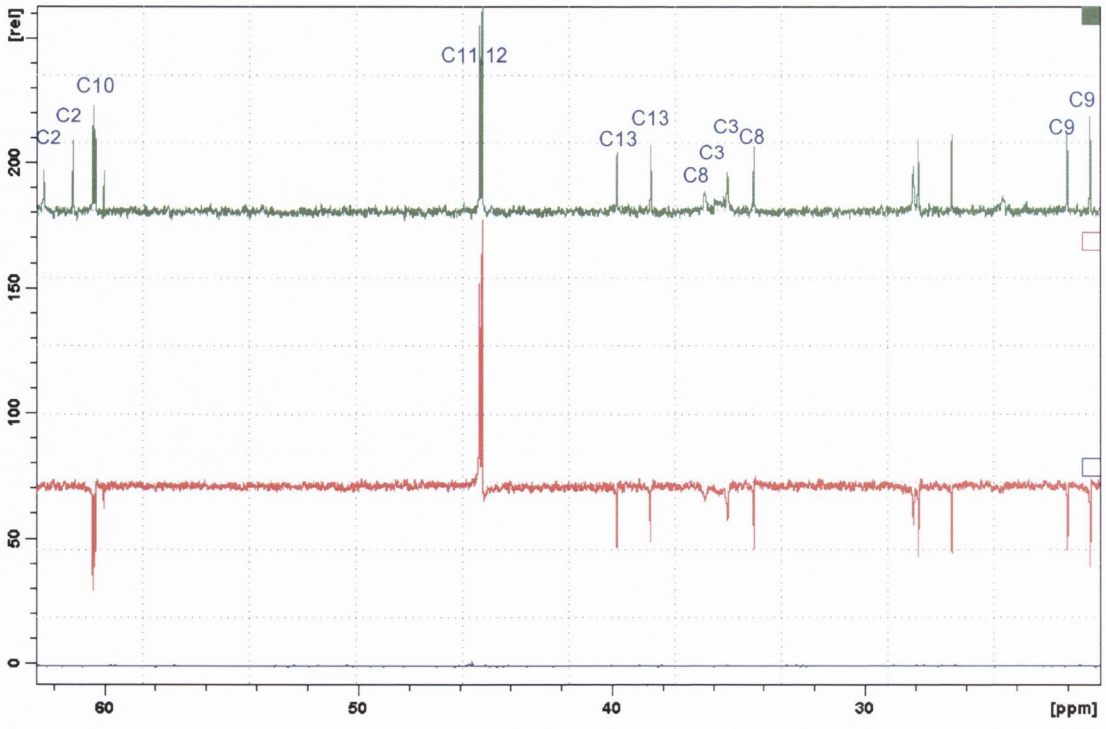


Fig. 4.23 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.10) from 21.5-62.5ppm

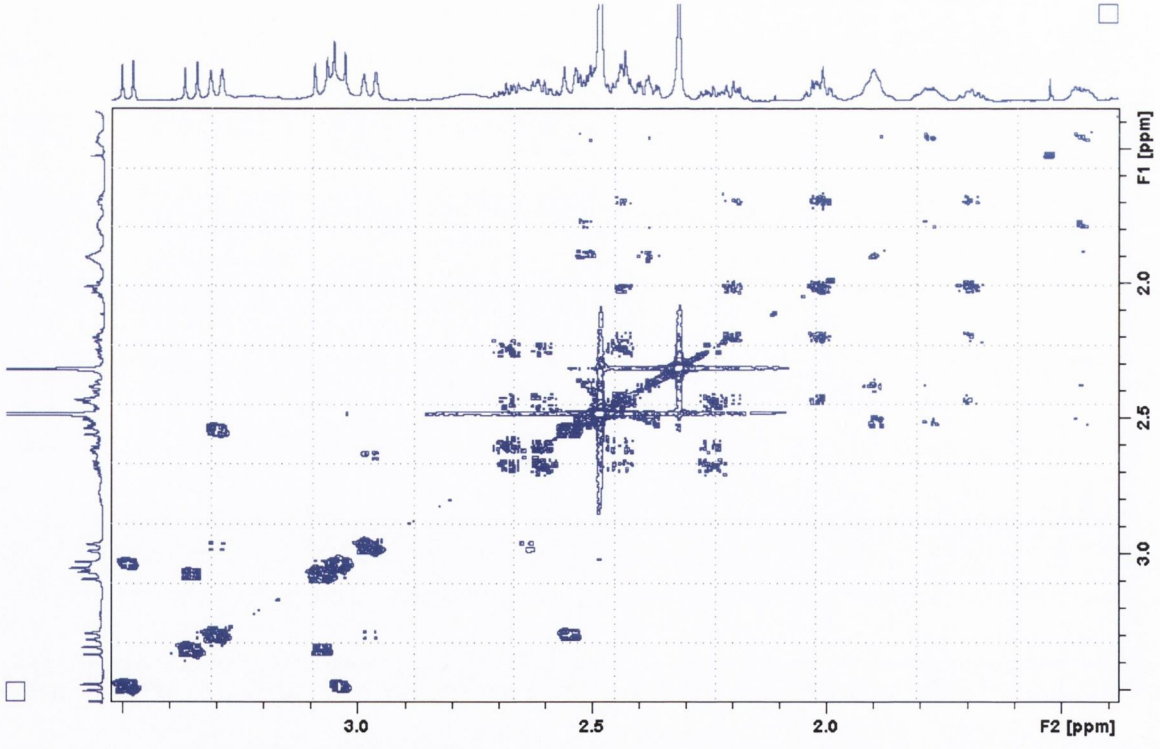


Fig. 4.24 H-H COSY NMR spectrum of compound (4.10) from 1.23-3.56ppm

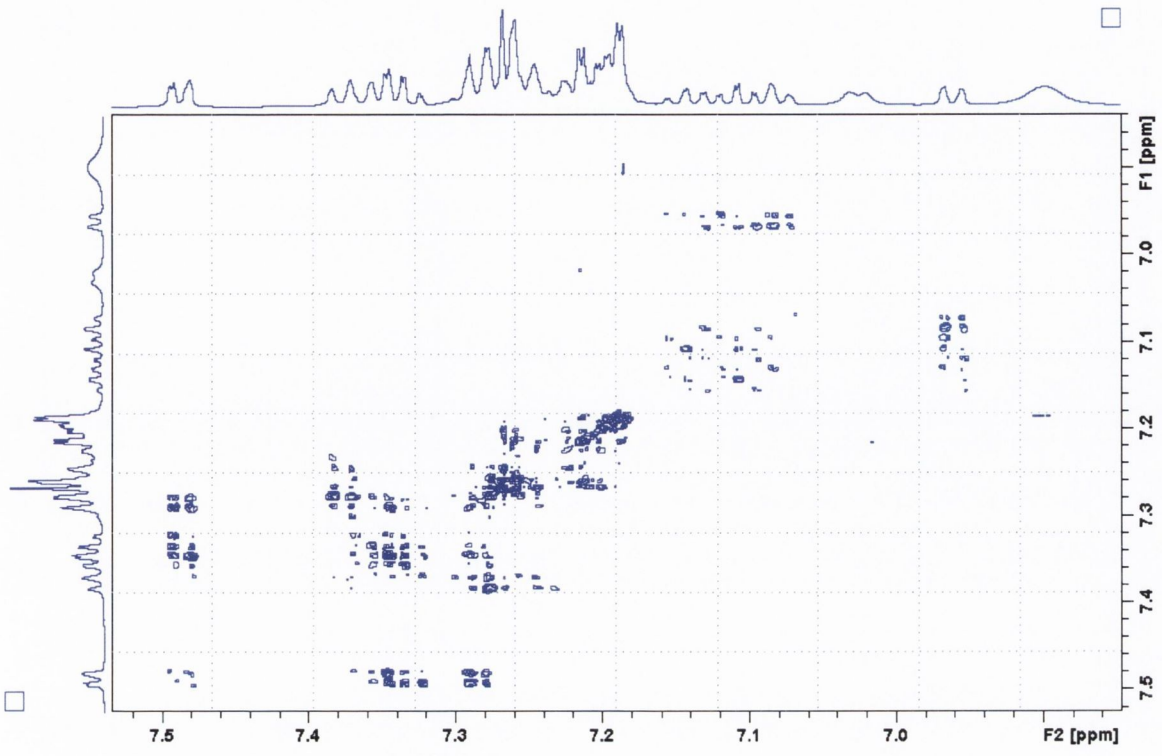


Fig. 4.25 H-H COSY NMR spectrum of compound (4.10) from 6.85-7.58ppm

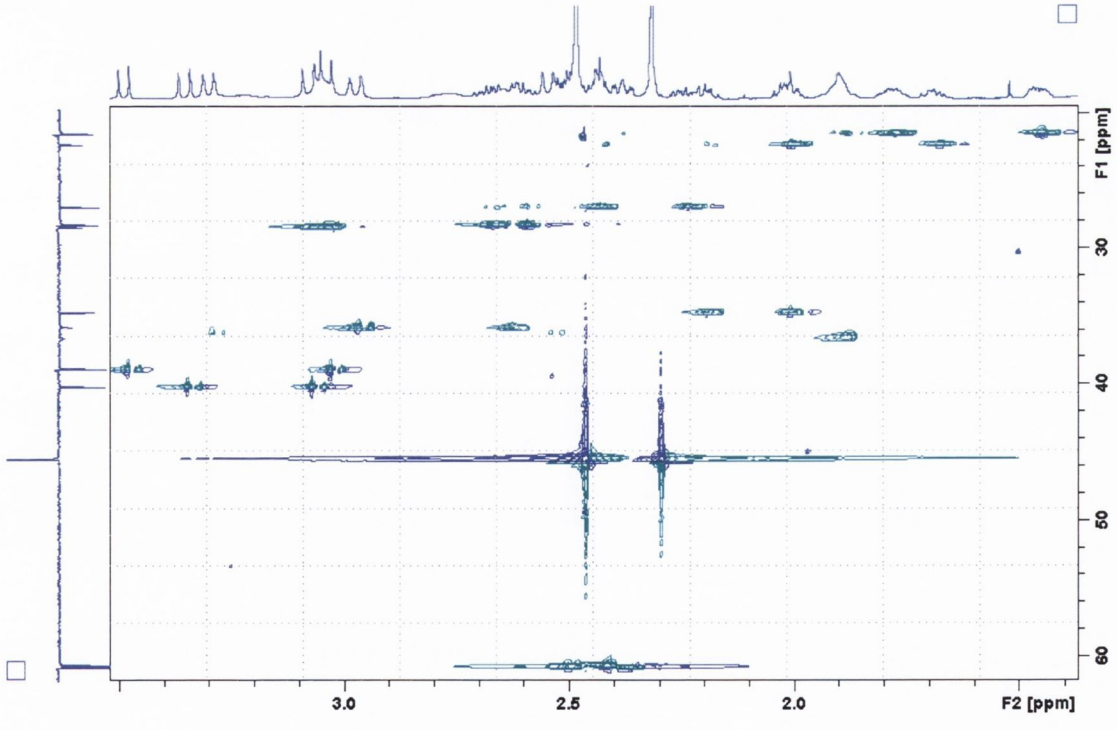


Fig. 4.26 C-H COSY NMR spectrum of compound (4.10) from 1.38-3.52ppm

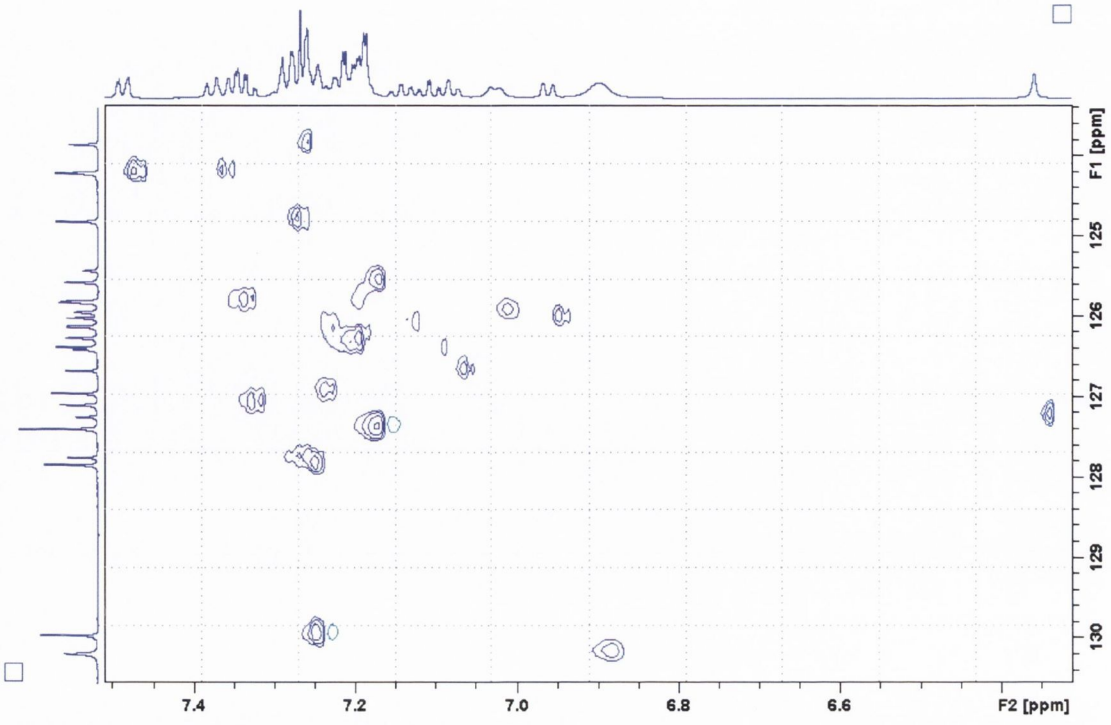
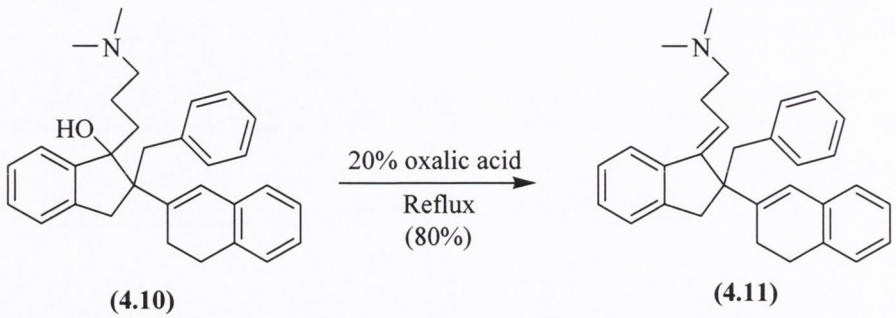


Fig. 4.27 C-H COSY NMR spectrum of compound (4.10) from 6.30-7.54ppm

For clarity, H-H COSY, C-H COSY spectra of compound **(4.10)** are shown in Fig. 4.24-Fig. 4.27. The infrared spectrum indicated the disappearance of the carbonyl group. The calculated molecular mass was 438.2797, which correlated well to the actual mass of 438.2795 ($+H^+$) of molecular formula $C_{31}H_{36}NO$.

4.4.3 Formation of the alkene (4.11)

Having secured the amino alcohol **(4.10)**, its conversion to the alkene **(4.11)** was accomplished by dissolving it in a 20% aq. oxalic acid and refluxing the resulting solution for two hours. The desired compound **(4.11)** was afforded in good yield (80%) after work-up and purification by flash column chromatography (Scheme 4.17).



(4.10) **(4.11)**

Scheme 4.17 Synthesis of compound (4.11)

The NMR spectra of the alkene **(4.11)** indicated that the alkene was isolated as a 0.7:1 mixture of E/Z stereoisomers. The ^{1}H NMR spectrum (Fig. 4.28) showed a similar pattern of signal overlap to that of its alcohol precursor. The methyl protons for one isomer resonated as a singlet at 2.15 while that of the other isomer was found at 2.36ppm also as a singlet. A number of broad multiplets were evident in the spectrum in the regions of 2.24-2.44ppm, 2.47-2.67ppm and 2.71-2.85ppm which accounted for the highly aliphatic signals in the molecule particularly those at positions C-9, 10, 21 and 22 positions, integrated for a total of sixteen protons. In the region between 3.10-3.50ppm, greater resolution between proton signals was evident. A doublet (J=16.74Hz) at 3.10ppm, multiplet at 3.17-3.24ppm, two overlapping doublets (J~13.14Hz and J~13.23Hz) at 3.29ppm and a doublet (J=12.96Hz) at 3.49ppm. The two alkenic protons at C-8 for both isomers featured as two triplets, located at 5.61ppm (J=7.11Hz) and 6.17ppm (J=7.41Hz). The two alkene singlets for C-19 resonated at 6.55 and 6.71ppm, respectively. In the aromatic region, twenty-five of the twenty-six protons resonated closely between 6.91-7.33ppm, with one isolated proton signal at 7.52ppm, as a doublet (J=7.50Hz). In the ^{13}C NMR spectrum (Fig. 4.30), the signals for those carbons at C-9, C-21 and C-22 were found at 25.6 and 25.7, 26.0 and 27.3, 28.7 and 28.8ppm. The four signals for the carbons at C-3 and C-13 positions resonated at 40.8, 42.1, 43.9 and 44.6ppm, while the signals for the quaternary carbon at C-2 were present at 54.3 and 56.2ppm. The signals for the remaining methylene carbon at C-10 were at 59.5 and 59.6ppm. The DEPT 135 NMR spectrum revealed the presence of twenty-six signals for the thirteen aromatic carbons as a stereoisomeric mixture between 119.3-130.5ppm. The signals for the tertiary carbons at C-8 position were at 120.1 and 122.6ppm, the one at C-20 position, at 120.7 and 122.2ppm, which was confirmed by C-H COSY NMR spectrum (Fig. 4.29 and Fig. 4.31).

HRMS analysis of compound **(4.11)** gave the accurate mass ($+H^{+}$) as 420.2709, calculated 420.2691 from molecular formula $C_{31}H_{34}N$.

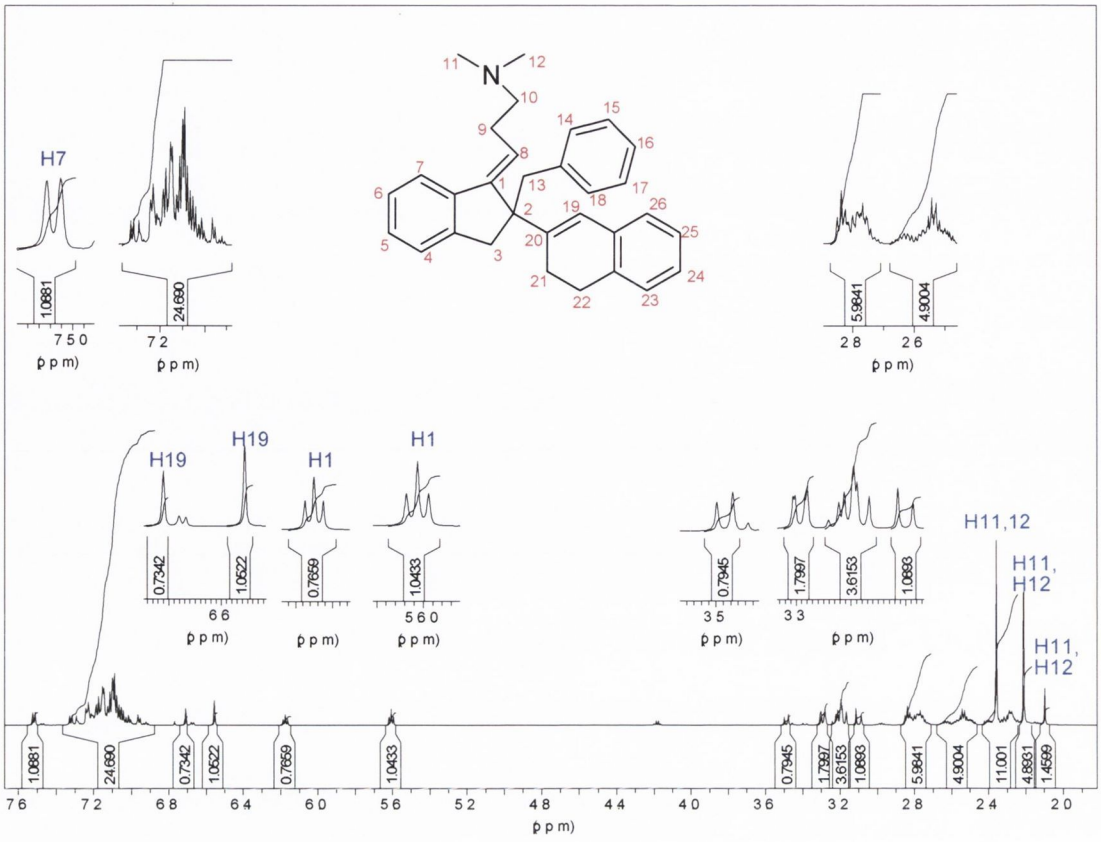


Fig. 4.28 ^{1}H NMR spectrum of compound (4.11)

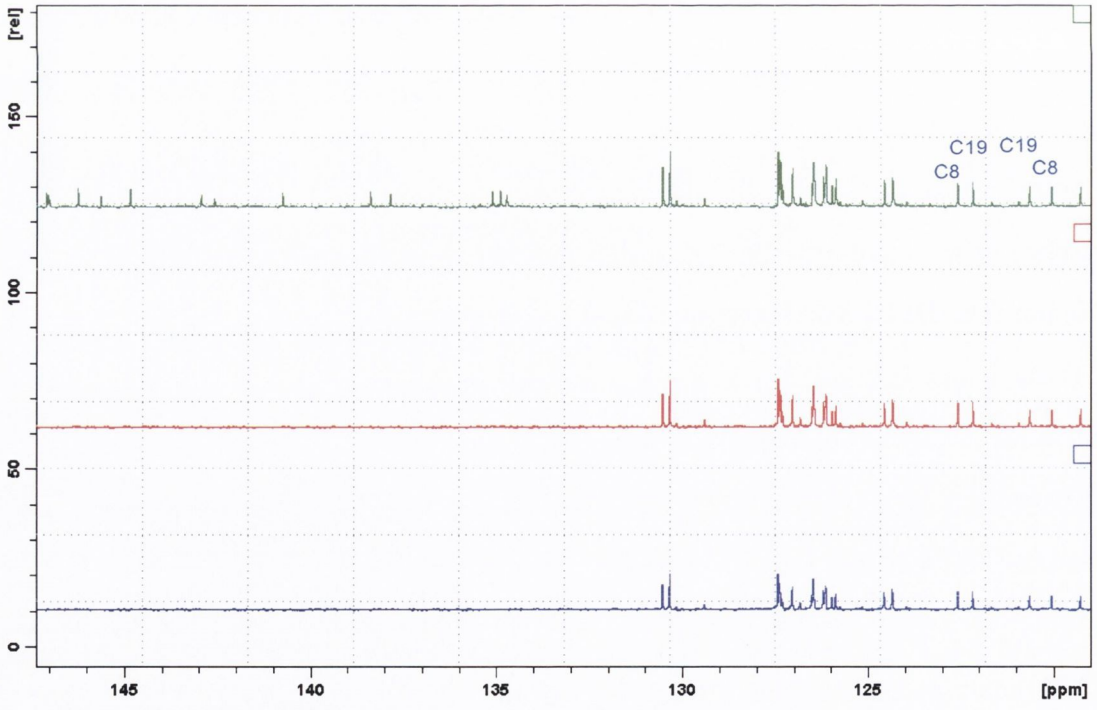


Fig. 4.29 ^{13}C, DEPT 135 and DEPT 90 NMR spectrum of compound (4.11) from 119.0-147.2ppm

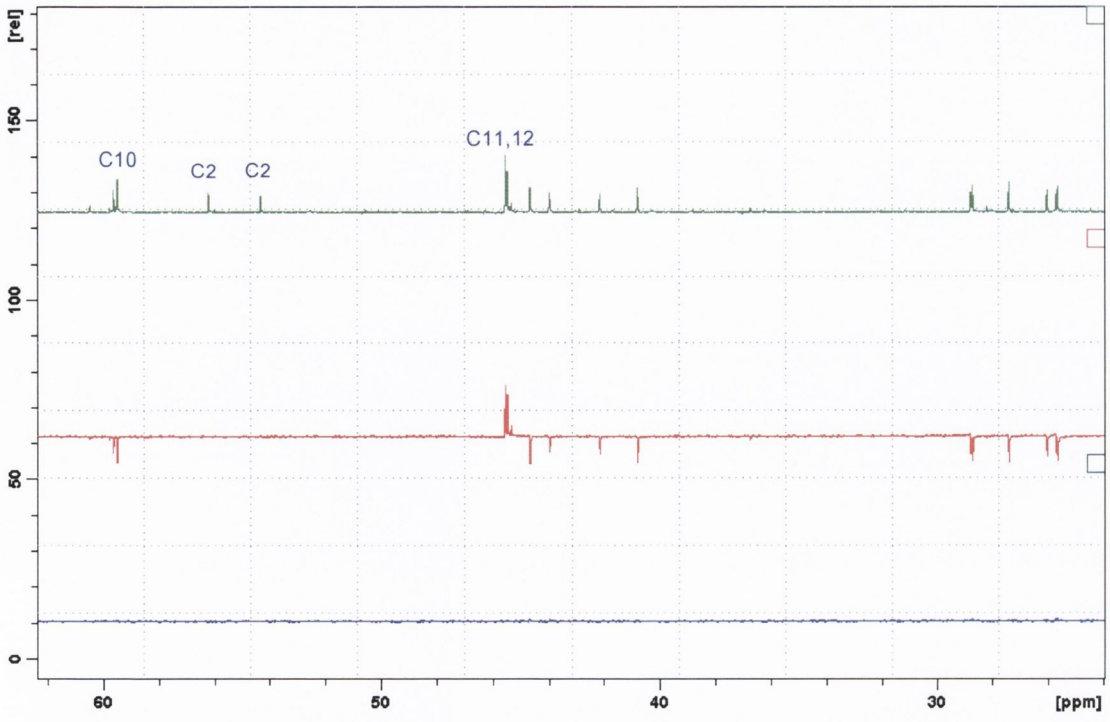


Fig. 4.30 ^{13}C, DEPT 135 and DEPT 90 NMR spectrum of compound (4.11) from 23.8-62.2ppm

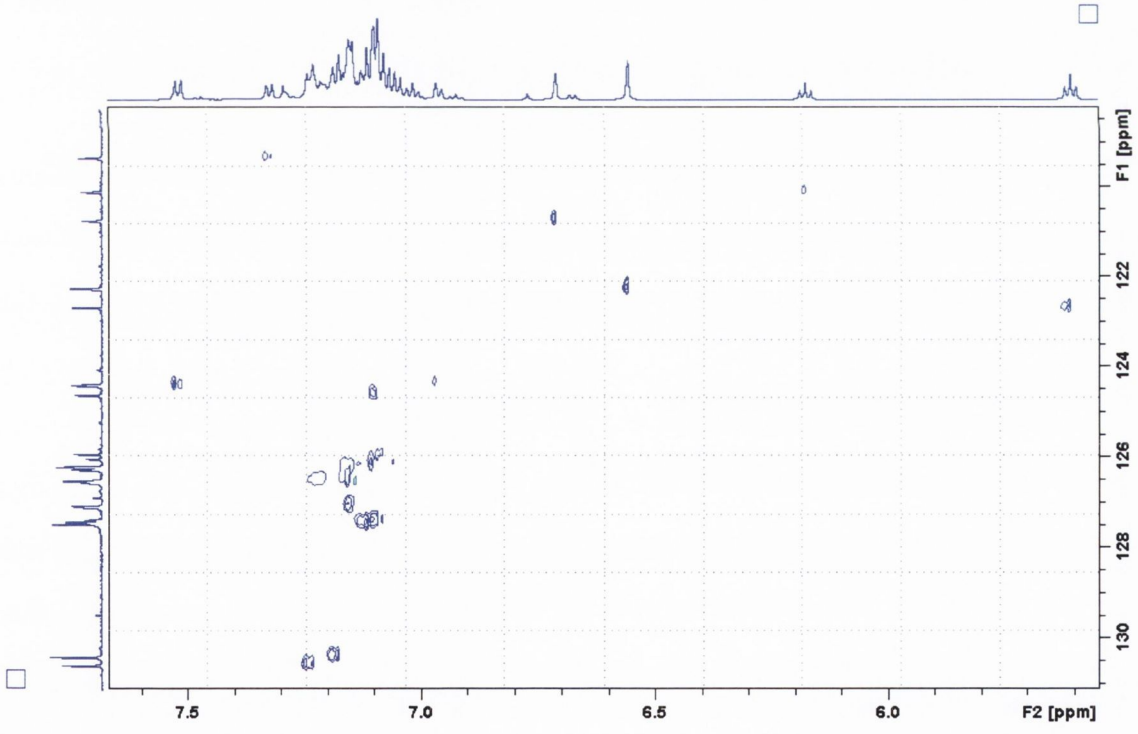


Fig. 4.31 C-H COSY NMR spectrum of compound (4.11) from 5.45-7.68ppm

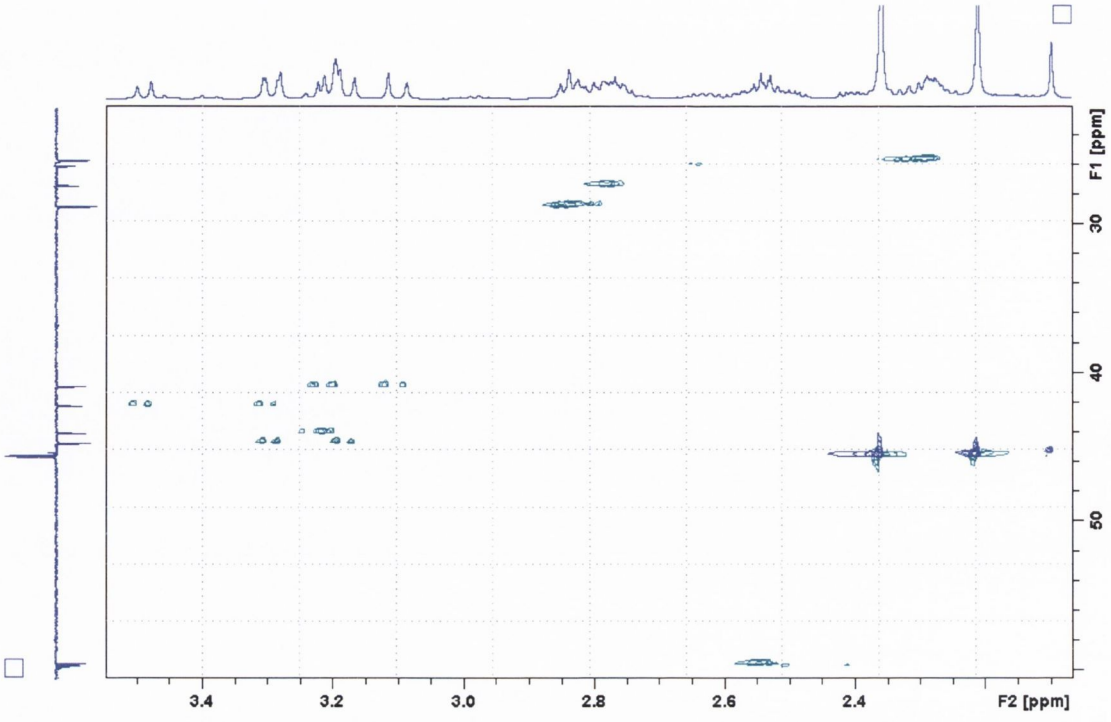


Fig. 4.32 C-H COSY NMR spectrum of compound (4.11) from 2.05-3.55ppm

4.5 Modification to the *para*-position of the benzyl ring

Of the series of compounds described in Chapter 2, those containing *para*-substituents on the benzyl ring possessed potent mast cell stabilisation *in vitro*. Indeed the methoxy and especially the trifluoromethoxy derivatives enjoyed good activity *in vivo*. In the closing section of this Chapter, the emphasis is largely based on the synthesis of compounds where the benzyl substituent is modified at the *para*-position. Aside from inclusion of the *p*-trifluoromethoxybenzyl group, the particular emphasis is on the design of molecules where *N,N*-dimethyl propylidene group is placed onto this position. It was felt that this could be achieved through the synthesis of precursors containing either nitrile or carboxy esters groups at this position.

Having selected the alkylating agents to be employed, the first step towards the synthesis of this series of target compounds was to effect benzylic alkylation of the aldol product **(4.2)**. In each case, the reaction was carried out through alkylation of compound **(4.2)**

with the appropriate benzyl halide using postassium *tert*-butoxide as base in a 1:1 mixture of *t*-butanol and diethyl ether. Once the synthesis of each benzylated derivative of compound **(4.2)** was complete, coupling of the *N,N*-dipropylamino group was effected through the Grignard method described for the synthesis of target compounds **(4.13)** and **(4.15)** (Fig. 4.33).

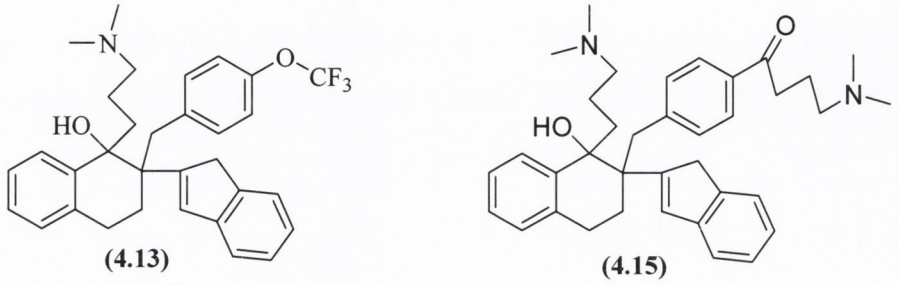
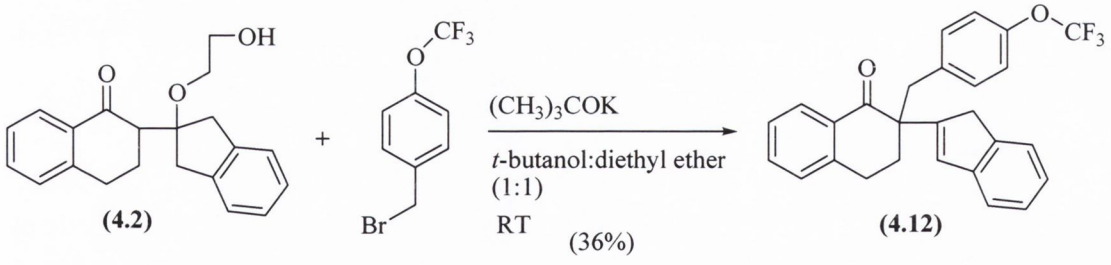


Fig. 4.33 Chemical structures of target compounds (4.13) and (4.15)

4.5.1 Synthesis of trifluoromethoxy benzyl derivative (4.13)

The synthesis of compound **(4.12)** was effected through alkylation of the aldol **(4.2)** with 4-(trifluoromethoxy)benzylbromide in a solution of *t*-butanol and diethyl ether with potassium *t*-butoxide as base. After two hours, the trifluoromethoxy derivative **(4.12)** was obtained in a 36% yield following work-up and purification by flash column chromatography (Scheme 4.18).



Scheme 4.18 Synthesis of compound (4.12)

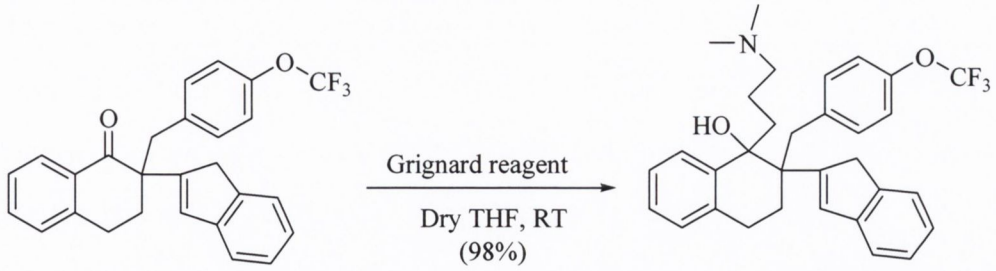
The ^{1}H NMR spectrum of compound **(4.12)** showed the two β-keto methylene protons on the tetralone ring as a double triplet (J_1=4.17Hz, J_2=13.04Hz) at 2.21ppm and a broad doublet (J=13.52Hz) at 2.37ppm. The first tetralone proton on the benzylic position resonated at 2.85ppm as a broad doublet (J=17.04Hz); the second one as a broad triplet

(J~14.78Hz) resonated in the region between 3.04-3.14ppm, overlapping with a doublet (J~45.40Hz) for one methylene proton on the benzyl group at 3.18ppm. The second methylene proton on the benzyl group was also found as a doublet (J~45.40Hz) at 3.23ppm. The remaining two methylene protons on the indene ring resonated as two doublets at 3.52ppm (J=18.06Hz) and 3.60ppm (J=18.06Hz), respectively. A singlet at 6.53ppm was considered to be the alkenic proton. The aromatic region was well resolved; two overlapping doublets (J=8.00Hz) were centred at 7.08ppm integrated for two protons, followed by two considerable overlapping multiplets in the regions between 7.14-7.20ppm and 7.24-7.30ppm, which represented a total of six protons. A triplet (J=7.52Hz) for one proton at 7.33ppm was followed by two overlapping triplets (J=6.78Hz, J=7.12Hz) at 7.41-7.47ppm, and integrated for two protons. The final aromatic proton nearest the carbonyl was found as a doublet (J=8.04Hz) at 8.20ppm. The DEPT 90 NMR spectrum contained four methylene carbon signals between 25.4-43.2ppm. The ^{13}C NMR spectrum confirmed the presence of the quaternary carbon on the tertralone ring at 52.9ppm and the carbon in the trifluoromethoxy group centred at 119.8ppm with $J_{F\text{-}C}$~258.4Hz. Twelve aromatic tertiary carbons were placed in the region between 119.9-135.9ppm, including one alkenic tertiary carbon at 129.7ppm, while the carbonyl resonated at 197.8ppm. The DEPT 135 NMR spectrum clearly demonstrated the absence of the six quaternary carbons and the carbonyl carbon signal at 197.8ppm. The existence of fluorine signal at -58.4ppm was confirmed by analysis of the ^{19}F NMR spectrum.

In the infrared spectrum, the $C{=}O_{str}$ signal was present at 1679.4cm^{-1}. HRMS analysis of compound **(4.12)** revealed the molecular mass ($+Na^+$) as 457.1376, that calculated being 457.1391.

Attachment of the *N,N*-dimethylpropyl substituent was completed through forming the corresponding Grignard reagent of *N,N*-dimethylproyl chloride and carrying out the coupling reaction onto compound **(4.12)** at room temperature. Following work-up and

purification by flash column chromatography, compound **(4.13)** was isolated in near quantitative yield as a mixture of stereoisomers.



(4.12) **(4.13)**

Scheme 4.19 Synthesis of compound (4.13)

The similar polarities of the two diastereoisomer pairs made their separation by a combination of flash column chromatography and preparative TLC very difficult. Nevertheless, it was possible to attain rich fractions of one pair of enantiomers. The NMR data described below was based on this enantiomer pair **(4.13)a** as when the mixture was analysed by NMR, very complex coupling patterns were observed with significant overlap of proton signals from each pair of isomers.

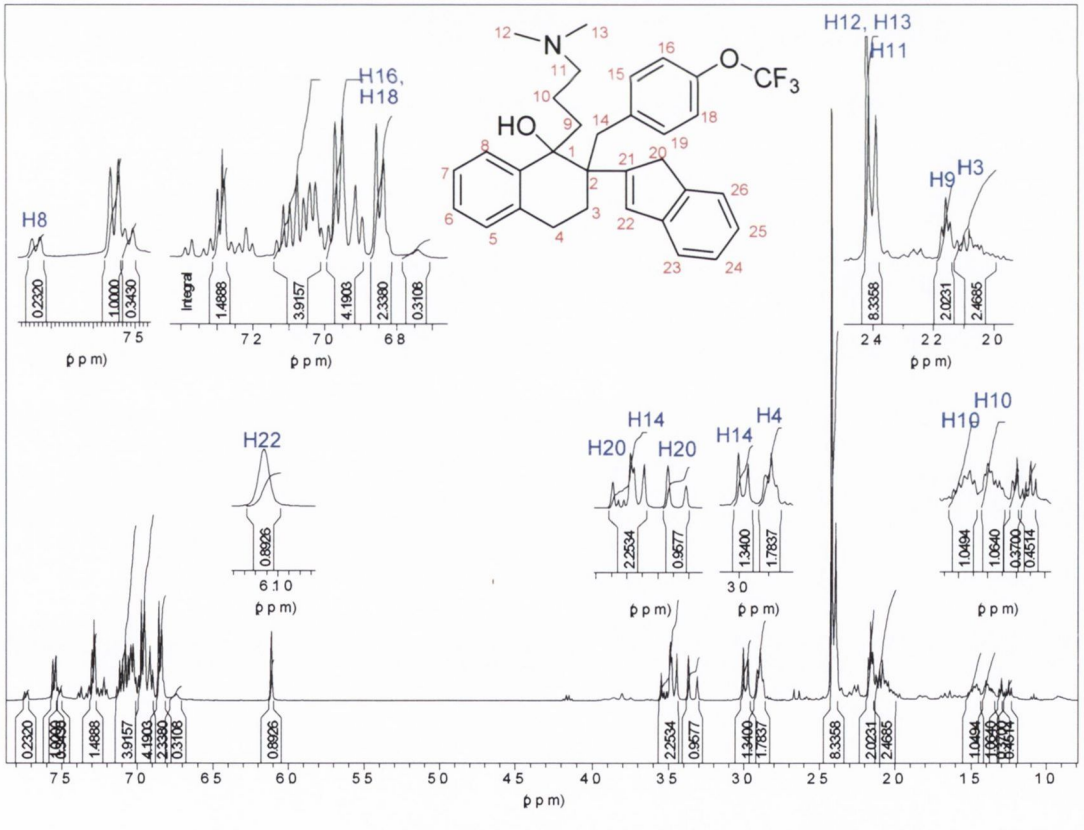


Fig. 4.34 ^{1}H NMR spectrum of compound (4.13)

In the ^{1}H NMR spectrum of compound **(4.13)a**, the first two aliphatic signals were attributed to the methylene protons on C-10, which occurred as two multiplets in the region between 1.36-1.41ppm and 1.45-1.53ppm, respectively. These signals were followed by three overlapping multiplets resonating from 1.97-2.17ppm which represented the two β–keto methylene protons at the C-3 position and the two methylene protons at the C-9 position. The signals for the two methylene protons on C-11 resonated as a multiplet overlapped slightly with two sharp singlet peaks for the six methyl protons in the region of 2.24-2.42ppm. The two benzylic protons (C-4) of the tetralone component resonated as a multiplet, occupied the region from 2.84-2.91ppm. The methylene protons on C-14 occurred separately, two doublets at 2.98ppm (J~15.28Hz) and 3.46ppm (J~15.28Hz). The remaining two methylene protons of the indene ring resonated as two separate doublets at 3.34ppm (J~25.30Hz) and 3.51ppm (J~25.30Hz). The vinylic proton appeared as a singlet at 6.11ppm. In the aromatic region, two overlapping doublets (J=8.24Hz) resonated at 6.84ppm and integrated for two protons. Seven protons resonated closely together as a multiplet from 6.89-7.13ppm, followed by a broad doublet between 7.20-7.34ppm. The terminal resonance was again that of that for the proton nearest the benzylic alcohol, resonating as a doublet (J=7.56Hz) at 7.55ppm. In the ^{13}C NMR spectrum, signals for seven methylene carbons of the predominant isomer **(4.13)a** were observed. The signals of two methyl carbons (C-12 and C-13) and the quaternary carbons (C-2 and C-1) were located at 45.1, 49.1 and 76.0ppm, respectively. The signal for the carbon in the trifluoromethoxy group was visible at 120.8ppm with $J_{F\text{-}C}$~260.3Hz. In the DEPT 135 NMR spectrum, thirteen tertiary carbons were found in the region of 119.3-131.3ppm, including the alkenic tertiary carbon (C-22) at 129.0ppm.

The ^{19}F NMR spectrum confirmed the existence of the fluorine at -58.4ppm as a singlet.

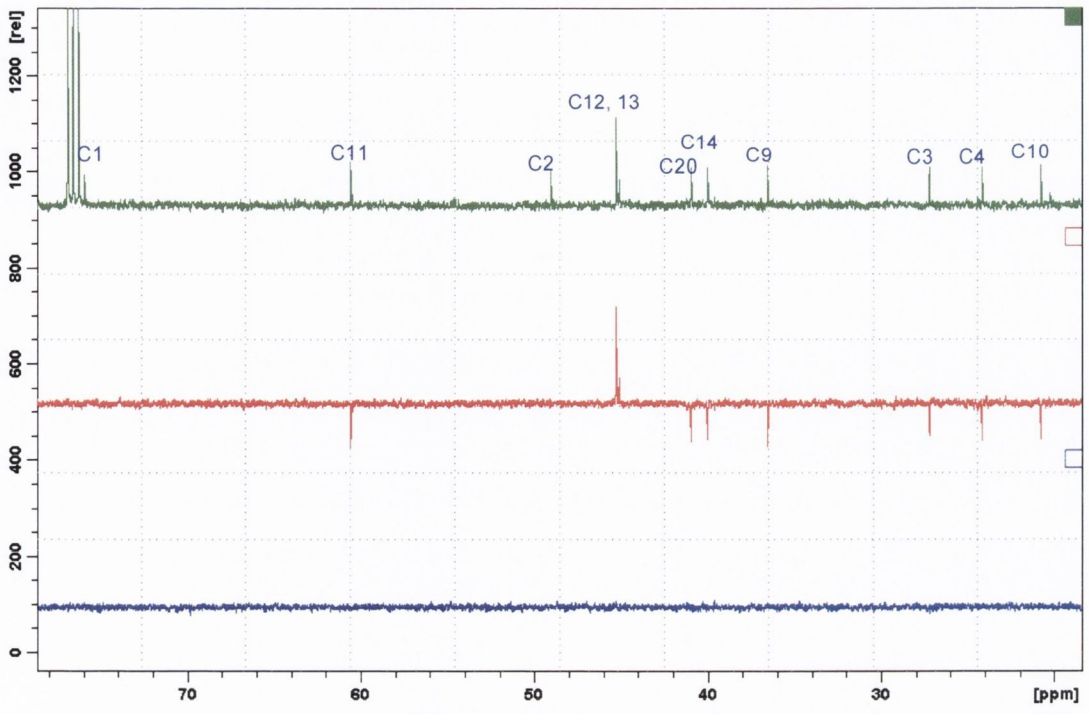


Fig. 4.35 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.13) from 18.0-78.5ppm

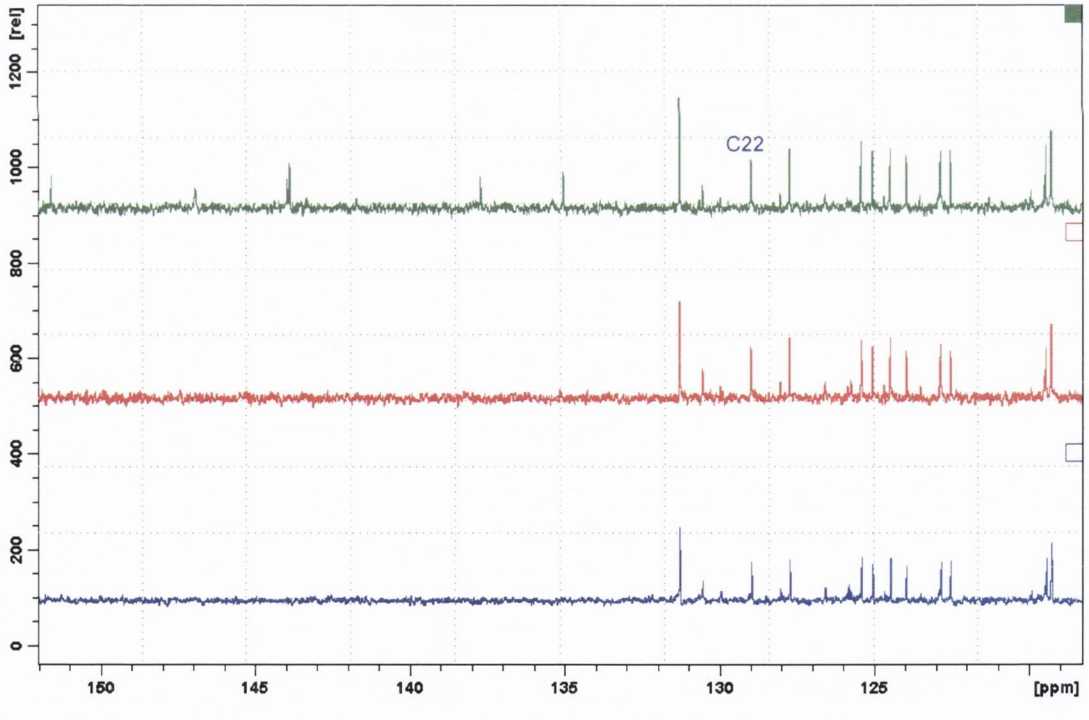


Fig. 4.36 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.13) from 118.7-152.1ppm

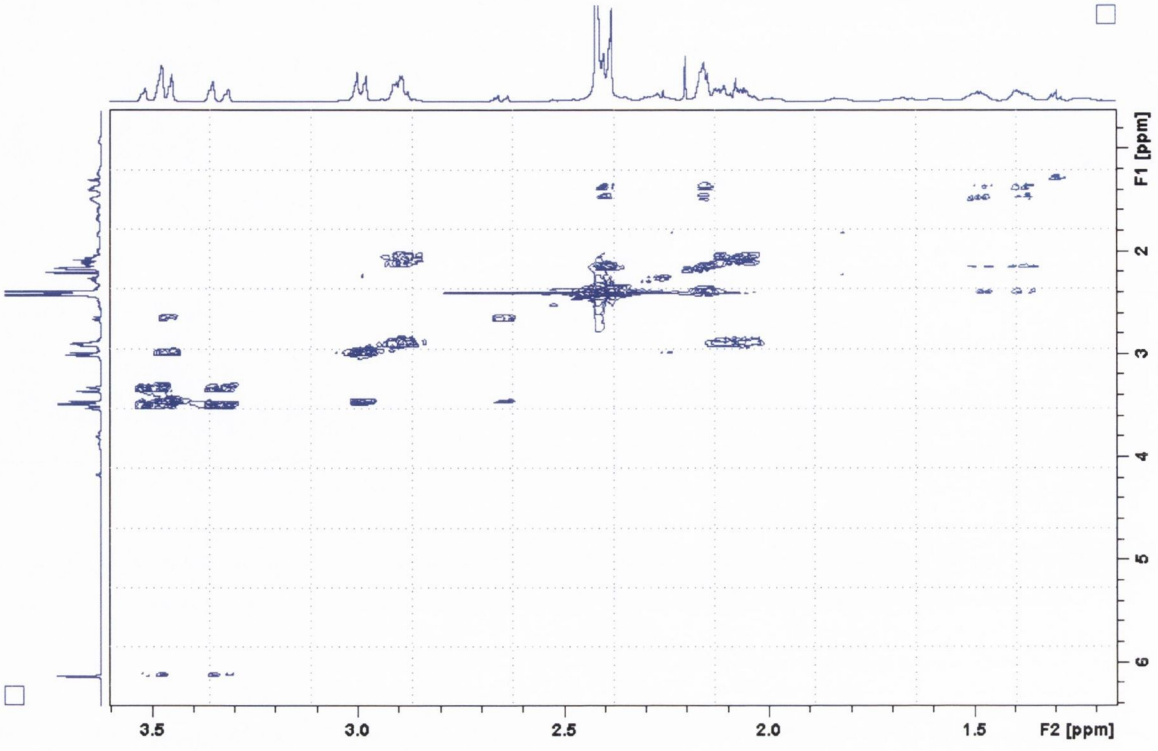


Fig. 4.37 H-H COSY NMR spectrum of compound (4.13) from 1.15-3.60ppm

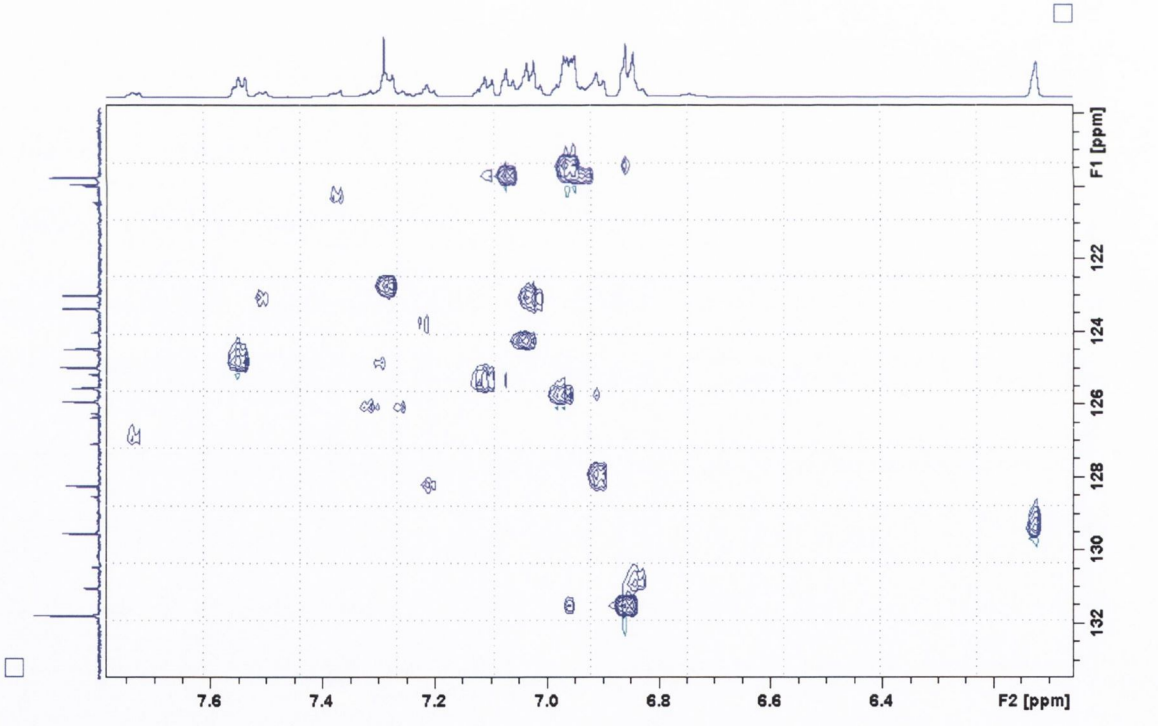


Fig. 4.38 C-H COSY NMR spectrum of compound (4.13), as stereoisomeric mixture, from 6.05-7.78ppm

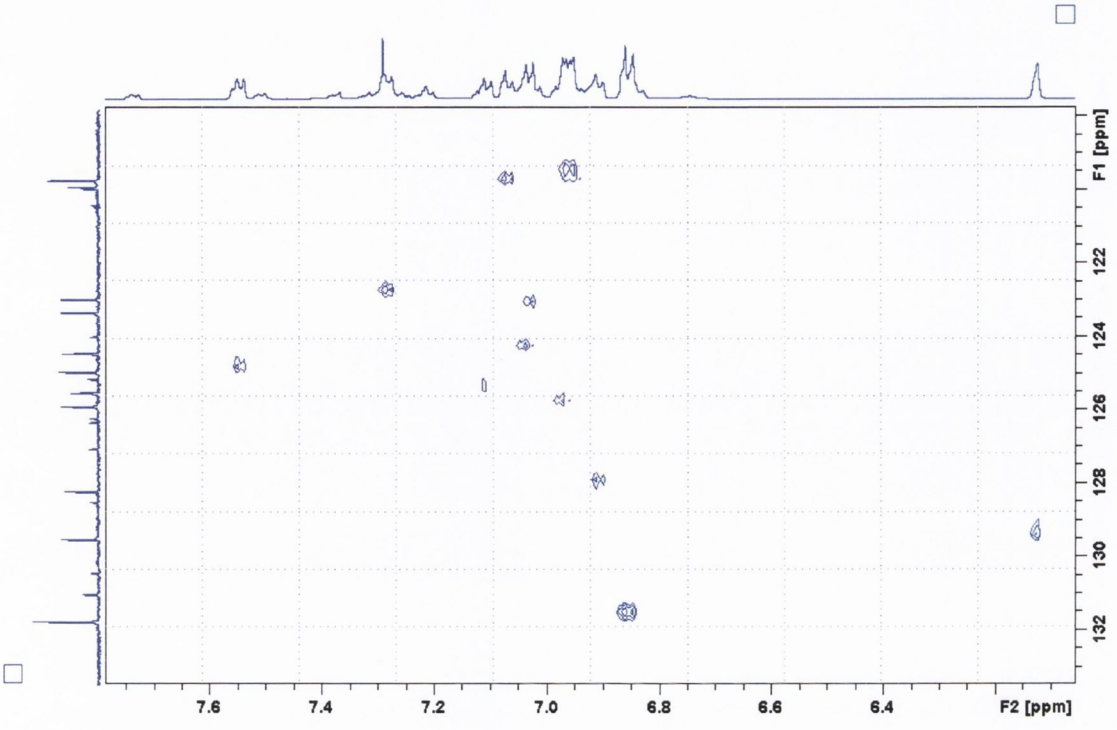


Fig. 4.39 C-H COSY NMR spectrum of compound (4.13)a, predominant isomer, from 6.05-7.78ppm

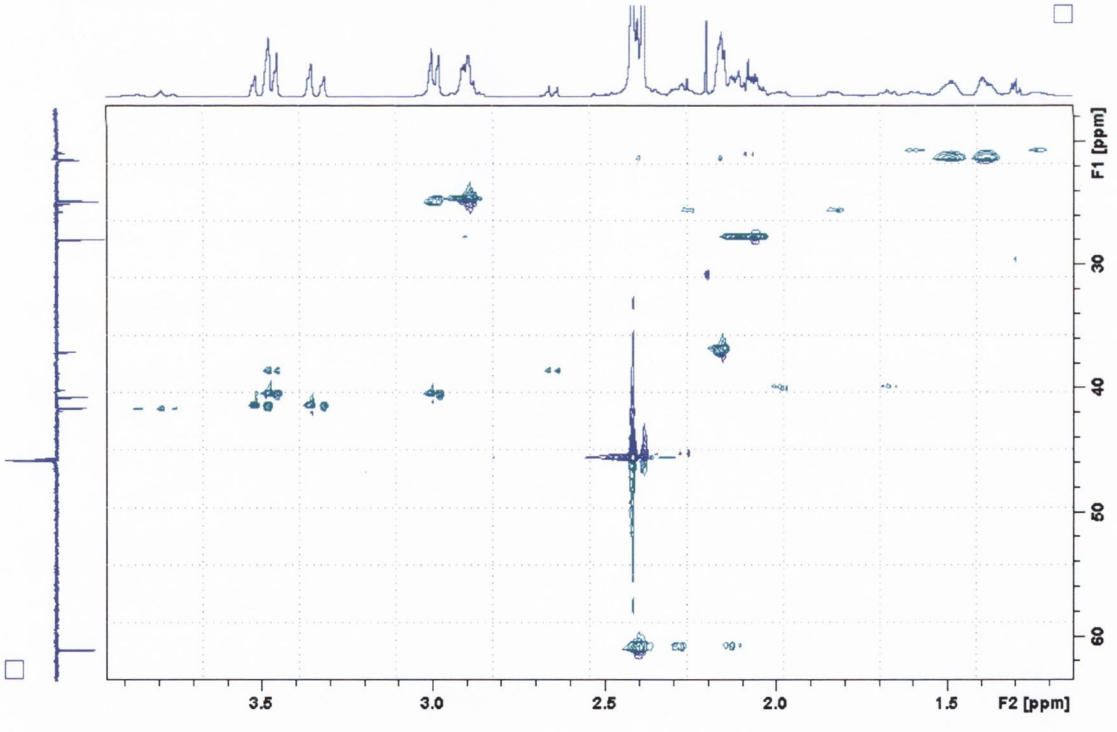


Fig. 4.40 C-H COSY NMR spectrum of compound (4.13), as stereoisomeric mixture from 1.16-3.95 ppm

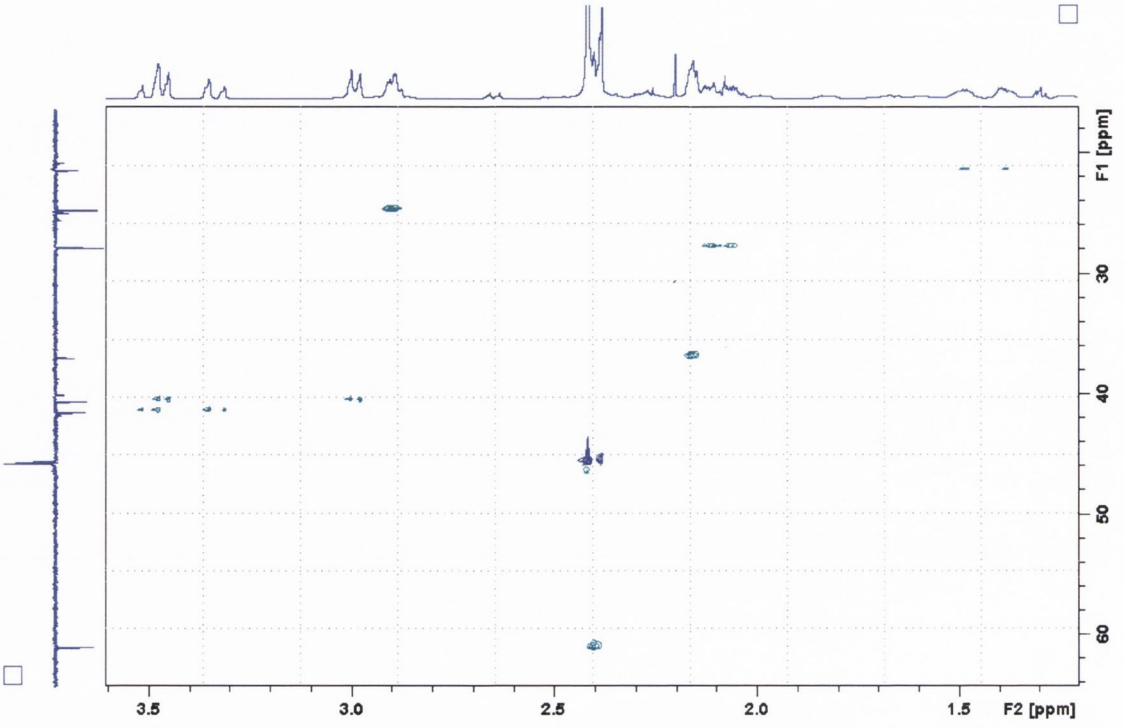


Fig. 4.41 C-H COSY NMR spectrum of compound (4.13)a, predominant isomer, from 1.20-3.60 ppm

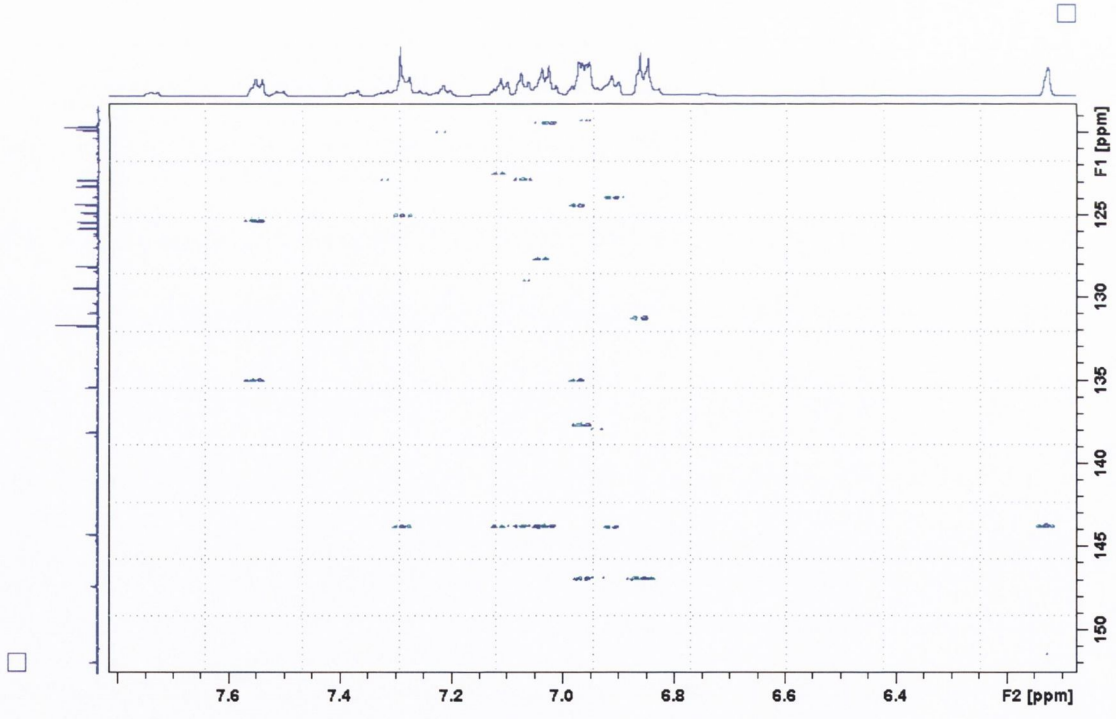


Fig. 4.42 HMBC NMR spectrum of compound (4.13) from 6.08-7.81ppm

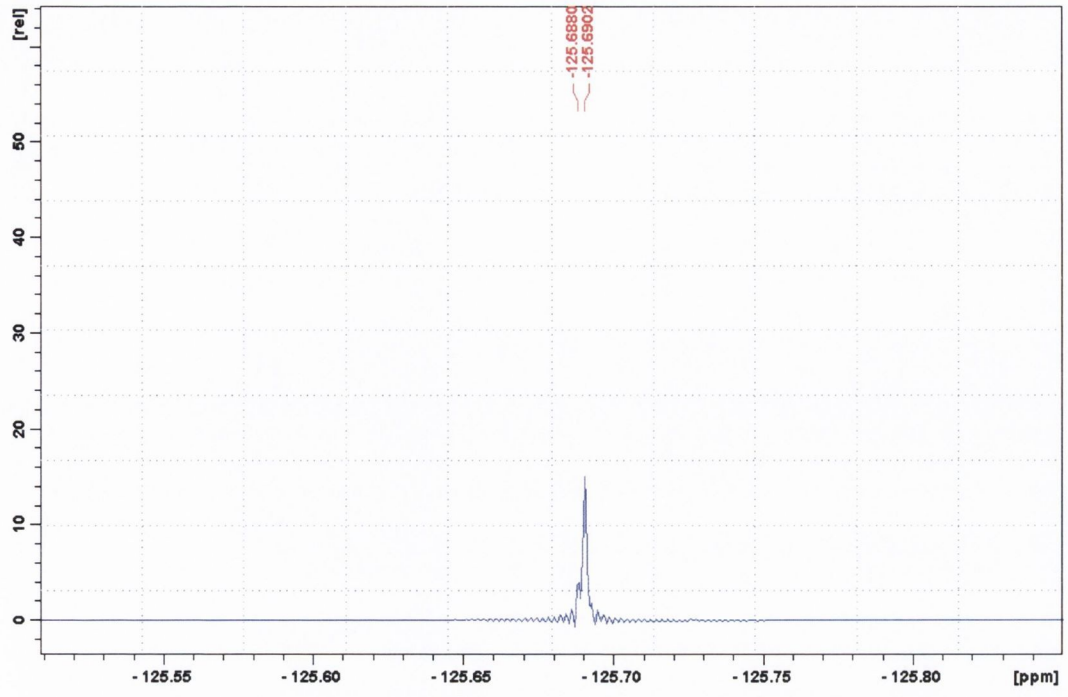
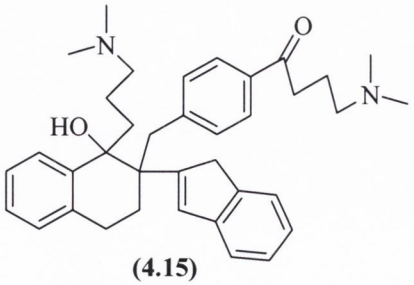


Fig. 4.43 ^{19}F *NMR spectrum of compound (4.13)*

In the infrared spectrum, compound **(4.13)** displayed the O-H_{str} at 3434.3cm^{-1}, while HRMS analysis gave the accurate molecular mass (+H^+) as 522.2618, that calculated being 522.2620, with molecular formula $C_{32}H_{35}NO_2F_3$.

4.5.2 Synthesis of amino ketone (4.15)

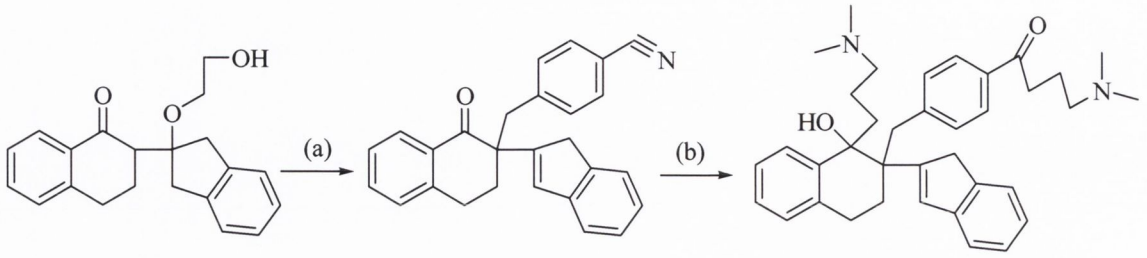
In a continuation of the thematic approach of placing electron withdrawing substituents onto the *para* position of the benzyl ring of compound **(4.4)**, it was decided to focus on the preparation of a suitable ketonic derivative that had in addition, the water-solubilising *N,N*-dimethylaminopropyl substituent attached onto its carbonyl, namely compound **(4.15)** (Fig. 4.44).



(4.15)

Fig. 4.44 Chemical structure of target compound (4.15)

In principal, it was expected that this target compound could be readily synthesised from the cyano derivative of compound **(4.4)**. The reaction of the aldol **(4.2)** with 4-(bromomethyl)benzonitrile with proceeded to give the nitrile **(4.14)** in an overall yield of 31% (Scheme 4.20).



(4.2) **(4.14)** **(4.15)a**, (30%); **(4.15)b**, (72%)

(a) 4-(bromomethyl)benzonitrile, $(CH_3)_3COK$, *t*-butanol:diethyl ether (1:1), RT, 3hrs, 31%
(b) Grignard reagent, dry THF, RT, 2.5hrs, **(4.11)a**, (30%); **(4.11)b**, (72%)

Scheme 4.20 Synthesis of compound (4.15)

Its ^{1}H NMR spectrum of compound **(4.14)** was very similar to compound **(4.12)** but with much better resolution of signals. Discrete peaks for each of the four protons of the tetralone ring were observed, a double triplet (J_1=4.59Hz, J_2=13.29Hz) at 2.18ppm, a double double doublet (J_1~2.75Hz, J_2~4.40Hz, J_3~13.76Hz) at 2.36ppm represented the two β-keto protons while a further two double double doublets at 2.86ppm (J_1~2.78Hz, J_2~4.24Hz, J_3~17.37Hz) and 3.10ppm (J_1~4.40Hz, J_2~12.76Hz, J_3~17.09Hz) accounted for the two benzylic tetralone protons. The following four doublets in the region 2.23-2.63ppm, with considerably overlapping signals, represented the remaining four methylene protons within the molecule. The vinylic proton resonated as a singlet at 6.53ppm, while all twelve aromatic proton signals were observed between 7.15-8.20ppm. Important features in the ^{13}C NMR spectrum were the signal for the nitrile carbon at 119.0ppm and the carbonyl at 198.0ppm.

The two diagnostic infrared characteristics of compound **(4.14)** were the $C{=}O_{str}$ at 1678.4cm^{-1} and the $C{\equiv}N_{str}$ at 2225.6cm^{-1}. HRMS analysis gave the accurate mass ($+H^+$) as 376.1715, calculated 376.1701 with molecular formula $C_{27}H_{22}NO$.

Having synthesised compound **(4.14)**, the next step was to perform the Grignard reaction using excess Grignard reagent to promote attack onto both the carbonyl and the cyano substituents. The reaction was complete within two hours as observed by TLC. Following work-up and purification by flash column chromatography, the corresponding alcohols, namely isomer **(4.15)a** (less polar) and isomer **(4.15)b** were isolated in moderate and good yields (Scheme 4.18).

The C-H COSY NMR spectrum proved invaluable when trying to identify the resonance position of each pair of methylene signals on compound **(4.15)a**. In the ^{1}H NMR spectrum of compound **(4.15)a**, a multiplet, slightly obscured by vacuum grease peak at 1.15-1.22ppm, was assigned to be one of the protons on C-10. A separate multiplet at 1.55-1.67ppm represented two methylene protons, presumably those protons on C-10 and C-9 (one from each position). One of the methylene protons on C-3 of the tetralin ring was seen as a multiplet in the region of 1.77-1.83ppm, followed by another multiplet at 1.84-1.88ppm considered to be the two methylene proton on C-29. This assignment was confirmed by inspection of the HMBC NMR spectrum showing a strong correlation between this signal and that of the carbonyl (Fig. 4.52). Two multiplets in the regions of 1.95-2.01ppm and 2.11-2.15ppm, accounted for the second proton on C-9 and one methylene proton attached to the carbon containing the amino substituents (C-11 or C30), respectively. The following four sharp singlets at 2.21, 2.23, 2.39 and 2.42ppm were assigned to be the four methyl groups, which integrated for twelve protons. The remaining methylene protons at C-3, C-11 and C-30 positions appeared as a multiplet in the region of 2.25-2.33ppm, integrated as four protons. The benzyl methylene protons (C-14) resonated as two doublets (J= 14.14Hz) at 2.69 and 3.51ppm, respectively. Those methylene protons on C-28 and C-4 are likely to be accounted for by the following four protons resonating between 2.92-3.09ppm, owing to their ability to freely rotate and the fact that the triplet couples to the carbonyl carbon observed in the HMBC NMR spectrum. The indene methylene signal resonates as a broad triplet at 3.86ppm. The aromatic and double bond regions of the spectrum were somewhat complex. However the vinyl proton was apparent at 6.71ppm resonating as a very broad singlet. The two

doublets at 6.90ppm (J=8.12Hz) and 7.69ppm (J=8.20Hz) were likely to represent the protons on carbons 15, 19 and 16, 18 respectively. The only other discrete signal in this region represented the proton on C-8, appeared as a doublet (J=7.28Hz) at 7.73ppm.

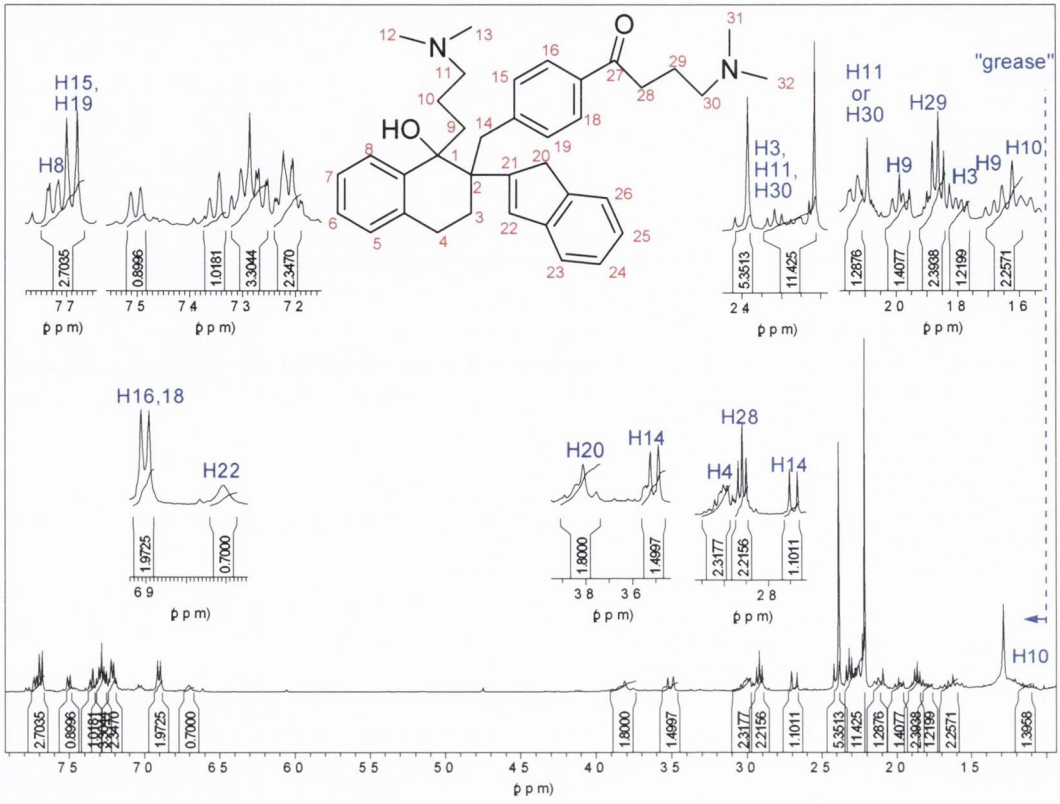


Fig. 4.45 ^{1}H NMR spectrum of compound (4.15)a

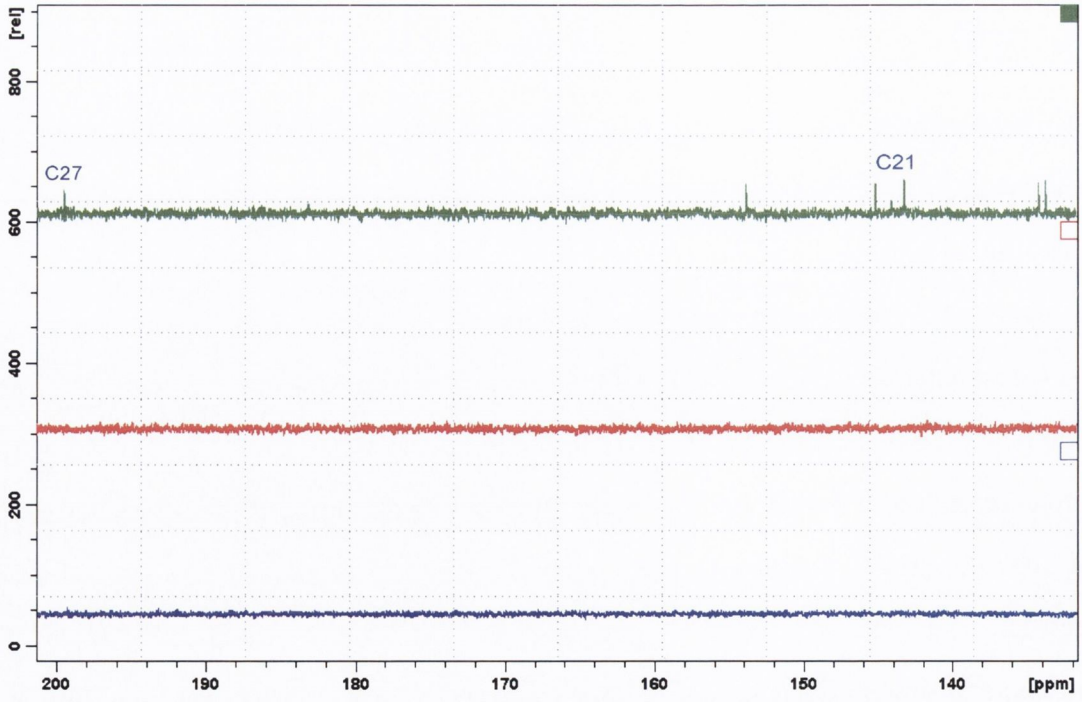


Fig. 4.46 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.15)a from 131.9-201.7ppm

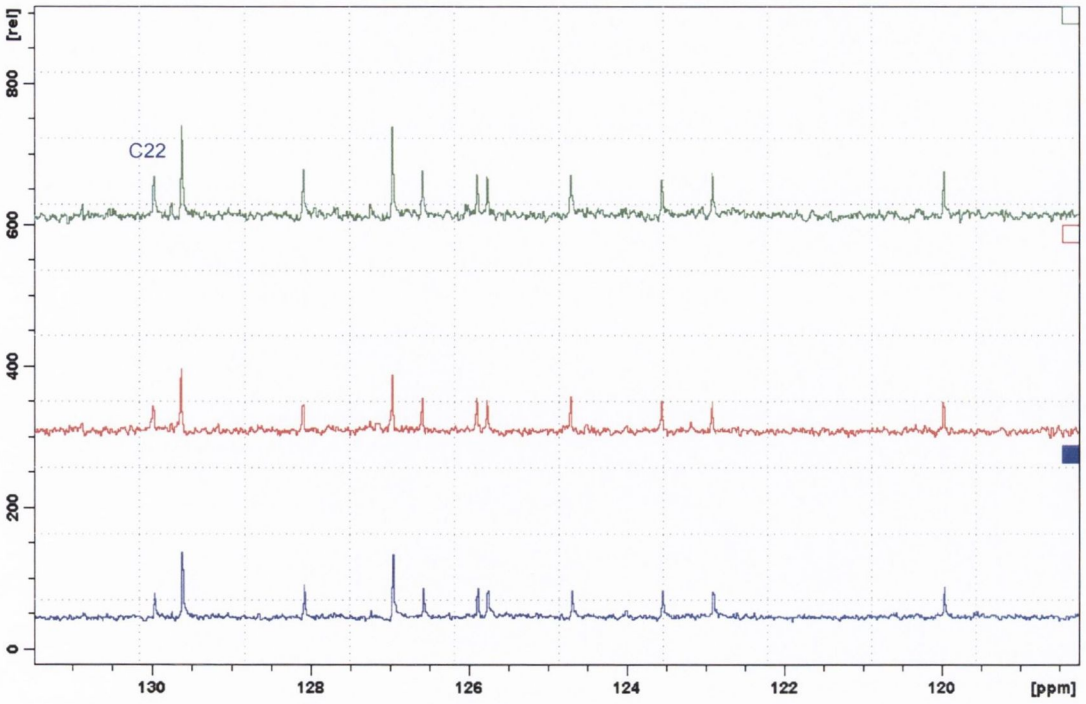


Fig. 4.47 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.15)a from 108.4-131.5ppm

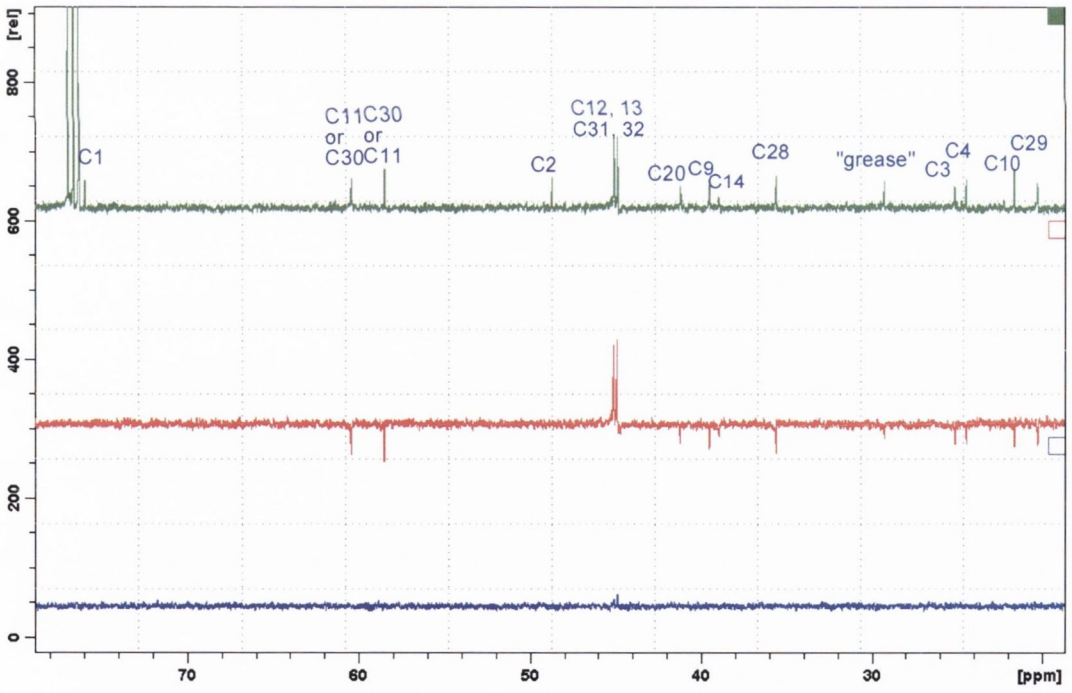


Fig. 4.48 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (4.15)a from 18.4-78.3ppm

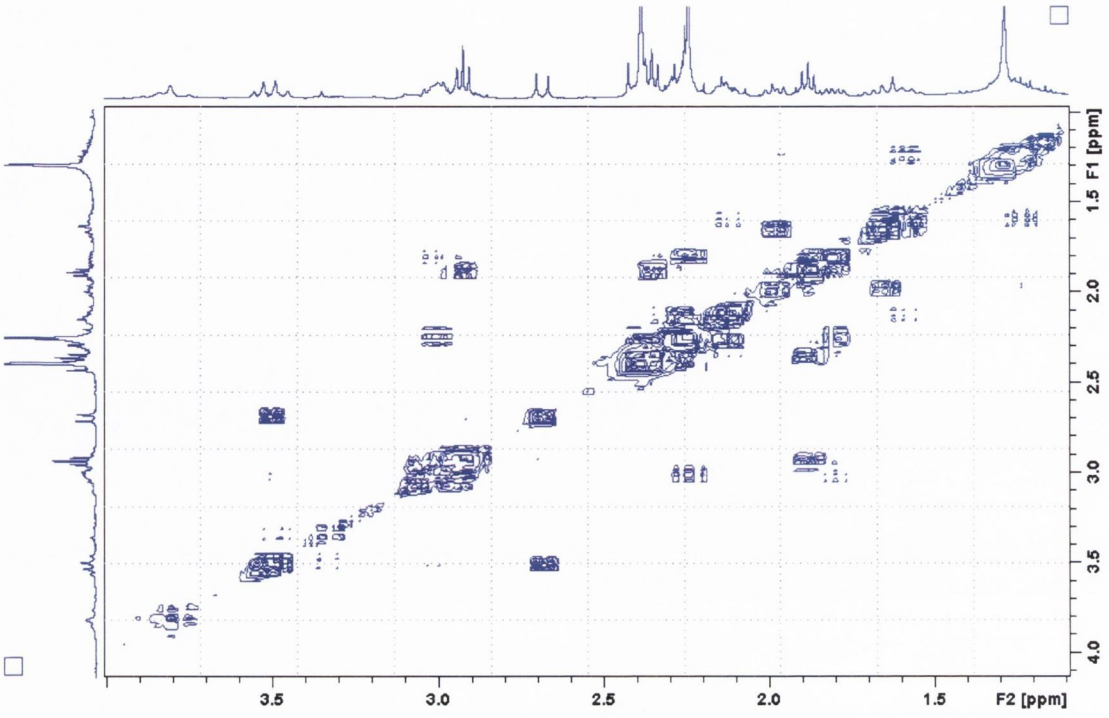


Fig. 4.49 H-H COSY NMR spectrum of compound (4.15)a from 1.10-4.00ppm

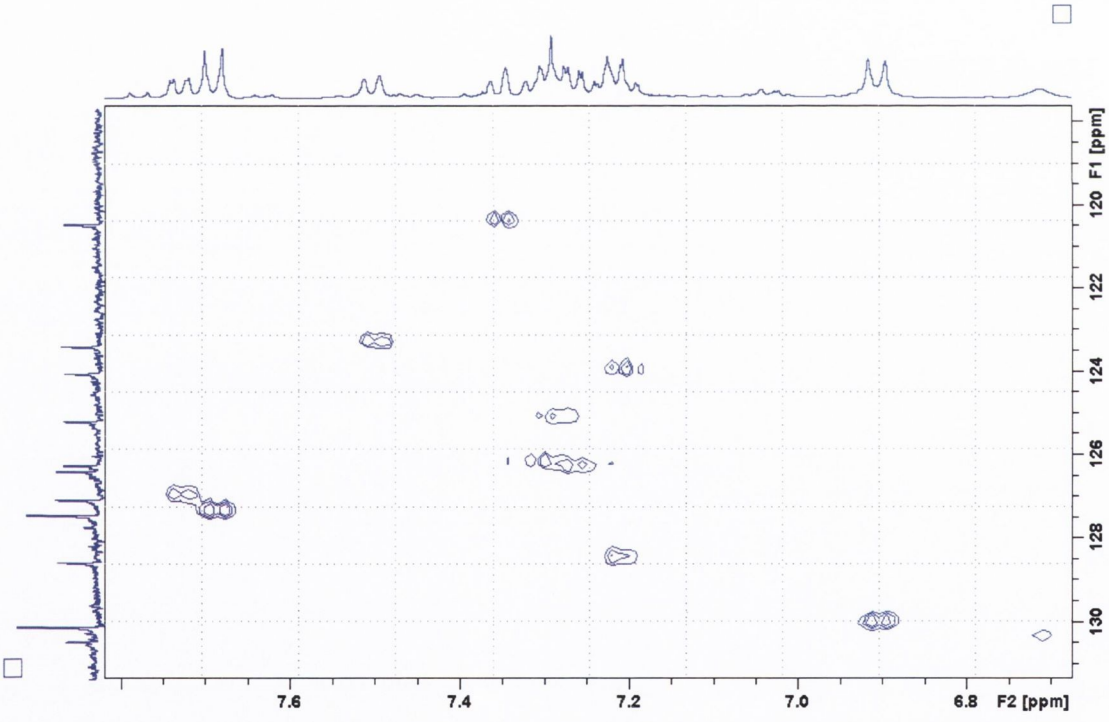


Fig. 4.50 C-H COSY NMR spectrum of compound (4.15)a from 6.75-7.84ppm

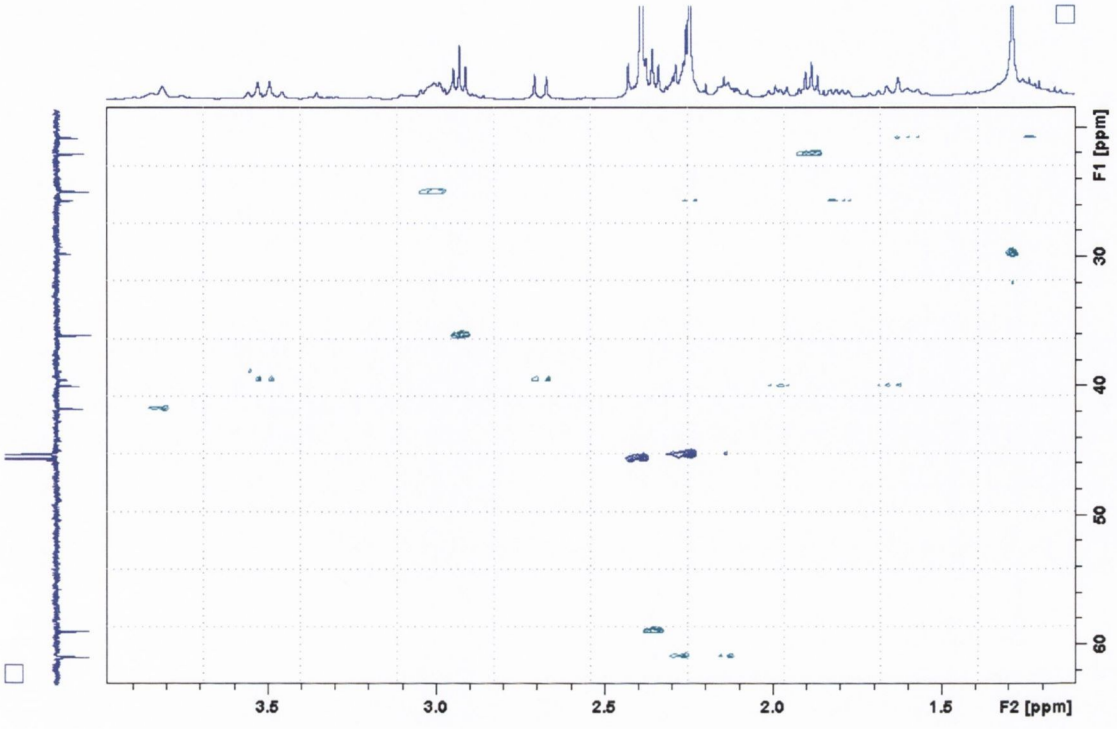


Fig. 4.51 C-H COSY NMR spectrum of compound (4.15)a from 1.10-3.96ppm

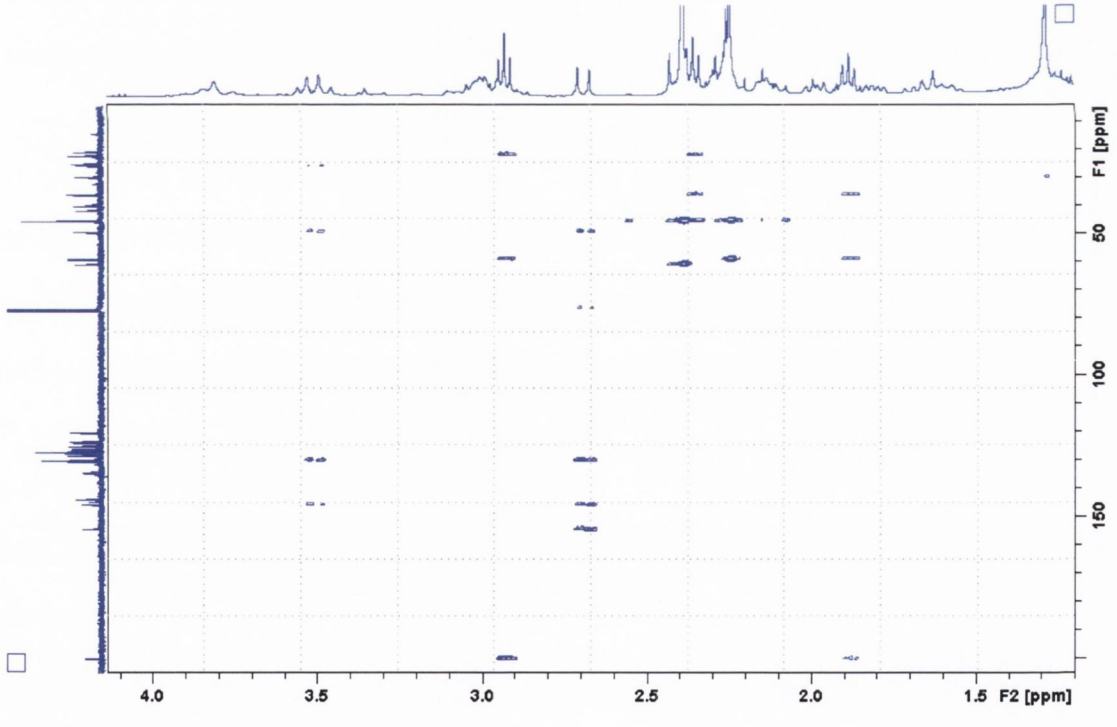


Fig. 4.52 HMBC NMR spectrum of compound (4.15)a from 1.20-4.15ppm

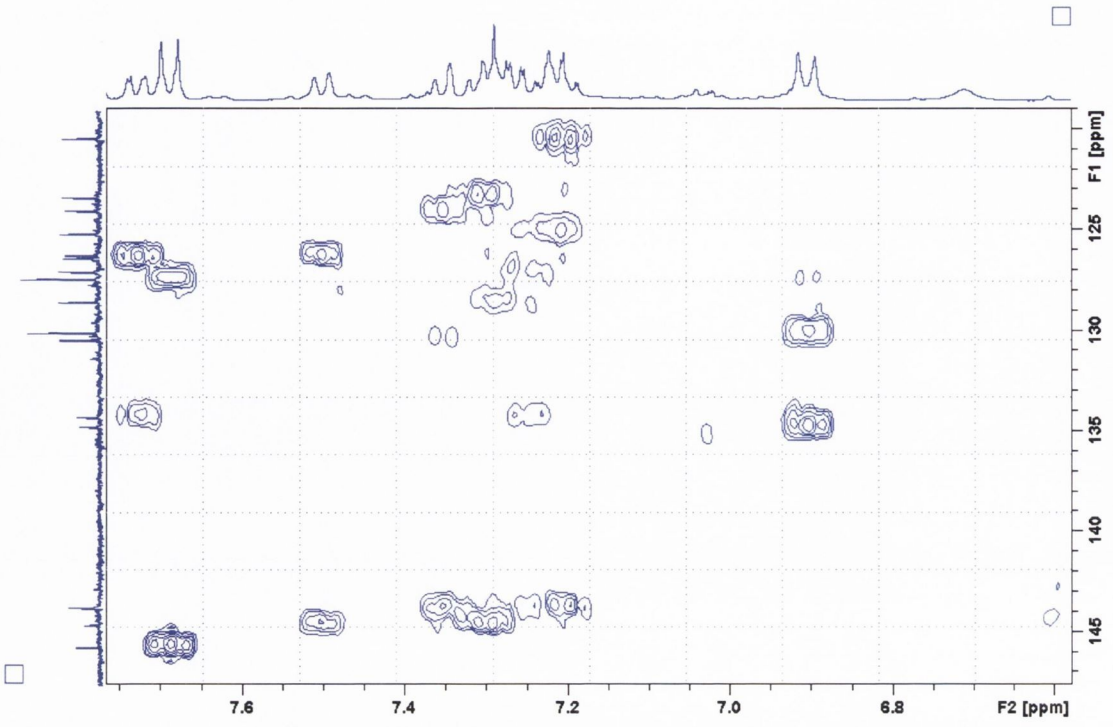


Fig. 4.53 HMBC NMR spectrum of compound (4.15)a from 6.64-7.78ppm

The DEPT 90 NMR spectrum of isomer **(4.15)a** contained eight methylene carbons at 20.3, 21.7, 24.5, 25.2, 35.6, 39.0, 39.5 and 41.2ppm, which represented those carbons at C-10, C-29, C-4, C-3, C-28, C-14, C-9 and C-20 respectively. The signals of the two set of dimethyl groups were located separately at 44.9 and 45.1ppm. The remaining two methylene carbon, those with the amino substituents attached, resonated at 58.5 and 60.5ppm (Fig. 4.48). The ^{13}C NMR spectrum confirmed the presence of the quaternary carbons (C-2) and (C-1), at 48.7 and 75.9ppm, respectively. Signals for the twelve aromatic tertiary carbons were also observed in between 120.0-129.6ppm while the alkenic tertiary carbon resonated at 130.0ppm. The absence of the six quaternary aromatic carbons in the region of 133.8-153.9ppm was verified by the DEPT 135 NMR spectrum. The carbonyl signal was at 199.5ppm. The above assignment of the spectra was confirmed by the H-H COSY, C-H COSY and HMBC experiments (Fig. 4.49-Fig. 4.53).

The infrared spectrum of compound **(4.15)a** gave the characteristic $C{=}O_{str}$ at 1679.7cm^{-1}, and the strong alcohol $O{-}H_{str}$ at 3410cm^{-1}. Accurate molecular mass ($+H^+$) as 551.3578,

calculated 551.3632 with molecular formula $C_{37}H_{47}N_2O_2$ was confirmed by HRMS analysis.

In the aliphatic region, the ^1H NMR spectrum of compound **(4.15)b** showed more complex coupling patterns and signals overlap than that of compound **(4.15)a**, two discrete broad multiplets resonated from 1.31-1.38ppm and 1.41-1.49ppm, integrated for a total of four protons. Four methylene groups resonated closely together as overlapping signals with two sharp singlets for the four methyl groups, from 2.05-2.40ppm, integrating for a total of twenty protons. The two benzylic tetralone protons appeared as a multiplet located between 2.84-2.89ppm, followed by two overlapping triplets (J=7.24Hz) at 2.93ppm, for the two protons at C-28 position. The remaining four methylene protons stretched from 3.20-3.31ppm and 3.48-3.55 as two discrete multiplets. The hydroxyl proton and the alkenic proton appeared as a very broad singlet and a very sharp singlet at 5.83 and 6.08ppm, respectively. The aromatic region of the ^1H NNMR spectrum was slightly better resolved than that of compound **(4.15)a**, with eight aromatic protons between 6.88-7.07ppm, followed by two separate doublets at 7.27ppm (J=7.16Hz) and 7.52ppm (J=7.56Hz) for two protons. The terminal signal appeared as two overlapping doublets (J=8.02Hz) centred at 7.70ppm, integrating for two protons. The ^{13}C NMR spectrum pattern of compound **(4.15)b** was slightly different to that of **(4.15)a**. The signal of the alkenic tertiary carbon was shifted upfield to 128.9ppm. The alkenic quaternary carbon signal resided at 143.8ppm with the other two aromatic quaternary carbons. The carbonyl signal was found at 199.6ppm. The structural determination was carried out with the aid of H-H COSY, C-H COSY and HMBC NMR spectra (Fig. 4.56-Fig. 4.58).

The infrared spectrum of compound **(4.15)b** contained $C=O_{str}$ at 1678.9cm^{-1}, and broad $O-H_{str}$, centred at 3339.1cm^{-1}, while HRMS analysis also give the accurate molecular mass ($+H^+$) as 551.3634, calculated 551.3638.

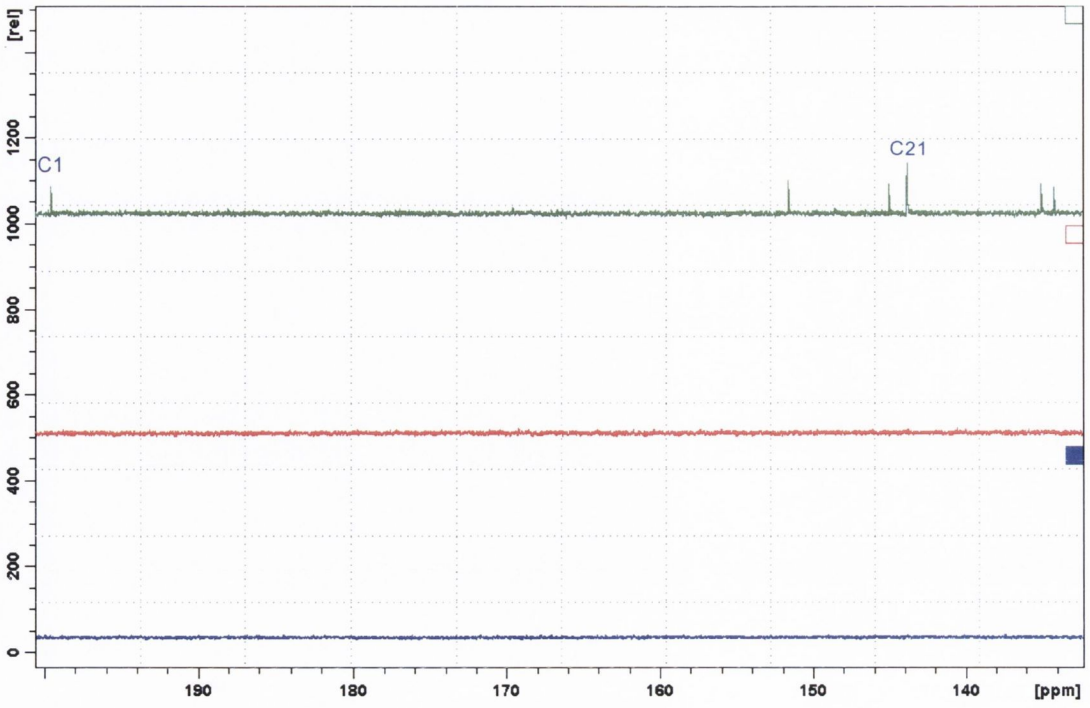


Fig. 4.54 ^{13}C, *DEPT 135 and DEPT 90 NMR spectra of compound (4.15)b from 136.3.-195.1ppm*

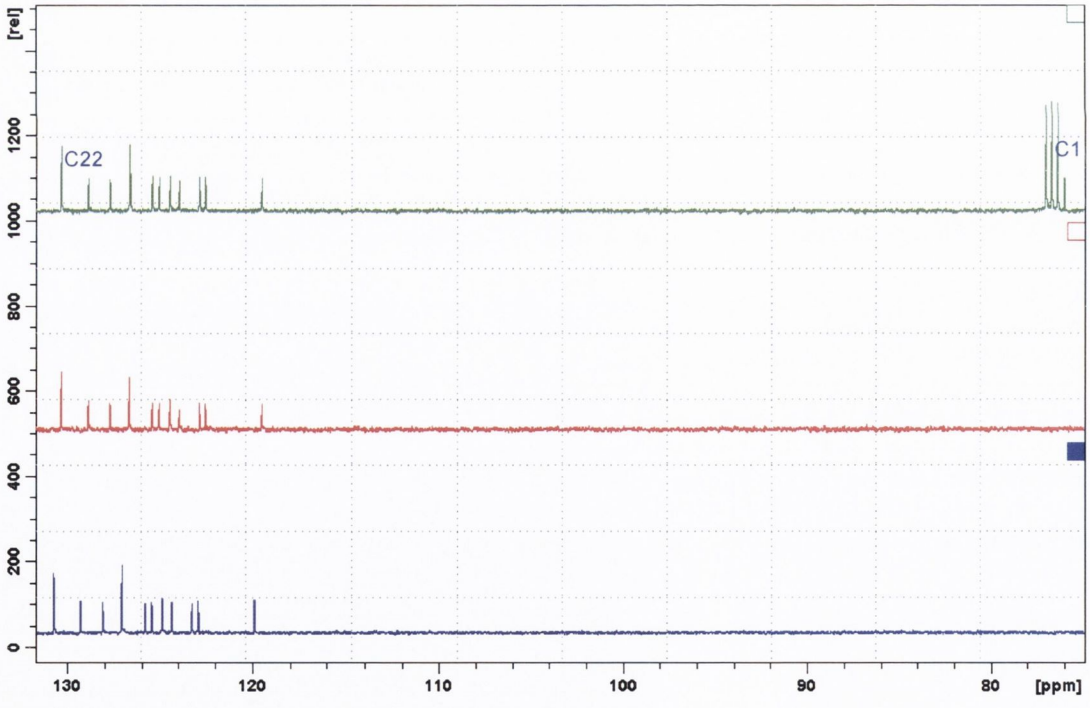


Fig. 4.55 ^{13}C, *DEPT 135 and DEPT 90 NMR spectra of compound (4.15)b from 85.3.-131.2ppm*

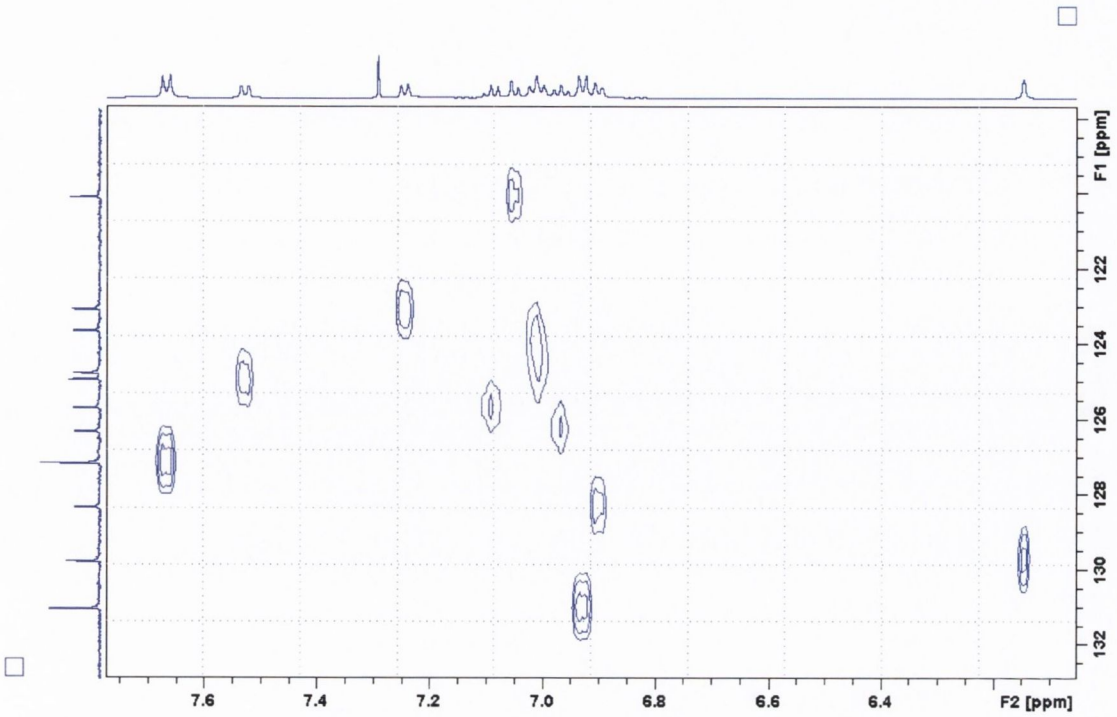


Fig. 4.56 C-H COSY NMR spectrum of compound (4.15)b from 6.05-7.78ppm

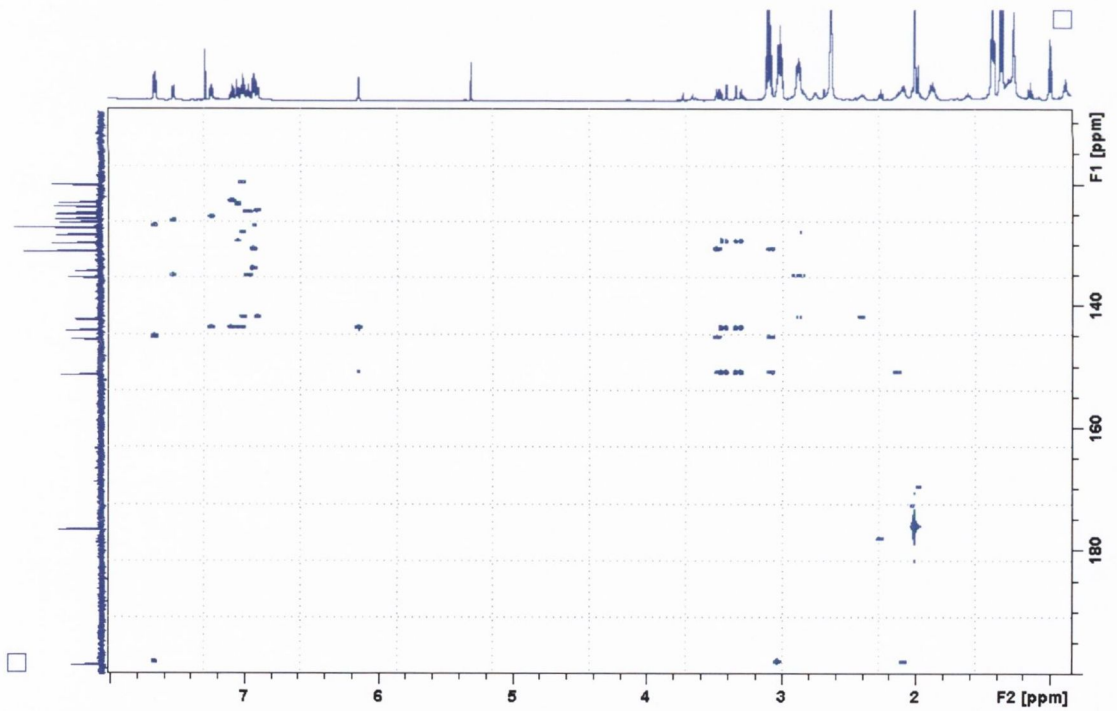


Fig. 4.57 HMBC NMR spectrum of compound (4.15)b from 0.80-8.00ppm

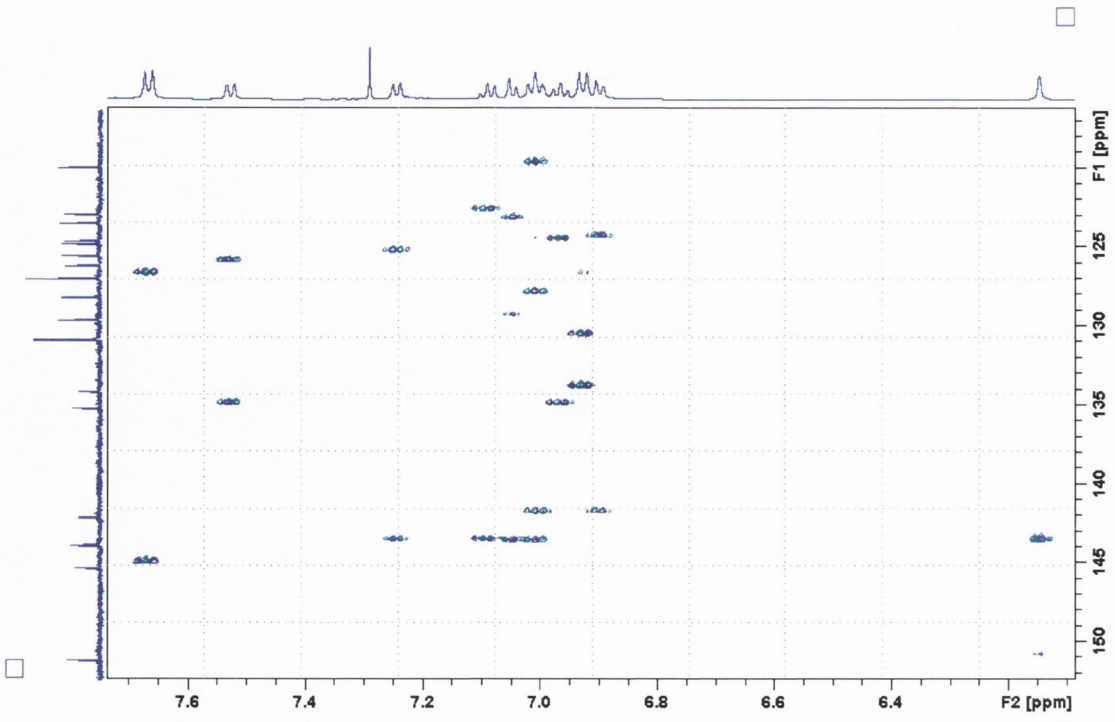


Fig. 4.58 HMBC NMR spectrum of compound (4.15)b from 6.59-7.95ppm

4.6 Conclusion

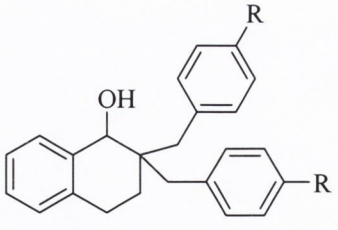
A series of indanol and tetrahydronapthol-based dimer compounds were synthesised by Barlow (2002), which showed impressive *in vitro* activity but poor activity *in vivo*. The purpose of the work described in this Chapter was to address this imbalance. The strategy employed was to retain the basic features of these compounds in the new design while at the same time incorporating into their structure a polar water-solubilising group. This was conveniently achieved by appending onto to the molecules, a *N,N*-dimethylaminopropyl side chain. The targeted compounds in question were synthesised through a directed aldol condensation reaction, followed by a benzylation reaction on the aldol product in each case. The final step in their synthesis involved attachment of the *N,N*-dimethylaminopropyl side chain onto the ketonic position of the alkylated products, which was conveniently achieved by a Grignard reaction to give the amino alcohols. All compounds synthesised were fully characterised by IR, NMR and HRMS. Of the compounds synthesised the only one to consistently demonstrate an effect *in vivo* was the amino alcohol **(4.10)**, which inhibited the PCA reaction by 63 ± 9.2% (Woods, 2009). It seems evident from the data obtained on these compounds that substitution on the *para*-position of the benzyl ring is detrimental towards their activity *in vivo*.

5.1 Introduction

Up to this point, the dimeric tetralin-indan compounds described in the preceding Chapter have been subjected to preliminary *in vivo* pharmacological evaluation as novel mast cell-stabilising agents, by Woods. Promising activity has been observed with compound **(4.10)** in particular. A common thread flowing through each compound described is their high aromatic content suggesting that π-rich centres are necessary for the observed activity. Although, their synthesis was achieved in five steps starting from the parent tetralone or indanone, it still yielded final compounds which at best could be presented as a mixture of enantiomers. Their isolation could only be achieved after extensive purification by a combination of flash column and preparative column chromatography.

5.2 Synthetic targets and strategy

Initial studies on the synthesis of compounds depicted in Fig 5.0 were performed by Finn (2007). Woods (2009) has demonstrated that compounds within this series display potent mass cell stabilisation activity, when compound 48/80 and A23187 were used to induce histamine release from rat peritoneal mast cells. In this Chapter, it was decided to expand upon this series, but place particular emphasis on the synthesis of compounds, namely structural types **(H)**, **(I)**, **(5.41)** and **(5.43)**, within Fig. 5.1, as the compounds depicted in Fig 5.0 were too insoluble to allow for their evaluation *in vivo*.



R = H, Cl, *t*-butyl, or O-CF_3

Fig. 5.0

The aim of the work outlined in this Chapter was to; (i) generate highly aromatic mast cell stabilising compounds analogous to those described in the preceding Chapter, but

which would require fewer steps to synthesise, (Fig. 5.1), (ii) reduce the number of potential asymmetric centres in the final compounds, (iii) build into their structure the 3-(dimethylamino)propylidene group for possible H1 binding/antihistamine activity, (iv) enhance the solubility of lead compounds by inclusion of a water-solubilising acetic acid group akin to that incorporated into the structure of the anti-allergy drug, Olapatadine, (v) use the acetic acid residue to promote through hydrogen bonding the formation of a single geometric isomer of the compound **(5.41)** depicted in Fig. 5.3, namely compound **(5.43)**.

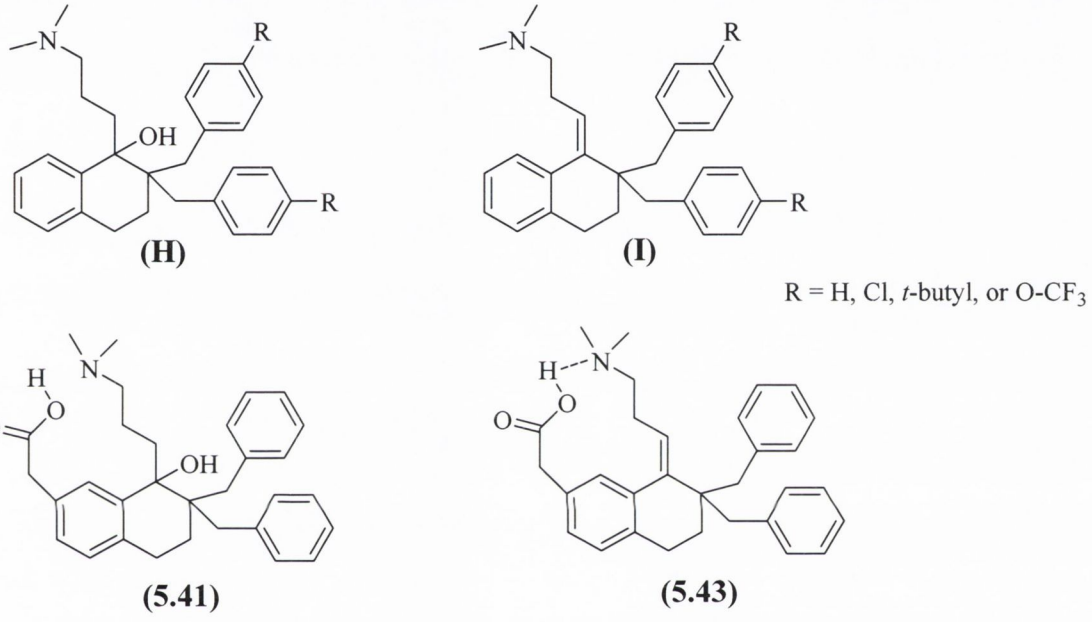
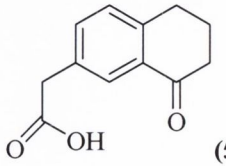


Fig. 5.1 Target compounds of structural types of (H), (I), (5.41) and (5.43)

In general, compounds that fall within Fig. 5.1 were synthesised in two and three steps respectively. Their synthesis commenced from α–tetralone and the appropriate benzyl halide and used potassium *tert*-butoxide as base, in a solvent mixture consisting of diethyl ether and *t*-butanol (*v:v,* 1:1). Introduction of the 3-(dimethylamino)propyl side chain onto the alkylated tetralone derivatives proceeded through a Grignard reaction to furnish the amino alcohols of structural type **(H)**. Subsequent dehydration of these alcohols afforded the amino alkenes of structural type **(I)**.



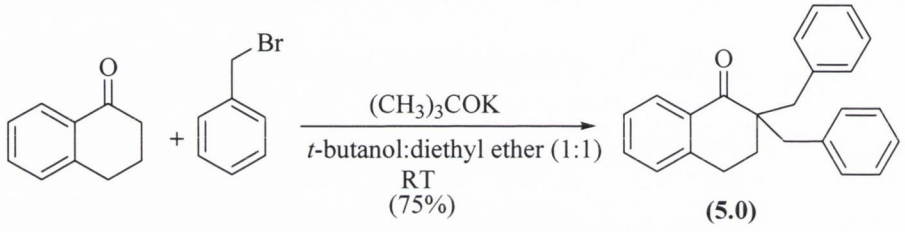
(5.34)

Fig. 5.2 Chemical structure of compound (5.34)

Although the same sequence of step was used to synthesise compounds **(5.41)** and **(5.43)**, it was nevertheless required to design an appropriate method to synthesise compound **(5.34)** (Fig. 5.2), namely 2-(1,2,3,4-tetrahydro-1-oxonaphthalen-7-yl)acetic acid, the basic building block used to synthesise the target compounds **(5.41)** and **(5.43)**.

5.3 Synthesis of dibenzyl amino alcohol (5.1)

The first compound to be synthesised within Fig 5.1 was the amino alcohol **(5.1)**. Its synthesis was complete in two steps. The first step involved alkylaton of α–tetralone with benzyl bromide using potassium *tert*-butoxide as base *t*-butanol to give ketone **(5.0)** in 75% yield (Scheme 5.0).

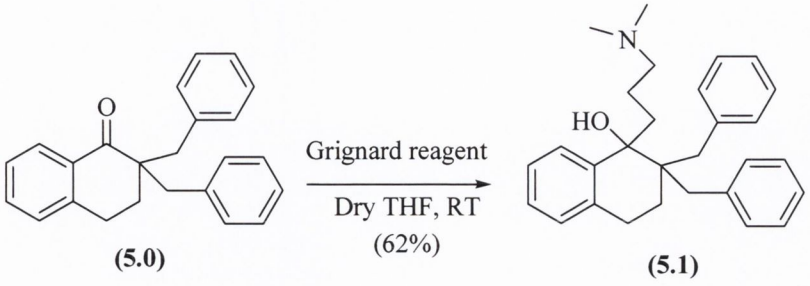


Scheme 5.0 Synthesis of dibenzyl (5.0)

The ^1H NMR data obtained on **(5.0)** was as expected in agreement with the literature data (Finn, 2008). Two overlapping triplets (J=6.40Hz) at 1.97ppm represented the two methylene protons at the β–keto position of the tetralone ring. This resonance was followed by two overlapping doublets (J=13.40Hz) at 2.71ppm corresponding to the two methylene protons from the benzyl group. The signals for the two benzylic protons of the tetralone ring were located at 3.07ppm as two overlapping triplets (J=6.36Hz). The remaining two methylene protons of the benzyl group were found at 3.37ppm as two overlapping doublets (J=13.40Hz). A total of thirteen aromatic protons resonated in the region of 7.16-7.47ppm, with a double doublet signal (J_1=1.26Hz, J_2=7.86Hz) at 8.14ppm for the aromatic proton nearest the carbonyl group. Compound **(5.0)** exhibited the characteristic peak of $C=O_{str}$ at 1677.6cm^{-1} in its infrared spectrum.

Having synthesised the ketone **(5.0)**, a Grignard reaction was used to attach the *N,N*-dimethylaminopropyl group onto the ketone **(5.0)**. Using dry THF as solvent,

coupling of [3-(dimethylamino)-propyl]magnesium chloride to the ketone **(5.0)** was performed at room temperature. This transformation occurred smoothly to produce the amino alcohol **(5.1)** in reasonable yield (62%) after fifteen hours.



(5.0) **(5.1)**

Scheme 5.1 Synthesis of compound (5.1)

^{1}H NMR analysis of compound **(5.1)** (see Fig. 5.3) showed two discrete multiplets integrating as two protons between 1.22-1.29ppm and 1.42-1.51ppm, respectively, corresponding to the two methylene protons on C-10 position. The following three multiplets each representing two protons were found at 1.77-1.89ppm, 1.92-2.05ppm and 2.26-2.37ppmm, respectively. The six methyl protons appeared as a sharp singlet at 2.43ppm. One of the methylene protons at C-14 (or C-20) position resonated as a doublet (J=14.04Hz) centred at 2.58ppm, followed by a multiplet at 2.65-2.74ppm integrating for one proton. In the region between 2.86-2.91ppm, a doublet signal (J=14.04Hz) representing one of the methylene protons at C-14 (or C-20) was overlapping with another proton signal. The signals of the remaining two protons at C-20 (or C-14) appeared as two doublets, at 3.07ppm (J=13.80Hz) and 3.26ppm (J=14.04Hz). In the aromatic region of the ^{1}H NMR spectrum, two protons overlapping as a multiplet at 6.74-6.76ppm, followed by a doublet (J=7.52Hz) at 7.07ppm integrating for one proton. Most of the remaining aromatic protons were in two complex patterns in the regions of 7.16-7.23ppm and 7.26-7.32ppm, integrating as ten protons. An isolated double doublet (J_1=1.24Hz, J_2=7.52Hz) was visible at 7.68ppm, representing the proton on C-8.

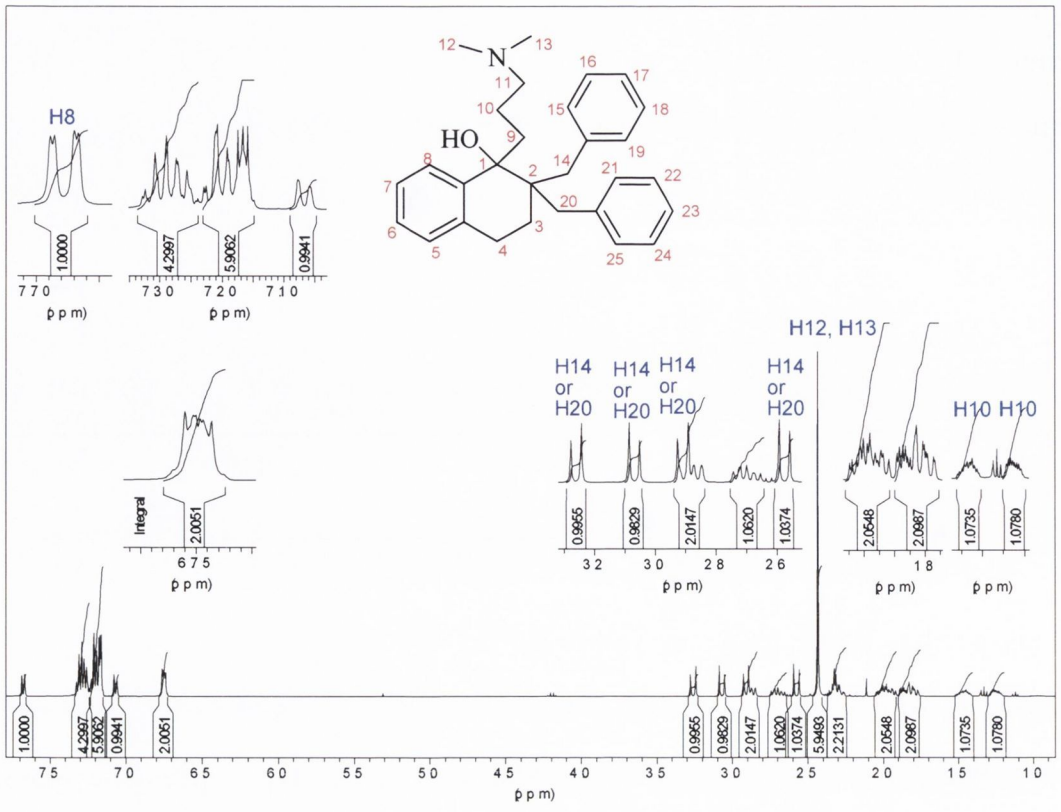


Fig. 5.3 H NMR spectrum of compound (5.1)

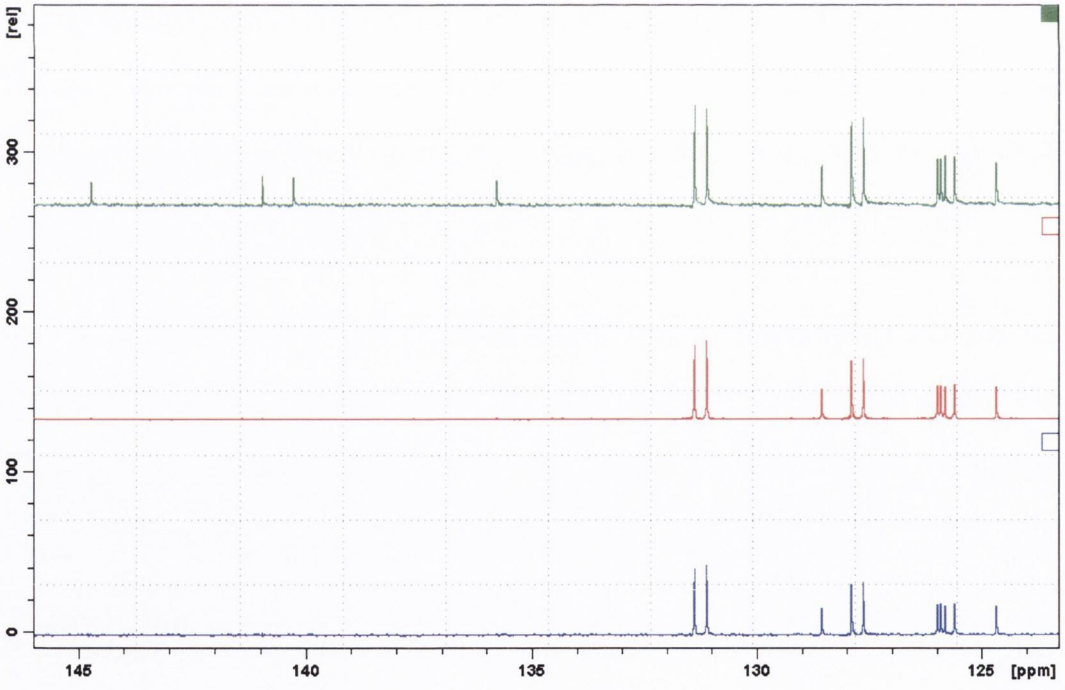


Fig. 5.4 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (5.1) from 123.5-146.1ppm

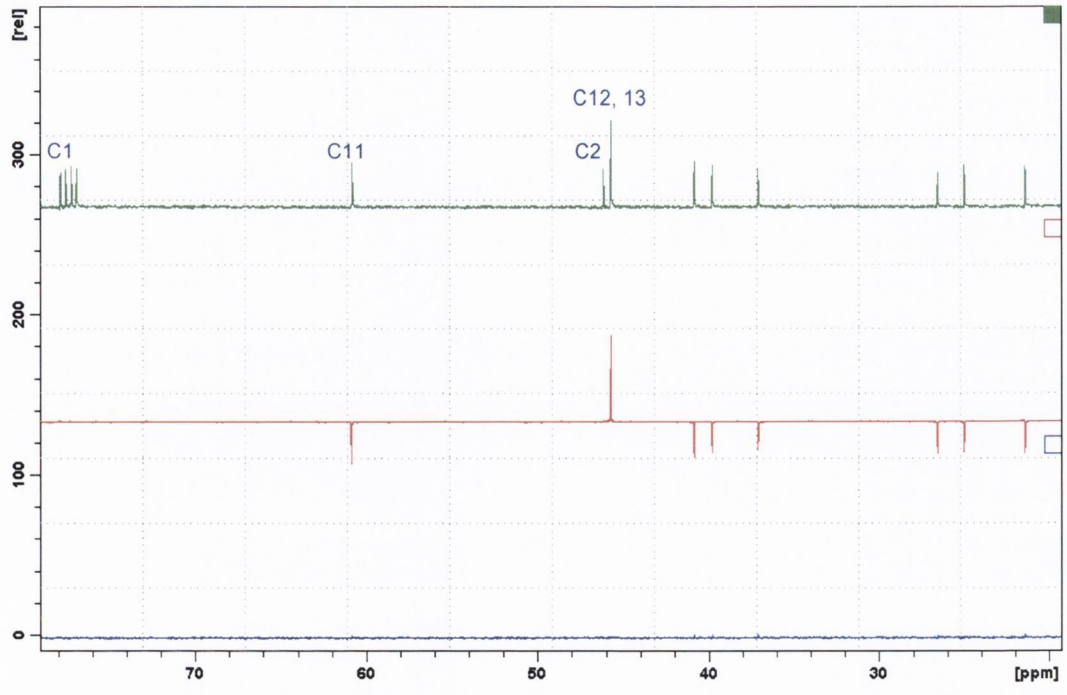


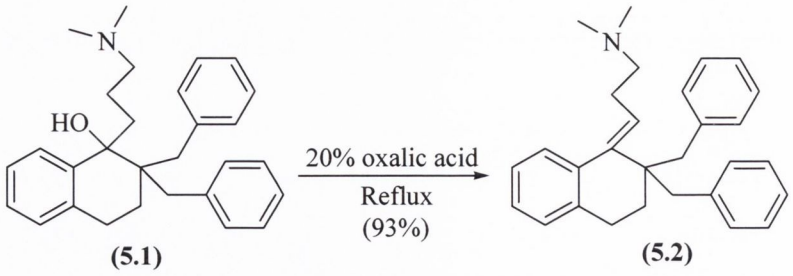
Fig. 5.5 ***^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (5.1) from 19.5-79.1ppm***

The DEPT 90 NMR spectrum (Fig. 5.4) showed ten signals resonated between 124.7-131.4ppm, for the fourteen aromatic tertiary carbons. It also confirmed the absence of the four aromatic quaternary carbons at 135.8, 140.3, 140.9 and 144.7ppm, respectively. The DEPT 135 NMR spectrum of compound **(5.1)** (Fig. 5.5) showed seven methylene signals from 21.3-60.8ppm and two methyl carbons at 45.7ppm were also found. The two quaternary carbon signals, at C-2 and C-1 positions, were found at 46.1 and 77.8ppm respectively in the ^{13}C NMR spectrum.

The infrared spectrum of compound **(5.1)** revealed the disappearance of the $C{=}O_{str}$ at 1677.6cm^{-1}. HRMS analysis gave the accurate molecular mass ($+H^+$) as 414.2811, calculated 414.2797 for molecular formula $C_{29}H_{36}NO$.

Dehydration of the alcohol **(5.1)** to the alkene **(5.2)** proceeded smoothly in a 20% aq. oxalic acid solution under reflux conditions. After eight hours, the target alkene **(5.2)** was obtained as a mixture of geometric isomers, about 10:1 ratio as determined by NMR, in excellent yield (93%), following acid-base work-up and purification. The two isomers

could not be separated completely by a combination of flash column chromatography and preparative TLC technique due to their almost identical R_f values on TLC.



(5.1) **(5.2)**

Scheme 5.2 Synthesis of compound (5.2)

The following structural elucidation study on compound **(5.2)** is based on its predominant *trans*-isomer. Confirmation that the predominant geometric isomer was the *trans* product was elucidated through NOE experiments, where the vinylic proton showed through coupling to both sets of benzyl protons on C-2 position of the tetralin ring (see Fig 5.14). The aliphatic region of the ^{1}H NMR spectrum of compound **(5.2)** was very well resolved. Two methylene protons at C-3 of the tetralin ring dominated at 1.81ppm and resonated as two overlapping triplets (J~6.24Hz). The six methyl protons were again evident as a sharp singlet at 2.33ppm. Two overlapping triplets (J=6.20Hz) at 2.44ppm represented the two benzylic methylene protons at C-4 position of the tetralin ring. The two methylene protons at C-11 were found at 2.50ppm, again, as two overlapping triplets (J~7.60Hz). A multiplet centred at 2.67ppm were attributable to the two methylene protons on C-10, and a pair of two overlapping doublets (J=13.50Hz) at 2.86ppm and 3.02ppm, respectively reflected the splitting of the four methylene protons at C-14 and C-20. The alkenic proton signal resonated at 5.70ppm as a triplet (J=7.12Hz). The aromatic region was intricate; a broad doublet (J=7.20Hz) integrating for one proton at 7.06ppm, followed by vast multiplet ranging from 7.12-7.33ppm, integrating for a total of thirteen protons.

The DEPT 135 NMR spectrum (Fig. 5.7) indicated the presence of fourteen aromatic tertiary carbon resonances between 125.0-131.2ppm in which the alkenic tertiary carbon was at 125.6ppm. The DEPT 90 NMR spectrum confirmed the absence of four aromatic

quaternary carbons from 139.1-140.8ppm, as well as the alkenic quaternary carbon at 135.6ppm. In the ^{13}C NMR spectrum (Fig. 5.8), all the important features were found and characterised; signals at 27.8, 28.7 and 33.7ppm represented the three methylene carbons at C-4, C-10 and C-3, respectively. The signal at 44.8ppm was considered to be the quaternary carbon at C-2 of the tetralin ring. The two methyl carbons were evident at 45.5ppm. The remaining two methylene peaks were found at 45.9 and 60.1ppm for the benzyl group carbons (C-14 and C-20) and carbon at C-11 position, respectively. The above proton and carbon assignments were confirmed by the use of H-H COSY, C-H COSY as well as HMBC data (Fig. 5.10- Fig. 5.13).

The diagnostic signal in the infrared spectrum of compound **(5.2)** was that of the $C{=}C_{wk}$ at 1674.6cm^{-1}. HRMS analysis provided accurate molecular mass ($+H^+$) as 396.2675, calculated 396.2691 with molecular formula $C_{29}H_{34}N$

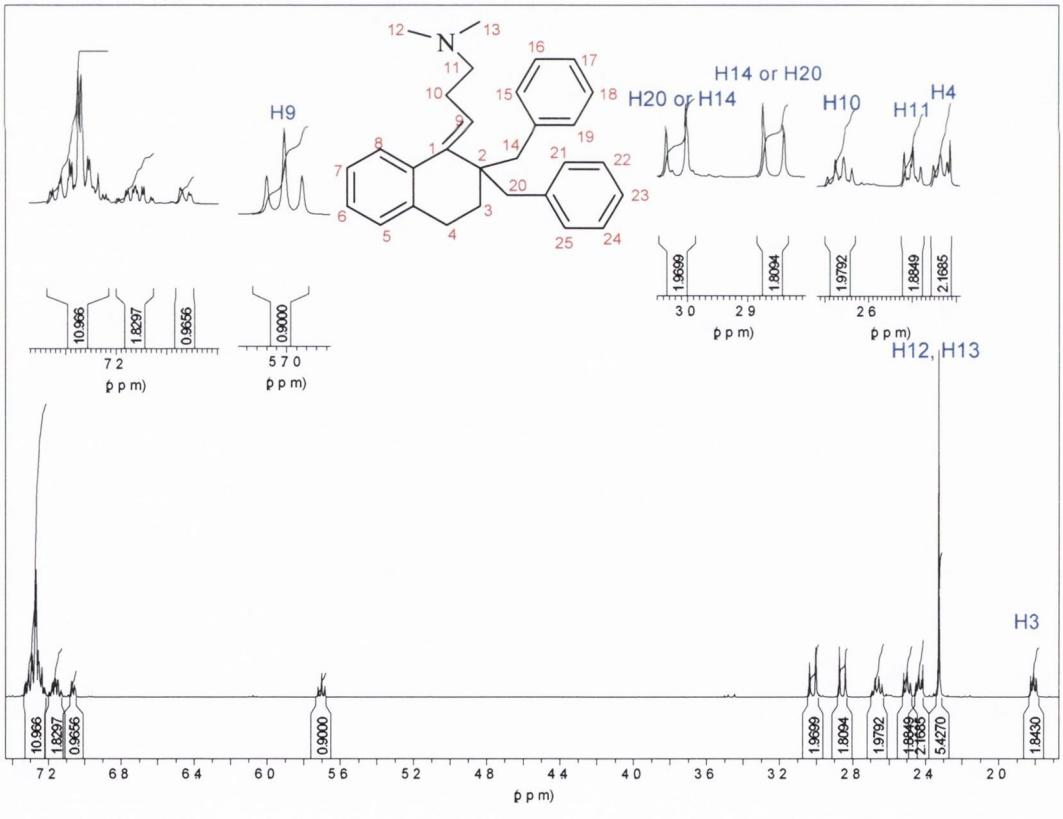


Fig. 5.6 ^1H NMR spectrum of compound (5.2)

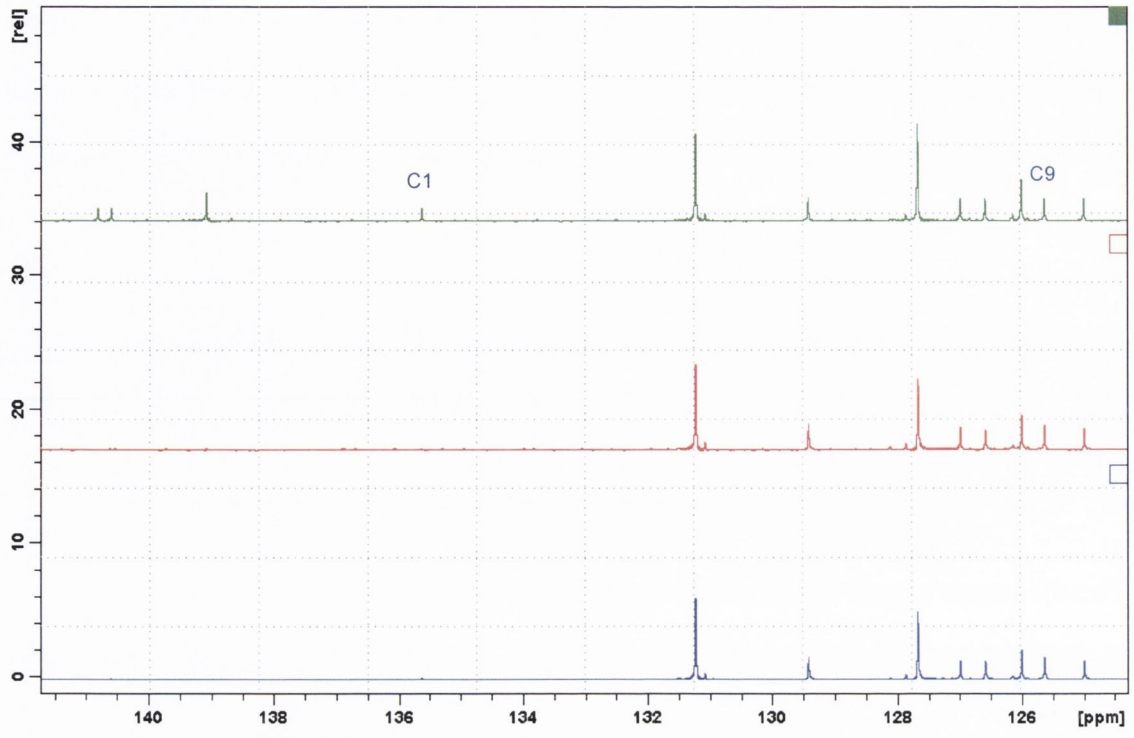


Fig. 5.7 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (5.2) from 123.8-141.5ppm

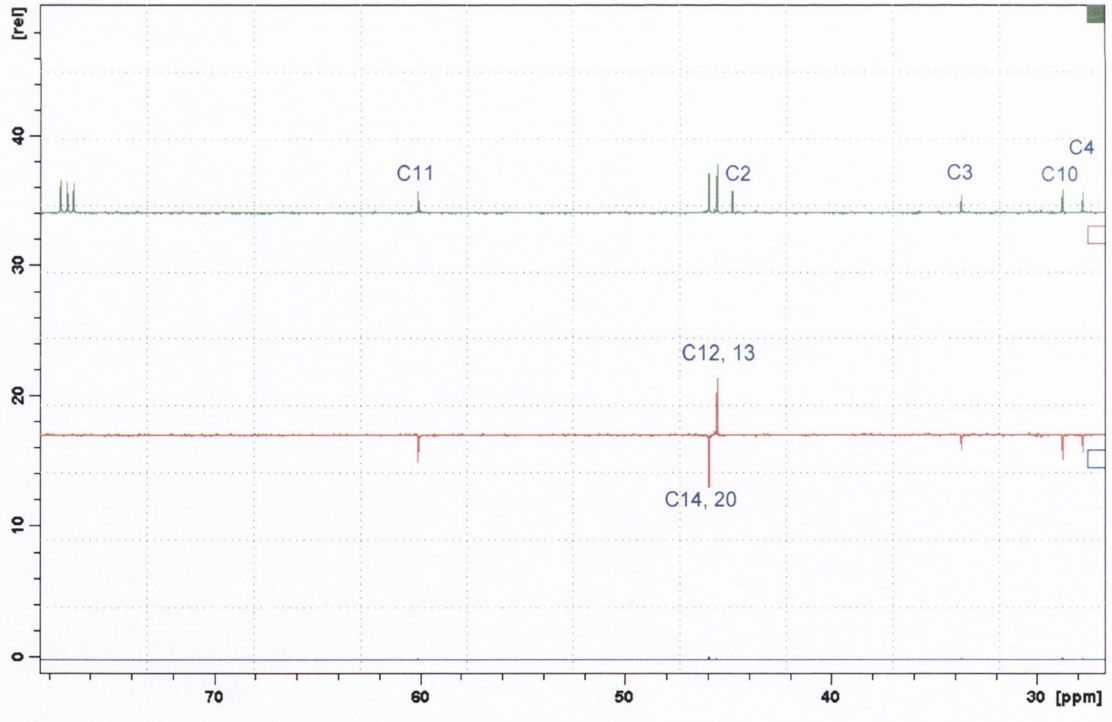


Fig. 5.8 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (5.2) from 27.3-78.1ppm

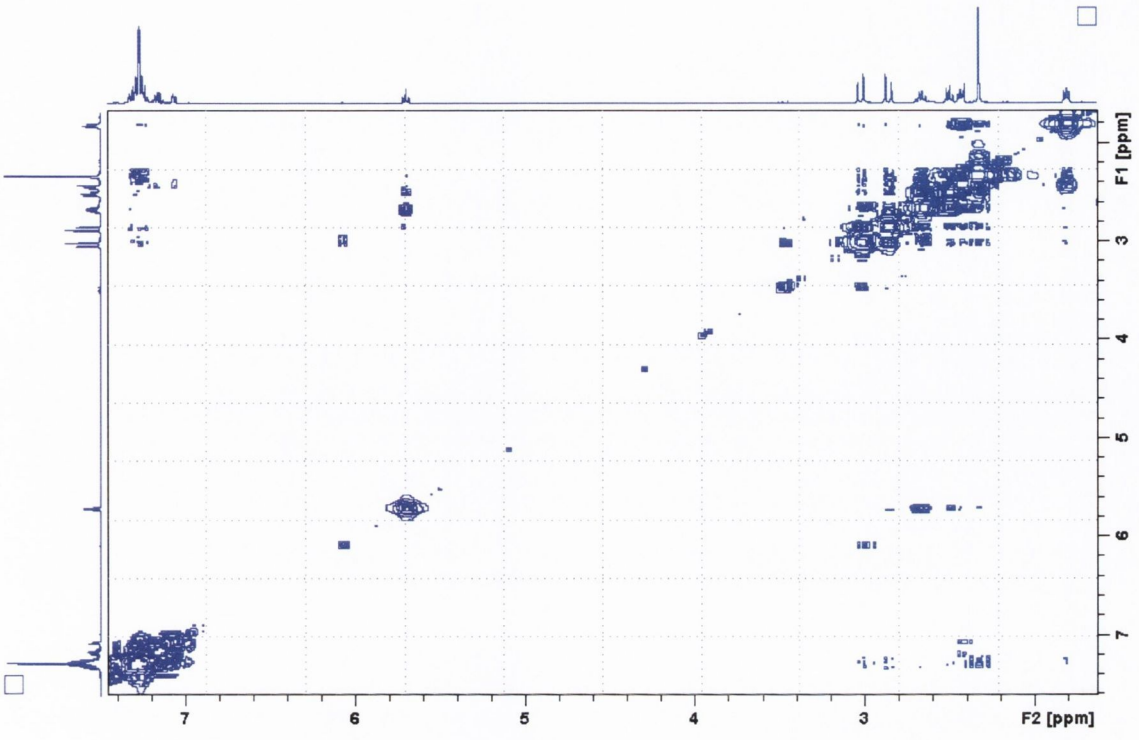


Fig. 5.9 H-H COSY NMR spectrum of compound (5.2)

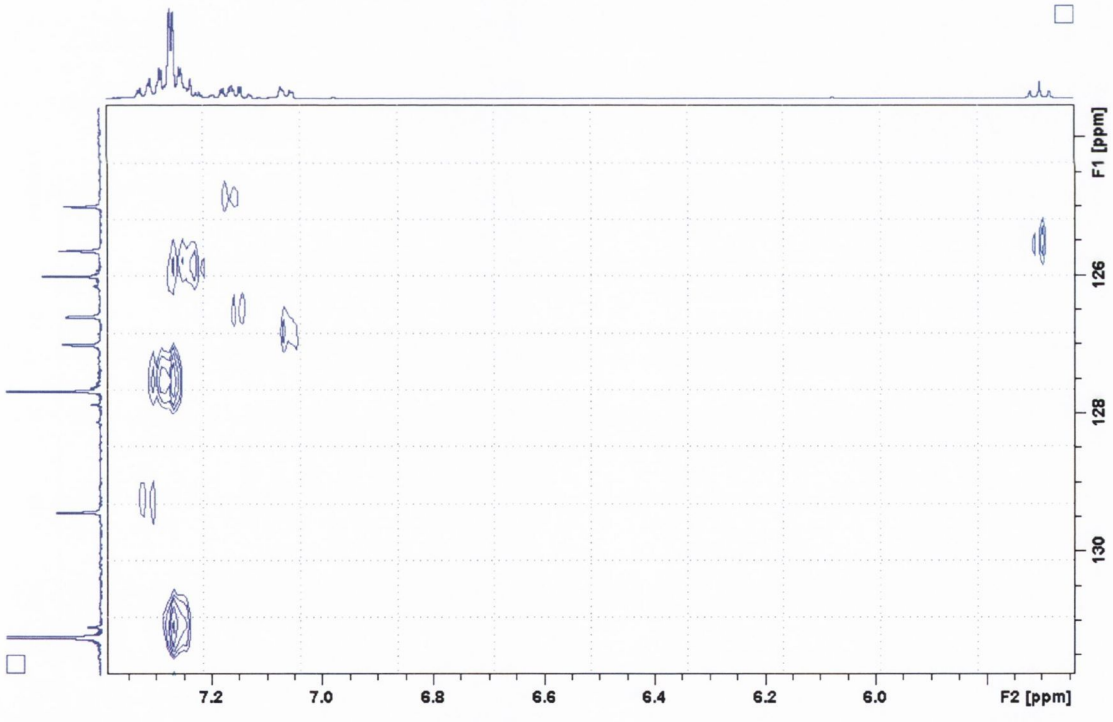


Fig. 5.10 C-H COSY NMR spectrum of compound (5.2) from 5.45-7.40ppm

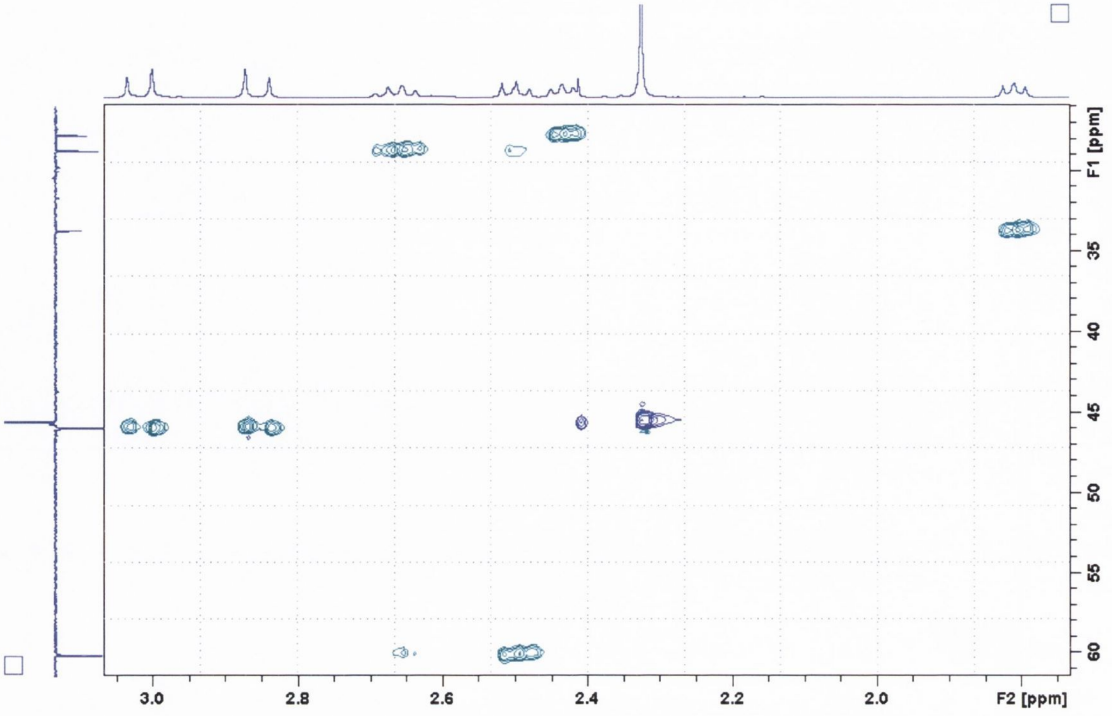


Fig. 5.11 C-H COSY NMR spectrum of compound (5.2) from 1.72-3.07ppm

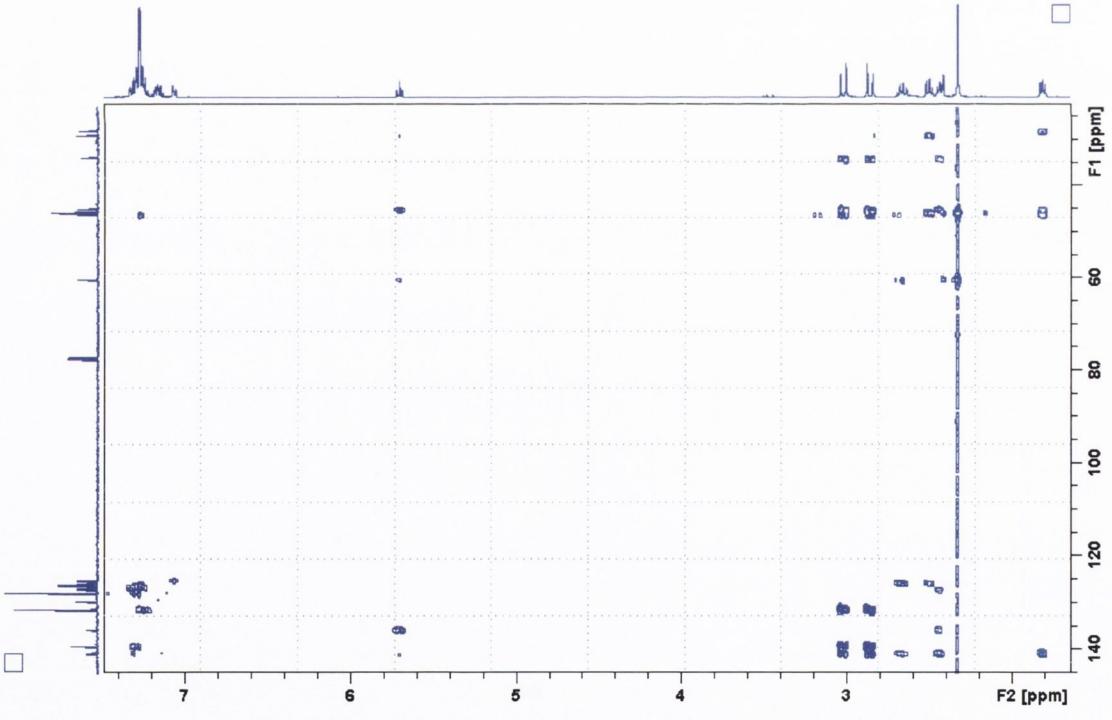


Fig. 5.12 HMBC NMR spectrum of compound (5.2)

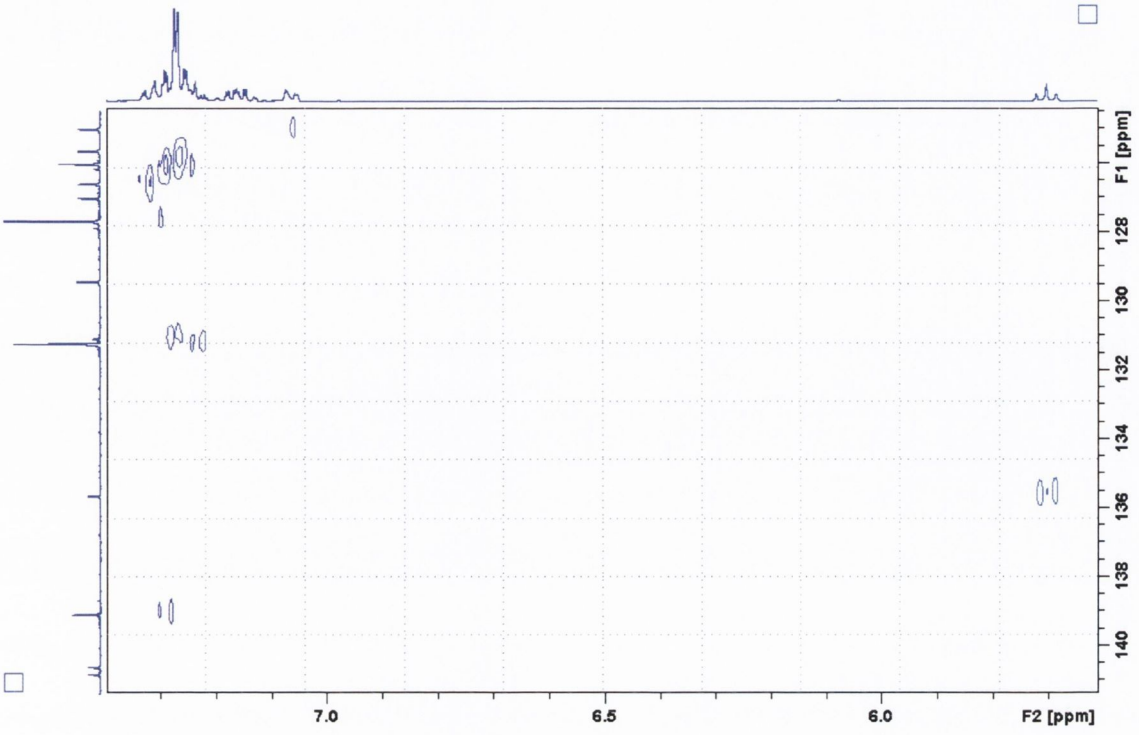


Fig. 5.13 HMBC NMR spectrum of compound (5.2) from 5.62-7.40ppm

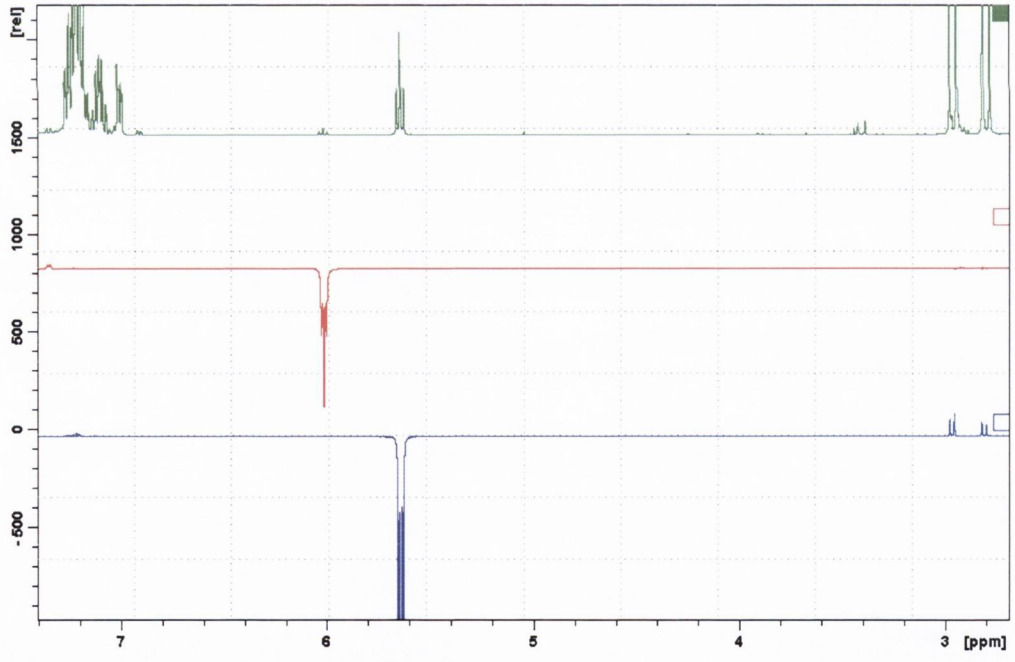


Fig 5.14 NOE NMR spectrum of compound (5.2)

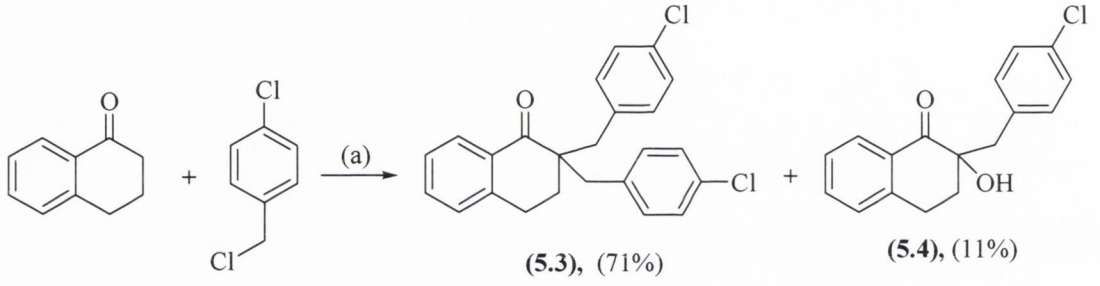
NOE experiment showed the coupling of the vinylic proton resonating at 5.64ppm to the benzyl protons at 2.75-3.00ppm with the vinylic proton of the minor isomer showing coupling to the aromatic proton H-8.

5.4 Formation of dichloro and di-*tert*-butyl derivatives of compounds (5.1) and (5.2)

It was felt that the *para* position of the benzyl ring may be a particularly vulnerable site for metabolism and masking it in the form of a chlorine or *tert*-butyl group might be sufficient in itself to prevent hydroxylation at this site.

5.4.1 Synthesis of the 4-chloro analogue

The synthetic approach started with alkylation of α-tetralone using 4-chlorobenzyl chloride (Scheme 5.3), which afforded the desired dialkylated product **(5.3)** in good yield (71%) together with a small percentage (11%) of monoalkylated product **(5.4)**



(5.3), (71%) **(5.4),** (11%)

(a) $(CH_3)_3COK$, *t*-butanol:diethyl ether (*v*:*v*, 1:1), RT

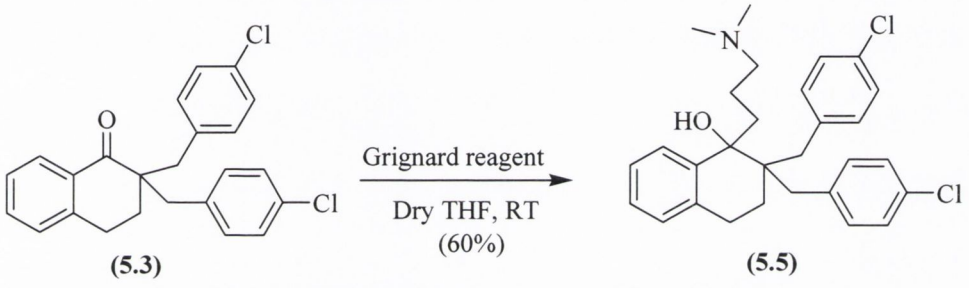
Scheme 5.3 Synthesis of compounds (5.3) and (5.4)

The aliphatic region of the ^1H NMR spectrum of compound **(5.3)** showed similar coupling pattern to that of compound **(5.0)**. In the aromatic region, most of the protons were individually visible (with the obvious exception of the symmetrical protons on the benzyl rings), a sharp doublet (J=7.80Hz) at 7.10ppm accounted for four protons, a doublet (J~7.84Hz) at 7.21ppm for one proton, another sharp doublet (J=7.84Hz) at 7.26ppm were attributable to the remaining four protons, *ortho* to the chlorine substituent, on the benzyl rings, a double doublet (J_1=7.84Hz, J_2=15.68Hz) at 7.34ppm represented one proton and a triplet (J=7.84Hz) at 7.47ppm also accounted for one proton. The terminal doublet (J=7.84Hz) representing the aromatic proton nearest the

carbonyl group resonated at 8.18ppm. The DEPT 135 NMR spectrum confirmed the presence of twelve aromatic tertiary carbons between 126.5-133.1ppm. The ^{13}C NMR spectrum indicated the signals of all six quaternary carbons from 131.7-142.3ppm. The carbonyl group was found at 199.4ppm.

Alkylation of α-tetralone to afford the desired ketone **(5.3)** did not greatly affect the spectral characteristics; in the infrared spectrum with the important $C=O_{str}$ signal, being located at 1678.0cm^{-1}.

The amino alcohol **(5.5)** was obtained following coupling of the Grignard reagent, [3-(dimethylamino)-propyl]magnesium chloride to the ketone **(5.3)**.



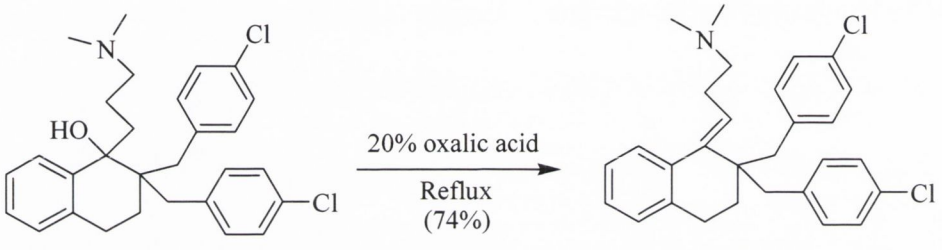
(5.3) **(5.5)**

Scheme 5.4 Synthesis of compound (5.5)

Compound **(5.5)** exhibited a similar ^{1}H NMR spectrum to compound **(5.1)**, especially in the aliphatic region of the spectrum. Better resolution of peaks was evident in the aromatic region possibly due to the symmetrical nature of the benzyl substituents containing the two chlorine atoms. Two overlapping doublets (J=8.80Hz) at 6.49ppm represented four protons on the benzyl rings. The signal for the proton at the C-5 position of the tetralin ring resonated as a doublet (J=7.84Hz) at 6.97ppm. The following pairs of two overlapping doublets showed almost identical coupling patterns at 7.05ppm (J=7.80Hz) and 7.09ppm (J=8.80Hz), corresponding to four aromatic protons on the benzyl rings. A triplet (J=7.34Hz) was found at 7.16ppm, representing one proton at C-6 (or C-7) of the tetralin ring. The remaining two protons of the benzyl groups and one proton at C-7 (or C-6) occurred as overlapping signals, between a triplet (J~10.72Hz) and a doublet (J=8.80Hz) in the region of 7.21-7.26ppm. The doublet (J=7.84Hz) representing the proton on C-8 of the tetralin ring resonated at 7.63ppm. The important

features of the ^{13}C NMR spectrum of compound **(5.5)** were the quaternary alcohol carbon at 76.9ppm. The signals for a total of twelve aromatic tertiary carbons resonated between 124.3-132.1ppm. The DEPT 135 NMR spectrum confirmed the absence of the six aromatic quaternary carbons between 130.9-144.0ppm. The significant observation in the infrared spectrum was the absence of $C{=}O_{str}$ signal, at 1678.0cm^{-1}. HRMS analysis revealed the molecular mass ($+H^+$) as 482.2022, calculated 482.2017.

Dehydration of the alcohol **(5.5)** with a 20% aq. oxalic acid solution under reflux conditions for ten hours, afforded an unequal geometric mixture of isomers of the alkene **(5.6)**, as confirmed by the ^1H NMR spectrum of compound **(5.6)**.



(5.5) **(5.6)**

Scheme 5.5 Synthesis of compounds (5.6)

Analysis of the NMR spectra is based on the predominant *trans* isomer of compound **(5.6)**. The aliphatic region of the ^1H NMR spectrum (Fig. 5.15) was considerably similar to that of the parent molecule **(5.2)**. The diagnostic signal was that of the alkenic methine proton at 5.70ppm, seen as a triplet (J=7.02Hz). Its corresponding carbon resonated at 125.6ppm in the DEPT 135 NMR spectrum. In the aromatic region, one proton of the tetralin ring resonated at 7.07ppm, as a doublet (J=7.00Hz), followed by two vast multiplets in the 7.15-7.20ppm and 7.24-7.27ppm regions, respectively, for a total of ten protons. The terminal resonance was again that of the proton at C-8 position of the tetralin ring, as a doublet (J=7.52Hz) at 7.33ppm. In the region between 124.8-139.8ppm, the ^{13}C NMR spectrum of compound **(5.6)** (Fig. 5.16) clearly confirmed the presence of twelve aromatic tertiary carbons and six aromatic quaternary carbons and one alkenic quaternary carbon, which corresponded well to the proposed structure.

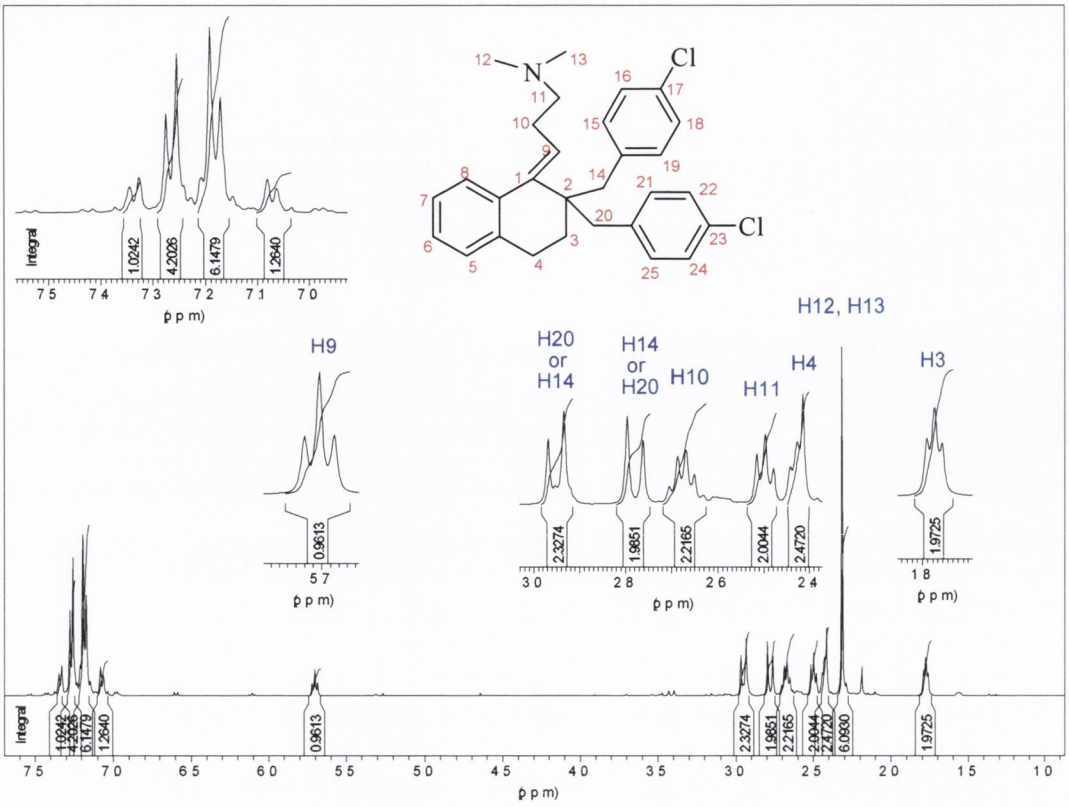


Fig. 5.15 ^{1}H NMR spectrum of compound (5.6)

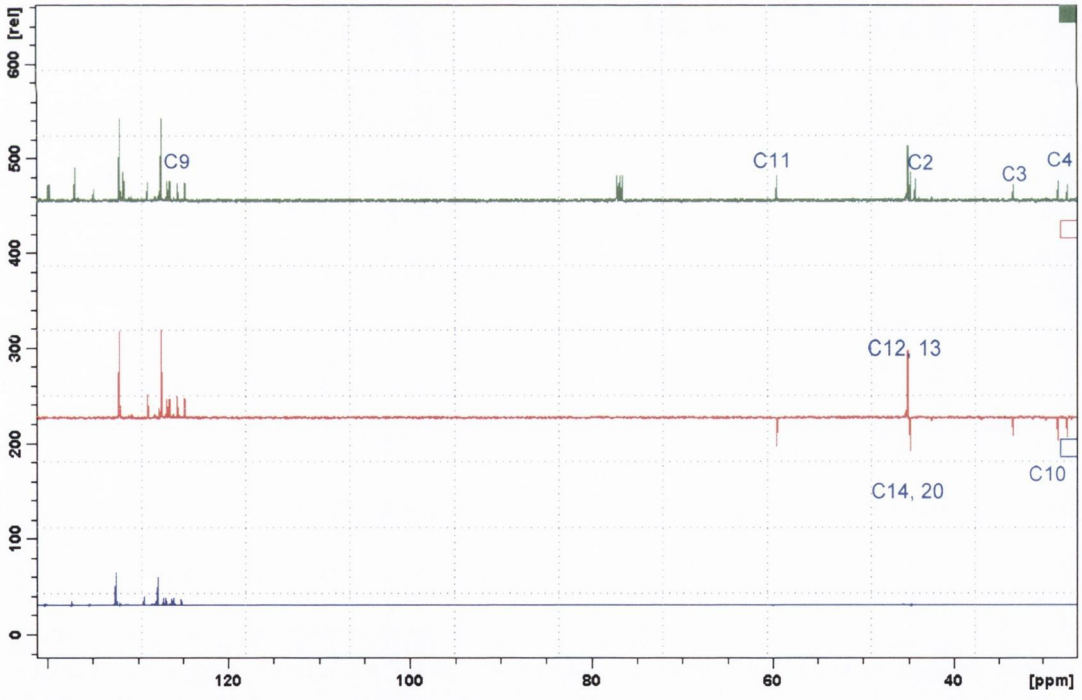


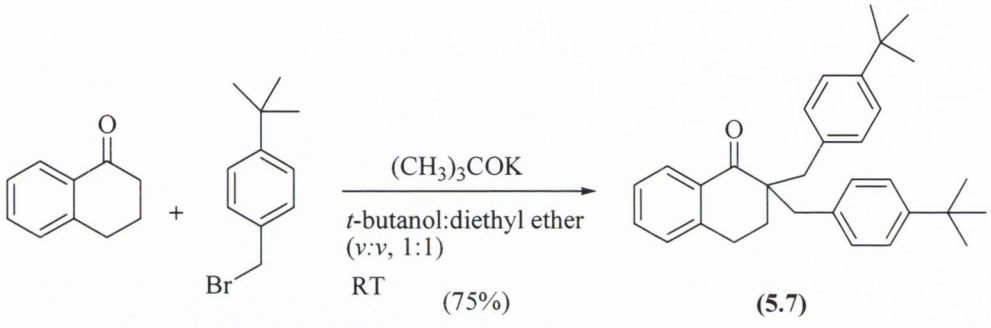
Fig. 5.16 ^{3}C, DEPT 135 and DEPT 90 NMR spectrum of compound (5.6)

The important feature of the infrared spectrum of compound **(5.6)** was the C=C_{wk} resonance from the 3-(dimethylamino)propylidendene side chain at 1679.4cm^{-1}. The

alkene **(5.6)** was also analysed by HRMS. The calculated molecular mass ($+H^+$) was 464.1912, which correlated well to the actual mass 464.1899. The following elemental composition for that peak, ($C_{29}H_{32}Cl_2N$), corresponded to the alkene **(5.6)**.

5.4.2 4-*tert*-butyl analogue

The attention was now drawn to the synthesis of further examples where the chlorine substituent was exchanged for a *t*-butyl group, a slightly bulky and also electron donating substituent. The 4-*tert*-butyl analogue **(5.9)** was synthesised starting with the initial alkylation of α–tetralone with 4-(*tert*-butyl)benzyl bromide, which gave the dialkylated ketone **(5.7)** after four hours in good yield (75%) as a white solid.

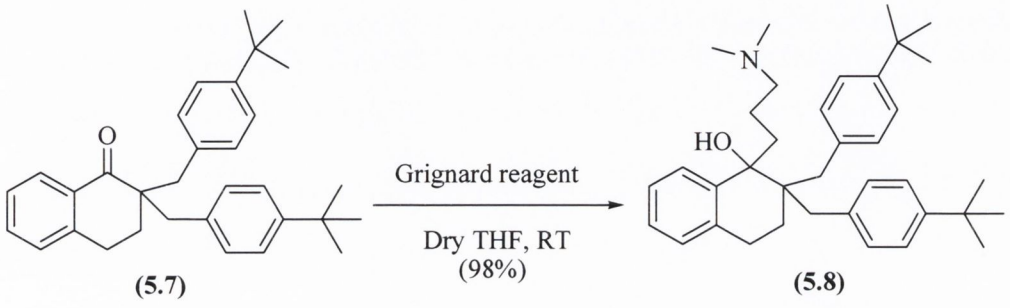


Scheme 5.6 Synthesis of compounds (5.7)

The diagnostic signal of the ^1H NMR spectrum of compound **(5.7)**, in comparison to the parent molecule **(5.0)**, was the methyl protons which resonated as a sharp singlet at 1.31ppm, integrating for a total of eighteen protons. The aromatic region was very well resolved. A pair of doublets, each considered to be four overlapping doublets, showed almost identical coupling patterns at 7.10 (J=8.04Hz) and 7.27ppm (J=8.00Hz), integrating for a total of eight protons corresponding to the benzyl group protons. The proton at C-5 position of the tetralone ring appeared as a doublet (J=7.52Hz) at 7.21ppm, followed by two triplets (J=7.28Hz) at 7.33 and7.46ppm respectively, representing the two protons of the tetralone ring. Again, the final doublet (J=7.52Hz) at 8.14ppm was due to the proton at C-8 position. The important features of the ^{13}C NMR spectrum were the signals of the methyl carbons at 30.9ppm, and the quaternary carbons of the *tert*-butyl carbons at 33.9ppm. The carbonyl peak was found at 200.4ppm. Alkylation of

α–tetralone had little effect on the infrared spectrum, the key signal of the $C=O_{str}$ was seen at 1678.5cm^{-1}.

The subsequent Grignard reaction of the ketone **(5.7)** proceeded smoothly to give the amino alcohol **(5.8)** in near quantitative yield following work-up and purification (Scheme 5.7).



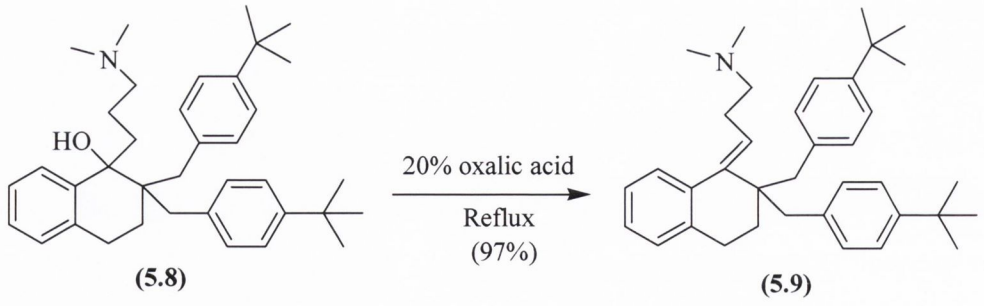
(5.7) **(5.8)**

Scheme 5.7 Synthesis of compounds (5.8)

The main changes in the ^{1}H NMR spectrum of the amino alcohol **(5.8)**, compared to that of the parent alcohol **(5.1)**, were the two sharp singlets for the *tert*-butyl groups at 1.41 and 1.46ppm integrating for eighteen protons, which were flanked on either side by the two methylene proton (C-10) singals at 1.31-1.35ppm and 1.49-1.62ppm, respectively. The ^{13}C NMR spectrum contained important signals at 31.3 and 31.2ppm representing the six methyl carbons of the *tert*-butyl groups, a quaternary signal of the *tert*-butyl groups at 33.9ppm and two methyl carbons of the side chain at 45.3ppm. The signal from the quaternary carbon at C-1 position resonated at 77.6ppm.

The infrared spectrum indicated the absence of the carbonyl signal at 1678.5cm^{-1}. HRMS analysis gave the accurate molecular mass ($+H^{+}$) as actual 526.4026, calculated 526.4049.

The final step, dehydration of the alkylated alcohol **(5.8)** with a 20% aq. oxalic acid solution (Scheme 5.8) gave an excellent yield (97%) of the geometric isomers of the desired tertiary amine **(5.9)**, which was isolated as a pale yellow crystalline solid. The isomers of the alkene **(5.9)** were unresolvable by TLC.



(5.8) **(5.9)**

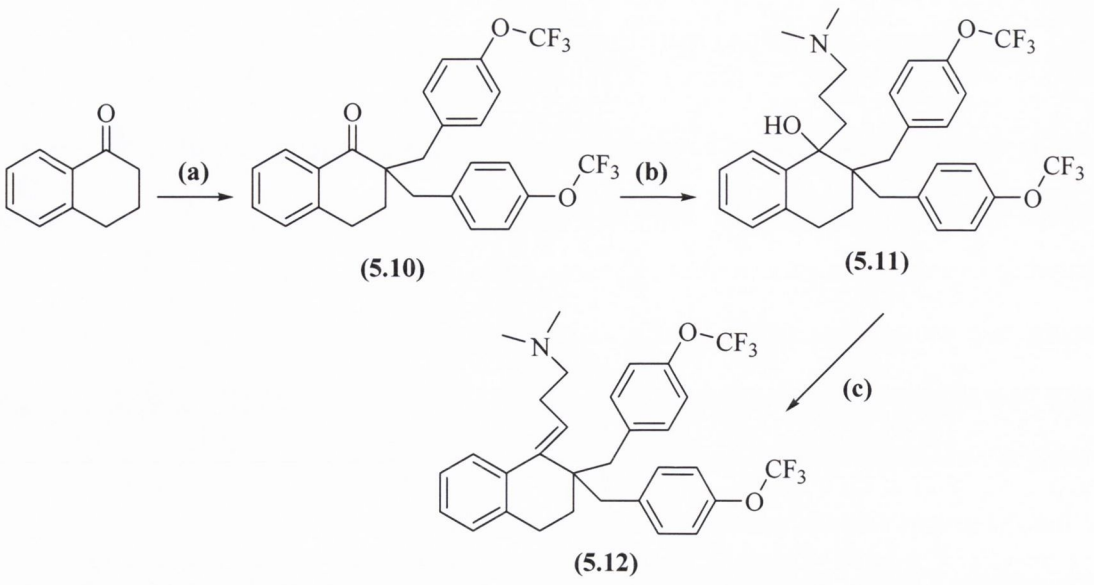
Scheme 5.8 Synthesis of compounds (5.9)

Therefore, spectra analysis and identification of the molecule were, again, based on the predominant *trans* isomer **(5.9)**. The ^{1}H NMR spectrum of alkene **(5.9)** closely resembled that of compound **(5.2)**; eighteen methyl protons of the two *tert*-butyl groups dominated at 1.39ppm. The two metylene protons at C-3 position were again evident as two broad overlapping triplets (J=5.84Hz) at 1.85ppm. The six methyl protons of the side chain were observed as a singlet at 2.34ppm. Two overlapping triplets (J=5.84Hz) at 2.45ppm represented the benzylic tetralin protons, and two overlapping triplets (J~7.60Hz) at 2.49ppm for the two protons at C-11 position of the side chain. A multiplet centred at 2.65ppm represented the two protons on C-10. A pair of two overlapping doublets at 2.84 (J=13.44Hz) and 2.99ppm (J=14.00Hz) reflected the splitting of the methylene protons (C-14 and C-20) of the benzyl groups. The alkenic proton appeared as a broad triplet (J=7.02Hz) at 5.65ppm. The aromatic region was intricate, ranging from 7.07-7.34ppm. Diagnostic signals in the ^{13}C NMR spectrum of the alkene **(5.9)** included the six methyl carbons and the two quaternary carbons of the *tert*-butyl groups at 31.1 and 33.9ppm, the alkenic tertiary carbon in the region of 124.5-126.0ppm and the alkenic quaternary carbon at 148.2ppm.

The important infrared characteristic peak of this compound was $C=C_{wk}$ absorption at 1662.5cm^{-1}. HRMS analysis gave an accurate mass ($+H^{+}$) as 508.3957, calculated 508.3943.

5.4.3 Synthesis of trifluoromethoxy analogues of compound (5.3)

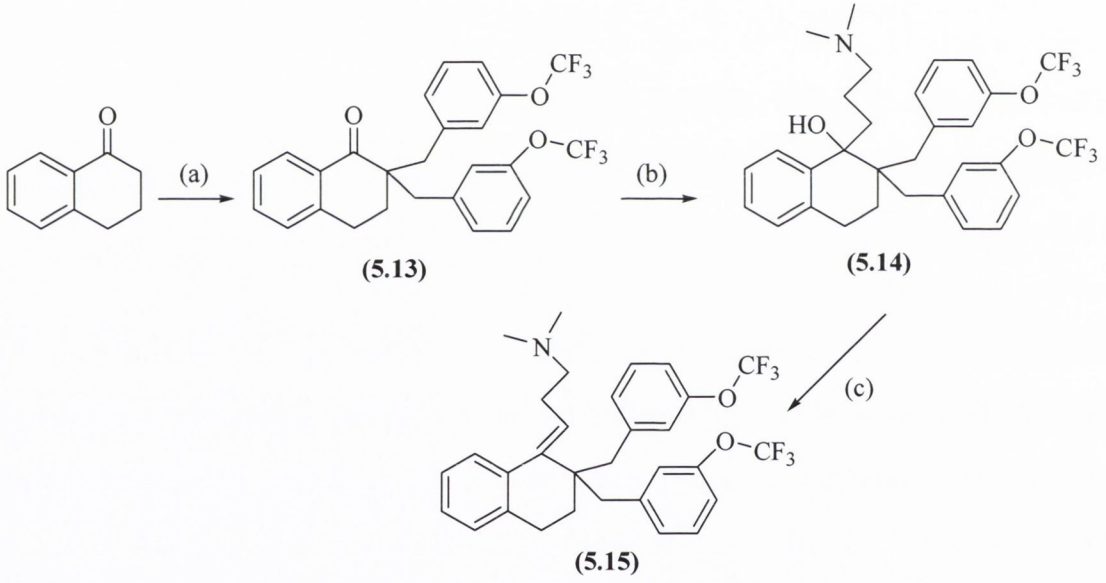
To date, the molecular designs have focused on introducing electron-withdrawing and or electron-donating substitutents onto the *para* position of the benzyl ring. In the following example with the trifluormethoxy analogues, it was decided to in addition to placing this group at position-4, to also secure the corresponding analogue where the group was attached onto position-3 of the benzyl ring. The generation of these analogues bearing the electron-withdrawing trifluoromethoxyl functionality at 4- or 3- positions on the benzyl group was approached through a similar sequence of reactions to those used for compound **(5.2)**. The starting material was α–tetralone, alkylation of which with alkylating agent, 4-(trifluoromethoxy)benzyl bromide or 3-(trifluoromethoxy)benzyl bromide gave the alkylated ketones **(5.10)** and **(5.13)**, respectively. Both alkylation reactions were accomplished after three hours at room temperature, resulting in approximately 75% yield for compound **(5.10)** and 79% yield for its regioisomer **(5.13)**. The subsequent Grignard reactions of the alkylated ketones **(5.10)** and **(5.13)**, with the Grignard reagent, [3-(dimethylamino)-propyl]magnesium chloride, was successful and yielded the required alcohols, namely compound **(5.11)** in reasonable yield and compound **(5.14)** in poor yield. The final step towards the desired trifluoromethoxyl analogues, namely alkenes **(5.12)** and **(5.15)**, was the dehydration of the alcohols **(5.11)** and **(5.14)** with a 20% aq. oxalic acid solution. These reactions proceeded with characteristic smoothness in reasonable yield to give their corresponding alkenes. Schemes 5.9 and 5.10 illustrate the sequence of steps involved in the preparation of the amino alkenes **(5.12)** and **(5.15)**.



(5.10) **(5.11)** **(5.12)**

(a) 4-(trifluoromethoxy)benzyl bromide, $(CH_3)_3COK$, *t*-butanol:diethyl ether (*v:v*, 1:1), RT, 3hrs, 75%
(b) Grignard reagent, dry THF, RT, 16hrs, 68%
(c) 20% aq. oxalic acid, reflux, 10hrs, 78%

Scheme 5.9 Synthesis of compound (5.12)



(5.13) **(5.14)** **(5.15)**

(a) 3-(trifluoromethoxy)benzyl bromide, $(CH_3)_3COK$, *t*-butanol:diethyl ether (*v:v*, 1:1), RT, 3hrs, 79%
(b) Grignard reagent, dry THF, RT, 10hrs, 39%
(c) 20% aq. oxalic acid, reflux, 8hrs, 71%

Scheme 5.10 Synthesis of compound (5.15)

5.4.3.1 Structure elucidation studies on compound (5.10) and its regioisomer (5.13)

In the ^{1}H NMR spectrum of compound **(5.10)**, two overlapping triplets (J=6.28Hz) at 1.99ppm, represented the two β–keto methylene protons of the tetralone ring, followed by two overlapping doublets (J=13.30Hz) at 2.71ppm for the two methylene protons of

the benzyl groups. The benzylic tetralin protons appeared as two overlapping triplets (J=6.02Hz) at 3.11ppm, followed by two overlapping doublets (J=13.30Hz) for the remaining methylene protons of the benzyl groups. One pair of four overlapping doublets (J=8.52Hz) was found at 7.14 and 7.20ppm, representing the eight aromatic protons on the benzyl rings. The latter signal was slightly overlapping with a doublet (J=7.52Hz) for the aromatic proton (C-5) of the tetralone ring at 7.24ppm. The following two discrete triplets were located at 7.36ppm (J=7.52Hz) and 7.50ppm (J=7.52Hz), integrating as one proton for each. The terminal doublet (J=8.04Hz) attributed to the aromatic proton nearest the carbonyl, resonated at 8.16ppm. The ^{13}C NMR spectrum of compound **(5.10)** indicated the presence of four methylene carbons at 24.8, 29.1 and 40.4ppm, respectively. The quaternary carbon, C-2, was present at 50.2ppm. A total of twelve aromatic tertiary carbons were located in the region of 120.0-133.1ppm. The quaternary carbon of the trifluoromethoxy group was observed at 120.2ppm, as a quartet ($J_{F\text{-}C}$~260.0Hz). The six aromatic quaternary carbons resonated between 131.7-147.5ppm. The signal of the carbonyl group was present at 199.5ppm. The ^{19}F NMR spectrum confirmed the presence of the fluorine at -58.3ppm.

The aliphatic region of ^{1}H NMR spectrum of its regio-isomer **(5.13)** showed similar coupling patterns to that of compound **(5.10)**. As expected the aromatic region was slightly more complex owing to the non-symmetrical placement of the tri-fluoromethoxy group. The main changes in the aromatic region, were sequence of overlapping signals from 7.09-7.13ppm representing six protons of the benzyl groups, followed by a doublet (J=7.52Hz) at 7.22ppm, for the proton on C-5 of the tetralone ring. The remaining two protons of the benzyl groups existed as two overlapping triplets (J=8.00Hz) at 7.29ppm. Similarly, The ^{13}C NMR spectrum revealed the presence of twelve aromatic tertiary carbons from 118.6-133.1ppm. The quaternary carbon of the trifluoromethoxy group appeared as a quartet ($J_{F\text{-}C}$~260.0Hz), at 119.9ppm. The presence of the fluorine at -58.3ppm was further confirmed by the ^{19}F NMR spectrum.

The main feature of the infrared spectra of these compounds was the carbonyl whose stretching frequency was observed at 1681.5cm^{-1} **(5.10)** and 1681.4cm^{-1} **(5.11)**. HRMS analysis, in both cases, gave the accurate molecule mass (+Na^{+}) as 517.1205 for compound **(5.10)** and 517.1224 for compound **(5.11)**, calculated 517.1214, molecular formula $C_{26}H_{20}F_6O_3Na$

5.4.3.2 Structure elucidation studies on compound (5.11) and its regioisomer (5.14)

The ^{1}H NMR spectrum of compound **(5.11)** contained two multiplets representing the two methylene protons at C-10 position, in the regions of 1.36-1.43ppm and 1.72-1.78ppm. Four multiplets for the six methylene protons stretched between 1.88-1.95ppm, 2.25-2.3ppm, 2.33-2.34ppm and 2.36-2.38ppm, respectively. The singlet for the six methylene protons resonated at 2.42ppm. A multiplet was found at 2.46-2.50ppm, integrating for one proton, followed by a doublet (J=14.04Hz) for one proton at 2.57ppm. Two methylene protons of the benzyl groups appeared as a doublet (J=13.71Hz) at 2.79ppm and a broad doublet (J=13.71Hz) at 2.84ppm. The remaining two protons of the benzyl group existed as two doublets, showing identical patterns due to the roofing effect, at 3.06ppm (J=13.71Hz) and 3.14ppm (J=13.71Hz). In the aromatic region, four protons of the benzyl group appeared as a pair of two overlapping doublets (J=8.52Hz) at 6.46ppm and 6.88ppm, respectively. A doublet (J=7.52Hz) for the proton at C-5 position of the tetralin ring was located at 6.92ppm, followed by a multiplet considered to be overlapping signals, integrating for five protons in the region of 7.11-7.17ppm. A triplet (J=7.54Hz) at 7.23ppm represented one aromatic tetralin proton. The final doublet (J=7.52H) located at 7.64ppm representing the proton at C-8 position. The ^{13}C NMR spectrum revealed the presence of a total of seven methylene carbons. The signal of the methyl carbons was found at 45.1ppm. The two aliphatic quaternary carbons at C-2 and C-1 positions were observed at 45.7 and 76.8ppm, respectively. The twelve aromatic tertiary carbons were found to be resonating between 119.4-131.9ppm. The quaternary carbon of the trifluoromethoxy group appeared as a quartet ($J_{F\text{-}C}$~258.8Hz), at 121.3ppm. The DEPT 90 NMR spectrum showed the absence of six

quaternary carbon signals at 135.1, 138.6, 139.0, 143.9, 146.7 and 147.1ppm. The fluorine signal was observed at -58.5ppm in the ^{19}F NMR spectrum.

The aliphatic region of the ^{1}H NMR spectrum of compound **(5.14)** gave a similar pattern to that of compound **(5.11)**. In the aromatic region, a singlet signal for the proton of the benzyl group was found at 6.34ppm, followed by a doublet (J=7.52Hz) at 6.40ppm, integrating for one proton. In the region of 6.93-7.00ppm, a multiplet was observed for three protons. Three triplets (J~7.52Hz, J~9.04Hz) were overlapping at 7.05 and 7.09ppm, integrated for three protons, followed by a double triplet (J_1=1.50Hz, J_2=7.54Hz) at 7.16 for one proton. Two discrete triplets resonated at 7.24ppm (J=7.54Hz) and 7.29ppm (J=7.78Hz), integrating as one proton each. Again, the terminal doublet (J=8.04Hz) at 7.65ppm represented the proton nearest the carbonyl group. The main changes in the ^{13}C NMR spectrum of compound **(5.14)** were the resonances of a total of twelve aromatic tertiary carbons, between 117.5- 129.2ppm. A quartet ($J_{F\text{-}C}$~258.8Hz) centred at 121.3ppm was believed to be the quaternary carbon of the trifluoromethoxy group. The ^{19}F NMR spectrum also revealed the presence of the fluorine at -58.20ppm.

As expected, the two molecules **(5.11)** and **(5.14)** showed the absence of the carbonyl absorption at around 1680cm^{-1} in their infrared spectra. HRMS analysis gave accurate molecule masses (+H^+) for both compounds, as 582.2449 for compound **(5.11)** and 582.2424 for compound **(5.14)**, calculated 582.2443, with the molecular formula $C_{31}H_{34}NO_3F_6$.

5.4.3.3 Structure elucidation studies on compound (5.12) and its regioisomer (5.15)

The NMR spectra confirmed that the resulting alkenes **(5.12)** and **(5.15)** were both obtained as unequal mixtures of stereoisomers. NMR analysis and structural elucidation of both compounds **(5.12)** and **(5.15)**, was again based on the predominant isomer and is summarised in Tables 5.0 and 5.1.

Proton	Compound (5.12)			Compound (5.15)		
	δ^1H ppm	mult (No.s of protons)	J (Hz)	δ^1H ppm	mult (No.s of protons)	J (Hz)
H3	1.78	2 x t, overlapping, (2H)	6.02	1.76	2 x t, overlapping, (2H)	6.52
H12,13	2.25 2.26	2 x s, (6H)		2.24	s, (6H)	
H4	2.38	2 x t, overlapping, (2H)	6.02	2.40 2.42	2 x t and 2 x t overlapping, (4H)	5.52 8.04
H11	2.44	2 x t, overlapping, (2H)	7.54			
H10	2.62	m, (2H)	7.04	2.59	m, (2H)	7.52
H14,20	2.79 2.97	2 x d, overlapping, (2H) 2 x d, overlapping, (2H)	13.56 13.52	2.79 2.92	2 x d, overlapping, (2H) 2 x d, overlapping, (2H)	13.56 13.56
H9	5.65	t, (1H)	7.02	5.64	t, (1H)	7.02
H5	7.04	broad d, (1H)	7.56			
H6,7 H16,18 H22,24	7.10-7.15	m, (6H)		7.01-7.14 m, (9H) 7.22-7.26 m, (3H)		
H8 H15,19 H21,25	7.21-7.29	m, (5H)				

Table 5.0 ^{1}H NMR characterisations of compound (5.12) and (5.15)

In brief, the alkene **(5.12)** displayed a similar ^{1}H NMR spectral pattern to the alkenes **(5.6)** and **(5.9)** in both the aliphatic and aromatic regions. In the aromatic region the ^{1}H NMR spectrum of compound **(5.12)**, showed a broad doublet for the proton on C-5 of

the tetralin ring. Identification of the exact resonance position of each aromatic proton was difficult, even when the 2-D spectra were employed. The signals for those protons on C6, C7, C16, C18, C22 and C24 positions closely overlapped in the region between 7.10-7.15ppm. The signals of the remaining five aromatic protons appeared as a complex multiplet ranging from 7.21-7.29ppm. As is evident from the ^{1}H NMR spectrum of **(5.15)** (Fig. 5.17) the aromatic protons were difficult to assign, as there was much signal overlap within this region. A particular notable feature of this spectrum was two vast multiplets between 7.01-7.14ppm and 7.22-7.26ppm which represented a total of twelve protons.

The aliphatic signals in the ^{13}C NMR spectra of compound **(5.12)** and **(5.15)** were observed at similar locations to those of compound **(5.6)** and **(5.9)**. In the aromatic region of the ^{13}C NMR spectrum of the alkene **(5.12)**, most aromatic tertiary carbons were able to be characterised due to their symmetrical properties. Important features of the spectrum were the quartet signal ($J_{F\text{-}C}$~259.5Hz) centred at 120.7ppm, which accounted for the quaternary carbon of the trifluoromethoxyl group, the alkenic tertiary carbon at 125.5ppm and the alkenic quaternary carbon at 134.8ppm.

However, the aromatic region of the ^{13}C NMR spectrum (Fig. 5.18) of the regio-isomer **(5.15)** was more complex. A total of twelve aromatic tertiary carbons were observed between 118.2-129.0ppm, in which the signal of the alkenic tertiary carbon was located at 125.4ppm. The alkenic quaternary carbon was found at 134.6ppm with the aid of the HMBC spectra (Fig. 5.21 and Fig. 5.22). Similarly, a quartet signal ($J_{F\text{-}C}$~259.8Hz) centred at 120.4ppm, represented the quaternary carbon of the trifluoromethoxyl functionality. The presence of the fluorine moiety in the molecule was confirmed by obtaining the ^{19}F NMR spectrum for both compounds **(5.12)** and **(5.15)**. The signals were located at -58.4 and -58.1ppm, respectively. The ^{1}H and ^{13}C resonance positions indicated in Tables **5.0** and **5.1** were identified through the use of the 2D spectra including H-H, C-H, HMBC spectra together with the ^{19}F NMR spectra of the regio-isomer **(5.15).** Selected spectra are shown in Fig. 5.17-Fig. 5.23.

	Compound (5.12)		Compound (5.15)	
	$\delta^{13}C$ (ppm)	No.s of carbons	$\delta^{13}C$ (ppm)	No.s of carbons
C4	27.2	1 x C	26.9	1 x C
C10	28.2	1 x C	28.1	1 x C
C3	33.3	1 x C	33.3	1 x C
C2	44.3	1 x C	44.2	1 x C
C14,20	44.8	2 x C	44.7	2 x C
C12,13	44.9	2 x C	44.9	2 x C
C11	59.5	1 x C	59.5	1 x C
$\underline{C}F_3$	120.7, quartet, $J_{F\text{-}C}$~259.5Hz		120.4, quartet, $J_{F\text{-}C}$~259.8Hz	
C16,18,22,24	119.7	4 x C	118.2, 123.0, 124.7, 126.4, 126.7, 128.4, 128.91, 129.0 for a total of 12 x C C9 at 125.4ppm 1 x C	
C6 or C7	124.7	1 x C		
C9	125.5	1 x C		
C7 or C6	126.4	1 x C		
C5	126.6	1 x C		
C8	128.8	1 x C		
C15,19,21,25	131.8	4 x C		
C1	134.8	1 x C	134.6	1 x C
Quat. carbons	137.1, 139.5, 139.8, 147.1	6 x C	139.5,139.7, 140.5, 148.3	6 x C

Table 5.1 ^{13}C NMR characterisation of compounds (5.12) and (5.15)

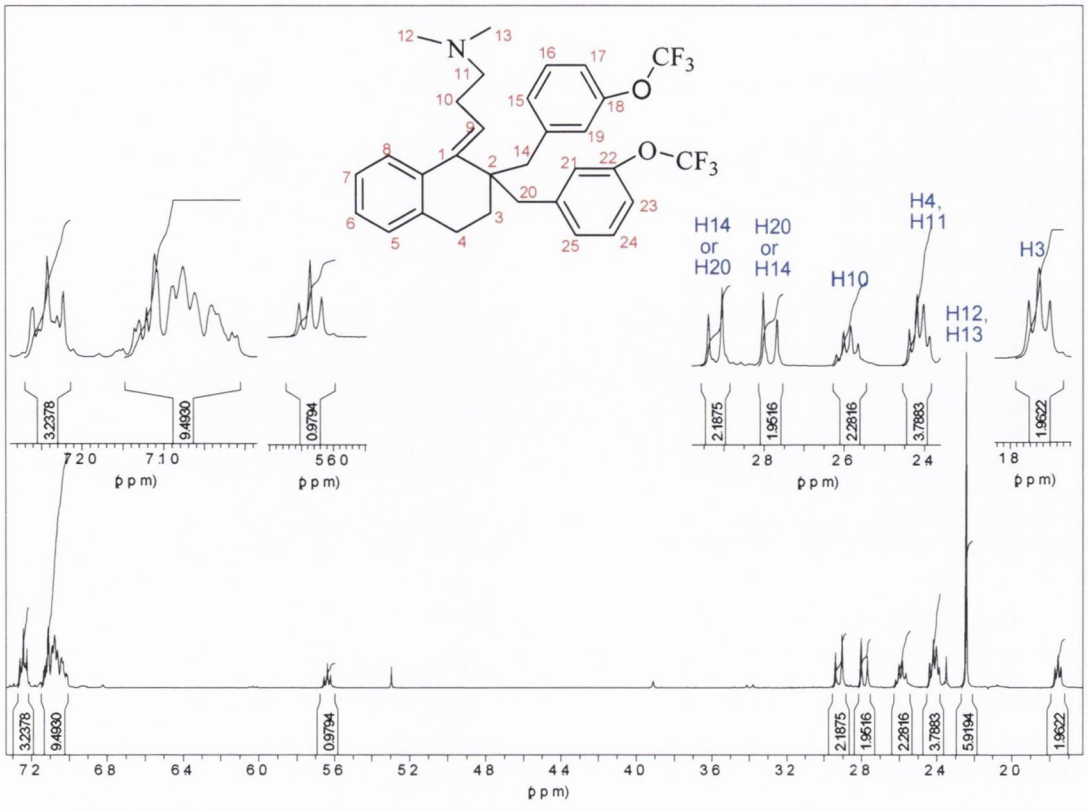


Fig. 5.17 ^{1}H NMR spectrum of compound (5.15)

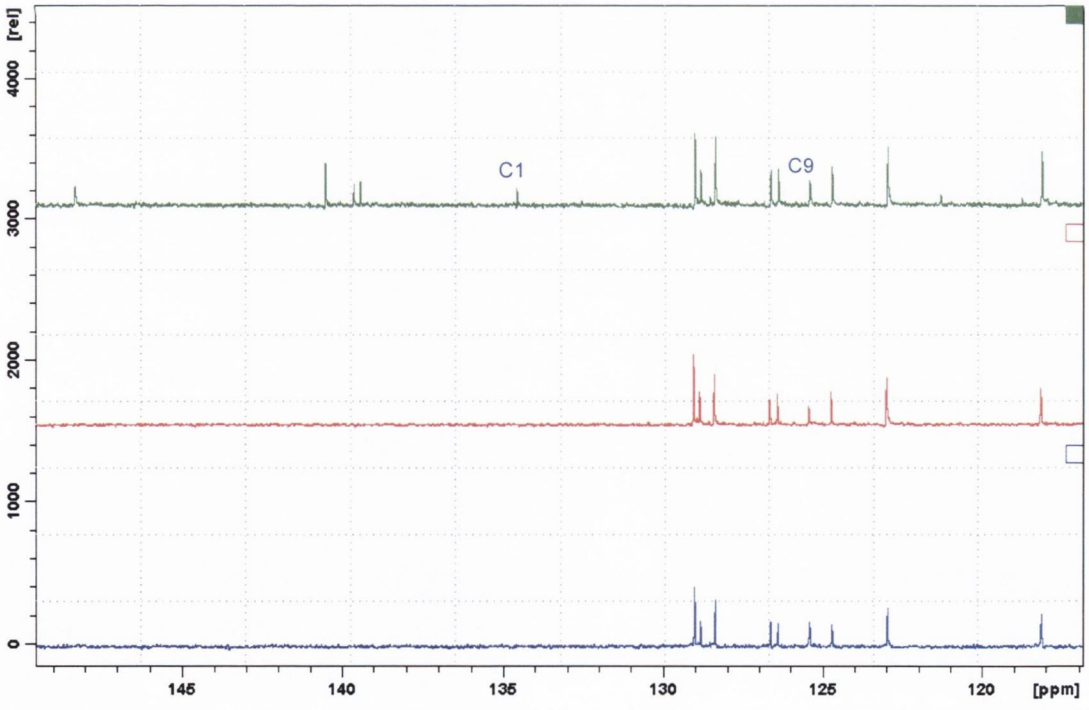


Fig. 5.18 ^{13}C, DEPT 135 and DEPT 90 NMR spectrum of compound (5.15) from 116.9-149.4ppm

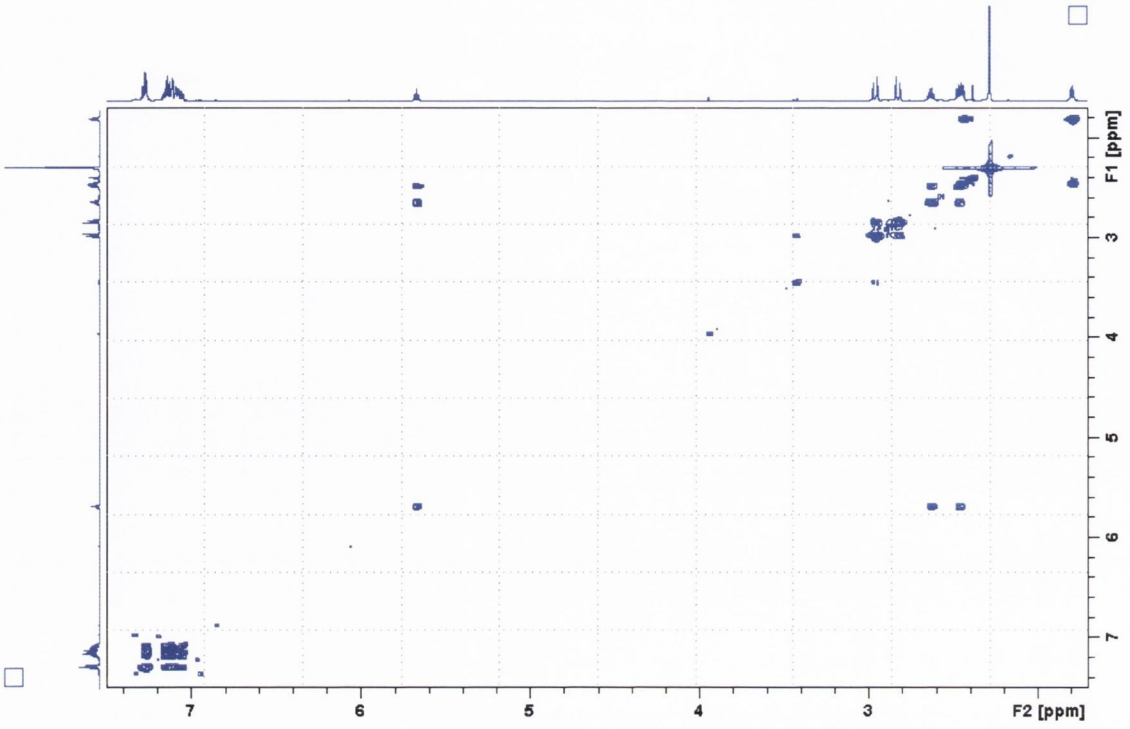


Fig. 5.19 H-H COSY NMR spectrum of compound (5.15)

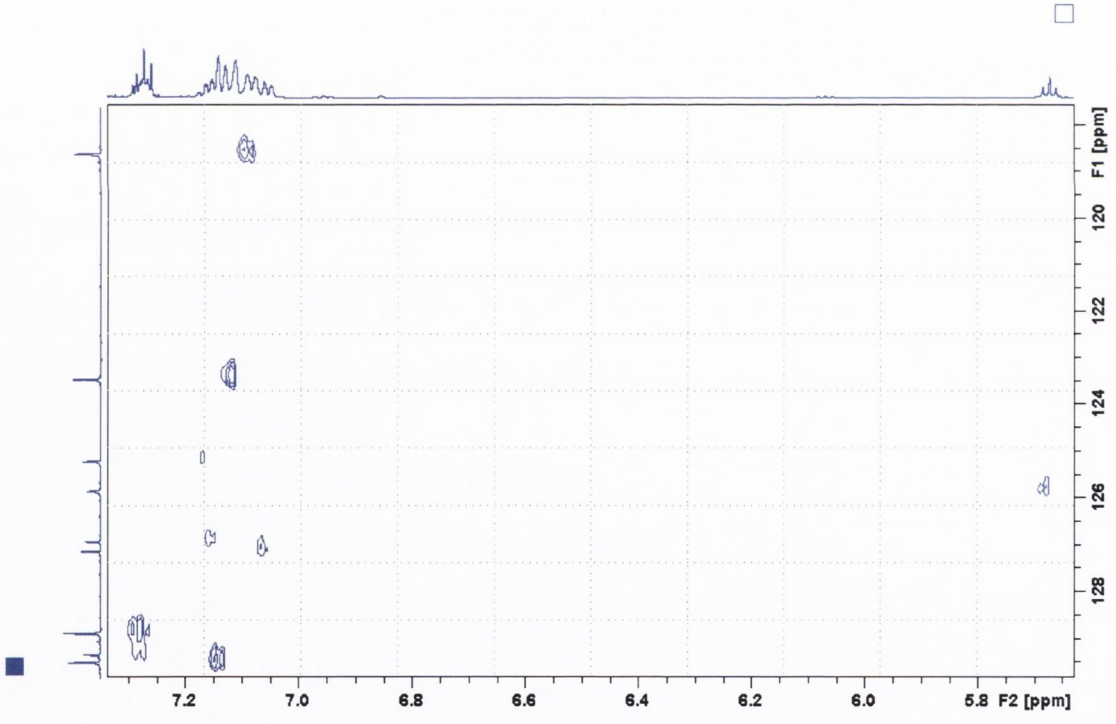


Fig. 5.20 C-H COSY NMR spectrum of compound (5.15) from 5.62-7.33ppm

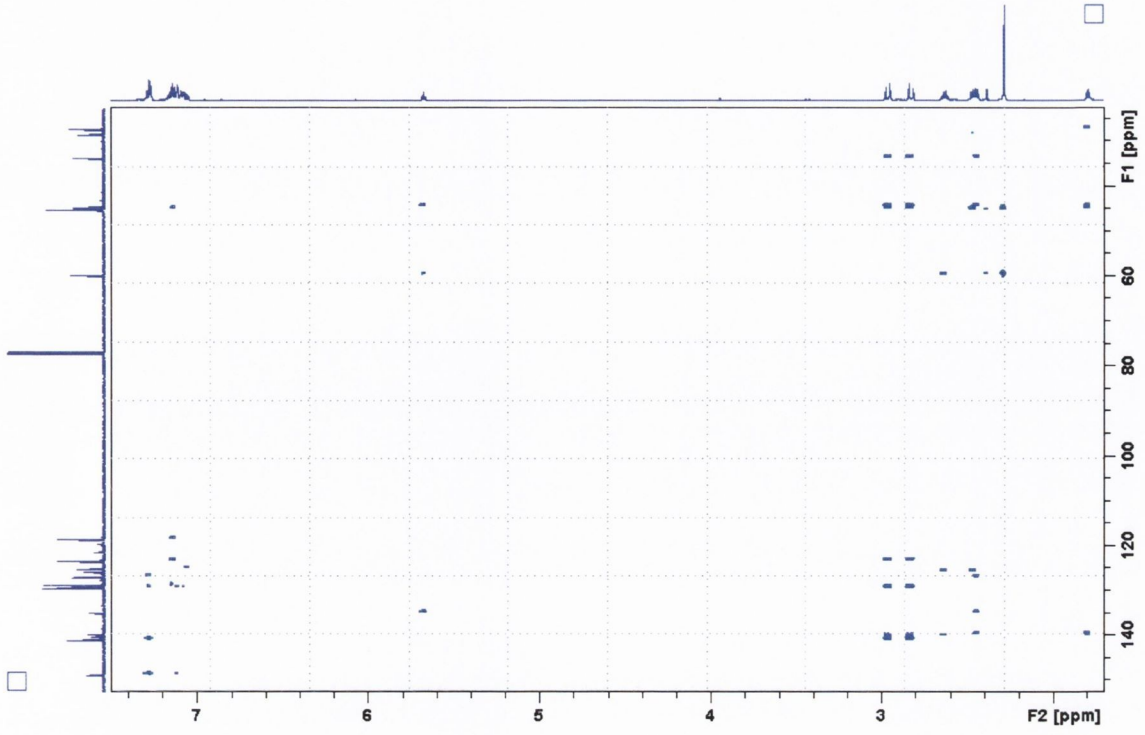


Fig. 5.21 HMBC NMR spectrum of compound (5.15)

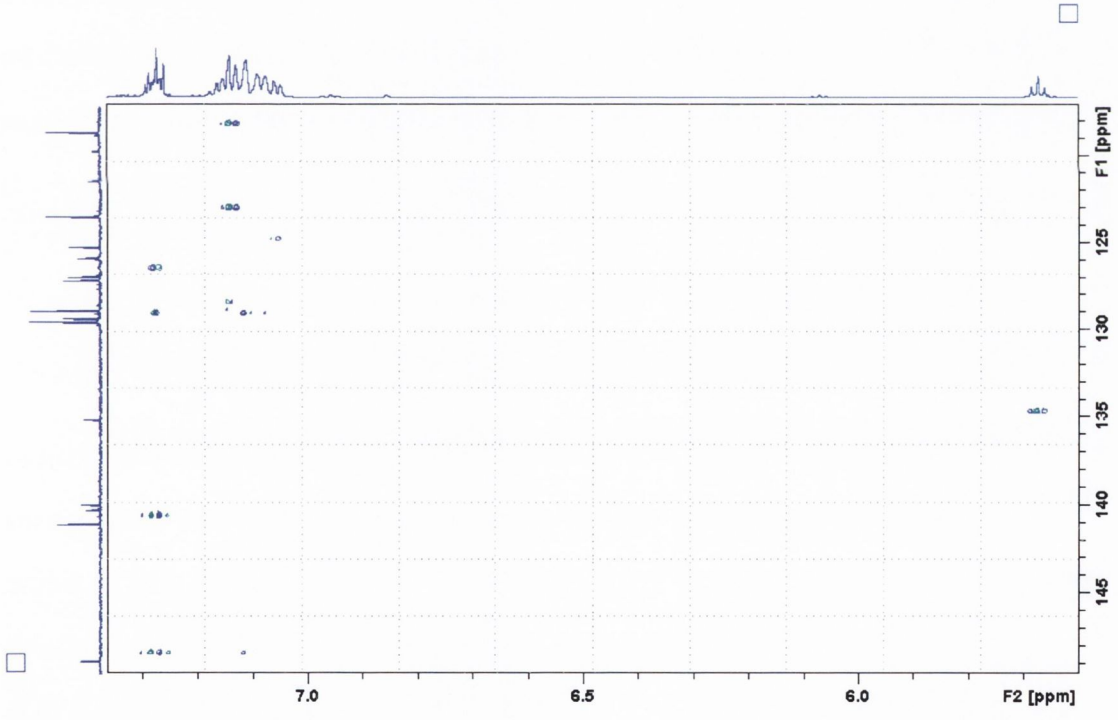


Fig. 5.22 HMBC NMR spectrum of compound (5.15) from 5.58-7.37ppm

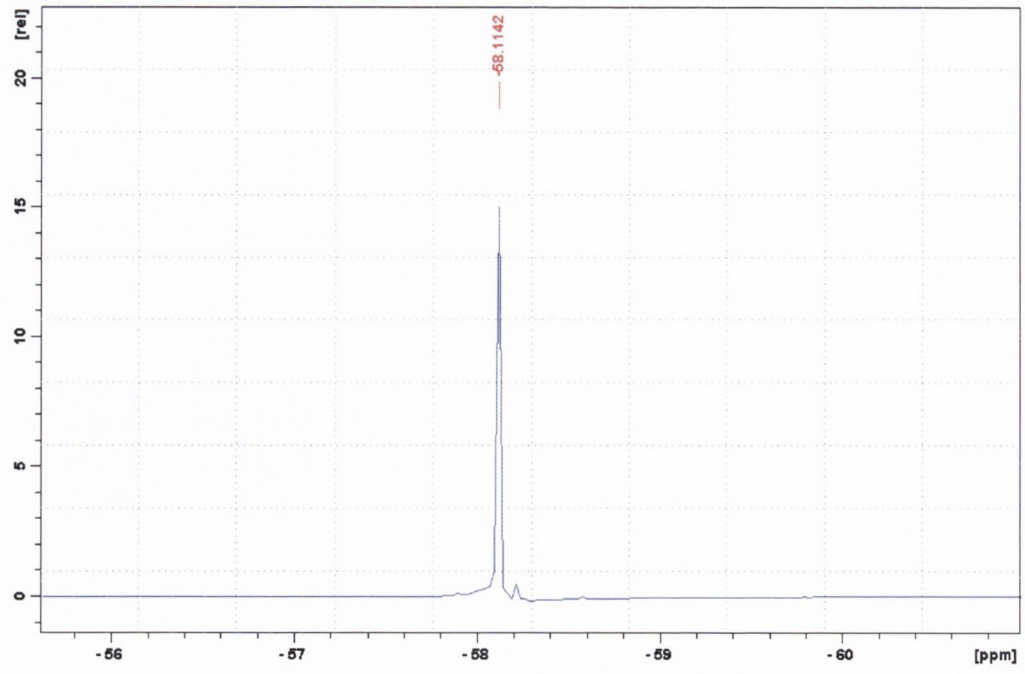


Fig. 5.23 ^{19}F *NMR spectrum of compound (5.15) from (-60.8)-(-56.6)ppm*

Dehydration of the alcohols **(5.11)** and **(5.14)** had virtually no effect on the infrared spectra; apart from the absorption peak for the $C=C_{wk}$ on the side chain at 1679.4cm^{-1} for compound **(5.12)** and 1683.3cm^{-1} for compound **(5.15)**. Both compounds gave satisfactory molecular masses ($+H^+$) on HRMS analysis: 564.2361 for compound **(5.12)** and 564.2341 for compound **(5.15)**, molecular formula $C_{31}H_{32}NO_2F_6$ requiring 564.2337.

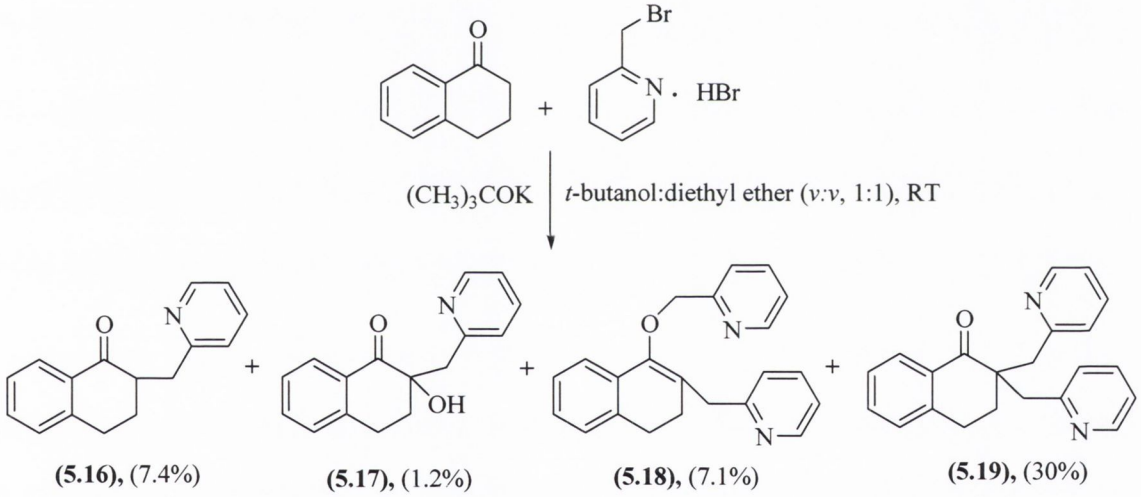
5.4.4 Synthesis of (pyridin-2-yl)methyl analogues of (5.1 and 5.2)

Having synthesised and evaluated compounds **(5.1)** and **(5.2)** and their derivatives described above, it was of interest to study the implications on their activities of replacing the aromatic ring of the benzyl group with a heterocycle ring. The pyridine functionality has been shown to be associated with various pharmacologic effects, such as anti-allergy and anti-inflammatory (Schwender et al., 1980; Youssefyeh, 1982; Blythin, 1986) and is presence on the structure of pyrilamine, an anti-allergy drug. Therefore, it was decided to synthesise (pyridin-2-yl)methyl analogues (Fig. 5.24) with the expectation that such a modification should retain some mast cell-stabilising activities.



Fig. 5.24 Chemical structure of (pyridin-2-yl)methyl analogues

Synthesis of these compounds was again approached through a similar synthetic route to that used for compounds **(5.1)** and **(5.2)**. The alkylating agent used in this case was 2-(bromomethyl)pyridine hydrobromide. Alkylation of α–tetralone yielded a mixture of products, but primarily (30%) the required alkylated ketone **(5.19)**, seen as the most polar compound among the reaction product mixture. The reaction and products are shown in Scheme 5.11.



(5.16), (7.4%) **(5.17),** (1.2%) **(5.18),** (7.1%) **(5.19),** (30%)

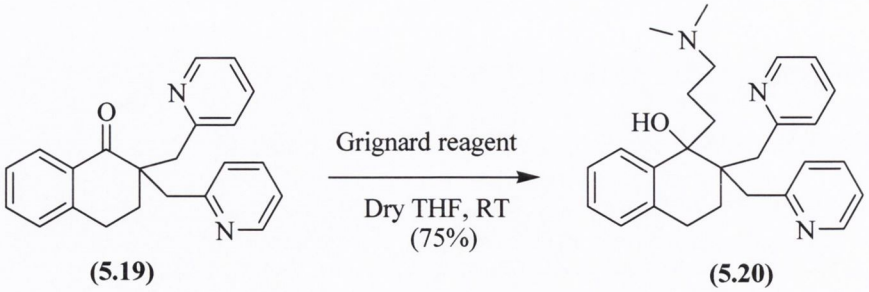
Scheme 5.11 Synthesis of compound (5.19)

^{1}H NMR analysis of compound **(5.19)** revealed that two β–keto methylene protons appeared as two overlapping triplets (J=6.28Hz) at 1.95ppm, followed by two overlapping doublets (J=13.30Hz) for two protons at 2.84ppm, representing one methylene group linked to the pyridine ring. The two benzylic tetralone protons resided at 2.98ppm, appearing as two overlapping triplets (J=6.28Hz). The remaining two methylene protons of the molecule existed as two overlapping doublets (J=13.30Hz) at 3.36ppm. The aromatic region was well resolved, each proton being observable as: two

overlapping double doublets (J_1~0.96Hz, J_2~5.03Hz) at 6.89ppm for two protons, one doublet (J=7.52Hz) at 7.03ppm for one proton, two overlapping doublets (J=7.52Hz) at 7.10ppm for two protons, one triplet (J=7.04Hz) at 7.13ppm for one proton, one double triplet (J_1=1.35Hz, J_2=7.28Hz) at 7.26ppm for one proton and two overlapping double triplets (J_1=1.83Hz, J_2=7.52Hz) at 7.35ppm for two protons. The aromatic tetralone proton nearest the carbonyl group resonated as a double doublet (J_1=1.05Hz, J_2=8.05Hz) at 7.98ppm, showing considerable *meta* coupling. The signal of the two aromatic pyridine protons linked to nitrogen atoms comprised two overlapping doublets (J=4.62Hz) centred at 8.33ppm. In the ^{13}C NMR spectrum, three signals for four methylene carbons were found at 24.9, 29.0 and 42.7ppm, followed by the signal of the α-keto quaternary carbon linked at 50.2ppm. A total of twelve aromatic tertiary carbons resonated between 120.8-148.3ppm. Signals for the four aromatic quaternary carbons resonated in the region of 131.7-157.6ppm. The carbonyl resonance was found at 199.7ppm.

The infrared spectrum of compound **(5.19)** contained $C{=}O_{str}$ at 1677.3cm^{-1}. HRMS analysis provided an accurate mass ($+H^+$) as 329.1661, that calculated being 329.1654 with molecular formula $C_{22}H_{21}N_2O$.

Treatment with the Grignard reagent, [3-(dimethylamino)-propyl]magnesium chloride in the presence of THF under anhydrous conditions gave the desired alcohol **(5.20)** in a good yield (Scheme 5.12).



(5.19) **(5.20)**

Scheme 5.12 Synthesis of compound (5.20)

The amino alcohol **(5.20)**, exhibited a similar pattern in the aliphatic region of the ^{1}H (Fig. 5.25) and ^{13}C NMR (Fig. 5.26 and Fig. 5.27) spectra to that of compound **(5.1)**.

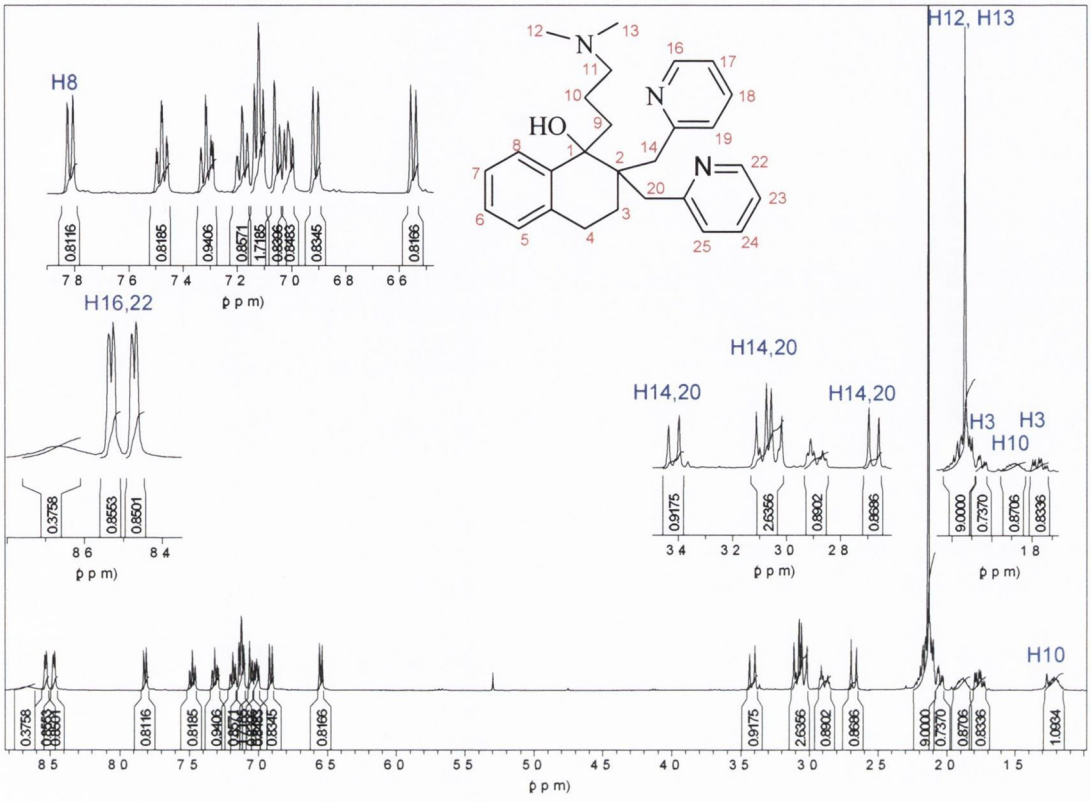


Fig. 5.25 ^{1}H NMR spectrum of compound (5.20)

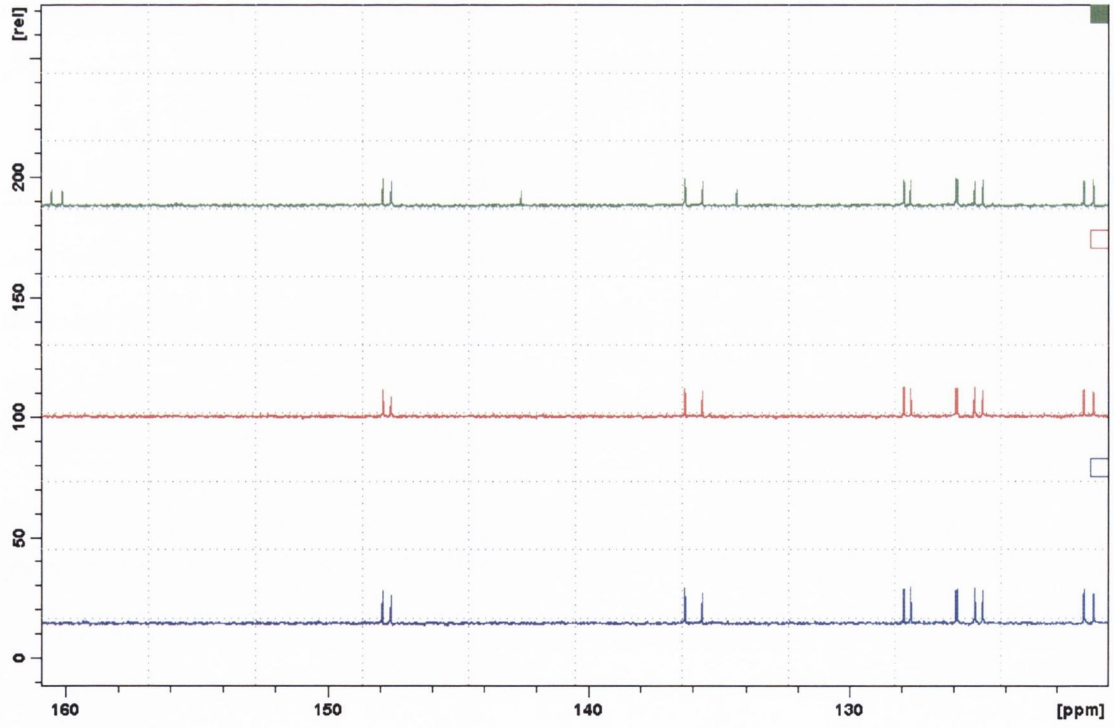


Fig. 5.26 ^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (5.20) from 120.0-161.1ppm

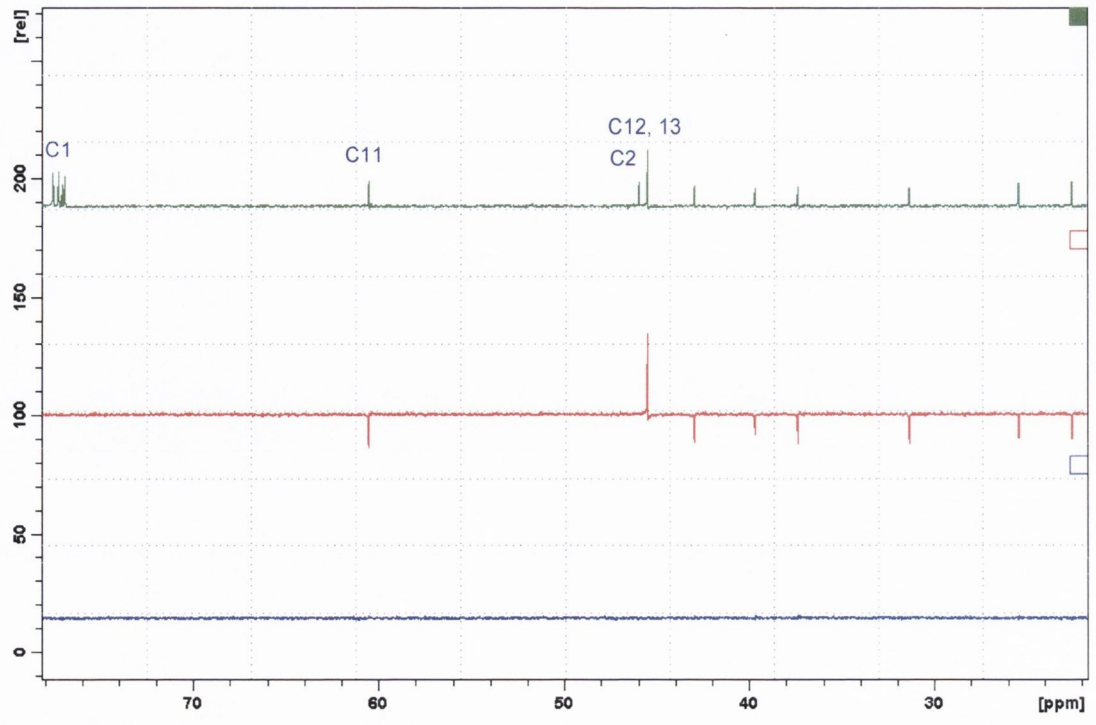


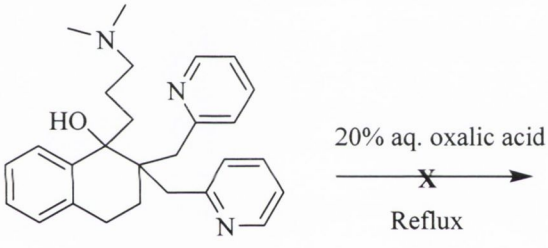
Fig. 5.27 ***^{13}C, DEPT 135 and DEPT 90 NMR spectra of compound (5.20) from 21.9-78.1ppm***

One β–keto proton of the tetralin ring was centred at 1.76ppm as a double triplet (J_1=4.52Hz, J_2=12.04Hz). It was flanked on either side by a methylene proton (C-10) appeared as a multiplet, ranging from 1.19-1.27ppm and 1.87-1.92ppm. The second β–keto proton at C-3 was found at 2.05ppm also appearing as a double triplet (J_1~3.78Hz, J_2~12.30Hz). Three methylene protons occupied the region from 2.10-2.22ppm as a multiplet, with slight overlap by a singlet of the six methyl protons at 2.12ppm. One methylene proton at C-14 (or C-20) was apparent as a doublet (J=15.04Hz) at 2.67ppm. A double triplet (J_1=4.52Hz, J_2=8.04Hz) at 2.89ppm was representative of the first benzylic tetralin proton, followed by the second, seen as a multiplet which was considered to be signals overlapping with those of two methylene protons at C-4 and C-20 between 3.02-3.11ppm. The remaining proton at C-20 (or C-4) resonated as a doublet (J=13.04Hz) at 3.38ppm. The aromatic region of the ^1H NMR spectrum of compound **(5.20)** was very well resolved, in comparison to that of the parent ketone **(5.19)**. All protons in this region were visible individually. The main changes were the two discrete symmetrical doublets (J=4.52Hz) at 8.47 and 8.53ppm respectively, representing the two protons at C-16 and C-22 positions, which were seen as

overlapping doublets in the parent molecule **(5.19)**. The key features of the ^{13}C NMR spectrum of compound **(5.20)** were the signals for the seven methylene carbons between 22.0-60.0ppm and the presence of the signals for the methyl carbons and the alcohol quaternary carbon, at 45.0 and 76.5ppm, respectively (Fig. 5.27).

The infrared spectrum of compound **(5.20)** confirmed the absence of the stretching frequency of the carbonyl group at 1677.3cm^{-1}. HRMS analysis gave an accurate molecular mass ($+H^+$) as 416.2705, calculated as 416.2702, molecular formula $C_{27}H_{34}N_3O$.

Disappointingly, dehydration of the alcohol **(5.20)** to the alkene was unsuccessful despite repeated attempts using the same conditions as used to generate the alkenes **(5.2)**, **(5.6)**, **(5.9)**, **(5.12)** and **(5.15)**.



(5.20)

Scheme 5.13 Attempted synthesis of (pyridin-2-yl)methyl analogue

5.5 Synthesis of compound (5.2) derivatives with either a seven- or a five-membered hydroaromatic ring

Akin to the series of compounds synthesised in the preceding Chapter, it was of interest to investigate the activity of a similar series of compounds where the tetralin ring was replaced by an indan (rigid) or benzosuberyl (more flexible) ring system. In principal, it was felt that compounds within this series could easily be furnished through the same sequence of steps involved in the synthesis of the tetralin series.

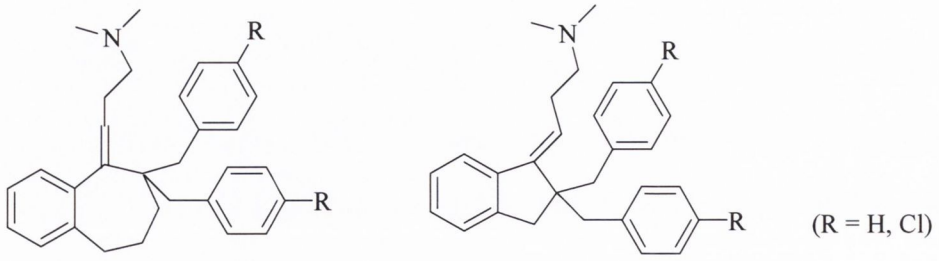
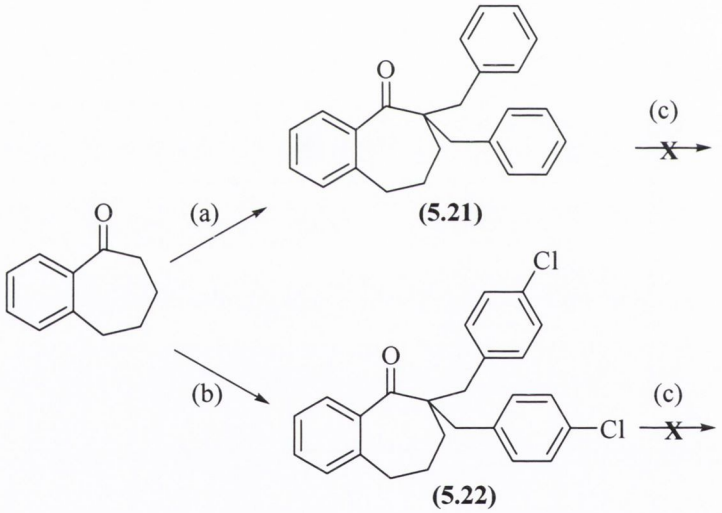


Fig.5.28 Benzosuberyl and indane analogues of compounds (5.2) and (5.6)

5.5.1 Attempted synthesis of benzosuberyl analogues of (5.2) and (5.6)

The synthesis of the bezosuberyl analogues commenced, by alkylation of benzosuberone with benzyl bromide and 4-chlorobenzyl chloride using potassium *tert*-butoxide as base in *t*-butanol for the benzylation steps (Scheme 5.15). Both compounds were isolated in reasonable yields, 59% for the benzyl **(5.21)** and 24% for the chlorobenzyl **(5.22)** respectively.



(a) Benzyl bromide, $(CH_3)_3COK$, *t*-butanol:diethyl ether (*v:v*, 1:1), RT, 4hrs, 59%
(b) 4-chlorobenzyl chloride, $(CH_3)_3COK$, *t*-butanol:diethyl ether (*v:v*, 1:1), RT, 5hrs, 24%
(c) Grignard reagent, dry THF, RT,

Scheme 5.15 Formation of compounds (5.21) and (5.22)

Compound **(5.21)** exhibited a similar ^1H NMR spectrum to that of compound **(5.0)**, but in addition displayed two overlapping pentets signals centred at 2.15ppm for the two γ–keto methylene protons. All the other signals in the aliphatic region were, however, shifted upfield by a considerable degree, yet they showed similar splitting patterns to

compound **(5.0)**. Signals in the aromatic region overlapped considerably in the ^{1}H NMR spectrum of compound **(5.21)**. A doublet (J=7.52Hz) was found at 6.92ppm for one proton, followed by two overlapping signals for a doublet (J~7.52Hz) and a triplet (J~7.75Hz), integrating for a total of two protons at 7.04 and 7.09ppm, respectively. The remaining eleven aromatic protons were closely assembled between 7.23-7.32ppm. The signals for the five methylene carbons were found to resonate from 23.0-43.0ppm in the ^{13}C NMR spectrum, followed by the signal for the tertiary carbon at 53.8ppm. The presence of a total of fourteen aromatic tertiary carbons was also confirmed in the region of 125.9-130.7ppm. The carbonyl carbon resonated at 213.9ppm.

The main changes in the ^{1}H NMR spectrum of the chloro derivative **(5.22)** were observed in the aromatic region. A broad doublet (J=7.52Hz) was located at 6.95ppm, integrating for one proton, followed by another broad doublet (J=7.52Hz) for one proton at 7.03ppm. The remaining ten protons were located close together, appearing as two multiplets, from 7.09-7.15ppm and 7.23-7.29ppm, respectively. Their corresponding carbon resonances were in the region of 126.1-131.8ppm in the DEPT 90 NMR spectrum of compound **(5.22)**. The terminal carbonyl carbon signal appeared at 213.0ppm in the ^{13}C NMR spectrum.

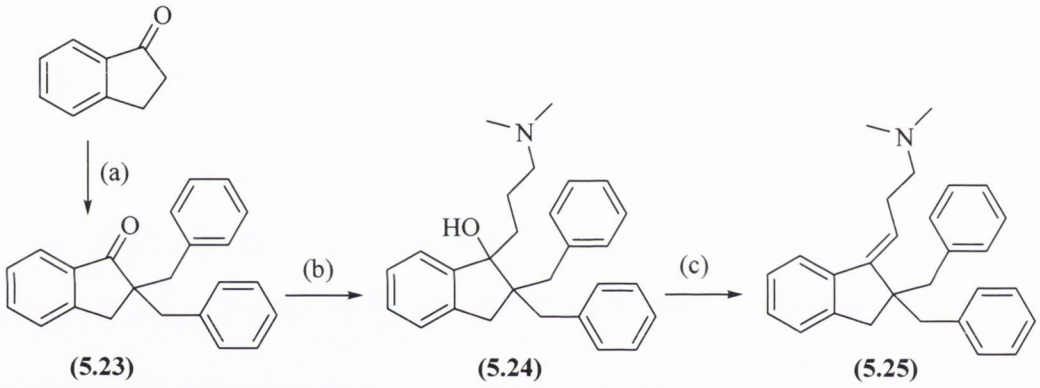
The key feature of the infrared spectra of compounds **(5.21)** and **(5.22)** were the $C{=}O_{str}$, at 1674.9cm^{-1} for compound **(5.21)** and 1673.8cm^{-1} for compound **(5.21)**. HRMS analysis gave the accurate molecular masses ($+Na^{+}$) as 363.1737, calculated 363.1725 with molecular formula $C_{25}H_{24}ONa$ for compound **(5.21)**; and 431.0959, calculated 431.0945 with molecular formula $C_{25}H_{22}Cl_{2}ONa$ for compound **(5.22)**.

It was somewhat surprising to find that despite repeated attempts, the coupling of the Grignard reagent to ketones **(5.21)** and **(5.22)** proved unsuccessful (Scheme 5.15); under the standard Grignard reaction conditions employed previously. No evidence to suggest formation of the desired amino alcohols could be detected by TLC, HRMS or by NMR analysis. In each case the starting material was recovered (Scheme 5.15). This suggests

that perhaps the flexible nature of the cycloheptanone ring results in the benzyl substituent being able to "crowd out" the carbonyl and thus prevent nucleophilic attack by the Grignard reagent.

5.5.2 Five membered hydroaromatic ring analogues

In order to fulfil the second objective, namely to determine the effect of indane ring analogues on anti-allergy activity, alkene **(5.25)** was synthesised in an analogous fashion that employed in the formation of compound **(5.2)**, using 1-indanone as starting material. Alkylation of 1-indanone with benzyl bromide, using potassium *tert*-butoxide as tertiary base in the presence of a mixture of *t*-butanol/diethyl ether, was successful in the production of compound **(5.23)** in good yield (84%).



(5.23) **(5.24)** **(5.25)**

(a) Benzyl bromide, $(CH_3)_3COK$, *t*-butanol:diethyl ether (*v:v*, 1:1), RT, 3hrs, 84%
(b) Grignard reagent, dry THF, RT, 4hrs, 98%
(c) 20% aq. oxalic acid, reflux, 8hrs, 87%

Scheme 5.16 Synthesis of compound (5.25)

The main features in the aliphatic region of the ^{1}H NMR spectrum of compound **(5.23)** was the benzylic indanone methylene singlet at 3.16ppm, flanked on either side by a benzyl methylene doublet signal, at 2.95ppm (J=13.54Hz) and 3.32ppm (J=13.54Hz). Signals for the twelve aromatic protons were overlapping between 7.13-7.21ppm, followed by a triplet (J=7.28Hz) at 7.34ppm for one proton. The doublet (J=8.04Hz) for the aromatic proton nearest the carbonyl resonated at 7.73ppm. The DEPT 90 NMR spectrum confirmed the presence of fourteen tertiary carbons in the region of

123.2-134.3ppm. The ^{13}C NMR spectrum indicated the existence of quaternary carbons, one at 54.8ppm and four at 136.6-152.7ppm. The carbonyl signal was at 210.0ppm. In the DEPT 135 NMR spectrum of compound **(5.23)**, the benzylic tetralone carbon and methylene carbons of the benzyl group were clearly located at 34.2 and 43.2ppm, respectively.

This molecule showed little change in the infrared spectrum, its principal absorption again that of the carbonyl stretching absorption, at 1705.5cm^{-1}.

Subsequent reaction of compound **(5.23)** with the Grignard reagent gave the desired indan amino alcohol **(5.24)** in quantitative yield. The main changes in the ^{1}H NMR spectrum of compound **(5.24)** related to the aliphatic region. Four discrete multiplets represented the six methylene protons on the side chain, resonating at 1.53-1.58ppm, 1.83-1.92ppm, 2.03-2.14ppm and 2.40-2.47ppm. The signal for the six methyl protons was found as a singlet at 2.50ppm. The remaining six methylene protons appeared as six separate doublets, at 2.69 (J=15.57Hz), 2.83 (J=13.56Hz), 2.91 (J=16.04Hz), 3.09 (J=16.04Hz), 3.19 (J=14.04Hz) and 3.24ppm (J=15.56Hz), respectively. In the aromatic region of the spectrum, a total of fourteen protons resided in the region of 6.86-7.69ppm. The key features of the ^{13}C NMR spectrum were the presence of six methylene carbons at 21.3, 35.5, 36.7, 39.6, 39.9 and 60.4ppm, respectively. The methyl carbon was located at 45.2ppm. Two aliphatic quaternary carbons were found at 56.3 (C-2) and 84.2ppm (C-1), respectively.

Examination of the infrared spectrum confirmed the absence of the $C{=}O_{str}$ at 1705.5cm^{-1}. HRMS analysis revealed the molecule mass ($+H^{+}$) as 400.2626, calculated as 400.2640 with molecular formula: $C_{28}H_{34}NO$.

In order to form the alkene **(5.25)**, alcohol **(5.24)** was treated with a 20% aq. oxalic acid solution under gentle reflux. The dehydration step was complete within eight hours and the desired product **(5.25)** was obtained in good yield (87%).

The NMR spectra of compound **(5.25)** indicated the presence of an unequal stereoisomeric mixture. Again, NMR characterisation of the molecule was based on the predominant isomer. The C-H COSY NMR spectrum allowed the correlation of the assignment of most proton and carbon signals detected in the ^{1}H and ^{13}C spectra of the alkene **(5.25)**. ^{1}H NMR analysis (Fig. 5.29) showed one singlet at 2.45ppm for the six methyl protons, followed by two overlapping triplets (J~7.76Hz) at 2.59ppm for the methylene protons at C-10 position. The two methylene protons on C-9 appeared as a multiplet at 2.84ppm. Two methylene protons (C-13 or C-19) occurred as two overlapping doublets (J=13.56Hz) at 2.95ppm. The two benzylic indan protons resonated as two overlapping doublets (J=6.52Hz) at 3.05ppm, overlapping with another two overlapping doublets (J=13.56Hz) for the remaining two protons at C-19 or C-13 at 3.08ppm. The alkenic proton was located at 5.40ppm as a triplet (J=7.02ppm). Thirteen of the fourteen aromatic protons resonated closely together form 7.23-7.36ppm.The final aromatic proton at C-7 position, appeared as a doublet (J=7.52Hz) at 7.70ppm.

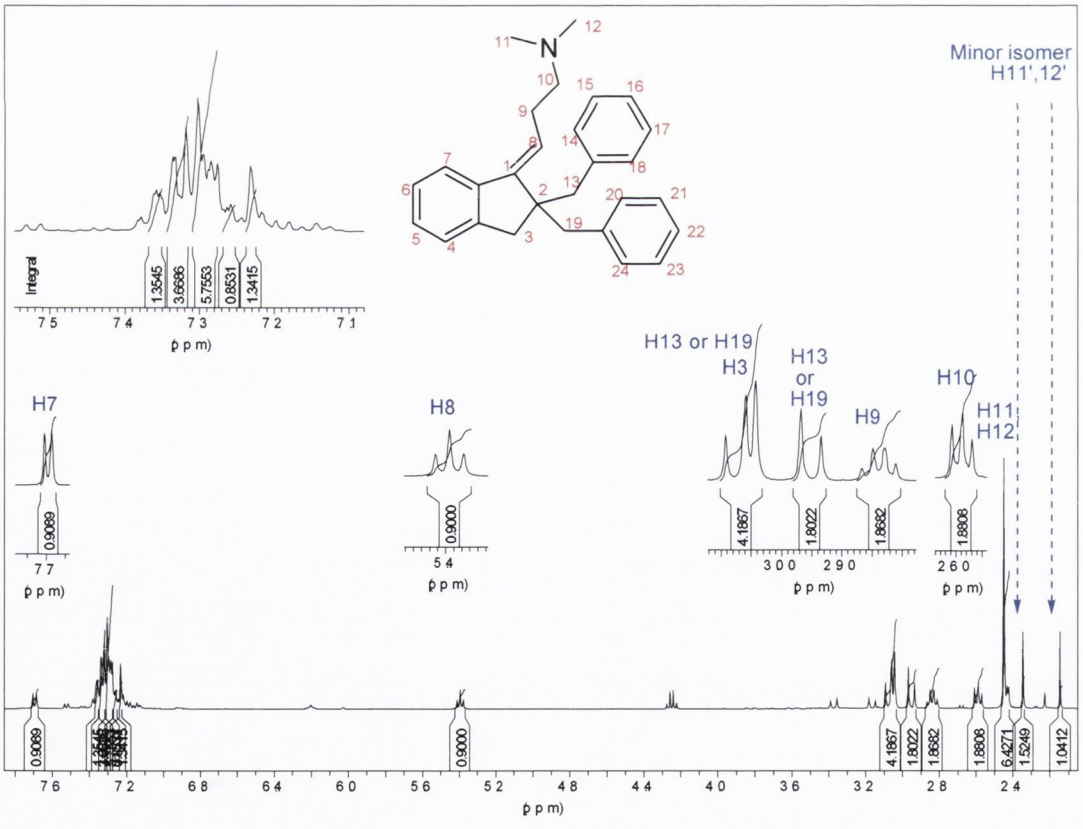


Fig. 5.29 ^{1}H NMR spectrum of compound (5.25)

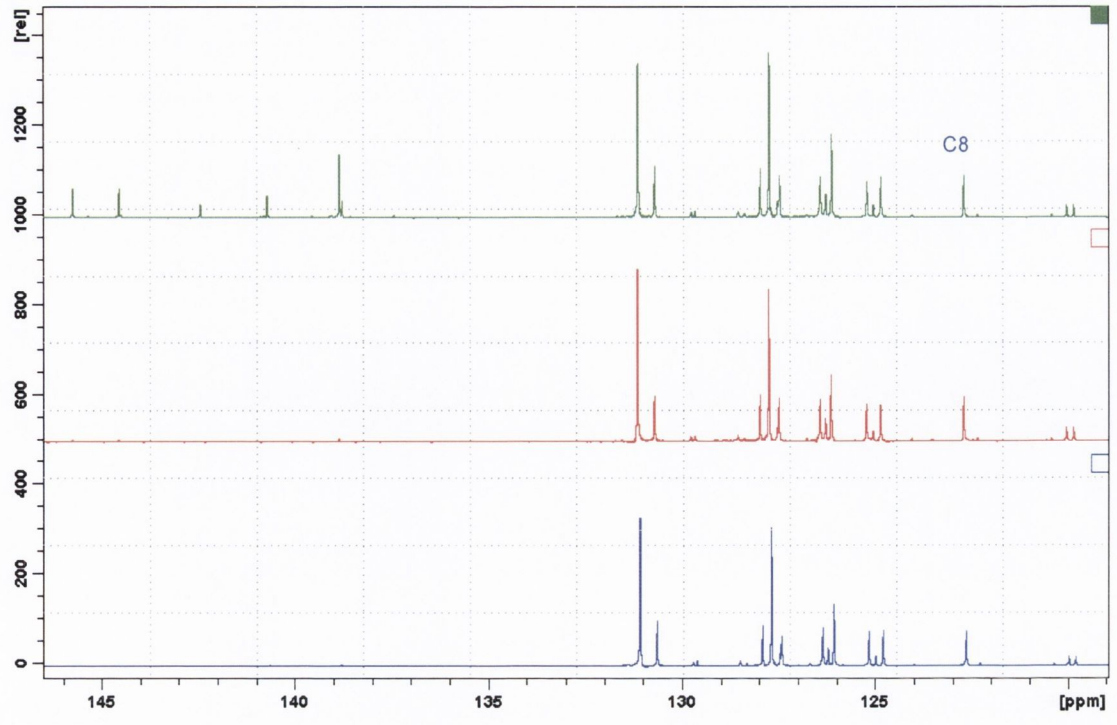


Fig. 5.30 ^{13}C, DEPT 135, DEPT 90 NMR spectra of compound (5.25) from 118.9-146.4ppm

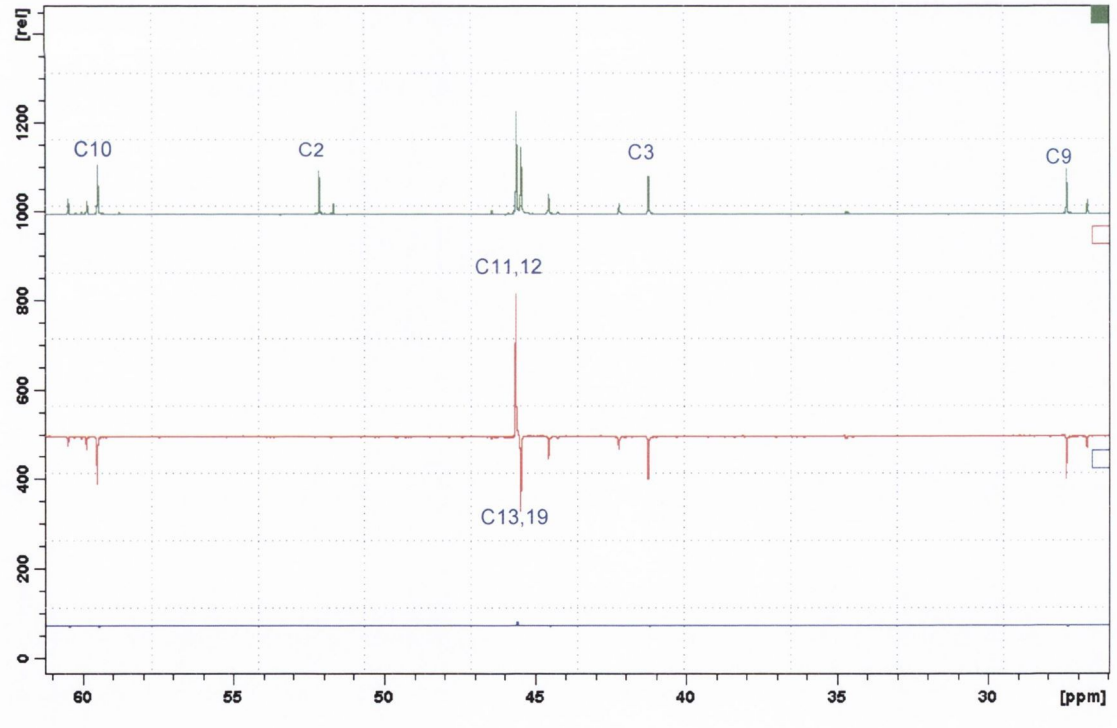


Fig. 5.31 ^{13}C, DEPT 135, DEPT 90 NMR spectra of compound (5.25) from 25.9-61.2ppm

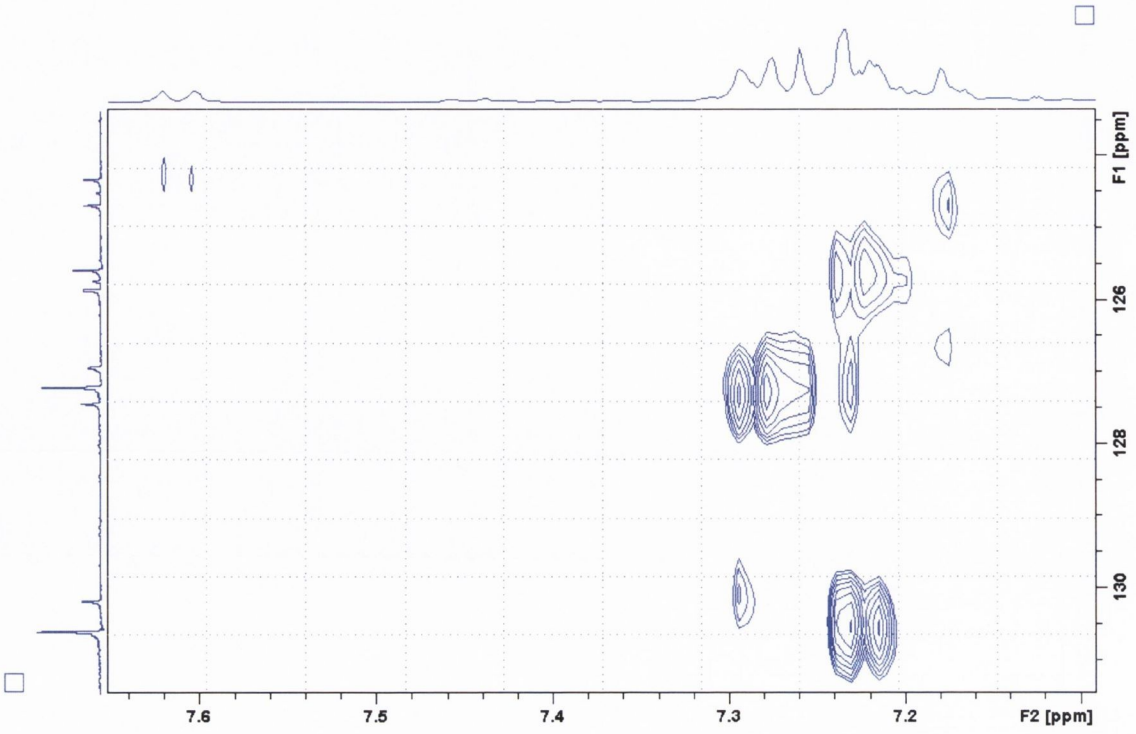


Fig. 5.32 C-H COSY NMR spectrum of compound (5.25) from 7.09-7.63ppm

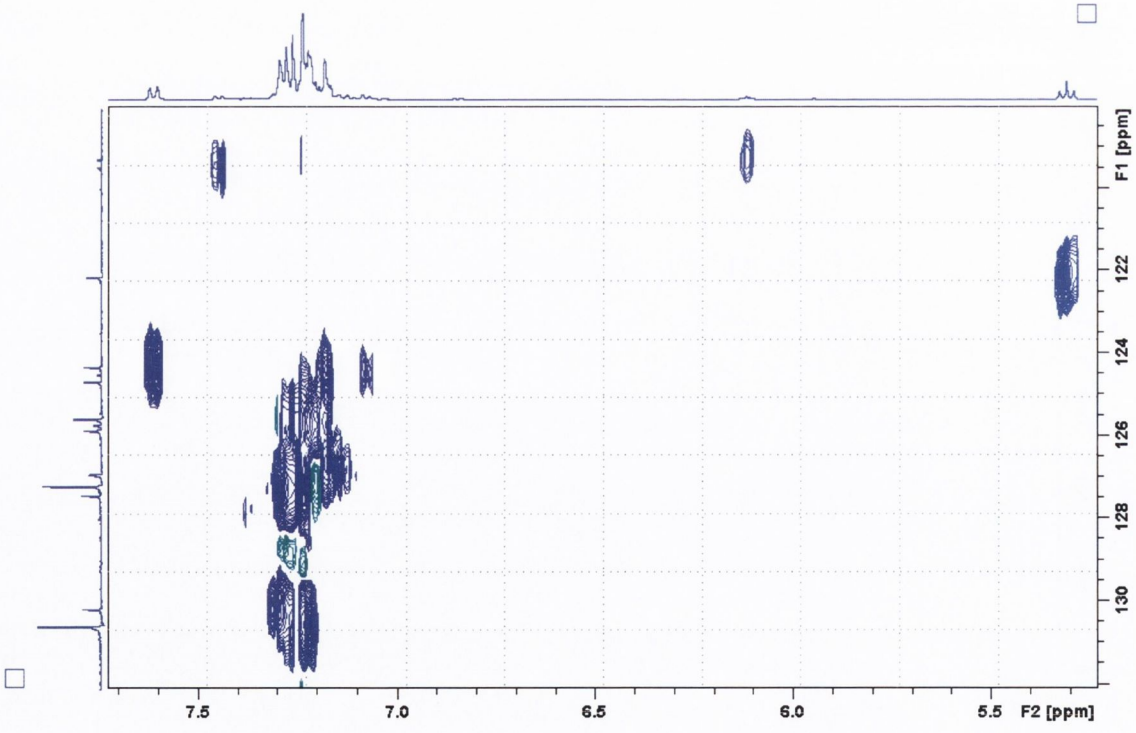


Fig. 5.33 C-H COSY NMR spectrum of compound (5.25) from 5.28-7.72ppm

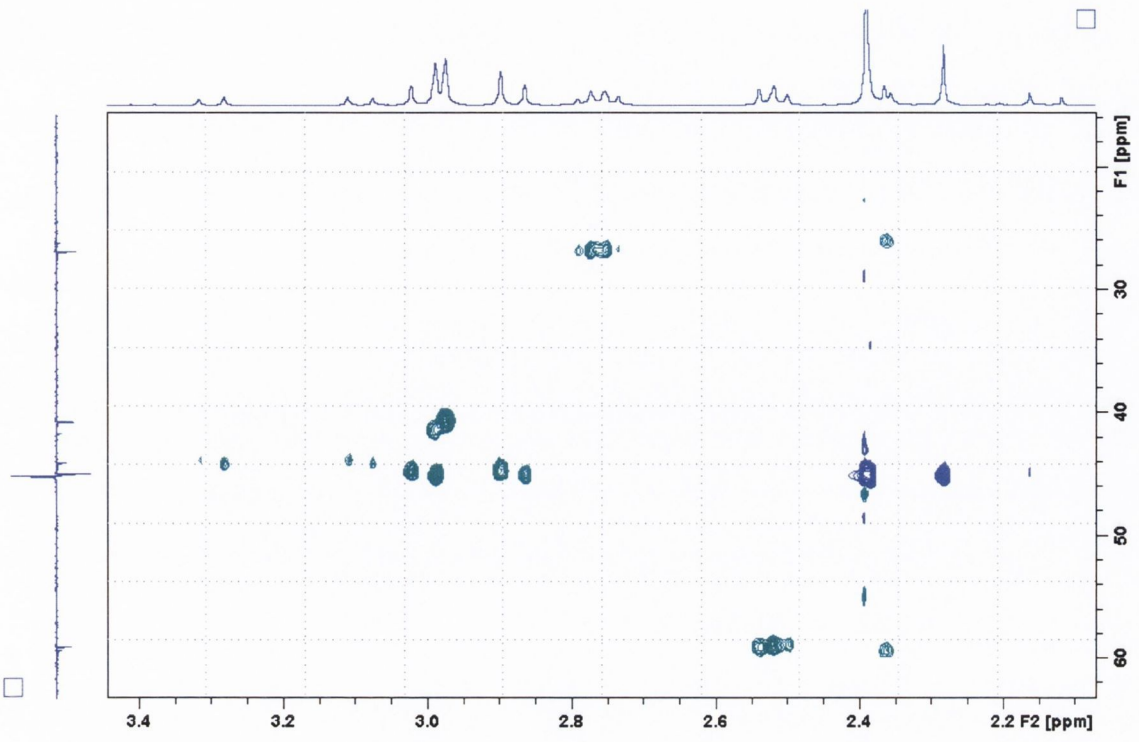


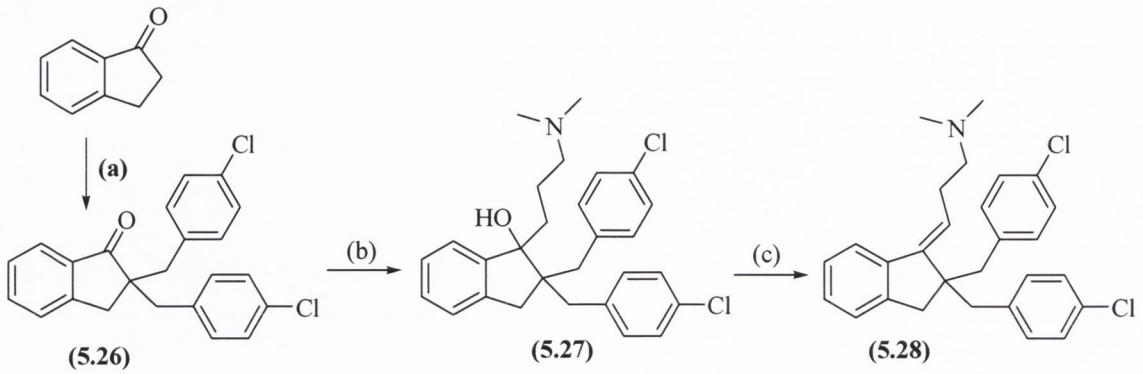
Fig. 5.34 C-H COSY NMR spectrum of compound (5.25) from 2.05-3.45ppm

The ^{13}C NMR spectrum of compound **(5.25)** confirmed the existence of five quaternary carbons between 138.4 and 145.3ppm. A total of fourteen aromatic tertiary carbons were identified in the region of 122.2-130.7ppm in the DEPT 90 NMR spectrum (Fig. 5.30). With the aid of C-H COSY NMR spectrum (Fig. 5.33), alkenic tertiary carbon was found at 122.2ppm. The DEPT 135 NMR spectrum (Fig. 5.31) revealed the presence of five methylene carbons at 26.9-59.0ppm. The methyl carbon signal resonated at 45.2ppm

Dehydration of the alcohol **(5.24)** showed the diagnostic signal alkene signal $C=C_{wk}$, at 1648.1cm^{-1} in the infrared spectrum of the alkene **(5.25)**. HRMS analysis gave satisfactory molecular mass ($+H^+$) as 382.2522, calculated as 382.2535 for molecular formula $C_{28}H_{32}N$, corresponded to the structure of compound **(5.25)**.

Substitution on the aromatic portion of the benzyl group by an electron-withdrawing chlorine group at the *para* position was approached through a similar sequence of reactions to those used for compound **(5.25)**. Alkylation of 1-indanone with

4-chlorobenzyl chloride gave the desired chloro derivative **(5.26)** as yellow crystalline solid, in good yield (88%).



(a) 4-chlorobenzyl chloride, $(CH_3)_3COK$, *t*-butanol:diethyl ether (*v:v*, 1:1), RT, 3hrs, 88%
(b) Grignard reagent, dry THF, RT, 4hrs, 99%
(c) 20% aq. oxalic acid, reflux, 8hrs, 90%

Scheme 5.17 Synthesis of compound (5.28)

The ^1H NMR spectrum of compound **(5.26)** displayed similar coupling patterns in the aliphatic region to that of compound **(5.23)**. The aromatic region was very well resolved. Two discrete sharp identical doublets (J=8.56Hz) were centred at 7.03ppm for the protons at C-14, 18, 20, 24, and at 8.56ppm for the protons at C-15, 17, 21, 23. Each doublet was considered to be four overlapping doublets. The proton on C-4 appeared as a doublet (J=8.00Hz) at 7.17ppm, followed by one triplet (J=7.28Hz) and one double triplet (J_1~1.00Hz, J_2~7.28Hz) at 7.23 and 7.40ppm for the two aromatic protons of the indanone ring. The remaining proton on C-7 was displayed as a doublet (J=7.52Hz) at 7.64ppm. The key features of the ^{13}C NMR spectrum were the presence of twelve aromatic tertiary protons in the region between 123.3-132.0ppm. The six aromatic quaternary carbons were located between 134.6-152.2ppm. The carbonyl group resonated at 209.4ppm.

This compound exhibited characteristic infrared absorption for the ketone at 1706.5cm^{-1}. HRMS analysis gave an accurate molecular mass ($+Na^+$) as 403.0627, calculated 403.0632 with molecular formula: $C_{23}H_{18}OCl_2Na$.

Treatment of compound **(5.26)** with the Grignard reagent in dry THF gave the amino alcohol **(5.27)** in quantitative yield. The ^{1}H NMR spectrum of compound **(5.27)** showed four methylene protons appearing as three multiplets centred at 1.47, 1.78 and 1.95ppm, respectively. Two broad overlapping triplets at 2.35ppm integrated for two protons, followed by a sharp singlet at 2.44ppm for the six methyl protons. Three separate doublets were observed at 2.64 (J=16.04Hz) 2.71 (J=5.04Hz) and 2.75ppm (6.52ppm), integrating as one proton for each. Two methylene protons appeared as two overlapping doublets at 2.90 (J=15.04Hz) and 2.93ppm (J=11.56Hz). The remaining methylene proton was located at 3.08ppm as a doublet (J=15.52Hz). The aromatic region showed different coupling patterns to the parent compound **(5.26)**. Two overlapping doublets (J=8.04Hz) resonated at 6.63ppm for two protons of the benzyl group. Another two protons of the benzyl group also appeared as two overlapping doublets (J=8.00Hz) at 7.01ppm, overlapping with a doublet (J=7.52Hz) at 7.03ppm for one aromatic indan proton. Two discrete triplets (J=7.28Hz) were located at 7.17 and 7.23ppm respectively, for the two aromatic indan protons. The remaining four protons of the benzyl groups existed as a pair of two overlapping doublets (J=8.52Hz) at 7.33 and 7.54ppm, respectively. Additional doublet (J=10.04Hz) was overlapping with the first doublet of the pair at 7.36ppm, identified as an aromatic indan proton. The key features in the ^{13}C NMR spectrum were the presence of the six methylene carbons from 21.1-60.3ppm and the methyl carbon signal at 45.1ppm. The quaternary alcohol carbon was found at 83.7ppm.

The absence of $C=O_{str}$ peak at 1706.5cm^{-1} was confirmed by the infrared spectrum of compound **(5.27)**. HRMS analysis gave an accurate molecular mass ($+H^{+}$) as 468.1628, calculated as 468.1631 with molecular formula: $C_{28}H_{32}NO$.

The final step toward the alkene **(5.28)**, namely dehydration of compound **(5.27)** was complete within eight hours and the product was obtained in good yield (90%) (Scheme 5.17). The ^{1}H and ^{13}C NMR spectra of compound **(5.28)**, like many of the molecules in this Chapter, confirmed the presence of an unequal geometric mixture of isomers. Identification of the alkene *via* NMR spectra analysis was, again, based on the

predominant single isomer. C-H COSY NMR spectrum (Fig. 5.38 and Fig. 5.39) allowed for the correlation and assignment of most of the proton and carbon signals detected in the ^{1}H and ^{13}C spectra of compound **(5.28)**.

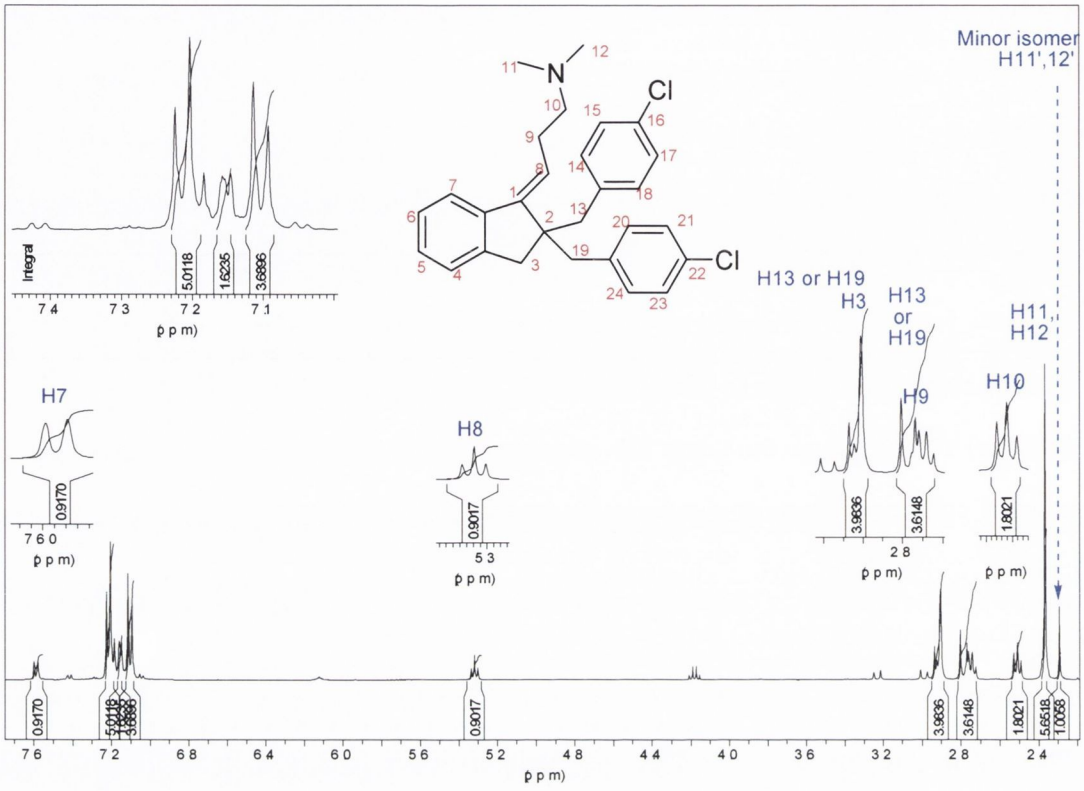


Fig. 5.35 ^{1}H NMR spectrum of compound (5.28)

In the ^{1}H NMR spectrum (Fig. 5.35) of compound **(5.28)**, the singlet at 2.37ppm was believed to be that of the six methyl protons. The two methylene protons on C-10 appeared as two overlapping triplets (J=7.52Hz) at 2.51ppm. The methylene protons on C-9 resonated as a multiplet at 2.75ppm, which were overlapping with a doublet (J~13.56Hz) at 2.79ppm integrating for two methylene protons on either C-13 or C-19. The two benzylic indan protons resonated as a singlet at 2.91ppm, overlapping with two overlapping doublets (J~12.08Hz) at 2.93ppm for the remaining two methylene protons. The alkenic proton was located at 5.32ppm as a triplet (J=7.04Hz). The aromatic region was well resolved. A doublet (J=8.52Hz) was found at 7.10ppm integrating for three protons, followed by two overlapping doublets (J=4.52Hz) at 7.15ppm for two protons. The following multiplet in the region of 7.18-7.22ppm, was considered as overlapping signals of two triplets (J~8.04Hz) and four doublets (J=8.52Hz) and integrated for six

protons in total. The final aromatic proton was located at 7.59ppm as a doublet (J=7.52Hz).

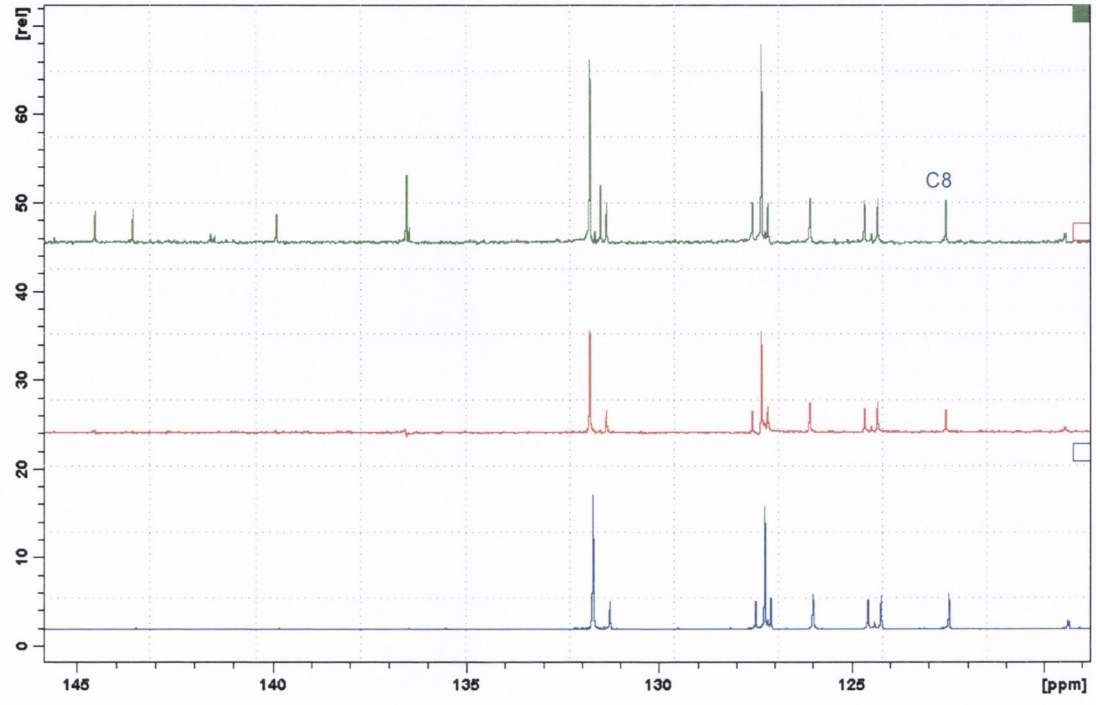


Fig. 5.36 ^{13}C, DEPT 135, DEPT 90 NMR spectra of compound (5.28) from 118.9-146.0ppm

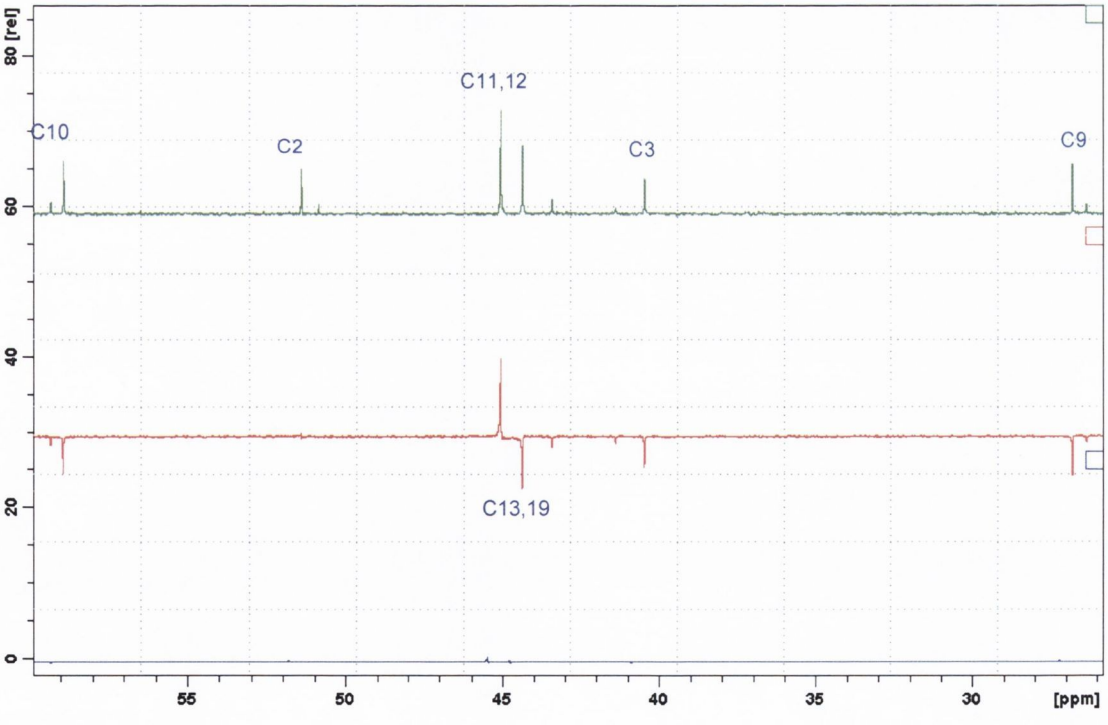


Fig. 5.37 ^{13}C, DEPT 135, DEPT 90 NMR spectra of compound (5.28) from25.9-60.0ppm

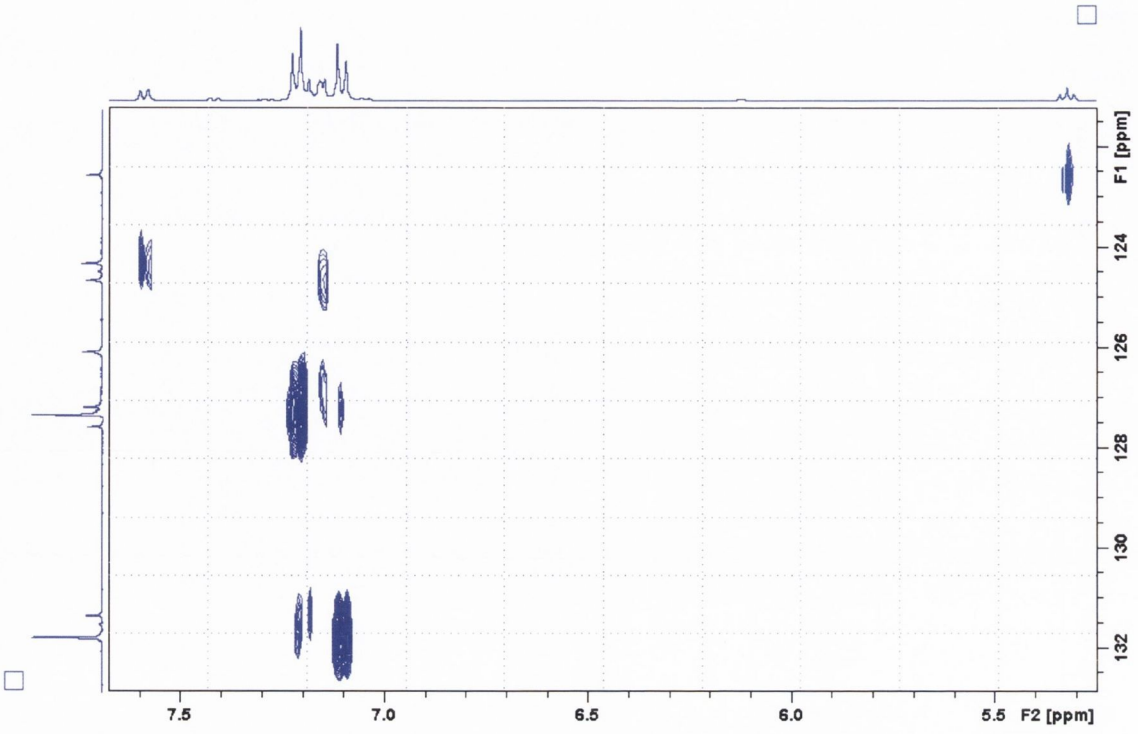


Fig. 5.38 C-H COSY NMR spectrum of compound (5.28)

from 5.25-7.68ppm

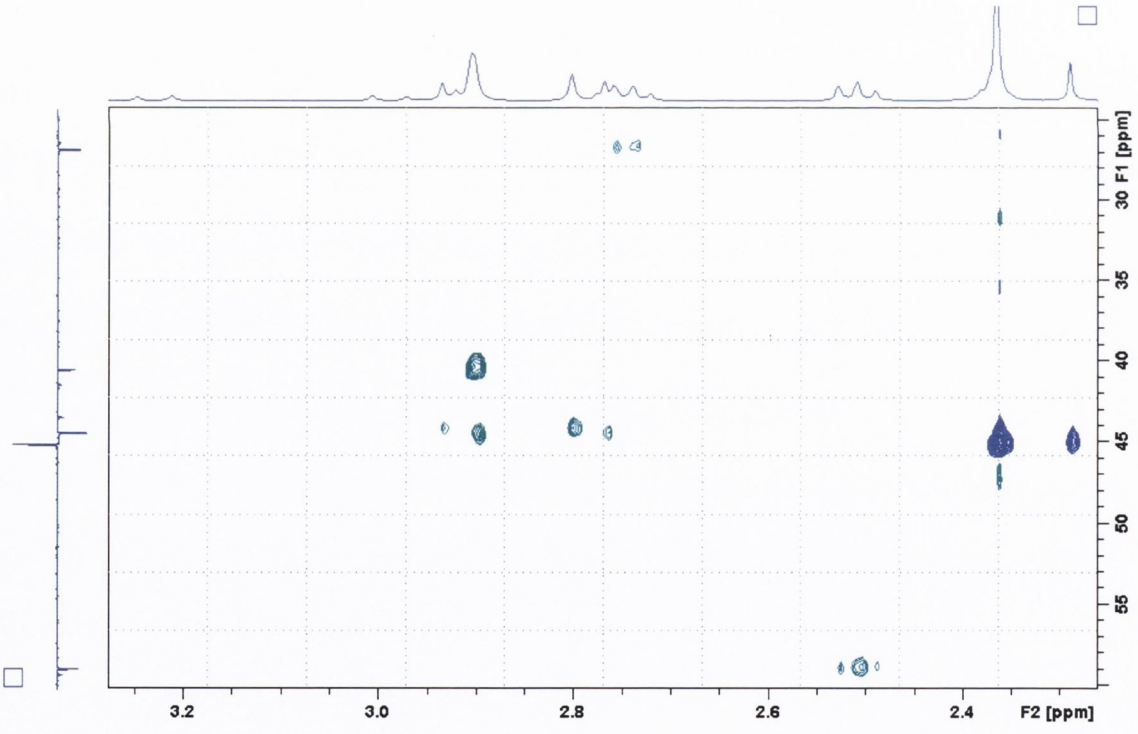


Fig. 5.39 C-H COSY NMR spectrum of compound (5.28)

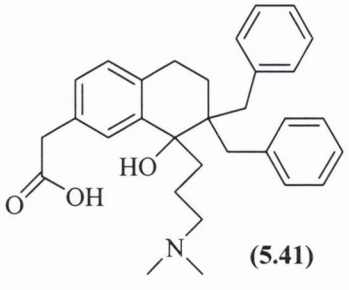
from 2.35-3.27ppm

Important features of the ^{13}C NMR spectrum of compound **(5.28)** were signals for a total of five methylene carbons between 26.8-58.9ppm and the alkenic tertiary carbon at 122.6ppm.

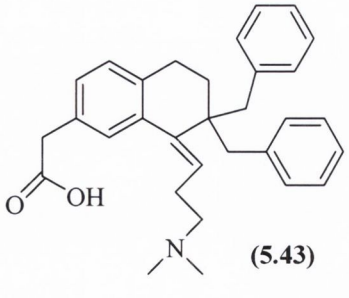
The only change for this compound in the infrared spectrum was the peak of the alkene group on the side chain, $C=C_{wk}$ at 1645.6cm^{-1}. HRMS analysis gave the actual molecular mass of 450.1739, which correlates well to the calculated mass of 450.1755. The elemental composition for that peak, $C_{28}H_{30}Cl_2N$, corresponded well to the designed structure of compound **(5.28)** ($+H^+$).

5.6 Synthesis of amino acid analogues

Several compounds synthesised within the tetralin series of compounds have demonstrated impressive activity *in vivo* by Woods (2009) in the Department. However, the highly lipophilic nature of the lead molecules somewhat comprises their true potential, especially when oral administration of these compounds is required for future studies. In addition, within the alkene series, it would be desirable to isolate a single geometric isomer of the alkene, although at present this proved difficult through chromatographic approaches. To address these concerns, it was decided to embark upon the synthesis of the amino acids **(5.41** and **5.43)**. It was hoped that by placing the acetic acid residue at position-7 of the tetralin ring that hydrogen bonding to the tertiary amine would favour the formation of the *trans* double bond.



(5.41)

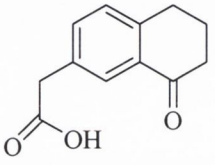


(5.43)

Fig. 5.40 Amino acid analogues (5.41) and (5.43)

5.6.1 Synthesis of tetralone acetic acid (5.34)

The synthesis of the amino acids **(5.41)** and **(5.43)** required us to generate the key tetralone acetic acid intermediate **(5.34)**. It was felt that once this compound was synthesised, its conversion into the amino acid **(5.41)** would follow the same sequence of steps as that used to synthesise compound **(5.3)**. The synthesis of compound **(5.34)** has been previously described, using 4-phenylbutyric acid as the starting material (Giardina et al., 1994). However, there was no experimental data provided for each intermediate step in its synthesis.

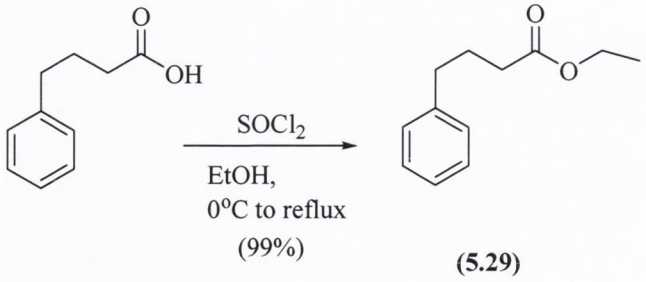


(5.34)

Fig. 5.41 Chemical structure of compound (5.34)

Step 1: Formation of the ethyl ester (5.29)

Firstly, 4-phenylbutyric acid was converted into its corresponding ethyl ester. The conversion was effected by the drop-wise addition of thionyl chloride to a solution of 4-phenylbutyric acid in ethanol at 0°C over thirty minutes. To facilitate complete conversion to the acid, the reaction was heated to reflux for a further four hours. The corresponding ethyl ester **(5.29)** was obtained quantitatively, as a pale yellow oil following purification (Scheme 5.18).

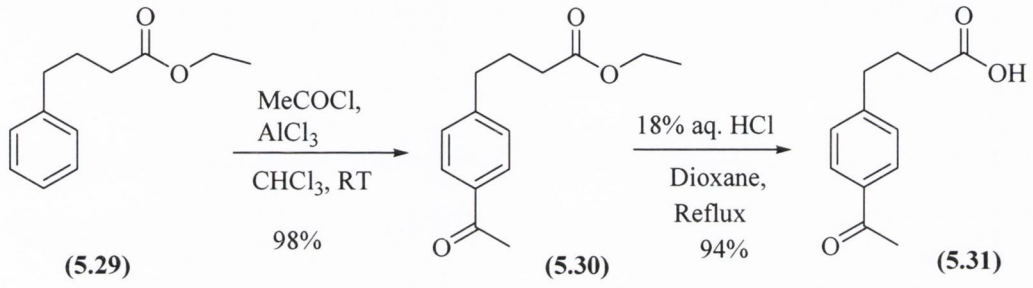


(5.29)

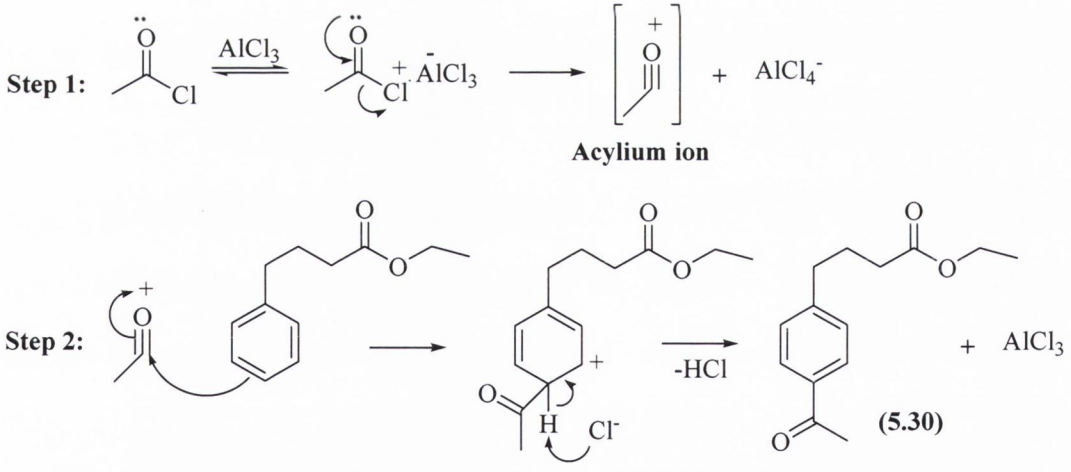
Scheme 5.18 Synthesis of compounds (5.29)

Steps 2 and 3: Acylation of ethyl ester (5.29) and hydrolysis of ethyl ester (5.30)

The next step involved inserting an acetyl substituent onto the *para*-position of the aromatic ring of the ethyl ester **(5.29)**. This transformation was completed through a Friedel-Crafts electrophilic aromatic substitution reaction using acetyl chloride and aluminium chloride as a Lewis acid catalyst and chloroform as solvent under reflux conditions. The acylated derivative **(5.30)** was obtained in near quantitative within four hours. Scheme 5.20 depicts the sequence of steps involved in the reaction.



Scheme 5.19 Synthesis of compounds (5.30) and (5.31)



Scheme 5.20 Mechanism of formation of compound (5.30)

The acyl group was easily identified by NMR analysis. A sharp singlet at 2.57ppm was identified as the three methyl protons of the acyl group in the ^{1}H NMR spectrum of compound **(5.30)** and the corresponding carbon was located at 26.2ppm in the ^{13}C NMR spectrum. The carbonyl of the acyl group was found at 197.3ppm. The infrared spectrum

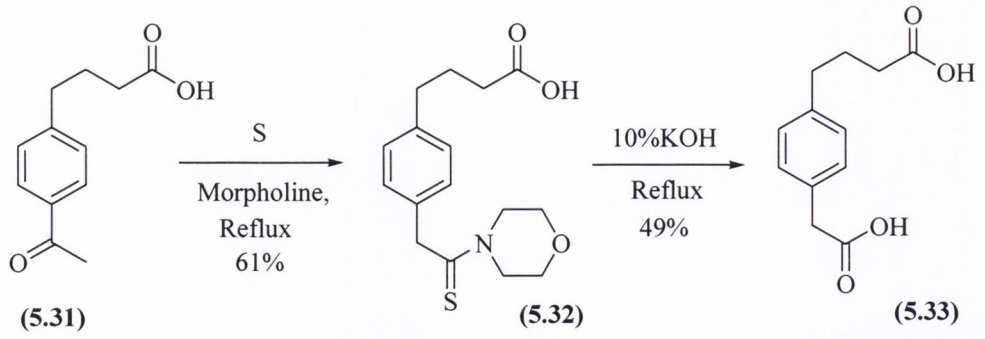
of the acylated compound **(5.29)** showed principal signals representing the C-O_{str} at 1268.3cm^{-1}, C=O_{str} at 1732.2cm-1 (ester) and 1682.8cm^{-1} (ketone). HRMS anaylysis gave satisfactory molecular mass (+2H^{+}) as 236.2012, calculated as 236.1407 with molecular formula $C_{14}H_{20}O_3$

Subsequent removal of the ethyl ester group was accomplished using 18% aq. HCl and dioxane. Its conversion was complete after the solution was refluxed for ten hours. After acid-base work-up and purification by flash column chromatography, 4-acetylphenylbutyric acid **(5.31)** was obtained, as a white solid, in excellent yield (94%) (Scheme 5.19).

The ^{1}H and ^{13}C NMR spectra of compound **(5.31)** displayed a similar coupling pattern to those of the parent compound **(5.30)**, apart from the absence of the signals associated with the ethyl ester group. Infrared analysis of compound **(5.31)** showed prominent C=O_{str} of the ketone group at 1681.7cm^{-1}, the carboxylic acid C=O_{str} stretching frequency at 1707.9cm^{-1} and O-H_{br} stretching at 3048.3cm^{-1}.

Steps 4 and 5: Formation of thioamide (5.32) and dicarboxylic acid (5.33)

Having successfully introduced the acetyl group onto 4-phenylbutyric acid, the synthesis of the intermediate thioamide **(5.32)** was carried out in order to generate the dicarboxylic acid **(5.33)** (Scheme 5.21). The use of thioamides as intermediates in medicinal, organic and peptide chemistry has widely been reported in the literature (Jurayj & Cushman, 1992; Metzner, 1992; Hoeeg-Jensen et al., 1994). Many synthetic approaches to the formation of thioamides have been made available. Recent developments include the use of microwave heating for the formation of the most popular type, namely thiomorpholides (King & McMillan, 1946; Nooshabadi et al., 1999; Aghapoor et al., 2002).

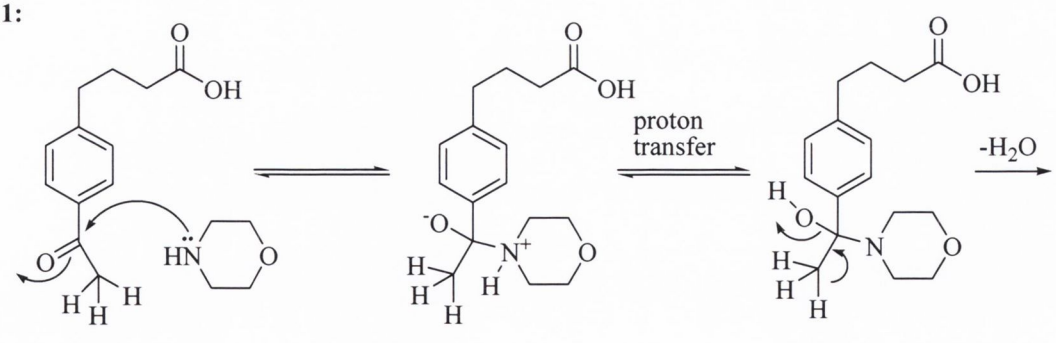


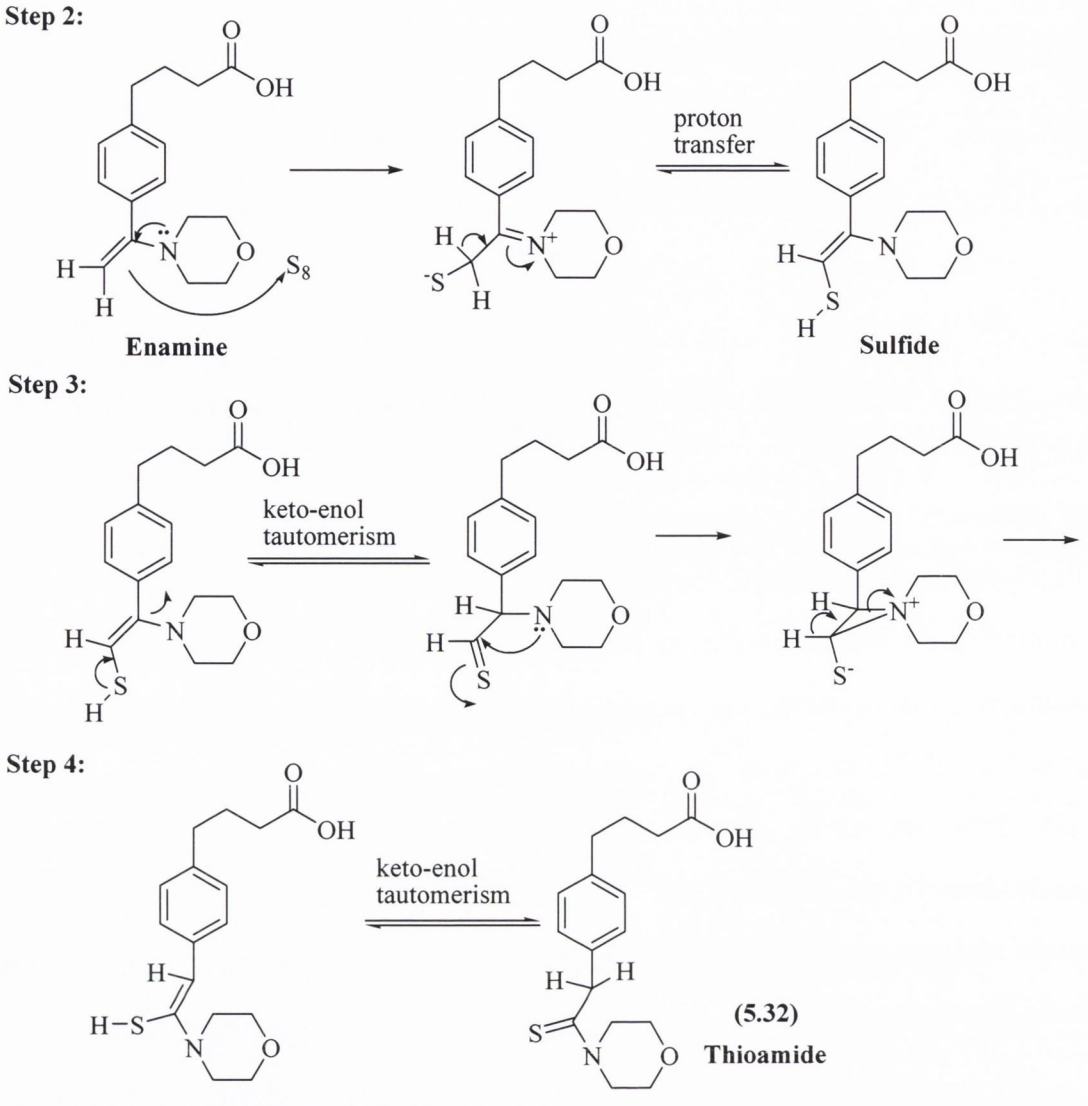
Scheme 5.21 Formation of compounds (5.32) and (5.33)

The approach used for the synthesis of thioamide **(5.32)** was to employ the Willgerodt-Kindler method (Brown, 1975) of using morpholine and sulphur to effect the formation of the thiomorpholide from the aryl alkyl ketone **(5.31)**. The progress of the reaction was monitored by TLC (mobile phase: ethyl acetate) and after three hours the starting acid **(5.31)** had been completely consumed. The method described by Giardina et al., (1994), of adding cold ethanol to the reaction mixture in order to facilitate crystallisation of thioamide **(5.32)** was unsuccessful in our hands. Instead the crude reaction mixture was isolated following work-up and extensive purification by flash column chromatography.

A proposed reaction mechanism for this Willgerodt-Kindler reaction is depicted in Scheme 5.22 (Carmack, 1989, Mundy et al., 2005):

Step 1:





Scheme 5.21 Mechanism of formation of compound (5.32)

The key steps involved are:

1 Interaction of the ketone moiety with the amine functionality of morpholine to generate the enamine.

2 Generation of the enesulphide intermediate through conjugate addition of sulphur onto the enamine.

3 Formation of the thiocarbonyl from suphide through enol to keto tautomerism. Aziridine ring formation by nucleophilic attack of amine functionality onto the thiocarbonyl.

4 Ring opening of the thio-substituted iminium-aziridinium enol-keto tautomerization to form the thiomorpholide **(5.32)**.

^{1}H NMR analysis of compound **(5.32)** revealed the presence of two overlapping pentets (J=7.49Hz) at 1.94ppm, two overlapping triplets (J=7.46Hz) at 2.29ppm and two overlapping triplets (J=7.52Hz) at 2.73ppm representing the six methylene protons of the carboxylic acid side chain, integrating for two protons each. Two overlapping doublets (J=4.76Hz) at 3.39ppm were identified as the two methylene protons beside the thiocarbonyl group, followed by four overlapping triplets (J=4.84Hz) for the four methylene protons close to the oxygen atom of the thioamide group at 3.72ppm. The remaining four protons of the thioamide group appear as another set of four overlapping triplets (J=4.84Hz) at 4.33ppm. In the aromatic region, two identical doublets were located at 7.13ppm (J=7.96Hz) and 7.21ppm (J=7.88Hz), each integrating for two protons. The proton of the carboxylic acid moiety appeared as a broad singlet at 7.29ppm. Two aromatic quaternary carbons were found at 132.8 and 140.0ppm, respectively in the ^{13}C NMR spectrum. The carbonyl signal for the acid appeared at 170.8ppm, while that of the thiocarbonyl resonated at 199.6ppm. The DEPT 90 NMR spectrum revealed the presence of four aromatic tertiary carbons at 127.3 and 128.5ppm, respectively. In the DEPT 135 NMR spectrum, three methylene carbons from the carboxylic acid side chain were located at 26.0, 31.6 and 34.4ppm, respectively. The signal for the two methylene carbons linked to the nitrogen atoms of the thioamide group was found at 49.7ppm, followed by the signal of the methylene carbon attached to the thiocarbonyl bond at 50.2ppm. The remaining two methylene carbons of the thioamide group appeared at 66.1 and 66.4ppm, respectively. The compound **(5.32)** showed many important infrared absorption, including those of ethereal C-O_{str}, thiocarbonyl C=S_{str}, carboxylic acid C=O_{str} and O-H_{br} at 1030.8cm^{-1}, 1113.3cm^{-1}, 1642.1cm^{-1} and 2921.3cm^{-1}.

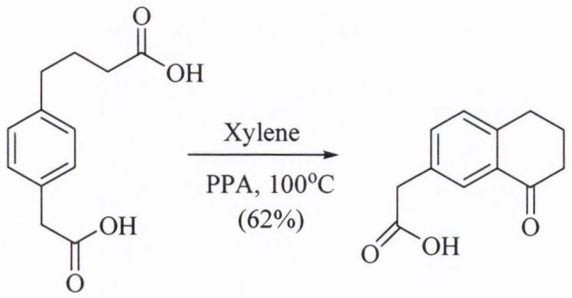
The subsequent hydrolysis step was performed following the treatment of the thiomorpholide intermediate **(5.32)** with 10% aq. KOH under reflux conditions. The progress of the reaction was monitored by TLC (ethyl acetate as mobile phase) to check for the disappearance of the starting material. This transformation was accomplished smoothly within three hours. Due to the fact that the product **(5.33)** is a dicarboxylic acid,

purification of the desired compound by flash column chromatogram was difficult. Bearing this in mind, the work-up procedure was believed to be essential for generation of compound **(5.33)** in pure from. Having reviewed the work-up procedure for hydrolysis of a thiomorpholide compound, reported by Giardina et al., (1994), a more sequential work-up procedure was carried out. The reaction mixture was initially acidified with 2M aq. HCl and extracted with ethyl acetate. The crude acid was then subjected to an acid-base extraction step using 5% aq. $NaHCO_3$, 2M aq. HCl and ethyl acetate as extraction solvent. The desired diacid **(5.33)** was then afforded as a pale yellow solid in improved yield (49%), compared to a 35% yield as stated in the literature (Giardina et al., 1994). The main changes in the ^1H NMR spectrum of compound **(5.33)** compared to its parent compound **(5.22)**, included the two methylene protons beside the newly formed carboxylic acid appearing as two overlapping singlets at 3.58ppm. The ^{13}C NMR spectrum contained signals for four methylene carbons at 25.9, 32.3, 33.8 and 39.7ppm, respectively. Signals for the two carbonyl moieties of both acid groups were found at 173.9 and 175.5ppm, respectively.

Important features in the infrared spectrum of compound **(5.33)** included carboxylic acids $C{=}O_{str}$ stretching and $O{-}H_{br}$ stretching, at 1702.0cm^{-1} and 2924.9cm^{-1}, while HRMS analysis gave the accurate molecular mass ($+Na^+$) as 245.0793, calculated 245.0784 with molecular formula $C_{12}H_{14}O_4Na$.

Step 6: Cyclisation of compound (5.33)

Formation of the tetralone acetic acid **(5.34)** was accomplished by heating compound **(5.33)** to 100°C in a two-phase mixture consisting of polyphosphoric acid (PPA) and xylene over three hours (Scheme 5.23). On completion the reaction was quenched by the addition of ice and extracted with ethyl acetate. The organic layer was then treated with base (5% $NaHCO_3$), the basic layer was acidified with 2M aq. HCl and extracted with ethyl acetate to afford **(5.34)** as an orange solid in moderate yield (62%).



(5.33) **(5.34)**

Scheme 5.23 Formation of compound (5.34)

Analysis of the ^{1}H NMR spectrum of **(5.34)** revealed similar coupling patterns in the aliphatic region to that encountered in NMR analysis of the parent compound **(5.33)**. In the aromatic region, a pair of doublets was observed at 7.24ppm (J=7.84Hz) and 7.42ppm (J=7.84Hz), each integrating as one proton. The remaining aromatic proton appeared as a singlet at 7.94ppm. The hydroxyl proton of the acid group was visible at 11.16ppm, as a broad singlet. The characteristic features of the ^{13}C NNR spectrum of compound **(5.34)** was the signal of the carboxylic acid carbonyl at 176.7ppm, and the carbonyl of the ketone at 198.3ppm. This data agreed well with the reported ^{1}H NMR data for compound **(5.34)**.

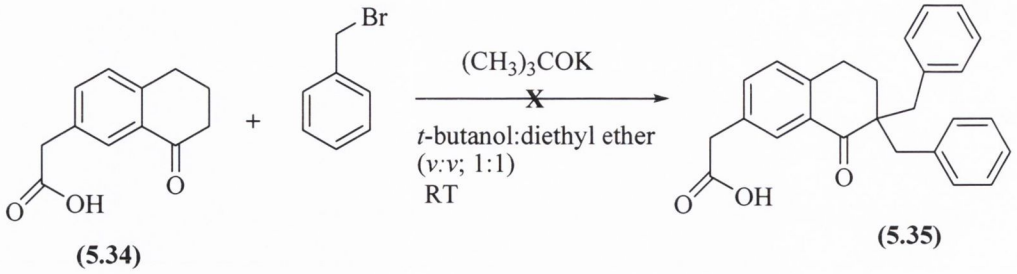
The diagnostic features of the infrared spectrum of compound **(5.34)** were those of the carbonyl and hydroxyl groups, $C{=}O_{str}$ at 1679.9cm^{-1} for ketone, $C{=}O_{str}$ at 1708.4cm^{-1} for acid and $O\text{-}H_{br}$ at 2944.6cm^{-1}. HRMS analysis gave an accurate molecular mass ($+H^{+}$) as 205.0855, calculated 205.0859, molecular formula $C_{12}H_{13}O_{3}$.

5.6.2 Benzylation of compound (5.34)

Method A:

Initially the method used to generate compound **(5.35)** was identical to that used to generate compound **(5.0)**. Thus compound **(5.34)** was treated with potassium *tert*-butoxide as base, benzyl bromide as alkylating agent, in the presence of a mixture of *t*-butanol and diethyl ether at room temperature. When the reaction was monitored by

TLC, there was no evidence of product even when excess potassium *tert*-butoxide and benzyl bromide were added to the reaction mixture. A precipitate did form which was identified as the potassium salt of the acid **(5.34)**.

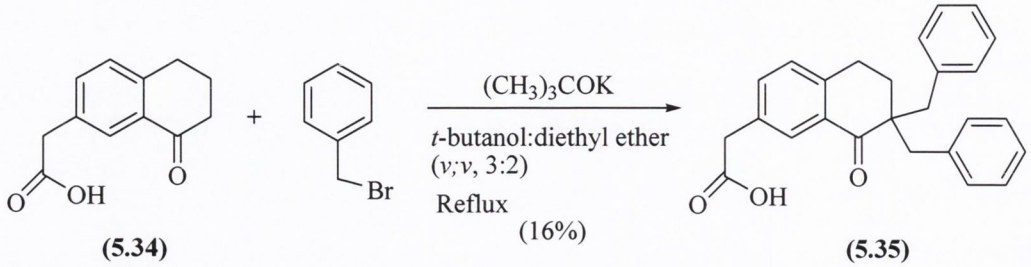


(5.34) **(5.35)**

Scheme 5.24 Attempted synthesis of compound (5.35)

Method B:

The conditions described for method A were again used, but on this occasion the reaction mixture was heated to reflux in order to drive the alkylation reaction. Under these conditions, the acid **(5.34)**, as its potassium salt, remained in solution and was alkylated albeit in low yield. After three hours, TLC analysis (mobile phase: ethyl acetate:hexane, 3:1) showed the presence of the product with a slightly higher R_f than the starting acid **(5.34)**. Separation of the acid **(5.34)** from the dibenzylated product **(5.35)** was achieved by taking into consideration their difference in lipophilicity. After work-up of the reaction, the crude oil thus obtained was re-dissolved in ether and extracted with a sat. aq. $NaHCO_3$ solution. The newly formed acid **(5.35)**, together with excess benzyl bromide from the reaction, remained in the organic layer while the slightly more polar acid was extracted into the basic solution. The ether layer was then extracted with 2.5M aq. NaOH. The basic layer thus obtained was washed with diethyl ether, acidified with 2M aq. HCl and extracted with ether to afford the desired acid **(5.35)** in a 16% yield.



(5.34) **(5.35)**

Scheme 5.25 Formation of compound (5.35)

The ^{1}H NMR spectrum of compound **(5.35)** contained two overlapping triplets (J=6.22Hz) at 1.94ppm, corresponding to the two methylene protons of the tetralone ring at β–keto position. Two overlapping doublets (J=13.38Hz) were found at 2.67ppm for two methylene protons of the benzyl group, followed by two overlapping triplets (J=6.14Hz) at 3.05ppm for the bezylic methylene of the tetralone ring. The remaining two methylene protons of the benzyl group also appeared as two overlapping doublets (J=13.38Hz) at 3.33ppm. The two methylene protons of the acetic acid group were located at 3.72ppm, as a singlet. In the aromatic region, eleven protons resonated closely together in the region of 7.15-7.29ppm, followed by a doublet at 7.41ppm integrating for one proton. The final signal was at 8.01ppm, integrating for one aromatic proton. The ^{13}C NMR spectrum confirmed the existence of six quaternary carbons, including the two carbonyl groups; that of the carboxylic acid moiety at 176.5ppm, and the carbon of the ketone at 200.4ppm. A total of thirteen aromatic tertiary carbons were found in the region of 126.4-134.2ppm in the DEPT 90 NMR spectrum. The DEPT 135 NMR spectrum revealed the presence of five methylene carbons in the region from 25.2-41.5ppm.

The characteristic features of the infrared spectrum of compound **(5.35)** included those of ketone $C=O_{str}$, acid $C=O_{str}$ and $O-H_{br}$ at 1681.9cm^{-1}, 1709.0cm^{-1} and 2932.4cm^{-1}. HRMS analysis revealed the accurate molecule mass ($+H^{+}$) as 385.1811, calculated 385.1798, molecular formula $C_{26}H_{25}O_3$

Method C:

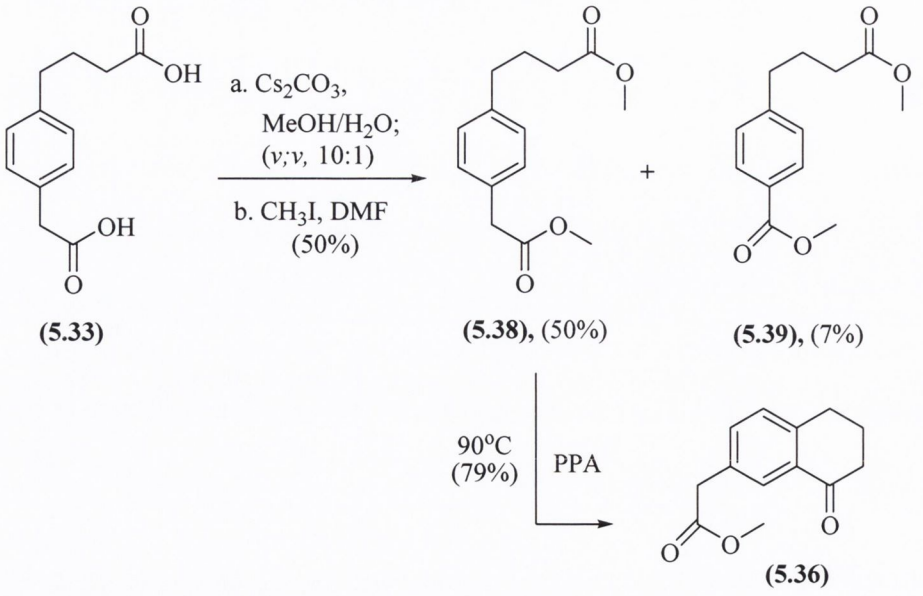
Although the cyclisation step produced a reasonable yield of the tetralone acetic acid **(5.34)**, it was nevertheless felt that one could improve this yield if the "carbonyl" of butanoic acid group possessed a good leaving group. Secondly, it was felt that the yield of the benzylated product could be enhanced if **(5.34)** was converted to its methyl ester derivative before attempting the benzylation step.

The approach used to address these concerns was to convert the acid **(5.33)** into its corresponding dimethyl ester derivative **(5.38)** and then effect the cyclisation and

benzylation steps. Formation of the di-caesium salt of the acid **(5.33)** was accomplised using caesium carbonate in the presence of a mixture of methanol and water (*v;v*; 10:1) at room temperature. This transformation was accomplished within ten minutes. Toluene was then used to azeotrope the water from the reaction mixture to yield the dry caesium salt as an off-white solid which was then dissolved in DMF and treated with excess iodomethane. The reaction was allowed to stir overnight and the desired dimethyl ester **(5.38)** was obtained in moderate yield (50%) together with a trace amount of compound **(5.39)**. The characteristic features of the ^{1}H NMR spectrum of compound **(5.38)** were the two sharp singlets at 3.65 and 3.75ppm, corresponding to the methyl protons of the methyl ester moieties, integrating as three protons for each singlet. The key features of the ^{13}C NMR spectrum were the two ester groups; the two methyl carbons at 51.0 and 51.5ppm, and the two carbonyls at 171.6 and 173.4ppm.

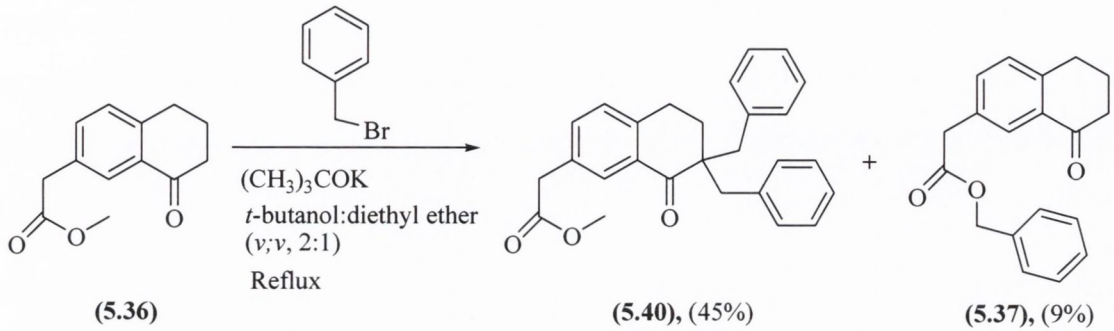
The ester **(5.38)** exhibited two C-O_{str} absorption peaks at 1155.2cm^{-1} and 1255.0cm^{-1}, and a sharp absorption peak of C=O_{str} at 1738.3cm^{-1} in the infrared spectrum.

The dicarboxylic acid methyl ester **(5.38)** was then added to neat polyphosphoric acid (PPA) and the mixture heated to ~90°C for two hours. Upon isolation, the methyl ester **(5.36)** was obtained in improved yield (79%).



Scheme 5.28 Formation of compounds (5.36) by method C

Alkylation of compound **(5.36)** with benzyl bromide and potassium *tert*-butoxide proceeded under reflux conditions in a solution of *t*-butanol and ether and afforded the di-benzylated product **(5.40)**, as a pale green oil, in 45% yield, together with a small amount of the benzyl ester **(5.37)** as a by product.

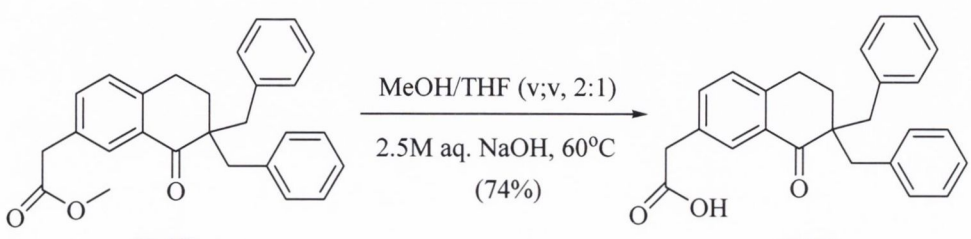


(5.36) **(5.40),** (45%) **(5.37),** (9%)

Scheme 5.29 Formation of compounds (5.40)

The ^1H NMR spectrum of compound **(5.40)** revealed two discrete doublets (J=13.40Hz) at 2.67 and 3.33ppm respectively, corresponding to the four methylene protons of the two benzyl groups. Twelve aromatic protons resonated very close together in the region of 7.15-7.44ppm, with an isolated doublet (J=7.96Hz) at 8.00ppm integrating for one proton. In the ^{13}C NMR spectrum, the two methylene carbons of the benzyl groups and the α–keto quaternary carbon of the tetralone ring were found at 41.1 and 50.5ppm, respectively. The presence of thirteen aromatic tertiary carbons was confirmed in the region of 126.1-133.9ppm.

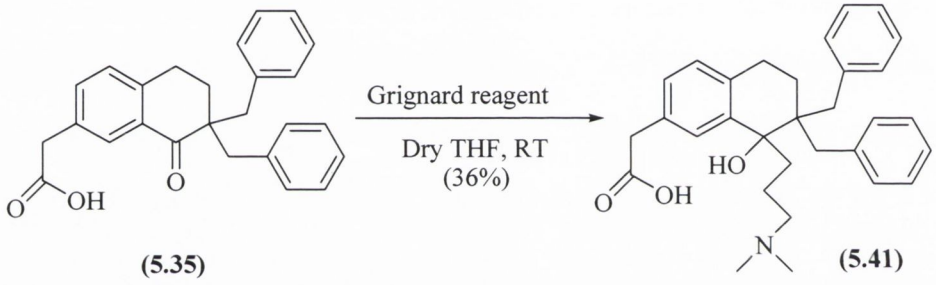
Hydrolysis of the ester **(5.40)** using strong base (2.5M aq. NaOH) in the presence of a mixture of methanol and THF (2:1) at 60°C proceeded smoothly. The reaction had gone to completion within four hours. Upon acid-base extraction, **(5.35)** was obtained in pure form in good yield (74%).



(5.40) **(5.35)**

Scheme 5.30 Hydrolysis of ester (5.40)

Following the successful synthesis of **(5.35)**, the Grignard reaction was carried out using freshly prepared Grignard reagent in dry THF at room temperature under anhydrous conditions. The Grignard reagent thus formed reacted smoothly with the ketone **(5.35)**. The starting ketone was totally consumed after four hours. Mindful of the amino acid nature of the desired alcohol **(5.41)**, a slightly different work-up procedure was chosen. The reaction was quenched by the addition of sat. aq. NH_4Cl solution and while still slightly basic was washed with diethyl ether to remove any amine by-products. The solution was then neutralised by adding 2M aq. HCl in a drop-wise fashion. The resultant aqueous solution was then extracted with DCM. Upon purification by a combination of methods, namely flash column chromatography (eluant: hexane:ethyl acetate:triethylamine, 10:10:1) and preparative TLC analysis (eluant: methanol), the resultant amino acid **(5.41)** was obtained in moderate yield (36%), as a pale yellow solid.



(5.35) **(5.41)**

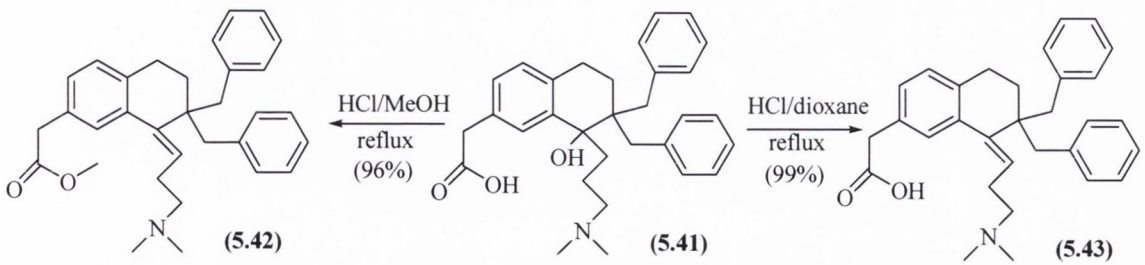
Scheme 5.31 Formation of compounds (5.41)

In the ^1H NMR spectrum of the alcohol **(5.41)**, seven multiplets were observed in the region of 1.28-2.26ppm, integrating for a total of nine methylene protons. In the region of 2.46-2.62ppm, a multiplet for one proton was overlapping with two singlets considered to be the six methyl protons. Four more methylene protons resonated as a multiplet at 2.67-2.82ppm, followed by a singlet at 3.11ppm for the two methylene protons of the acid group. Thirteen aromatic protons resonated from 6.73-7.27ppm. The two hydroxyl moieties of the acid and alcohol groups were located at 4.59 and 7.54ppm, respectively. The ^{13}C NMR spectrum confirmed the presence of a total of thirteen aromatic tertiary carbons and five quaternary carbons in the region of 125.9-139.6ppm. The carbonyl carbon of the acid appeared at 174.2ppm. The DEPT 135 NMR spectrum revealed signals of eight methylene carbons from 19.3-58.2ppm, and the two methyl

carbons at 43.0ppm. The quaternary carbon linked to the benzyl group was located at 45.7ppm in the ^{13}C NMR spectrum, and the quaternary alcohol carbon at 79.1ppm.

The important features of this molecule in the infrared spectrum were C-O_{str} (alcohol), C=O_{str} (acid), O-H_{br} (acid), O-$H_{br,str}$ (alcohol) at 1166.5cm^{-1}, 1713.1cm^{-1}, 2953.2cm^{-1}, 3368.9cm^{-1}, respectively. HRMS analysis gave an actual molecular mass as 472.2842, which correlated well to the calculated mass of 472.2852. The following elemental composition for that peak, ($C_{31}H_{38}O_3N$), corresponded to the structure of the alcohol **(5.41)** ($+H^+$).

In order to produce the desired molecule **(5.43)**, the dehydration reaction was performed by heating a methanol/HCl solution of the amino acid alcohol **(5.41)** for five minutes. Under these conditions, the alkene formed in near quantitative yield. Unsurprisingly, analysis of the NMR spectrum of the alkene also confirmed complete conversion of the carboxylic acid to the methyl ester **(5.42)** (Scheme 5.32). Nevertheless, purification of the alkene **(5.42)**, by preparative TLC was more convenient than that of the amino acid precursor **(5.41)**. Changing the solvent to 1,4-dioxane afforded the target amino acid **(5.43)** as a very clean product, which was simply obtained by removal of the solvent under high vacuum. Analysis of the NMR spectra of the amino acid **(5.43)** indicated that the *trans*-isomer formed almost exclusively. A very minor amount of the *cis*-isomer (less than 1%) was evident in the ^1H spectrum.



Scheme 5.32 Formation of compounds (5.42)

When the ^1H NMR spectrum of the amino acid **(5.43)** was recorded in $CDCl_3$, ^1H signals were generally very broad and poorly resolved. It was therefore decided to change the solvent to deuterated dimethylsulfoxide. The signals in the spectra obtained using this

solvent were very well resolved. With an aid of 1D H-H COSY spectrum (Fig. 4.45), a triplet (J=6.02Hz) at 1.61ppm was believed to be for the two protons on C-3 while the triplet at 2.32ppm (J=6.02Hz) accounted for the protons on C-4. The benzyl methylene protons on C-14 and C-20 resonated as two overlapping doublets, one set at 2.74ppm (J=13.56Hz) while the other set resonated at 2.95ppm (J=13.56Hz). The tall singlet at 2.78ppm, its sharpness somewhat comprised by the protons on C-10 also resonating at this position, represented the methyl protons on C-12 and C-13. A broad triplet at 3.21ppm (J=7.91Hz) represented the protons on C-11. The protons on C-26 resonated as singlet at 3.56ppm while the double bond proton resonated as triplet at 5.66ppm (J=7.15Hz). The aromatic region was slightly less clear cut although assignment was facilitated by the use of the HMBC spectrum in particular. The proton attached to C-5 resonated as a doublet at 6.96ppm (J=7.53Hz) while the proton on C-6 also resonated as a doublet at 7.00ppm (J=7.61Hz). The slightly unequal intensity of the doublet perhaps accounted for by the roofing effect. The singlet at 7.12ppm represented the proton on C-8. The remaining peaks in the aromatic region were can be assigned to the benzyl aromatic protons.

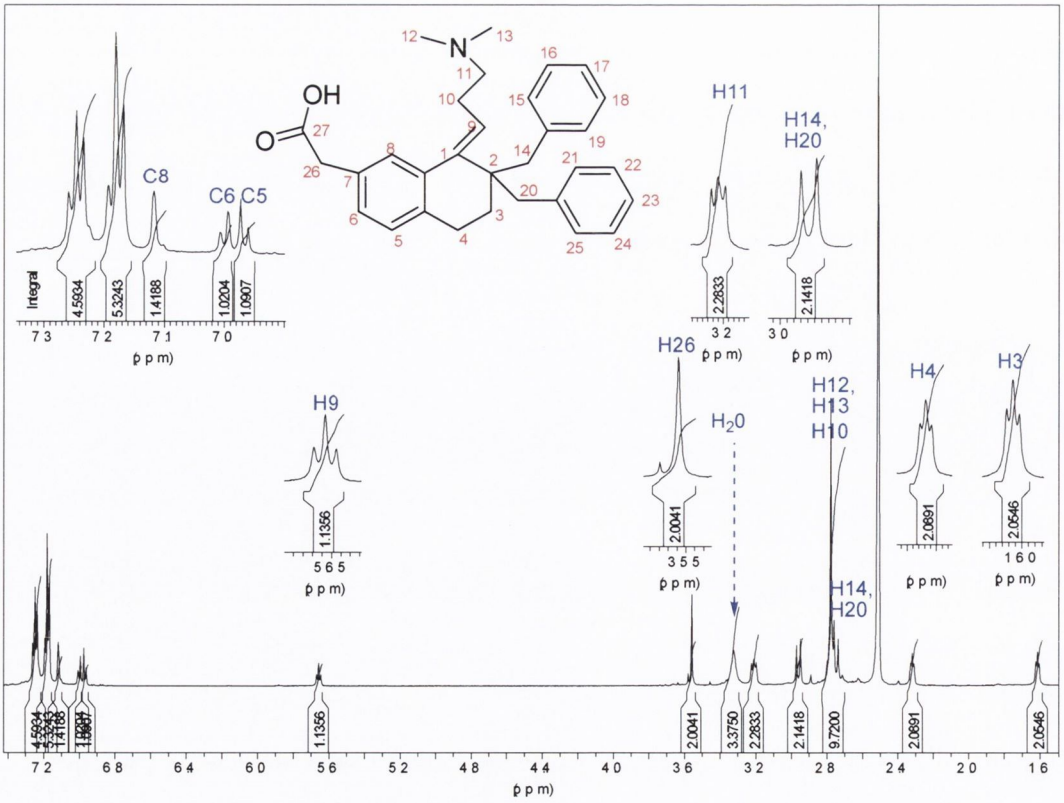


Fig. 5.42 ^{1}H NMR spectrum of compound (5.43)

Having assigned most of the protons signals, assignment of the carbon signals was conveniently achieved following inspection of the C-H COSY NMR spectra (Fig. 5.46-Fig. 5.47). The methylene carbon resonating at 25.0ppm was C-10, while C-4 resonated at 26.4ppm, C-3 at 32.2ppm, C-26 at 40.2ppm, C-14 and C-20 at 45.1 and finally C-11 at 55.8ppm. The methyl carbons, C-12 and C-13 resonated together at 41.9ppm. In the aromatic/double bond region of the spectrum, C-9 resonated at 122.1ppm, while C-5 resonated at 126.8ppm, C-6 at 127.9, C-8 at 130.0ppm. C-17 and C-23 resonated together at 126.0ppm while the remaining tertiary carbons in on C-16, 18, 22, 24 and C-15, 19, 21, 25 resonated as two sharp signals at 127.6 and 130.9ppm. The carboxylic acid carbon resonated at 173.2ppm.

The ketonic $C{=}O_{str}$ was located at 1716.7cm^{-1} in the infrared spectrum. The HRMS analysis gave the actual molecular mass as 454.2766, which correlated well to the calculated mass as 454.2741. The following elemental composition for that peak, ($C_{31}H_{36}O_2N$), corresponded to the structure of the alcohol **(5.43)** ($+H^+$).

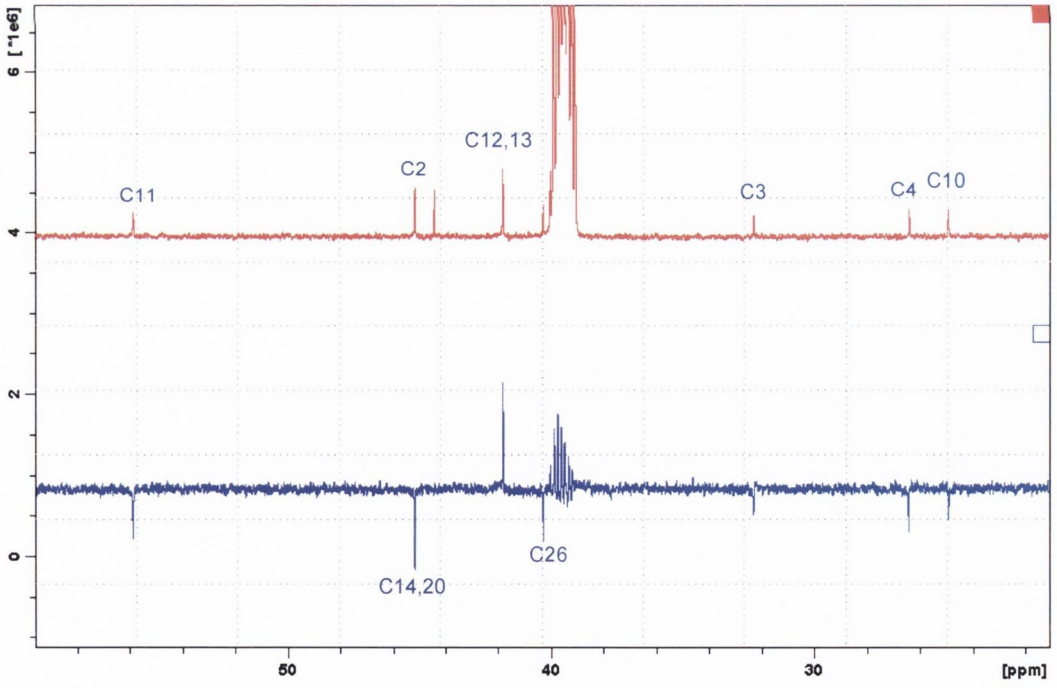


Fig. 5.43 ^{13}***C, DEPT 135 and DEPT 90 NMR spectra of compound (5.43) from 20.5-60.0ppm***

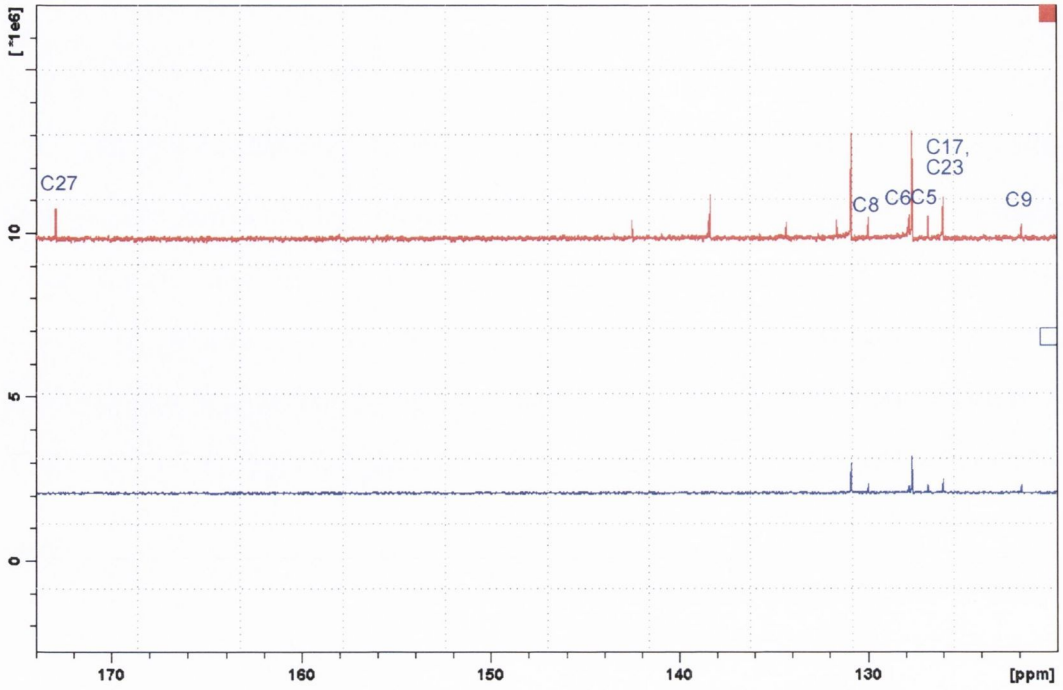


Fig. 5.44 ^{13}C, *DEPT 135 and DEPT 90 NMR spectra of compound (5.43) from 120.0-174.0ppm*

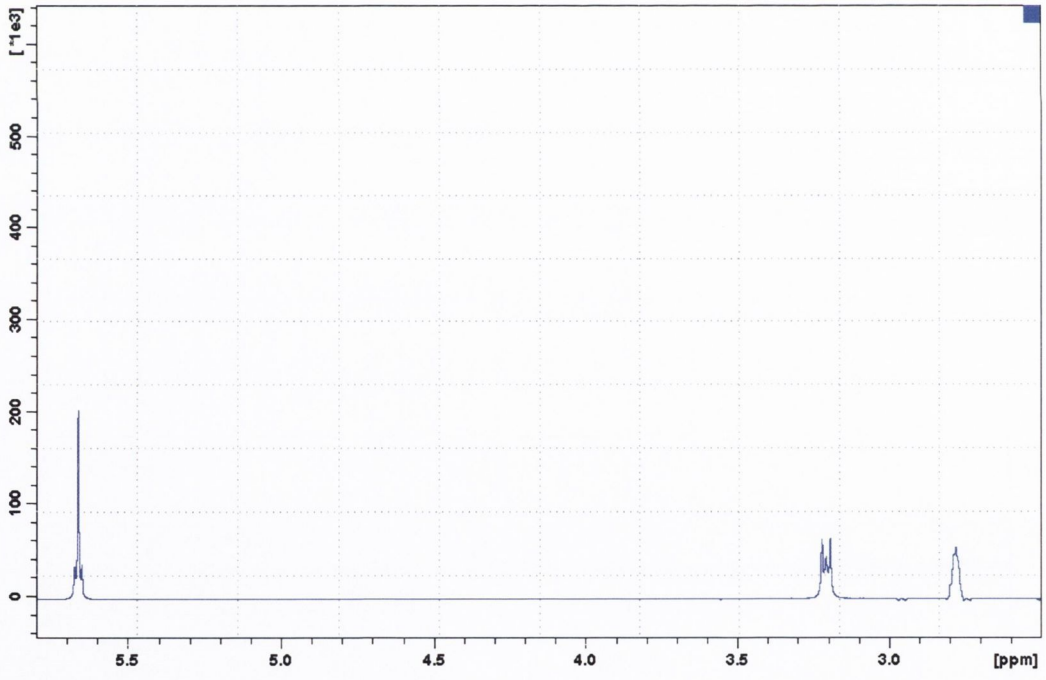


Fig. 5.45 1D H-H COSY NMR spectrum of compound (5.43) from 2.40-5.80ppm

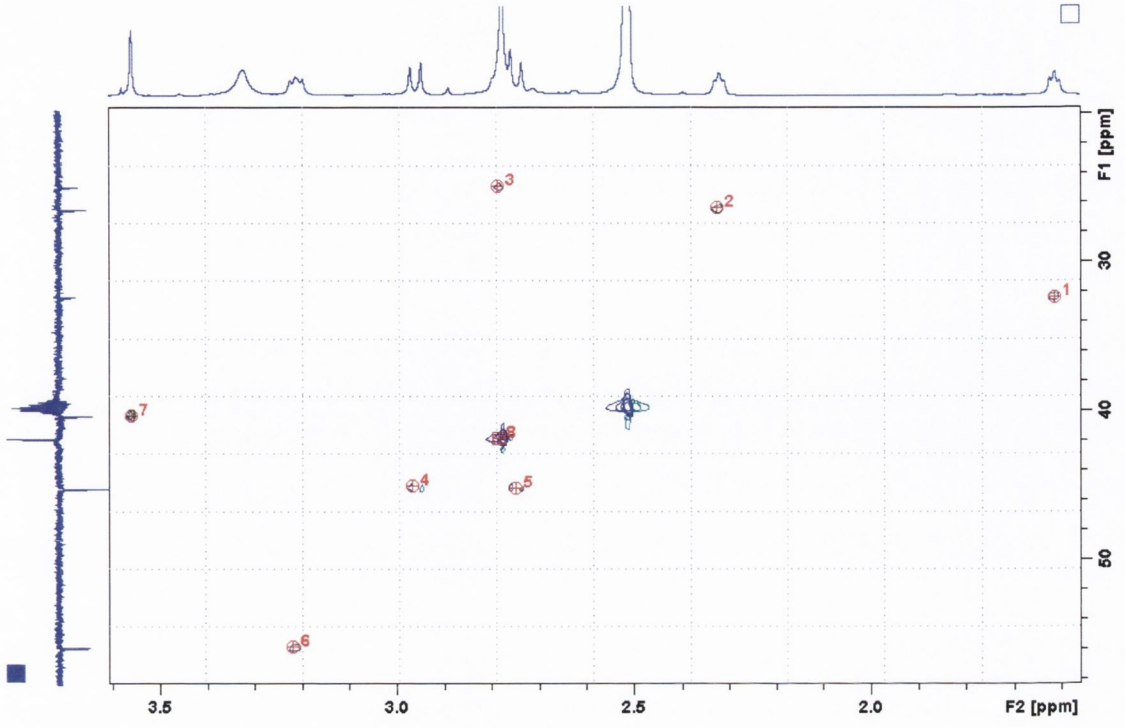


Fig. 5.46 C-H COSY NMR spectrum of compound (5.43)

from 1.55-3.60ppm

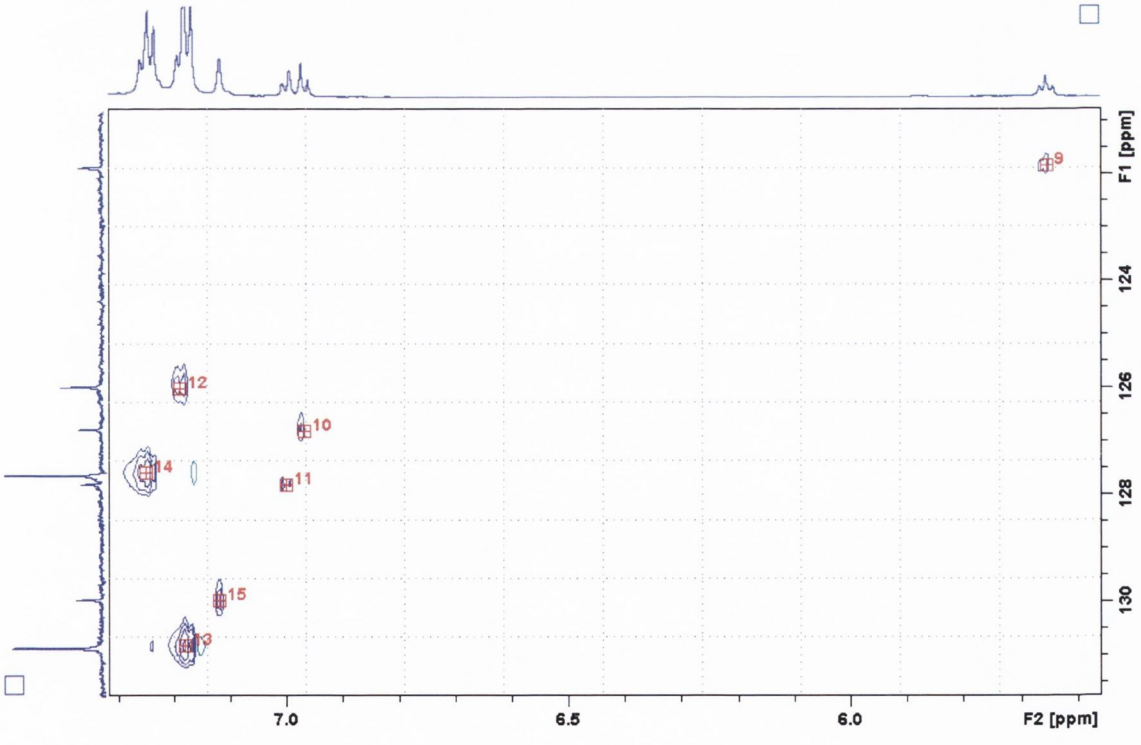


Fig. 5.47 C-H COSY NMR spectrum of compound (5.43)

from 5.55-7.31ppm

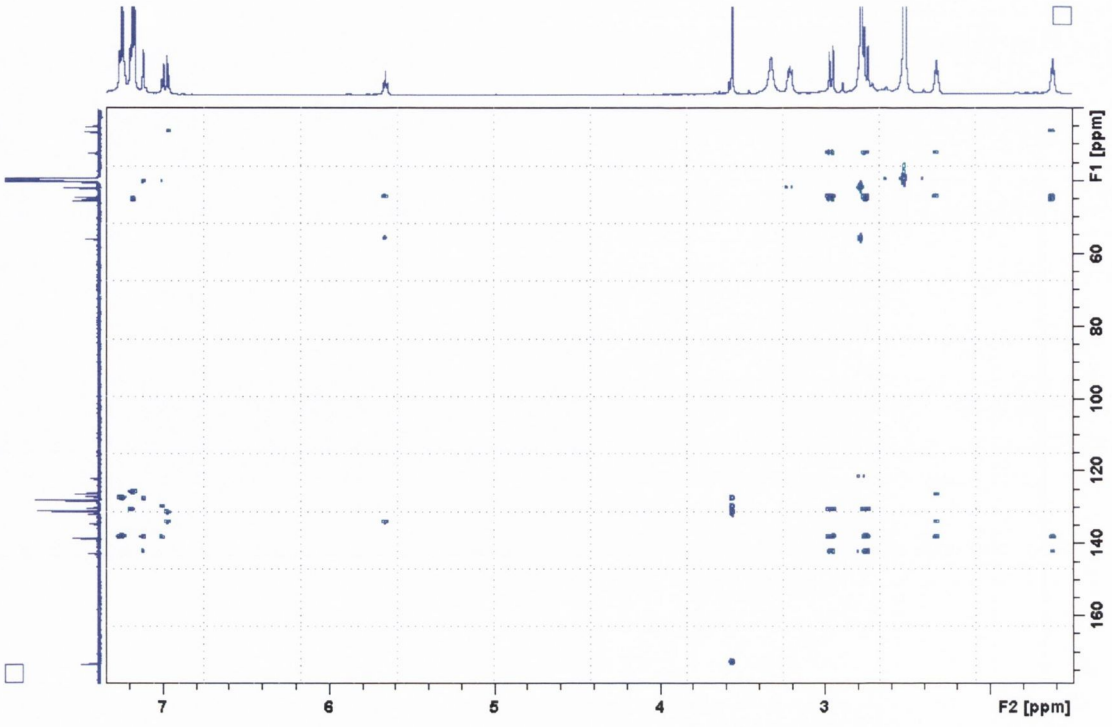


Fig. 5.48 HMBC NMR spectrum of compound (5.43)

5.7 Biological data on compounds synthesised

The *in vivo* data obtained by Woods on the ability of test compounds to inhibit passive cutaneous anaphylaxis was impressive on alcohols; compounds **(5.1)** and **(5.5)** and on alkenes **(5.3)**, **(5.6)** and **(5.9)**. Data analysis completed by MDS Pharma also indicates that compound **(5.2)** is not only a histamine H1 antagonist but also a histamine H4 antagonist. In these assays at 10uM, compound **(5.2)** inhibited histamine binding to the H1 receptor by 94% and by 64% to the H4 receptor. As H1 and H4 receptors are involved in the onset of allergic conditions, this was an important additional property of these compounds. A similar set of data clearly showed the same pattern of activity with **(5.6)**. At 10uM this compound showed 95% and 63% inhibition of histamine binding to both the H1 and H4 receptors. The *in vivo* data obtained on the analogous series of compounds within the indane series was disappointing as all compounds tested were essentially inactive. A similar pattern was observed with the trifluoro compound **(5.11)** and the pyridyl alcohol **(5.20)**. As the amino acid **(5.43)** was just synthesised prior to submission, data on this compound is not yet available. The data generated on the test compounds synthesised to date clearly indicates that only minor modifications to the structure is tolerated especially with; (i) respect to the size of the B-ring (tetralin ring is preferred) and (ii) to the nature of benzylic groups that are allowed.

Test Compound (Vehicle)	**% Inhibition of PCA** (mean ± S.E.M)
(5.1) (W/I)	84.49 ± 16.37
(5.2) (W/I)	84.49 ± 16.37
(5.5) (W/I)	76.31 ± 8.689
(5.5) (2% Tween in W/I)	85.54 ± 22.26
(5.6) (2% Tween in W/I)	89.68 ± 53.96
(5.8) (W/I)	30.00 ± 29.64
(5.9) (2.5% Tween in W/I)	78.89 ± 8.766
DSCG (W/I)	89.02 ± 6.264

Test Compound (Vehicle)	**% Inhibition of PCA** (mean ± S.E.M)
DSCG (2% Tween in W/I)	87.83 ± 13.13
DSCG (2.5% Tween in W/I)	92.15 ± 5.817

Table 5.3: Test compounds showing % inhibition of in vivo Passive Cutaneous Anaphylaxis (PCA). W/I, water for injection; Tween, Tween80; DSCG, disodium cromoglycate. Tween 80 was used to increase solubility of certain test compounds.

It was decided to select compound **(5.6)** for a pharmacokinetic study, based on the fact that its activity *in vivo* was similar to that of DSCG and the fact that this compound is also an inhibitor of histamine binding to both the H1 and H4 receptors. The study was carried out by Charles River on the alkene **(5.6)**. Following oral administration of (5.6) at 3mg/kg, it maximum concentration in plasma (C_{max}) was observed at 4 h post dose, following administration. Following intravenous administration at 2mg/kg, the maximum plasma concentration of compound **(5.6)** was observed at the first sampling time point. Following intravenous administration, clearance estimates (1946 mL/kg/h) were greater than the values for hepatic and renal plasma flow rates in rats, suggesting possible elimination of compound **(5.6)** by active processes. The alkene **(5.6)** was still measurable at 24h post dosing. Apparent terminal elimination half life ($T_{½}$) estimates were generally similar following oral compared to intravenous dose administration. The mean half lives were 9.98±0.86 h and 11.07±0.85 h for intravenous and oral administration, respectively. Oral bioavailability was calculated to be 57%, indicating good absorption of compound **(5.6)**.

5.8 Conclusion

This Chapter describes the methodology used to synthesise a range of novel anti-allergy compounds. A range of test compounds were synthesised based on the tetralin structure. Initially the focus was on the synthesis of test compounds that contained an aminopropyl group and a dibenzyl substituent at positions 1 and 3 of the tetralin structure respectively. Having synthesised a range of compounds containing these basic structures, this Chapter describes the synthesis of analogous indane series of test compounds. Significant emphasis in the closing discussions within this Chapter describes the synthesis of test compounds containing the acetic acid residue on position-7 of the tetralin ring. The synthesis of compounds within this series was not without its difficulties but nevertheless the targeted compound was synthesised, and almost exclusively so as one single geometric isomer. Of the compounds synthesised, five show a similar level of activity to DSCG *in vivo*, while some have the additional properties of being H1 and H4 antagonists. The *in vivo* efficacy data and the pharmacokinetic study demonstrates that the key objective of the work described in this chapter has been achieved, namely to identify a potent anti-allergy compounds with a good bioavailability, stability and metabolic profile.

Experimental

All chemicals and reagents were obtained from Sigma-Aldrich Ltd. and were not characterised.

Uncorrected melting points (MP) were determined on an Electrotherm® melting point

Spectral data were obtained on the following instruments:

Infra Red (IR) spectral data were obtained using a Perkin Elmer FT-IR spectrophotometer Paragon 100. Band positions are given in cm^{-1}. Solid samples were analysed by potassium bromide (KBr) discs and oils were analysed as neat films on NaCl plates.

Nuclear Magnetic Resonance (NMR): All ^{1}H and ^{13}C spectra were recorded at 27°C on Bruker MSL 400 and 600. 1D NMR spectra were analysed with Bruker WIN-NMR software and 2D NMR spectra were analysed with Bruker Topspin 2.0 software. All samples were dissolved in $CDCl_3$ prior to analysis (except where indicated). Peak positions were assigned relative to $CDCl_3$ resonances at 7.28ppm for the ^{1}H NMR spectra and 78.16ppm, 76.90ppm and 75.62ppm for the ^{13}C NMR spectra. Coupling constants were reported in hertz (Hz). Abbreviations used were as follows: s=singlet, d=doublet, t=triplet, q=quartet, dd=double doublet, p=pentet, dt=doublet triplet, td=triple doublet, ddd=doublet of double doublets, m=multiplet, br=broad, tert.=tertiary, quant.=quaternary.

High Resolution Mass Spectroscopy (HRMS): All high resolution mass spectra were obtained from a Miromass LCT Mass Spectrophotometer with electrospray ionisation. Data analysis was performed using MassLynx software version 3.5.

Column chromatography was carried out using silica gel 60 (230-400mesh) and thin layer chromatography with silica gel GF-254 pre-coated aluminium sheets (Merck Laboratories). Compounds were visually detected by *UV* at 254 and 365nm, and a variety of spray reagents (i.e. potassium permanganate, vanillin staining solution, ninhydrin solution).

Anhydrous THF was prepared by refluxing over powered lithium aluminium hydride for two hours, then collecting the distillate and allowing it to fall back down over sodium wire and benzophenone. The resulting mixture was refluxed till a purple colour was obtained. The colourless distillate was used immediately.

Anhydrous DCM was prepared by distillation over powered calcium hydride and rejecting the wet forerun (~5%).

Preparation of Jones Reagent:

The Jones reagent was prepared by dissolving chromium trioxide (CrO_3) (5g, 50mmol) in distilled water (10ml). Sulphuric acid (4.3ml) was added into the reaction mixture by drop-wise fashion, after which distilled water (9.7ml) was then added to effect dissolution of any precipitated CrO_3.

Preparation of Grignard reagent

The Grignard reagent, [3-(dimethylamino)propyl]magnesium chloride, was prepared under an atmosphere of nitrogen by adding anhydrous THF (5ml) to magnesium turnings (0.40g, 16.67mmol). To this stirred reaction mixture was added 3-dimethylaminopropyl chloride*(2ml), in the presence of 1,2-dibromoethane (0.4ml). The resulting mixture was heated to 90°C for 2mins, and then allowed to stir at room temperature for further use.

(*3-dimethylaminopropyl chloride was generated by the treatment of 3-dimethylamino-1-propyl chloride hydrochloride with KOH pellets, followed by distillation of the reaction mixture.)

9-bromo-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (2.0)

To a stirred solution of 1-benzosuberone (2.50g, 15.60mmol) in carbon tetrachloride (15ml) was added *N*-bromosuccinimide (6.94g, 39.01mmol) and a catalytic quantity of dibenzoyl peroxide. The resulting mixture was heated under reflux for two hours, and on reaching reflux careful monitoring the progress of the reaction by thin layer chromatography (TLC) (hexane:ethyl acetate, 8:1). The reaction mixture was then cooled to room temperature and filtered with dichloromethane (DCM) to remove the excess starting material (NBS). The solvent was evaporated *in vacuo.* The resulting compound was partitioned between water (25ml) and diethyl ether (3 x 20ml). The combined organic layers were dried over magnesium sulphate, filtered and evaporated *in vacuo* to give crude product. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 50:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(2.0)** as a yellow oil (3.11g, 83%) with the following physical properties;

IR (DCM, v); cm^{-1} 2936.2, 1686.5, 1596.7, 1447.4, 1256.7, 1209.3

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.02-2.12 (m, 1H, 1H of C$\underline{H}_2$), 2.25-2.37 (m, 2H, C$\underline{H}_2$), 2.47-2.56 (m, 1H, 1H of C$\underline{H}_2$), 2.72-2.78 (m, 1H, 1H of C$\underline{H}_2$), 3.24 (ddd, J_1=14.06Hz, J_2=9.54Hz, J_3=4.65Hz, 1H, 1H of C$\underline{H}_2$), 5.58 (dd, J_1=6.02Hz, J_2=2.02Hz, 1H, C$\underline{H}$Br), 7.38 (dt, J_1=7.26Hz, J_2=1.26Hz, 1H, Ar-$\underline{H}$), 7.42 (d, J=7.00Hz, 1H, Ar-$\underline{H}$) 7.47 (dt, J_1=7.28Hz, J_2=1.39Hz, 1H, Ar-$\underline{H}$), 7.62 (d, J=7.52Hz, 1H, COAr-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 21.6 ($\underline{C}H_2$), 34.2 ($\underline{C}H_2$), 41.6 ($\underline{C}H_2$), 54.1 ($\underline{C}$HBr), 128.3 (tert. C), 128.5 (tert. C), 129.2 (tert. C), 131.2 (tert. C), 139.2 (quat. C), 139.4 (quat. C), 204.5 ($\underline{C}$=O)

9-azido-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (2.1)

To a stirred solution of compound **(2.0)** (0.98, 4.10mmol) in DMF (10ml) was added sodium azide (1.33g, 20.49mmol). The reaction was allowed to stir at 60°C for two and half hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 6:1) to check for the disappearance of the starting material. The reaction mixture was

extracted between water (50ml) and diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The product was purified by flash column chromatography on silica gel 230-400mesh (eluent, hexane:ethyl acetate, 20:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(2.1)** as a dark brown oil (0.67g, 81%) with the following physical properties;

IR (DCM, v); cm^{-1} 2940.9, 2104.1, 1680.6, 1598.1, 1449.4, 1250.0

HRMS ($+H^+$): actual 202.0977, calculated 202.0980, molecular formula $C_{11}H_{12}N_3O$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.81-1.91 (m, 2H of CH_2), 2.0-2.08 (m, 1H of CH_2), 2.13-2.22 (m, 1H of CH_2), 2.93 (ddd, J_1=12.32Hz, J_2=8.37Hz, J_3=4.52Hz, 1H, 1H of CH_2), 2.65 (ddd, J_1=12.04Hz, J_2=7.77Hz, J_3=4.27Hz, 1H, 1H of CH_2), 4.91 (t, J=5.52Hz, 1H, CH-N_3), 7.37 (d, J=7.52Hz, 1H, Ar-H); 7.42 (dt, J_1=7.54Hz, J_2=1.01Hz, 1H, Ar-H), 7.52 (dt, J_1=7.52Hz, J_2=1.52Hz, 1H, Ar-H), 7.65 (dd, J_1=7.52Hz, J_2=1.52Hz, 1H, COAr-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 19.0 (CH_2), 30.6 (CH_2), 40.4 (CH_2), 63.7 (CHN_3), 126.9 (tert. C), 128.3 (tert. C), 128.6 (tert. C), 131.6 (tert. C), 136.7 (quat. C), 137.7 (quat. C), 205.4 (C=O)

9-amino-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (2.2)

To a stirred solution of compound **(2.1)** (1.19g, 5.89mmol) in acetic acid (10ml) was added zinc dust (3.08g, 46.15mmol). The reaction mixture was stirred at 0°C for the initial half hour and was allowed to warm to room temperature for seven hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to observe the disappearance of the starting material. The reaction mixture was filtered to remove the zinc dust and the solvent was evaporated *in vacuo* using toluene to aid in the removal of acetic acid. The resulting residue was partitioned between 2M aq. HCl (25ml) and diethyl ether (3 x 20ml each), the aqueous layer was removed, and basified with 2M aq. NaOH. The basic aqueous layers were further extracted with diethyl ether (3 x 20ml), the combined organic layer was dried over magnesium sulphate, filtered and

concentrated *in vacuo* to yield the title amine **(2.2)** as a white solid (0.63g, 56%) with the following physical properties;

Mp: 138-141°C

IR (KBr, v); cm^{-1} 2940.9, 2104.1, 1680.6, 1598.1, 1449.4, 1250.0

HRMS ($+H^+$): actual 175.1076, calculated 175.1075, molecular formula $C_{11}H_{14}NO$

9-(cyclohex-2-enylamino)-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (2.3)

To a stirred solution of compound **(2.2)** (0.20g, 1.14mmol) in acetone (5ml), was added 3-bromocyclohexene (0.26ml, 2.28mmol) and potassium carbonate (0.47g, 3.42mmol). The resulting mixture was heated to reflux for twelve hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1). The reaction mixture was partitioned between 2M aq. HCl (25ml) and diethyl ether (3 x 20ml each), the organic layers were discarded. The aqueous layer was then basified with 2.5M aq. NaOH and was extracted with diethyl ether (3 x 20ml). The organic layers were combined and the solvent removed *in vacuo*. The resulting oil was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 5:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title amine **(2.3)** as a yellow oil (0.18g, 62%) with the following physical properties;

IR (DCM, v); cm^{-1} 3322.4, 3019.5, 2928.8, 1681.3, 1599.7, 1448.1, 1243.3, 1102

HRMS ($+H^+$): actual 256.1691, calculated 256.1701, molecular formula $C_{17}H_{22}NO$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.21-2.08 (6 x m, 10H, 5 x $C\underline{H}_2$), 2.48-2.57 (m, 1H, 1H of $C\underline{H}_2CO$), 2.77-2.86 (broad m, 1H, $NC\underline{H}CH{=}CH$), 2.89-2.98 (m, 1H, 1H of $C\underline{H}_2CO$), 4.23 and 4.25 (1 x t, J~5.04Hz and 1 x t, J~5.52Hz, signals overlapping, 1H, $NC\underline{H}Ar$), 5.54-5.77 (m, 2H, $C\underline{H}{=}C\underline{H}$), 7.31-7.38 (m, 2H, 2 x Ar-$\underline{H}$), 7.43 (2 x d, J=7.52Hz, signals overlapping, 1H, Ar-$\underline{H}$), 7.46 and 7.47 (1 x t, J=7.54Hz and 1 x t, J=3.02Hz, signals overlapping, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 19.8, 20.1, 20.2, 20.5 (2 x $\underline{C}H_2$), 25.2 and 25.3 ($\underline{C}H_2$), 28.1 and 30.1 ($\underline{C}H_2$), 32.6 and 32.7 ($\underline{C}H_2$), 40.8 and 41.0 ($\underline{C}H_2$), 49.0 and 49.4 ($\underline{C}H$), 56.3 and 56.9 ($\underline{C}H$), 126.2 and 126.4 (tert. C), 127.1, 2 x 127.2, 127.4, 128.3, 2 x

128.4 (CH=CH and 2 x tert. C), 130.7 and 130.8 (tert. C), 138.6 and 138.7 (quat. C), 140.9 and 141.4 (quat. C).

(2,3,4-trimethoxyphenyl)methanol (2.4)

To a stirred solution of 2,3,4-trimethoxybenzaldehyde (5.00g, 25.48mmol) in methanol (50ml), was slowly added sodium borohydride (0.96g, 25.48mmol) at 0°C. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to observe the disappearance of the starting material. After two hours, the reaction was quenched by the addition of water (50ml) to aid in the removal of methanol *in vacuo*. The resulting residue was partitioned between water and diethyl ether (3 x 30ml each). The combined organic layers were dried over magnesium sulphate, filtered and concentrated *in vacuo* to give a crude oil. The oil was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 4:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(2.4)** as a colourless oil (4.90g, 98%) with the following physical properties;

IR (DCM, v); cm^{-1} 3410.2, 2940.3, 1602.6, 1496.2, 1276.6, 1098.3, 1016.5

HRMS ($+Na^+$): actual 221.0789, calculated 221.0790, molecular formula $C_{10}H_{14}O_4Na$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 3.85 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 4.89 (s, 2H, CH_2), 6.62 (d, J=8.20Hz, 1H, Ar-H), 6.97 (d, J=8.20Hz, 1H, Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 55.5 (OCH_3), 60.3 (OCH_3), 60.7 (OCH_3), 61.1 (CH_2), 106.6 (tert. C), 122.9 (tert. C), 126.4 (quat. C), 141.5 (quat. C), 151.2 (quat. C), 153.0 (quat. C)

The spectral data was consistent with that reported in the literature data.

(3,4,5-trimethoxyphenyl) methanol (2.5)

Compound **(2.5)** was synthesised from 3,4,5-trimethoxybenaldehyde (5.00g, 25.48mmol), using the method outlined for the preparation of compound **(2.4)**. The

procedure yielded the title compound **(2.5)** as a light yellow oil (4.14g, 82%) with the following physical properties;

IR (DCM, v); cm^{-1} 3410.0, 2940.2, 1602.6, 1467.4, 1274.6, 1098.3, 1016.8

HRMS ($+Na^+$): actual 221.0789, calculated 221.0790, molecular formula $C_{10}H_{14}O_4Na$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.78 (broad s, 1H, O<u>H</u>), 3.85 (s, 3H, C<u>H</u>$_3$), 3.89 (2 x s, signals overlapping, 6H, 2 x C<u>H</u>$_3$), 4.66 (s, 2H, C<u>H</u>$_2$), 6.62 (2 x s, signals overlapping, 2H, 2 x Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 2 x 56.1 (2 x O<u>C</u>H$_3$), 60.9 (O<u>C</u>H$_3$), 65.6 (<u>C</u>H$_2$), 2 x 103.7 (2 x tert. C), 126.4 (quat. C), 141.5 (quat. C), 151.2 (quat. C), 153.0 (quat. C), 2 x 136.2 (2 x quat. C), 2 x 152.9 (2 x quat. C)

The spectral data was consistent with that reported in the literature data.

1-(bromomethyl)-2,3,4-trimethoxybenzene (2.6)

To a stirred solution of compound **(2.4)** (4.90g, 24.60mmol) in diethyl ether (50ml) at -20°C was added phosphorous tribromide (9.99g, 36.90mmol) drop-wise, *via* a syringe over ten minutes. The reaction was stirred at this temperature for ninety minutes. On completion, the reaction was quenched by pouring the contents of the round bottomed flask into a beaker containing a mixture of ice and water. The resulting mixture was then extracted using diethyl ether (1 x 50ml). The ether extract was then washed with ice water (1 x 50ml) following by 5% aq. $NaHCO_3$ (2 x 50ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo* at low temperature. The product was not purified by flash column chromatography and was used within two hours of preparation. The title compound **(2.6)** was obtained as a colourless oil (4.90g, 77%) with the following physical properties;

IR (DCM, v); cm^{-1} 2938.0, 1599.2, 1495.5, 1469.2, 1094.1

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 3.88 (s, 3H, OC<u>H</u>$_3$), 3.89 (s, 3H, OC<u>H</u>$_3$), 4.05 (s, 3H, OC<u>H</u>$_3$), 4.55 (s, 2H, C<u>H</u>$_2$), 6.65 (d, J=8.50Hz, 1H, Ar-<u>H</u>), 7.06 (d, J=8.50Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 28.8 ($\underline{C}H_2$), 55.6 ($O\underline{C}H_3$), 60.3 ($O\underline{C}H_3$), 60.7 ($O\underline{C}H_3$), 106.8 (tert. C), 123.7 (quat. C), 123.9 (tert. C), 141.8 (quat. C), 151.8 (quat. C), 154.0 (quat. C)

5-(bromomethyl)-1,2,3-trimethoxybenzene (2.7)

Compound **(2.7)** was synthesised from compound **(2.5)** (1.56g, 7.87mmol) using the same method as described for the synthesis of compound **(2.6)**. The procedure yielded the title compound **(2.7)** as a white crystalline solid (1.52g, 76%) with the following physical properties;

Mp: 115-120°C

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 3.79 (3 x s, signals overlapping, 9H, 3 x $C\underline{H}_3$), 4.40 (s, 2H, $C\underline{H}_2$), 6.57 (s, 2H, 2 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 34.0 ($Br\underline{C}H_2$), 2 x 55.7 (2 x $O\underline{C}H_3$), 60.33 ($O\underline{C}H_3$), 2 x 105.6 (2 x tert. C), 132.8 (quat. C), 137.5 (quat. C), 2 x 152.7 (2 x quat. C)

9-[*N*-(2,3,4-trimethoxybenzyl)-N-(cyclohex-2-enyl)amino]-6,7,8,9-tetrahydrobenzo [7]annulen-5-one (2.8)

To a stirred solution of compound **(2.3)** (0.26g, 1.02mmol) in acetone (10ml) was added compound **(2.6)** (1.33g, 5.10mmol) and potassium carbonate (0.14g, 1.02mmol). The mixture was heated to gentle reflux and the progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1). After sixteen days the reaction was partitioned between water (25ml) and diethyl ether (3 x 20ml each). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 10:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title amine **(2.8)** as a yellow oil (0.24g, 54%) with the following physical properties;

IR (DCM, v); cm^{-1} 3396.1, 3062.8, 2937.5, 1682.9, 1600.0, 1465.5, 1287.2, 1101.0

HRMS ($+H^+$): actual 436.2480, calculated 436.2488, molecular formula $C_{27}H_{34}NO_4$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.37-2.23 (4 x m, 10H, 5 x C$\underline{H_2}$), 2.52-2.65 (m, 1H, 1H of C$\underline{H_2}$CO), 2.68-2.75 (m, 1H, 1H of C$\underline{H_2}$CO), 3.31 (broad m, 1H, NC$\underline{H}$CH=CH), 3.68-3.86 (m, 2H, NC$\underline{H_2}$), 3.87-3.89 (s, 9H, 3 x OC$\underline{H_3}$), 4.13-4.18 (m, 1H, NC$\underline{H}$Ar), 5.65-5.80 (m, 2H, C$\underline{H}$=C$\underline{H}$), 6.72 (d, J=9.04Hz, 1H, Ar-$\underline{H}$), 7.27 and 7.28 (1 x d, J=4.52Hz and 1 x d, J= 4.0Hz, signals overlapping, 1H, Ar-$\underline{H}$), 7.31-7.36 (m, 1H, Ar-$\underline{H}$), 7.44-7.59 (m, 2H, 2 x Ar-$\underline{H}$), 7.82 and 7.89 (2 x d, J=7.76Hz, signals overlapping, 1H, COAr-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.5 and 20.8 ($\underline{C}H_2$), 22.1 and 22.2 ($\underline{C}H_2$), 25.1 and 25.2 ($\underline{C}H_2$), 25.4 and 27.1 ($\underline{C}H_2$), 29.2 and 30.2 ($\underline{C}H_2$), 40.6 and 40.7 ($\underline{C}H_2$), 44.3 and 44.9 ($\underline{C}H_2$), 55.3 and 55.4 ($\underline{C}H$), 55.5, 60.3, 60.4 (3 x O$\underline{C}H_3$), 60.7 and 61.1 ($\underline{C}H$), 2 x 106.8 (tert. C), 123.3 and 123.5 (tert. C), 126.4 and 126.5 (tert. C), 126.6, 127.1, 127.2, 127.3 (1 x tert. C and 1 x quat. C), 127.4 and 127.5 (tert. C), 129.8, 129.9, 130.1, 130.3 ($\underline{C}H$=$\underline{C}H$), 2 x 131.3 (tert. C), 138.6 and 138.8 (quat. C), 2 x 141.4 (quat. C), 142.9 and 143.8 (quat. C), 150.8 and 151.1 (quat. C), 151.6 and 151.7 (quat. C), 206.1 and 206.9 ($\underline{C}$=O)

9-[*N*-(3,4,5-trimethoxybenzyl)-N-(cyclohex-2-enyl)amino]-6,7,8,9-tetrahydrobenzo [7]annulen-5-one (2.9)

To a stirred solution of compound **(2.3)** (0.10g, 0.39mmol) in acetone (6ml) was added compound **(2.7)** (0.50g, 1.95mmol) and potassium carbonate (0.05g, 0.39mmol). The mixture was heated to gentle reflux and the progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1). After sixteen days the reaction was partitioned between water (25ml) and diethyl ether (3 x 20ml each). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 12:1). All homogenous fractions were collected and the solvent was evaporated to afford a diastereomeric mixture of the title amine **(2.9)** as a yellow oil (0.10g, 59%) with the following physical properties;

IR (DCM, v); cm^{-1} 3400.4, 3053.1, 2938.7, 1683.2, 1593.5, 1463.3, 1253.9, 1125.8

HRMS (+Na^+): actual 458.2322, calculated 458.2307, molecular formula $C_{27}H_{33}NO_4Na$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.40-2.16 (5 x m, 10H, 5 x C$\underline{H}_2$), 2.53-2.58 (m, 1H, 1H of C$\underline{H}_2$CO), 2.73-2.80 (m, 1H, 1H of C$\underline{H}_2$CO), 3.34 (broad m, 1H, NC$\underline{H}$CH=CH), 3.67-3.83 (m, 2H, NC$\underline{H}_2$), 3.85 (s, 3H, OC$\underline{H}_3$), 3.89 (s, 6H, 2 x OC$\underline{H}_3$), 4.14-4.19 (m, 1H, NC$\underline{H}$Ar), 5.65-5.79 (m, 2H, C$\underline{H}$=C$\underline{H}$), 6.63 and 6.66 (2 x s, 2H, 2 x Ar-$\underline{H}$), 7.32 and 7.34 (1 x d, J=7.52Hz and 1 x d, J=7.56Hz, signals overlapping, 1H, Ar-$\underline{H}$), 7.45-7.55 (m, 2H, 2 x Ar-$\underline{H}$), 7.76 and 7.78 (2 x d, J=7.78Hz, signals overlapping, 1H, COAr-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.4 and 20.6 ($\underline{C}H_2$), 22.0 and 22.1 ($\underline{C}H_2$), 25.1 and 25.2 ($\underline{C}H_2$), 25.4 and 27.1 ($\underline{C}H_2$), 29.2 and 29.9 ($\underline{C}H_2$), 40.6 and 40.7 ($\underline{C}H_2$), 51.7 and 52.4 ($\underline{C}H_2$), 55.7 and 55.8 ($\underline{C}H$), 3 x 56.0 (3 x O$\underline{C}H_3$), 60.9 and 62.2 ($\underline{C}H$), 2 x 103.8 (tert. C), 2 x 104.0 (tert. C), 2 x 126.8 (tert. C), 127.0 and 127.2 (tert. C), 127.4 and 127.6 (tert. C), 129.0, 130.0, 130.1 and 130.4 ($\underline{C}H$=$\underline{C}H$), 130.7 and 130.8 (tert. C), 2 x 135.8 (quat. C), 137.0 and 137.6 (quat. C), 138.8 and 140.0 (quat. C), 142.4 and 143.0 (quat. C), 4 x 152.6 (2 x quat. C), 206.2 and 206.8 ($\underline{C}$=O)

9-[*N*-benzyl-N-(cyclohex-2-enyl)amino]-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (2.10)

To a stirred solution of compound **(2.2)** (1.00g, 3.92mmol) in acetonitrile (10ml) was added benzyl bromide (0.51g, 4.31mmol) and *N,N*-diisopropylethylamine (0.76g, 5.88mmol). The resulting mixture was heated to gentle reflux and kept under an atmosphere of nitrogen gas for seven days. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 4:1). The reaction mixture was then allowed to cool to room temperature and partitioned between 2M aq. HCl (25ml) and diethyl ether (3 x 20ml each). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 10:1). All homogenous fractions were collected and the solvent was evaporated to yield a stereoisomeric mixture of the title compound **(2.10)** as a clear oil (0.85g, 63%) with the following physical properties;

IR (DCM, v); cm^{-1} 2933.2, 2864.7, 1686.3, 1599.0, 1495.7, 1453.8, 1280.9, 1124.4

HRMS ($+H^+$): actual 346.2186, calculated 346.2171, molecular formula $C_{24}H_{27}NO$

^1H NMR ($CDCl_3$, 400MHz) δ_H (ppm) 1.37-2.28 (4 x m, 10H, 5 x C$\underline{H_2}$), 2.60 and 2.75 (2 x m, 2H, C$\underline{H_2}$CO), 3.35 and 3.42 (2 x broad m, 1H, NC$\underline{H}$CH=CH), 3.76-4.02 (m, 2H, NC$\underline{H_2}$), 4.20 (m, 1H, NC$\underline{H}$Ar), 5.69-5.87 (m, 2H, C$\underline{H}$=C$\underline{H}$), 7.25 (m, 1H, Ar-$\underline{H}$), 7.32-7.38 (m, 3H, 3 x Ar-$\underline{H}$), 7.44-7.63 (m, 4H, 4 x Ar-$\underline{H}$), 7.86 and 7.96 (2 x d, J=7.50Hz, signals overlapping, COAr-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc (ppm): 20.0 and 20.3 ($\underline{C}H_2$), 21.6 and 21.7 ($\underline{C}H_2$), 24.7 and 24.8 ($\underline{C}H_2$), 25.0 and 26.5 ($\underline{C}H_2$), 29.1 and 29.9 ($\underline{C}H_2$), 40.2 and 40.3 ($\underline{C}H_2$), 50.8 and 51.6 (N$\underline{C}H_2$), 55.2 and 55.3 ($\underline{C}$H), 60.9 and 61.5 ($\underline{C}$H), 2 x 126.1 (tert. C), 126.5 and 126.6 (1x tert. C), 127.2, 127.3, 127.4, 127.5, 2 x 127.7, 127.8, 129.8, 129.9, 130.2, ($\underline{C}$H=$\underline{C}$H and 6 x tert. C), 2 x 130.9 (tert. C), 138.8 and 138.9 (quat. C), 141.1 and 141.8 (quat. C), 142.7 and 143.7 (quat. C), 205.6 and 206.5 ($\underline{C}$=O)

9-{*N*-[4-(trifluoromethoxy)benzyl]-N-(cyclohex-2-enyl)amino}-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (2.11)

To a stirred solution of compound **(2.3)** (0.50g, 1.96mmol) in acetonitrile (8ml) was added 4-(trifluoromethoxy)benzyl bromide (0.35ml, 2.16mmol) and *N,N*-diisopropylethylamine (0.38g, 2.94mmol). The resulting mixture was heated to gentle reflux and kept under an atmosphere of nitrogen gas. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 4:1). After twelve days, the reaction mixture was quenched by the addition of 2M aq. HCl (20ml) and the product was extracted with diethyl ether (3 x 25ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 25:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title compound **(2.11)** as a yellow oil (0.44g, 81%) with the following physical properties;

IR (DCM, v); cm^{-1} 3400.8, 3058.0, 2922.7, 1682.4, 1450.6, 1295.5, 1161.2

HRMS ($+H^+$): actual 452.1813, calculated 452.1813, molecular formula $C_{25}H_{26}NO_2F_3$

^1H NMR ($CDCl_3$, 400MHz) δH(ppm) 1.39-2.18 (4 x m, 10H, 5 x $C\underline{H}_2$), 2.55-2.63 (m, 1H, 1H of $C\underline{H}_2CO$), 2.67-2.75 (m, 1H, 1H of $C\underline{H}_2CO$), 3.35-3.41 (broad m, 1H, NC<u>H</u>CH=CH), 3.72-3.93 (m, 2H, $NC\underline{H}_2$), 4.14-4.17 (m, 1H, NC<u>H</u>Ar), 5.64-5.79 (m, 2H, C<u>H</u>=C<u>H</u>), 7.16 (2 x t, J=7.04Hz, signals overlapping, 2H, 2 x Ar-<u>H</u>), 7.30 (dd, J_1=13.56Hz, J_2=6.52Hz, 1H, Ar-<u>H</u>), 7.38 (d, J=8.52Hz, 1H, Ar-H), 7.42-7.53 (m, 3H, 3 x Ar-<u>H</u>), 7.76 and 7.82 (2 x d, J=8.04Hz, signals overlapping, 1H, COAr-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 19.9 and 20.1 ($\underline{C}H_2$), 21.4 and 21.5 ($\underline{C}H_2$), 24.6 and 24.7 ($\underline{C}H_2$), 24.8 and 26.1 ($\underline{C}H_2$), 29.0 and 29.6 ($\underline{C}H_2$), 40.2 and 40.3 ($\underline{C}H_2$), 50.3 and 50.9 ($\underline{C}H_2$), 55.3 and 55.4 (<u>C</u>H), 61.4 and 61.7 (<u>C</u>H), 116.2, 118.8 ($O\underline{C}F_3$), 4 x 120.3 (2 x tert. C), 121.3, 123.9 ($O\underline{C}F_3$), 126.7, 126.8, 127.0, 127.2, 127.3, 127.7, (3 x tert. C), 2 x 128.5 (tert. C), 2 x 128.7 (tert. C), 129.4, 129.8, 130.2, 130.5 (<u>C</u>H=<u>C</u>H) 2 x 130.9 (tert. C), 138.7 and 138.9 (quat. C), 139.9 and 140.7 (quat. C), 142.2 and 143.0 (quat. C), 2 x 147.3 (Ar-$\underline{C}OCF_3$), 205.8 and 206.6 (<u>C</u>=O)

9-{N-[3-(trifluoromethoxy)benzyl]-N-(cyclohex-2-enyl)amino}-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (2.12)

To a stirred solution of compound **(2.3)** (0.50g, 1.96mmol) in acetonitrile (8ml) was added 3-(trifluoromethoxy) benzyl bromide (0.55g, 2.16mmol) and *N',N'*-diisopropylethylamine (0.38g, 2.94mmol), using the method outlined for the preparation of compound **(2.11)**. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 40:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title compound **(2.12)** as a yellow oil (0.61g, 73%) with the following physical properties;

IR (DCM, v); cm^{-1} 3062.8, 2925.2, 1687.6, 1450.1, 1260.8, 1215.9, 1163.6

HRMS ($+H^+$): actual 452.1813, calculated 452.1813, molecular formula $C_{25}H_{26}NO_2F_3$

^1H NMR ($CDCl_3$, 400MHz) δH(ppm) 1.40-2.21 (4 x m, 10H, 5 x $C\underline{H}_2$), 2.55-2.66 (m, 1H, 1H of $C\underline{H}_2CO$), 2.69-2.77 (m, 1H, 1H of $C\underline{H}_2CO$), 3.37-3.41 (broad m, 1H, NC<u>H</u>C=C), 3.75-3.98 (m, 2H, $NC\underline{H}_2$), 4.15-4.19 (m, 1H, NC<u>H</u>Ar), 5.65-5.82 (m, 2H,

C<u>H</u>=C<u>H</u>), 7.07-7.09 (m, 1H, Ar-<u>H</u>), 7.27-7.39 (m, 4H, 4 x Ar-<u>H</u>), 7.48-7.56 (m, 2H, 2 x Ar-<u>H</u>), 7.79 and 7.85 (2 x d, J=7.76Hz, signals overlapping, 1H, COAr-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.3 and 20.6 (<u>C</u>H_2), 21.9 and 22.0 (<u>C</u>H_2), 25.1 and 25.2 (<u>C</u>H_2), 25.4 and 26.7 (<u>C</u>H_2), 29.7 and 30.3 (<u>C</u>H_2), 40.6 and 40.8 (<u>C</u>H_2), 50.9 and 51.5 (<u>C</u>H_2), 55.8 and 55.9 (<u>C</u>H), 61.7 and 61.9 (<u>C</u>H), 2 x 118.9 (tert. C), 119.3 and 119.5 (O<u>C</u>F_3), 120.1 and 120.3 (tert. C), 120.8 and 121.8 (O<u>C</u>F_3), 126.1 and 126.2 (tert. C), 127.2, 127.3, 127.4, 127.6, 127.8, 128.1 (3 x tert. C), 2 x 129.5 (tert. C) 130.2, 130.9, 131.1 (<u>C</u>H=<u>C</u>H) 131.4 and 131.5 (tert. C), 139.1 and 139.3 (quat. C), 142.3 and 143.4 (quat. C), 144.3 and 145.1 (quat. C), 2 x 149.3 (Ar-<u>C</u>OCF_3), 206.3 and 207.1 (<u>C</u>=O)

9-[*N*-(2,3,6-trifluorobenzyl)-N-(cyclohex-2-enyl)amino]-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (2.13)

To a stirred solution of compound **(2.3)** (0.50g, 1.96mmol) in acetonitrile (10ml) was added 2,3,6-trifluorobenzyl bromide (0.49g, 2.16mmol) and *N,N*-diisopropylethylamine (0.38g, 2.94mmol), using the method outlined for the preparation of compound **(2.11)**. The product was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 16:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title compound **(2.13)** as a yellow oil (0.54g, 70%) with the following physical properties;

IR (DCM, v); cm^{-1} 3400.4, 3058.0, 3019.3, 2930.4, 1684.2, 1495.7, 1245.6, 1033.2

HRMS ($+H^+$): actual 400.1877, calculated 400.1697, molecular formula $C_{25}H_{25}NOF_3$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.39-2.42 (3 x m, 10H, 5 x C<u>H</u>$_2$), 2.61-2.76 (m, 2H, C<u>H</u>$_2$CO), 3.27-3.47 (broad m, 1H, NC<u>H</u>CH=CH), 3.81-4.06 (2 x m, 3H, 2H of NC<u>H</u>$_2$ and 1H of NC<u>H</u>Ar), 5.74-5.85 (m, 2H, C<u>H</u>=C<u>H</u>), 6.72-6.81 (m, 1H, Ar-<u>H</u>), 6.97-7.07 (m, 1H, Ar-<u>H</u>), 7.23-7.29 (m, 1H, Ar-H), 7.42 (t, J=7.34Hz, 1H, Ar-<u>H</u>), 7.52 (dd, J_1=2.51Hz, J_2=5.02Hz, 1H, Ar-<u>H</u>), 7.83 and 7.88 (2 x d, J=7.56Hz, signals overlapping, 1H, COAr-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.6 and 20.7 (<u>C</u>H_2), 22.0 and 22.2 (<u>C</u>H_2), 25.0 and 25.1 (<u>C</u>H_2), 25.4 and 27.1 (<u>C</u>H_2), 28.8 and 30.1 (<u>C</u>H_2), 2 x 39.1 (<u>C</u>H_2), 40.8 and 41.0 (<u>C</u>H_2), 55.7 and 55.9 (<u>C</u>H), 61.1 and 61.7 (<u>C</u>H), 2 x 110.2 (tert. C), 2 x 115.4 (tert. C), 2

x 118.0 (quat. C), 2 x 118.5 (quat. C), 126.7 and 126.2 (tert. C), 127.6 and 127.8 (tert. C), 2 x 128.4 (Ar-CHCCO), 129.6, 130.4, 130.6 (CH=CH), 2 x 130.9 (tert. C), 2 x 131.6 (CH=CH), 139.1 and 139.3 (quat. C), 143.7 and 144.1 (quat. C), 146.1 and 147.7 (quat. C), 148.3 and 149.2 (quat. C), 156.2 and 157.8 (quat. C), 206.3 and 207.1 (C=O)

9-{*N*-[methyl 4-(benzoate)benzyl]-N-(cyclohex-2-enyl)amino}-6,7,8,9,-tetrahydrobe nzo [7] annulen-5-one (2.14)

To a stirred solution of compound **(2.3)** (0.50g, 1.96mmol) in acetonitrile (10ml) was added methyl 4-(bromomethyl) benzoate (0.49g, 2.15mmol) and *N,N*-diisopropylethylamine (0.38g, 2.94mmol). The resulting mixture was heated till gentle reflux and kept under an atmosphere of nitrogen gas for thirteen days. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 4:1). The reaction mixture was then partitioned between water (25ml) and diethyl ether (3 x 20ml each). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 10:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title compound **(2.14)** as a yellow oil (0.43g, 54%) with the following physical properties;

IR (DCM, v); cm^{-1} 3430, 3058.0, 3024.2, 2931.1, 1721.2, 1682.96, 1434.9, 1278.5

HRMS ($+Na^+$): actual 426.2053, calculated 426.2045, molecular formula $C_{26}H_{29}NO_3Na$

^1H NMR ($CDCl_3$, 400MHz) δH(ppm): 1.34-2.11 (4 x m, 10H, 5 x CH_2), 2.46-2.58 (m, 1H, 1H of CH_2CO), 2.63-2.72 (m, 1H, 1H of CH_2CO), 3.31 (broad d, 1H, NCHCH=CH), 3.72-3.76 (m, 1H, NCHAr), 3.83-3.97 (s and m, signals overlapping, 4H, 3H of CH_3OCO- and 1 H of NCH_2), 4.06-4.13 (m, 1H, 1H of NCH_2), 5.62-5.76 (m, 2H, CH=CH), 7.25-7.29 (m, 1H, Ar-H), 7.42-7.50 (m, 4H, 4x Ar-H), 7.74 and 7.80 (2 x d, J=7.52Hz, signals overlapping, 1H, Ar-H), 7.96 and 7.97 (2 x d, J=8.04Hz, signals overlapping, 2H, 2 x Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 19.8 and 20.1 (CH_2), 21.4 and 21.5 (CH_2), 24.5, 24.6, 24.7, 26.4 (2 x CH_2), 29.1 and 29.7 (CH_2), 40.1 and 40.2 (CH_2), 2 x 50.8 (CH_2),

50.7 and 51.6 ($\underline{C}H_2$), 51.5 ($\underline{C}H_3$), 55.4 and 55.5 ($\underline{C}H$), 61.5 and 61.8 ($\underline{C}H$), 126.7 and 126.8 (tert. C), 126.9 and 127.1 (tert. C), 127.2, 2 x 127.3, 2 x 127.4, 127.6 (3 x tert. C), 2 x 128.0 (2 x quat. C), 4 x 129.1 (2 x tert. C), 129.1, 129.9, 2 x 130.3, 130.5 ($\underline{C}H=\underline{C}H$), 2 x 130.9 (tert. C), 138.7 and 138.8 (quat. C), 141.9 and 142.8 (quat. C), 146.9 and 147.7 (quat. C), 2 x 166.5 ($\underline{C}OOCH_3$), 205.7 and 206.6 ($\underline{C}$=O)

Methyl 2-(4-(bromomethyl)phenyl)acetate (2.15)

To a stirred solution of 4-bromomethyl phenylacetic acid (0.70g, 3.06mmol) in anhydrous DCM (15ml) was added HPLC grade methanol (1.4ml) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (1.17g, 4.58mmol) under an atmosphere of nitrogen gas. The reaction was stirred at room temperature for six hours. An additional 1.4ml of HPLC grade methanol was placed into the reaction mixture. The reaction was stirred for a further eighteen hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to check for the disappearance of the starting material. On completion, the reaction mixture was extracted between water (25ml) and diethyl ether (3 x 20ml). The combined organic layers were dried over magnesium sulphate, filtered and concentrated *in vacuo* to give crude product. The resulting product was purified by flash column chromatography on silica gel 230-400mesh (eluent, hexane:ethyl acetate, 20:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(2.15)** as a colourless oil (0.73g, 98%) with the following physical properties;

IR (DCM, v); cm^{-1} 2951.5, 1736.3, 1514.3, 1435.6, 1229.5, 1160.5, 1013.6

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 3.65 (s, 2H, $C\underline{H}_2$), 3.72 (s, 3H, $C\underline{H}_3$), 4.51 (s, 2H, $C\underline{H}_2$), 7.29 (2 x d, J=8.00Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.38 (2 x d, J=8.04Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 51.70 ($\underline{C}H_3$), 32.78 ($Br\underline{C}H_2$), 40.39 ($\underline{C}H_2$), 2 x 129.2 (2 x tert. C), 2 x 130.1 (2 x tert. C), 133.8 (quat. C), 136.2 (quat. C), 171.3 (C=O)

The spectral data was consistent with that reported in the literature data.

9-{*N*-[methyl-2-(4-phenylacetate) benzyl]-*N*-(cyclohex-2-enyl)amino}-6,7,8,9,-tetra hydrobenzo[7]annulen-5-one (2.16)

To a stirred solution of compound **(2.3)** (0.60g, 2.35mmol) in acetonitrile (10ml) was added compound **(2.15)** (0.63g, 2.59mmol) and *N,N*-diisopropylethylamine (0.46g, 3.53mmol). The resulting mixture was heated till gentle reflux and kept under an atmosphere of nitrogen gas for twenty four hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 4:1) to observe the disappearance of the starting material. The reaction mixture was then partitioned between water (25ml) and diethyl ether (3 x 20ml each). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 12:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title compound **(2.16)** as a yellow oil (0.12g, 12%) with the following physical properties;

IR (DCM, v); cm^{-1} 3399.9, 3058.0, 3019.3, 2928.4, 1738.8, 1682.37, 1435.2, 1262.2

HRMS ($+Na^+$): actual 440.2211, calculated 440.2202, molecular formula $C_{27}H_{31}NO_3Na$

^1H NMR ($CDCl_3$, 400MHz) δH(ppm): 1.28-2.22 (3 x m, 10H, 5 x C$\underline{H}_2$), 2.56-2.60 (m, 1H, 1H of C$\underline{H}_2$CO), 2.65-2.75 (m, 1H, 1H of C$\underline{H}_2$CO), 3.35 (broad d, 1H, NC$\underline{H}$CH=CH), 3.64 (s, 2H, ArC$\underline{H}_2$COO), 3.71 (s, 3H, C$\underline{H}_3$OCO), 3.73-3.97 (m, 2H, NC$\underline{H}_2$), 4.15-4.18 (m, 1H, NC$\underline{H}$Ar), 5.65-5.80 (m, 2H, C$\underline{H}$=C$\underline{H}$), 7.25 (2 x d, J=8.02Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.32 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.39 and 7.41 (1 x d, J=8.00Hz and 1 x d, J=9.56Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.48-7.63 (m, 2H, 2 x Ar-$\underline{H}$), 7.84 and 7.94 (2 x d, J=8.02Hz, signals overlapping, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.4 and 20.7 ($\underline{C}H_2$), 22.0 and 22.7 ($\underline{C}H_2$), 25.1 and 25.2 ($\underline{C}H_2$), 25.4 and 26.9 ($\underline{C}H_2$), 29.6 and 30.4 ($\underline{C}H_2$), 40.6 and 40.7 ($\underline{C}H_2$), 2 x 40.8 ($\underline{C}H_2$), 50.7 and 51.6 ($\underline{C}H_2$), 52.1 ($\underline{C}H_3$), 55.6 and 55.8 ($\underline{C}H$), 61.0 and 61.6 ($\underline{C}H$), 126.5 and 126.6 (tert. C), 2 x 127.1 ($\underline{C}H$=CH), 2 x 127.3 (tert. C), 2 x 127.8 (CH=$\underline{C}H$), 4 x 128.7 (2 x tert. C), 129.9 and 130.0 (tert. C), 130.2 and 130.3 (tert. C), 2 x 130.9 (tert. C),

131.6 and 131.7 (quat. C), 138.6 and 138.8 (quat. C), 139.9 and 140.8 (quat. C), 142.7 and 143.8 (quat. C), 2 x 171.8 (COOCH3), 205.8 and 206.8 (C=O)

9-[*N*-(4-nitrobenzyl)-*N*-(cyclohex-2-enyl)amino]-6,7,8,9-tetrahydrobenzo[7]annulen -5-one (2.17)

To a stirred solution of compound **(2.3)** (0.50g, 1.96mmol) in acetonitrile (8ml) was added 4-nitrobenzyl bromide (0.47g, 2.16mmol) and *N,N*-diisopropylethylamine (0.38g, 2.94mmol). The resulting mixture was heated to gentle reflux and kept under an atmosphere of nitrogen gas. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 4:1). After ten days, the reaction was quenched by the addition of 2M aq. HCl (20ml) and the product was extracted with diethyl ether (3 x 25ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 6:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title compound **(2.17)** (0.40g, 64%) as a yellow oil with the following physical properties;

IR (DCM, v); cm^{-1} 3405.8, 3062.8, 3014.5, 2928.9, 1682.7, 1598.5, 1518.2, 1343.6

HRMS ($+H^+$): actual 391.2013, calculated 391.2314, molecular formula $C_{24}H_{27}N_2O_3$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.37-2.11 (2 x m, 10H, 5 x CH_2), 2.55-2.67 (m, 1H, 1H of CH_2CO), 2.68-2.71 (m, 1H, 1H of CH_2CO), 3.37-3.45 (broad m, 1H, NCHCH=CH), 3.81-3.97 (m, 2H, NCH_2), 4.14-4.17 (m, 1H, NCHAr), 5.64-5.82 (m, 2H, CH=CH), 7.25-7.31 (m, 1H, Ar-H), 7.41-7.50 (m, 3H, 3 x Ar-H), 7.53 (d, J=8.8Hz, 1H, Ar-H), 7.70 (d, J=7.76Hz, 1H, Ar-H), 8.08-8.15 (m, 2H, 2 x Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.2 and 20.4 (CH_2), 2 x 21.9 (CH_2), 2 x 25.1 (CH_2), 25.2 and 26.3 (CH_2), 29.6 and 30.0 (CH_2), 40.7 and 40.9 (CH_2), 51.5 and 51.9 (CH_2), 2 x 56.1 (CH), 62.7 and 62.9 (CH), 2 x 123.4 (tert. C), 2 x 123.5 (tert. C), 127.4 and 127.7 (tert. C), 2 x 127.5 (tert. C), 127.9 and 128.2 (tert. C), 2 x 128.3 (tert. C), 2 x 128.5 (tert. C), 129.3, 129.7, 131.2 (CH=CH) 2 x 131.4 (tert. C), 139.2 and 139.5 (quat.

C), 141.9 and 142.5 (quat. C), 146.6 and 146.7 (quat. C), 149.9 and 150.7 (quat. C), 206.4 and 207.1 (C=O)

9-[*N*-(4-(nitrilbenzyl)-*N*-(cyclohex-2-enyl)amino]-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (2.18)

To a stirred solution of compound **(2.3)** (1.00g, 3.92mmol) in acetonitrile (10ml) was added 4-(bromomethyl)-benzonitrile (0.85g, 4.31mmol) and *N,N*-diisopropylethylamine (0.76ml, 5.88mmol). The resulting mixture was heated till gentle reflux and kept under an atmosphere of nitrogen for twelve days. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 4:1) to observe the disappearance of the starting material. The reaction mixture was then partitioned between 2M aq. HCl (25ml) and diethyl ether (3 x 20ml each). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 10:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title compound **(2.18)** as a yellow oil (1.23g, 85%) with the following physical properties;

IR (DCM, v); cm^{-1} 3428.4, 3067.6, 2929.1, 2226.3, 1682.6, 1606.3, 1448.5, 1099.6

HRMS ($+Na^+$): actual 393.1926, calculated 393.1943, molecular formula $C_{25}H_{26}N_2ONa$

^1H NMR ($CDCl_3$, 400MHz) δH(ppm): 1.37-2.11 (3 x m, 10H, 5 x CH_2), 2.50-2.60 (m, 1H, 1H of CH_2CO), 2.65-2.75 (m, 1H, 1H of CH_2CO), 3.39 (broad m, 1H, NCHCH=CH), 3.75-3.93 (m, 2H, NCH_2), 4.11-4.14 (m, 1H, NCHAr), 5.62-5.80 (m, 2H, CH=CH), 7.28 (2 x t, J=8.00Hz, 1H, signals overlapping, Ar-H), 7.39-7.49 (m, 4H, 4 x Ar-H), 7.54 and 7.58 (1 x d, J=8.04Hz and 1 x d, J=8.52Hz, signals overlapping, 2H, 2 x Ar-H), 7.68 and 7.70 (2 x d, J=3.52Hz, signals overlapping, 1H, COAr-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 19.8 and 20.0 (CH_2), 21.4 and 21.5 (CH_2), 3 x 24.7, 25.9 (2 x CH_2), 29.1 and 29.5 (CH_2), 40.2 and 40.4 (CH_2), 51.2 and 51.6 (CH_2), 2 x 55.6 (CH), 62.2 and 62.3 (CH), 109.5 and 109.6 (quat. C), 118.7 (CN), 126.9, 2 x 127.0, 127.2, 127.3, 127.7 (3 x tert. C), 2 x 127.9 (tert. C), 2 x 128.0 (tert. C), 128.9, 129.3,

130.7, 130.8 (CH=CH), 130.9 and 131.0 (tert. C), 2 x 131.5 (tert. C), 2 x 131.6 (tert. C), 138.7 and 139.0 (quat. C), 141.5 and 142.1 (quat. C), 147.2 and 148.0 (quat. C), 205.9 and 206.7 (C=O)

9-{*N*-[4-(aminomethyl)benzyl]-*N*-(cyclohex-2-enyl)amino}-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-ol (2.19)

To a stirred solution of compound **(2.18)** (0.56g, 1.51mmol) in anhydrous tetrahydrofuran (THF) (40ml) was slowly added lithium aluminium hydride ($LiAlH_4$) (0.40g, 10.54mmol) at 0°C under anhydrous conditions. The temperature of the reaction was slowly increased to room temperature and maintained at this temperature under an atmosphere of nitrogen gas for an additional hour. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 2:1) to observe the disappearance of the starting material. The reaction mixture was partitioned between 2M aq. HCl (25ml) and diethyl ether (3 x 20ml each). The acidic solution was basified with 2.5M. aq. NaOH. The basic fraction was extracted with diethyl either (3 x 30ml). The combined etheral extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo* to give a yellow oil. The resulting residue **(2.19)** (0.45g, 79%) was not purified by flash column chromatography, with the following physical properties;

IR (DCM, v); cm^{-1} 3381.6, 2920.9, 1443.2, 1093.6

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.28-2.29 (6 x m, 12H, 6 x CH_2), 3.32 (broad m, 2H, NH_2), 3.51-3.57 (m, 1H, NCHCH=CH), 3.74-3.94 (m, 4H, 2H of NCH_2 and 2H of NH_2CH_2), 4.00-4.02 (m, 1H, NCHAr), 4.64-4.67 (m, 1H, CHOH), 5.71-5.87 (m, 2H, CH=CH), 7.13-7.37 (2 x m, 8H, 8 x Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 21.7, 21.9, 24.4, 24.5, 24.9, 25.2 (3 x CH_2), 29.3, 30.7, 30.8, 33.4 (2 x CH_2), 2 x 45.7 (CH_2), 53.1 and 53.5 (CH_2), 56.2 and 56.5 (CH), 66.4 and 67.5 (CH_2), 2 x 69.1 (CH), 75.8 and 75.9 (CHOH), 2 x 126.6, 126.8, 127.0 (2 x tert. C), 2 x 127.5 (tert. C), 2 x 127.9, 2 x 128.6 (2 x tert. C), 2 x 128.9 (tert. C), 2 x 129.5, 129.7, 129.8 (CH=CH), 4 x 130.0 (2 x tert. C), 2 x 140.5 (quat. C), 141.2 and 141.4 (quat. C), 4 x 144.2 (2 x quat. C)

9-[*N*-(2-phenylbenzyl)-*N*-(cyclohex-2-enyl)amino]-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (2.20)

To a stirred solution of compound **(2.3)** (0.50g, 1.96mmol) in acetonitrile (10ml) was added 2-phenylbenzyl bromide (0.39ml, 2.16mmol) and *N,N*-diisopropylethylamine (0.38g, 2.94mmol), using the method outlined for the preparation of compound **(2.11)**. The crude product was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 40:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title compound **(2.20)** as a yellow oil (0.34g, 41%) with the following physical properties;

IR (DCM, v); cm^{-1} 3396.13, 3053.1, 2917.87, 1684.2, 1451.0, 1038.2

HRMS ($+H^+$): actual 422.2478, calculated 422.2484, molecular formula $C_{30}H_{32}NO$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.26-1.95 (4 x m, 10H, 5 x C$\underline{H}_2$), 2.44-2.65 (m, 2H, C$\underline{H}_2$CO), 3.22 (broad m, 1H, NC$\underline{H}$CH=CH), 3.62-3.69 (m, 1H, NC$\underline{H}$Ar), 3.83-3.98 (m, 2H, NC$\underline{H}_2$), 5.48-5.73 (m, 2H, C$\underline{H}$=C$\underline{H}$), 7.18 (d, J=7.32Hz, 1H, Ar-$\underline{H}$), 7.27 (d, J=5.84Hz, signals overlapping, 4H, 4 x Ar-$\underline{H}$), 7.35-5.55 (m, 6H, 6 x Ar-$\underline{H}$), 7.75 and 7.92 (2 x d, J=7.28Hz, Ar-$\underline{H}$), 7.82 and 7.85 (1 x d, J=7.32Hz and 1 x d, J=8.04Hz, signals overlapping, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.3 and 20.6 ($\underline{C}H_2$), 22.0 and 22.1 ($\underline{C}H_2$), 2 x 25.1 ($\underline{C}H_2$), 25.4 and 27.0 ($\underline{C}H_2$), 29.1 and 30.1 ($\underline{C}H_2$), 40.6 and 40.7 ($\underline{C}H_2$), 47.7 and 48.3 ($\underline{C}H_2$), 55.2 and 55.3 ($\underline{C}H$), 60.6 and 60.9 ($\underline{C}H$), 125.6 and 125.7 (tert. C), 126.4 and 126.5 (tert. C), 2 x 127.0 (tert. C), 2 x 127.2 (tert. C), 127.4, 127.5, 127.6, 128.6, 128.7, 8 x 128.9, 129.4, 129.5, 2 x 129.9, 130.1, 130.4, 130.9 ($\underline{C}H$=$\underline{C}H$ and 9 x tert. C), 138.1 and 138.6 (quat. C), 138.8 and 138.9 (quat. C), 140.0 and 140.6 (quat. C), 140.9 and 141.0 (quat. C), 142.7 and 143.7 (quat. C), 206.0 and 206.9 ($\underline{C}$=O)

9-[*N*-benzhydryl-*N*-(cyclohex-2-enyl)amino]-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (2.21)

To a stirred solution of compound **(2.3)** (1.00g, 3.92mmol) in acetonitrile (10ml) was added bromodiphenylmethane (1.07g, 4.31mmol) and *N,N*-diisopropylethylamine (0.76g,

5.88mmol), The resulting mixture was heated till gentle reflux and kept under an atmosphere of nitrogen gas for four days. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 4:1). The reaction mixture was then partitioned between water (25ml) and diethyl ether (3 x 20ml each). The combined organic fractions were dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 10:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title compound **(2.21)** as a yellow oil (0.53g, 32%) with the following physical properties;

IR (DCM, v); cm^{-1} 3391.3, 3062.8, 3029.0, 2927.6, 1681.1, 1595.0, 1448.7, 1278.5

HRMS ($+Na^+$): actual 444.2318, calculated 444.32303, molecular formula $C_{30}H_{31}NONa$

^1H NMR ($CDCl_3$, 600Hz) δ_H(ppm): 1.45-2.20 (5 x m, 10H, 5 x C$\underline{H}_2$), 2.22-2.50 (m, 1H, 1H of C$\underline{H}_2$CO), 2.54-2.66 (m, 1H, 1H of C$\underline{H}_2$CO), 3.95 and 4.03 (2 x broad m, 1H, NC$\underline{H}$CH=CH), 4.47-4.51 (m, 1H, NC$\underline{H}$(Ar)CH_2), 5.41 and 5.50 {2 x s, 1H, NCH(Ar)Ar}, 5.79-5.87 (m, 1H, C$\underline{H}$=CH), 5.91-5.99 (m, 1H, CH=C$\underline{H}$), 7.14-7.42 (m, 9H, 9 x Ar-$\underline{H}$), 7.53 (t, J=7.70Hz, 1H, Ar-$\underline{H}$), 7.57 (m, 1H, Ar-$\underline{H}$), 7.63 (t, J=7.40Hz, 1H, Ar-$\underline{H}$), 7.78, 7.84, 7.95 (3 x d, J=7.92Hz, J=7.14Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 150Hz) δc(ppm): 20.1 and 20.3 ($\underline{C}H_2$), 22.5 and 22.8 ($\underline{C}H_2$), 25.0 and 25.1 ($\underline{C}H_2$), 30.3 and 30.7 ($\underline{C}H_2$), 32.1 and 32.2 ($\underline{C}H_2$), 40.4 and 40.6 ($\underline{C}H_2$), 55.5 and 55.7 ($\underline{C}H$), 58.7 and 59.0 ($\underline{C}H$), 65.7 and 65.9 ($\underline{C}H$), 125.9, 2 x 126.3, 126.5, 126.7, 126.8, 127.0, 127.3, 127.4, 127.5, 127.7, 127.8, 127.9, 128.0, 2 x 128.1, 3 x 128.2, 128.3, 128.6, 128.9, 129.4, 130.1, 130.3, 130.4, 131.1, 131.4, 131.7, 132.4, 2 x 133.0 ($\underline{C}H$=$\underline{C}H$ and 14 x tert. C), 138.1, 138.3, 143.5, 143.6, 144.0, 144.8, 144.9, 145.2 (4 x quat. C), 206.3 and 206.7 ($\underline{C}$=O)

Attempted alkylation of secondary amine (2.3) with 4-(bromomethyl)pyridine

Method A:

To a stirred solution of compound **(2.3)** (0.08g, 0.31mmol) in DMF (5ml) was added 4-(bromomethyl)pyridine (0.24g, 0.94mmol) and caesium carbonate (0.41g, 1.25mmol) at room temperature. The reaction was allowed to stir for three days, during which time

three additions of both 4-(bromomethyl)pyridine (0.24g, 0.94mmol) and caesium carbonate (0.41g, 1.25mmol) were made. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 2:1). Upon completion, the reaction mixture was partitioned between water (25ml) and diethyl ether (3 x 20ml each). The combined organic fractions were dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: ethyl acetate:methanol, 2:1). All homogenous fractions were collected and the solvent was evaporated to afford two isolated products. TLC analysis of the most polar product indicated the decomposition had occurred during the solvent evaporation. NMR analysis of the less polar product revealed that the reaction had not proceeded as desired.

Method B:

To a stirred solution of compound **(2.3)** (0.05g, 0.20mmol) in acetone (5ml) was added 4-(bromomethyl)pyridine (0.15g, 0.59mmol) and caesium carbonate (0.26g, 0.78mmol). The reaction was allowed to stir for two days under reflux condition. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 2:1). The reaction mixture was then partitioned between water (25ml) and diethyl ether (3 x 20ml each). The combined organic fractions were dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: ethyl acetate:methanol, 1:1). NMR analysis indicated that the reaction had not proceeded as desired.

Method C:

To a stirred solution of compound **(2.3)** (0.20g, 0.78mmol) in acetonitrile (5ml) was added 4-(bromomethyl)pyridine (0.22g, 0.86mmol) and *N,N*-diisopropylethylamine (0.15g, 1.18mmol), The resulting mixture was allowed to stir at room temperature and kept under an atmosphere of nitrogen gas for two days. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 2:1). The reaction mixture was then partitioned between water (25ml) and diethyl ether (3 x 20ml each). The combined organic fractions were dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The

residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: ethyl acetate:methanol, 1:1). NMR analysis indicated that the reaction had not proceeded as desired. Similar results were obtained when the reaction temperature was changed to reflux condition from room temperature.

3,4-dihydro-1,1-dimethylnaphthalen-2(1H)-one (3.0)

To a stirred solution of β–tetralone (5.00g, 34.2mmol) in *t*-butyl alcohol (30ml) was slowly added methyl iodide (3.40ml, 68.40mmol) and potassium *tert*-butoxide (7.68g, 68.40mmol) at 0°C. The temperature was allowed to increase to ambient after twenty minutes and the reaction was left stirring for an additional half hour. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to observe the disappearance of the starting material. On completion, the reaction mixture was partitioned between 2M aq. HCl (30ml) and diethyl ether (3 x 25ml each). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 30:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(3.0)** as a yellow oil (5.11g, 86%), with the following physical properties;

IR (DCM, v); cm^{-1} 3413.6, 2972.6, 1712.6, 1644.2, 1450.5, 1245.5, 1092.9

HRMS ($+Na^+$): actual 197.0940, calculated 197.0942, molecular formula $C_{12}H_{14}ONa$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.48 (2 x s, signals overlapping, 6H, 2 x $C\underline{H}_3$), 2.72 (t, J=7.04Hz, 2H, $C\underline{H}_2$), 3.13 (t, J=6.52Hz, 2H, $C\underline{H}_2$), 7.19-7.25 (m, 2H, 2 x Ar-$\underline{H}$), 7.30 (broad dt, J_1=2.24Hz, J_2=7.16Hz, 1H, Ar-$\underline{H}$), 7.38 (d, J=8.00Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 2 x 26.5 (2 x $\underline{C}H_3$), 28.2 ($\underline{C}H_2$), 36.8 ($\underline{C}H_2$), 47.3 ($CH_3\underline{C}CH_3$), 127.7 (tert. C), 126.7 (tert. C), 126.0 (tert. C), 125.7 (tert. C), 143.1 (quat. C) 134.7 (quat. C), 214.2 (C=O)

The spectral data was consistent with that reported in the literature data.

1,2,3,4-tetrahydro-1,1-dimethylnaphthalen-2-ol (3.1)

To a stirred solution of compound **(3.0)** (3.36g, 19.31mmol) in methanol (50ml) was added $NaBH_4$ (0.73g, 19.31mmol) at 0°C. The reaction was allowed to stir at this temperature for an hour. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to observe the disappearance of the starting material. On completion, the reaction mixture was quenched by the addition of water (1 x 30ml). The methanol was removed from the reaction mixture under reduced pressure and the product was partitioned between water (25ml) and diethyl ether (3 x 25ml each). The combined organic extracts were dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 3:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(3.1)** as a white solid (3.18g, 94%) with the following physical properties;

Mp: 78-81°C

IR (KBr, v); cm^{-1} 3324.5, 2936.9, 1488.8, 1644.2, 1445.5, 1060.1

HRMS ($+H^+$): actual 176.1110, calculated 176.1196, molecular formula $C_{12}H_{16}O$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.39 (s, 3H, $C\underline{H}_3$), 1.33 (s, 3H, $C\underline{H}_3$), 1.94-2.02 (m, 2H, $C\underline{H}_2$), 2.84-3.04 (m, 2H, $C\underline{H}_2$), 3.80 (dd, J_1=3Hz, J_2=9.04Hz, 1H, C<u>H</u>OH), 7.09 (d, J=7.12Hz, 1H, Ar-<u>H</u>), 7.13 (dt, J_1=1.28Hz, J_2=7.37Hz, 1H, Ar-<u>H</u>), 7.20 (broad t, J=13.6Hz, 1H, Ar-<u>H</u>), 7.37 (d, J=7.72Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100Hz) δc(ppm): 24.6 ($\underline{C}H_3$), 26.4 ($\underline{C}H_2$), 26.6 ($\underline{C}H_2$), 28.5 ($\underline{C}H_3$), 38.7 ($CH_3\underline{C}CH_3$), 75.1 (<u>C</u>HOH), 125.2 (tert. C), 125.7 (tert. C), 126.4 (tert. C), 128.3 (tert. C), 134.1 (quat. C) 143.8 (quat. C)

1,2,3,4-tetrahydro-1,1-dimethylnaphthalen-2-yl methanesulfonate (3.2)

To a stirred solution of the alcohol **(3.1)** (0.83g, 4.71mmol) in dry DCM (15ml) was added methanesulphonyl chloride (0.54g, 4.71mmol) at 0°C under anhydrous conditions. To the resultant solution was added *N,N*-diisopropylethylamine (0.91g, 7.07mmol), drop-wise by syringe, over twenty minutes. The reaction mixture was maintained at this

temperature under an atmosphere of nitrogen gas for one hour. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 5:1) to observe the disappearance of the starting material. On completion, the reaction mixture was quenched with 5% aq. $NaHCO_3$ (20ml) and extracted with DCM (3 x 20ml). The combined organic fractions were further extracted with water (3 x 20ml). The resulting organic fractions were collected, dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 9:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(3.2)** as a colourless oil (1.19g, 99%) with the following physical properties;

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.33 (s, 3H, $C\underline{H}_3$), 1.40 (s, 3H, $C\underline{H}_3$), 2.22-2.35 (m, 2H, $C\underline{H}_2$), 2.86-2.94 (m, 1H, 1H of $C\underline{H}_2$), 3.03-3.11 (s and m, signals overlapping, 4H, $SO_2C\underline{H}_3$ and 1H of $C\underline{H}_2$), 4.87 (dd, J_1=3.38Hz, J_2=7.42Hz, 1H, $C\underline{H}OSO_2$), 7.10 (d, J=7.28Hz, 1H, Ar-$\underline{H}$), 7.15 (dt, J_1=1.23Hz, J_2=7.32Hz, 1H, Ar-$\underline{H}$), 7.21 (broad t, J=7.11Hz, 1H, Ar-$\underline{H}$), 7.34 (d, J=8.16Hz, 1H, Ar-$\underline{H}$)

2-azido-1,2,3,4-tetrahydro-1,1-dimethylnaphthalene (3.3)

To a stirred solution of the mesylate **(3.2)** (1.20g, 4.72mmol) in DMF (20ml) was added sodium azide (6.14g, 94.46mmol) at room temperature. The reaction mixture was heated to 60°C. After sixteen days the reaction was quenched by the addition of water (1 x 50ml) and the product was extracted with diethyl ether (3 x 30ml). The combined ethereal fractions were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 15:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(3.3)** as a yellow oil (0.21g, 22%) with the following physical properties;

IR (DCM, v); cm^{-1} 3022.1, 2961.4, 2098.2, 1492.1, 1447.0, 126.5

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.29 (s, 3H, $C\underline{H}_3$), 1.39 (s, 3H, $C\underline{H}_3$), 2.04-2.22 (m, 2H, $C\underline{H}_2$), 2.87-3.03 (m, 2H, $C\underline{H}_2$), 3.62 (dd, J_1=3.00Hz, J_2=9.26Hz, 1H, $C\underline{H}N_3$), 7.08 (d,

J=7.52Hz, 1H, Ar-H), 7.14 (broad t, J=7.30Hz, 1H, Ar-H), 7.20 (broad t, J=7.00Hz, 1H, Ar-H), 7.34 (d, J=8.00Hz, 1H, Ar-H)

1,2,3,4-tetrahydro-1,1-dimethylnaphthalen-2-amine (3.4)

To a stirred solution of azide **(3.3)** (0.21g, 1.04mmol) in acetic acid (10ml) was added zinc dust (0.54g, 8.04mmol). The reaction mixture was stirred at 0°C for the initial half hour and was allowed to warm to room temperature for seventy-two hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to observe the disappearance of the starting material. The reaction mixture was filtered to remove the zinc dust and the solvent was evaporated *in vacuo* using toluene to aid in the removal of acetic acid. The resulting residue was partitioned between 2M aq. HCl (25ml) and diethyl ether (3 x 20ml each). The combined organic fractions were discarded. The aqueous layer was basified with 2.5M aq. NaOH, and further extracted with diethyl ether (3 x 20ml). The combined organic layers were concentrated under reduced pressure to yield the title primary amine **(3.4)** as a yellow oil (0.09g, 53%) with the following physical properties;

IR (DCM, v); cm^{-1} 3388.8, 2937.2, 1488.9, 1606.2, 1520.3

HRMS ($+H^+$): actual 175.1356, calculated 175.2592, molecular formula $C_{12}H_{17}N$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.27, 1.38 and 1.54 (3 x s, 6H, 2 x CH_3), 1.62-1.72, 1.76-1.88 and 1.97-2.04 (3 x m, 3H, 2H of CH_2 and 1H of CH), 2.82-3.00 (m, 2H, CH_2), 7.01-7.42 (m, 4H, 4 x Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 2 x 24.7 (2 x CH_3), 27.2 (CH_2), 27.3 (CH_2), 38.2 (CH_3CCH_3), 55.8 (CH), 125.1 (tert. C), 125.7 (tert. C), 126.6 (tert. C), 128.5 (tert. C), 134.4 (quat. C), 144.4 (quat, C)

Attempted synthesis of 2-bromo-1,2,3,4-tetrahydro-1,1-dimethylnaphthalene

To a stirred solution of the alcohol **(3.1)** (0.20g, 1.14mmol) in diethyl ether (20ml) at 0°C was added phosphorous tribromide (0.61g, 2.27mmol) drop-wise, *via* a syringe over ten minutes. The reaction was stirred at this temperature for one hour. On completion,

the reaction was quenched by pouring the contents of the round bottomed flask into a beaker containing a mixture of ice and water. The resulting mixture was then extracted using diethyl ether (1 x 50ml). The ether extract was then washed with ice water (1 x 50ml) following by 5% aq. $NaHCO_3$ (2 x 50ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo* at low temperature. NMR analysis of the crude product indicated that the reaction had not proceeded as required.

3,4-dihydro-1,1-dimethylnaphthalen-2(1H)-oxime (3.5)

To a stirred solution of the ketone **(3.0)** (0.20g, 1.15mmol) in ethanol/water (50ml, *v:v*, 2:1), was added sodium acetate (0.10g, 1.15mmol) and hydroxylamine hydrochloride (0.09g, 1.30mmol) at 60°C for two hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to observe the disappearance of the starting material. On completion, the reaction mixture was washed with ethanol and solvent was removed *in vacuo* afterwards. The resulting residue was then partitioned between water (1 x 25ml) and DCM (3 x 25ml each). The combined organic layers were dried over magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 15:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(3.5)** as a white crystalline solid (0.22g, 99%) with the following physical properties;

Mp: 92-94°C

IR (KBr, v); cm^{-1} 3264.4, 2973.4, 1655.3 1446.5, 936.6

HRMS ($+H^+$): actual 190.1299, calculated 190.1226, molecular formula $C_{12}H_{16}ON$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.38 (s, 1H, N-O<u>H</u>), 1.70 (s, 6H, 2 x C<u>H</u>$_3$), 2.97 (m, 2H, C<u>H</u>$_2$), 3.05 (m, 2H, C<u>H</u>$_2$), 7.20-7.25 (m, 2H, 2 x Ar-<u>H</u>), 7.31 (t, J=7.34Hz, 1H, Ar-<u>H</u>), 7.46 (d, J=7.52Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 21.5(<u>C</u>H$_2$), 27.5 (<u>C</u>H$_2$), 2 x 27.6 (2 x <u>C</u>H$_3$), 40.5 (CH$_3$<u>C</u>CH$_3$), 125.1 (tert. C), 126.3 (tert. C), 126.9 (tert. C), 128.4 (tert. C), 135.8 (quat. C), 143.2 (quat, C), 164.7 (<u>C</u>=N-OH)

Reduction of oxime (3.5)

To a stirred solution of the oxime **(3.5)** (1.14g, 6.03mmol) in dry THF (22ml) was very slowly added $LiAlH_4$ (1.37g, 36.10mmol) at 0°C under anhydrous reaction conditions. After ten minutes the reaction temperature was allowed to increase to ambient and the reaction was allowed to proceed for a further five days under an atmosphere of nitrogen. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to observe the disappearance of the starting material. On completion, the reaction mixture was quenched by the addition of ice. The resultant solution was washed with 2M aq. HCl (1 x 30ml) and extracted with diethyl ether (3 x 25ml). The acidic aqueous solution was basified with 2.5M aq. NaOH (1 x 50ml) and extracted again with diethyl ether (3 x 25ml). The organic fractions were combined, dried over magnesium sulphate and evaporated *in vacuo* to afford the corresponding primary amine (**3.4**) as a yellow oil (0.97g, 92%).

Data previously described

Attempted oxidation of primary amine (3.4)

To a stirred, ice-cooled solution of the primary amine **(3.4)** (0.50g, 2.87mmol) in glacial acetic acid (60ml) was added a 10% solution of chromium trioxide in glacial acetic acid (11ml, 17.60mmol), drop-wise, over a period of thirty minutes. The cooled reaction mixture was stirred vigorously during the addition of Jones reagent. The temperature of the reaction was maintained between 0-15°C throughout the reaction. The progress of the reaction was monitored by TLC (MeOH:ethyl acetate, 1:2) to observe the disappearance of the starting material. After eight hours, the reaction was quenched by the addition of sat. aq. Na_2CO_3 (80ml) and the resultant solution was extracted with diethyl ether (3 x 25ml). The organic fractions were combined and solvent was removed *in vacuo*. The crude product was then partitioned between 2M aq. HCl (20ml) and diethyl ether (3 x 25ml each). The combined organic fractions were discarded. The acidic layer was then basified with 2.5M aq. NaOH (30ml) and extracted with diethyl ether (3 x 30ml). The organic fractions were combined, dried over magnesium sulphate, filtered and

concentrated *in vacuo* to afford a yellow oil. The resulting residue was not further purified by flash column chromatography. TLC analysis indicated that a complex mixture of products had resulted. NMR data indicated that the reaction had not proceeded as required.

Tert-butyl 1,2,3,4-tetrahydro-1,1dimethylnaphthalen-2-ylcarbamate (3.7)

Method A:

To a stirred solution of the primary amine (**3.4**) (0.20g, 1.14mmol) in DCM (7ml) was added di-*tert*-butyl dicarbonate (0.50g, 2.28mmol) and *N,N*-diisopropylethylamine (0.29g, 2.28mmol) followed by 4-(dimethylamino)-pyridine (DMAP) (catalytic quantity). The reaction was allowed to stir at room temperature for an hour. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 5:1) to observe the disappearance of the starting material. On completion, the reaction was quenched by the addition of water (1 x 30ml) and extracted with diethyl ether (3 x 30ml). The organic fractions were combined, dried over magnesium sulphate and evaporated off *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 20:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(3.7)** as a yellow oil (0.16g, 53%) with the following physical properties;

IR (DCM, v); cm^{-1} 3347.8, 2973.8, 1701.6, 1500.9, 1365.6, 1171.1

HRMS ($+Na^+$): actual 298.1781, calculated 298.1783, molecular formula $C_{17}H_{25}NO_2Na$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.31 (s, 3H, $C\underline{H}_3$), 1.38 (s, 3H, $C\underline{H}_3$), 1.49 (s, 9H, 3 x $C\underline{H}_3$), 1.87-1.94 (m, 1H, 1H of $C\underline{H}_2$), 2.02-2.08 (m, 1H, 1H of $C\underline{H}_2$), 2.92-2.94 (m, 2H, $C\underline{H}_2$), 3.88 (t, J=8.02Hz, 1H, C<u>H</u>NH), 4.65 (d, J=9.52Hz, 1H, N<u>H</u>), 7.10 (d, J=7.52Hz, 1H, Ar-<u>H</u>), 7.15 (t, J=7.52Hz, 1H, Ar-<u>H</u>), 7.21 (t, J=7.04Hz, 1H, Ar-<u>H</u>), 7.38 (d, J=7.56Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc (ppm): 24.6 ($\underline{C}H_2$), 25.4 (ArC$\underline{C}H_3$), 26.8 ($\underline{C}H_2$), 3 x 28.0 (3 x $\underline{C}H_3$), 29.7 (ArC$\underline{C}H_3$), 37.7 ($CH_3\underline{C}CH_3$), 54.7 ($\underline{C}H$), 80.1 {$O\underline{C}(CH_3)_3$}, 125.4 (tert. C), 125.8 (tert. C), 126.5 (tert. C), 128.5 (tert. C), 134.0 (quat. C), 143.7 (quat. C), 155.4 ($\underline{C}$=O)

Method B:

To a stirred solution of the primary amine (**3.4**) (2.13g, 12.18mmol) in methanol (67ml) was added di-*tert*-butyl dicarbonate (5.31g, 24.36mmol) followed by caesium carbonate (7.95g, 24.36mmol). The reaction was allowed to stir at room temperature for four hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 5:1) to observe the disappearance of the starting material. On completion, the reaction was quenched by the addition of water (1 x 30ml) and extracted with diethyl ether (3 x 30ml). The organic fractions were combined, dried over magnesium sulphate and evaporated off *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 20:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(3.7)** as a yellow oil (2.37g, 71%).

Data previously described

Method C:

To a stirred solution of the primary amine **(3.4)** (5.67g, 32.30mmol) in DCM (70ml) was added di-*tert*-butyl dicarbonate (14.10g, 64.60mmol) followed by *N,N*-diisopropylethylamine (8.35g, 64.60mmol). The reaction was allowed to stir at room temperature over night. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 5:1) to observe the disappearance of the starting material. On completion, the reaction was quenched by the addition of water (1 x 30ml) and extracted with diethyl ether (3 x 30ml). The organic fractions were combined, dried over magnesium sulphate and evaporated off *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 20:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(3.7)** as a yellow oil (8.51g, 95%).

Data previously described

Method D:

To a stirred solution of the primary amine (**3.4**) (3.61g, 20.60mmol) in a mixture of water (20ml) and *t*-butanol (90ml) was added 2M aq. NaOH (10ml). Di-*tert*-butyl dicarbonate (8.99g, 41.20mmol) was then added to the stirred solution. The reaction was allowed to stir at room temperature for four hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 5:1) to observe the disappearance of the starting material. On completion, the basic aqueous fraction was extracted with diethyl ether (1 x 30ml). The combined organic fractions were discarded. The basic aqueous fraction was then acidified using 2M aq. HCl (1 x 50ml) and the product was extracted with diethyl ether (3 x 25ml). The organic fractions were combined, dried over magnesium sulphate and evaporated off *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 20:1). All homogenous fractions were collected and the solvent was evaporated to afford the title *BOC*-protected amine **(3.7)** as a yellow oil (5.47g, 96%).

Data previously described

Tert-butyl 1,2,3,4-tetrahydro-1,1dimethyl-4-oxonaphthalen-2-ylcarbamate (3.8)

Method A:

To a stirred solution of the *BOC*-protected amine **(3.7)** (1.77g, 6.44mmol) in DCM (80ml) was added meta-chloroperoxybenzoic acid (MCPBA) (2.78g, 16.09mmol) and sodium hydrogen carbonate (1.08g, 12.87mmol). The reaction was stirred at room temperature for seven days. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 5:1). The reaction was quenched by the addition of sat. aq. $NaHCO_3$ (1 x 50ml) and the product was extracted with DCM (3 x 30ml). The combined organic extracts were washed with brine (1 x 50ml) again. The organic fraction was dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 8:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(3.8)** as a yellow oil (0.44g, 24%) with the following physical properties;

IR (DCM, v); cm^{-1} 3341.2, 2975.7, 1683.0, 1524.7, 1366.5, 1298.7, 1168.9

HRMS ($+Na^+$): actual 312.1562, calculated 312.1576, molecular formula $C_{17}H_{23}NO_3Na$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.25 (s, 3H, C$\underline{H_3}$), 1.30 (2 x s, signals overlapping, 12H, 4 x C$\underline{H_3}$), 2.65 and 2.69 (2 x d, J=8.04Hz, signals overlapping, 1H, 1H of C$\underline{H_2}$), 2.85 (dd, J_1=4.00Hz, J_2=17.56Hz, 1H, 1H of C$\underline{H_2}$), 4.06 (broad t, 1H, C$\underline{H}$NH), 5.09 (d, J=9.52Hz, 1H, N$\underline{H}$CH), 7.18 (t, J=7.26Hz, 1H, Ar-$\underline{H}$), 7.35 (d, J=8.04Hz, 1H, Ar-$\underline{H}$), 7.43 (t, J=7.28Hz, 1H, Ar-$\underline{H}$), 7.87 (d, J=7.87Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 24.2 ($\underline{C}H_3CCH_3$), 27.8 (3 x $\underline{C}H_3$), 27.9 ($CH_3C\underline{C}H_3$), 38.4 ($CH_3\underline{C}CH_3$), 41.0 ($\underline{C}H_2$), 54.2 ($\underline{C}$HNH), 78.7 {$\underline{C}(CH_3)_3$}, 125.8 (tert. C), 126.2 (tert. C), 126.7 (tert. C), 130.3 (quat. C), 134.0 (tert. C), 149.6 (quat. C), 155.0 (NH$\underline{C}$OO), 195.5 ($\underline{C}$=O)

Method B:

To a stirred solution of the *BOC*-protected amine **(3.7)** (0.40g, 1.45mmol) in acetone (3.60ml) at 0°Cwas added Jones reagent (3.34ml), drop-wise, over a period of thirty minutes. The temperature of the reaction was maintained between 5-15°C throughout the reaction. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 7:1) to observe the disappearance of the starting material. After forty-five minutes, the reaction was quenched by the addition of sat. aq. Na_2CO_3 (1 x 20ml) and the product was extracted with diethyl ether (3 x 25ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 8:1). All homogenous fractions were collected and the solvent was evaporated to afford the title compound **(3.8)** as a yellow oil (0.09g, 22%).

Data previously described

4,4-dimethylnaphthalen-1(4H)-one (3.9)

To a stirred solution of the *BOC*-protected amine **(3.8)** (0.60g, 2.07mmol) in DCM (3ml) was added TFA (3ml) at 0°C. The temperature of the reaction mixture was maintained between 0-5°C throughout the reaction. The progress of the reaction was monitored by

TLC (hexane:ethyl acetate, 3:1) to observe the disappearance of the starting material. After an hour, the reaction mixture was quenched by the addition of water (1 x 20ml) and the product was extracted with DCM (3 x 20ml). The organic fractions were collected and discarded. The acidic aqueous solution was adjusted to pH 13 with 2.5M aq. NaOH and extracted with DCM (3 x 20ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo* to afford the title primary **(3.9)** as a white crystalline solid (0.33g, 92%) with the following physical properties;

MP: 46-50°C

IR (KBr, v); cm^{-1} 2963.9, 1661.4, 1600.6, 1307.6

HRMS ($+H^+$): actual 173.1000, calculated 173.0961, molecular formula $C_{12}H_{13}O$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.51 (s, 6H, 2 x $C\underline{H}_3$), 6.40 (d, J=10.04Hz, 1H, $C\underline{H}$=CH), 6.95 (d, J=9.52Hz, 1H, CH=$C\underline{H}$), 7.41 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.55-7.62 (m, 2H, 2 x Ar-$\underline{H}$), 8.21 (d, J=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 2 x 29.2 ($\underline{C}H_3C\underline{C}H_3$), 37.1 ($CH_3\underline{C}CH_3$), 2 x 125.9, 126.3, 126.4, 132.2, 157.1 (4 x tert. C, $\underline{C}H$=$\underline{C}H$), 130.1 (quat. C), 149.3 (quat. C), 184.7 (C=$\underline{O}$)

***Tert*-butyl 1,2,3,4-tetrahydro-4-hydroxy-1,1-dimethylnaphthalen-2-yl carbamate (3.10)**

To a stirred solution of compound **(3.8)** (0.62g, 2.15mmol) in methanol (50ml) was added sodium borohydride (0.20g, 5.36mmol) at 0°C. The temperature of the reaction mixture was maintained between 0-5°C throughout the reaction. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to observe the disappearance of the starting material. After twenty-two hours, the reaction was quenched by the addition of water (1 x 20ml) and the methanol was removed from the reaction mixture under vacuum. The resulting residue was extracted between water (30ml) and diethyl ether (3 x 25ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 4:1). All

homogenous fractions were collected and the solvent was evaporated to afford the title compound **(3.10)** as a white solid (0.41g, 66%) with the following physical properties;

Mp: 72-78°C

IR (KBr, v); cm^{-1} 3373.9, 2973.8, 1693.4, 1513.6, 1170.7, 1073.8

HRMS ($+Na^+$): actual 314.1742, calculated 314.1732, molecular formula $C_{17}H_{25}NO_3Na$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.30 (s, 3H, $C\underline{H}_3$), 1.33 (s, 3H, $C\underline{H}_3$), 1.46 (s, 9H, 3 x $C\underline{H}_3$), 2.01 (m, 1H, 1H of $C\underline{H}_2$), 2.30 (broad m, 1H, 1H of $C\underline{H}_2$), 3.04 (broad s, 1H, O<u>H</u>), 3.89 (broad t, 1H, C<u>H</u>NH), 4.91 (broad t, 1H, C<u>H</u>OH), 5.50 (d, J=9.56Hz, 1H, N<u>H</u>CH), 7.22 (t, J=7.28Hz, 1H, Ar-<u>H</u>), 7.35 (d, J=7.28Hz, 1H, Ar-<u>H</u>), 7.43 (d, J=8.00Hz, 1H, Ar-<u>H</u>), 7.87 (d, J=7.52Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 2 x 25.1 ($\underline{C}H_3C\underline{C}H_3$), 3 x 28.0 (3 x $\underline{C}H_3$), 33.5 ($\underline{C}H_2$), 38.6 ($CH_3\underline{C}CH_3$), 52.9 (<u>C</u>HNH), 78.7(<u>C</u>HOH),78.7 {$\underline{C}(CH_3)_3$}, 125.9 (tert. C), 126.3 (tert. C), 127.6 (tert. C), 127.8 (tert. C), 136.3 (quat. C), 143.3 (quat. C), 155.6 (NH<u>C</u>OO)

1,2-dihydro-1,1-dimethylnaphthalen-2-amine (3.11)

To a stirred solution of the alcohol **(3.10)** (1.47g, 5.02mmol) was added a 20% of solution of oxalic acid in water (130ml). The reaction was refluxed for two days. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to observe the disappearance of the starting material. On completion, the reaction was allowed to reach room temperature. The reaction mixture was quenched by the addition of water (1 x 20ml) and extracted with diethyl ether (3 x 20ml). The combined ethereal fractions were discarded. The acidic aqueous solution was adjusted to pH 13 with 2.5M aq. NaOH and extracted with diethyl ether (3 x 20ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo* to afford the title primary **(3.11)** as a yellow oil (0.54g, 62%) with the following physical properties;

IR (DCM, v); cm^{-1} 3405.8, 1446.8, 1038.2

HRMS ($+H^+$): actual 174.1279, calculated 174.1283, molecular formula $C_{12}H_{16}N$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.27 (s, 3H, $C\underline{H}_3$), 1.35 (s, 3H, $C\underline{H}_3$), 3.24 (d, J=4.52Hz, 1H, $C\underline{H}NH_2$), 6.01 (dd, J_1=4.52Hz, J_2=9.52Hz, 1H, $C\underline{H}$=CH), 6.46 (d, J=9.56Hz, 1H, CH=$C\underline{H}$), 7.09 (dd, J_1=1.5Hz, J_2=7.02Hz, 1H, Ar-$\underline{H}$), 7.19 (dt, J_1=1.52Hz, J_2=7.52Hz, 1H, Ar-$\underline{H}$), 7.24 (dt, J_1=1.48Hz, J_2=7.52Hz, 1H, Ar-$\underline{H}$), 7.35 (d, J=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 22.1 ($\underline{C}H_3CCH_3$), 27.6 ($CH_3C\underline{C}H_3$), 38.9 ($CH_3\underline{C}$ CH_3), 56.3 ($\underline{C}HNH_2$), 125.0, 126.4, 126.9, 127.1, 128.0 (4 x tert. C, $\underline{C}$H=CH), 132.0 (CH=$\underline{C}$H), 132.0 (quat. C), 143.1 (quat. C)

(Z)-6,7-dihydrobenzo[7]annulen-5-one (3.14)

To a stirred solution of compound **(2.0)** (0.30g, 1.26mmol) in DMF (15ml) was added sodium iodide (0.19g, 1.26mmol) and cyclopentylamine (0.14ml, 1.38mmol) followed by *N,N*-diisopropylethylamine (0.24g, 1.88mmol) at 60°C. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 4:1) to observe the disappearance of the starting material. The reaction was allowed to stir at this temperature for three hours. On completion, the reaction was quenched by the addition of water (1 x 25ml) and extracted with diethyl ether (3 x 25ml). The organic fractions were combined, dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 8:1). All homogenous fractions were collected and the solvent was evaporated *in vacuo*. Compounds **(3.13)** and **(3.14)** were obtained, both as yellow oil, with the following physical properties;

Compound (3.14)

IR (DCM, v); cm^{-1} 3401.5, 1679.3, 1598.8, 1282.8

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.49-2.54 (m, 2H, $C\underline{H}_2$), 2.94-2.97 (m, 2H, $C\underline{H}_2$), 6.16-6.21 (m, 1H, $CH_2C\underline{H}$=CH), 6.47 (d, J=11.52Hz, 1H, CH_2CH=$C\underline{H}$), 7.23 (d, J=7.64Hz, 1H, Ar-$\underline{H}$), 7.29 (t, J=7.34Hz, 1H, Ar-$\underline{H}$), 7.47 (dt, J_1=1.32Hz, J_2=7.50Hz, 1H, Ar-$\underline{H}$), 7.91 (dd, J_1=0.74Hz, J_2=7.62Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 23.9 ($\underline{C}H_2$), 41.4 ($\underline{C}H_2$), 127.0, 129.4, 131.2, 131.7, 132.4, 133.1 (4 x tert. C, $\underline{C}H=\underline{C}H$), 135.4 (quat. C), 136.2 (quat. C), 201.7 ($\underline{C}=O$)

6-bromo-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (3.15)

6,6-dibromo-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (3.16)

To a stirred solution of benzosuberone (4.00g, 24.96mmol) in acetic acid (35ml) was slowly added bromine (0.32ml, 24.96mmol) at room temperature. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 10:1) until complete consumption of the starting material was observed. After fifty minutes, the acetic acid was removed from the reaction mixture under vacuum by the aid of using toluene (1 x 20ml). The resulting residue was extracted between water (1 x 30ml) and diethyl ether (3 x 25ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:toluene, 1:2). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the desired compound **(3.15)** as a pale purple oil (5.53g, 93%) and a side product **(3.16)** as a yellow oil (0.32g, 4%) with the following physical properties;

Compound (3.15)

IR (DCM, v); cm^{-1} 2939.8, 1683.1, 1597.9, 1447.7, 1274.4, 1191.8

HRMS ($+Na^+$): actual 260.9885, calculated 260.9891, molecular formula $C_{11}H_{11}OBrNa$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.87-1.94 (m, 2H, $C\underline{H_2}$), 2.12-2.21 (m, 1H, 1H of $C\underline{H_2}$), 2.25-2.33 (m, 1H, 1H of $C\underline{H_2}$), 2.77-2.84 (m, 1H, 1H of $C\underline{H_2}$), 2.89-2.96 (m, 1H, 1H of $C\underline{H_2}$), 4.80 (dd, J_1=4.02Hz, J_2=8.02Hz, 1H, $C\underline{H}Br$), 7.12 (d, J=7.56Hz, 1H, Ar-$\underline{H}$), 7.21 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.33 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.52 (d, J=7.56Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 23.6 ($\underline{C}H_2$), 32.8 ($\underline{C}H_2$), 33.2 ($\underline{C}H_2$), 54.2 ($\underline{C}HBr$), 126.2 (tert. C), 129.0 (tert. C), 129.4 (tert. C), 131.8 (tert. C), 137.1 (quat. C), 139.3 (quat. C), 199.2 ($\underline{C}=O$)

Compound (3.16)

IR (DCM, v); cm^{-1} 2944.7, 1703.8, 1597.5, 1446.9, 1240.4, 951.6

HRMS ($+H^+$): actual 317.1810, calculated 316.9171, molecular formula $C_{11}H_{11}OBr_2$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.01-2.06 (m, 2H, C$\underline{H_2}$), 2.77-2.83 (m, 4H, 2 x C$\underline{H_2}$), 7.19 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.34 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.48 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.52 (d, J=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.0 ($\underline{C}H_2$), 30.7 ($\underline{C}H_2$), 44.6 ($\underline{C}H_2$), 69.1 ($\underline{C}Br_2$), 126.6 (tert. C), 128.4 (tert. C), 128.8 (tert. C), 132.0 (tert. C), 135.5 (quat. C), 136.4 (quat. C), 196.2 ($\underline{C}$=O)

Synthesis of (Z)-8,9-dihydrobenzo[7]annulen-5-one (3.17)

Method A:

To a stirred solution of compound **(3.15)** (0.50g, 2.09mmol) in DMF (10ml) was added caesium carbonate (0.82g, 2.51mmol) at room temperature. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 10:1) to observe the disappearance of the starting material. The reaction was allowed to stir for sixteen hours. On completion, the reaction mixture was extracted between water (1 x 30ml) and diethyl ether (3 x 25ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 20:1). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the compound **(3.18)** (0.14g, 18%) as a dark yellow oil with the following physical properties;

(Z)-6-(8-bromo-5,6,7,8-tetrahydrobenzo[7]annulen-9-ylidene)-6,7,8,9-tetrahydro benzo[7]annulen-5-one (3.18)

HRMS ($+K^+$): actual 419.0423, calculated 419.0413, molecular formula $C_{22}H_{21}OBrK$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.87-1.93 (m, 1H, 1H of C$\underline{H_2}$), 2.05-2.29 (m, 5H, 5H of 3 x C$\underline{H_2}$), 2.36-2.39 (m, 2H, C$\underline{H_2}$), 2.62-2.67 (m, 1H, 1H of C$\underline{H_2}$), 2.80-2.85 (m, 2H, C$\underline{H_2}$), 3.24-3.31 (m, 1H, 1H of C$\underline{H_2}$), 4.53 (dd, J_1=4.68Hz, J_2=9.40Hz, 1H, C$\underline{H}$Br),

7.12 (d, J=7.60Hz, 1H, Ar-H), 7.23-7.27 (m, 4H, 4 x Ar-H), 7.31-7.33 (m, 1H, Ar-H), 7.40 (dt, J_1=1.76Hz, J_2=7.30Hz, 1H, Ar-H), 7.52 (dd, J_1=1.18Hz, J_2=8.18Hz, 1H, Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 22.5 (CH_2), 29.3 (CH_2), 31.2 (CH_2), 33.3 (CH_2), 33.5 (CH_2), 33.6 (CH_2) 82.1 (CHBr), 106.7 (quat. C), 2 x 125.9 (2 x tert. C), 126.1 (tert. C), 128.2 (tert. C), 128.5 (tert. C), 129.1 (tert. C), 129.2 (tert. C), 131.5 (tert. C), 133.5 (quat. C), 137.2 (quat. C), 140.8 (quat. C), 141.6 (quat. C), 148.5 (quat. C), 202.0 (C=O)

Method B:

To a round bottomed flask containing compound **(3.15)** (1.47g, 6.15mmol) was added 2,4,6-trimethylpyridine (10ml) at room temperature. The reaction mixture was refluxed at 170°C for six hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 10:1) until the complete consumption of the starting material was observed. The reaction was quenched by the addition of 6N aq. HCl (1 x 30ml) and extracted with diethyl ether (3 x 25ml). The organic fractions were combined and washed with water (1 x 30ml). The resulting ethereal solution was dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 20:1). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the desired compound **(3.17)** (0.45g, 46%) as a yellow oil, and a trace of compound **(3.19)** as a dark orange oil with the following physical properties;

Compound (3.17)

IR (DCM, v); cm^{-1} 3396.1, 1644.8, 1449.4, 1295.2, 1030.9

HRMS ($+H^+$): actual 159.0807, calculated 159.0810, molecular formula $C_{11}H_{11}O$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.59-2.63 (m, 2H, CH_2), 3.08 (t, J=6.02Hz, 2H, CH_2), 6.30 (d, J=12.04Hz, 1H, CH=CH), 6.74-6.78 (m, 1H, CH=CH), 7.21 (t, J=7.52Hz, 1H, Ar-H), 7.32 (t, J=7.52Hz, 1H, Ar-H), 7.43 (t, J=7.52Hz, 1H, Ar-H), 7.56 (d, J=7.52Hz, 1H, Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 29.3 (<u>C</u>H_2), 34.0 (<u>C</u>H_2), 126.3, 128.5, 129.2, 131.8, 132.0 (4 x tert. C, <u>C</u>H=CH), 139.3 (quat. C), 139.5 (quat. C), 146.7 (CH=<u>C</u>H), 194.6 (<u>C</u>=O)

(6Z,8Z)-5H-benzo[7]annulen-5-one (3.19)

IR (DCM, v); cm^{-1} 3449.3, 1700.0, 1587.0, 1335.9

HRMS ($+H^+$): actual 157.0646, calculated 157.0653, molecular formula $C_{11}H_9O$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 6.73 (dd, J_1=8.04Hz, J_2=11.52Hz, 2H, CH=CH-CH=C<u>H</u>), 7.00 (d, J=12.04Hz, 1H, CH=CH-C<u>H</u>=CH), 7.10 (dd, J_1=8.00Hz, J_2=12.04Hz, 1H, CH=C<u>H</u>-CH=CH), 7.36 (d, J=11.56Hz, 1H, C<u>H</u>=CH-CH=CH), 7.67-7.82 (m, 3H, 3 x Ar-<u>H</u>), 8.53 (d, J=8.02Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 126.3, 130.1, 130.5, 131.9, 133.5, 135.1, 135.5, 135.9, (4 x tert. C, 1 x quat. C and <u>C</u>H=<u>C</u>H-<u>C</u>H=CH), 138.5 (quat. C), 139.1 (CH=CH-CH=<u>C</u>H), 188.0 (<u>C</u>=O)

<u>Method C:</u>

To a stirred solution of 2-iodobenzoic acid (1.00g, 4.03mmol) in distilled water (13ml) was added oxone (3.22g, 5.24mmol). The reaction mixture was mechanically stirred and heated to 70-75°C over twenty minutes. The texture of the reaction mixture went from a viscous slurry to a liquid suspension as temperature rose. After three hours, the reaction was allowed to cool to 5°C and stirred at this temperature for further one and half hours. The resulting mixture was filtered through a porosity sintered-glass funnel under reduced pressure, and the solid was then repeatedly washed with water (5 x 30ml) and acetone (2 x 30ml). The desired compound **(IBX)** as a white crystalline solid (0.87g, 77%), was left to dry at room temperature for one day for further use. The melting point, IR and NMR were in agreement with the literature data. To a stirred solution of benzosuberone (1.00g, 6.25mmol) in a mixture of toluene:DMSO (*v:v*, 2:1; 10ml) was added IBX (2.28g, 8.13mmol). The reaction mixture was mechanically stirred and heated to 70-75°C. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 10:1). However, it was difficult to drive the reaction to completion. After two

days, the reaction mixture was diluted with diethyl ether (1 x 30ml) and extracted with 5% aq. $NaHCO_3$ (1 x 25ml), water (1 x 25ml), and brine (1 x 25ml). The organic fraction was dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 20:1). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the desired title compound **(3.17)** as a yellow oil (0.48g, 49%).

Data previously described

Attempted the coupling of amine (3.11) and enone (3.17)

Method A:

To a stirred solution of compound **(3.17)** (0.05g, 0.32mmol) in DMF (10ml) was added compound **(3.11)** (0.10g, 0.63mmol) followed by caesium carbonate (0.20g, 0.63mmol) at room temperature. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1). The starting material **(3.17)** was rapidly disappeared as soon the caesium carbonate was added. After ten minutes the other starting material **(3.11)** was recovered unchanged.

Method B:

To a stirred solution of compound **(3.17)** (0.05g, 0.32mmol) in DMF (7ml) was added compound **(3.11)** (0.10g, 0.63mmol) followed by triethylamine (0.06g, 0.63mmol) at room temperature. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1). After fifteen hours, no progress of the reaction was observed. An additional triethylamine (0.09ml) was added. Due to the very slow progress of the reaction, another 0.09ml of triethylamine was added after three days. The reaction was allowed to stirred at room temperature for a further ten days until the complete consumption of compound **(3.17)** observed by TLC. The reaction mixture was extracted between water (1 x 30ml) and diethyl ether (3 x 25ml). The organic fractions were combined, dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The two major products were isolated by means of preparative thin layer chromatography on silical gel G_F-254

pre-coated aluminium sheets (eluent: hexane:ethyl acetate, 15:1). The reactions did not generate the desired product as confirmed by NMR analysis.

8,9-epoxy-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-one (3.20)

To a stirred solution of compound **(2.0)** (1.31g, 5.40mmol) in DMF (65ml) was added caesium carbonate (4.43g, 13.60mmol) at room temperature. The reaction mixture was then heated to 30°C. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 8:1). After two days, the reaction was quenched by the addition of water (1 x 200ml) and extracted with diethyl ether (3 x 25ml). The resulting ethereal fractions were combined, dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 20:1). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the compound **(3.14)** as a pale yellow oil (0.35g, 40%). To a stirred solution of compound **(3.14)** (0.20g, 1.27mmol) in DCM (1.3ml) was added a 50% solution of MCPBA (0.22g, 1.27mmol) dissolved in DCM (0.5ml). The resulting mixture was stirred at room temperature over night. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 8:1). On completion, the suspension was quenched by the addition of sat. aq. $NaHCO_3$ (1 x 30ml) and extracted with DCM (3 x 25ml). The combined organic fractions were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 100:15). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the compound **(3.20)** as a white solid (0.17g, 77%) with the following physical properties;

Mp: 113-118°C

IR (KBr, v); cm^{-1} 3376.8, 1665.9, 1282.7

HRMS ($+Na^+$): actual 197.0582, calculated 197.0578, molecular formula $C_{11}H_{10}O_2Na$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.10-2.18 (m, 1H, 1H of C$\underline{H}_2$), 2.26-2.34 (m, 1H, 1H of C$\underline{H}_2$), 2.51 (td, J_1=4.26Hz, J_2=17.56Hz, 1H, 1H of C$\underline{H}_2$), 2.89 (ddd, J_1=4.52Hz, J_2=13.04Hz, J_3=17.56Hz, 1H, 1H of C$\underline{H}_2$), 3.63-3.66 (m, 1H, C$\underline{H}$OCH), 3.92 (d,

J=4.52Hz, 1H, CHOC<u>H</u>), 7.36 (dt, J_1=1.52Hz, J_2=7.28Hz, 1H, Ar-<u>H</u>), 7.42-7.48 (m, 2H, 2 x Ar-<u>H</u>), 7.52 (d, J=8.04Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.4 (<u>C</u>H_2), 37.4 (<u>C</u>H_2), 56.3 (<u>C</u>HOCH), 57.5 (CHO<u>C</u>H), 128.3 (tert. C), 129.0 (tert. C), 129.7 (tert. C), 131.1 (tert. C), 134.2 (quat. C), 137.2 (quat. C), 204.3 (<u>C</u>=O)

6,7,8,9-tetrahydro-8-hydroxybenzo[7]annulen-5-one (3.21)

6,7,8,9-tetrahydro-9-hydroxybenzo[7]annulen-5-one (3.22)

To a stirred solution of compound **(3.20)** (0.55g, 3.16mmol) in ethyl acetate (16ml) was added methanol (0.13ml, 3.16mmol) followed by 10% Pd/C (0.06g) under an atmosphere of hydrogen gas. The resulting mixture was stirred at room temperature for four hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 2:1). On completion, the reaction mixture was filtered and the solvent was removed *in vacuo* to afford a yellow oil. The resulting residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 2:1). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the compound **(3.21)** as a yellow oil (0.42g, 75%), and a mixture of the compounds **(3.22** and **3.23)** with identical R_f values (0.05g, 8.9%) as a yellow oil with the following physical properties;

Compound (3.21)

IR (DCM, v); cm^{-1} 3409.7, 2943.1, 1671.7, 1599.0, 1450.8, 1266.6, 1051.9

HRMS (+Na^+): actual 199.0739, calculated 199.0735, molecular formula $C_{11}H_{12}O_2Na$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.66-1.73 (m, 1H, 1H of C<u>H</u>$_2$), 2.01-2.09 (m, 1H, 1H of C<u>H</u>$_2$), 2.55 (ddd, J_1=2.0Hz, J_2=9.28Hz, J_3=18.56Hz, 1H, 1H of C<u>H</u>$_2$), 2.82 (ddd, J_1=2.0Hz, J_2=9.78Hz, J_3=18.04Hz, 1H, 1H of C<u>H</u>$_2$), 2.95 (dd, J_1=6.78Hz, J_2=14.3Hz, 1H, 1H of C<u>H</u>$_2$), 3.03 (dd, J_1=5.02Hz, J_2=14.06Hz, 1H, 1H of C<u>H</u>$_2$), 4.22-4.28 (m, 1H, C<u>H</u>OH), 7.18 (d, J=7.52Hz, 1H, Ar-<u>H</u>), 7.31 (dt, J_1=1.0Hz, J_2=7.54Hz, 1H, Ar-<u>H</u>), 7.41 (dt, J_1=1.33Hz, J_2=7.26Hz, 1H, Ar-<u>H</u>), 7.69 (dd, J_1=1.0Hz, J_2=7.52Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 29.3 ($\underline{C}H_2$), 37.2 ($\underline{C}H_2$), 40.4 ($\underline{C}H_2$), 67.7 ($\underline{C}HOH$), 126.9 (tert. C), 128.3 (tert. C), 130.2 (tert. C), 132.1 (tert. C), 136.3 (quat. C), 137.7 (quat. C), 205.6 ($\underline{C}$=O)

Mixture of compounds (3.22) and (3.23)

IR (DCM, v); cm^{-1} 3391.2, 2949.0, 1711.2, 1675.8, 1321.0, 1284.0, 1017.1

(3.22) HRMS ($+Na^+$): actual 199.0729, calculated 199.0735, molecular formula $C_{11}H_{12}O_2Na$

(3.23) HRMS ($+H^+$): actual 175.0438, calculated 175.0754, molecular formula $C_{11}H_{11}O_2$

6,7-dihydro-9H-benzo[7]annulene-5,8-dione (3.23)

To a stirred solution of compound **(3.20)** (2.08g, 11.95mmol) in ethyl acetate (60ml) was added 10% Pd/C (0.21g) under an atmosphere of hydrogen gas. The resulting mixture was stirred at room temperature for five hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 2:1). On completion, the reaction mixture was filtered and the solvent was removed *in vacuo* to afford a yellow oil. The resulting residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 2:1). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the compound **(3.21)** as a yellow oil (2.06g, 98%) and the compound **(3.23)** as a dark orange oil (0.02g, 1.0%) with the following physical properties;

Compound (3.21)

Data previously described

Compound (3.23)

IR (DCM, v); cm^{-1} 3414.7, 2922.4, 1716.3, 1675.5, 1321.0, 1284.0, 1017.1

HRMS ($+H^+$): actual 175.1396, calculated 175.0754, molecular formula $C_{11}H_{11}O_2$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.61-2.64 (m, 2H, $C\underline{H}_2$), 3.03-3.06 (m, 2H, $C\underline{H}_2$), 3.98 (s, 2H, $ArC\underline{H}_2CO$), 7.17 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.35 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.45 (dt, J_1=1.19Hz, J_2=7.40Hz, 1H, Ar-$\underline{H}$), 7.93 (d, J=7.76Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 35.3 ($\underline{C}H_2$), 39.0 ($\underline{C}H_2$), 50.3 ($\underline{C}H_2$), 128.0 (tert. C), 130.0 (tert. C), 130.8 (tert. C), 133.7 (tert. C), 2 x 135.8 (2 x quat. C), 200.4 ($\underline{C}$=O), 207.0 ($\underline{C}$=O)

6,7,8,9-tetrahydro-5-oxo-5H-benzo[7]annulen-8-yl methanesulfonate (3.24)

8-azido-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (3.25)

1,1a,7,7a-tetrahydro-cyclopropa[b]naphthalen-2-one (3.26)

To a stirred solution of the alcohol **(3.21)** (1.18g, 6.70mmol) in dry DCM (34ml) was added methanesulphonyl chloride (1.30g, 11.39mmol) at 0°C under anhydrous reaction conditions. To the resultant solution was added *N,N*-diisopropylethylamine (1.30g, 10.05mmol), drop-wise by syringe, over twenty minutes. The reaction mixture was maintained at this temperature under an atmosphere of nitrogen gas for four hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 2:1) to observe the disappearance of the starting material. On completion, the reaction mixture was quenched with 5% aq. $NaHCO_3$ (1 x 20ml) and extracted with DCM (3 x 20ml). The combined organic fractions were further extracted with water (3 x 20ml). The resulting organic fraction was dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 3:1). All homogenous fractions were collected and the solvent was evaporated to afford the product **(3.24)** as a white solid (1.70g, 92%). The mesylate **(3.24)** (1.56g, 6.13mmol) was dissolved in DMF (80ml) and sodium azide (3.98g, 61.3mmol) was added to the stirred solution at room temperature. The reaction mixture was heated to 60°C. After fifteen hours the reaction was quenched by the addition of water (1 x 200ml) and the product was extracted with diethyl ether (3 x 30ml). The combined ethereal fractions were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 10:1). All homogenous fractions were collected and the solvent was evaporated to afford a mixture of the keto azide **(3.25)** and **(3.26)** as a yellow oil (0.87g, 71%).

Mixture of compounds (3.25) and (3.26)

IR (DCM, v); cm^{-1} 3414.7, 2922.4, 1716.3, 1675.5, 1321.0, 1284.0, 1017.1

(3.25) HRMS ($+H^+$): actual 202.2404, calculated 202.5340, molecular formula $C_{11}H_{11}ON_3$

(3.26) HRMS ($+H^+$): actual 159.5181, calculated 159.7684, molecular formula $C_{11}H_{10}O$

***Tert*-butyl 6,7,8,9-tetrahydro-5-oxo-5H-benzo[7]annulen-8-ylcarbamate (3.27)**

To a stirred solution of the compounds mixture **(3.25)** and **(3.26)** (0.87g, 4.33mmol) in a 1:1 mixture of ethanol and ethyl acetate (48ml) was added di-*tert*-butyl dicarbonate (1.42g, 6.49mmol) and 10% Pd/C (catalytic amount). The mixture was stirred at room temperature under an atmosphere of hydrogen gas. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 8:1). The reaction mixture was allowed to stir over night. On completion, 10% Pd/C was removed from the reaction mixture by filtration using DCM (100ml). The solvent was removed from the flask under vacuum. The resulting residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 12:1). All homogenous fractions were collected and the solvent was evaporated to afford the desired product **(3.27)** as a yellow oil (0.47g, 84%) and compound **(3.26)** from the mixture of starting material as a yellow oil (0.46g), with the following physical properties;

Compound (3.26)

IR (DCM, v); cm^{-1} 3421.4, 3024.8, 2908.1, 1669.0, 1605.8, 1351.1, 1288.9

HRMS ($+Na^+$): actual 181.0607, calculated 181.0588, molecular formula $C_{11}H_{10}ONa$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 0.60-0.63 (m, 1H, 1H of C$\underline{H}_2$), 1.11-1.16 (m, 1H, 1H of C$\underline{H}_2$), 1.73-1.80 (m, 1H, C$\underline{H}$), 1.93-1.98 (m, 1H, C$\underline{H}$), 3.00-3.15 (broad m, 2H, C$\underline{H}_2$), 6.99 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.12 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.27 (t, J=7.28Hz, 1H, Ar-$\underline{H}$), 7.69 (d, J=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 12.3 ($\underline{C}H_2$), 13.4 ($\underline{C}H$), 24.8 ($\underline{C}H$), 27.3 ($\underline{C}H_2$), 126.2 (tert. C), 126.4 (tert. C), 128.4 (tert. C), 130.5 (quat. C), 132.5 (tert. C), 138.0 (quat. C), 197.5 ($\underline{C}$=O)

Compound (3.27)

IR (DCM, v); cm^{-1} 3341.3, 2975.0, 2932.4, 1713.1, 1678.0, 1519.9, 1250.5, 1168.4

HRMS ($+H^+$): actual 276.1811, calculated 276.1594, molecular formula $C_{16}H_{22}O_3N$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.48 (s, 9H, 3 x $C\underline{H}_3$), 1.52-1.55 (m, 1H, 1H of $C\underline{H}_2$), 2.17-2.24 (m, 1H, 1H of $C\underline{H}_2$), 2.63 (ddd, J_1=1.76Hz, J_2=10.8Hz, J_3=18.56Hz, 1H, 1H of $C\underline{H}_2$), 2.77 (ddd, J_1=2.01Hz, J_2=8.01Hz, J_3=18.58Hz, 1H, 1H of $C\underline{H}_2$), 2.93 (dd, J_1=5.04Hz, J_2=14.04Hz, 1H, 1H of $C\underline{H}_2$), 3.19 (dd, J_1=5.04Hz, J_2=14.04Hz, 1H, 1H of $C\underline{H}_2$), 4.17 (broad m, 1H, C$\underline{H}$NH), 4.51 (broad s, 1H, N$\underline{H}$), 7.21 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.38 (dt, J_1=1.0Hz, J_2=7.52Hz, 1H, Ar-$\underline{H}$), 7.49 (dt, J_1=1.32Hz, J_2=7.52Hz, 1H, Ar-$\underline{H}$), 7.74 (dd, J_1=1.0Hz, J_2=7.56Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 26.9 ($\underline{C}H_2$), 28.0 (3 x $\underline{C}H_3$), 37.7 ($\underline{C}H_2$), 38.2 (CH_2), 47.7 ($\underline{C}$HNH), 79.2 {$\underline{C}(CH_3)_3$}, 127.1 (tert. C), 128.4 (tert. C), 130.1 (tert. C), 132.2 (tert. C), 135.8 (quat. C), 138.0 (quat. C), 154.6 (NH$\underline{C}$=O), 204.8 ($\underline{C}$=O)

8-amino-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (3.28)

To a stirred solution of compound **(3.27)** (0.68g, 2.47mmol) in dry DCM (2ml) was added trifluoroacetic acid (2ml) drop-wise and at 0°C. The reaction was left stirring for ten minutes after which time the flask was removed from the ice and was allowed to increase to room temperature. The reaction proceeded for an additional hour. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to observe the disappearance of the starting material. On completion, trifluoroacetic acid was removed from the mixture under vacuum. The residue was dissolved in diethyl ether (10ml) and the resulting mixture was partitioned between water (1 x 30ml) and diethyl ether (3 x 25ml). The organic fractions were discarded. The acidic solution was basified with 2.5M aq. NaOH (30ml) and extracted again using diethyl ether (3 x 25ml). The combined ethereal fractions were dried over magnesium sulphate, filtered and concentrated *in*

vacuo to afford the desired product **(3.28)** as a yellow oil (0.35g, 81%) with the following physical properties;

IR (DCM, v); cm^{-1} 3244.9, 3094.0, 2944.7, 1674.2, 1452.5, 1135.6.9

HRMS ($+H^+$): actual 176.1070, calculated 176.1075, molecular formula $C_{11}H_{14}ON$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.74-1.85 (broad m, 2H, C$\underline{H_2}$), 2.33-2.42 (broad m, 2H, C$\underline{H_2}$), 2.91 (broad s, 2H, N$\underline{H_2}$), 3.59-3.68 (broad m, 3H, C$\underline{H}NH_2$ and C$\underline{H_2}$), 7.13 (d, J=7.04Hz, 1H, Ar-$\underline{H}$), 7.26 (t, J=7.54Hz, 1H, Ar-$\underline{H}$), 7.33 (t, J=7.28Hz, 1H, Ar-$\underline{H}$), 7.74 (d, J=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 28.8 ($\underline{C}H_2$), 39.1 ($\underline{C}H_2$), 39.4 (CH_2), 49.5 ($\underline{C}HNH_2$), 125.9 (tert. C), 126.4 (tert. C), 129.5 (tert. C), 130.1 (tert. C), 134.5 (quat. C), 139.9 (quat. C), 210.5 ($\underline{C}$=O)

8-(cyclohex-2-enylamino)-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (3.29)

To a stirred solution of compound **(3.28)** (0.32g, 1.83mmol) in DMF (15ml), was added 3-bromocyclohexene (0.59g, 3.66mmol) and caesium carbonate (1.79g, 5.49mmol) at room temperature. The progress of the reaction was monitored by TLC (ethyl acetate). After three days, the reaction mixture was partitioned between water (1 x 200ml) and DCM (3 x 20ml each). The organic layers were combined and the solvent removed *in vacuo*. The resulting oil was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 2:1). All homogenous fractions were collected and the solvent was evaporated to afford a stereoisomeric mixture of the title amine **(3.29)** as a yellow oil (0.31g, 67%) with the following physical properties;

IR (DCM, v); cm^{-1} 3327.6, 2929.7, 1675.2, 1599.4, 1449.2, 1108.4

HRMS ($+H^+$): actual 256.1696, calculated 256.1701, molecular formula $C_{17}H_{22}NO$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.23-1.26 (broad s, 1H, N$\underline{H}$), 1.38-1.45 (m, 1H, 1H of C$\underline{H_2}$), 1.50-1.57 (m, 2H, C$\underline{H_2}$), 1.72-1.73 (m, 1H, 1H of C$\underline{H_2}$), 1.86-1.88 (m, 1H, 1H of C$\underline{H_2}$), 1.99 (broad m, 3H, 3H of 2 x C$\underline{H_2}$), 2.60 (ddd, J_1=2.27Hz, J_2=9.53Hz, J_3=17.81Hz, 1H, 1H of C$\underline{H_2}$), 2.81-2.99 (m, 3H, 3H of 2 x C$\underline{H_2}$), 3.32 (broad m, 2H, HNC$\underline{H}$C=C and C$\underline{H}$NH), 5.64-5.67 (m, 1H, CH=C$\underline{H}$), 5.75-5.78 (m, 1H, C$\underline{H}$=CH), 7.19

(d, J=7.52Hz, 1H, Ar-H), 7.31 (t, J=7.54Hz, 1H, Ar-H), 7.43 (t, J=7.28Hz, 1H, Ar-H), 7.72 (d, J=7.52Hz, 1H, Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 19.6 and 19.9 (CH_2) 2 x 24.8 (CH_2), 27.3 and 27.8 (CH_2), 29.4 and 29.6 (CH_2), 2 x 37.4 (CH_2), 2 x 38.1 (CH_2), 49.2 and 49.3 (CH), 2 x 51.2 (CH), 2 x 126.6 (tert. C), 2 x 128.1 (tert. C), 128.5, 128.7, 129.3, 129.4 (CH=CH), 2 x 130.1 (tert. C), 2 x 131.8 (tert. C), 137.3 and 137.5 (quat. C), 137.9 and 140.0 (quat. C)

8-[*N*-benzyl-*N*-(cyclohex-2-enyl)amino]-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (3.30)

8-(dibenzylamino)-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (3.31)

To a stirred solution of compound **(3.29)** (0.23g, 0.90mmol) in DMF (10ml) was added benzyl bromide (0.31g, 1.80mmol) and caesium carbonate (0.59g, 1.80mmol) at room temperature. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1). After three weeks, the reaction mixture was partitioned between 2M aq. HCl (1 x 30ml) and diethyl ether (3 x 20ml each). The ethereal fractions were discarded. The acidic solution was then basified by 2.5M aq. NaOH and extracted with diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 25:1). All homogenous fractions were collected and the solvent was evaporated to yield a stereoisomeric mixture of the title compound **(3.30)** as a yellow oil (0.22g, 71%) and compound **(3.31)** as a yellow oil (0.09g, 28%) with the following physical properties;

Compound (3.30)

IR (DCM, v); cm^{-1} 3386.5, 2928.5, 1745.6, 1676.1, 1454.9, 1263.6

HRMS ($+H^+$): actual 346.2161, calculated 346.2171, molecular formula $C_{24}H_{27}NO$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm) 1.43-1.54 (m, 2H, CH_2), 1.59-1.66 (m, 1H, 1H of CH_2), 1.75-1.85 (m, 1H, 1H of CH_2), 1.90-2.04 (m, 4H, 2 x CH_2), 2.58-2.67 (m, 1H, 1H of CH_2), 3.86 (ddd, J_1=4.09Hz, J_2=9.53Hz, J_3=16.35Hz, 1H, 1H of CH_2), 3.09-3.20 (m,

2H, C$\underline{H}_2$), 3.22-3.32 and 3.39-3.51 (2 x m, 2H, 2 x NC$\underline{H}$), 3.76-3.86 (m, 2H, NC$\underline{H}_2$), 5.60-5.84 (m, 2H, C$\underline{H}$=C$\underline{H}$), 7.20-7.27 (m, 2H, 2 x Ar-$\underline{H}$), 7.28 (m, 1H, Ar-$\underline{H}$), 7.31-7.32 (m, 2H, 2 x Ar-$\underline{H}$), 7.36-7.39 (m, 2H, 2 x Ar-$\underline{H}$), 7.43 (dt, J_1=1.05Hz, J_2=14.72Hz, 1H, Ar-$\underline{H}$), 7.77 (2 x d, J=7.66Hz, signals overlapping, 1H, COAr-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 2 x 22.0 ($\underline{C}H_2$), 2 x 25.1 ($\underline{C}H_2$), 26.2 and 26.7 ($\underline{C}H_2$), 26.9 and 27.9 ($\underline{C}H_2$), 39.1 and 39.3 ($\underline{C}H_2$), 39.8 and 39.9 ($\underline{C}H_2$), 50.5 and 50.9 ($\underline{C}H_2$), 55.3 and 55.4 ($\underline{C}H$), 56.2 and 56.4 ($\underline{C}H$), 2 x 126.6 (tert. C), 2 x 126.7 (tert. C), 2 x 127.7 (tert. C), 4 x 128.2 (2 x tert. C), 128.4 and 128.5 (tert. C), 2 x 128.7 (tert. C), 130.2 and 130.3 ($\underline{C}H$=$\underline{C}H$), 2 x 130.7 (tert. C), 131.1 and 131.7 ($\underline{C}H$=$\underline{C}H$), 132.4 and 132.5 (tert. C), 137.9 and 138.1 (quat. C), 140.2 and 140.4 (quat. C), 141.8 and 142.0 (quat. C), 2 x 204.9 ($\underline{C}$=O)

Compound (3.31)

HRMS (+H^+): actual 356.2006, calculated 356.1949, molecular formula $C_{25}H_{26}NO$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm) 2.05-2.09 (m, 2H, C$\underline{H}_2$), 2.06-2.67 (m, 1H, 1H of C$\underline{H}_2$), 2.86-2.93 (m, 1H, 1H of C$\underline{H}_2$), 3.08 and 3.12 (2 x d, J=4.66Hz, 1H, 1H of ArC$\underline{H}_2$CH), 3.17-3.24 (m, 1H, NC$\underline{H}$), 3.28 and 3.32 (2 x d, J=6.30Hz, 1H, 1H of ArC$\underline{H}_2$CH), 3.65-3.73 (m, 2H, NC$\underline{H}_2$), 7.22-7.25 (m, 2H, 2 x Ar-$\underline{H}$), 7.31 (6 x dt, J_1=1.74Hz, J_2=7.10Hz, signals overlapping, 6H, 6 x Ar-$\underline{H}$), 7.38 (4 x d, J=7.10Hz, signals overlapping, 4H, 4 x Ar-$\underline{H}$), 7.43 (broad dt, J_1~1.61Hz, J_2~7.77Hz, 1H, Ar-$\underline{H}$), 7.78 (d, J=7.92Hz, 1H, COAr-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 23.3 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 34.8 ($\underline{C}H_2$), 2 x 54.0 (2 x $\underline{C}H_2$), 55.8 ($\underline{C}H$), 126.3 (tert. C), 2 x 126.5 (2 x tert. C), 4 x 127.8 (4 x tert. C), 4 x 128.0 (4 x tert. C), 128.5 (tert. C), 130.5 (tert. C), 132.1 (tert. C), 137.6 (quat. C), 2 x 139.5 (2 x quat. C), 139.6 (quat. C), 204.2 ($\underline{C}$=O)

(1,2-dihydronaphthalen-4-yloxy)trimethylsilane (4.0)

To a stirred solution of α–tetralone (2.00g, 13.70mmol) in dry DCM (10ml) at 0°C was added triethylamine (1.52g, 15.07mmol) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) (3.04g, 13.70mmol). The solution was allowed to stir at 0°C for two hours.

The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 5:1). The solvent was removed *in vacuo*. The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford the silyl enol ether **(4.0)** as a colourless oil (2.79g, 93%), with the following physical properties. The title compound **(4.0)** was stored in a dry flask under nitrogen at 0°C until further use.

IR (DCM, v); cm^{-1} 2961.2, 2835.8, 1403.1, 1339.4, 1253.6, 1189.8, 1094.7

HRMS ($+H^+$): actual 219.1193, calculated 219.1200, molecular formula $C_{13}H_{19}OSi$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 0.32 (s, 9H, 3 x $C\underline{H}_3$), 2.35-2.40 (m, 2H, $ArCH_2C\underline{H}_2$), 2.82 (t, J=7.96Hz, 2H of $ArC\underline{H}_2CH_2$), 5.25 (t, J=4.60Hz, 1H, C=C$\underline{H}$), 7.15-7.29 (m, 3H, 3 x Ar-$\underline{H}$), 7.48 (d, J=7.40Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 3 x -0.18 (3 x $\underline{C}H_3$), 21.8 ($\underline{C}H_2$), 27.8 ($\underline{C}H_2$), 104.9 (C=$\underline{C}$H), 121.4 (tert. C), 125.8 (tert. C), 126.5 (tert. C), 126.9 (tert. C), 133.1 (quat. C), 136.6 (quat. C), 147.7 (O$\underline{C}$=C)

The spectral data was consistent with that reported in the literature data.

1',3'-dihydro-spiro{[1,3]dioxolane-2,2'-indene} (4.1)

To a solution of 2-indanone (1.00g, 7.58mmol) in ethylene glycol (5ml) was added triethylorthoformate (5ml) and *p*-ToSH (0.35g, 1.58mmol). The reaction was stirred at room temperature overnight, after which time the reaction was quenched by the addition of water (100ml) and the product extracted with diethyl ether (3 x 25ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo,* and the residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 6:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford the dioxane **(4.1)** as a pale yellow oil (0.95g, 71%) with the following physical properties;

IR (DCM, v); cm^{-1} 2955.1, 2885.4, 1403.2, 1331.9, 1292.3, 1226.8, 1104.2, 1030.7

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 3.32 (s, 4H, 2 x $C\underline{H}_2$), 4.05 (s, 4H, 2 x $OC\underline{H}_2$), 7.29-7.33 (m, 4H, 4 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 2 x 42.9 (2 x $\underline{C}H_2$), 2 x 64.1 (2 x $O\underline{C}H_2$), 117.1 ($O\underline{C}O$), 2 x 124.2 (2 x tert. C), 2 x 126.3 (2 x tert. C), 2 x 139.6 (2 x quat. C)

The spectral data was consistent with that reported in the literature data.

2-[2-(2-hydroxyethoxy)-2,3-dihydro-1H-inden-2-yl]-3,4-dihydronaphthalen-1 (2H)-one (4.2)

To a stirred solution of compound **(4.0)** (1.59g, 9.03mmol) in anhydrous DCM (20ml) in an evacuated three necked flask, was added compound **(4.1)** (1.97g, 9.03mmol) and tin(IV) tetrachloride (2.35g, 9.03mmol) at -78°C under anhydrous conditions and an atmosphere of nitrogen. After an hour, the reaction was quenched by the addition of iced water and the product was extracted using DCM (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The crude product was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 4:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford the title aldol product **(4.2)** as a golden brown oil (2.41g, 83%) with the following physical properties;

IR (DCM, v); cm^{-1} 3500.2, 3061.7, 2956.4, 1686.3, 1638.3, 1251.5, 1188.6, 1093.1

HRMS ($+Na^+$): actual 345.1459, calculated 345.1467, molecular formula $C_{21}H_{22}O_3Na$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.03 (s, 1H, O$\underline{H}$), 2.16-2.24 (m, 1H, 1H of $CH_2C\underline{H}_2CH$), 2.39-2.44 (m, 1H, 1H of $CH_2C\underline{H}_2CH$), 2.82-2.84 (m, 2H, $C\underline{H}_2$), 2.96-3.09 (m, 2H, $C\underline{H}_2$), 3.20-3.51 (m, 7H, 6H of 3 x $C\underline{H}_2$ and 1H of C$\underline{H}$), 7.16-7.29 (m, 6H, 6 x Ar-$\underline{H}$), 7.44 (t, J=7.28Hz, 1H, Ar-$\underline{H}$), 8.02 (d, J=7.56Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 24.9 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 42.3 ($\underline{C}H_2$), 42.6 ($\underline{C}H_2$), 54.8 ($\underline{C}H$), 61.5 ($OH\underline{C}H_2$), 63.7 ($O\underline{C}H_2CH_2OH$), 86.6 (quat. C), 123.6 (tert. C), 123.7 (tert. C), 2 x 126.1 (2 x tert. C), 126.2 (tert. C), 126.9 (tert. C), 128.2 (tert. C), 132.8 (tert. C), 133.3 (quat. C), 140.5 (quat. C), 141.7 (quat. C), 143.6 (quat. C), 198.1 ($\underline{C}$=O)

The spectral data was consistent with that reported in the literature data.

2-benzyl-3,4-dihydro-2-(1H-inden-2-yl)naphthalen-1(2H)-one (4.3)

To a stirred solution of the aldol **(4.2)** (1.00g, 3.10mmol) in *t*-butanol/diethyl ether (20ml, *v:v*, 1:1) was added benzyl bromide (2.66g, 15.53mmol). To this solution was added potassium *tert*-butoxide (0.35g, 3.10mmol) as a solution in *t*-butanol (10ml), adding the base drop-wise over two hours. The reaction was stirred at room temperature and the progress of the reaction was monitored by TLC (hexane:ethyl acetate, 5:1) to check for the disappearance of the starting material. After a total of four hours, the *t*-butanol was removed by the extraction between water (100ml) and diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo,* and the residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the title alkylated product **(4.3)** as yellow crystals (0.6g, 55%) with the following physical properties;

Mp: 125-127°C

IR (KBr, v); cm^{-1} 3026.2, 2926.6, 1678.0, 1599.5, 1453.4, 1288.9, 1231.3

HRMS ($+Na^+$): actual 373.1573, calculated 373.1568, molecular formula $C_{26}H_{22}ONa$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.30-2.38 (m, 1H, 1H of OCCC$\underline{H_2}CH_2$), 2.48-2.52 (m, 1H, 1H of OCCC$\underline{H_2}CH_2$), 2.89-2.94 (m, 1H, 1H of OCCC$H_2C\underline{H_2}$), 3.13-3.24 (m, 1H, 1H of OCCC$H_2C\underline{H_2}$), 3.34 and 3.68 (2 x d, J=13.56Hz, 2H, C$\underline{H_2}$), 3.42 and 3.75 (2 x d, J=22.82Hz, 2H, C$\underline{H_2}$), 6.70 (s, 1H, C$\underline{H}$=C), 7.22 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.31 (3 x dt, J_1=1.52Hz, J_2=7.04Hz, signals overlapping, 3H, 3 x Ar-$\underline{H}$), 7.34-7.45 (m, 6H, 6 x Ar-$\underline{H}$), 7.50-7.57 (m, 2H, 2 x Ar-$\underline{H}$), 8.40 (d, J=8.00Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.6 ($\underline{C}H_2$), 31.0 ($\underline{C}H_2$), 33.4 ($\underline{C}H_2$), 44.2 ($\underline{C}H_2$), 53.1 (CO$\underline{C}CH_2$), 120.5, 123.3, 124.3, 2 x 126.2, 126.3, 2 x 127.7, 127.8, 128.4, 129.7, 2 x 130.7 (12 x tert. C and C=$\underline{C}$H), 132.0 (quat. C), 133.0 (tert. C), 137.4 (quat. C), 142.8 (quat. C), 143.3 (quat. C), 144.0 (quat. C), 146.7 (quat. C), 198.1 ($\underline{C}$=O)

2-benzyl-1-[3-(dimethylamino)propyl]-1,2,3,4-tetrahydro-2-(1H-inden-2-yl)-naphthalen-1-ol (4.4)

To a dry three-necked round bottomed flask containing Grignard reagent (2.00g, 13.79mmol) in anhydrous THF (5ml), which was synthesized according to the method described previously, was added a solution of compound **(4.3)** (1.00g, 2.86mmol) in anhydrous THF (10ml). The resulting mixture was stirred at room temperature under an atmosphere of nitrogen and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. After a total of fifteen hours aq. NH_4Cl (50ml) was added and the reaction was extracted with diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford a stereoisomeric mixture of the title compound **(4.4)a** and **(4.4)b** as a colourless oil (1.15g, 93 %). Compounds **(4.4)a** and **(4.4)b** were isolated by means of preparative thin layer chromatography on silica gel (eluent: hexane:ethyl acetate:triethylamine, 10:10:1), both as a colourless oil with the following physical properties;

Compound (4.4)a

IR (DCM, v); cm^{-1} 3435.8, 2946.1, 1643.7, 1462.8, 1262.4, 1021.7

HRMS ($+H^+$): actual 438.2782, calculated 438.2797, molecular formula $C_{31}H_{36}NO$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.20-1.28 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 1.56-1.65 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 1.66-1.72 (m, 1H, 1H of $NCH_2CH_2C\underline{H}_2$), 1.87 (dd, J_1=8.02Hz, J_2=14.04Hz, 1H, 1H of $ArCH_2C\underline{H}_2C$), 2.00 (dd, J_1=8.54Hz, J_2=12.54Hz, 1H, 1H of $NCH_2CH_2C\underline{H}_2$), 2.10-2.30 (m, 3H, 1H of $NC\underline{H}_2CH_2CH_2$, 1H of $ArCH_2C\underline{H}_2C$ and 1H of $NC\underline{H}_2CH_2CH_2$), 2.39 (s, 6H, 2 x $C\underline{H}_3$), 2.65 (d, J=14.56Hz, 1H, 1H of $OHCCC\underline{H}_2Ar$), 2.97 (dd, J_1=8.52Hz, J_2=18.04Hz, 1H, 1H of $ArC\underline{H}_2CH_2$), 3.08 (dd, J_1=9.04Hz, J_2=18.56Hz, 1H, 1H of $ArC\underline{H}_2CH_2$), 3.50 (d, J=14.56Hz, 1H, 1H of $OHCCC\underline{H}_2Ar$), 3.85 (2 x d, J~12.56Hz, signals overlapping, 2H, $CH{=}CC\underline{H}_2$), 6.74 (s, 1H,

C<u>H</u>=C), 6.85-6.86 (m, 2H, 2 x Ar-<u>H</u>), 7.09-7.11 (m, 3H, 3 x Ar-<u>H</u>), 7.20-7.34 (m, 5H, 5 x Ar-<u>H</u>), 7.38 (d, J=7.00Hz, 1H, Ar-<u>H</u>), 7.52 (d, J=7.04Hz, 1H, Ar-<u>H</u>), 7.76 (d, J=7.52Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.3 ($NCH_2\underline{C}H_2CH_2$), 24.5 ($Ar\underline{C}H_2CH_2$), 25.2 ($ArCH_2\underline{C}H_2$), 38.9 ($OHCC\underline{C}H_2Ar$), 39.6 ($NCH_2CH_2\underline{C}H_2$), 41.3 ($CH{=}C\underline{C}H_2$), 2 x 45.1 (2 x $\underline{C}H_3$), 48.6 ($CH_2\underline{C}CH_2$), 60.5 ($N\underline{C}H_2CH_2CH_2$), 76.1 ($OH\underline{C}CH_2$), 119.9 (tert. C), 122.9 (tert. C), 123.4 (tert. C), 124.6 (tert. C), 125.2 (tert. C), 125.7 (tert. C), 125.8 (tert. C), 126.6 (tert. C), 2 x 127.1 (2 x tert. C), 128.1 (tert. C), 2 x 129.5 (2 x tert. C), 129.7 (C=<u>C</u>H), 134.1, 139.2, 143.4, 143.5, 144.4, 154.6 (5 x quat. C and <u>C</u>=CH)

Compound (4.4)b

IR (DCM, v); cm^{-1} 3061.9, 2946.1, 2824.2, 1642.5, 1463.3, 1381.8, 1266.2, 1022.5

HRMS ($+H^+$): actual 438.2792, calculated 438.2797, molecular formula $C_{31}H_{36}NO$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.41-1.45 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 1.55-1.62 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 2.13-2.26 (m, 4H, $NCH_2CH_2C\underline{H}_2$, and $C\underline{H}_2$), 2.43-2.47 (1x s and 1 x m, signals overlapping, 8H, 2 x $C\underline{H}_3$ and $C\underline{H}_2$), 2.93-2.97 (m, 2H, $ArC\underline{H}_2CH_2$), 3.08 (d, J=13.04Hz, 1H, 1H of $OHCCC\underline{H}_2Ar$), 3.43 (d, J=23.08Hz, 1H, 1H of $CH{=}CC\underline{H}_2$), 3.54 (d, J=13.04Hz, 1H, 1H of $OHCCC\underline{H}_2Ar$), 3.62 (d, J=23.08Hz, 1H, 1H of $CH{=}CC\underline{H}_2$), 6.22 (s, 1H, C<u>H</u>=C), 6.93-7.20 (4 x m, 11H, 11 x Ar-<u>H</u>), 7.36 (d, J=7.04Hz, 1H, Ar-<u>H</u>), 7.63 (d, J=7.52Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.9 ($\underline{C}H_2$), 24.4 ($\underline{C}H_2$), 27.4 ($\underline{C}H_2$), 36.6 ($\underline{C}H_2$), 40.9 ($\underline{C}H_2$), 41.1 ($\underline{C}H_2$), 2 x 45.4 (2 x $\underline{C}H_3$), 49.2 ($CH_2\underline{C}CH_2$), 60.6 ($\underline{C}H_2$), 76.7 ($OH\underline{C}CH_2$), 119.5, 122.6, 122.8, 124.0, 124.6, 125.1, 125.3, 125.4, 2 x 127.0, 127.8, 128.9 (11 x tert. C and C=<u>C</u>H), 2 x 130.3 (2 x tert. C), 135.2, 139.0, 144.1, 2 x 144.2, 152.2 (5 x quat. C and <u>C</u>=CH)

(3Z)-3-(3-benzyl-2,3-dihydro-3-(1H-inden-2-yl)naphthalen-4(1H)-ylidene)-*N,N*-dimethylpropan-1-amine (4.5)

To a round bottomed flask containing **(4.4)b** (2.00g, 0.41mmol) was added a 20% solution of oxalic acid in water (20ml). The resulting mixture was heated till gently

refluxing for two hours and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. The reaction mixture was then partitioned between water (25ml) and diethyl ether (3 x 20ml each). The aqueous layer was basified with 2.5M aq. NaOH and the product was extracted with diethyl ether (3x 30ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford a stereoisomeric mixture of the title compound **(4.5)a** and **(4.5)b** as a dark brown oil (0.12g, 72 %). Compound **(4.5)b**, was isolated by means of preparative thin layer chromatography on silica gel (eluent: hexane:ethyl acetate:triethylamine, 10:10:1) as a pink oil with the following physical properties;

Compound (4.5)b

IR (DCM, v); cm^{-1} 3400.2, 3058.0, 2690.8, 1708.3, 1655.9, 1595.0, 1455.5

HRMS ($+H^+$): actual 420.2697, calculated 420.2691, molecular formula $C_{31}H_{34}N$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.30-1.34 (m, 1H, 1H of C$\underline{H}_2$), 1.97-2.04 (m, 1H, 1H of C$\underline{H}_2$), 2.23-2.34 (1x s and 1 x m, signals overlapping, 8H, 6H of C$\underline{H}_3$NC$\underline{H}_3$ and 2H of C$\underline{H}_2$), 2.49-2.58 (m, 2H, C$\underline{H}_2$), 2.60-2.75 (m, 2H, C$\underline{H}_2$), 2.80-2.92 (m, 2H, C$\underline{H}_2$), 3.13-3.26 (1 x d and 1 x m, signals overlapping, 3H, 3H of 2 x C$\underline{H}_2$), 3.47 (d, J=22.40Hz, 1H, 1H of C$\underline{H}_2$), 5.77 (t, J=7.06Hz, 1H, C=C$\underline{H}$CH$_2$CH$_2$N), 6.28 (s, 1H, C$\underline{H}$=C), 6.96-7.34 (5 x m, 12H, 12 x Ar-$\underline{H}$), 7.38 (d, J=7.24Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.9 (C$\underline{H}_2$), 28.1 (C$\underline{H}_2$), 32.1 (C$\underline{H}_2$), 38.9 (C$\underline{H}_2$), 2 x 45.1 (C$\underline{H}_3$NC$\underline{H}_3$), 45.4 (C$\underline{H}_2$), 46.4 (CH$_2$C$\underline{C}$H$_2$), 59.9 (C$\underline{H}_2$), 60.6 (C$\underline{H}_2$), 119.8, 122.6, 122.9, 123.3, 124.3, 125.6, 125.7, 126.4, 2 x 127.0, 127.6, 128.4, 128.8 (11 x tert. C and 2 x C=C$\underline{H}$), 2 x 130.6 (2 x tert. C), 135.2, 137.8, 137.9, 142.7, 142.8, 144.6, 152.1 (5 x quat. C and 2 x C$\underline{=}$CH)

3',4'-dihydro-1'*H*-spiro{[1,3]dioxolane-2,2'naphthalene} (4.6)

To a solution of β-tetralone (2.00g, 13.70mmol) in ethylene glycol (8ml) was added triethylorthoformate (5ml) and *p*-ToSH (0.35g, 1.58mmol). The reaction was stirred at room temperature for three hours, after which time the reaction was quenched by the addition of water (100ml) and the product was extracted with diethyl ether (3 x 25ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 12:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford the title compound **(4.6)** as colourless oil (2.06g, 79%) with the following physical properties;

IR (DCM, v); cm^{-1} 2940.9, 2872.7, 1493.9, 1364.5, 1260.1, 1124.0, 1094.5, 1060.4

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.05 (t, J=7.00Hz, 2H, $CH_2C\underline{H_2}C$), 3.07-3.09 (m, 4H, 2H of $C\underline{H_2}CH_2C$ and 2H of Ar-$C\underline{H_2}C$), 4.06-4.11 (m, 4H, $OC\underline{H_2}C\underline{H_2}O$), 7.15-7.22 (m, 4H, 4 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 27.7 ($\underline{C}H_2$), 31.4 ($\underline{C}H_2$), 38.7 ($\underline{C}H_2$), 2 x 64.2 ($O\underline{C}H_2\underline{C}H_2O$), 107.9 ($O\underline{C}O$), 125.5 (tert. C), 125.7 (tert. C), 128.1 (tert. C), 128.9 (tert. C), 134.0 (quat. C), 134.9 (quat. C)

The spectral data was consistent with that reported in the literature data.

(1H-inden-3-yloxy)trimethylsilane (4.7)

To a stirred solution of 1–indanone (2.00g, 15.13mmol) in anhydrous DCM (10ml) at 0°C was added triethylamine (2.32ml, 16.64mmol) and trimethylsilyl trifluoromethanesulphonate (TMSOTf) (2.74g, 15.13mmol). The solution was allowed to stir at 0°C for two hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 5:1). The solvent was removed *in vacuo*. The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford the title silyl enol ether **(4.7)** as a colourless oil (2.23g, 71%), with the following

physical properties. The title compound **(4.7)** was stored in a dry flask under nitrogen at 0°C until further use.

IR (DCM, v); cm^{-1} 3430.0, 2960.7, 2897.3, 1602.3, 1575.7, 1363.2, 1253.8, 1179.2

HRMS ($+H^+$): actual 205.1031, calculated 205.1043, molecular formula $C_{12}H_{17}OSi$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 0.49 (s, 9H, 3 x $C\underline{H}_3$), 3.42 (2 x d, , J=2.32Hz, signals overlapping, 2H, $C\underline{H}_2$), 5.59 (t, J=2.38Hz, 1H, C=C$\underline{H}$), 7.37 (dt, J_1=0.80Hz, J_2=7.45Hz, 1H, Ar-$\underline{H}$), 7.46 (t, J=7.36Hz, 1H, Ar-$\underline{H}$), 7.55 (d, J=11.44Hz, Ar-$\underline{H}$), 7.56 (d, J=11.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 3 x 0.34 (3 x $\underline{C}H_3$), 33.6 ($\underline{C}H_2$), 105.8 (C=$\underline{C}$H), 117.8 (tert. C), 123.4 (tert. C), 124.8 (tert. C), 125.7 (tert. C), 141.5 (quat. C), 142.4 (quat. C), 153.2 (O$\underline{C}$=CH)

The spectral data was consistent with that reported in the literature data.

2-[2-(2-hydroxyethoxy)-1,2,3,4-tetrahydronaphthalen-2-yl]-2,3-dihydroinden-1-one (4.8)

To a stirred solution of compound **(4.12)** (2.05, 10.79mmol) in anhydrous DCM (20ml) in an evacuated three necked flask, was added compound **(4.7)** (2.20g, 10.79mmol) and tin(IV) tetrachloride (2.81g, 10.79mmol) at -78°C under anhydrous conditions and an atmosphere of nitrogen. After an hour, the reaction was quenched by the addition of iced water and the product was extracted using DCM (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The crude product was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 3:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford a stereoisomeric mixture of the title aldol product **(4.8)** as a yellow solid (2.95, 85%) with the following physical properties;

Mp: 78-81°C

IR (KBr, v); cm^{-1} 3415.1, 2928.9, 1699.0, 1606.6, 1495.8, 1464.2, 1276.8, 1062.4

HRMS ($+Na^+$): actual 345.1457, calculated 345.1467, molecular formula $C_{21}H_{22}O_3Na$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.90-1.95 (m, 1H, 1H of C<u>H</u>$_2$), 2.09-2.31 (2 x m, 1H, 1H of C<u>H</u>$_2$), 2.51 (broad s, 1H, O<u>H</u>), 2.74-3.65 (6 x m, 11H, 10H of 5 x C<u>H</u>$_2$ and 1H of COC<u>H</u>CH$_2$), 7.01-7.15 (2 x m, 4H, 4 x Ar-<u>H</u>), 7.41 (t, J=7.40Hz, 1H, Ar-<u>H</u>), 7.51-7.54 (m, 1H, Ar-<u>H</u>), 7.61-7.66 (m, 1H, Ar-<u>H</u>), 7.77 (broad d, J=7.66Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.6 and 25.7 (<u>C</u>H$_2$), 28.1, 28.9, 29.0, 29.5 (2 x <u>C</u>H$_2$), 35.4 and 36.0 (<u>C</u>H$_2$), 51.8 and 52.5 (<u>C</u>H), 2 x 62.0 (<u>C</u>H$_2$), 63.1 and 63.3 (<u>C</u>H$_2$), 77.1 and 77.2 (O<u>C</u>CH), 2 x 123.9 (tert. C), 2 x 125.8 (tert. C), 2 x 126.0 (tert. C), 126.4 and 126.5 (tert. C), 2 x 127.5 (tert. C), 2 x 128.5 (tert. C), 2 x 129.4 (tert. C), 133.8 and 134.0 (quat. C), 2 x 135.0 (tert. C), 2 x 135.9 (quat. C), 2 x 137.7 (quat. C), 153.5 and 153.6 (quat. C), 2 x 206.6 (<u>C</u>=O)

The spectral data was consistent with that reported in the literature data.

2-benzyl-2,3-dihydro-2-(1,2-dihydronaphthalen-3-yl)inden-1-one (4.9)

To a stirred solution of the aldol **(4.8)** (2.95g, 9.16mmol) in *t*-butanol/diethyl ether (40ml, *v:v*, 1:1) was added benzyl bromide (7.83g, 45.81mmol). To this solution was added potassium *tert*-butoxide (1.03g, 9.16mmol) as a solution in *t*-butanol (20ml), adding the base dropwise over an hour. The reaction was stirred at room temperature and the progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1) to check for the disappearance of the starting material. After a total of one and a half hours, *t*-butanol was removed by the extraction between water (100ml) and diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 50:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the title alkylated product **(4.9)** as a white solid (2.83g, 88%) with the following physical properties;

Mp: 126-139°C

IR (KBr, v); cm^{-1} 3396.1, 3024.2, 2917.9, 1707.1, 1604.7, 1453.5, 1269.6, 1030.9

HRMS (+Na$^+$): actual 373.1575, calculated 373.1568, molecular formula $C_{26}H_{22}ONa$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.31-2.39 (m, 1H, 1H of C$\underline{H}_2$CH$_2$), 2.48-2.52 (m, 1H, 1H of C$\underline{H}_2$CH$_2$), 2.81 and 2.84 (1 x d, J~1.68Hz and 1 x d, J~6.72Hz, signals overlapping, 2H, C$\underline{H}_2$), 3.29-3.48 (m, 4H, 2 x C$\underline{H}_2$), 6.58 (s, 1H, C$\underline{H}$=C), 7.07 (dd, J_1=2.04Hz, J_2=6.52Hz, 1H, Ar-$\underline{H}$), 7.12-7.23 (m, 8H, 8 x Ar-$\underline{H}$), 7.32-7.38 (m, 2H, 2 x Ar-$\underline{H}$), 7.53 (dt, J_1=1.08Hz, J_2=7.46Hz, 1H, Ar-$\underline{H}$), 7.79 (d, J=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 24.9 ($\underline{C}H_2$), 28.5 ($\underline{C}H_2$), 36.7 ($\underline{C}H_2$), 40.7 ($\underline{C}H_2$), 59.6 (CO$\underline{C}$CH$_2$), 124.1, 124.3, 126.0, 2 x 126.5, 126.6, 127.0, 127.1, 127.5 (9 x tert. C and C=$\underline{C}$H), 2 x 128.2 (2 x tert. C), 2 x 130.2 (2 x tert. C), 134.2 (quat. C), 134.8 (quat. C), 134.9 (tert. C), 136.5 (quat. C), 137.3 (quat. C), 140.6 (quat. C), 152.7 (quat. C), 207.2 ($\underline{C}$=O)

2-benzyl-1-(3-(dimethylamino)propyl)-2,3-dihydro-2-(1,2-dihydronaphthalen-3-yl)-1H-inden-1-ol (4.10)

To a dry three-necked round bottomed flask containing Grignard reagent (3.00g, 24.68mmol) in anhydrous THF (7ml), which was synthesized according to the method described previously, was added a solution of compound **(4.9)** (1.50g, 4.29mmol) in anhydrous THF (15ml). The resulting mixture was stirred at room temperature under an atmosphere of nitrogen and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. After a total of fifteen hours aq. NH_4Cl (50ml) was added and the reaction was extracted with diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford a stereoisomeric mixture of the title compound **(4.10)** as a pale yellow oil (1.91g, 99%) with the following physical properties;

IR (DCM, v); cm^{-1} 3025.9, 2947.6, 2825.9, 1602.7, 1454.1, 1265.6, 1021.4

HRMS (+H$^+$): actual 438.2795, calculated 438.2797, molecular formula $C_{31}H_{36}NO$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.44-1.48 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 1.69-1.77 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 1.78-1.81 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 1.90 (broad m, 2H, $NCH_2CH_2C\underline{H}_2$), 1.99-2.04 (m, 2H, 1H of $NCH_2C\underline{H}_2CH_2$ and 1H of $NCH_2CH_2C\underline{H}_2$), 2.18-2.26 (m, 2H, 1H of $NCH_2CH_2C\underline{H}_2$ and 1H $CH{=}CC\underline{H}_2CH_2$), 2.31-2.73 (2 x s and 4 x m, signals overlapping, 23H, 12H of 2 x $C\underline{H}_3NC\underline{H}_3$ and 11H of 6 x $C\underline{H}_2$), 2.97 (d, J=10.16Hz, 1H, 1H of $C\underline{H}_2$), 3.02-3.09 (m, 4H, 2H of $CH{=}CCH_2C\underline{H}_2$ and 2H of $C\underline{H}_2$), 3.30 (d, J=9.36Hz, 1H, 1H of $C\underline{H}_2$), 3.35 (d, J=10.52Hz, 1H, 1H of $C\underline{H}_2$), 3.49 (d, J=9.40Hz, 1H, 1H of $C\underline{H}_2$), 6.12 (broad s, 1H, $C\underline{H}$=C), 6.36 (s, 1H, $C\underline{H}$=C), 6.90 (broad m, 2H, 2 x Ar-$\underline{H}$), 6.96 (d, J=4.68Hz, 1H, Ar-$\underline{H}$), 7.03 (broad d, J=4.16Hz, 1H, Ar-$\underline{H}$), 7.08 (broad t, J=4.56Hz, 1H, Ar-$\underline{H}$), 7.11 (dt, J_1=0.92Hz, J_2=4.81Hz, 1H, Ar-$\underline{H}$), 7.14 (dt, J_1=0.96Hz, J_2=4.84Hz, 1H, Ar-$\underline{H}$), 7.19-7.30 (2 x m, 15H, 15 x Ar-$\underline{H}$), 7.32-7.38 (m, 3H, 3 x Ar-$\underline{H}$), 7.49 (broad d, J=4.92Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 21.4 and 22.2 (2 x $NCH_2\underline{C}H_2CH_2$), 26.9 ($CH{=}C\underline{C}H_2CH_2$), 2 x 28.2 ($CH{=}C\underline{C}H_2CH_2$ and $CH{=}CCH_2\underline{C}H_2$), 28.4 ($CH{=}CCH_2\underline{C}H_2$), 34.6 ($NCH_2CH_2\underline{C}H_2$), 35.7 ($\underline{C}H_2$), 36.2 ($\underline{C}H_2$), 36.5 ($NCH_2CH_2\underline{C}H_2$), 38.8 ($\underline{C}H_2$), 40.1 ($\underline{C}H_2$), 2 x 45.4 (2 x $\underline{C}H_3N\underline{C}H_3$), 60.6 and 60.7 (2 x $\underline{C}H_2$), 61.5 and 62.7 (2 x $CH_2\underline{C}CH_2$), 85.0 and 85.9 (2 x $OH\underline{C}CH_2$), 123.8 (tert. C), 2 x 124.2 (2 x tert. C), 124.8 (tert. C) 125.4 (C=$\underline{C}$H), 125.6 (tert. C), 2 x 125.8 (2 x tert. C), 125.9 (tert. C), 2 x 126.0 (2 x tert. C), 2 x 126.1 (2 x tert. C), 126.3 (2 x tert. C), 2 x 126.4 (2 x tert. C), 126.7 (tert. C), 126.9 (tert. C), 127.1 (tert. C), 127.2 (C=$\underline{C}$H), 2 x 127.4 (2 x tert. C), 127.7 (tert. C), 127.8 (tert. C), 130.0 (tert. C), 2 x 130.2 (2 x tert. C), 134.4, 134.8, 135.0, 135.2, 138.8, 139.3, 139.4, 140.7, 142.3, 143.4, 147.5, 149.0 (10 x quat. C and 2 x $\underline{C}$=CH)

(3Z)-3-(2-benzyl-1,2-dihydro-2-(1,2-dihydronaphthalen-3-yl)inden-3-ylidene)-*N,N*-dimethylpropan-1-amine (4.11)

To a round bottomed flask containing compound **(4.10)** (1.91g, 4.37mmol) was added a 20% solution of oxalic acid in water (100ml). The resulting mixture was heated till gently refluxing for two hours and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the

starting material. On completion, the reaction was allowed to reach room temperature. The reaction mixture was then partitioned between water (50ml) and diethyl ether (3 x 25ml each). The aqueous layer was basified with 2.5M aq. NaOH and the product was extracted with diethyl ether (3x 25ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford a stereoisomeric mixture of the title compound **(4.11)** as a yellow oil (1.47g, 80 %) with the following physical properties;

IR (DCM, v); cm^{-1} 3388.2, 3025.5, 2937.2, 2765.1, 1634.5, 1458.9, 1268.6, 1032.6

HRMS ($+H^+$): actual 420.2709, calculated 420.2691, molecular formula $C_{31}H_{34}N$

^1H NMR ($CDCl_3$, 600MHz) δ_H(ppm): 2.10 (s, 1H, 1H of $C\underline{H}_3$), 2.21 (s, 5H, 5H of $C\underline{H}_3NC\underline{H}_3$), 2.24-2.44 (1 x s and 1 x m, signals overlapping, 11H, 6H of $C\underline{H}_3NC\underline{H}_3$ and 5H of 3 x $C\underline{H}_2$), 2.47-2.67 (m, 5H, 5H of 3 x $C\underline{H}_2$), 2.71-2.85 (m, 6H, 3 x $C\underline{H}_2$), 3.10 (d, J=16.74Hz, 1H, 1H of $C\underline{H}_2$), 3.17-3.24 (m, 4H, 2 x $C\underline{H}_2$), 3.29 (1 x d, J~13.14Hz and 1 x d, J~13.23Hz, signals overlapping, 2H, 1H of $C\underline{H}_2$ and 1H of $C\underline{H}_2$), 3.49 (d, J=12.96Hz, 1H, 1H of $C\underline{H}_2$), 5.61 (t, J=7.11Hz, 1H, C=C$\underline{H}$CH$_2$CH$_2$N), 6.17 (t, J=7.41Hz, 1H, C=C$\underline{H}$CH$_2$CH$_2$N), 6.55 (s, 1H, ArC$\underline{H}$=C), 6.71 (s, 1H, ArC$\underline{H}$=C), 6.91-7.33 (m, 25H, 25 x Ar-$\underline{H}$), 7.52 (d, J=7.50Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 150MHz) δc(ppm): 25.6 and 25.7 ($\underline{C}H_2$), 26.0 and 27.3 ($\underline{C}H_2$), 28.7 and 28.8 ($\underline{C}H_2$), 40.8 and 42.1 ($\underline{C}H_2$), 43.9 and 44.6 ($\underline{C}H_2$), 45.3, 45.4 and 45.5 (2 x $\underline{C}H_3N\underline{C}H_3$), 54.3 and 56.2 ($CH_2\underline{C}CH_2$), 59.5 and 59.6 (N$\underline{C}H_2CH_2$CH=C), 119.3 (tert. C), 120.1 (C=$\underline{C}$HCH$_2$CH$_2$N), 120.7 (Ar$\underline{C}$H=C), 122.2 (Ar$\underline{C}$H=C), 122.6 (C=$\underline{C}$HCH$_2$CH$_2$N), 2 x 124.4 (2 x tert. C), 124.6 (tert. C), 125.9 (tert. C), 126.0 (tert. C), 3 x 126.2 (3 x tert. C), 3 x 126.5 (3 x tert. C), 126.8 (tert. C), 2 x 127.0 (2 x tert. C), 2 x 127.3 (2 x tert. C), 4 x 127.4 (4 x tert. C), 2 x 130.4 (2 x tert. C), 130.5 (tert. C), 134.7, 134.9, 135.1, 137.9, 138.4, 140.8, 142.6, 143.0, 144.8, 145.6, 146.2, 2 x 147.0, 147.1 (10 x quat. C and 4 x $\underline{C}$=CH)

2-[4-(trifluoromethoxy)benzyl]-3,4-dihydro-2-(1H-inden-2-yl)naphthalen-1(2H)-one (4.12)

To a stirred solution of compound **(4.2)** (1.00g, 3.11mmol) in *t*-butanol/diethyl ether (20ml, *v:v*, 1:1) was added 4-(trifluoromethoxy) benzyl bromide (1.58g, 6.20mmol). To this solution was added potassium *tert*-butoxide (0.35g, 3.13mmol) as a solution in *t*-butanol (5ml), adding the base drop-wise over an hour. The reaction was stirred at room temperature and the progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1). After a total of two hours, *t*-butanol was removed by the extraction between water (100ml) and diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 18:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the title compound **(4.12)** as a yellow oil (0.49g, 36%) with the following physical properties;

IR (DCM, v); cm^{-1} 3067.8, 2925.6, 1679.4, 1600.8, 1507.9, 1455.2, 1260.6, 1222.6, 1164.6, 1157.2, 1020.0

HRMS ($+Na^+$): actual 457.1376, calculated 457.1391, molecular formula $C_{27}H_{21}O_2F_3Na$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.21 (dt, J_1=4.17Hz, J_2=13.04Hz, 1H, 1H of COCC$\underline{H}_2$CH$_2$), 2.37 (broad d, J~13.52Hz, 1H, 1H of COCC$\underline{H}_2$CH$_2$), 2.85 (broad d, J=17.04Hz, 1H, 1H of COCCH$_2$C$\underline{H}_2$), 3.04-3.17 (1 x broad t, J~14.78Hz and 1 x d, J=45.40Hz, signals overlapping, 2H, 1H of COCCH$_2$C$\underline{H}_2$ and 1H of COCC$\underline{H}_2$Ar), 3.23 (d, J=45.40Hz, 1H, 1H of COCC$\underline{H}_2$Ar), 3.52 (d, J=18.06Hz, 1H, 1H of CH=CC$\underline{H}_2$), 3.60 (d, J=18.06Hz, 1H, 1H of CH=CC$\underline{H}_2$), 6.54 (s, 1H, C$\underline{H}$=C), 7.08 (2 x d, J=8.00Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.14-7.20 (m, 4H, 4 x Ar-$\underline{H}$), 7.24-7.30 (m, 2H, 2 x Ar-$\underline{H}$), 7.33 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.41-7.47 (1 x t, J=6.78Hz and 1 x t, J=7.12Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 8.20 (d, J=8.04Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.4 ($\underline{C}H_2$), 30.8 ($\underline{C}H_2$), 38.8 ($\underline{C}H_2$), 43.2 ($\underline{C}H_2$), 52.9 (CO$\underline{C}$CH$_2$), 2 x 119.9 (2 x tert. C), 120.4 (tert. C), 119.8 (q, $J_{F\text{-}C}$~258.4Hz, $\underline{C}F_3$), 123.1 (tert. C), 124.3 (tert. C), 126.1 (tert. C), 126.2 (tert. C), 127.7 (tert. C), 128.2 (tert.

C), 129.7 (C=CH), 2 x 131.8 (2 x tert. C), 133.0 (tert. C), 135.9 (quat. C), 142.5, 143.0, 143.7, 2 x 146.0 (4 x quat. C and C= CH), 147.5 (quat. C), 197.8 (C=O)

^{19}F NMR ($CDCl_3$, 400MHz) δc(ppm): -58.4 (s, CF_3)

2-[4-(trifluoromethoxy)benzyl]-1-[3-(dimethylamino)propyl]-1,2,3,4-tetrahydro-2-(1H-inden-2-yl)naphthalen-1-ol (4.13)

To a dry three-necked round bottomed flask containing Grignard reagent (1.00g, 8.23mmol) in anhydrous THF (5ml), which was synthesized according to the method described previously, was added a solution of compound **(4.12)** (0.40g, 0.92mmol) in anhydrous THF (5ml). The resulting mixture was stirred at room temperature under an atmosphere of nitrogen and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. After a total of two and half hours aq. NH_4Cl (50ml) was added and the reaction was extracted by diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford a stereoisomeric mixture of the title compound **(4.13)a** and **(4.13)b** as a pale yellow solid (0.47g, 98%) with the following physical properties;

Mp: 151-158°C

IR (KBr, v); cm^{-1} 3434.3, 1644.0, 1507.3, 1463.8, 1259.5, 1223.1, 1165.4, 1020.7

HRMS ($+H^+$): actual 522.2618, calculated 522.2620, molecular formula $C_{32}H_{35}NO_2F_3$

Major stereoisomer (4.13)a

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.36-1.41 (m, 1H, 1H of $NCH_2CH_2CH_2$), 1.45-1.53 (m, 1H, 1H of $NCH_2CH_2CH_2$), 1.97-2.17 (3 x m, 4H, 2H of $ArCH_2CH_2$ and 2H of $NCH_2CH_2CH_2$), 2.24-2.42 (m and 2 x s, signals overlapping, 8H, 2H of $NCH_2CH_2CH_2$ and 6H of CH_3NCH_3), 2.84-2.91 (m, 2H, $ArCH_2CH_2$), 2.98 (d, J=15.28Hz, 1H, 1H of $OHCCCH_2Ar$), 3.34 (d, J=23.16Hz, 1H, 1H of $CH=CCH_2$), 3.46 (d,

J=15.28Hz, 1H, 1H of OHCCC$\underline{H}_2$Ar), 3.51 (d, J=27.44Hz, 1H, 1H of CH=CC$\underline{H}_2$), 6.11 (s, 1H, C$\underline{H}$=C), 6.84 (2 x d, J=8.24Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 6.89-7.13 (m, 7H, 7 x Ar-$\underline{H}$), 7.20-7.34 (m, 2H, 2 x Ar-$\underline{H}$), 7.55 (d, J=7.56Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.8 (NCH$_2\underline{C}$H$_2$CH$_2$), 24.2 (Ar$\underline{C}$H$_2$CH$_2$), 27.3 (ArCH$_2\underline{C}$H$_2$), 36.6 (NCH$_2$CH$_2\underline{C}$H$_2$), 40.0 (OHCC$\underline{C}$H$_2$), 41.0 (CH=C$\underline{C}$H$_2$), 2 x 45.1 ($\underline{C}$H$_3$N$\underline{C}$H$_3$), 49.1 (CH$_2\underline{C}$CH$_2$), 60.6 (N$\underline{C}$H$_2$CH$_2$CH$_2$), 76.0 (CH$_2\underline{C}$OH), 2 x 119.3 (2 x tert. C), 119.5 (tert. C), 120.8 (q, $J_{F\text{-}C}$~260.3Hz, $\underline{C}$F$_3$), 122.5 (tert. C) 122.9 (tert. C), 124.0 (tert. C), 124.5 (tert. C), 125.1 (tert. C), 125.4 (tert. C), 127.8 (tert. C), 129.0 (C=C$\underline{H}$), 2 x 131.3 (2 x tert. C), 135.0 (quat. C), 137.7 (quat. C), 2 x 143.9 (quat. C and $\underline{C}$=CH), 144.0 (quat. C), 147.0 (quat. C), 151.6, (quat. C)

^{19}F NMR ($CDCl_3$, 376MHz) δ_F(ppm): -58.4 (s, C$\underline{F}_3$)

4-{[1,2,3,4-tetrahydro-2-(1H-inden-2-yl)-1-oxonaphthalen-2-yl]methyl}benzo-nitrile (4.14)

To a stirred solution of compound **(4.2)** (0.50g, 1.55mmol) in *t*-butanol/diethyl ether (10ml, *v:v*, 1:1) was added 4-(bromomethyl)benzonitrile (1.22g, 6.21mmol). To this solution was added potassium *tert*-butoxide (0.18g, 1.61mmol) as a solution in *t*-butanol (5ml), adding the base drop-wise over two hours. The reaction was stirred at room temperature and the progress of the reaction was monitored by TLC (hexane:ethyl acetate, 3:1). After a total of three hours, *t*-butanol was removed by the extraction between water (100ml) and diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 20:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the title compound **(4.14)** as a yellow oil (0.18g, 31%) with the following physical properties;

IR (DCM, v); cm^{-1} 3400.8, 2922.7, 2225.6, 1678.4, 1599.8, 1455.2, 1288.9, 1074.3

HRMS (+H$^+$): actual 376.1715, calculated 376.1701, molecular formula $C_{27}H_{22}NO$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.18 (dt, J_1=4.59Hz, J_2=13.29Hz, 1H, 1H of COCC$\underline{H}_2$CH$_2$), 2.36 (ddd, J_1~2.75Hz, J_2~4.40Hz, J_3~13.76Hz, 1H, 1H of COCC$\underline{H}_2$CH$_2$),

2.86 (ddd, J_1~2.78Hz, J_2~4.24Hz, J_3~17.37Hz, 1H, 1H of $COCH_2C\underline{H}_2$), 3.10 (ddd, J_1~4.40Hz, J_2~12.76Hz, J_3~17.09Hz, 1H, 1H of $COCH_2C\underline{H}_2$), 3.23 (d, J=13.32Hz, 1H, 1H of $C\underline{H}_2$), 3.26 (d, J=22.64Hz, 1H, 1H of $C\underline{H}_2$), 3.53-3.63 (1 x d, J=13.44Hz and 1 x d, J~22.88Hz, signals overlapping, 2H, $C\underline{H}_2$), 6.53 (s, 1H, $C\underline{H}$=C), 7.15 (d, J=7.76Hz, 1H, Ar-$\underline{H}$), 7.19 (2 x dt, J_1=2.00Hz, J_2=7.02Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.24-7.31 (m, 4H, 4 x Ar-$\underline{H}$), 7.34 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.42-7.48 (1 x t, J~7.04Hz and 1 x dt, J_1~1.52Hz, J_2~7.52Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.53 (2 x d, J~8.28Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 8.20 (dd, J_1=1.26Hz, J_2=8.02Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.8 ($\underline{C}H_2$), 31.4 ($\underline{C}H_2$), 39.2 ($\underline{C}H_2$), 44.5 ($\underline{C}H_2$), 53.4 ($CO\underline{C}CH_2$), 119.0 (Ar-$\underline{C}$N), 120.9, 123.6, 124.9, 126.6, 126.8, 128.2, 128.7, 130.4, (7 x tert. C and C=$\underline{C}$H), 2 x 131.6 (2 x tert. C), 2 x 131.7 (2 x tert. C), 132.1 (quat. C), 133.6 (tert. C), 142.9, 143.3, 143.5, 144.0, 145.8 (4 x quat. C and $\underline{C}$= CH), 198.0 ($\underline{C}$=O)

4-(dimethylamino)-1-{4-[(1-(3-(dimethylamino)propyl)-1,2,3,4-tetrahydro-1-hydroxy-2-(1H-inden-2-yl)naphthalen-2-yl)methyl]phenyl}butan-1-one (4.15)

To a dry three-necked round bottomed flask containing Grignard reagent (1.00g, 8.23mmol) in anhydrous THF (5ml), which was synthesized according to the method described previously, was added a solution of compound **(4.14)** (0.18g, 0.48mmol) in anhydrous THF (8ml). The resulting mixture was stirred at room temperature under an atmosphere of nitrogen and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:2) to check for the disappearance of the starting material. After a total of two and half hours aq. NH_4Cl (50ml) was added and the product was extracted by diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:2). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford the title compound **(4.15)a** as a dark brown oil (0.08g, 30%), and its stereoisomer compound **(4.15)b** as a dark brown oil (0.19g, 72%) with the following physical properties;

Compound (4.15)a (less polar)

IR (DCM, v); cm^{-1} 3418.0, 2926.3, 2772.9, 1679.7, 1605.5, 1462.8, 1370.8, 1273.7, 1020.6

HRMS ($+H^+$): actual 551.3578, calculated 551.3632, molecular formula $C_{37}H_{47}N_2O_2$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.15-1.22 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2COH$), 1.55-1.67 (m, 2H, 1H of $NCH_2C\underline{H}_2CH_2COH$ and 1H of $NCH_2CH_2C\underline{H}_2COH$), 1.77-1.83 (m, 1H, 1H of $ArCH_2C\underline{H}_2$), 1.84-1.88 (m, 2H, $NCH_2C\underline{H}_2CH_2CO$), 1.95-2.01 (m, 1H, 1H of $NCH_2CH_2C\underline{H}_2COH$), 2.11-2.15 (m, 1H, $NC\underline{H}_2CH_2CH_2$), 2.21 and 2.23 (2 x s, 6H, $C\underline{H}_3NC\underline{H}_3$), 2.25-2.33 (m, 4H, 1H of $ArCH_2C\underline{H}_2$ and 3H of 2 x $NC\underline{H}_2CH_2CH_2$), 2.39 and 2.42 (2 x s, 6H, $C\underline{H}_3NC\underline{H}_3$), 2.69 (d, J=14.14Hz, 1H, 1H of $OHCCC\underline{H}_2Ar$), 2.92 (2 x t, J=7.30Hz, signals overlapping, 2H, $COC\underline{H}_2CH_2CH_2N$), 2.96-3.09 (m, 2H, $ArC\underline{H}_2CH_2$), 3.51 (d, J=14.14Hz, 1H, 1H of $OHCCC\underline{H}_2Ar$), 3.74-3.95 (m, signals overlapping, 2H, $CH{=}CC\underline{H}_2$), 6.61 (broad s, 1H, O<u>H</u>), 6.71 (broad s, 1H, C=C<u>H</u>), 6.90 (2 x d, J=8.12Hz, signals overlapping, 2H, 2 x Ar-<u>H</u>), 7.18-7.23 (m, 2H, 2 x Ar-<u>H</u>), 7.24-7.30 (m, 3H, 3 x Ar-<u>H</u>), 7.32-7.37 (m, 1H, Ar-<u>H</u>), 7.50 (d, J=7.32Hz, 1H, Ar-<u>H</u>), 7.69 (d, J~8.20Hz, 1H, Ar-<u>H</u>), 7.73 (broad d, J~7.28Hz, 1H, Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.3 ($\underline{C}H_2$), 21.7 ($\underline{C}H_2$), 24.5 ($\underline{C}H_2$), 25.2 ($\underline{C}H_2$), 35.6 ($\underline{C}H_2$), 39.0 ($\underline{C}H_2$), 39.5 ($\underline{C}H_2$), 41.2 ($\underline{C}H_2$), 44.9 ($\underline{C}H_3N\underline{C}H_3$), 45.1 ($\underline{C}H_3N\underline{C}H_3$), 48.7 ($CH_2\underline{C}CH_2$), 58.5 ($\underline{C}H_2$), 60.5 ($\underline{C}H_2$), 75.9 ($OH\underline{C}CH_2$), 120.0 (tert. C), 122.9 (tert. C), 123.6 (tert. C), 124.7 (tert. C), 125.8 (tert. C), 125.9 (tert. C), 126.6 (tert. C), 2 x 127.0 (2 x tert. C), 128.1 (tert. C), 2 x 129.6 (2 x tert. C), 130.0 (C=C<u>H</u>), 133.8 (quat. C), 134.3 (quat. C), 2 x 143.3 (2 x quat. C), 144.2 (<u>C</u>=CH), 145.2 (quat. C), 153.9 (quat. C), 199.5 (<u>C</u>=O)

Compound (4.15)b (more polar)

IR (DCM, v); cm^{-1} 3339.1, 2946.9, 2779.7, 1678.9, 1605.5, 1462.9, 1373.8, 1274.6, 1227.2, 1020.6

HRMS ($+H^+$): actual 551.3634, calculated 551.3638, molecular formula $C_{37}H_{47}N_2O_2$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.31-1.38 (m, 2H, $NCH_2C\underline{H}_2CH_2$), 1.41-1.49 (m, 2H, $NCH_2C\underline{H}_2CH_2$), 2.05-2.40 (2 x s and 4 x m, signals overlapping, 20H, 12H of 2 x

$C\underline{H_3}NC\underline{H_3}$ and 8H of 4 x $C\underline{H_2}$), 2.84-2.89 (m, 2H, $ArC\underline{H_2}CH_2$), 2.93 (2 x t, J=7.24Hz, signals overlapping, 2H, $OCC\underline{H_2}CH_2CH_2N$), 3.20-3.31 (m, 2H, $C\underline{H_2}$), 3.48-3.55 (m, 2H, $C\underline{H_2}$), 5.83 (broad s, C-O$\underline{H}$), 6.08 (s, 1H, C$\underline{H}$=C), 6.88, 6.91 and 6.94 (1 x t, J~7.44Hz, 2 x d, J~8.24Hz and 1 x t, J~7.36Hz, signals overlapping, 4H, 4 x Ar-$\underline{H}$), 6.98-7.03 and 7.07 (1 x m and 1 x dd, J_1~7.28Hz, J_2~14.44Hz, 4H, 4 x Ar-$\underline{H}$), 7.27 (d, J=7.16Hz, 1H, Ar-$\underline{H}$), 7.52 (d, J=7.56Hz, 1H, Ar-$\underline{H}$), 7.70 (2 x d, J=8.02Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.8 ($\underline{C}H_2$), 21.6 ($\underline{C}H_2$), 24.2 ($Ar\underline{C}H_2CH_2$), 27.3 ($ArCH_2\underline{C}H_2$), 35.6 ($OHC\underline{C}H_2CH_2CH_2N$), 36.5 ($OC\underline{C}H_2CH_2CH_2N$), 40.8 ($\underline{C}H_2$), 41.0 ($\underline{C}H_2$), 44.8 ($\underline{C}H_3N\underline{C}H_3$), 45.2 ($\underline{C}H_3N\underline{C}H_3$), 49.3 ($CH_2\underline{C}CH_2$), 58.5 ($N\underline{C}H_2CH_2$), 60.5 ($N\underline{C}H_2CH_2$), 76.0 ($OH\underline{C}CH_2$), 119.4 (tert. C), 122.5 (tert. C), 122.9 (tert. C), 124.0 (tert. C), 124.5 (tert. C), 125.0 (tert. C), 125.4 (tert. C), 2 x 126.7 (2 x tert. C), 127.8 (tert. C), 128.9 (C=C$\underline{H}$), 2 x 130.4 (2 x tert. C), 134.2 (quat. C), 135.0 (quat. C), 3 x 143.8 (2 x quat. C and $\underline{C}$=CH), 145.0 (quat. C), 151.6, (quat. C), 199.6 ($\underline{C}$=O)

2,2-dibenzyl-3,4-dihydronaphthalen-1(2H)-one (5.0)

To a stirred solution of α-tetralone (2.00g, 13.68mmol) in *t*-butanol/diethyl ether (40ml, *v:v*, 1:1) was added benzyl bromide (4.68g, 27.36mmol). To this solution was added potassium *tert*-butoxide (3.07g, 27.36mmol) as a solution in *t*-butanol (20ml), adding the base dropwise over two hours. The reaction was stirred at room temperature and the progress of the reaction was monitored by TLC (hexane:ethyl acetate, 4:1). After a total of six hours, the *t*-butanol was removed by the extraction between water (200ml) and diethyl ether (3 x 30ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo,* and the residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 16:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the title compound **(5.0)** as a pale yellow crystalline solid (3.34g, 75%). 0.85g of starting material, α-tetralone, was recovered. Compound **(5.0)** was with the following physical properties.

Mp: 56-68°C

IR (KBr, v); cm^{-1} 3381.6, 3027.2, 2928.1, 1677.6, 1599.7, 1494.8, 1453.9, 1289.7, 1227.9, 1031.1

HRMS ($+Na^+$): actual 349.1559, calculated 349.1568, molecular formula $C_{24}H_{22}ONa$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm) 1.97 (t, J=6.40Hz, signals overlapping, 2H, $ArCH_2C\underline{H_2}$), 2.71 (2 x d, J=13.40Hz, signals overlapping, 2H, $CH_2CC\underline{H_2}$), 3.07 (2 x t, J=6.36Hz, signals overlapping, 2H, $ArC\underline{H_2}CH_2$), 3.37 (2 x d, J=13.40Hz, signals overlapping, 2H, $C\underline{H_2}CCH_2$), 7.16-7.23 (3 x m, 6H, 6 x Ar-H), 7.24 (t, J=1.60Hz, 1H, Ar-H), 7.25-7.26 (m, 2H, 2 x Ar-H), 7.27-7.28 (m, 2H, 2 x Ar-H), 7.34 (broad t, J~7.56Hz, 1H, Ar-H), 7.47 (dt, J_1=1.45Hz, J_2=7.46Hz, 1H, Ar-H), 8.14 (dd, J_1=1.26Hz, J_2=7.86Hz, 1H, Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.5 ($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 2 x 41.6 (2 x $\underline{C}H_2$), 50.9 ($CH_2\underline{C}CH_2$), 2 x 126.4 (2 x tert. C), 126.7 (tert. C), 4 x 128.0 (4 x tert. C), 128.1 (tert. C), 128.6 (tert. C), 4 x 131.0 (4 x tert. C), 132.5 (quat. C), 133.2 (tert. C), 2 x 137.4 (2 x quat. C), 143.0 (quat. C), 200.7 ($\underline{C}$=O).

2,2-dibenzyl-1-[3-(dimethylamino)propyl]-1,2,3,4-tetrahydronaphthalen-1-ol (5.1)

To a dry three-necked round bottomed flask containing Grignard reagent (3.00g, 16.45mmol) in anhydrous THF (5ml), which was synthesized according to the method described previously, was added a solution of compound **(5.0)** (2.00g, 6.13mmol) in anhydrous THF (10ml). The resulting mixture was stirred at room temperature under an atmosphere of nitrogen and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. After a total of fifteen hours aq. NH_4Cl (50ml) was added and the reaction was extracted with diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent removed *in vacuo* to afford the title compound **(5.1)** as a yellow oil

(1.57g, 62%) with the following physical properties;

IR (DCM, v); cm^{-1} 3560.4, 3026.0, 2947.2, 2822.2, 1601.5, 1493.1, 1451.5, 1021.8

HRMS ($+H^+$): actual 414.2811, calculated 414.2797, molecular formula $C_{29}H_{36}NO$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.22-1.29 (m, 1H, 1H of $NCH_2C\underline{H_2}CH_2$), 1.42-1.51 (m, 1H, 1H of $NCH_2C\underline{H_2}CH_2$), 1.77-2.05 (2 x m, 4H, 2 x $C\underline{H_2}$), 2.26-2.37 (m, 2H, $C\underline{H_2}$), 2.43 (s, 6H, $C\underline{H_3}NC\underline{H_3}$), 2.58 (d, J=14.04Hz, 1H, 1H of $CH_2CC\underline{H_2}$), 2.65-2.74 (m, 1H, 1H of $C\underline{H_2}$), 2.86-2.91 (1 x m and 1 x d, J=14.04Hz, signals overlapping, 2H, 1H of $C\underline{H_2}$ and 1H of $CH_2CC\underline{H_2}$), 3.07 (d, J=13.80Hz, 1H, 1H of $C\underline{H_2}CCH_2$), 3.26 (d, J=14.04Hz, 1H, 1H of $C\underline{H_2}CCH_2$), 6.74-6.76 (m, 2H, 2 x Ar-$\underline{H}$), 7.07 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.16-7.23 (m, 6H, 6 x Ar-$\underline{H}$), 7.26-7.32 (m, 4H, 4 x Ar-$\underline{H}$), 7.68 (dd, J_1=1.24Hz, J_2=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 21.3 ($\underline{C}H_2$), 25.0 ($\underline{C}H_2$), 26.5 ($\underline{C}H_2$), 37.1 ($\underline{C}H_2$), 39.8 ($\underline{C}H_2$), 40.8 ($\underline{C}H_2$), 2 x 45.7 ($\underline{C}H_3N\underline{C}H_3$), 46.1 ($CH_2\underline{C}CH_2$), 60.8 ($\underline{C}H_2$), 77.8 (Ar$\underline{C}$OH), 124.7 (tert. C), 125.6 (tert. C), 125.8 (tert. C), 125.9 (tert. C), 126.0 (tert. C), 2 x 127.6 (2 x tert. C), 2 x 127.9 (2 x tert. C), 128.5 (tert. C), 2 x 131.1 (2 x tert. C), 2 x 131.4 (2 x tert. C), 135.8 (quat. C). 140.3 (quat. C), 140.9 (quat. C), 144.7 (quat. C)

(Z)-3-[3,3-dibenzyl-2,3-dihydronaphthalen-4(1H)-ylidene]-*N,N*-dimethylpropan-1-amine (5.2)

To a round bottomed flask containing compound **(5.1)** (1.00g, 2.42mmol) was added a 20% solution of oxalic acid in water (50ml). The resulting mixture was heated till gently refluxing for eight hours and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. On completion, the reaction was allowed to reach room temperature. The reaction mixture was then partitioned between water (1 x 50ml) and diethyl ether (3 x 25ml each). The aqueous layer was basified with 2.5M aq. NaOH and the product was extracted with diethyl ether (3x 25ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl

acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent removed *in vacuo* to afford a stereoisomeric mixture of the title compound **(5.2)** as a pale yellow oil (0.89g, 93 %) with the following physical properties;

IR (DCM, v); cm^{-1} 3381.6, 3060.1, 3026.5, 2937.2, 2854.8, 2777.2, 1674.6, 1601.6, 1494.6, 1453.6, 1031.4

HRMS ($+H^+$): actual 396.2675, calculated 396.2691, molecular formula $C_{29}H_{34}N$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.81 (2 x t, J~6.24Hz, signals overlapping, 2H, $ArCH_2C\underline{H_2}$), 2.33 (s, 6H, $C\underline{H_3}NC\underline{H_3}$), 2.44 (2 x t, J=6.20Hz, signals overlapping, 2H, $ArC\underline{H_2}CH_2$), 2.50 (2 x t, J~7.60Hz, 2H, $CH_2C\underline{H_2}NCH_3$), 2.67 (m, 2H, $C\underline{H_2}CH_2NCH_3$), 2.86 (2 x d, J=13.50Hz, signals overlapping, 2H, $ArC\underline{H_2}C$), 3.02 (2 x d, J=13.50Hz, signals overlapping, 2H, $ArC\underline{H_2}C$), 5.70 (t, J=7.12Hz, 1H, C=C$\underline{H}$), 7.06 (broad d, J=7.20Hz, 1H, Ar-$\underline{H}$), 7.12-7.19 (m, 2H, 2 x Ar-$\underline{H}$), 7.22-7.33 (m, 11H, 11 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 27.8 ($Ar\underline{C}H_2CH_2$), 28.7 ($C{=}CH\underline{C}H_2$), 33.7 ($ArCH_2\underline{C}H_2$), 44.8 ($CH_2\underline{C}CH_2$), 2 x 45.5 ($\underline{C}H_3N\underline{C}H_3$), 2 x 45.9 ($Ar\underline{C}H_2C\underline{C}H_2Ar$), 60.1 ($\underline{C}H_2NCH_3$), 125.0 (tert. C), 125.6 (C=C$\underline{H}$), 2 x 125.9 (2 x tert. C), 126.6 (tert. C), 127.0 (tert. C), 4 x 127.7 (4 x tert. C), 129.4 (tert. C), 4 x 131.2 (4 x tert. C), 135.6 ($\underline{C}$=CH). 2 x 139.1 (2 x quat. C), 140.6 (quat. C), 140.8 (quat. C)

2,2-bis(4-chlorobenzyl)-3,4-dihydronaphthalen-1(2H)-one (5.3)

2-(4-chlorobenzyl)-3,4-dihydro-2-hydroxynaphthalen-1(2H)-one (5.4)

To a stirred solution of α-tetralone (2.00g, 13.68mmol) in *t*-butanol/diethyl ether (40ml, 1:1) was added 4-chlorobenzyl chloride (11.01g, 68.40mmol). To this solution was added potassium *tert*-butoxide (3.07g, 27.36mmol) as a solution in *t*-butanol (20ml), adding the base dropwise over several hours. The reaction was stirred for a total of five hours, and the *t*-butanol was removed by extraction between water (200ml) and diethyl ether (3 x 30ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 15:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the

title compound **(5.3)** as a yellow oil (3.82g, 71%), and the compound **(5.4)** as a off-white solid (0.42g, 11%) with the following physical properties;

Compound (5.3)

IR (DCM, v) cm^{-1} 2962.1, 1903.0, 1737.9, 1678.0, 1598.8, 1491.9, 1265.6, 1093.6

HRMS ($+H^+$): actual 417.0781, calculated 417.0789, molecular formula $C_{24}H_{20}Cl_2O$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.95 (broad t, J=5.84Hz, 2H, $ArCH_2C\underline{H_2}$), 2.64 (2 x d, J=13.22Hz, signals overlapping, 2H, $CH_2CC\underline{H_2}$), 3.06 (broad t, J=5.84Hz, 2H, $ArC\underline{H_2}CH_2$), 3.26 (2 x d, J=13.22Hz, signals overlapping, 2H, $C\underline{H_2}CCH_2$), 7.10 (2 x d, J=7.80Hz, signals overlapping, 4H, 4 x Ar-H), 7.21 (d, J~7.84Hz, 1H, Ar-H), 7.26 (2 x d, J~7.84Hz, 4H, 4 x Ar-H), 7.34 (dd, J_1=7.84Hz, J_2=15.68Hz, 1H, Ar-H), 7.47 (t, J=7.84Hz, 1H, Ar-H), 8.18 (d, J=7.84Hz, 1H, Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 24.9 ($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 2 x 40.41 (2 x $\underline{C}H_2$), 50.3 ($CH_2\underline{C}CH_2$), 126.5 (tert. C), 127.7 (tert. C), 4 x 127.8 (4 x tert. C), 128.4 (tert. C), 131.7 (quat. C), 4 x 131.9 (4 x tert. C), 2 x 132.0 (2 x quat. C), 133.1 (tert. C), 2 x 135.3 (2 x quat. C), 142.3 (quat. C), 199.4 ($\underline{C}$=O)

Compound (5.4)

Mp: 120-128°C

IR (KBr, v) cm^{-1} 3479.2, 2929.3, 1682.9, 1602.6, 1491.2, 1289.5, 1226.5, 1091.9

HRMS ($+Na^+$): actual 309.0662, calculated 309.0658, molecular formula $C_{17}H_{15}O_2ClNa$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.16-2.21 (m, 1H, 1H of $C\underline{H_2}$), 2.23-2.30 (m, 1H, 1H of $C\underline{H_2}$), 2.95 (2 x dd, J_1=13.74Hz, J_2=24.86Hz, signals overlapping, 2H, $C\underline{H_2}$), 3.34 and 3.08 (2 x dd, J_1~1.88Hz, J_2~5.44Hz, 1H, 1H of $C\underline{H_2}$), 3.22 and 3.27 (2 x dd, J_1=5.24Hz, J_2=12.28Hz, 1H, 1H of $C\underline{H_2}$),7.11 (2 x d, J=8.820Hz, signals overlapping, 2H, 4 x Ar-H), 7.25 (d, J=8.16Hz, signals overlapping, 2H, 2 x Ar-H), 7.33 (d, J=7.60Hz, 1H, Ar-H), 7.40 (t, J=7.60Hz, 1H, Ar-H), 7.59 (t, J=7.32Hz, 1H, Ar-H), 8.04 (d, J=8.16Hz, 1H, Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.9 ($\underline{C}H_2$), 33.4 ($\underline{C}H_2$), 40.8 ($\underline{C}H_2$), 75.5 ($CH_2\underline{C}OH$), 126.7 (tert. C), 127.6 (tert. C), 2 x 127.8 (2 x tert. C), 128.8 (tert. C), 129.8 (quat. C), 2 x 131.3 (2 x tert. C), 132.3 (quat. C), 133.5 (quat. C), 133.9 (tert. C), 142.7

(quat. C), 200.2 (C=O)

2,2-bis(4-chlorobenzyl)-1-[3-(dimethylamino)propyl]-1,2,3,4-tetrahydro-naphthalen -1-ol (5.5)

To a dry three-necked round bottomed flask containing Grignard reagent (2.00g, 13.70mmol) in anhydrous THF (5ml), which was synthesized according to the method described previously, was added a solution of compound **(5.3)** (2.00g, 5.08mmol) in anhydrous THF (15ml). The resulting mixture was stirred at room temperature under an atmosphere of nitrogen and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. After a total of sixteen hours aq. NH_4Cl (50ml) was added and the reaction was extracted with diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford a stereoisomeric mixture of the title compound **(5.5)** as a green oil (1.46g, 60%) with the following physical properties;
IR (DCM, v) cm^{-1} 3063.2, 2948.0, 2782.2, 1490.8, 1464.1, 1264.7, 1094.1, 1015.4
HRMS ($+H^+$): actual 482.2022, calculated 482.2017, molecular formula $C_{29}H_{34}NOCl_2$

Compound (5.5)a (predominant isomer)

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.24-1.31 (m, 1H, 1H of C$\underline{H}_2$), 1.36-1.39 (m, 1H, 1H of C$\underline{H}_2$), 1.69-1.75 (m, 1H, 1H of C$\underline{H}_2$), 1.87-1.95 (m, 3H, 3H of 2 x C$\underline{H}_2$), 2.24-2.26 (m, 1H, 1H of C$\underline{H}_2$), 2.29-2.36 (m, 1H, 1H of C$\underline{H}_2$), 2.41 (s, 6H, C$\underline{H}_3$NC$\underline{H}_3$), 2.50-2.58 (1 x d, J=14.16Hz, and 1 x m, signals overlapping, 2H, 1H of $CH_2CC\underline{H}_2$ and 1H of C$\underline{H}_2$), 2.77 (d, J=14.16Hz, 1H, 1H of $CH_2CC\underline{H}_2$), 2.81-2.86 (m, 1H, 1H of C$\underline{H}_2$), 2.96 (d, J=13.70Hz, 1H, 1H of C$\underline{H}_2CCH_2$), 3.13 (d, J=13.70Hz, 1H, 1H of C$\underline{H}_2CCH_2$), 6.49 (2 x d, J=8.80Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 6.97 (d, J=7.84Hz, 1H, Ar-$\underline{H}$), 7.05 (2 x d, J=7.80Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.09 (2 x d, J=8.80Hz, signals

overlapping, 2H, 2 x Ar-H), 7.16 (t, J=7.34Hz, 1H, Ar-H), 7.21-7.26 (1 x t, J~10.72Hz, and 1 x d, J=8.80Hz, signals overlapping, 3H, 3 x Ar-H), 7.63 (d, J=7.84Hz, 1H, Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.9 ($\underline{C}H_2$), 24.4 ($\underline{C}H_2$), 26.0 ($\underline{C}H_2$), 36.7 ($\underline{C}H_2$), 38.6 ($\underline{C}H_2$), 40.3 ($\underline{C}H_2$), 45.2 ($\underline{C}H_3N\underline{C}H_3$), 2 x 45.7 ($CH_2\underline{C}CH_2$), 60.3 ($\underline{C}H_2$), 76.9 (Ar$\underline{C}$OH), 124.3 (tert. C), 125.4 (tert. C), 125.6 (tert. C), 2 x 127.1 (2 x tert. C), 2 x 127.5 (2 x tert. C), 128.1 (tert. C), 130.9 (quat. C), 131.3 (quat. C), 2 x 131.7 (2 x tert. C), 2 x 132.1 (2 x tert. C), 135.1 (quat. C), 138.2 (quat. C), 138.8 (quat. C), 144.0 (quat. C)

(Z)-3-[3,3-bis(4-chlorobenzyl)-2,3-dihydronaphthalen-4(1H)-ylidene]-*N,N*-dimethyl propan-1-amine (5.6)

To a round bottomed flask containing compound **(5.5)** (0.97g, 2.02mmol) was added a 20% solution of oxalic acid in water (100ml). The resulting mixture was heated till gently refluxing for ten hours and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. On completion, the reaction was allowed to reach room temperature. The reaction mixture was then partitioned between water (1 x 50ml) and diethyl ether (3 x 25ml each). The combined etheral fractions were discarded. The aqueous layer was basified with 2.5M aq. NaOH and the product was extracted with diethyl ether (3x 25ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent removed *in vacuo* to afford the title compound **(5.6)** as a yellow oil (0.69g, 74 %) with the following physical properties;

IR (DCM, v); cm^{-1} 3391.3, 2938.5, 2777.7, 1679.4, 1490.9, 1264.1, 1092.9, 1015.1

HRMS ($+H^+$): actual 464.1899, calculated 464.1912, molecular formula, $C_{29}H_{32}Cl_2N$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.78 (t, J=6.02Hz, 2H, $ArCH_2C\underline{H_2}$), 2.31 (s, 6H, $C\underline{H_3}NC\underline{H_3}$), 2.43 (2 x t, J~6.04Hz, signals overlapping, 2H, $ArC\underline{H_2}CH_2$), 2.50 (2 x t, J=7.28Hz, signals overlapping, 2H, $NC\underline{H_2}CH_2CH$), 2.66 (m, 2H, $NCH_2C\underline{H_2}CH$), 2.78 (2 x d, J=13.56Hz, signals overlapping, 2H, $ArC\underline{H_2}C$), 2.95 (2 x d, J=13.56Hz, signals

overlapping, 2H, ArC$\underline{H_2}$C), 5.70 (t, J=7.02Hz, 1H, C=C$\underline{H}$), 7.07 (d, J=7.00Hz, 1H, Ar-$\underline{H}$), 7.15-7.20 (m, 6H, 6 x Ar-$\underline{H}$), 7.24-7.27 (m, 4H, 4 x Ar-$\underline{H}$), 7.33 (d, J=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 27.3 (ArC$\underline{H_2}$CH$_2$), 28.3 (C=CH$\underline{C}$H$_2$), 33.3 (ArCH$_2\underline{C}$H$_2$), 44.3 (CH$_2\underline{C}$CH$_2$), 2 x 44.8 (Ar$\underline{C}$H$_2$C$\underline{C}$H$_2$Ar), 45.1 ($\underline{C}$H$_3$N$\underline{C}$H$_3$), 59.6 ($\underline{C}$H$_2$NCH$_3$), 124.8 (tert. C), 125.6 (C=C$\underline{H}$), 126.4 (tert. C), 126.7 (tert. C), 4 x 127.4 (4 x tert. C), 128.9 (tert. C), 2 x 131.5 (2 x quat. C), 4 x 132.0 (4 x tert. C), 134.8, 2 x 136.9 (2 x quat. C and $\underline{C}$=CH), 139.7 (quat. C), 139.8 (quat. C)

2,2-bis(4-*tert*-butylbenzyl)-3,4-dihydronaphthalen-1(2H)-one (5.7)

To a stirred solution of α-tetralone (2.00g, 13.68mmol) in *t*-butanol/diethyl ether (40ml, 1:1) was added 4-(*tert*-butyl)benzyl bromide (6.22g, 27.36mmol). To this solution was added potassium *tert*-butoxide (3.07g, 27.36mmol) as a solution in *t*-butanol (20ml), adding the base dropwise over several hours. The reaction was stirred for a total of four hours, and the *t*-butanol was removed by extraction between water (200ml) and diethyl ether (3 x 30ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 50:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the title compound **(5.7)** as a white solid (4.52g, 75%) with the following physical properties;

Mp: 86-97°C

IR (KBr, v); cm^{-1} 3026.5, 2962.0, 1678.5, 1600.1, 1511.7, 1453.8, 1362.4, 1109.9

HRMS (+Na$^+$): actual 461.2804, calculated 461.2820, molecular formula $C_{32}H_{38}ONa$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.31 (s, 18H, 6 x C$\underline{H_3}$), 1.97 (2 x t, J=6.26Hz, signals overlapping, 2H, C$\underline{H_2}$), 2.65 (2 x d, J=13.56Hz, signals overlapping, 2H, C$\underline{H_2}$), 3.09 (2 x t, J=6.28Hz, signals overlapping, 2H, C$\underline{H_2}$), 3.30 (2 x d, J=13.04Hz, signals overlapping, 2H, C$\underline{H_2}$), 7.10 (4 x d, J=8.04Hz, signals overlapping, 4H, 4 x Ar-$\underline{H}$), 7.21 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.27 (4 x d, J=8.00Hz, signals overlapping, 4H, 4 x Ar-$\underline{H}$), 7.33 (t, J=7.28Hz, 1H, Ar-$\underline{H}$), 7.46 (t, J=7.28Hz, 1H, Ar-$\underline{H}$), 8.14 (d, J=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.1 ($\underline{C}H_2$), 28.6 ($\underline{C}H_2$), 6 x 30.9 (6 x $\underline{C}H_3$), 2 x 33.9 {2 x $\underline{C}(CH_3)_3$}, 2 x 40.4 (2 x $\underline{C}H_2$), 50.4 ($CH_2\underline{C}CH_2$), 4 x 124.4 (4 x tert. C), 126.2 (tert. C), 127.7 (tert. C), 128.2 (tert. C), 4 x 130.2 (4 x tert. C), 132.0 (quat. C), 132.6 (tert. C), 2 x 133.8 (2 x quat. C), 142.6 (quat. C), 2 x 148.6 (2 x quat. C), 200.4 ($\underline{C}$=O)

2,2-bis(4-tert-butylbenzyl)-1-[3-(dimethylamino)propyl]-1,2,3,4-tetrahydronaphthalen-1-ol (5.8)

To a dry three-necked round bottomed flask containing Grignard reagent (2.00g, 13.70mmol) in anhydrous THF (5ml), which was synthesized according to the method described previously, was added a solution of compound **(5.7)** (2.00g, 4.57mmol) in anhydrous THF (15ml). The resulting mixture was stirred at room temperature under an atmosphere of nitrogen and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. After a total of sixteen hours aq. NH_4Cl (50ml) was added and the reaction was extracted with diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford the title compound **(5.8)** as a pale yellow crystalline solid (2.61g, 98%) with the following physical properties;

Mp: 79-84°C

IR (KBr, v); cm^{-1} 3556.9, 3025.9, 2960.3, 2781.7, 1512.7, 1463.6, 1268.3, 1021.6

HRMS ($+H^+$): actual 526.4026, calculated 526.4049, molecular formula $C_{37}H_{52}NO$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.31-1.35 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 1.41 {s, 9H, $C(C\underline{H}_3)_3$}, 1.46 {s, 9H, $C(C\underline{H}_3)_3$}, 1.49-1.62 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 1.84-2.00 (m, 3H, 3H of 2 x $C\underline{H}_2$), 2.12-2.17 (m, 1H, 1H of $C\underline{H}_2$), 2.32-2.35 (m, 1H, 1H of $C\underline{H}_2$), 2.36-2.41 (m, 1H, 1H of $C\underline{H}_2$), 2.45 (s, 6H, $C\underline{H}_3NC\underline{H}_3$), 2.62 (d, J=14.06Hz, 1H, 1H of $CH_2CC\underline{H}_2$), 2.75-2.82 (m, 1H, 1H of $C\underline{H}_2$), 2.90-2.94 (m, 1H, 1H of $C\underline{H}_2$), 2.98 (d, J=14.06Hz, 1H, 1H of $CH_2CC\underline{H}_2$), 3.16 (d, J=14.06Hz, 1H, 1H of $C\underline{H}_2CCH_2$), 3.27 (d, J=14.06Hz, 1H, 1H of $C\underline{H}_2CCH_2$), 6.75 (2 x d, J=8.52Hz, signals overlapping, 2H, 2 x

Ar-H), 7.12 (d, J=7.00Hz, 1H, Ar-H), 7.21-7.27 (m, 5H, 5 x Ar-H), 7.32 (t, J=7.54Hz, 1H, Ar-H), 7.40 (2 x d, J=8.52Hz, signals overlapping, 2H, 2 x Ar-H), 7.74 (d, J=7.52Hz, 1H, Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 21.0 ($\underline{C}H_2$), 24.6 ($\underline{C}H_2$), 26.7 ($\underline{C}H_2$), 3 x 31.1 {$C(\underline{C}H_3)_3$}, 3 x 31.2 {$C(\underline{C}H_3)_3$}, 2 x 33.9 {2 x $\underline{C}(CH_3)_3$}, 36.6 ($\underline{C}H_2$), 38.5 ($\underline{C}H_2$), 39.8 ($\underline{C}H_2$), 2 x 45.3 ($\underline{C}H_3N\underline{C}H_3$), 45.7 ($CH_2\underline{C}CH_2$), 60.4 ($\underline{C}H_2$), 77.6 (Ar$\underline{C}$OH) 2 x 124.1 (2 x tert. C), 124.2 (tert. C), 2 x 124.4 (2 x tert. C), 2 x 125.6 (2 x tert. C), 128.1 (tert. C), 2 x 130.3 (tert. C), 2 x 130.6 (2 x tert. C), 135.3 (quat. C), 136.7 (quat. C), 137.3 (quat. C), 144.2 (quat. C), 147.8 (quat. C), 148.1 (quat. C).

(Z)-3-[3,3-bis(4-tert-butylbenzyl)-2,3-dihydronaphthalen-4(1H)-ylidene]-*N,N*-dimethylpropan-1-amine (5.9)

To a round bottomed flask containing compound **(5.8)** (2.00g, 3.81mmol) was added a 20% solution of oxalic acid in water (50ml). The resulting mixture was heated till gentle reflux for eight hours and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. On completion, the reaction was allowed to reach room temperature. The reaction mixture was then partitioned between water (1 x 50ml) and diethyl ether (3 x 25ml each). The combined etheral fractions were discarded. The aqueous layer was basified with 2.5M aq. NaOH and the product was extracted with diethyl ether (3x 25ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent removed *in vacuo* to afford the title compound **(5.9)** as a pale yellow crystalline solid (1.87g, 97%) with the following physical properties;

Mp: 72-76°C

IR (KBr, v); cm^{-1} 3391.3, 2962.3, 2778.1, 1662.5, 1513.5, 1457.5, 1268.5, 1042.1

HRMS ($+H^+$): actual 508.3957, calculated 508.3943, molecular formula, $C_{37}H_{50}N$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.39 {s, 18H, 2 x C(C$\underline{H_3}$)$_3$}, 1.85 (2 x broad t, J=5.84Hz, signals overlapping, 2H, ArCH$_2$C$\underline{H_2}$), 2.34 (s, 6H, C$\underline{H_3}$NC$\underline{H_3}$), 2.45 (2 x t, J=5.84Hz, signals overlapping, 2H, ArC$\underline{H_2}$CH$_2$), 2.49 (2 x t, J~7.60Hz, signals overlapping, 2H, NC$\underline{H_2}$CH$_2$CH), 2.65 (m, 2H, NCH$_2$C$\underline{H_2}$CH), 2.84 (2 x d, J=13.44Hz, signals overlapping, 2H, C$\underline{H_2}$), 2.99 (2 x d, J=14.00Hz, signals overlapping, 2H, C$\underline{H_2}$), 5.65 (broad t, J=7.02Hz, 1H, C$\underline{H}$=C), 7.07-7.09 (m, 1H, Ar-$\underline{H}$), 7.15-7.18 (m, 2H, 2 x Ar-$\underline{H}$), 7.18-7.23 (m, 4H, 4 x Ar-$\underline{H}$), 7.29-7.34 (m, 5H, 5 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 27.4 (C$\underline{H_2}$), 28.3 (C$\underline{H_2}$), 2 x 31.1 {2 x C(C$\underline{H_3}$)$_3$}, 33.5 (C$\underline{H_2}$), 2 x 33.9 {2 x C$\underline{(CH_3)_3}$}, 44.3 (CH$_2$C$\underline{C}$H$_2$), 2 x 44.9 (2 x C$\underline{H_2}$), 2 x 45.1 (C$\underline{H_3}$NC$\underline{H_3}$), 59.7 (C$\underline{H_2}$NCH$_3$), 4 x 124.0 (4 x tert. C), 124.5, 124.9, 126.0 (2 x tert. C and C=C$\underline{H}$), 126.5 (tert. C), 129.0 (tert. C), 4 x 130.4 (4 x tert. C), 135.3 (quat. C), 2 x 135.6 (2 x quat. C), 140.2 (quat. C), 140.4 (quat. C), 2 x 148.2 (quat. C and C=CH)

2,2-bis[4-(trifluoromethoxy)benzyl)]-3,4-dihydronaphthalen-1(2H)-one (5.10)

To a stirred solution of α-tetralone (1.00g, 6.84mmol) in *t*-butanol/diethyl ether (20ml, *v:v*, 1:1) was added 4-(trifluoromethoxy)benzyl bromide (3.49g, 13.68mmol). To this solution was added potassium *tert*-butoxide (1.53g, 13.68mmol) as a solution in *t*-butanol (20ml), adding the base dropwise over an hour. The reaction was stirred for a total of three hours, and the *t*-butanol was removed by extraction between water (100ml) and diethyl ether (3 x 30ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 15:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the title compound **(5.10)** as a yellow oil (2.52g, 75%) with the following physical properties;

IR (DCM, v); cm^{-1} 3068.9, 2931.5, 1681.5, 1601.6, 1508.8, 1455.1, 1163.4, 1020.7

HRMS(+Na$^+$): actual 517.1205, calculated 517.1214, molecular formula $C_{26}H_{20}F_6O_3Na$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.99 (2 x broad t, J=6.28Hz, signals overlapping, 2H, ArCH$_2$C$\underline{H_2}$), 2.71 (2 x d, J=13.30Hz, signals overlapping, 2H, CH$_2$CC$\underline{H_2}$), 3.11 (2 x

broad t, J=6.02Hz, signals overlapping, 2H, ArC$\underline{H}_2$CH$_2$), 3.33 (2 x d, J=13.30Hz, signals overlapping, 2H, CH$_2$CCH$_2$), 7.14 (4 x d, J=8.52Hz, signals overlapping, 4H, 4 x Ar-H), 7.20 and 7.24 (4 x d, J=8.52Hz and 1 x d, J=7.52Hz, signals overlapping, 5H, 5 x Ar-H), 7.36 (t, J=7.52Hz, 1H, Ar-H), 7.50 (t, J=7.52Hz, 1H, Ar-H), 8.16 (d, J=8.04Hz, 1H, Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 24.8 ($\underline{C}H_2$), 29.1 ($\underline{C}H_2$), 2 x 40.4 (2 x $\underline{C}H_2$), 50.2 ($CH_2\underline{C}CH_2$), 4 x 120.0 (4 x tert. C), 120.2 (q, $J_{F\text{-}C}$~260.0Hz, $\underline{C}F_3$), 126.5 (tert. C), 127.7 (tert. C), 128.3 (tert. C), 4 x 131.7 (tert. C), 131.7 (quat. C), 133.1 (tert. C), 2 x 135.4 (2 x quat. C), 142.3 (quat. C), 2 x 147.5 (quat. C), 199.5 ($\underline{C}$=O)

^{19}F NMR ($CDCl_3$, 400MHz) δ_F(ppm): -58.3 (s, $C\underline{F}_3$)

2,2-bis[4-(trifluoromethoxy)benzyl]-1-[3-(dimethylamino)propyl]-1,2,3,4-tetrahydro naphthalen-1-ol (5.11)

To a dry three-necked round bottomed flask containing Grignard reagent (2.00g, 13.70mmol) in anhydrous THF (5ml), which was synthesized according to the method described previously, was added a solution of compound **(5.10)** (1.72g, 3.48mmol) in anhydrous THF (15ml). The resulting mixture was stirred at room temperature under an atmosphere of nitrogen and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. After a total of sixteen hours aq. NH_4Cl (50ml) was added and the reaction was extracted by diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford the title compound **(5.11)** as an amber oil (1.37g, 68%) with the following physical properties;

IR (DCM, v); cm^{-1} 3066.6, 2949.9, 2826.6, 1508.0, 1465.9, 1262.6, 1163.7, 1021.6

HRMS (+H$^+$): actual 582.2449, calculated 582.2443, molecular formula $C_{31}H_{34}NO_3F_6$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.36-1.43 (m, 1H, 1H of NCH$_2$C$\underline{H}_2$CH$_2$),

1.72-1.78 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 1.88-1.95 (m, 3H, 3H of 2 x $C\underline{H}_2$), 2.25-2.32 (m, 1H, 1H of $C\underline{H}_2$), 2.33-2.34 (m, 1H, 1H of $C\underline{H}_2$), 2.36-2.38 (m, 1H, 1H of $C\underline{H}_2$), 2.42 (s, 6H, $C\underline{H}_3NC\underline{H}_3$), 2.46-2.50 (m, 1H, 1H of $C\underline{H}_2$), 2.57 (d, J=14.04Hz, 1H, 1H of $C\underline{H}_2$), 2.79 (d, J=13.71Hz, 1H, 1H of $C\underline{H}_2CCH_2$), 2.84 (broad d, J=13.71Hz, 1H, 1H of $C\underline{H}_2CCH_2$), 3.06 (d, J=13.71Hz, 1H, 1H of $CH_2CC\underline{H}_2$), 3.14 (d, J=13.71Hz, 1H, 1H of $CH_2CC\underline{H}_2$), 6.46 (2 x d, J=8.52Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 6.88 (2 x d, J=8.52Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 6.92 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.11-7.17 (m, 5H, 5 x Ar-$\underline{H}$), 7.23 (t, J=7.54Hz, 1H, Ar-$\underline{H}$), 7.64 (d, J=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.9 ($\underline{C}H_2$), 24.3 ($\underline{C}H_2$), 26.3 ($\underline{C}H_2$), 36.7 ($\underline{C}H_2$), 38.4 ($\underline{C}H_2$), 40.5 ($\underline{C}H_2$), 2 x 45.1 ($\underline{C}H_3N\underline{C}H_3$), 45.7 ($CH_2\underline{C}CH_2$), 60.4 ($\underline{C}H_2$), 76.8 (Ar$\underline{C}$OH), 2 x 119.4 (2 x tert. C), 2 x 120.0 (2 x tert. C), 121.3 (q, $J_{F\text{-}C}$~258.8Hz, $\underline{C}F_3$), 124.3 (tert. C), 125.5 (tert. C), 125.6 (tert. C), 128.0(tert. C), 2 x 131.4 (2 x tert. C), 2 x 131.9 (2 x tert. C), 135.1 (quat. C), 138.6 (quat. C), 139.0 (quat. C), 143.9 (quat. C), 146.7 (quat. C), 147.1 (quat. C)

^{19}F NMR ($CDCl_3$, 400MHz) δ_F(ppm): -58.5 (s, $C\underline{F}_3$)

(Z)-3-{3,3-bis[4-(trifluoromethoxy)benzyl]-2,3-dihydronaphthalen-4(1H)-ylidene}-*N,N*-dimethylpropan-1-amine (5.12)

To a round bottomed flask containing compound **(5.11)** (0.70g, 1.20mmol) was added a 20% solution of oxalic acid in water (50ml). The resulting mixture was heated till gently refluxing for ten hours and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. On completion, the reaction was allowed to reach room temperature. The reaction mixture was then partitioned between water (50ml) and diethyl ether (3 x 25ml each). The combined etheral fractions were discarded. The aqueous layer was basified with 2.5M aq. NaOH and the product was extracted with diethyl ether (3x 25ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All

homogenous fractions were collected and the solvent was removed *in vacuo* to afford the title compound **(5.12)** as a colourless oil (0.53g, 78%) with the following physical properties;

IR (DCM, v); cm^{-1} 3391.3, 2940.9, 2779.2, 1679.4, 1508.2, 1458.4, 1260.9, 1020.0

HRMS ($+H^+$): actual 564.2361, calculated 564.2337, molecular formula, $C_{31}H_{32}NO_2F_6$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm) 1.78 (2 x t, J=6.02Hz, signals overlapping, 2H, $ArCH_2C\underline{H_2}$), 2.27 and 2.25 (2 x s, 6H, $C\underline{H_3}NC\underline{H_3}$), 2.38 (2 x t, J=6.02Hz, signals overlapping, 2H, $ArC\underline{H_2}CH_2$), 2.44 (2 x t, J=7.54Hz, signals overlapping, 2H, $NC\underline{H_2}CH_2CH$), 2.62 (m, 2H, $NCH_2C\underline{H_2}CH$), 2.79 (2 x d, J=13.54Hz, signals overlapping, 2H, $ArC\underline{H_2}CCH_2Ar$), 2.97 (2 x d, J=13.54Hz, signals overlapping, 2H, $ArCH_2CC\underline{H_2}Ar$), 5.65 (t, J=7.02Hz, 1H, ArC=C<u>H</u>), 7.04 (broad d, J~7.56Hz, 1H, Ar-<u>H</u>), 7.10-7.15 (m, 6H, 6 x Ar-<u>H</u>), 7.21-7.29 (m, 5H, 5 x Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 27.2 ($Ar\underline{C}H_2CH_2$), 28.2 ($NCH_2\underline{C}H_2CH$), 33.3 ($ArCH_2\underline{C}H_2$), 44.3 ($CH_2\underline{C}CH_2$), 2 x 44.8 ($Ar\underline{C}H_2C\underline{C}H_2Ar$), 2 x 44.9 ($\underline{C}H_3N\underline{C}H_3$), 59.5 ($N\underline{C}H_2CH_2CH$), 4 x 119.7 (4 x tert. C), 120.7 (q, $J_{F\text{-}C}$~259.5Hz, $\underline{C}F_3$), 124.7 (tert. C), 125.5 (ArC=<u>C</u>H), 126.4 (tert. C), 126.6 (tert. C), 128.8, (tert. C), 4 x 131.8 (4 x tert. C), 134.8 (Ar-<u>C</u>=CH), 2 x 137.1 (2 x quat. C), 139.5 (quat. C), 139.8 (quat. C), 2 x 147.1 (quat. C)

^{19}F NMR ($CDCl_3$, 400MHz) δ_F(ppm): -58.4 (s, $C\underline{F_3}$)

2,2-bis[3-(trifluoromethoxy)benzyl]-3,4-dihydronaphthalen-1(2H)-one (5.13)

To a stirred solution of α-tetralone (1.00g, 6.84mmol) in *t*-butanol/diethyl ether (20ml, 1:1) was added 3-(trifluoromethoxy)benzyl bromide (3.49g, 13.68mmol). To this solution was added potassium *tert*-butoxide (1.53g, 13.68mmol) as a solution in *t*-butanol (20ml), adding the base dropwise over an hour. The reaction was stirred for a total of three hours, and the *t*-butanol was removed by extraction between water (100ml) and diethyl ether (3 x 30ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 15:1).

All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the title compound **(5.13)** as a yellow oil (2.68g, 79%) with the following physical properties;

IR (DCM, v); cm^{-1} 3072.1, 2932.1, 1681.4, 1602.5, 1488.2, 1453.7, 1261.9, 1161.3

HRMS($+Na^+$): actual 517.1224, calculated 517.1214, molecular formula $C_{26}H_{20}F_6O_3Na$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.00 (2 x t, J=6.02Hz, signals overlapping, 2H, $ArCH_2C\underline{H_2}$), 2.73 (2 x d, J=13.04Hz, signals overlapping, 2H, $CH_2CC\underline{H_2}$), 3.08 (2 x t, J=6.02Hz, signals overlapping, 2H, $ArC\underline{H_2}CH_2$), 3.35 (2 x d, J=13.04Hz, signals overlapping, 2H, $C\underline{H_2}CCH_2$), 7.09-7.13 (m, 6H, 6 x Ar-$\underline{H}$), 7.22 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.29 (2 x t, J=8.00Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.34 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.47 (t, J=7.26Hz, 1H, Ar-$\underline{H}$), 8.16 (d, J=8.04Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 24.7 ($\underline{C}H_2$), 29.1 ($\underline{C}H_2$), 2 x 40.8 (2 x $\underline{C}H_2$), 50.3 ($CH_2\underline{C}CH_2$), 2 x 118.6 (2 x tert. C), 119.9 (q, $J_{F\text{-}C}$~259.8Hz, $\underline{C}F_3$), 2 x 122.9 (2 x tert. C), 126.5 (tert. C), 127.7 (tert. C), 128.3 (tert. C), 4 x 131.7 (4 x tert. C), 131.7 (quat. C), 133.1 (tert. C), 2 x 139.0 (2 x quat. C), 142.2 (quat. C), 2 x 148.5 (2 x quat. C), 199.2 ($\underline{C}$=O)

^{19}F NMR ($CDCl_3$, 400MHz) δ_F(ppm):-58.3 (s, $C\underline{F_3}$)

2,2-bis[3-(trifluoromethoxy)benzyl]-1-[3-(dimethylamino)propyl]-1,2,3,4-tetrahydro naphthalen-1-ol (5.14)

To a dry three-necked round bottomed flask containing Grignard reagent (2.00g, 13.70mmol) in anhydrous THF (5ml), which was synthesized according to the method described previously, was added a solution of compound **(5.13)** (1.85g, 3.48mmol) in anhydrous THF (10ml). The resulting mixture was stirred at room temperature under an atmosphere of nitrogen and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. After a total of ten hours aq. NH_4Cl (50ml) was added and the reaction was extracted by diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then

purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford the title compound **(5.14)** as a green oil (0.68g, 39%) with the following physical properties;

IR (DCM, v); cm^{-1} 3066.7, 2950.7, 2826.7, 1609.8, 1487.3, 1447.1, 1261.9, 1022.1

HRMS ($+H^+$): actual 582.2424, calculated 582.2443, molecular formula $C_{31}H_{34}NO_3F_6$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm) 1.36-1.45 (m, 1H, 1H of $NCH_2C\underline{H_2}CH_2$), 1.75-1.81 (m, 1H, 1H of $NCH_2C\underline{H_2}CH_2$), 1.89-1.94 (m, 3H, 3H of 2 x $C\underline{H_2}$), 2.25-2.41 (m, 3H, 3 of 2 x $C\underline{H_2}$), 2.43 (s, 6H, $C\underline{H_3}NC\underline{H_3}$), 2.46-2.59 (m, 2H, $C\underline{H_2}$), 2.82 (2 x d, J=14.04Hz, 2H, $C\underline{H_2}CCH_2$), 3.07 (d, J=13.54Hz, 1H, 1H of $CH_2CC\underline{H_2}$), 3.17 (d, J=13.54Hz, 1H, 1H of $C\underline{H_2}CCH_2$), 6.34 (s, 1H, Ar-$\underline{H}$), 6.40 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 6.93-7.00 (m, 3H, 3 x Ar-$\underline{H}$), 7.05 and 7.09 (3 x t, J~7.52Hz, J~9.04Hz, signals overlapping, 3H, 3 x Ar-$\underline{H}$), 7.16 (dt, J_1=1.50Hz, J_2=7.54Hz, 1H, Ar-$\underline{H}$), 7.24 (t, J=7.54Hz, 1H, Ar-$\underline{H}$), 7.29 (t, J=7.78Hz, 1H, Ar-$\underline{H}$), 7.65 (d, J=8.04Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 20.9 ($\underline{C}H_2$), 24.3 ($\underline{C}H_2$), 26.1 ($\underline{C}H_2$), 36.7 ($\underline{C}H_2$), 39.0 ($\underline{C}H_2$), 40.9 ($\underline{C}H_3NCH_3$), 45.0 ($CH_3N\underline{C}H_3$), 45.9 ($CH_2\underline{C}CH_2$), 60.4 ($\underline{C}H_2$), 76.9 (Ar$\underline{C}$OH), 117.5 (tert. C), 117.9 (tert. C), 120.6 (q, $J_{F\text{-}C}$~260.0Hz, $\underline{C}F_3$), 122.7 (tert. C), 123.3 (tert. C), 124.3 (tert. C), 125.5 (tert. C), 125.7 (tert. C), 2 x 128.1 (2 x tert. C), 128.6 (tert. C), 128.8 (tert. C), 129.2 (tert. C), 135.0 (quat. C), 142.1 (quat. C), 142.5 (quat. C), 143.9 (quat. C), 148.1 (quat. C), 148.5 (quat. C)

^{19}F NMR ($CDCl_3$, 400MHz) δ_F(ppm): -58.20 (s, $C\underline{F_3}$)

(Z)-3-{3,3-bis[3-(trifluoromethoxy)benzyl]-2,3-dihydronaphthalen-4(1H)-ylidene}-*N,N*-dimethylpropan-1-amine (5.15)

To a round bottomed flask containing compound **(5.14)** (0.58g, 1.03mmol) was added a 20% solution of oxalic acid in water (40ml). The resulting mixture was heated till gently refluxing for eight hours and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. On completion, the reaction was allowed to reach room temperature.

The reaction mixture was then partitioned between water (50ml) and diethyl ether (3 x 25ml each). The combined etheral fractions were discarded. The aqueous layer was basified with 2.5M aq. NaOH and the product was extracted with diethyl ether (3x 25ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent removed *in vacuo* to afford the title compound **(5.15)** as a colourless oil (0.56g, 71%) with the following physical properties;

IR (DCM, v); cm^{-1} 3400.8, 2941.94, 2770.0, 1683.3, 1611.2, 1487.9, 1447.6, 1260.3

HRMS ($+H^+$): actual 564.2341, calculated 564.2337, molecular formula, $C_{31}H_{32}NO_2F_6$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.76 (2 x t, J=6.52Hz, signals overlapping, 2H, $ArCH_2C\underline{H_2}$), 2.24 (s, 6H, $C\underline{H_3}NC\underline{H_3}$), 2.40 and 2.42 (2 x t, J~5.52Hz and 2 x t, J~8.04Hz, signals overlapping, 4H, 2H of $ArC\underline{H_2}CH_2$ and 2H of $NC\underline{H_2}CH_2CH$), 2.59 (m, 2H, $NCH_2C\underline{H_2}CH$), 2.79 (2 x d, J=13.56Hz, signals overlapping, 2H, $ArCH_2CC\underline{H_2}Ar$), 2.92 (2 x d, J=13.56Hz, signals overlapping, 2H, $ArC\underline{H_2}CCH_2Ar$), 5.64 (t, J=7.02Hz, 1H, Ar-C=C<u>H</u>), 7.01-7.14 (m, 9H, 9 x Ar-<u>H</u>), 7.22-7.26 (m, 3H, 3 x Ar-<u>H</u>);

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 26.9 ($ArC\underline{H_2}CH_2$), 28.1 ($NCH_2C\underline{H_2}CH$), 33.3 ($ArCH_2C\underline{H_2}$), 44.2 ($CH_2C\underline{C}H_2$), 2 x 44.7 ($ArC\underline{H_2}C\underline{C}H_2Ar$), 2 x 44.9 ($C\underline{H_3}NC\underline{H_3}$), 59.5 ($NC\underline{H_2}CH_2CH$), 2 x 118.2 (2 x tert. C), 120.4 (q, $J_{F\text{-}C}$~259.8Hz, $C\underline{F_3}$), 2 x 123.0 (2 x tert. C), 124.7 (tert. C), 125.4 (Ar-C=<u>C</u>H), 126.4 (tert. C), 126.7 (tert. C), 2 x 128.4 (2 x tert. C), 128.91 (tert. C), 2 x 129.0 (2 x tert. C), 134.6 (Ar-<u>C</u>=CH), 139.5 (quat. C), 139.7 (quat. C), 2 x 140.5 (2 x quat. C), 2 x 148.3 (2 x quat. C)

^{19}F NMR ($CDCl_3$, 400MHz) δ_F(ppm): -58.1 (s, $C\underline{F_3}$)

3,4-dihydro-2-[(pyridin-2-yl)methyl]naphthalen-1(2H)-one (5.16)

3,4-dihydro-2-hydroxy-2-[(pyridin-2-yl)methyl]naphthalen-1(2H)-one (5.17)

2-{[1,2-dihydro-3-((pyridin-2-yl)methyl)naphthalen-4-yloxy]methyl}pyridine (5.18)

3,4-dihydro-2,2-bis[(pyridin-2-yl)methyl]naphthalen-1(2H)-one (5.19)

To a stirred solution of α-tetralone (1.00g, 6.84mmol) in *t*-butanol/diethyl ether (20ml,

1:1) was added 2-(bromomethyl)pyridine hydrobromide (3.46g, 13.68mmol). To this solution was added potassium *tert*-butoxide (2.30g, 20.52mmol) as a solution in *t*-butanol (10ml), adding the base dropwise over an hour. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 1:1). After two hours, an additional solution of potassium *tert*-butoxide (0.76g, 6.84mmol) in *t*-butanol (3ml) was added to the reaction. The reaction mixture was stirred at room temperature for a total of ten hours, and the *t*-butanol was removed by extraction between water (100ml) and diethyl ether (3 x 30ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 3:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the title compounds **(5.16)** as a brown oil (0.12g, 7.4%), **(5.17)** as a orange solid (0.02g, 1.2%), **(5.18)** as a dark brown oil (0.16g, 7.1%) and **(5.19)** as a dark brown oil (0.67g, 30%) with the following physical properties;

Compound (5.16)

IR (DCM, v); cm^{-1} 3361.0, 2928.6, 1681.9, 1592.2, 1474.0, 1434.5, 1294.2, 1223.0

HRMS ($+H^+$): actual 238.1237, calculated 238.1232, molecular formula $C_{16}H_{16}NO$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.81-1.94 (m, 1H, 1H of $CHC\underline{H}_2CH_2$), 2.13-2.20 (m, 1H, 1H of $CHC\underline{H}_2CH_2$), 2.84 (dd, J_1=8.54Hz, J_2=14.06Hz, 1H, 1H of $C\underline{H}_2$), 2.92-3.07 (m, 2H, $C\underline{H}_2$), 3.11-3.19 (m, 1H, 1H of $CH_2C\underline{H}CH_2$), 3.62 (dd, J_1=5.04Hz, J_2=14.04Hz, 1H, 1H of $C\underline{H}_2$), 6.89 (dd, J_1~5.52Hz, J_2~7.04Hz, 1H, Ar-$\underline{H}$), 7.22-7.32 (m, 3H, 3 x Ar-$\underline{H}$), 7.46 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.62 (dt, J_1=1.67Hz, J_2=7.66Hz, 1H, Ar-$\underline{H}$), 8.05 (d, J=8.04Hz, 1H, Ar-$\underline{H}$), 8.54 (d, J=5.00Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 28.2 ($\underline{C}H_2$), 28.5 ($\underline{C}H_2$), 37.8 ($\underline{C}H_2$), 47.7 ($CH_2\underline{C}HCH_2$), 120.8 (tert. C), 123.5 (tert. C), 126.1 (tert. C), 127.0 (tert. C), 128.3 (tert. C), 132.0 (quat. C), 132.8 (tert. C), 135.9 (tert. C), 143.7 (quat. C), 148.8 (tert. C), 159.8 (quat. C), 199.0 ($\underline{C}$=O)

Compound (5.17)

Mp: 55-58°C

IR (KBr, v); cm^{-1} 3425.1, 2922.7, 1687.6, 1592.9, 1435.3, 1289.1, 1221.4

HRMS ($+H^+$): actual 254.1188, calculated 254.1181, molecular formula $C_{16}H_{16}NO_2$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.20 and 2.22 (1 x t, J~4.89Hz and 1 x t, J~3.80Hz, signals overlapping, 2H, OHCC$\underline{H_2}$CH$_2$), 3.08-3.14 (m, 1H, 1H of OHCCH$_2$C$\underline{H_2}$), 3.15 (s, 2H, NCC$\underline{H_2}$COH), 3.30-3.39 (m, 1H, 1H of OHCCH$_2$C$\underline{H_2}$), 7.20 (t, J=6.19Hz, 1H, Ar-$\underline{H}$), 7.29 (1 x t, J~7.00Hz and 1 x t, J~4.04Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.36 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.54 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.65 (dt, J_1=1.33Hz, J_2=7.66Hz, 1H, Ar-$\underline{H}$), 8.03 (d, J=8.04Hz, 1H, Ar-$\underline{H}$), 8.52 (d, J=5.00Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.8 ($\underline{C}H_2$), 33.8 ($\underline{C}H_2$), 42.5 ($\underline{C}H_2$), 75.5 (CH$_2\underline{C}$CH$_2$), 121.4 (tert. C), 124.5 (tert. C), 126.4 (tert. C), 127.7 (tert. C), 128.5 (tert. C), 130.2 (quat. C), 133.4 (tert. C), 136.0 (tert. C), 143.1 (quat. C), 148.2 (tert. C), 156.9 (quat. C), 199.5 ($\underline{C}$=O)

Compound (5.18)

IR (DCM, v); cm^{-1} 3400.3, 3064.3, 2930.3, 1650.5, 1590.6, 1434.4, 1298.4, 1080.0

HRMS ($+H^+$): actual 329.1648, calculated 329.1654, molecular formula $C_{22}H_{21}N_2O$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.28 (2 x t, J=8.02Hz, signals overlapping, 2H, C=CC$\underline{H_2}$CH$_2$), 2.76 (2 x t, J=8.04Hz, signals overlapping, 2H, C=CCH$_2$C$\underline{H_2}$), 3.87 (s, 2H, C=CC$\underline{H_2}$CN), 4.99 (s, 2H, COC$\underline{H_2}$CN), 7.07 (t, J=6.26Hz, 1H, Ar-$\underline{H}$), 7.11-7.21 (m, 5H, 5 x Ar-$\underline{H}$), 7.46 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.53 (broad t, J~7.78Hz, 1H, Ar-$\underline{H}$), 7.65 (d, J=8.00Hz, 1H, Ar-$\underline{H}$), 7.71 (broad t, J~7.78Hz, 1H, Ar-$\underline{H}$), 8.49 (d, J=5.00Hz, 1H, Ar-$\underline{H}$), 8.57 (d, J=4.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 26.1 ($\underline{C}H_2$), 27.5 ($\underline{C}H_2$), 38.6 ($\underline{C}H_2$), 73.9 (CO$\underline{C}$H$_2$CN), 120.8 (tert. C), 121.1 (tert. C), 121.2 (tert. C), 122.2 (tert. C), 122.3 (tert. C), 123.2 (quat. C), 126.0 (tert. C), 126.7 (tert. C), 127.0 (tert. C), 130.8 (quat. C), 136.0 (tert. C), 136.3 (tert. C), 136.4 (quat. C), 147.7 (quat. C), 2 x 148.7 (2 x tert. C), 157.2 (quat. C), 159.6 (quat. C)

Compound (5.19)

IR (DCM, v); cm^{-1} 3386.5, 3062.7, 2927.5, 1677.3, 1588.3, 1434.3, 1292.0, 1223.1

HRMS($+H^+$): actual 329.1661, calculated 329.1654, molecular formula $C_{22}H_{21}N_2O$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm) 1.95 (2 x t, J=6.28Hz, signals overlapping, 2H, $COCC\underline{H_2}CH_2$), 2.84 (2 x d, J=13.30Hz, signals overlapping, 2H, $CC\underline{H_2}CN$), 2.98 (2 x t, J=6.28Hz, signals overlapping, 2H, $COCCH_2C\underline{H_2}$), 3.36 (2 x d, J=13.30Hz, signals overlapping, 2H, $CC\underline{H_2}CN$), 6.89 (2 x dd, J_1~0.96Hz, J_2~5.03Hz, signals overlapping, 2H, 2 x Ar-H), 7.03 (d, J=7.52Hz, 1H, Ar-H), 7.10 (2 x d, J=7.52Hz, signals overlapping, 2H, 2 x Ar-H), 7.13 (t, J=7.04Hz, 1H, Ar-H), 7.26 (dt, J_1=1.35Hz, J_2=7.28Hz, 1H, Ar-H), 7.35 (2 x dt, J_1=1.83Hz, J_2=7.52Hz, signals overlapping, 2H, 2 x Ar-H), 7.98 (dd, J_1=1.05Hz, J_2=8.05Hz, 1H, Ar-H), 8.33 (2 x d, J=4.62Hz, signals overlapping, 2H, 2 x Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 24.9 ($\underline{C}H_2$), 29.0 ($\underline{C}H_2$), 2 x 42.7 ($\underline{C}H_2C\underline{C}H_2$), 50.2 ($CH_2\underline{C}CH_2$), 2 x 120.8 (2 x tert. C), 2 x 124.9 (2 x tert. C), 126.0 (tert. C), 127.5 (tert. C), 128.2 (tert. C), 131.7 (quat. C), 132.6 (tert. C), 2 x 135.4 (2 x tert. C), 142.9 (quat. C), 2 x 148.3 (2 x tert. C), 2 x 157.6 (2 x quat. C), 199.7 ($\underline{C}$=O)

1-[3-(dimethylamino)propyl]-1,2,3,4-tetrahydro-2,2-bis[(pyridin-2-yl)methyl]naphthalen-1-ol (5.20)

To a dry three-necked round bottomed flask containing Grignard reagent (2.00g, 13.70mmol) in anhydrous THF (5ml), which was synthesized according to the method described previously, was added a solution of compound **(5.19)** (0.67g, 2.04mmol) in anhydrous THF (10ml). The resulting mixture was stirred at room temperature under an atmosphere of nitrogen and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. After a total of eight hours aq. NH_4Cl (50ml) was added and the reaction was extracted by diethyl ether (3 x 20ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford the title compound **(5.20)** as a yellow oil (0.64g, 75%) with the following physical properties;

IR (DCM, v); cm^{-1} 3376.8, 3067.0, 2939.3, 1594.2, 1569.1, 1472.4, 1433.5, 1039.8

HRMS ($+H^+$): actual 416.2705, calculated 416.2702, molecular formula $C_{27}H_{34}N_3O$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.19-1.27 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 1.76 (dt, J_1=4.52Hz, J_2=12.04Hz, 1H, 1H of $ArCH_2C\underline{H}_2$), 1.87-1.92 (m, 1H, 1H of $NCH_2C\underline{H}_2CH_2$), 2.06 (dt, J_1~3.78Hz, J_2~12.30Hz, 1H, 1H of $ArCH_2C\underline{H}_2$), 2.10-2.22 (1 x s and 1 x m, singals overlapping, 9H, 6H of $C\underline{H}_3NC\underline{H}_3$ and 3H of 2 x $C\underline{H}_2$), 2.67 (d, J=15.04Hz, 1H, 1H of $C\underline{H}_2CCH_2$), 2.89 (dt, J_1=4.52Hz, J_2=8.04Hz, 1H, 1H of $ArC\underline{H}_2CH_2$), 3.02-3.11 (m, signals overlapping, 3H, 2H of $C\underline{H}_2CC\underline{H}_2$ and 1H of $ArC\underline{H}_2CH_2$), 3.38 (d, J=13.04, 1H, 1H of $CH_2CC\underline{H}_2$), 6.55 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 6.91 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.00-7.06 (m, 2H, 2 x Ar-$\underline{H}$), 7.12 (2 x t, J=5.76Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.18 (t, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.32 (dt, J_1=1.52Hz, J_2=7.54Hz, 1H, Ar-$\underline{H}$), 7.48 (dt, J_1=1.70Hz, J_2=7.57Hz, 1H, Ar-$\underline{H}$), 7.82 (d, J=8.04Hz, 1H, Ar-$\underline{H}$), 8.47 (d, J=4.52Hz, 1H, Ar-$\underline{H}$), 8.53 (d, J=4.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 22.0 ($\underline{C}H_2$), 24.9 ($\underline{C}H_2$), 30.9 ($\underline{C}H_2$), 36.9 ($\underline{C}H_2$), 39.2 ($\underline{C}H_2$), 42.5 ($\underline{C}H_2$), 2 x 45.0 ($\underline{C}H_3N\underline{C}H_3$), 45.5 ($CH_2\underline{C}CH_2$), 60.0 ($\underline{C}H_2$), 76.5 ($Ar\underline{C}OH$), 120.2 (tert. C), 120.5 (tert. C), 124.4 (tert. C), 124.8 (tert. C), 125.4 (tert. C), 125.5 (tert. C), 127.2(tert. C), 127.5 (tert. C), 133.9 (quat. C), 135.2 (tert. C), 135.9 (tert. C), 142.1 (quat. C), 147.1 (tert. C), 147.4 (tert. C), 159.7 (quat. C), 160.1 (quat. C)

Attempted synthesis of (Z)-3-{2,3-dihydro-3,3-bis[(pyridin-2-yl)methyl]naphthal ene-4(1H)-ylidene}-*N,N*-dimethylpropan-1-amine

To a round bottomed flask containing compound **(5.20)** (0.40g, 0.96mmol) was added a 20% solution of oxalic acid in water (30ml). The resulting mixture was heated till gently refluxing and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1). After ten hours the reaction was allowed to reach room temperature. The reaction mixture was then partitioned between water (50ml) and diethyl ether (3 x 25ml each). The combined etheral fractions were discarded. The aqueous layer was basified with 2.5M aq. NaOH and the product was extracted with diethyl ether (3x 25ml). The combined organic extracts were dried over magnesium

sulphate, filtered and concentrated *in vacuo.* The residue was then purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate:triethylamine, 10:10:1). All homogenous fractions were collected and the solvent was removed *in vacuo* to afford the starting materials unchanged.

6,6-dibenzyl-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (5.21)

The procedure for compound **(5.0)** was repeated for compound **(5.21)**, using the following quantities of 1-benzosuberone (2.00g, 12.50mmol), *t*-butanol/diethyl ether (40ml, 1:1), benzyl bromide (4.28g, 25.00mmol), potassium *tert*-butoxide (2.81g, 25.00mmol) as a solution in *t*-butanol (20ml); to yield the title compound **(5.21)** as a white crystalline solid (2.52g, 59%) with the following physical properties;

Mp: 83-87°C

IR (KBr); cm^{-1} 3396.1, 2942.9, 1674.9, 1600.6, 1494.7, 1452.9, 1235.6, 1031.0

HRMS ($+Na^+$): actual 363.1737, calculated 363.1725, molecular formula $C_{25}H_{24}ONa$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.82 (2 x t, J=6.54Hz, signals overlapping, 2H, $COCC\underline{H_2}CH_2CH_2$) 2.15 (2 x p, J=6.85Hz, signals overlapping, 2H, $COCCH_2C\underline{H_2}CH_2$), 2.82 (2 x t, J=7.04Hz, signals overlapping, 2H, $COCCH_2CH_2C\underline{H_2}$), 2.88 (2 x d, J=13.30Hz, signals overlapping, 2H, $COCC\underline{H_2}Ar$), 3.25 (2 x d, J=13.30Hz, signals overlapping, 2H, $COCC\underline{H_2}Ar$), 6.92 (d, J=7.52Hz, 1H, Ar-$\underline{H}$), 7.04 and 7.09 (1 x d, J~7.52Hz and 1 x t, J~7.75Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.23-7.32 (2 x m, 11H, 11 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 23.0 ($\underline{C}H_2$), 28.0 ($\underline{C}H_2$), 30.9 ($\underline{C}H_2$), 2 x 43.0 (2 x $\underline{C}H_2$), 53.8 ($CH_2\underline{C}CH_2$), 125.9 (tert. C), 2 x 126.1 (2 x tert. C), 126.9 (tert. C), 127.3 (tert. C), 4 x 127.6 (4 x tert. C), 130.5 (tert. C), 4 x 130.7 (4 x tert. C), 136.5 (quat. C), 2 x 136.6 (2 x quat. C), 141.3 (quat. C), 213.9 ($\underline{C}$=O)

Attempted synthesis of 6,6-dibenzyl-5-[3-(dimethylamino)propyl]-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-ol

The procedure for compound **(5.1)** was repeated for 6,6-dibenzyl-5-[3-(dimethylamino)propyl]-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-ol using the following quantities of Grignard reagent (2.00g, 13.70mmol), anhydrous THF (15ml), compound **(5.1)** (1.00g, 2.45mmol). The resultant solution was allowed to stir at room temperature for three days. The reaction was monitored by TLC and the starting material was recovered unchanged.

6,6-bis(4-chlorobenzyl)-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (5.22)

The procedure for compound **(5.3)** was repeated for compound **(5.22)**, using the following quantities of 1-benzosuberone (2.00g, 12.50mmol), *t*-butanol/diethyl ether (40ml, 1:1), 4-chlorobenzyl chloride (4.0g, 25.00mmol), potassium *tert*-butoxide (2.81g, 25.00mmol) as a solution in *t*-butanol (20ml); to yield the title compound **(5.22)** as a yellow oil (1.22g, 24%) with the following physical properties;

IR (DCM, v); cm^{-1} 3400.4, 2941.6, 1673.8, 1597.8, 1491.1, 1234.2, 1093.7, 1015.2

HRMS ($+Na^+$): actual 431.0959, calculated 431.0945, molecular formula $C_{25}H_{22}Cl_2ONa$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.73 (2 x t, J=6.52Hz, signals overlapping, 2H, $COCC\underline{H_2}CH_2$), 2.05-2.11 (m, 2H, $COCCH_2C\underline{H_2}$), 2.76 (2 x d, J=13.28Hz, signals overlapping, 2H, $CC\underline{H_2}Ar$), 2.78 (2 x t, J~7.02Hz, signals overlapping, 2H, $COCCH_2CH_2C\underline{H_2}$), 3.15 (2 x d, J=13.28Hz, signals overlapping, 2H, $CC\underline{H_2}Ar$), 6.95 (broad d, J~7.52Hz, 1H, Ar-H), 7.03 (broad d, J~7.52Hz, 1H, Ar-H), 7.09-7.15 (m, 5H, 5 x Ar-H), 7.23-7.29 (m, 5H, 5 x Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 22.8 ($\underline{C}H_2$), 28.2 ($\underline{C}H_2$), 31.0 ($\underline{C}H_2$), 2 x 42.3 (2 x $\underline{C}H_2$), 53.6 ($CH_2\underline{C}CH_2$), 126.1 (tert. C), 127.0 (tert. C), 127.6 (tert. C), 4 x 127.8 (4 x tert. C), 130.8 (tert. C), 4 x 131.8 (4 x tert. C), 2 x 132.1 (2 x quat. C), 2 x 134.9 (quat. C), 136.6 (quat. C), 140.9 (quat. C), 213.0 ($\underline{C}$=O)

Attempted synthesis of 6,6-bis(4-chlorobenzyl)-5-[3-(dimethylamino)propyl]-6,7, 8,9-tetrahydro-5H-benzo[7]annulen-5-ol

The procedure for compound **(5.5)** was repeated for 6,6-bis(4-chlorobenzyl)-5-[3-(dimet hylamino)propyl]-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-ol using the following quantities of Grignard reagent (2.00g, 13.70mmol), anhydrous THF (15ml), compound **(5.22)** (1.00g, 2.45mmol). The resultant solution was allowed to stir at room temperature for three days. The reaction was monitored by TLC and the starting material was recovered unchanged.

2,2-dibenzyl-2,3-dihydroinden-1-one (5.23)

The procedure for compound **(5.0)** was repeated for compound **(5.23)**, using the following quantities of 1-indanone (2.00g, 15.15mmol), *t*-butanol/diethyl ether (20ml, 1:1), benzyl bromide (5.18g, 30.30mmol), potassium *tert*-butoxide (3.39g, 30.30mmol) as a solution in *t*-butanol (5ml); to yield the title compound **(5.23)** as a pale yellow crystalline solid (3.96g, 84%) with the following physical properties;

Mp: 87-95°C

IR (KBr, v); cm^{-1} 3400.5, 3028.4, 2917.1, 1705.5, 1607.4, 1495.2, 1296.2, 1029.5

HRMS ($+Na^+$): actual 335.1410, calculated 335.1412, molecular formula: $C_{23}H_{20}ONa$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.95 (2 x d, J=13.54Hz, signals overlapping, 2H, $ArC\underline{H_2}CCH_2Ar$), 3.16 (2 x s, signals overlapping, 2H, $ArC\underline{H_2}CCO$), 3.32 (2 x d, J=13.54Hz, signals overlapping, 2H, $ArCH_2CC\underline{H_2}Ar$), 7.13-7.21 (m, 12H, 12 x Ar-$\underline{H}$), 7.34 (t, J=7.28Hz, 1H, Ar-$\underline{H}$), 7.73 (d, J=8.04Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 34.2 ($Ar\underline{C}H_2CCO$), 2 x 43.2 ($Ar\underline{C}H_2C\underline{C}H_2Ar$), 54.8 ($CH_2\underline{C}CH_2$), 123.2 (tert. C), 125.8 (tert. C), 2 x 126.2 (2 x tert. C), 126.7 (tert. C), 4 x 127.7 (4 x tert. C), 4 x 130.0 (4 x tert. C), 134.3 (tert. C), 136.6 (quat. C), 2 x 136.8 (2 x quat. C), 152.7 (quat. C), 210.0 ($\underline{C}$=O)

2,2-dibenzyl-1-(3-(dimethylamino)propyl)-2,3-dihydro-1H-inden-1-ol (5.24)

The procedure for alcohol **(5.1)** was repeated for alcohol **(5.24)**, using the following

quantities of Grignard reagent (2.00g, 13.70mmol), anhydrous THF (15ml), compound **(5.23)** (1.00g, 3.21mmol); to yield the title compound **(5.24)** as a colourless oil (1.28g, 98%) with the following physical properties;

IR (DCM, v); cm^{-1} 3405.8, 3025.5, 2824.2, 1601.9, 1495.2, 1454.7, 1265.2, 1033.0

HRMS ($+H^+$): actual 400.2626, calculated 400.2640, molecular formula: $C_{28}H_{34}NO$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.53-1.58 (m, 1H, 1H of C$\underline{H}_2$), 1.83-1.92 (m, 1H, 1H of C$\underline{H}_2$), 2.03-2.14 (m, 2H, C$\underline{H}_2$), 2.40-2.47 (m, 2H, C$\underline{H}_2$), 2.50 (s, 6H, 2 x C$\underline{H}_3$), 2.69 (d, J=15.57Hz, 1H, 1H of C$\underline{H}_2$), 2.83 (d, J=13.56Hz, 1H, 1H of C$\underline{H}_2$), 2.91 (d, J=16.04Hz, 1H, 1H of C$\underline{H}_2$), 3.09 (d, J=16.04Hz, 1H, 1H of C$\underline{H}_2$), 3.19 (d, J=14.04Hz, 1H, 1H of C$\underline{H}_2$), 3.24 (d, J=15.56Hz, 1H, 1H of C$\underline{H}_2$), 6.86 (2 x t, J=3.50Hz, signals overlapping 2H, 2 x Ar-$\underline{H}$), 7.17 and 7.18 (4 x d, J~2.52Hz and J~4.00Hz, signals overlapping, 4H, 4 x Ar-$\underline{H}$), 7.27 (t, J=7.02Hz, 1H, Ar-$\underline{H}$), 7.34 (2 x dd, J_1=7.52Hz, J_2=15.08Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.44 (2 x t, J=7.28Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.48 (t, J=7.02Hz, 1H, Ar-$\underline{H}$), 7.69 (2 x d, J=7.52Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 21.3 ($\underline{C}H_2$), 35.5 ($\underline{C}H_2$), 36.7 ($\underline{C}H_2$), 39.6 ($\underline{C}H_2$), 39.9 ($\underline{C}H_2$), 2 x 45.2 ($\underline{C}H_3N\underline{C}H_3$), 56.3 ($CH_2\underline{C}CH_2$), 60.4 ($N\underline{C}H_2CH_2CH_2$), 84.2 (Ar$\underline{C}$OH), 123.9 (tert. C), 124.3 (tert. C), 125.1 (tert. C), 125.3 (tert. C), 125.4 (tert. C), 126.8 (tert. C), 2 x 127.0 (2 x tert. C), 2 x 127.8 (2 x tert. C), 2 x 130.3 (2 x tert. C), 2 x 130.6 (2 x tert. C), 139.1 (quat. C), 139.6 (quat. C), 139.7 (quat. C), 149.2 (quat. C)

(Z)-3-(2,2-dibenzyl-1,2-dihydroinden-3-ylidene)-*N,N*-dimethylpropan-1-amine (5.25)

The procedure for compound **(5.2)** was repeated for compound **(5.25)**, using the following quantities of compound **(5.24)** (0.74g, 1.85mmol), a 20% solution of oxalic acid in water (20ml); to yield the title compound **(5.25)** as a yellow oil (0.62g, 87%) with the following physical properties;

IR (DCM, v); cm^{-1} 3400.7, 2854.6, 1648.1, 1601.9, 1494.2, 1453.9, 1154.6, 1041.6

HRMS ($+H^+$): actual 382.2522, calculated 382.2535, molecular formula $C_{28}H_{32}N$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.45 (s, 6H, $C\underline{H_3}NC\underline{H_3}$), 2.59 (2 x t, J~7.76Hz, signals overlapping, 2H, $NC\underline{H_2}CH_2CH$), 2.84 (m, 2H, $NCH_2C\underline{H_2}CH$), 2.95 (2 x d, J=13.56Hz, signals overlapping, 2H, $ArCH_2CC\underline{H_2}Ar$), 3.05 and 3.08 (2 x d, J=6.52Hz and 2 x d, J=13.56Hz, signals overlapping, 4H, $ArC\underline{H_2}C$ and $ArC\underline{H_2}CCH_2Ar$), 5.40 (t, J=7.02Hz, 1H, $C\underline{H}$=C), 7.23-7.36 (2 x m, 13H, 13 x Ar-$\underline{H}$), 7.70 (d, J=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 26.9 ($NCH_2\underline{C}H_2CH$), 40.8 ($Ar\underline{C}H_2C$), 2 x 45.0 ($Ar\underline{C}H_2C\underline{C}H_2Ar$), 2 x 45.2 ($\underline{C}H_3N\underline{C}H_3$), 51.7 ($CH_2\underline{C}CH_2$), 59.0 ($N\underline{C}H_2CH_2CH$), 122.2 (C=$\underline{C}$H), 124.4 (tert. C), 124.7 (tert. C), 2 x 125.7 (2 x tert. C), 125.9 (tert. C), 127.0 (tert. C), 3 x 127.4 (3 x tert. C), 127.5 (tert. C), 130.2 (tert. C), 3 x 130.7 (3 x tert. C), 2 x 138.4 (2 x quat. C), 140.3, 144.1, 145.3 (2 x quat. C and $\underline{C}$=CH)

2,2-bis(4-chlorobenzyl)-2,3-dihydroinden-1-one (5.26)

The procedure for compound **(5.3)** was repeated for compound **(5.26)**, using the following quantities of 1-indanone (2.00g, 15.15mmol), *t*-butanol/diethyl ether (20ml, *v:v*, 1:1), 4-chlorobenzyl chloride (4.88g, 30.30mmol), potassium *tert*-butoxide (3.39g, 30.30mmol) as a solution in *t*-butanol (5ml); to yield the title compound **(5.26)** as a yellow crystalline solid (5.07g, 88%) with the following physical properties;

Mp: 88-95°C

IR (KBr, v); cm^{-1} 3395.6, 3030.6, 2919.0, 1706.5, 1607.6, 1491.8, 1297.1, 1094.5

HRMS ($+Na^+$): actual 403.0627, calculated 403.0632, molecular formula: $C_{23}H_{18}OCl_2Na$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.79 (2 x d, J=13.56Hz, signals overlapping, 2H, $CC\underline{H_2}Ar$), 3.02 (s, 2H, $ArC\underline{H_2}CCO$), 3.18 (2 x d, J=13.56Hz, signals overlapping, 2H, $CC\underline{H_2}Ar$), 7.03 (2 x d, J=8.56Hz, signals overlapping, 4H, 4 x Ar-$\underline{H}$), 7.12 (2 x d, J=8.56Hz, signals overlapping, 4H, 4 x Ar-$\underline{H}$), 7.17 (d, J=8.00Hz, 1H, Ar-$\underline{H}$), 7.23 (t, J=7.28Hz, 1H, Ar-$\underline{H}$), 7.40 (dt, J_1~1.00Hz, J_2~7.28Hz, 1H, Ar-$\underline{H}$), 7.64 (d, J=7.52Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 34.0 ($\underline{C}H_2$), 2 x 42.4 (2 x $\underline{C}H_2$), 54.5 ($CH_2\underline{C}CH_2$), 123.3 (tert. C), 125.7 (tert. C), 126.9 (tert. C), 4 x 127.8 (4 x tert. C), 4 x 131.1 (4 x tert. C), 132.0 (tert. C), 2 x 134.6 (2 x quat. C), 2 x 135.0 (2 x quat. C), 136.2 (quat. C), 152.2

(quat. C), 209.4 (C=O)

2,2-bis(4-chlorobenzyl)-1-(3-(dimethylamino)propyl)-2,3-dihydro-1H-inden-1-ol (5.27)

The procedure for compound **(5.5)** was repeated for compound **(5.27)**, using the following quantities of Grignard reagent (2.00g, 13.70mmol), anhydrous THF (15ml), compound **(5.26)** (1.00g, 2.63mmol); to yield the title alcohol **(5.27)** as a yellow oil (1.28g, 99%) with the following physical properties;

IR (DCM, v); cm^{-1} 3401.0, 3068.6, 2948.4, 1672.2, 1491.3, 1459.4, 1096.3, 1016.0

HRMS ($+H^+$): actual 468.1628, calculated 468.1631, molecular formula: $C_{28}H_{32}NO$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm) 1.47 (m, 1H, 1H of CH_2), 1.78 and 1.95 (2 x m, 3H, 3H of 2 x CH_2), 2.35 (2 x t, J~9.78Hz, signals overlapping, 2H, CH_2), 2.44 (s, 6H, CH_3NCH_3), 2.64 (d, J=16.04Hz, 1H, 1H of CH_2), 2.71 (d, J=5.04Hz. 1H, 1H of CH_2), 2.75 (d, J=6.52Hz, 1H, 1H of CH_2), 2.90 and 2.93 (1 x d, J=15.04Hz and 1 x d, J=11.56Hz, signals overlapping, 2H, CH_2), 3.08 (d, J=15.52Hz, 1H, 1H of CH_2), 6.63 (2 x d, J=8.04Hz, signals overlapping, 2H, 2 x Ar-H), 7.01 and 7.03 (2 x d, J=8.00Hz and 1 x d, J=7.52Hz, signals overlapping, 3H, 3 x Ar-H), 7.17 (t, J=7.28Hz, 1H, Ar-H), 7.23 (t, J=7.28Hz, 1H, Ar-H), 7.33 and 7.36 (2 x d, J=8.52Hz and 1 x d, J=10.04Hz, signals overlapping, 3H, 3 x Ar-H), 7.54 (2 x d, J=8.52Hz, signals overlapping, 2H, 2 x Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 21.1 (CH_2), 35.7 (CH_2), 35.9 (CH_2), 39.1 (CH_2), 39.8 (CH_2), 2 x 45.1 (CH_3NCH_3), 56.0 (CH_2CCH_2), 60.3 (NCH_2CH_2), 83.7 (ArCOH), 123.8 (tert. C), 124.2 (tert. C), 125.5 (tert. C), 126.8 (tert. C), 2 x 126.9 (2 x tert. C), 2 x 127.8 (2 x tert. C), 130.7 (quat. C), 131.2 (quat. C), 2 x 131.5 (2 x tert. C), 2 x 131.7 (2 x tert. C), 137.8 (quat. C), 138.0 (quat. C), 138.5 (quat. C), 149.0 (quat. C)

(Z)-3-[2,2-bis(4-chlorobenzyl)-1,2-dihydroinden-3-ylidene]-*N,N*-dimethylpropan-1-amine (5.28)

The procedure for compound **(5.6)** was repeated for compound **(5.28)**, using the

following quantities of compound **(5.27)** (1.02g, 2.18mmol), 20% solution of oxalic acid in water (80ml); to yield the title compound **(5.28)** as a yellow oil (0.88g, 90%) with the following physical properties;

IR (DCM, v); cm^{-1} 3401.0, 2936.5, 2766.5, 1645.6, 1491.2, 1460.2, 1095.4, 1015.1

HRMS ($+H^+$): actual 450.1739, calculated 450.1755, molecular formula, $C_{28}H_{30}Cl_2N$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm) 2.37 (s, 6H, $C\underline{H_3}NC\underline{H_3}$), 2.51 (2 x t, J=7.52Hz, signals overlapping, 2H, $NC\underline{H_2}CH_2CH$), 2.75 and 2.79 (m and 2 x d, J~13.56Hz, signals overlapping, 4H, 2H of $NCH_2C\underline{H_2}CH$ and 2H of $CH{=}CCC\underline{H_2}ArCl$), 2.91 and 2.93 (1 x s and 2 x d, J~12.08Hz, signals overlapping, 4H, 2H of $ArC\underline{H_2}C$ and 2H of $CH{=}CCC\underline{H_2}ArCl$), 5.32 (t, J=7.04Hz, 1H, C=C<u>H</u>), 7.10 (3 x d, J=8.52Hz, signals overlapping, 3H, 3 x Ar-<u>H</u>), 7.15 (2 x d, J=4.52Hz, signals overlapping, 2H, 2 x Ar-<u>H</u>), 7.20 and 7.21 (2 x t , J~8.04Hz and 4 x d, J=8.52Hz, signals overlapping, 6H, 6 x Ar-<u>H</u>), 7.59 (d, J=7.52Hz, signals overlapping, 1H, 1 x Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 26.8 ($NCH_2\underline{C}H_2CH$), 40.5 ($Ar\underline{C}H_2C$), 2 x 44.4 ($ClAr\underline{C}H_2C\underline{C}H_2ArCl$), 2 x 45.1 ($\underline{C}H_3N\underline{C}H_3$), 51.4 ($ClArCH_2\underline{C}CH_2ArCl$), 58.9 ($N\underline{C}H_2CH_2CH$), 122.6 (C=<u>C</u>H), 124.3 (tert. C), 124.7 (tert. C), 126.1 (tert. C), 127.2 (tert. C), 3 x 127.3, (3 x tert. C), 127.6 (tert. C), 131.3 (tert. C), 2 x 131.5 (2 x quat. C), 3 x 131.8 (3 x tert. C), 2 x 136.5 (2 x quat. C), 139.9, 143.5, 144.5 (2 x quat. C and <u>C</u>=CH)

Ethyl 4-phenylbutanoate (5.29)

To a stirred solution of 4-phenylbutyric acid (2.00g, 12.18mmol) in ethanol (16ml) was added thionyl chloride (0.98ml, 13.40mmol) drop-wise at 0°C. After thirty minutes the flask was removed from the ice bath and the reaction was heated till gently refluxing. The solution was allowed to stir at this temperature for four hours. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 6:1) to observe the disappearance of the starting material. On completion, the reaction was allowed to reach room temperature. The reaction mixture was quenched by the addition of water (40ml) and extracted with diethyl ether (3 x 20ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was purified by

flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 6:1). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the title compound **(5.29)** as a pale yellow oil (2.41g, 103%) with the following physical properties;

IR (DCM, v); cm^{-1} 3027.6, 2937.7, 1733.6, 1497.0, 1200.7, 1030.9

HRMS($+H^+$): actual 193.1061, calculated 193.1223, molecular formula $C_{12}H_{17}O_2$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.30 (3 x t, J=7.08Hz, signals overlapping, 3H, $COOCH_2C\underline{H_3}$), 2.01 (2 x p, J=7.52Hz, signals overlapping, 2H, $COCH_2C\underline{H_2}CH_2Ar$), 2.37 (2 x t, J=7.48Hz, signals overlapping, 2H, $COC\underline{H_2}CH_2CH_2Ar$), 2.70 (2 x t, J=7.60Hz, signals overlapping, 2H, $COCH_2CH_2C\underline{H_2}Ar$), 4.17 (2 x q, J=7.13Hz, signals overlapping, 2H, $COOC\underline{H_2}CH_3$), 7.23 and 7.24 (1 x d, J~7.56Hz and 1 x t, J~5.84Hz, signals overlapping, 3H, 3 x Ar-H), 7.33 (2 x t, J~7.48Hz, signals overlapping, 2H, 2 x Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 13.8 ($\underline{C}H_3$), 26.1 ($\underline{C}H_2$), 33.2 ($\underline{C}H_2$), 34.7 ($\underline{C}H_2$), 59.8 ($COO\underline{C}H_2CH_3$), 125.9 (tert. C), 2 x 128.4 (2 x tert. C), 2 x 128.5 (2 x tert. C), 141.0 (quat. C), 173.1 ($\underline{C}OOCH_2CH_3$)

Ethyl 4-(4-acetylphenyl)butanoate (5.30)

To a round bottomed flask containing compound **(5.29)** (2.10g, 10.93mmol) was added chloroform (32ml) followed by aluminium chloride (5.83g, 43.70mmol). Acetyl chloride (1.72g, 21.85mmol) was added dropwise to the stirred solution at room temperature. After fifteen minutes, the reaction mixture was quenched by pouring into a mixture of ice (50.90g) and 37% aq. HCl (11.56ml) and the product was extracted with DCM (3 x 25ml). The combined DCM extracts were then washed with 5% aq. $NaHCO_3$, dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 10:1). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the title ester **(5.30)** as a colourless oil (2.75g, 107%) with the following physical properties;

IR (DCM, v); cm^{-1} 3055.8, 2980.8, 1732.2, 1682.8, 1606.9, 1413.2, 1268.3

HRMS(+2H$^+$): actual 236.2012, calculated 236.1407, molecular formula $C_{14}H_{20}O_3$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.25 (3 x t, J=7.08Hz, signals overlapping, 3H, $COOCH_2C\underline{H_3}$), 1.96 (2 x p, J=7.34Hz, signals overlapping, 2H, $COCH_2C\underline{H_2}CH_2Ar$), 2.31 (2 x t, J=7.40Hz, signals overlapping, 2H, $COC\underline{H_2}CH_2CH_2Ar$), 2.57 (s, 3H, $COC\underline{H_3}$), 2.70 (2 x t, J=7.64Hz, signals overlapping, 2H, $COCH_2CH_2C\underline{H_2}Ar$), 4.12 (2 x q, J=7.38Hz, signals overlapping, 2H, $COOC\underline{H_2}CH_3$), 7.27 (2 x d, J=8.08Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.88 (2 x d, J=8.16Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 13.8 ($COOCH_2\underline{C}H_3$), 25.7 ($\underline{C}H_2$), 26.2 ($CO\underline{C}H_3$), 33.0 ($\underline{C}H_2$), 34.6 ($\underline{C}H_2$), 59.9 ($COO\underline{C}H_2CH_3$), 2 x 128.1 (2 x tert. C), 2 x 128.2 (2 x tert. C), 134.7 (quat. C), 146.8 (quat. C), 172.8 ($\underline{C}OOCH_2CH_3$), 197.3 ($\underline{C}OCH3$)

4-(4-acetylphenyl)butanoic acid (5.31)

To a stirred solution of ester **(5.30)** (2.65g, 11.32mmol) in 18% aq. HCl (21ml) was added dioxane (3ml) at room temperature. The resultant mixture was refluxed for ten hours and the progress of the reaction was monitored by TLC (hexane:ethyl acetate, 1:1). On completion, the reaction mixture was then cooled to room temperature and 2.5M aq. NaOH was added to adjust pH of the reaction solution to 13. The product was extracted with diethyl ether (3 x 30ml) and the combined organic extracts were discarded. The basic aqueous layer was then acidified with 2M aq. HCl and the resulting solution was extracted with diethyl ether (3 x 30ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 1:1). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the title compound **(5.31)** as a white solid (2.19g, 94%) with the following physical properties;

Mp: 43-44°C

IR (KBr, v); cm^{-1} 3048.3, 2932.6, 1707.9, 1681.7, 1413.6, 1269.5

HRMS (+H$^+$): actual 207.3089, calculated 207.1016, molecular formula $C_{12}H_{15}O_3$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.98 (2 x p, J=7.50Hz, signals overlapping, 2H, $COCH_2C\underline{H_2}CH_2Ar$), 2.38 (2 x t, J=7.34Hz, signals overlapping, 2H, $COC\underline{H_2}CH_2CH_2Ar$), 2.58 (s, 3H, $COC\underline{H_3}$), 2.73 (2 x t, J=7.64Hz, signals overlapping, 2H, $COCH_2CH_2C\underline{H_2}Ar$), 7.28 (2 x d, J=8.12Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.79 (broad s, $COO\underline{H}$), 7.89 (2 x d, J=8.20Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.4 ($\underline{C}H_2$), 26.1 ($CO\underline{C}H_3$), 32.8 ($\underline{C}H_2$), 34.5 ($\underline{C}H_2$), 2 x 128.2 (2 x tert. C), 2 x 128.4 (2 x tert. C), 134.7 (quat. C), 146.7 (quat. C), 178.7 ($\underline{C}OOH$), 197.8 ($\underline{C}OCH_3$)

4-[4-(2-morpholino-2-thioxoethyl)phenyl]butanoic acid (5.32)

To a stirred solution of compound **(5.31)** (7.27g, 35.29mmol) in morpholine (15.37g, 176.46mmol) was added sulphur (5.65g, 176.56mmol) at room temperature. The resultant mixture was refluxed for three hours and the progress of the reaction was monitored by TLC (ethyl acetate). On completion, cold ethanol (20ml) was slowly added to the reaction mixture during the subsequent cooling to observe any precipitated materials. The reaction mixture was then partitioned between water (60ml) and diethyl ether (3 x 20ml each). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 1:1). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the title morpholide **(5.32)** as a yellow solid (6.61g, 61%) with the following physical properties;

Mp: 73-76°C

IR (KBr, v); cm^{-1} 3487.9, 2921.3, 1642.1, 1491.2, 1434.8, 1272.5, 1113.3, 1030.8

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.94 (2 x p, J=7.49Hz, signals overlapping, 2H, $COCH_2C\underline{H_2}CH_2Ar$), 2.29 (2 x t, J=7.46Hz, signals overlapping, 2H, $COC\underline{H_2}CH_2CH_2Ar$), 2.73 (2 x t, J=7.52Hz, signals overlapping, 2H, $COCH_2CH_2C\underline{H_2}Ar$), 3.39 (2 x d, J=4.76Hz, signals overlapping, 2H, $ArC\underline{H_2}C{=}S$), 3.72 (4 x t, J=4.84Hz, signals overlapping, 4H, $C\underline{H_2}NCH_2$), 4.33 (4 x t, J=4.84Hz, signals overlapping, 4H, $CH_2SC\underline{H_2}$),

7.13 (2 x d, J=7.96Hz, signals overlapping, 2H, 2 x Ar-H), 7.21 (2 x d, J=7.88Hz, signals overlapping, 2H, 2 x Ar-H), 7.29 (broad s, COOH)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 26.0 ($\underline{C}H_2$), 31.6 ($\underline{C}H_2$), 34.4 ($\underline{C}H_2$), 2 x 49.7 ($\underline{C}H_2N\underline{C}H_2$), 50.2 ($Ar\underline{C}H_2C{=}S$), 66.1 ($CH_2O\underline{C}H_2$), 66.4 ($\underline{C}H_2OCH_2$), 2 x 127.3 (2 x tert. C), 2 x 128.5 (2 x tert. C), 132.8 (quat. C), 140.0 (quat. C), 170.8 (COOH), 199.6 ($\underline{C}{=}S$)

4-[4-(carboxymethyl)phenyl]butanoic acid (5.33)

To a round bottomed flask containing compound **(5.32)** (4.66g, 15.18mmol) was added a 10% solution of KOH in water (30ml). The reaction mixture was heated till gently refluxing for three hours and the progress of the reaction was monitored by TLC (ethyl acetate) to check for the disappearance of the starting material. On completion, it was removed from the hot oil bath and allowed to decrease to room temperature. The mixture was acidified with 2M aq. HCl and the product was extracted with ethyl acetate (3 x 50ml). The combined organic extracts were concentrated under reduced pressure to afford the crude carboxylic acid. The resulting residue was then redissolved in 5% aq. $NaHCO_3$ (50ml) and extracted with ethyl acetate (3 x 30ml). The organic fractions were combined and discarded. The resulting basic aqueous solution was reacidified with 2M aq. HCl (100ml). A mixture of water (150ml) and methanol (40ml) was also added into the acidic suspension and the product was extracted with ethyl acetate (3 x 50ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo* to afford the title compound **(5.33)** as a pale yellow solid (1.65g, 49%) with the following physical properties. The product was not further purified.

Mp: 158-162°C

IR (KBr, v); cm^{-1} 3401.0, 3096.6, 2924.9, 1702.0, 1407.0, 1267.2, 1192.5

HRMS ($+Na^+$): actual 245.0793, calculated 245.0784, molecular formula $C_{12}H_{14}O_4Na$

^1H NMR (CD_3OD, 400MHz) δ_H(ppm): 1.91 (2 x p, J=7.51Hz, signals overlapping, 2H, $COCH_2C\underline{H}_2CH_2Ar$), 2.31 (2 x t, J=7.40Hz, signals overlapping, 2H, $COC\underline{H}_2CH_2CH_2Ar$), 2.65 (2 x t, J=7.60Hz, signals overlapping, 2H, $COCH_2CH_2C\underline{H}_2Ar$), 3.58 (s, 2H, $ArC\underline{H}_2COOH$), 4.98 (broad s, 2H, 2 x COO**H**), 7.17 (2 x d, J=8.04Hz, signals

overlapping, 2H, 2 x Ar-H), 7.22 (2 x d, J=8.04Hz, signals overlapping, 2H, 2 x Ar-H)

^{13}C NMR (CD_3OD, 100MHz) δc(ppm): 25.9 ($\underline{C}H_2$), 32.3 ($\underline{C}H_2$), 33.8 ($\underline{C}H_2$), 39.7 ($\underline{C}H_2$), 2 x 127.7 (2 x tert. C), 2 x 128.5 (2 x tert. C), 131.7 (quat. C), 139.7 (quat. C), 173.9 ($\underline{C}OOH$), 175.5 ($\underline{C}OOH$)

2-(1,2,3,4-tetrahydro-1-oxonaphthalen-7-yl)acetic acid (5.34)

To a stirred solution of polyphosphoric acid (PPA) (2.90g, 3.09mmol) in xylene (7ml) was added a suspension of compound **(5.33)** (1.00g, 4.50mmol) in xylene (3ml) drop-wise over ten minutes at room temperature. The resultant mixture was refluxed at 100°C for three hours and the progress of the reaction was monitored by TLC (ethyl acetate). On completion, the reaction mixture was poured into ice (50g) and the product was extracted with diethyl ether (3 x 20ml). The combined ethereal fractions were concentrated to half-volume and the resulting solution was then extracted with 5% aq. $NaHCO_3$ (3 x 20ml). The ethereal fraction was discarded. The basic aqueous solution was acidified with 2M aq. HCl and the product was extracted with diethyl ether (3 x 20ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: ethyl acetate). All homogenous fractions were collected and the solvent was evaporated *in vacuo* to afford the title acetic acid **(5.34)** as an orange solid (0.57g, 62%) with the following physical properties;

Mp: 67-74°C

IR (KBr, v); cm^{-1} 3207.7, 3038.6, 2944.6, 1708.4, 1679.9, 1610.6, 1431.9, 1168.4

HRMS ($+H^+$): actual 205.0855, calculated 205.0859, molecular formula $C_{12}H_{13}O_3$

^{1}H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.12 (2 x p, J=6.20Hz, signals overlapping, 2H, $COCH_2C\underline{H_2}CH_2Ar$), 2.66 (2 x t, J=6.46Hz, signals overlapping, 2H, $C\underline{H_2}$), 2.94 (2 x t, J=5.98Hz, signals overlapping, 2H, $C\underline{H_2}$), 3.68 (s, 2H, $ArC\underline{H_2}COOH$), 7.24 (d, J=7.84Hz, 1H, Ar-H), 7.42 (d, J=7.84Hz, 1H, Ar-H), 7.94 (s, 1H, Ar-H), 11.16 (broad s, 1H, COOH)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 22.7 ($\underline{C}H_2$), 28.9 ($\underline{C}H_2$), 38.5 ($\underline{C}H_2$), 40.0 ($\underline{C}H_2$),

127.6 (tert. C), 128.8 (tert. C), 131.5 (quat. C), 132.1 (quat. C), 2 x 134.2 (2 x tert. C), 143.3 (quat. C), 176.7 (COOH), 198.3 (C=O)

Synthesis of 2-(2,2-dibenzyl-1,2,3,4-tetrahydro-1-oxonaphthalen-7-yl)acetic acid (5.35)

Method A:

The procedure for compound **(5.0)** was repeated for compound **(5.35)**, using the following quantities of the acetic acid **(5.34)** (0.10g, 0.49mmol), *t*-butanol/diethyl ether (10ml, *v:v*, 1:1), benzyl bromide (0.42g, 2.45mmol), potassium *tert*-butoxide (0.11g, 0.98mmol) as a solution in *t*-butanol (2ml). The reaction was left to stir at room temperature for twenty minutes, after which time insoluble material was observed in the reaction mixture. TLC analysis indicated that the reaction solution only contained benzyl bromide and the starting acetic acid had precipitated from the reaction mixture. The resultant reaction mixture was allowed to stir at room temperature for a further three hours. The reaction was monitored by TLC (ethyl acetate:hexane, 3:1) and compound **(4.53)** was not present in the reaction mixture. The reaction had not proceeded as desired and starting materials were recovered unchanged.

Method B:

The procedure for compound **(5.0)** was repeated for compound **(5.35)**, using the following quantities of the acetic acid **(5.34)** (0.75g, 3.68mmol), *t*-butanol/diethyl ether (50ml, *v:v*, 3:2), benzyl bromide (6.28g, 36.76mmol), potassium *tert*-butoxide (0.82g, 7.35mmol) as a solution in *t*-butanol (8ml). The reaction mixture was heated till gently refluxing. The progress of the reaction was monitored by TLC (ethyl acetate:hexane, 3:1). After three hours the reaction was then removed from the hot oil bath and allowed to decrease to room temperature. The reaction was quenched by the addition of water (1 x 50ml) and the pH of the reaction solution was adjusted to 1 by using 2M aq. HCl. The resulting acidic solution was then basified with sat. aq. $NaHCO_3$ and the product was extracted with diethyl ether (3 x 50ml). The combined ethereal fractions were concentrated under reduced pressure to afford an oily residue. The oil was dissolved in

diethyl ether (1 x 5ml) and the resulting mixture was extracted between 2.5M aq. NaOH (1 x 30ml) and diethyl ether (3 x 25ml). The combined organic fractions were discarded. The basic solution was reacidified with 2M aq. HCl and the product was extracted with diethyl ether (3 x 20ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo* to afford the starting acetic acid **(5.34)** (0.18g) recovered. The original basic ($NaHCO_3$) aqueous extract was acidified with 2M. aq. HCl. The product was extracted with diethyl ether (3 x 50ml). The organic fractions were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo* to afford the title compound **(5.35)** as a yellow oil (0.17g, 16%) with the following physical properties. The product was not further purified by flash column chromatography.

IR (DCM, v); cm^{-1} 3434.8, 3148.2, 2932.4, 1709.0, 1681.9, 1495.3, 1431.1, 1158.7

HRMS ($+H^+$): actual 385.1811, calculated 385.1798, molecular formula $C_{26}H_{25}O_3$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.94 (2 x t, J=6.22Hz, signals overlapping, 2H, $ArCH_2C\underline{H_2}$), 2.67 (2 x d, J=13.40Hz, signals overlapping, 2H, $ArCH_2C\underline{H_2}Ar$), 3.05 (2 x t, J=6.14Hz, signals overlapping, 2H, $ArC\underline{H_2}CH_2$), 3.33 (2 x d, J=13.36Hz, signals overlapping, 2H, $ArCH_2CCH_2Ar$), 3.72 (s, 2H, $ArC\underline{H_2}COOH$), 7.15-7.29 (2 x m, 11H, 11 x Ar-H), 7.41 (d, J=6.64Hz, 1H, Ar-H), 8.01 (s, 1H, Ar-H)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.2 ($\underline{C}H_2$), 29.1 ($\underline{C}H_2$), 40.3 ($\underline{C}H_2$), 2 x 41.5 (2 x $\underline{C}H_2$), 50.8 ($ArCH_2\underline{C}CH_2Ar$), 2 x 126.4 (2 x tert. C), 4 x 128.0 (4 x tert. C), 128.9 (tert. C), 129.1 (tert. C), 4 x 130.9 (4 x tert. C), 131.9 (quat. C), 132.4 (quat. C), 134.2 (tert. C), 2 x 137.3 (2 x quat. C), 142.1 (quat. C), 176.5 ($\underline{C}OOH$), 200.4 ($\underline{C}$=O)

Method C:

Step 1 **Synthesis of methyl 4-{4-[(methoxycarbonyl)methyl]phenyl}butanoate (5.38) and Methyl 4-(3-(methoxycarbonyl)propyl)benzoate (5.39)**

To a stirred solution of compound **(5.33)** (1.00g, 4.50mmol) in a mixture of methanol (30ml) and water (3ml) was added caesium carbonate (2.94g, 9.00mmol) at room temperature. The resultant mixture was allowed to stir at this temperature for twenty minutes after which the solvent was removed under reduced pressure. Toluene was used to azeotrope the water from the reaction mixture. Compound **(5.33)** as a crude caesium

salt was obtained, as an off-white solid. The resulting salt was dissolved in DMF (5ml) and iodomethane (1.40ml, 22.50mmol) was added drop-wise to the stirred reaction mixture over ten minutes at room temperature. The reaction was left stirring at this temperature over night and it was then quenched by the addition of water (1 x 50ml). The product was extracted with diethyl ether (3 x 50ml). The combined ethereal fractions were concentrated under reduced pressure to afford the title methyl ester **(5.38)** as a pale yellow oil (0.56g, 50%) and methyl ester **(5.39)** as a yellow oil (0.07g, 6.6%) with the following physical properties;

Compound (5.38)

IR (DCM, v); cm^{-1} 3455.6, 3010.4, 2952.4, 1738.3, 1515.8, 1436.1, 1255.0, 1155.2, 1014.2

HRMS ($+Na^+$): actual 273.1111, calculated 273.1097, molecular formula $C_{14}H_{18}O_4Na$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.94 (2 x p, J=7.52Hz, signals overlapping, 2H, $CH_2C\underline{H_2}CH_2$), 2.33 (2 x t, J=7.44Hz, signals overlapping, 2H, $C\underline{H_2}$), 2.963 (2 x t, J=7.58Hz, signals overlapping, 2H, $C\underline{H_2}$), 3.59 (s, 2H, $ArC\underline{H_2}COOCH_3$), 3.65 (s, 3H, $COOC\underline{H_3}$), 3.67 (s, 3H, $COOC\underline{H_3}$), 7.14 (2 x d, J=8.08Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.20 (2 x d, J=8.00Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 26.0 ($\underline{C}H_2$), 32.8 ($\underline{C}H_2$), 34.2 ($\underline{C}H_2$), 40.3 ($\underline{C}H_2$), 51.0 ($COO\underline{C}H_3$), 51.5 ($COO\underline{C}H_3$), 2 x 128.2 (2 x tert. C), 2 x 128.7 (2 x tert. C), 131.2 (quat. C), 139.7 (quat. C), 171.6 ($\underline{C}OOCH_3$), 173.4 ($\underline{C}OOCH_3$)

Compound (5.39)

IR (DCM, v); cm^{-1} 3516.9, 3043.5, 2954.6, 1737.7, 1685.4, 1605.3, 1437.1, 1213.9, 1171.2

HRMS ($+H^+$): actual 237.1092, calculated 237.1121, molecular formula $C_{13}H_{17}O_4$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.00 (2 x p, J=7.57Hz, signals overlapping, 2H, $CH_2C\underline{H_2}CH_2$), 2.36 (2 x t, J=7.34Hz, signals overlapping, 2H, $C\underline{H_2}$), 2.76 (2 x t, J=7.66Hz, signals overlapping, 2H, $C\underline{H_2}$), 3.69 (s, 3H, $COOC\underline{H_3}$), 4.00 (s, 3H, $COOC\underline{H_3}$),), 7.35 (2 x d, J=8.12Hz, signals overlapping, 2H, 2 x Ar-$\underline{H}$), 7.97 (2 x d, J=8.08Hz, 2H, 2 x Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 25.5 ($\underline{C}H_2$), 32.7 ($\underline{C}H_2$), 34.8 ($\underline{C}H_2$), 51.2 ($COO\underline{C}H_3$), 52.3 ($COO\underline{C}H_3$), 2 x 128.6 (2 x tert. C), 2 x 129.9 (2 x tert. C), 149.1 (quat. C), 163.7 (quat. C), 173.1 ($\underline{C}OOCH_3$), 185.2 ($\underline{C}OOCH_3$)

Step 2 **Synthesis of methyl 2-(1,2,3,4-tetrahydro-1-oxonaphthalen-7-yl)acetate (5.36)**

To a round bottomed flask containing compound **(5.38)** was added PPA (5.00g, 14.79mmol) at room temperature. The reaction was heated to 90°C and allowed to stir for two hours at this temperature. The progress of the reaction was monitored by TLC (hexane:ethyl acetate, 5:1) to observe the disappearance of the starting material. The reaction mixture was then partitioned between water (50ml) and diethyl ether (3 x 25ml each). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: hexane:ethyl acetate, 10:1). All homogenous fractions were collected and the solvent was evaporated to afford compound **(5.36)** as a yellow oil (0.69g, 79%) with the following physical properties;

IR (DCM, v); cm^{-1} 3449.3, 2951.4, 1736.3, 1683.8, 1612.6, 1435.8, 1257.2, 1165.5

HRMS: actual 219.1016, calculated 219.1016, molecular formula $C_{13}H_{14}O_3$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.08 (2 x p, J=6.34Hz, signals overlapping, 2H, $CH_2\underline{C}H_2CH_2$), 2.59 (2 x t, J=6.88Hz, signals overlapping, 2H, $C\underline{H}_2$), 2.90 (2 x t, J=6.10Hz, signals overlapping, 2H, $C\underline{H}_2$), 3.60 (s, 2H, $ArC\underline{H}_2COOCH_3$), 3.64 (s, 3H, $ArCH_2COOC\underline{H}_3$), 7.19 (d, J=7.84Hz, 1H, Ar-$\underline{H}$), 7.37 (dd, J_1=1.96Hz, J_2=7.84Hz, 1H, Ar-$\underline{H}$), 7.87 (d, J=1.80Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 23.2 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 39.1 ($\underline{C}H_2$), 40.5 ($\underline{C}H_2$), 52.0 ($COO\underline{C}H_3$), 127.8 (tert. C), 129.2 (tert. C), 132.5 (quat. C), 132.6 (quat. C), 134.3 (tert. C), 143.3 (quat. C), 171.6 ($\underline{C}OOH$), 198.1 ($\underline{C}$=O)

Step 3 **Synthesis of methyl 2-(2,2-dibenzyl-1,2,3,4-tetrahydro-1-oxonaphthalen-7--yl)acetate (5.40) and benzyl 2-(1,2,3,4-tetrahydro-1- oxonaphthalen-7-yl) acetate (5.37)**

To a stirred solution of acetic acid methyl ester **(5.36)** (0.50g, 2.29mmol) in *t*-butanol/diethyl ether (30ml, *v:v*, 2:1) was added benzyl bromide (3.92g, 22.94mmol). To this solution was added potassium *tert*-butoxide (0.51g, 4.59mmol) as a solution in *t*-butanol (5ml), adding the base drop-wise over twenty minutes. The reaction was then heated to gently refluxing and the progress of the reaction was monitored by TLC (hexane:ethyl acetate, 5:1). The reaction was left stirring at this temperature for two hours, after which time additional benzyl bromide (7.84g, 45.85mmol) and water (10ml) was added into the reaction suspension. The resulting mixture became clear and was allowed to stir under reflux conditions for a further six hours. On completion, the reaction was quenched by the addition of 2M aq. HCl (1 x 60ml) and the product was extracted with diethyl ether (3 x 30ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo,* and the residue was purified by flash column chromatography on silica gel 230-400mesh (eluent: ethyl acetate). All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the alkylated acetic acid methyl ester **(5.40)** as a pale green oil (0.41g, 45%), and compound (**5.37**) as a pale yellow oil with the following physical properties;

Compound (5.37)

IR (DCM, v); cm^{-1} 3420.3, 3033.8, 2946.5, 1735.7, 1682.5, 1610.7, 1496.9, 1433.5, 1153.6

HRMS ($+Na^+$): actual 317.1161, calculated 317.1154, molecular formula $C_{19}H_{18}O_3Na$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 2.16 (2 x p, J=6.34Hz, signals overlapping, 2H, $CH_2C\underline{H_2}CH_2$), 2.67 (2 x t, J=3.44Hz, signals overlapping, 2H, $C\underline{H_2}$), 2.97 (2 x t, J=6.06Hz, signals overlapping, 2H, $C\underline{H_2}$), 3.72 (s, 2H, $ArC\underline{H_2}COOCH_2Ar$), 5.16 (s, 2H, $ArCH_2COOC\underline{H_2}Ar$), 7.25 (d, J=7.92Hz, 1H, Ar-$\underline{H}$), 7.29-7.40 (m, 4H, 4 x Ar-$\underline{H}$), 7.44 (dd, J_1=2.00Hz, J_2=7.84Hz, 1H, Ar-$\underline{H}$), 7.97 (d, J=1.80Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 23.2 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 39.1 ($\underline{C}H_2$), 40.7 ($\underline{C}H_2$), 66.8 ($COO\underline{C}H_2Ar$), 127.9 (tert. C), 2 x 128.2 (2 x tert. C), 128.3 (tert. C), 2 x 128.6 (2 x tert. C), 129.2 (tert. C), 132.4 (quat. C), 132.7 (quat. C), 134.4 (tert. C), 135.7 (quat. C), 143.4 (quat. C), 171.2 ($\underline{C}OOCH_2Ar$), 198.2 ($\underline{C}$=O)

Compound (5.40)

IR (DCM, v); cm^{-1} 3412.8, 3028.6, 2927.6, 1737.8, 1679.5, 1496.1, 1454.7, 1152.3

HRMS ($+H^+$): actual 397.1436, calculated 397.1798, molecular formula $C_{27}H_{25}O_3$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.94 (2 x t, J=6.36Hz, signals overlapping, 2H, $C\underline{H}_2$), 2.67 (2 x d, J=13.40Hz, signals overlapping, 2H, $ArCH_2C\underline{C}H_2Ar$), 3.04 (2 x t, J=6.32Hz, signals overlapping, 2H, $C\underline{H}_2$), 3.33 (2 x d, J=13.40Hz, signals overlapping, 2H, $ArC\underline{H}_2CCH_2Ar$), 3.72 (s, 2H, $ArC\underline{H}_2COOCH_3$), 5.17 (s, 3H, $ArCH_2COOC\underline{H}_3$), 7.15-7.44 (3 x m, 12H, 12 x Ar-$\underline{H}$), 8.00 (d, J=7.96Hz, 1H, Ar-$\underline{H}$)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 24.7 ($\underline{C}H_2$), 28.9 ($\underline{C}H_2$), 40.3 ($\underline{C}H_2$), 2 x 41.1 (2 x $\underline{C}H_2$), 49.0 ($COO\underline{C}H_3$), 50.5 ($ArCH_2\underline{C}CH_2Ar$), 2 x 126.1 (2 x tert. C), 4 x 127.7 (4 x tert. C), 128.4 (tert. C), 128.7 (tert. C), 4 x 130.6 (4 x tert. C), 132.0 (quat. C), 132.1 (quat. C), 133.9 (tert. C), 2 x 136.9 (2 x quat. C), 142.6 (quat. C), 170.7 ($\underline{C}OOH$), 200.0 ($\underline{C}$=O)

Step 3

Hydrolysis of compound (5.40)

To a stirred solution of compound **(5.40)** (0.50g, 1.26mmol) in 2.5M aq. NaOH (40ml) was added a mixture of methanol/THF (75ml, *v:v*, 2:1) at room temperature. The resultant mixture was allowed to stir at 60°C for four hours and the progress of the reaction was monitored by TLC (ethyl acetate:hexane, 3:1). On completion, the reaction quenched by water (1 x 60ml) and the product was extracted with diethyl ether (3 x 30ml). The combined organic extracts were discarded. The basic aqueous solution was acidified with 2M aq. HCl and the product was extracted with diethyl ether (3 x 30ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo* to yield the alkylated acetic acid **(5.35)** as a yellow oil (0.35g, 74%).

Data previously described.

2-{2,2-dibenzyl-1-[3-(dimethylamino)propyl]-1,2,3,4-tetrahydro-1-hydroxynaphthalen-7-yl}acetic acid (5.41)

To a dry three-necked round bottomed flask containing Grignard reagent (2.00g, 13.70mmol) in anhydrous THF (5ml), which was synthesized according to the method described previously, was added a solution of compound **(5.35)** (0.14g, 0.36mmol) in anhydrous THF (15ml) under dry reaction conditions. The resulting mixture was stirred at room temperature under an atmosphere of nitrogen and the progress of the reaction was monitored by TLC (hexane:ethyl acetate:triethylamine, 10:10:1) to check for the disappearance of the starting material. After a total of four hours the reaction was quenched by the addition of sat. aq. NH_4Cl solution (50ml) and the product was extracted with diethyl ether (3 x 20ml). The combined organic extracts were discarded. 2M aq. HCl was added drop-wise into the basic aqueous solution until the pH was neutral and the product was then extracted with DCM (3x 50ml). The combined organic extracts were washed with water (1 x 50ml), dried over magnesium sulphate, filtered and concentrated *in vacuo.* The resulting residue was purified by means of a combination of flash column chromatography (eluent: methanol:ethyl acetate, 2:1) and preparative TLC analysis (eluent: methanol). All homogenous fractions were collected and the solvent was removed *in vacuo* to yield the target amino acid **(5.41)** as a pale yellow solid (0.06g, 35%) with the following physical properties;

Mp: 45-48°C

IR (KBr, v); cm^{-1} 3368.9, 2953.2, 2705.3, 1713.1, 1567.8, 1494.4, 1385.8, 1166.5

HRMS ($+H^+$): actual 472.2842, calculated 472.2852, molecular formula $C_{31}H_{38}O_3N$

^1H NMR ($CDCl_3$, 600MHz) δ_H(ppm): 1.28-1.39 (m, 2H, C$\underline{H_2}$), 1.45-1.89 (4 x m, 5H, 5H of 3 x C$\underline{H_2}$), 2.07 (broad m, 1H, 1H of C$\underline{H_2}$), 2.26 (broad m, 1H, 1H of C$\underline{H_2}$), 2.46-2.62 (2 x s and m, signals overlapping, 7H, 6H of C$\underline{H_3}$NC$\underline{H_3}$ and 1H of C$\underline{H_2}$), 2.67-2.82 (m, 4H, 2 x C$\underline{H_2}$), 3.11 (s, 2H, ArC$\underline{H_2}$COOH), 6.73-6.79 (2 x m, 2H, 2 x Ar-$\underline{H}$), 4.59 (broad s, O$\underline{H}$), 6.98 (t, J=5.10Hz, 1H, Ar-$\underline{H}$), 7.05-7.27 (4 x m, 10H, 10 x Ar-$\underline{H}$), 7.54 (broad s, O$\underline{H}$)

^{13}C NMR ($CDCl_3$, 150MHz) δc(ppm): 19.3 ($\underline{C}H_2$), 24.2 ($\underline{C}H_2$), 27.7 ($\underline{C}H_2$), 29.5 ($\underline{C}H_2$), 34.3 ($\underline{C}H_2$), 38.7 ($\underline{C}H_2$), 39.3 ($\underline{C}H_2$), 2 x 43.0 ($\underline{C}H_3N\underline{C}H_3$), 45.7 ($ArCH_2\underline{C}CH_2Ar$), 58.2 ($N\underline{C}H_2$), 79.1 ($OH\underline{C}CH_2$), 125.9 (tert. C), 126.0 (tert. C), 127.6 (tert. C), 127.7 (tert. C), 2 x 127.8 (2 x tert. C), 2 x 127.9 (2 x tert. C), 128.5 (tert. C), 2 x 130.8 (2 x tert. C), 2 x

131.1 (2 x tert. C), 133.0 (quat. C), 133.8 (quat. C), 139.4 (quat. C), 2 x 139.6 (2 x quat. C), 174.2 (<u>C</u>OOH)

Methyl 2-{(Z)-2,2-dibenzyl-1-[3-(dimethylamino)propylidene]-1,2,3,4-tetrahydro naphthalen-7-yl}acetate (5.42)

To a solution of compound **(5.41)** (0.03g, 0.06mmol) in methanol (3ml) was introduced conc. HCl and conc. H_2SO_4 gas for ten seconds. The resulting mixture was then heated between 70-80°C for five minutes after which the solvent was removed under reduced pressure. The crude product was washed with diethyl ether (3 x 5ml). The etheral layer was removed manually each time. The target amino acid methyl ester **(5.42)** was obtained as an off-white solid (0.03g, 96%) with the following physical properties;

Mp: 52-58°C

IR (KBr, v); cm^{-1} 3392.1, 2928.7, 2675.1, 1734.4, 1491.4, 1453.2, 1259.5, 1162.8

HRMS ($+H^+$): actual 468.2810, calculated 468.2832, molecular formula $C_{32}H_{38}O_2N$

^1H NMR ($CDCl_3$, 400MHz) δ_H(ppm): 1.77 (2 x t, J=6.02Hz, signals overlapping, 2H, $ArCH_2C\underline{H_2}$), 2.45 (2 x t, J=5.98Hz, signals overlapping, 2H, $NCH_2C\underline{H_2}CH$), 2.79-3.15 (1 x s and 1 x m, signals overlapping, 14H, 6H of $C\underline{H_3}NC\underline{H_3}$ and 8H of 4 x $C\underline{H_2}$), 3.47-3.52 (m, 1H, 1H of $ArC\underline{H_2}COOH$),), 3.60 (s, 1H, 1H of $ArC\underline{H_2}COOH$), 3.71 (s, 3H, $ArCH_2COOC\underline{H_3}$), 5.50 (broad s, 1H, C=C<u>H</u>), 6.96-7.36 (4 x m, 13H, 13 x Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 100MHz) δc(ppm): 24.5 ($Ar\underline{C}H_2CH_2$), 26.5 ($NCH_2\underline{C}H_2CH$), 32.9 ($ArCH_2\underline{C}H_2$), 40.0 ($Ar\underline{C}H_2COOCH_3$), 2 x 42.2 ($\underline{C}H_3N\underline{C}H_3$), 44.4 ($ArCH_2\underline{C}CH_2Ar$), 2 x 44.6 ($Ar\underline{C}H_2C\underline{C}H_2Ar$), 51.7 ($COO\underline{C}H_3$), 56.8 ($N\underline{C}H_2CH_2CH$), 119.6 (C=<u>C</u>H), 2 x 125.7 (2 x tert. C), 127.0 (tert. C), 4 x 127.3 (4 x tert. C), 127.8 (tert. C), 129.5 (tert. C), 130.2 (quat. C), 4 x 130.5 (4 x tert. C), 134.3 (<u>C</u>=CH), 2 x 138.0 (2 x quat. C), 138.9 (quat. C), 144.7 (quat. C), 172.2 (<u>C</u>OOCH$_3$)

2-{(Z)-2,2-dibenzyl-1-[3-(dimethylamino)propylidene]-1,2,3,4-tetrahydronaphthalen-7-yl}acetic acid (5.43)

To a solution of compound **(5.41)** (0.10g, 0.21mmol) in 1,4-dioxane (5ml) was introduced conc. HCl and conc. H_2SO_4 gas for ten seconds. The resulting mixture was then heated about 90°C for five minutes after which the solvent was removed under reduced pressure. The crude product was washed with diethyl ether (3 x 5ml). The etheral layer was removed manually each time. The target amino acid **(5.43)** was obtained as an off-white solid (0.09g, 99%) with the following physical properties;

Mp: 178-181°C

IR (KBr, v); cm^{-1} 3407.7, 2922.7, 1716.7, 1734.4, 1493.6

HRMS ($+H^+$): actual 454.2766, calculated 454.2741, molecular formula $C_{31}H_{36}O_2N$

^1H NMR ($CDCl_3$, 600MHz) δ_H(ppm): 1.61 (t, J=6.02Hz, 2H, $ArCH_2C\underline{H_2}$), 2.32 (t, J=6.02Hz, 2H, $ArC\underline{H_2}CH_2$), 2.73-2.77 {1 x d (J=13.56Hz), 1 x s, 1 x m, signals overlapping, 10H, 2H of $ArC\underline{H_2}CC\underline{H_2}Ar$, 6H of $C\underline{H_3}NC\underline{H_3}$ and 2H of $CHC\underline{H_2}CH_2N$}, 2.95 (d, J=13.56Hz, 2H, $ArC\underline{H_2}CC\underline{H_2}Ar$), 3.21 (broad t, J=7.91Hz, 2H, $NC\underline{H_2}$), 3.56 (s, 2H, $ArC\underline{H_2}COOCH$), 5.66 (t, J=7.15Hz, 1H, C=C<u>H</u>), 6.96 (d, J=7.53Hz, 1H, Ar-<u>H</u>), 7.00 (d, J=7.61Hz, 1H, Ar-<u>H</u>), 7.17-7.19 (m, 5H, 5 x Ar-<u>H</u>), 7.25 (m, 5H, 5 x Ar-<u>H</u>)

^{13}C NMR ($CDCl_3$, 150MHz) δc(ppm): 25.0 ($NCH_2\underline{C}H_2CH$), 26.4 ($Ar\underline{C}H_2CH_2$), 32.2 ($ArCH_2\underline{C}H_2$), 40.2 ($Ar\underline{C}H_2COOH$), 2 x 45.1 ($\underline{C}H_3N\underline{C}H_3$), 44.4 ($ArCH_2\underline{C}CH_2Ar$), 2 x 45.1 ($Ar\underline{C}H_2C\underline{C}H_2Ar$), 55.8 ($N\underline{C}H_2CH_2CH$), 122.1 (C=<u>C</u>H), 2 x 126.0 (2 x tert. C), 126.8 (tert. C), 4 x 127.6 (4 x tert. C), 127.9 (tert. C), 130.0 (tert. C), 4 x 130.9 (4 x tert. C), 131.6, 134.3, 2 x 138.3, 138.4 and 142.5 (5 x quat. C and <u>C</u>=CH), 172.9 (<u>C</u>OOH)

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