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Towards Carbohydrate Recognition in Aqueous Media

by

Theo J. Ryan



A Thesis Presented to the University of Dublin
for the Degree of Doctor of Philosophy.

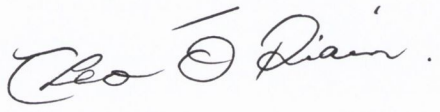
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Theo J. Ryan

October 2001.

"There's the porter's lodge and a parking lot and in this building I see the contortions of glass, bubbling pots and skylights poking out of the roof. I want so much to learn. To know what you do with acids and esters and make my experiments go pop at the right time like the rest of you. From the very first word you tell me I'm going to remember. On my way to my tutor. Through these playing fields, flat green and velvet. How lovely with benches where I can sit watching, reading, or anything under these old trees. I think late summer is still hanging in the sky. And by these flower beds still smelling, into this pretty square where the opulent members of college live behind granite and big windows. That's for me."

Sebastian B. Dangerfield.

For My Parents

Acknowledgements

I would firstly like to thank my supervisor, Professor Tony Davis, for all his advice, guidance and help over the last four years and for giving me the opportunity of working on a very interesting project.

A big thank you to Dr. Richard Wareham for being an invaluable help with the project work and for pointing the way out of the laboratory towards O'Neill's and to the many race tracks and mostly for doing things "his way". Cheers Rich.

Thanks also to Trini, Andrew and Greg who worked on the carbohydrate project and to Trini and Andrew for being excellent company on TMR trips.

I would like to thank the Chemistry Department, Trinity College Dublin and the School of Chemistry, University of Bristol, for the opportunity of working in both places. Thanks to everyone who made our move to Bristol a smooth(ish) one especially everyone in N314.

Thanks to all the technical staff in both Trinity and Bristol for all their assistance throughout the course of this project. Thanks especially to Dr. John O'Brien in Trinity for all the NMRs he ran and for digging out and shipping old files at awkward times. Thanks also to Dr. Martin Murray and Dr. Ken MacNeil for NMR and MS spectra. Special thanks to Fred Cowzer for extracurricular chats and for sourcing everything from chemicals, to golf courses, to fan belts.

Thanks also to Dr. Yiu-Fai Ng, Dr. Jean-Christophe Meillon and Prof. Jeremy Sanders for their involvement in the collaboration with the pentyl macrotricycle binding sweet-beads.

Thanks to all the backlab gang both past and present especially Adrían B, Adrian R (the audio-visual extravaganza), Alan (you'll never putt like me), Amy, Eileen Mc, Fionn, Jens, John Barry, John Clare, Julie, Killian, Larry, Mark, Mary Mc, Mary-Rose, Michael, Nieves, Trevor (Maythers), Tríona and Shay.

Thanks to Rosey- I finally got it written up.

To all my proof readers: Rich, Trevor, Trini (and Cormac), thank you.

I must also mention the original college gang: Bríd, Brídín, Lorraine, Maureen, Sharon and Sinéad. Cheers to Colin Belts , Donal, Naoise (Clowndog) and Pádraig (PMXITH) and to Dave (Dr. Love), Rich and Co. for quality week(end) entertainment.

Thanks to Flo for everything and for putting up with me while I was writing up.

Finally, I would like to thank my parents, Jim and Maureen, and my sister Ethel for all their love and support throughout my University years.

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Summary

Chapter One provides a brief introduction to supramolecular chemistry. The types of interactions involved in host-guest chemistry are discussed as well as the principles behind the design of a receptor.

Chapter Two examines carbohydrate recognition in organic media. Contributions from various laboratories including the Davis laboratory provide a variety of receptors with different carbohydrate binding abilities.

Chapter Three describes the phenomena of carbohydrate recognition in aqueous media. Contributions within this area from various laboratories are described, as well as the aims of this project.

Chapter Four gives an account of the synthesis of the pentyl macrotricycle **2.57** as previously carried out by Wareham. Improvements made to this synthesis are described in detail.

Chapter Five relates the syntheses of two new organic-soluble macrotricyclic receptors, namely benzyl macrotricycle **5.6** and polybenzyl macrotricycle **5.17**. Also discussed is the synthesis of the water-soluble macrotricycle **5.23**.

Chapter Six details testing carried out on the pentyl macrotricycle **2.57** by means of ^{1}H magic angle spinning NMR on a solid supported glucoside. Also described are ^{1}H NMR binding studies of polybenzyl macrotricycle **5.17** for octyl-β-D-glucoside in various solvents as well as the extraction of various carbohydrates from aqueous into organic media by **5.17**.

Chapter Seven is a detailed account of the experimental procedures for the syntheses of the reported compounds.

Abbreviations

Ac	acetyl
ACCN	1,1′-azobis(cyclohexanecarbonitrile)
AIBN	1,1′-azobis(isobutyronitrile)
Ala	alanine
aq.	aqueous
Asp	aspartic acid
atm	atmosphere
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
br	broad
ca.	*circa*
calcd	calculated
Cbz	benzyloxycarbonyl
CI	chemical ionisation
Cys	cysteine
d	doublet
DCC	*N*,*N*′-dicyclohexylcarbodiimide
DCM	dichloromethane
DCU	dicyclohexylurea
DMF	*N*,*N*-dimethyl formamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dppf	diphenylphosphino ferrocene
DSS	2,2-dimethyl-2-silapentane-5-sulphonate, sodium salt
EI	electron impact
eq	equivalent
ES	electrospray
Et	ethyl
FAB	fast atom bombardment
Glu	glutamic acid
Gly	glycine

h	hour
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IR	infra red
J	coupling constant
K_a	association constant
K_d	dissociation constant
LCMS	liquid chromatography mass spectrometry
lit.	literature
Lys	lysine
m	*meta*
m	multiplet
m.p.	melting point
m/z	mass to charge ratio
MAS	magic angle spinning
Me	methyl
MS	mass spectrometry
N,*N*-DIPEA	*N*,*N*-diisopropylethylamine
NBS	*N*-bromosuccinimide
NMP	*N*-methyl-pyrollidinone
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
o	*ortho*
p	*para*
PEG	polyethylene glycol
Pfp	pentafluorophenol
Ph	phenyl
Phe	phenylalanine
ppm	parts per million
q	quartet
R_f	distance travelled relative to solvent front
RT	room temperature
s	singlet
sat.	saturated

t	*tertiary*
t	triplet
Tf	triflate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSI	trimethylsilyl iodide
Trp	tryptophan
Tyr	tyrosine
UV	ultraviolet
δ	chemical shift
ν_{max}	wave number

CHAPTER ONE

1.1 Supramolecular Chemistry and Molecular Recognition

Supramolecular chemistry has been defined by one of the pioneers of the area, Jean-Marie Lehn, as "chemistry beyond the molecule."[1] Other definitions have described supramolecular chemistry as "the chemistry of the non-covalent bond" and "non-molecular chemistry."[2] In the broadest sense, supramolecular chemistry involves intermolecular interaction or recognition between at least two molecules, given the terms "host" and "guest", resulting in a "host-guest complex" or supramolecule. Molecular recognition itself may be defined as the branch of chemistry concerned with the non-covalent interactions between molecules such as hydrogen bonding, ion pairing, van der Waal's forces, metal-to-ligand interaction, π-stacking, *etc.* Molecular receptors, the organic molecules engaged in molecular recognition "are able to bind selectively ionic or molecular substrates (or both) by means of various intermolecular interactions, leading to an assembly of two or more species, a supermolecule."[1]

In order for molecules to recognise and bind to each other several molecular architectural factors are important.[3] Firstly, there must be a complementary stereoelectronic arrangement between the host and guest molecules *i.e.* the molecules must be able to structurally fit together as well as possess complementary binding sites. Secondly, high structural organisation requires multiple binding sites due to the inherent weakness of non-covalent interactions. Finally, strong interaction between host and guest is important.

1.2 Preorganisation, Thermodynamic Factors and Solvent Effects

In 1894, Emil Fischer used the analogy of the "lock and key" to describe the receptor-substrate binding by enzymes. As with enzymes, synthetic receptors also need to be rationally designed so as to fit with a target substrate. More specifically a receptor molecule must be well preorganised for the successful recognition and binding of a substrate. A receptor molecule is deemed to be preorganised if it does not undergo significant conformational change upon binding of a guest. A higher degree of recognition is a result of higher binding free energy, as determined by the second law of thermodynamics, **Equation 1.1**:

$$\Delta G = \Delta H - T\Delta S$$

Equation 1.1

The preorganisation of a host is essential as it is a major factor in the overall free energy of the complex.[2] In terms of the thermodynamics, a typical complexation may be thought of as occurring in two stages. In the first stage, the host and guest must reorganise their conformations for the optimum arrangement of their binding sites for complementarity with each other. This is energetically unfavourable as ΔS is negative. The second stage is complexation itself. This is energetically favourable due to the attraction between host and guest being enthalpically stabilising. The overall free energy of complexation is the difference between these two processes. Therefore, if the host is well preorganised, the energy costs due to reorganisation will be small and the overall binding free energy will be higher allowing for better host-guest complexation.

The association of the host and guest molecules may be written as shown in **Equation 1.2**:

$$\text{Host.Solvent} + \text{Guest.Solvent} \rightleftharpoons \text{Host.Guest} + \text{Solvent.Solvent}$$

Equation 1.2

The binding forces between the host and solvent as well as between the guest and solvent molecules are frequently the same as those between host and guest molecules. Therefore, competition exists for the host and guest binding sites between the solvent molecules and the host and guest molecules. Consequently, for complexation to occur, solvent molecules must be displaced from the binding sites. Again, as indicated in **Equation 1.1**, the solvation free energy comprises an enthalpically favourable solvation term and an entropically unfavourable solvation term. For complexation to occur, any free energy costs from desolvation must be countered by a lower free energy of complexation, further illustrating the importance of a well-preorganised host molecule.

From a design point of view, both the host and guest should possess the necessary complementary features without promoting the affinity of either for solvent molecules. For example, hydrogen bonding is particularly effective in non-

polar media as there is little competition on the part of the solvent for the available binding sites. Complexation must be driven either by intrinsic binding (strong interaction between host and guest) or by solvophobic binding (poor interaction between host and/or guest and the solvent).

1.3 Measuring Binding Constants

The measure of a host's ability to bind a particular guest is given by its binding constant, K_a. This is a thermodynamic equilibrium constant for the process:

$$\text{Host} + \text{Guest} \rightleftharpoons \text{Host.Guest}$$

The binding constant, K_a, is measured in M^{-1} and is represented by **Equation 1.3**, where [HG] denotes concentration of host-guest complex, [H] denotes concentration of free host and [G] denotes concentration of free guest at equilibrium.

$$K_a = \frac{[HG]}{[H][G]}$$

Equation 1.3

From **Equation 1.3**, it can be seen that a higher binding constant therefore signifies a higher equilibrium concentration of the complexed material.

The binding of two molecules, H and G, may be analysed by any experimental technique that can give information concerning the concentration of the complex, as a function of changing concentration of the guest. A binding study involves the titration of a host against a guest, with the monitoring of changes in a particular host property after each addition of guest. The most common properties monitored include conductivity, NMR, fluorescence, UV and calorimetry. NMR binding studies are particularly useful as they not only supply a binding constant after data analysis but can also yield structural information concerning the binding modes involved. Most host-guest equilibria are fast, relative to the NMR time-scale and the resonance being followed in the titration is an average of the chemical shifts of the bound and unbound species. A typical NMR binding study involves the addition of aliquots of guest to a known concentration of host and following the changes of a particular resonance ($\Delta\delta$) which is influenced by the guest binding. A

plot of Δδ vs. Guest Concentration will give a binding curve as in **Figure 1.1**, which yields quantitative information regarding the binding. A computer based least-squares curve fitting program then calculates the binding constant for the complexation.

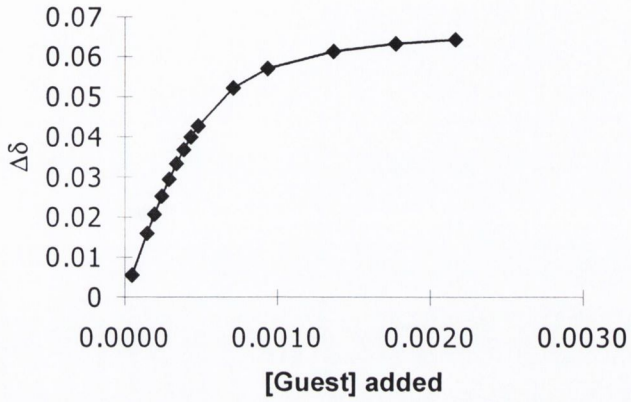


Figure 1.1 A typical binding curve with Δδ plotted against [Guest]

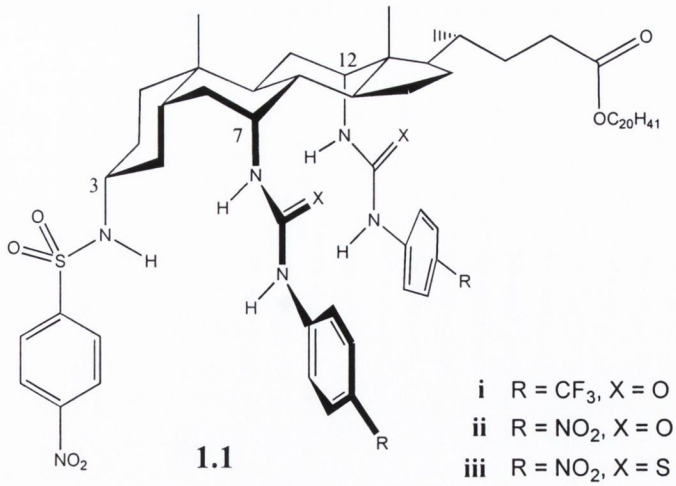
1.4 The Nature of Host-Guest Interactions

Generally, supramolecular interactions are of a non-covalent nature, the most important of which are outlined below:

1.4.1 Hydrogen Bonding

When a hydrogen atom is covalently bonded to an electronegative atom such as O, the bond is polarised and the hydrogen may be thought of as being acidic. The hydrogen may then form a weak bond with a proximal atom, which has a source of negative charge such as a lone pair of electrons. The energy of a H-bond is typically 4–120 $kJmol^{-1}$.

Hydrogen bonding is one of the most important molecular interactions in host-guest chemistry. It is seen throughout the natural world being largely responsible for the double-helical structure of DNA, the three dimensional conformation of many proteins, as well as the recognition of substrates by enzymes.



1.1

The synthetic receptor **1.1**, designed within the Davis group[4] for the recognition of anions, illustrates the potential for H-bonding between a host and a guest. The steroid backbone acts as a scaffold, allowing the sulfonamide in the 3- position and the urea moieties in the 7- and 12- positions to form a cavity into which an anion can fit. The sulfonamide with a *para* nitrophenyl appendage in the 3- position, together with the various electron withdrawing groups **i**, **ii** and **iii**, make the amino hydrogens more acidic and therefore better H-bond donors. In case **iii**, **1.1** has been shown to bind Cl^- with a K_a value of 1.03×10^{11} M^{-1} and Br^- with a K_a value of 2.59×10^{10} M^{-1}, in chloroform.

1.4.2 Van der Waal's Forces

Van der Waal's forces are weak electrostatic attractions arising from the polarisation of an electron cloud by an adjacent nucleus. They are typically of the order of less than 5 $kJmol^{-1}$, and operate over a short range. The forces may arise from dipole-dipole, dipole-induced dipole or induced dipole-induced dipole interactions (also known as dispersion or London forces).[5] The total potential energy for two atoms, may be written as **Equation 1.4**:

$$V = A/r^{12} - B/r^{6}$$

Equation 1.4

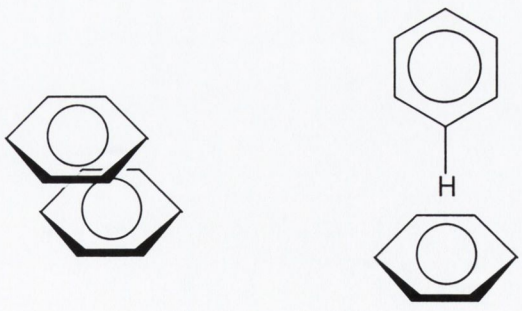
The first term represents the repulsion potential due to the exclusion of electrons from regions of space where electron clouds overlap and follows an inverse twelfth

order. The second term represents the attractive potential due to dispersion forces and following an inverse sixth power, the attraction decreases rapidly with increasing distance apart. The attractive forces are additive with every bond thus contributing to the overall interaction between molecules.

An important factor in determining the strength of van der Waal's forces is the relative polarisability of the electrons in the atoms involved. The less tightly the electrons are held to the nucleus of an atom the more polarisable they are, so for example, lone pairs of electrons are more polarisable than bonded pairs.

1.4.3 Aromatic Interactions

Aromatic interactions between π systems, commonly referred to as π-stacking, π-π interactions or aromatic interactions, are ubiquitous in nature. They contribute to the tertiary structure of proteins, the base stacking interactions which stabilise DNA as well as the intercalation of drugs into DNA. By investigating the configurations of co-facial porphyrin dimers, Hunter and Sanders have proposed a model for π-π interactions.[6], [7] Their model consists of a positively charged δ-framework sandwiched between two clouds of negatively charged π-electrons. Based on their experimental work previous models, involving solvophobic effects, electron donor-acceptor and charge transfer models, were rejected in favour of electrostatic interactions involving the out-of-plane π electron density. These electrostatic effects govern the geometry of the interaction. Edge-to-face interaction is believed to be due to the attraction between the partially negative charged π system of a face and the partially positively charged hydrogen on an edge.



Face-to-Face **Edge-to-Face**

Figure 1.2 Aryl-aryl interactions

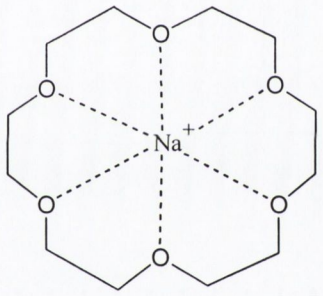
Closely related to π-π interaction is CH-π interaction between a hydrogen chain and an aromatic system.[8] CH-π interactions are much less documented than aryl-aryl interactions, yet they are believed to be more advantageous as regards molecular recognition in water due to alkanes being less soluble than aromatic moieties. Examples of this will be illustrated in later chapters.

1.4.4 Electrostatic Interactions

Electrostatic interactions occur between species of opposite charges. More specifically, electrostatic interactions can be ion-ion, ion-dipole or dipole-dipole.

Ion-ion attraction is strong and comparable in energy to covalent bonding, being in the range of 100–350 $kJmol^{-1}$.

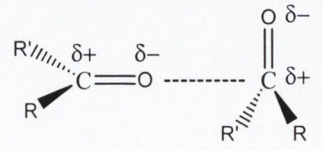
Ion-dipole interaction is typically in the range of 100–250 $kJmol^{-1}$. A good illustration of this type of bonding can be seen in Pederson's crown ether **1.2**, one of the original supermolecules.



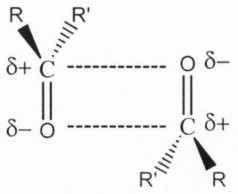
1.2

Dipole-dipole interactions are the weakest of all electrostatic interactions, being of the order of of 5–50 $kJmol^{-1}$. The alignment of one dipole, such as a carbonyl group, with another can result in attraction between the two groups. The dipoles may align in two different fashions:

Type I Alignment of a single pair of poles from adjacent molecules.



Type II Opposing alignment of dipoles.



1.5 Design of a Carbohydrate Receptor

As mentioned earlier, many aspects are important in the binding of a guest by a host. In the rational design of a host, a number of factors must be adhered to for it to be complementary and preorganised for a particular guest:

1.5.1 Purpose of Complexation

The applications which lie behind the desire to bind various guests include membrane transport, phase transfer catalysis, sensors and catalysis. Not all of these applications require the same type or strength of binding. For example, the extraction of a substrate from one phase into another will require strong binding. However, for catalysis or the transport of a substrate across a biological membrane the receptor needs to be able to bind the substrate, allow for a reaction to occur (in the case of catalysis) and then release the substrate. Binding of the guest too strongly would therefore effect its rôle.

1.5.2 Steric Factors

The host binding site must be of a complementary size to the guest if strong binding is to occur. Rigid receptors which are not the correct size may prove to be poor binders due to their inability to adapt to the size of the guest. Size is also critical as regards the selectivity of the host for one guest over another. Rigid receptors will exclude certain molecules which are too large for their binding site but may exhibit poor host-guest interaction if the cavity is too large for the guest. This is where the use of computerised molecular modelling packages can be beneficial.

1.5.3 Electronic Factors

For strong binding, complementarity between the electrostatic charges must be accomplished. For a negatively charged guest, such as a halide, the host must provide complementary positive charge sites such as H-bond donors. Conversely,

for positively charged guests, such as the Group I elements, a source of negative charge must be supplied, for example lone pairs of electrons on N and O atoms. Non-polar CH units and aromatic systems in a guest will require a more subtle approach such as CH-π or π-π interaction. Whatever the type of binding mode employed, it must be incorporated into the host in such a way as to maximise the number of host-guest interactions.

1.5.4 Solvent Factors

The solvent in which complexation is to be achieved is also a critical factor. Whether the binding is to take place in non-polar media or polar media, such as water, will be what dictates the solubilising groups placed on the host. For the promotion of organic solubility, alkyl chains or non-polar aromatic surfaces may be built into the receptor. Conversely, for the promotion of aqueous solubility, charged groups such as ammonium or carboxylate moieties may be incorporated, or for neutral aqueous solubility a number of polar groups such as hydroxyls may be employed.

CHAPTER TWO

2.1 Carbohydrate Recognition in Organic Media

Carbohydrates are complex molecules, having a three dimensional arrangement of functionality. X-ray crystal structures of protein-carbohydrate complexes have shown an extensive array of H-bonds between host and guest. **Figure 2.1** shows glucose being bound by an *E.coli* galactose chemoreceptor protein.[9] Polar residues surrounding the carbohydrate are depicted in yellow and non-polar residues in green. H-bonds are represented as broken green lines.

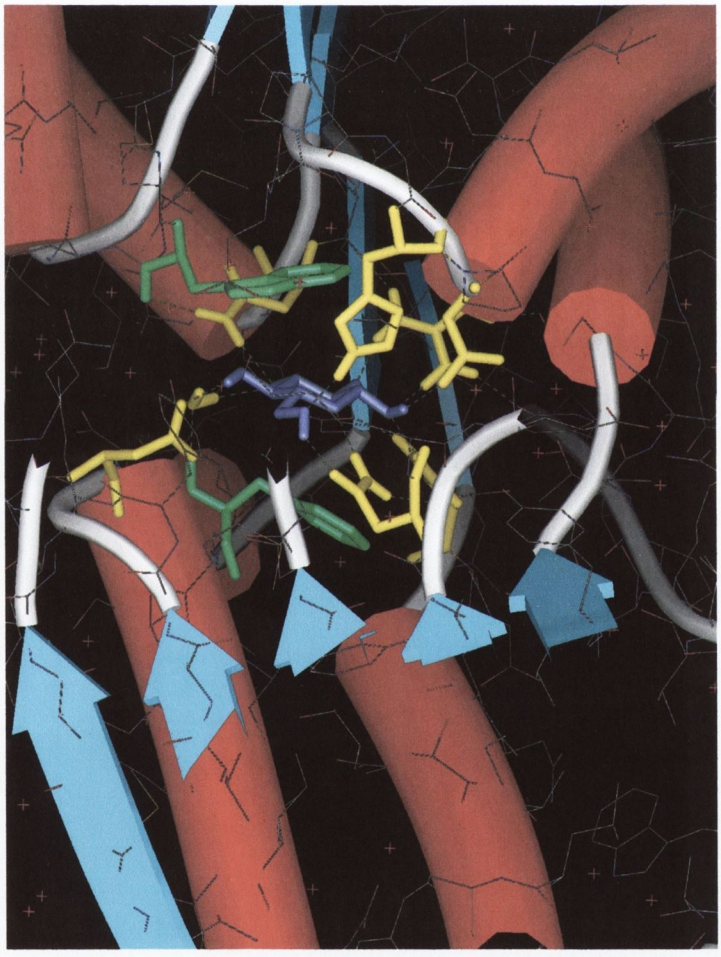


Figure 2.1 X-ray crystal structure of glucose bound to the *E.coli* galactose chemoreceptor protein

Receptor design should follow this example, as it is provided by nature and also because of the knowledge that H-bonding is very effective in non-polar media due to the lack of competition for binding sites by the solvent. By providing a preorganised

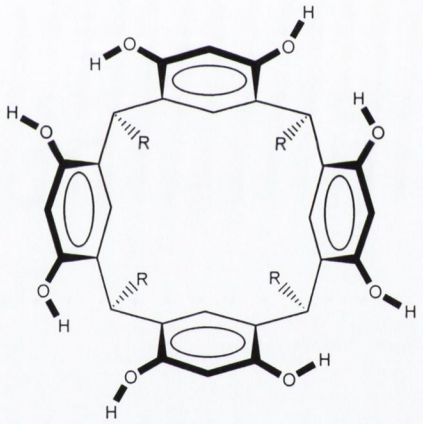
complementary arrangement of H-bond donor and/or acceptor functionality, success in recognition should be possible in organic solvents.

Due to the size and complex three-dimensional arrangement of the carbohydrate functional groups the design and synthesis of receptors is quite a challenge. A receptor with a binding site of a suitable size is therefore required, but not at the cost of maintaining rigidity and the subsequent preorganisation. Rigidity is also important in preventing a receptor from H-bonding with its own donor and acceptor groups and thereby collapsing upon itself.

2.2 Face-to-Face Recognition

2.2.1 Contribution from Aoyama *et al.*

In 1988, Aoyama *et al.* reported the first successful carbohydrate receptor, a "calixresorcinol", namely octahydroxy calix[4]arene **2.1**.[10], [11]



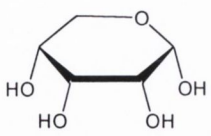
2.1

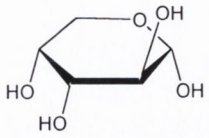
R = *n*-$C_{11}H_{23}$

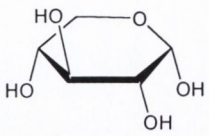
It consists of a crown or bowl-shaped conformation and binds a variety of carbohydrates in a face-to-face fashion. The employment of this mode of binding, as a first success in the area, is not surprising considering the difficulties in fully encapsulating a carbohydrate, as demonstrated by nature's best receptors, the bacterial periplasmic proteins. Receptor **2.1** is well preorganised, having eight hydroxy groups predisposed as H-bond donor/acceptors. **2.1** was shown to extract 4 equivalents of water into a CCl_4 solution and was also capable of extracting certain carbohydrates from water into CCl_4 but only at high concentrations of the guest. As seen in **Table 2.1**, pentoses were extracted from aqueous solutions with more success than hexoses.

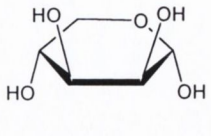
Sugar	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	2.10	2.11
Sugar/**2.1**	0.5	0.1	≤0.03	≤0.03	0.8	≤0.03	≤0.03	≤0.03	1.0	0.1

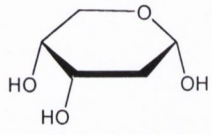
Table 2.1 Extraction values in equivalents for pentoses and hexoses **2.2-2.10** by **2.1**

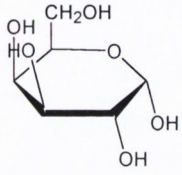

D-ribose (**2.2**)

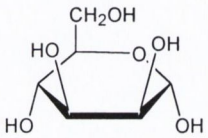

D-arabinose (**2.3**)

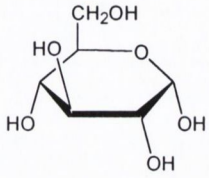

D-xylose (**2.4**)

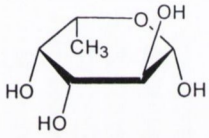

D-lyxose (**2.5**)

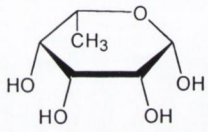

2-deoxy-D-ribose (**2.6**)


D-galactose (**2.7**)


D-mannose (**2.8**)


D-glucose (**2.9**)

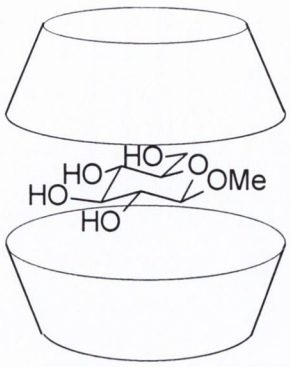

L-fucose (**2.10**)


L-rhamnose (**2.11**)

Sugar solution concentrations in the above extractions were 2.4 M. D-ribose and D-arabinose were the sugars extracted with most success from the pentoses tested, with D-xylose and D-lyxose proving difficult. Of the fully oxygenated aldohexoses tested, none were extracted in any significant quantity. This is most probably due to each of them having a hydrophilic CH_2OH group in their pyranose form, which would be exocyclic upon complexation and therefore not involved in H-bonding with the host and being only exposed to the bulk solvent.[12]

Although aldohexose sugars were not extracted, the deoxyhexoses L-fucose **2.10** and L-rhamnose **2.11** were extracted highly and moderately respectively. Also, the ketohexose D-fructose was extracted from a 3.5 M aqueous solution in a 13 % sugar/host percentage. The most extensively studied of the sugars was D-ribose which formed a complex of the form **2.1**:**2.2**:$2H_2O$. From ^{13}C NMR data it was shown that the predominantly bound form was the α-pyranoside despite the excess presence of the β-pyranoside. These experimental results led Aoyama to believe that binding was occurring through a ditopic interaction with the carbohydrate hydroxy group at C-1 and C-4 in a *cis* arrangement promoted by a third *cis* OH at C-3. [12], [13]

Also tested were the alkyl glucosides methyl-β-D-glucoside and its α anomer. This time however, the stoichiometry of binding was 2:1 (host:guest) implying a sandwich like complex, **2.12**.

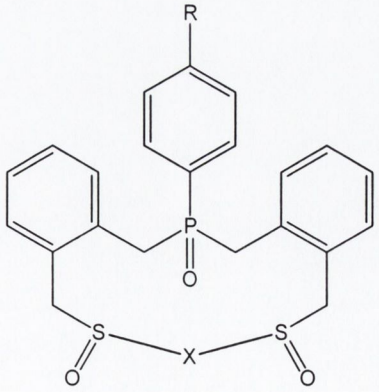


2.12

This was observed only with the methyl-β-D-glucoside as none of the α anomer was extracted. The selectivity of β over α is presumably due to the steric hindrance caused by the axial methoxy group and the subsequent prevention of two host molecules being allowed to come together.

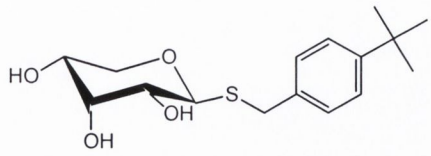
2.2.2 Contribution from Gellman *et al.*

Gellman and co-workers also designed receptors which would bind carbohydrates in a face-to-face manner. Their macrocycles employed sulfoxide and phosphine oxide groups as multipoint H-bond acceptors.[14] The macrocycles **2.13** and **2.14** were tested for their respective carbohydrate affinities by passing them down monosaccharide functionalised stationary phases on a HPLC column. The sugars used for the stationary phase were xylose **2.15** and ribose **2.16**:

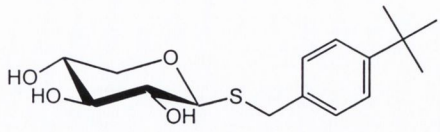


2.13 X = $(CH_2)_3$, R = H
2.14 X = *o*-xylyl, R = *t*Bu

Elution was with MeOH/DCM (2:98) and it was observed that **2.13** was retained most strongly on the xylose functionalised column while **2.14** was retained most strongly on the ribose functionalised column. However, when ^{1}H NMR titrations were carried out in CD_2Cl_2 on the organic-soluble derivatives of xylose **2.15** and ribose **2.16**, binding constants of only 30-40 M^{-1} were obtained.



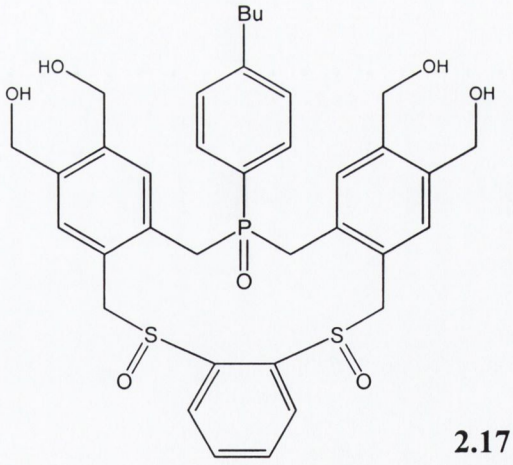
derivatised xylose **2.15**



derivatised ribose **2.16**

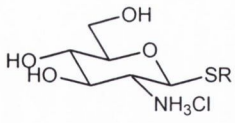
On testing Aoyama's macrocycle **2.1** on the xylose functionalised stationary phase, they found it was retained by approximately 8 minutes longer than **2.13** and **2.14**, highlighting the benefits of HPLC as a test for carbohydrate recognition.

Other work by Gellman *et al.* showed that the three oxygens of their macrocycles were effective at binding primary ammonium cations in organic media.[15] This prompted the addition of peripheral hydroxyl groups to the macrocycle, resulting in **2.17,** with a view to binding protonated amino sugars *via* H-bonding with both ammonium and hydroxyl groups.[16] The affinity of **2.17** for hexose ammonium salts **2.18–2.22** was tested by ^{1}H NMR titrations.

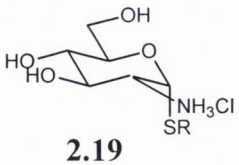


2.17

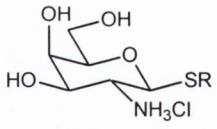
Ammonium hexoses tested:



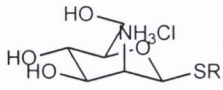
2.18



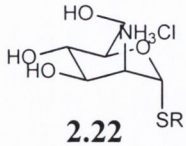
2.19



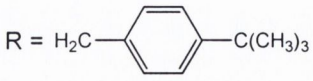
2.20



2.21



2.22



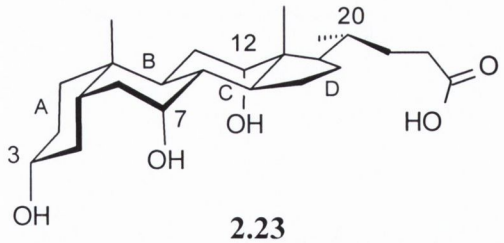
Hexose ammonium ion	**2.18**	**2.19**	**2.20**	**2.21**	**2.22**
K_a M^{-1}	3000	2800	1400	1000	2200

Table 2.2 Association constants between **2.17** and hexoses **2.18-2.22** in $CD_3OD/CDCl_3$ (10:90)

Despite the competitive environment of $CD_3OD/CDCl_3$ (10:90) the results of **Table 2.2** suggests H-bonding between host and guest. A three-fold selectivity was observed between **2.18** and **2.21** in favour of the α-epimer, and approximately a two fold selectivity between **2.18** and **2.20** in favour of the 4-OH being in a β orientation.

2.3 Encapsulation Steroidal based Receptors

The binding site in the face-to-face type receptors are co-directed rather than convergent. As explained, this is suitable for certain carbohydrates but it poses the problem of host molecules self-associating as well not bonding in a 1:1 stoichiometry. Another drawback in face-to-face recognition is the lack of simultaneous interaction with all of the binding sites of the carbohydrate, which will limit its potency as a binder. This can only be achieved by a framework which fully encapsulates the sugar, as exhibited by the bacterial periplasmic proteins. One such framework which has been successfully employed as a synthetic host is the steroid, cholic acid **2.23**.[17] It is an ideal candidate as a host molecule as it possesses a number of important features.

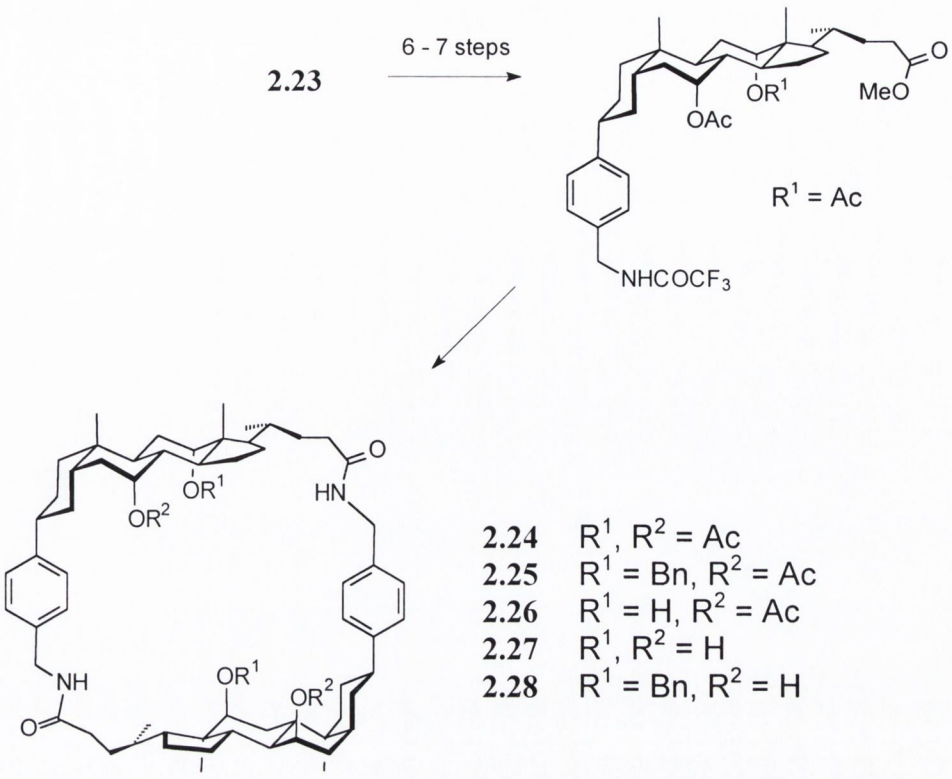


2.23

The steroid is a rigid framework and the *cis* ring junction between rings A and B gives a concave shape to the molecule. It contains four functional groups which are well spaced out over the molecule; hydroxy groups at the 3-, 7- and 12- positions and a carboxylic acid attached to the C-20 side chain. The inherent asymmetry of the molecule allows for strong control over the synthetic manipulations of the individual groups. Also axial arrangement of the functionality on the same side (α side) of the steroid leaves them well orientated for supramolecular interaction. Finally, cholic acid is readily available and is a cheap starting material.

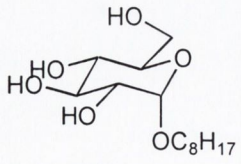
2.3.1 Contribution from Davis *et al.*

The synthesis of the first "cholaphanes", macrocyclic cholic acid based receptors, by the Davis group were reported in 1989.[18], [19], [20] Starting with cholic acid **2.23** the steroidal 3α-OH was converted to a protected *p*-aminomethyl phenyl spacer. Deprotection of the amino and carboxyl groups followed by cyclodimerisation resulted in macrocycles **2.24**-**2.28**.

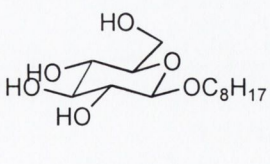


2.24 R^1, R^2 = Ac
2.25 R^1 = Bn, R^2 = Ac
2.26 R^1 = H, R^2 = Ac
2.27 R^1, R^2 = H
2.28 R^1 = Bn, R^2 = H

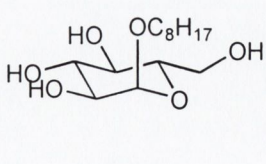
Two series of macrocycles were obtained, one in which the 7- and 12- positions had the same functionality (**2.24** and **2.27**) and one where these positions were differentiated by means of selective acylations.[19] A large cavity was formed which had inward co-directed functionality at positions 7- and 12-. ^{1}H NMR titrations were carried out to test the affinities of the macrocycles for the organic-soluble sugar derivative, dodecyl-β-D-glucoside in $CDCl_3$. **2.27** had a binding constant (K_a) of 1740 M^{-1} while the K_a of **2.28** was 700 M^{-1} and both exhibited 1:1 complexation. As mentioned, the steroid has an inherent chirality which prompted the testing of **2.27** for enantioselective and diastereoselective recognition of octyl-α-D-glucoside **2.29**, octyl-β-D-glucoside **2.30**, octyl-α-L-glucoside **2.31** and octyl-β-L-glucoside **2.32**.



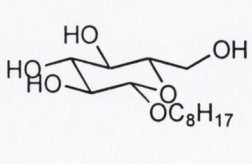
2.29



2.30



2.31



2.32

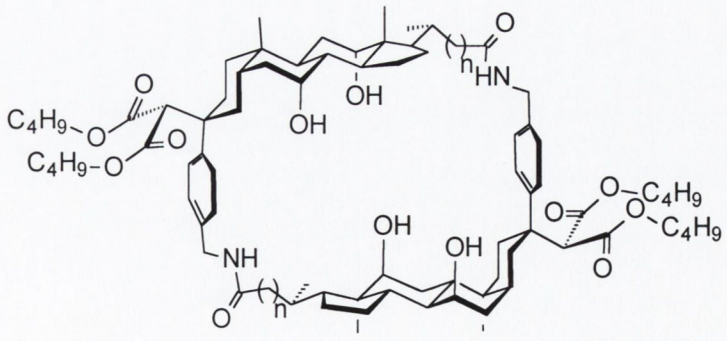
As shown in **Table 2.3**, diastereoselectivity was observed between the α-D and β-D sugars **2.29** and **2.30** by a factor of 5.5 and enantioselectivity was observed between the β-D and β-L sugars **2.30** and **2.32** by a factor of 3.

Sugar	**2.29** (α-D)	**2.30** (β-D)	**2.31** (α-L)	**2.32** (β-L)
K_a [M^{-1}]	545	3100	1030	1000

Table 2.3 Association constants between **2.27** and octyl-glucosides **2.29-2.32** in $CDCl_3$

Although the benzyl amide spacer group in macrocycles **2.24-2.28** restricts the conformation somewhat, it was thought that this could be bettered. Molecular modelling had shown that a number of different and undesirable conformations were possible due to rotation about the carbon-carbon single bonds in the side chain. Using this information the molecule was made more rigid by reducing the number of carbon atoms at this position.

A second problem encountered with **2.27** was its solubility in organic solvents. In $CDCl_3$ it was *ca.* 2 mM, which was barely sufficient for binding study measurements. The solubility properties were improved by introducing an externally directed dibutylmalonyl group at the 3 position. This was executed chemoselectively allowing the butyl esters to have a β orientation and the aryl spacer unit an α orientation thus enabling cyclodimerisation. This gave rise to the more preorganised and organic-soluble receptors, **2.33-2.35**.[21]



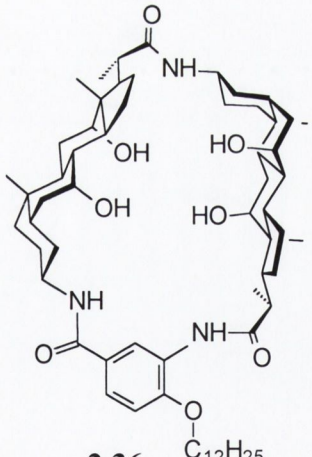
2.33 n = 2
2.34 n = 1
2.35 n = 0

^{1}H NMR titrations against octyl-β-D-glucoside showed that **2.33-2.35** bound the sugar with K_a values of 1305, 600 and 1560 M^{-1} respectively. Although these results

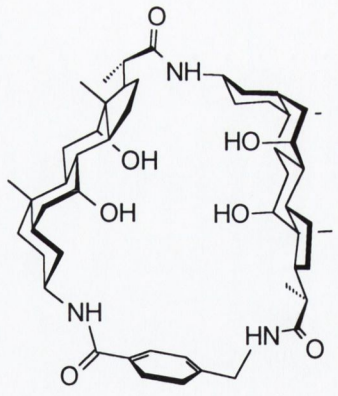
show an affinity of these cholaphanes for carbohydrates, the results are lower than expected for such a seemingly suitable substrate.

Extractions of methyl-β-D-glucoside from aqueous phase (1–2.5 M) into chloroform were carried out using **2.33-2.35**, and also with receptor **2.27**. The results were quantified by TLC and the success of extraction was **2.35** > **2.34** > **2.27** > **2.33**. The receptors having fewer carbons in the side chain proved to be better but surprisingly **2.35** extracted more of the sugar than **2.27,** considering that **2.27** had a higher binding constant when measured by ^{1}H NMR titration. However, this may be attributed to a difference in the substrate (octyl vs. methyl glucosides) as well as the conditions (dry vs. water-saturated chloroform).

Further use of the shortened side chain building block, bis-nor cholic acid, was made by Walsh giving rise to the cyclocholamides **2.36–2.37**.[22], [23] Like compounds **2.33–2.35**, these compounds have restricted conformational freedom. Macrocycle **2.36**, while having a rigid preorganised cavity with inward directed polar functionality, proved to be unsuccessful as a carbohydrate receptor having very low binding constants due to the small cavity size. The insertion of an extra carbon atom creating a benzyl amide spacer increased the cavity size enough for **2.37** to bind octyl-β-D-glucoside in $CDCl_3$ with a binding constant of 750 M^{-1}.



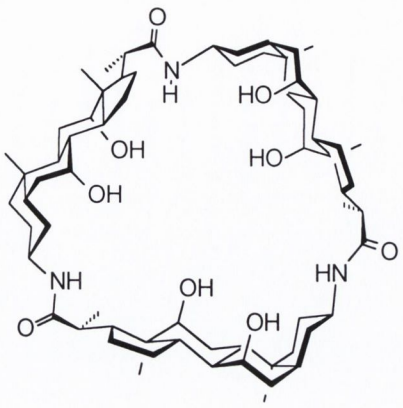
2.36



2.37

The cyclocholamide **2.38** was synthesised by cyclotrimerisation of monomer units, reacting the 3α amine of one monomer with a pentafluorothiophenol active ester of another. This resulted in a macro-trilactam composed of three steroidal units with six hydroxy and three amide NH groups pointing into the cavity. When tested

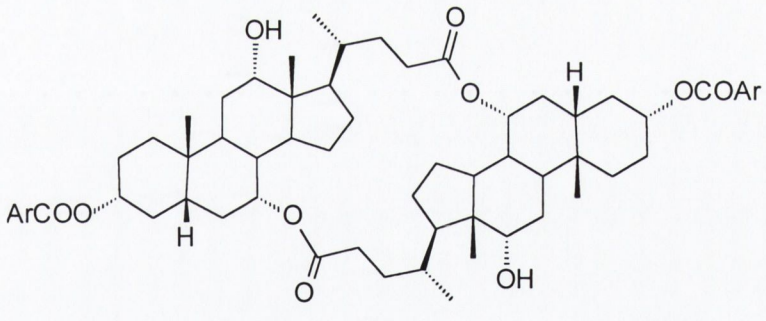
by ^{1}H NMR titration against octyl-β-D-glucoside in $CDCl_3$, evidence of binding was observed, but complex stoichiometries prevented a binding constant being calculated.



2.38

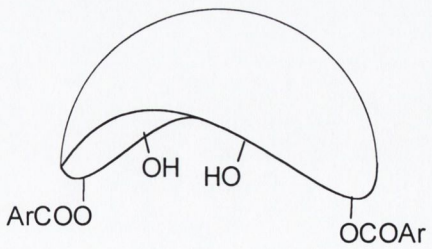
2.3.2 Contribution from Sanders and Bonar-Law *et al.*

Sanders and Bonar-Law further exploited cholic acid by forming an ester linkage between the 7-OH and the carboxylic acid groups of two monomers, resulting in cyclocholate **2.39**.[24], [25] The binding properties were moderate as the system contains only two hydroxyl groups. Binding studies were carried out against a range of sugars in $CDCl_3$ (**Table 2.4**).

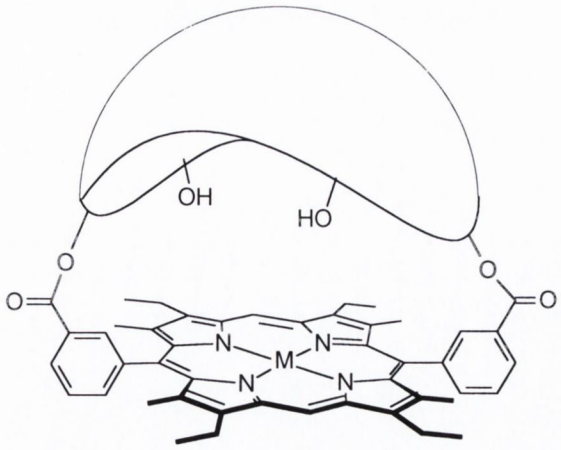


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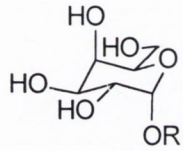
2.39



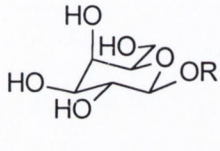
The receptor was made more rigid by the addition of a porphyrin unit to the two aryl esters in the 3- positions, giving macrocycles **2.40** and **2.41**.



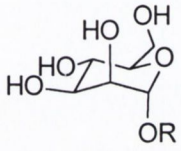
2.40 M = H_2
2.41 M = Zn



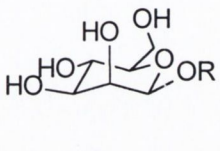
2.42



2.43



2.44



2.45

R = $C_{10}H_{23}$

UV binding studies in $CHCl_3$ showed the expected improvement on **2.39**. However, in CH_2Cl_2 the K_a values were noticeably lower and it was argued this may be due to greater solvation of the host and/or guest. The addition of the Zn metal ion into the porphyrin **2.41** was expected to increase binding through additional Lewis acid coordination. This was observed for α-D-glucoside and β-D-glucoside, with a slight decrease in binding for α-D-mannoside as shown in **Table 2.4**.

	2.39	**2.40**		**2.41**	
	($CDCl_3$)	($CHCl_3$)	(CH_2Cl_2)	(CH_2Cl_2)	(+ H_2O)
α-D-galactoside **2.42**	39			490	
β-D-galactoside **2.43**				200	
α-D-glucoside **2.29**	450	1740	370	910	
α-L-glucoside **2.31**				260	
β-D-glucoside **2.30**	680	3900	770	1480	6700
α-D-mannoside **2.44**	2300	30000	7900	7000	12300
β-D-mannoside **2.45**				5200	18500

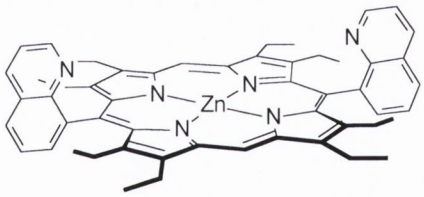
Table 2.4 Association constants between receptors **2.39-2.41** and organic-soluble glucosides

Surprisingly, the addition of small amounts of water or methanol to the solvents used for binding studies increased binding. Usually the addition of a polar cosolvent to a host-guest complex in non-polar media will decrease binding due to the solvent competing for the polar binding sites of both host and guest. The observed increase in binding which was a factor of 4.5 for β-D-glucoside, is presumably due to the water or methanol molecules filling the gap between host and guest thereby extending the intermolecular hydrogen-bonding network. When bulky alcohols, such as isopropyl alcohol or *tert*-butyl alcohol were added, binding decreased. The limited space between host and guest will allow a small polar molecule such as water or methanol to fit in, but not larger ones. This intercalation of polar molecules between host and guest is a well-known phenomenon in biological processes.[26]

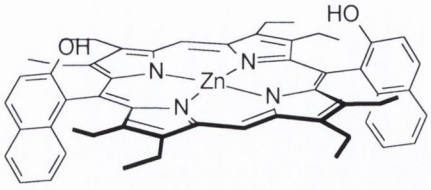
2.4 Alternative Structures – U-Shapes, Bowls and Cages

2.4.1 Contribution from Mizutani and Ogoshi *et al.*

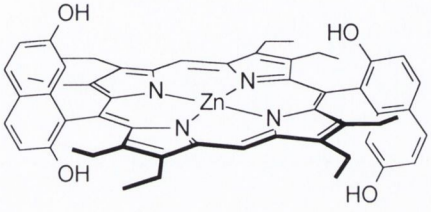
The use of porphyrins in carbohydrate receptors was also exploited by Mizutani, Ogoshi and co-workers, this time creating U-shaped molecules by the addition of aromatic structures at the 5- and 15- positions of the porphyrin.[27], [28] Again, a Zn metal ion was incorporated into the porphyrin for Lewis acid coordination with a guest carbohydrate. Receptor **2.46** has two basic nitrogen atoms which can direct their lone electron pairs into the cavity and act as H-bonding acceptors. Receptor **2.47** has two phenolic hydroxy groups *cis* to each other, while **2.48** has four hydroxy groups in total and both sets of groups can act as H-bond donors due to their acidity.



2.46



2.47



2.48

Binding studies were carried out in chloroform on a range of monools and diols, which showed that the latter bound much more strongly than monools and *trans* diols bound more strongly than *cis* diols. The highest binding constants were obtained for octyl-β-D-glucoside, with **2.46**, **2.47** and **2.48** having K_a values of 41400, 180 and 2090 M^{-1} respectively. It was argued that the Lewis base/Lewis acid/Lewis base combination of **2.46** created a better binding pocket compared with the Lewis acid/Lewis acid/Lewis acid combinations of **2.47** and **2.48**. An analogue of **2.46** where the nitrogen atoms of the quinolyl group are *trans* to each other showed weaker binding, which pointed to cooperative binding of the carbohydrate by the two quinolyl groups.

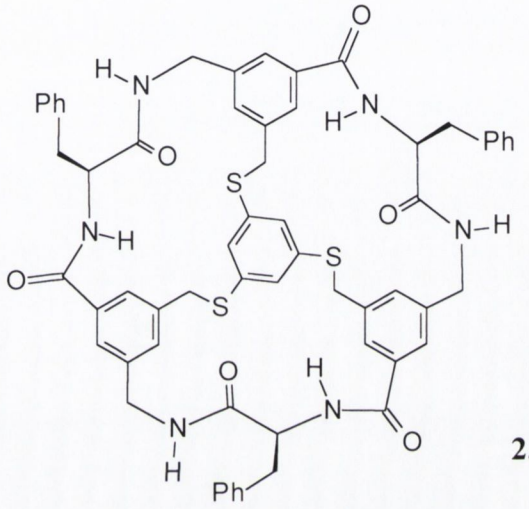
When Zn was replaced by two protons the binding of octyl-β-D-glucoside decreased from 41400 to 600 M^{-1} demonstrating the importance of Zn as a Lewis acid in this receptor.

With **2.47** and **2.48** it was shown that the addition of small amounts of water and various alcohols increased binding in $CHCl_3$, as had previously been reported by Sanders and Bonar-Law with compound **2.41**. Initially, small additions of MeOH and *tert*-butanol increased binding of octyl-β-D-glucoside by **2.48** in $CHCl_3$ but further additions of the alcohols decreased the binding as expected. However, when this was tried with receptor **2.46**, binding was inhibited. This was explained by the

fact that small additions of polar molecules will only assist in binding if the molecule has acidic recognition sites, as with **2.47** and **2.48**.

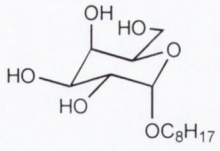
2.4.2 Contribution from Still *et al.*

A novel receptor by Still and co-workers, which has a bowl-shaped conformation, had been tested and proved successful for the recognition of peptides and amino acid derivatives.[29] This C_3 symmetrical molecule has alternating H-bond donors and acceptors present at the "mouth" of the receptor **2.49**.

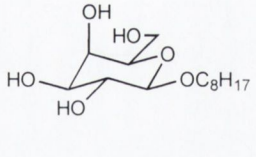


2.49

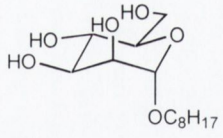
Having this array of polar functionality as well as a cavity large enough for a monosaccharide, **2.49** was titrated against a range of octyl glycosides in $CDCl_3$ as shown in **Table 2.5**.



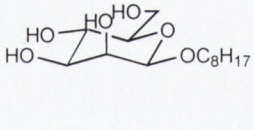
2.50



2.51



2.52



2.53

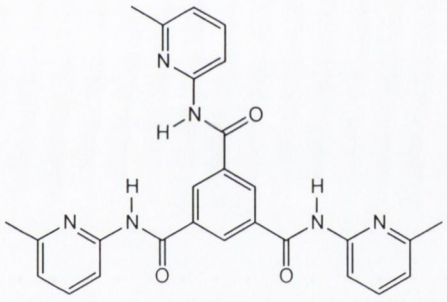
Pyranoside	K_a [M^{-1}]	Pyranoside	K_a [M^{-1}]
α-D-galactoside **2.50**	270	β-D-galactoside **2.51**	640
α-D-glucoside **2.29**	380	α-L-glucoside **2.31**	160
β-D-glucoside **2.30**	1770	β-L-glucoside **2.32**	450
α-D-mannoside **2.52**	5800	β-D-mannoside **2.53**	230

Table 2.5 Association constants between **2.49** and pyranosides **2.29-2.32** and **2.50-2.53** in $CDCl_3$

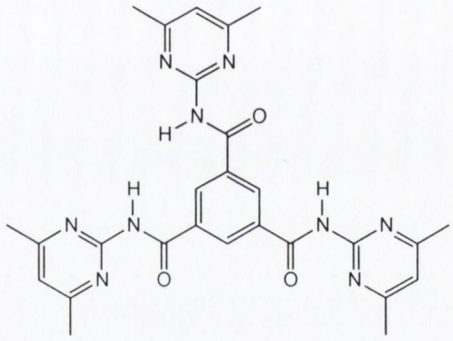
It is evident from these results that **2.49** exhibits enantioselective binding by a factor of nearly 4 in favour of β-D over β-L glucoside. The greatest selectivity is seen between α-D and β-D-mannoside, favouring the α anomer by a factor of 25. This selectivity is not due to the stereochemistry at the anomeric position as the β-glucosides (both D and L) are favoured over the α anomers.

2.4.3 Contribution from Mazik *et al.*

Two recently reported receptors by Mazik and co-workers for carbohydrate recognition make use of a three dimensional binding mode and yet are readily accessible.[30] Both structures **2.54** and **2.55** are acyclic (unlike most other receptors which exhibit three dimensional recognition) and consist of either pyridine-amide or pyrimidine-amide moieties linked by a central phenyl spacer. Both receptors are capable of cooperative binding, having both H-bond donor and acceptor groups as well as aromatic units for CH-π interaction.



2.54



2.55

^{1}H NMR measured binding constants of **2.54** and **2.55** for *n*-octyl-α– and *n*-octyl-β-D-glucoside in $CDCl_3$ are given in **Table 2.6**:

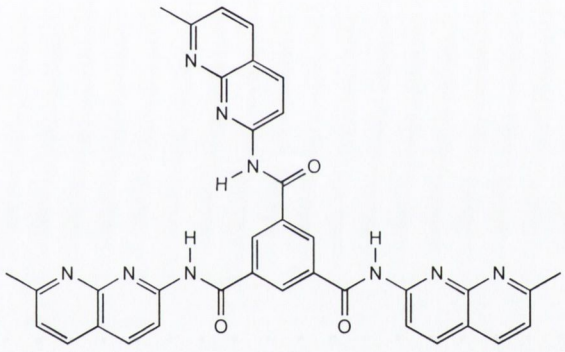
	K_a [M^{-1}] in $CDCl_3$	
	n-octyl-α-D-glucoside	*n*-octyl-β-D-glucoside
2.54	4,000	8,700
2.55	12,500	13,700

Table 2.6 Association constants between **2.54** and **2.55** and *n*-octyl-D-glucosides (α and β) in $CDCl_3$

H-bonding by the amide protons was evident by the observed downfield shift ($\Delta\delta$ = 0.7). Receptor **2.54** formed a suspension in $CDCl_3$ but became fully soluble upon addition of octyl-β-D-glucoside, pointing to complex formation. The importance of the ring nitrogens as H-bond acceptors was demonstrated with **2.55** exhibiting higher binding than **2.54,** due to the three extra ring nitrogens.

Molecular modelling suggested the importance of having three pyridine-amide arms rather than just the two, as the third arm is capable of orientating itself so as to promote CH-π interaction. This was further compounded by the synthesis and testing of a two arm analogue of **2.54** which resulted in an eight-fold reduced binding ability of the octyl-pyranosides.

To improve upon these receptors, the pyridine and pyrimidine units were replaced by naphthyridine units to give **2.56** which has extra H-bond acceptor ability while also increasing the aromatic surface area for additional CH-π interaction.[31]



2.56

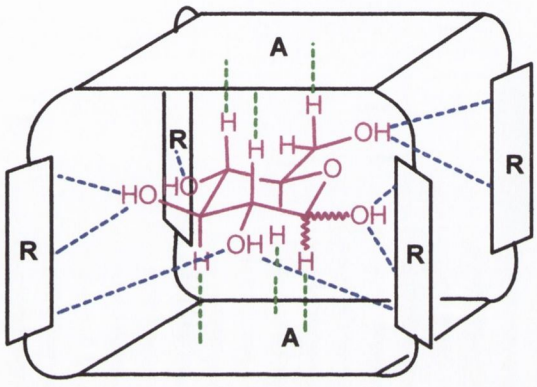
2.56 showed improved binding of octyl-β-D-glucoside with a binding constant of 26,500 M^{-1}, a three-fold increase on that of **2.54**. This improved binding suggested that along with cooperative binding, secondary H-bonding was occurring between the carbohydrate hydroxy groups and the two nitrogen atoms of the naphthyridine, also referred to as the "three-centre two-hydrogen" bonds.

The significance of the amide protons was illustrated by the synthesis and testing of an analogue of **2.54** where the three amide groups were replaced with esters. This only allowed for H-bond accepting by the receptor, rather than cooperative binding as in **2.54** and was reflected in the lower binding constant of 800 M^{-1} for octyl-β-D-glucoside, a factor of 11 lower than the K_a for **2.54**.

While the receptors **2.54**, **2.55** and **2.56** are relatively straightforward to synthesise and are capable of binding to saccharides in a three dimensional fashion, they do not fully encapsulate the saccharide, which would enhance binding.

2.5 A Recent Receptor from the Davis Group

From the information obtained from carbohydrate recognition by proteins, nature greatly aided in the design of the following schematic representation of a synthetic receptor for carbohydrates (**Figure 2.2**):



A = Aromatic, apolar surface for hydrophobic contacts

R = Rigid spacer prevents macrocycle collapsing

Provides H-bonding sites

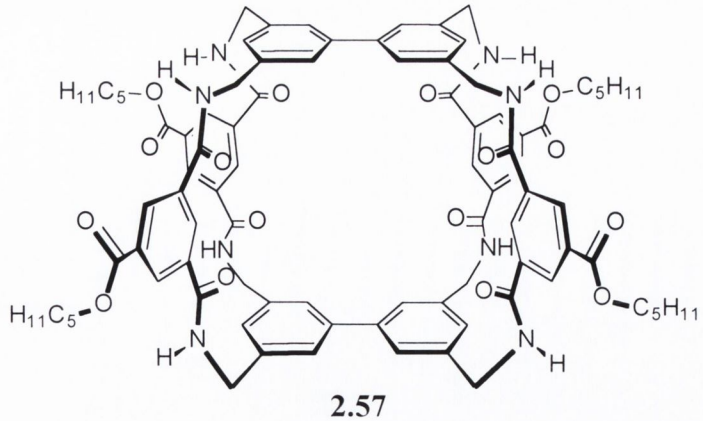
Water Solubilising groups

----- = H-bond

----- = Hydrophobic / CH-π interaction

Figure 2.2 Schematic representation of a receptor for β-D-glucosides

The two units designated **A** represent the apolar surfaces for hydrophobic contacts with the hydrophobic region of the carbohydrate, in this case glucose. The four units designated **R** have a threefold functionality: firstly, they must act as rigid spacers preventing the macrocycle from collapsing upon itself, secondly, they should possess H-bonding sites and finally, there must be externally orientated water-solubilising groups. Wareham designed and synthesised the macrotricycle **2.57**:[32], [13]



2.57

The molecule consists of two biaryl units, top and bottom, and four bis-amide linkers, forming a cage-like structure enabling full encapsulation of a monosaccharide. The criterion of having aromatic units is met, allowing for CH-π interactions as noted earlier and there is also some flexibility about the biaryl C–C bonds enabling for a better fit with a sugar molecule. Each linker group consists of two amide groups providing H-bond donor/acceptor sites. The amide groups are separated by an aromatic spacer incorporating externally orientated pentyl ester groups which may be cleaved or manipulated to promote water solubility. The molecule is extremely well preorganised, with eight amide moieties in total surrounding the cavity and aromatic hydrophobic patches orientated top and bottom.

2.57 was modelled as the tetra-methyl ester with β-D-glucopyranose as guest using macromodel version 5.0 with amber force field.[32] As shown in **Figure 2.3**, six intermolecular hydrogen bonds between the host and guest are represented by broken lines and hydrogen atoms on C1, C2 and C3 of the carbohydrate form close CH-π interactions with the host.

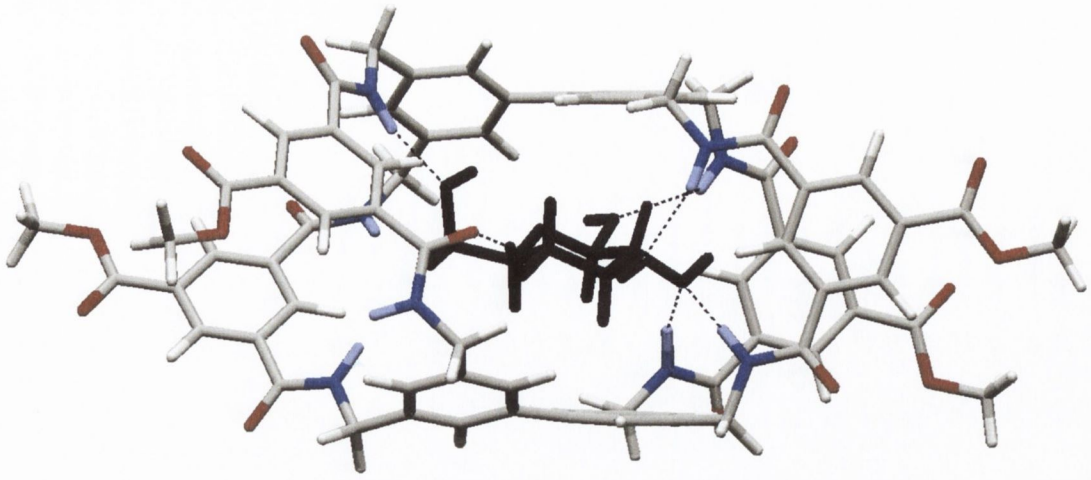


Figure 2.3 Monte Carlo (1000 step) energy minimised structure of **2.57** plus β-D-glucopyranoside. The host was modelled as the tetra-methyl ester for simplicity.

2.57 proved to be an excellent receptor for *n*-octyl-pyranosides, as listed in **Table 2.7**. ^{1}H NMR binding studies were not possible in $CDCl_3$ as the spectrum was unresolved in this solvent. Therefore, testing in $CHCl_3$ was measured by fluorescence titration. As anticipated, *n*-octyl-β-D-glucoside was bound the strongest and had a binding constant of 300,000 M^{-1} in $CHCl_3$ and 980 M^{-1} in the quite competitive medium of $CD_3OH/CDCl_3$ (8:92).

	K_a M^{-1}	
	$CD_3OH/CDCl_3$ (8:92)	$CHCl_3$
n-octyl-β-D-glucoside **2.30**	980	300,000
n-octyl-α-D-glucoside **2.29**	20	13,000
n-octyl-β-D-galactoside **2.51**	220	110,000

Table 2.7 Association constants between **2.57** and octyl pyranosides

Selectivity of β-D-glucoside over its α anomer by **2.57** is of the order of 50 in $CD_3OH/CDCl_3$ (8:92). This selectivity is attributable to the axial orientation of the *n*-octyl ether chain of the α-D-glucoside molecule, making it sterically much more difficult to enter the cavity, compared with *n*-octyl-β-D-glucoside.

CHAPTER THREE

3.1 Carbohydrate Recognition in Nature

It has been realised in recent years that carbohydrates have not been given the deserved attention and research in the past and this has fuelled much interest in the biological, as well as chemical, disciplines.[13] Molecular recognition between different biomolecules is of fundamental importance to life itself. The information-carrying potential of oligosaccharides has been realised to be greater than that of proteins and nucleic acids of comparable molecular weight, and their presence as appendages on cell surfaces and proteins may be suggestive of important biological functions. Carbohydrate recognition in the body is important for metabolism as well as transport across cell membranes. The supplementation of such natural systems by synthetic models is therefore useful in extracting information and may prove to be of importance in influencing biochemical activities.[33], [34], [35] Synthetic receptors could be used as drugs (*e.g.* anti-infective agents) to target cell types, thus acting as "synthetic antibodies". Another possibility is the transport of carbohydrates and pharmaceutical derivatives across cell membranes.[13]

The challenge to the supramolecular chemist is the architectural design as well as the synthesis of a truly biomimetic carbohydrate receptor. Mimicking nature amounts to "simplifying the processes involved".[36] In other words, what is required is a receptor with the properties capable of binding carbohydrates in an aqueous environment. Success on this front would enable the answering of fundamental and important questions concerning the principles behind carbohydrate binding, which have eluded scientists to date. Carbohydrates present a difficult task for receptor design, having a complex three dimensional array of functionality. X-ray crystallography of protein-carbohydrate complexes has shown the expected polar and hydrophobic interactions. More precisely, the polar groups of the carbohydrate are bonded by H-bond donor and acceptor groups, while the hydrophobic regions are complemented by non-polar surfaces.[37], [38], [39] It is noteworthy that these non-polar surfaces of the proteins are almost always aromatic, suggestive of CH-π type interactions.[40] There are three main classes of proteins which are important as regards carbohydrate binding: lectins, antibodies and bacterial periplasmic proteins. The structural analysis of lectins has revealed a binding site for sugar appendages on

cell surfaces.[39] For lectins, typical binding constants for monosaccharides are in the region 10^3-10^4 M^{-1}, which are considered weak in biological terms.[41] Similarly, structural studies of antibody-carbohydrate complexes have shown clefts on protein surfaces for carbohydrates.[42] However, the greatest binding constants are achieved by bacterial periplasmic proteins, with K_a values of 10^6-10^7 M^{-1}, due to their ability to fully encapsulate the monosaccharide.[37]

3.2 The Driving Force behind Recognition in Aqueous Media

Despite the availability of much structural data on carbohydrate-protein complexes, the driving force behind their complexation is not fully understood.[13] The molecular recognition of polar molecules such as carbohydrates in organic media is largely based on intrinsic binding, due to the insignificant competition from solvent molecules for the binding sites of both the host and guest. For the recognition of non-polar molecules in aqueous media, the binding is driven by hydrophobic or solvophobic effects. However, when it comes to recognition of carbohydrates in aqueous media the situation is a much more complex and challenging one and it remains uncertain as to whether intrinsic or hydrophobic binding is more important.

Initially it was believed that binding may be entropy driven, as highly ordered water molecules escape from hydrophobic patches of both host and guest upon complexation.[43] In addition to hydrophobic binding, attractive dispersion forces between complementary non-polar surfaces was also suggested as a driving force.[44] Crystallographic evidence,[37] on the other hand, focussed attention on H-bonding networks which pointed to intrinsic binding. It is quite obvious, however, that crystallography by itself cannot distinguish between entropy and H-bonding as a driving force.

Experiments were carried out by Lemieux and co-workers to investigate the changes in thermodynamic parameters upon binding of a tetra-saccharide by a lectin.[43] If hydrophobic binding was to be the driving force then an increase in entropy would be expected. However, this was not the case and the observed decrease in entropy upon binding led to the abandonment of a dominant hydrophobic effect. These results prompted the belief that the thermodynamic changes could only be explained by changes in the dynamics of the water structure. Lemieux coined the

term "hydraphobic" which refers to hydrophobicity at polyamphiphilic surfaces, as opposed to hydrophobic, which indicates hydrophobicity at non-polar surfaces.

When, upon binding, water molecules are released into the bulk water from a non-polar surface, an increase in entropy is observed. Conversely, when strained water molecules at polyamphiphilic surfaces are released into bulk water, a decrease in both enthalpy and entropy occurs. Water at a polyamphiphilic surface has been shown to be perturbed over approximately three water layers. As this occurs at both the receptor and substrate, many water molecules are involved and therefore the release into the bulk of these high energy water molecules will contribute significantly to a negative enthalpy. If unaccompanied by a significant entropy decrease, this can result in complex formation.

Chervenak and Toone,[45] investigating the thermodynamics of binding between lectins and carbohydrates in H_2O and D_2O, observed a decrease in enthalpy of binding in D_2O. This is further evidence that solvent reorganisation provides an enthalpic driving force for binding.

3.3 Carbohydrate Recognition in Aqueous Media

3.3.1 Contribution from Sugimoto *et al.*

Some recent work by Sugimoto and co-workers[46] described the synthesis of a library of small peptides, of which some had the ability to bind a monosaccharide in aqueous solution. Their work is based upon the principle that X-ray crystallography has revealed that high selectivity for a saccharide is achieved through a combination of hydrogen bonding networks between protein and carbohydrate hydroxy groups and CH-π interaction between hydrophobic patches on the carbohydrate and aromatic surfaces of amino acids. The carbohydrate is therefore sandwiched between the apolar aromatic surfaces while simultaneously having its hydroxy groups involved in hydrogen bonding. The model described herein was established by testing a number of alanine based peptides of varying length to see what length of peptide chain would give optimum sandwiching of D-galactose between two tryptophan indole units. The peptides initially tested were:

(**a**) Lys-Trp

(**b**) Lys-Trp-Trp

(**c**) Lys-Trp-Ala-Trp

(**d**) Lys-Trp-Ala-Ala-Trp

(**e**) Lys-Trp-Ala-Ala-Ala-Trp

(**f**) Lys-Trp-Ala-Ala-Ala-Ala-Trp

(**g**) Lys-Trp-Ala-Ala-Ala-Ala-Ala-Trp

The differences in fluorescence (ΔF) of the test peptides **b-g** in the presence and absence of D-galactose were measured in aqueous buffer solution and **Figure 3.1** shows that peptide (**e**) has the strongest binding.

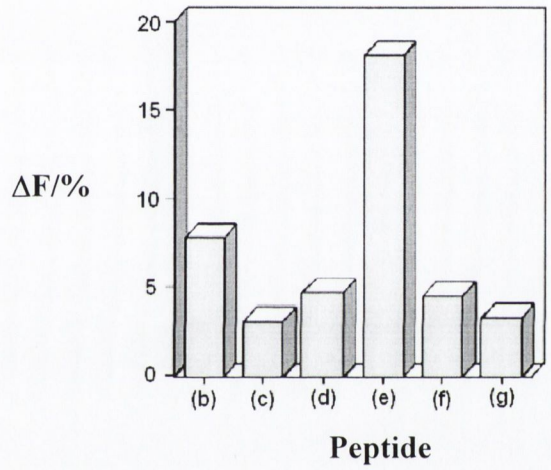


Figure 3.1 Fluorescence of test-peptides **b-g**

Based on these results, a solid phase combinatorial library of *ca.* 62,000 members of structure Aro-X-X-X-Aro was synthesised. The two terminal Aro groups represent L-aromatic amino acids and X any natural L-amino acid, except Cys. The sugar tested for was D-erythrose in an aqueous buffer, as it is light yellow in colour and the beads could therefore be selected by visualisation after being washed with buffer solution to remove non-specific interactions.

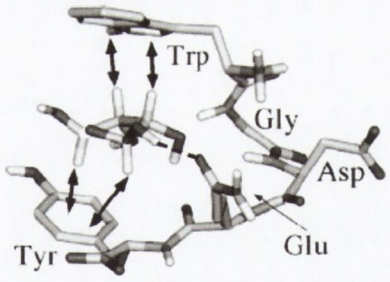
15 of the 62,000 beads were selected on the basis of a positive yellow colour and peptide sequencing revealed that 10 of these 15 beads were Trp-Z-Z-Z-(Phe/Thr), where two of the three Z amino acids were either Asp or Glu.

Two of the ten peptides, **3.1** and **3.2**, as well as test-peptide **e** were titrated against D-galactose and D-erythrose in aqueous buffer and the fluorescence binding constants were then calculated (**Table 3.1**).

Peptide	K_a 10^4 M^{-1}	
	D-erythrose	D-galactose
Trp-Gly-Asp-Glu-Tyr **3.1**	35.0	5.22
Trp-Ala-Asp-Glu-Phe **3.2**	26.5	3.81
Lys-Trp-Ala-Ala-Ala-Trp **e**	0.41	3.89

Table 3.1 Association constants between **3.1**, **3.2** and **e** and D-erythrose and D-galactose in aqueous buffer.

The binding constant of **3.1** for D-erythrose is 3.5×10^5 M^{-1}, which is of the order when a lectin binds a monosaccharide in an aqueous environment.[41] Molecular modelling of the **3.1**-D-erythrose complex suggests that binding occurs through sandwiching of the sugar molecule between the aromatic units of Trp and Thr as well as forming an H-bond with Glu.



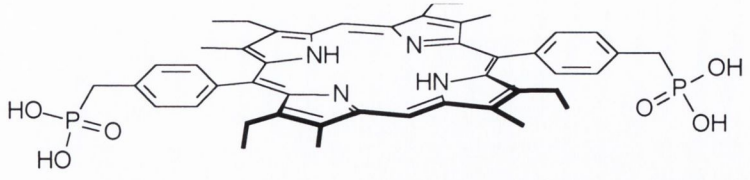
3.1

This binding of a monosaccharide in an aqueous environment seems very impressive, however it should be pointed out that no NMR evidence has been reported for this system. The receptor is not rigid either, which makes such high binding of D-erythrose in water quite surprising.

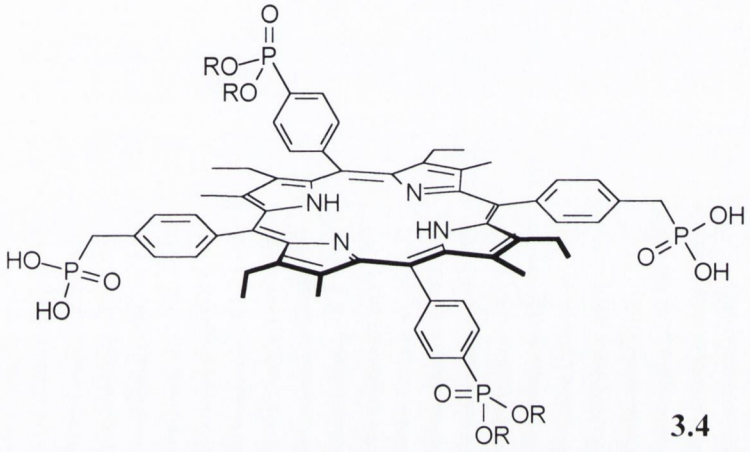
3.3.2 Contribution from Král *et al.*

Král and co-workers recently reported the synthesis of two porphyrin based water-soluble receptors capable of binding saccharides in aqueous media.[47] The incorporation of phosphonate esters into diphenyl porphyrin **3.3** and

tetraphenylporphyrin **3.4** allows H-bonding between the saccharide and the P=O groups, which are known to be good H-bond acceptors. The porphyrin units also act as a chromophore for UV-vis binding studies.



3.3



3.4

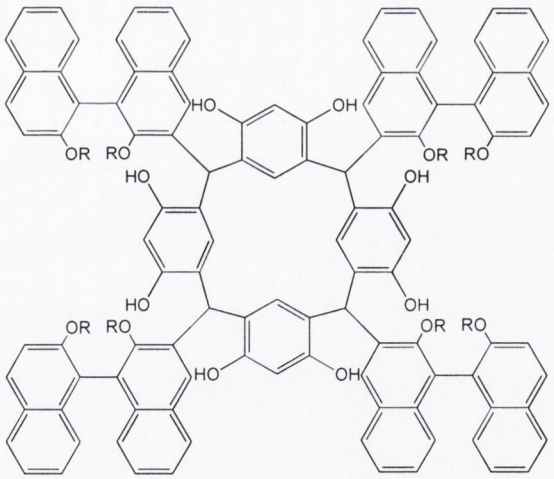
A number of mono- as well as disaccharides were tested in 5 % methanol in water following by UV-vis titration (**Table 3.2**).

	K_a 10^4 [M^{-1}]	
	3.3	**3.4**
D(+) galactose	1.35	1.97
α-D-glucose	1.90	1.76
α-D-arabinose	1.38	1.50
D(+) mannose	1.40	1.20
D(–) fructose	1.33	1.18
D(–) ribose	0.60	0.78
D-trehalose	1.94	2.12
D(+) maltose	2.17	2.42
α-D-lactose	2.13	2.50

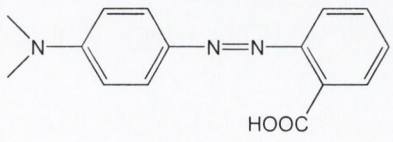
Table 3.2 Association constants between **3.3** and **3.4** and substrates in MeOH/Water (5:95)

These results are all of the order of 10^4 M^{-1} which are similar to the affinities lectins exhibit for a monosaccharide. The only ^1H NMR data to supplement the UV binding study was carried out in d_6-DMSO, rather than D_2O or D_2O with deuterated methanol as cosolvent, which would have been consistent with the UV binding studies. ^1H NMR showed that interaction of the receptor **3.3** with α-D-glucose in d_6-DMSO caused broadening of the anomeric proton as well as hydroxy group protons. A shift of 17 ppm in the ^{31}P NMR spectra between compound **3.3** and the equimolar α-D-glucose:**3.3** complex in water was reported. IR spectroscopy was also used for further evidence of hydrogen bonding in the complex of α-D-glucose with **3.3**. Saccharide -OH stretches shifted from 3390 to 3408 cm^{-1} and C–O from 1025 to 1071 cm^{-1}. With regard to the receptor, the P=O band broadened and the P–O stretch shifted from 1180 to 1158 cm^{-1}.

Two further water-soluble receptors were reported by Král and co-workers, on this occasion based on 1,1′-binaphthyl calixresorcinols **3.5** and **3.6**.[48] The authors make use of a non-covalently bound chromophore, methyl red **3.7**, which acts as an indicator, to follow complexations between **3.5** and **3.6** and their guest saccharides.



3.5: R = Me
3.6: R = H



3.7 (methyl red)

Equimolar complexes of **3.5** and **3.6** with methyl red **3.7** exhibit strong absorbances in the visible spectral range. The receptor structure is described as cup-shaped with

the binaphthyl substituents forming a deep cavity with the lower rim being polyhydroxylated. The principle behind the binding is that competition is set up between the saccharide guest and the methyl red indicator. Initially the receptor and the methyl red form a 1:1 complex and changes in absorbance are measured as the guest sugar is titrated into the mixture, competing with methyl red **3.7** for the receptor binding site.

An ^{1}H NMR binding study showed that methyl red indicator binds to **3.5** and **3.6** in acetonitrile with low binding constants of 60 and 80 M^{-1} respectively. Shifts of the aromatic protons of the receptors and **3.7**, as well as shifts of the dimethyl amino protons of **3.7**, were observed. For **3.5**, the hydroxy protons of the lower rim and the methyl protons of **3.7** broadened, suggesting interaction between these two sites of the molecules. For **3.6**, the binaphthyl hydroxy groups shifted by *ca.* 0.5 ppm upon complexation with **3.7**.

Complexes **3.5-3.7** and **3.6-3.7** were tested for sugar binding by UV-vis titrations in MeOH/H_2O (1:99) due to the insolubility of **3.5**, **3.6** and **3.7** in pure water. Upon complexation with a saccharide, **3.5-3.7** changed colour from orange to yellow and **3.6-3.7** changed from violet to yellow. The binding constants for various saccharides are listed in **Table 3.3**:

	K_a 10^2 [M^{-1}]	
	3.5	**3.6**
Me-α-D-glucoside	11.9	15.0
D(+) galactose	9.0	9.5
D(–) fructose	7.7	7.5
D(–) ribose	8.1	7.3
α-D-glucose	9.9	11.0
D(+) maltose	45.2	45.5
maltotriose	39.9	40.2
maltotetraose	28.4	40.0
α-D-lactose	18.7	35.3
β-D-lactose	16.4	33.0

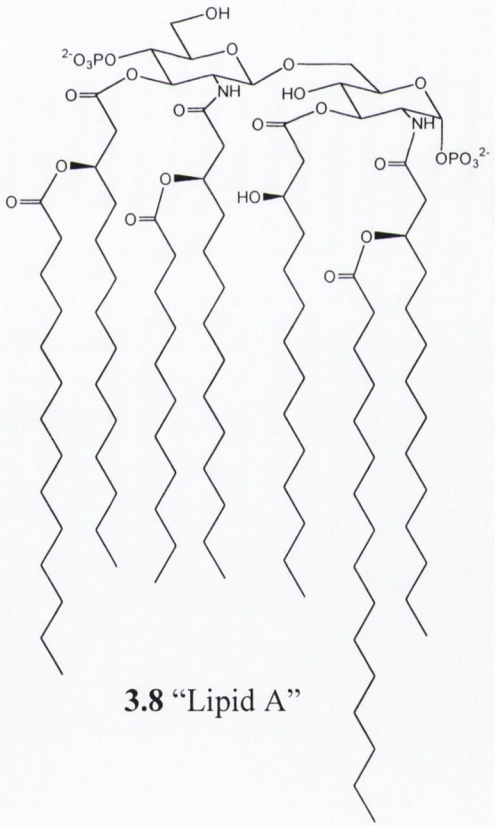
Table 3.3 Association constants between **3.5** and **3.6** and substrates in MeOH/H_2O (1:99)

Again, these results are of the order of which lectins bind carbohydrates. No ^{1}H NMR evidence is reported, but this may be due in part to aggregation of host molecules at NMR concentrations, a problem often observed with apolar hosts in aqueous solution, as pointed out by the authors.

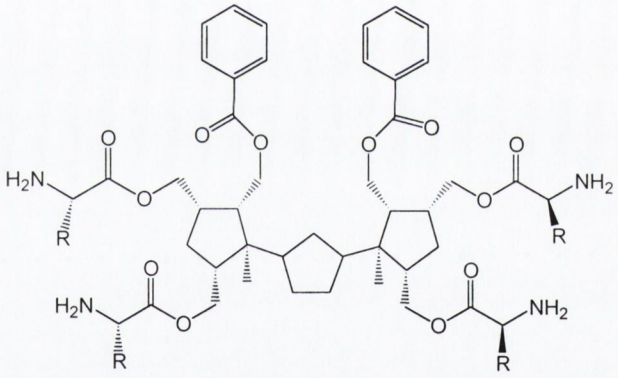
3.3.3 Contribution from Miller *et al.*

A recently reported receptor by Miller and co-workers is based on a *ter*-cyclopentane structure which is highly substituted and contains ten stereogenic centres.[49] The target molecules are simple monosaccharides, oligosaccharides and liposaccharides, with particular emphasis on liposaccharide **3.8**, known as Lipid A.

Lipid A is a portion of lipopolysaccharide (LPS) which constitutes the outer cell membrane of Gram negative bacteria and is responsible for causing bacterial sepsis in the body.[50] Antibiotics have been developed but have a poor side effect profile and so make a receptor for Lipid A a desirable goal.



3.8 "Lipid A"



3.9 R = H
3.10 R = CH_2Ph
3.11 R = indole

3.9-3.11 were derived from reacting the tetra-ol peripheral groups of the intermediates with amino acids Gly, Phe and Trp respectively. The affinities of the receptors for Lipid A were measured by UV titration in phosphate buffered saline pH

7.4. **3.10** and **3.11** bound to **3.8** with dissociation constants of 587 and 592 nM respectively, which are comparable to those seen for the antibiotic polymyxin B, designed for Lipid A recognition. Binding of Lipid A to **3.9** was not observed below the critical micelle concentration, pointing to the importance of the amino acid side chain.

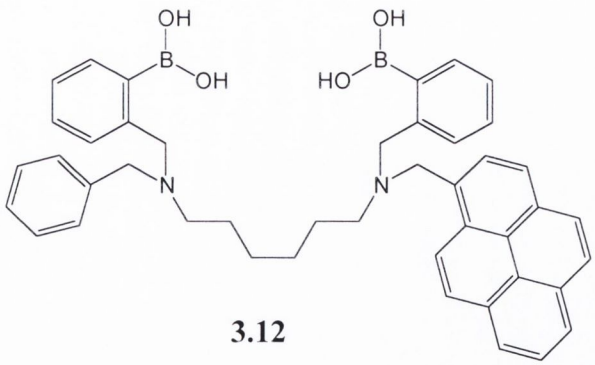
Separate control experiments were carried out to show that the interaction between receptors **3.10** and **3.11** with the phospholipid chain of Lipid A were not significant. Titrations of dipalmitoyl phosphatidylcholine, a lipid-containing molecule, into solutions of **3.10** and **3.11** showed only very weak interaction. Also, to demonstrate that interactions were not due only to amino acid functionality, the methyl ester of Trp was titrated against Lipid A in aqueous solution. Again, non-specific interaction was observed.

An ^{1}H NMR binding study between **3.11** and Lipid A was undertaken in D_2O/d_6-DMSO (5:95). Upon addition of Lipid A, all resonances broadened and a precipitate appeared in the tube, which prevented any structural information being obtained. While these receptors show affinity for carbohydrates, no binding of **3.9-3.11** to a monosaccharide was reported.

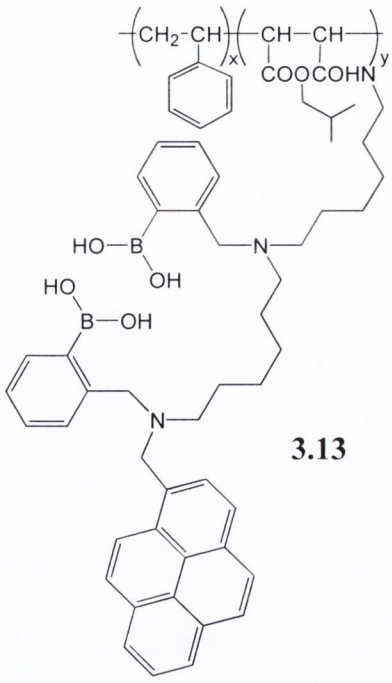
3.3.4 Contribution from Shinkai and James *et al.*

There are many examples in the literature of aryl boronic acids acting as carbohydrate receptors. These include mono- as well as diboronic acids and recognition occurs through covalent bond formation between the boronic acids and hydroxy groups of the sugar, which form readily in water.[51] As recognition occurs through covalent bond formation, interaction between the sugar and the receptor is therefore very strong which enables molecules of this type to be useful as sugar sensors.

Two recent receptors from James and co-workers use a modular approach to receptor design in which the extent of interaction with a sugar is determined by fluorescence.[52] Two receptors are reported, one a solution based receptor **3.12** and the second a polymer supported receptor **3.13**, which consist of two boronic acid units each which enables saccharide selectivity:



3.12

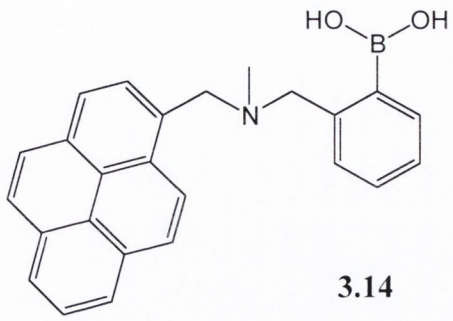


3.13

Interaction between the boronic acid and the amines of **3.12** and **3.13** creates a photoinduced electron transfer (PET) sensory system for saccharides. The boronic acid, acting as a lewis acid and the amine acting as a lewis base exhibit increased interaction upon binding of a saccharide. This boronic acid-amine interaction is capable of modulating the PET from the amine to the fluorophore with the consequence of these compounds showing increased fluorescence upon saccharide binding, due to suppression of the PET from the amine to the fluorophore. Therefore an increase in fluorescence is a direct result of stronger boronic acid-amine interaction.

The receptors **3.12** and **3.13** consist of three main units: a boronic acid receptor unit; a spacer unit and finally a pyrenyl fluorophore unit. The author points out that it is desirable to have the option of varying the linker as well as the fluorophore so that different sugars may be bound and sensed. The use of a hexamethylene linker, as is present in **3.12** and **3.13**, was shown by Shinkai to be an important feature of these type of receptors in obtaining glucose selectivity.[53] While polymer supported boronic acid based sensors had been synthesised previously, none had been based on systems which exhibited selectivity in solution. This led to the synthesis of receptor **3.13**.

To determine any possible selectivites, compound **3.14** containing only one boronic acid unit, was synthesised and tested as a reference for **3.12** and **3.13**.



3.14

Receptors **3.12-3.14** were tested for binding of four saccharides in aqueous methanolic buffer solution (52.1 weight % at pH 8.21) and the stability constants of the resulting complexes calculated as shown in **Table 3.4**:

	3.12 $K\ M^{-1}$	**3.12/3.14**	**3.13** $K\ M^{-1}$	**3.13/3.14**	**3.14** $K\ M^{-1}$
D-glucose	962	22	385	9	44
D-galactose	657	13	778	15	51
D-fructose	784	2	1124	3	395
D-mannose	74	2	79	2	36

Table 3.4 Stability constants K for complexes **3.12-3.14** and various saccharides in aqueous methanolic buffer, pH 8.21

Cooperative binding of both boronic acid groups is occurring as the stability constants of **3.12** and **3.13** are higher for all four sugars than those of the monoboronic acid receptor **3.14**. The binding affinity of **3.12** for D-glucose and D-galactose is 22 and 13 times greater respectively than that of the monoboronic acid **3.14**. However, the stability constant of **3.12** with D-fructose is only twice as strong as that of **3.12**. This is explained by the fact that D-glucose forms 1:1 cyclic complexes with diboronic acids whereas D-fructose forms 2:1 acyclic complexes.

Selectivity for D-glucose is lower with the polymer supported receptor **3.13** compared with the solution-based receptor **3.12** but selectivity for the other

saccharides is similar. This selectivity difference is possibly due to the proximity of the receptor to the polymer backbone.

3.4 Aims of this Project

The aims of this project were to design, synthesise and test a receptor capable of recognizing carbohydrates in both organic and aqueous media based on Wareham's macrotricycle **2.57**. Initially, this involved improving upon the synthesis of **2.57** as, owing to its success, future analogues were to have the same core features. Once achieved, the solubilisation of the macrotricycle in an aqueous media and subsequent testing of carbohydrate recognition, were objectives. This was to be attained by the hydrolysis of the pentyl ester side chains of **2.57** or by employing alternative water-solubilising groups in the aryl spacer units.

As outlined in Chapter Two, macrotricycle **2.57** was capable of recognising carbohydrates in organic media with very high affinities indeed. However, Wareham found that due to solubility problems at the organic aqueous interface, extraction of sugars from water into an organic medium proved to be impossible. Therefore, the synthesis and testing of an organic-soluble macrotricycle capable of extracting sugars from water was another goal of the project.

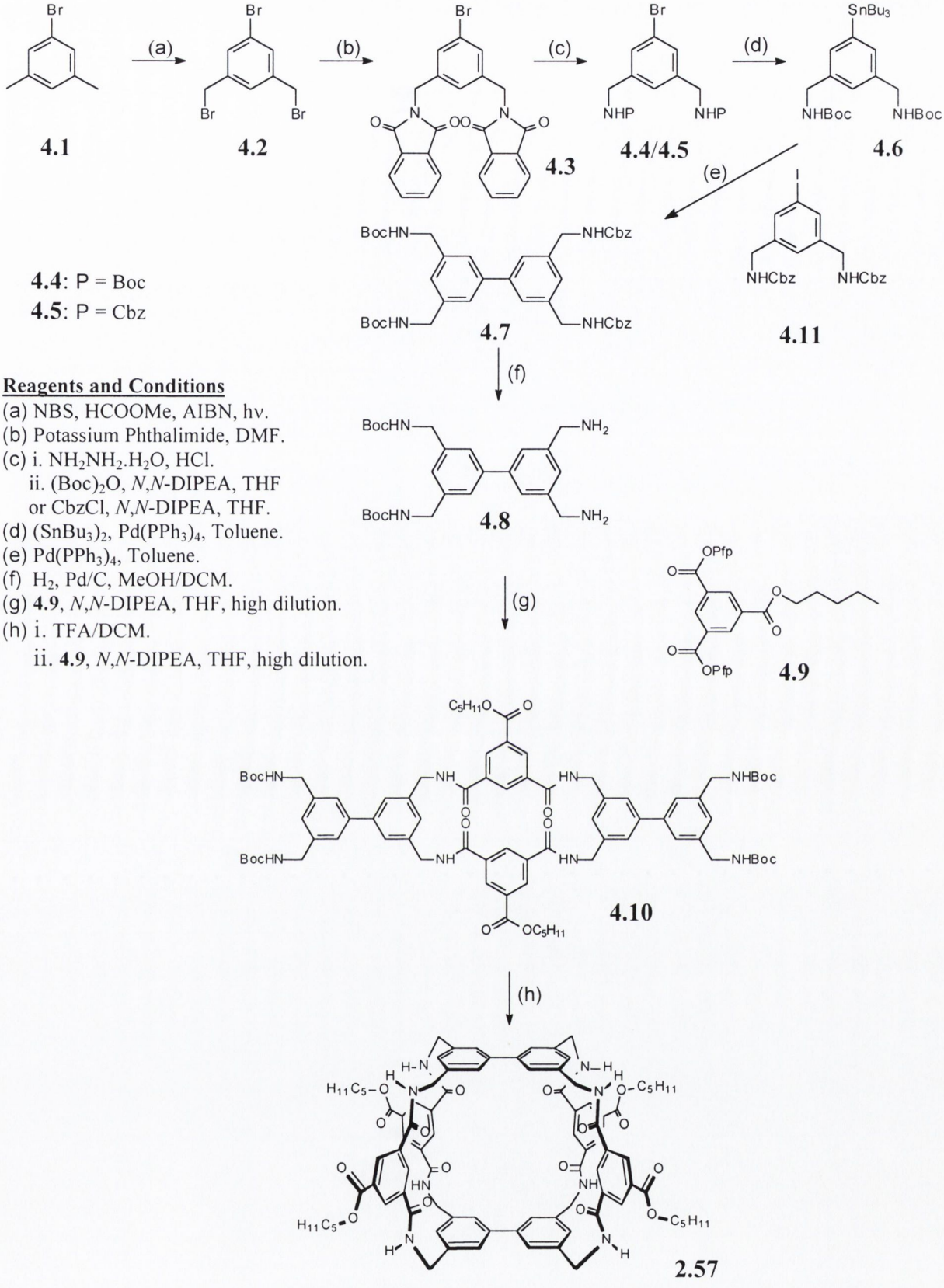
CHAPTER FOUR

Preamble

As outlined in Chapter Two, Wareham and Davis designed and synthesised the pentyl macrotricycle **2.57**, which was subsequently tested for carbohydrate recognition in organic media. The initial aim for this project was the optimisation of Wareham's synthesis of **2.57**. This chapter will deal with the original synthesis by Wareham, in detail, before describing the improvements made to it.

4.1 Synthesis of Pentyl Macrotricycle 2.57 by Wareham

Reactions and conditions for the synthesis of **2.57** are outlined in **Scheme 4.1**. The first main goal is the synthesis of the unsymmetrically protected biaryl **4.7**. The biaryl benzylic amines need to be differentially protected, as this will allow for control to be exercised over the cyclisation of the biaryl with the bis-pentafluorophenyl activated ester **4.9**. Deprotection of the amine groups at one end of the biaryl **4.7**, followed by cyclisation with **4.9** in a [2 + 2] cyclisation, affords the tetra-Boc compound **4.10**. [1 + 1] cyclisation between **4.8** and **4.9** is not possible due to steric/electronic reasons. Deprotection of the Boc protected amines followed by a second cyclisation, this time [2 + 1], yields the desired pentyl macrotricycle **2.57**. Wareham synthesised the unsymmetrical biaryl **4.7** by means of a Stille coupling reaction between two orthogonally protected aryl derivatives **4.4** and **4.5**. These two compounds were synthesised following an analogous route as they differ only in their amine protecting groups.



Scheme 4.1 Synthesis of the pentyl macrotricycle **2.57** as carried out by Wareham[32], [54]

4.1.1 Bromination of 5-Bromo-*m*-xylene

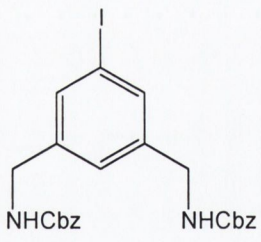
Employing 5-bromo-*m*-xylene **4.1** as starting material, bromination at the benzylic position was carried out using an excess of NBS, in methyl formate, with AIBN acting as a radical initiator in the presence of a 100 W lamp which induced reflux. Reaction times were typically 4 hours and work-up involved extracting the desired material into CCl_4 and removing undesired succinimide by filtration. Recrystallisation from hexane, of the solid obtained after concentration of the filtrate *in vacuo*, yielded the desired product **4.2** with typical yields being 20-30 %.

4.1.2 Phthalimide Formation 4.3 and 4.21

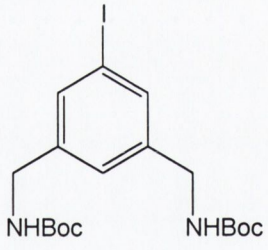
Introduction of the benzylic nitrogens into **4.2** and **4.19** was achieved using the Gabriel synthesis[55], with DMF as solvent. Reaction times were short, *ca.* 5 minutes and purification was by recrystallisation from ethyl acetate/hexane and typical yields were *ca.* 70 %.

4.1.3 Phthalimide Deprotection and Amino Protection 4.3 and 4.21

Removal of the phthalimide groups of **4.3** and **4.21** to give the free amine compound was achieved by refluxing hydrazine hydrate in MeOH/DCM (4:1) at 80 °C. Reflux time was 2 hours and the unwanted phthalazine-1,4-dione side product was removed by filtration.



4.11



4.12

The bis-amine product **4.22** (*cf.* section 4.2.5) was isolated by acid/base extractions and the two free amines were either bis-Cbz or bis-Boc protected to give **4.11** and **4.12** respectively. Boc protection was achieved using Boc anhydride and Cbz protection using Cbz-Cl. The aryl halides were purified by recrystallisation from ethyl acetate/hexane and yields for **4.11** and **4.12** were typically 70 %.

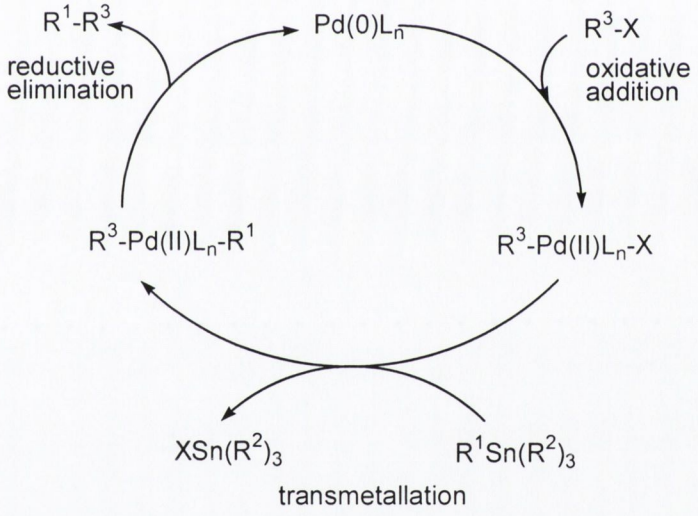
4.1.4 Stille Biaryl Coupling

Coupling of the differentially protected aryl halides **4.11** and **4.12** was accomplished using a Stille biaryl coupling procedure. The Stille reaction is a palladium catalysed coupling between an arylstannane and an aryl halide or triflate and is a means of carbon-carbon bond formation (**Equation 4.1**). The reaction is very versatile as it proceeds under neutral conditions and can therefore tolerate a wide range of functional groups. Also, the reagents employed are not particularly moisture or air sensitive.

$$R^1Sn(R^2)_3 + R^3X \xrightarrow{Pd(0)L_n} R^1\text{-}R^3 + (R^2)_3SnX$$

Equation 4.1

The generally accepted mechanism for the Stille reaction is outlined in **Scheme 4.2**:

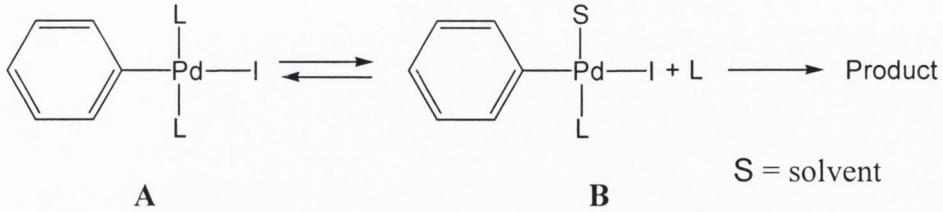


Scheme 4.2 Mechanism for the Stille biaryl coupling reaction

Insertion of Pd(0) into the aryl halide results in a Pd(II) species which then undergoes transmetallation with the organostannane. Reductive elimination gives the desired carbon-carbon bond formation as well as regenerating the Pd(0) catalyst. Mechanistic studies have shown the rate-determining step to be transmetallation. Farina has investigated ways of accelerating this particular step. The two improvements introduced concerned the ligand and the use of a copper salt.[56]

Ligand Effects:

The extent to which the ligand donates electrons to the Pd centre was found to be of great importance. It was discovered that the use of ligands with low donicity enhances the electrophilicity of the Pd(II) intermediate **A**, (**Scheme 4.3**), and therefore reactivity towards the organostannane.



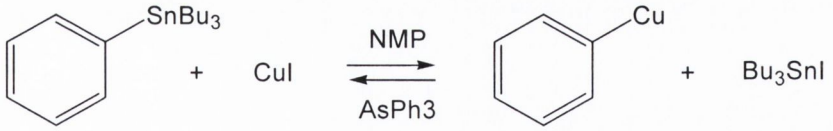
A **B**

Scheme 4.3 Ligand effects in the Stille reaction

On the other hand, strong donors such as PPh_3 inhibit the reaction. The use of $AsPh_3$ as ligand enhances the reaction 1000-fold, as it is only weakly donating.

Copper Salt Effect:

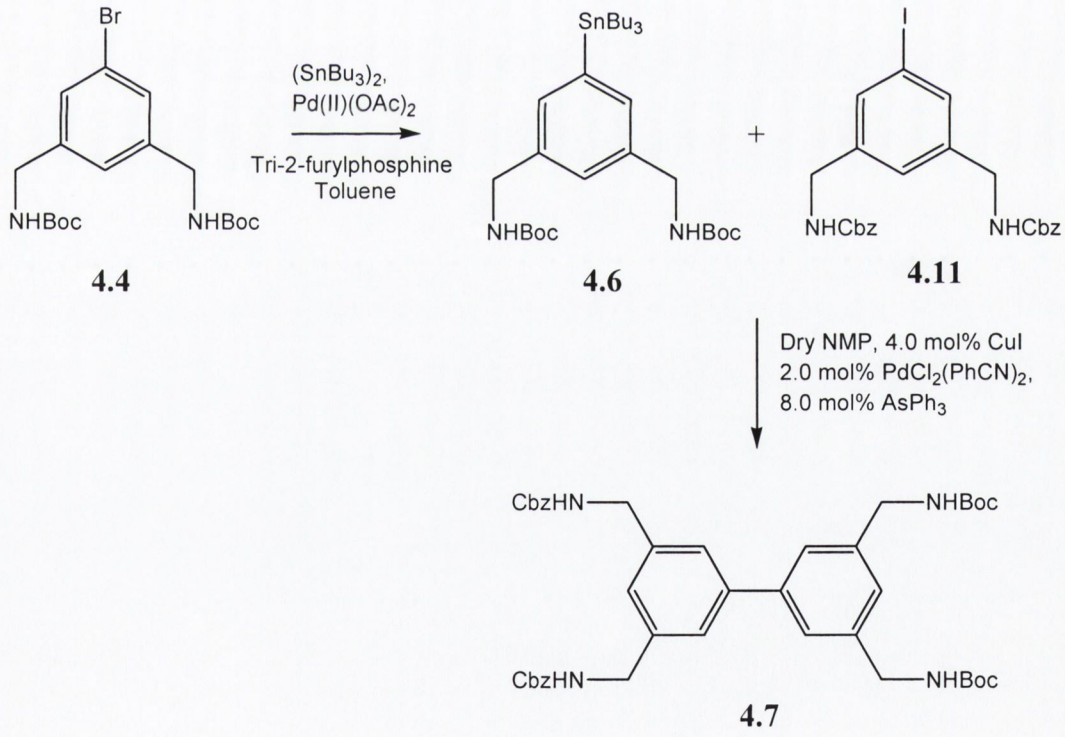
The use of Cu(I) salts were shown to increase the reactivity by acting as "phosphine scavengers" in cases where PPh_3 was used as a ligand. The dissociation of a ligand from **A** (**Scheme 4.3**) is important in the transmetallation process. Since PPh_3 shows high donicity, a metal ion such as Cu(I) can mop-up the dissociated PPh_3 ligands, pushing equilibrium to the right and forming more of the reactive species **B** (**Scheme 4.3**). Under the same conditions, this effect was not seen with $AsPh_3$ as ligand, however when using NMP as solvent, the use of copper salts have shown increased reactivity. The aryl stannane reacts with Cu(I) in NMP to give an organocopper species. The reaction is in equilibrium and proceeds only in the presence of low donor ligands. It was postulated by the author that NMP, being a polar solvent, stabilises the trialkyltin halide which forms upon reaction of the organostannane with Cu(I) (**Scheme 4.4**). The copper species may then contribute in the transmetallation step of the Stille reaction.



Scheme 4.4 Cu(I) salt as a reagent in the Stille coupling

In Wareham's synthesis, formation of the aryl stannane **4.6** was achieved using hexabutyl di-tin in a palladium catalysed coupling with the bis-Boc protected aryl bromide **4.4**. Typical yields for stannane formation were *ca.* 50 %.

The Stille coupling of the aryl stannane with the bis-Cbz protected aryl iodide **4.11** (synthesised from 5-iodo-*m*-xylene, see section **4.2.6**) was carried out as outlined in **Scheme 4.5**, employing 4 mol % CuI and 8 mol % $AsPh_3$ in NMP. The desired asymmetric biaryl **4.7** was obtained in yields of *ca.* 30 %, or *ca.* 15 % from **4.4** *i.e.* for the two steps . The toxicity of the tin reagents together with the poor yields for both steps made the unsymmetrical biaryl formation a problematic step in the synthesis.



Scheme 4.5 Wareham's synthesis of unsymmetrical biaryl by Stille coupling

4.1.5 Removal of Amine Protecting Groups

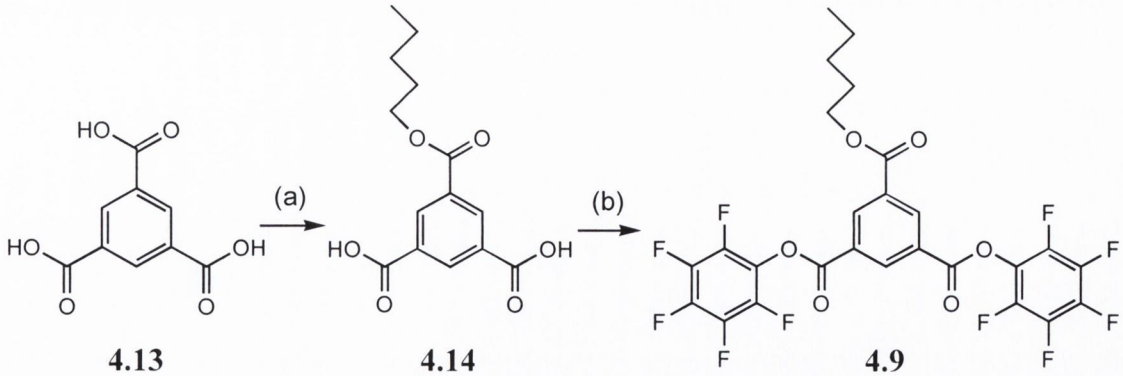
With the required biaryl **4.7** synthesised, the next stage was the stepwise removal of the amine protecting groups followed by cyclisation with the active ester **4.9**. The deprotection of the Cbz groups, followed by [2 + 2] cyclisation with **4.9** to give the tetra-Boc macrotricyclic precursor **4.10**, proved to be better than the alternative pathway of initial Boc group removal followed by cyclisation to a tetra-Cbz protected macrocyclic precursor. This was due to the tetra-Cbz macrotricyclic precursor being less soluble than **4.10** and therefore more difficult to purify prior to further reaction.

Cbz removal was achieved by hydrogenolysis in MeOH/DCM (1:1) with 10 % Pd/C as catalyst. Some difficulties were encountered in the Cbz removal with reactions taking longer than anticipated. Reactions were undertaken using *ca.* a half weight equivalent of 10 % Pd/C with respect to the bis-Cbz bis-Boc biaryl **4.7**, but extra additions of catalyst often needed to be made to bring the reaction to completion. Reaction mixtures were filtered through pre-washed celite to remove Pd/C and the bis-amino bis-Boc biaryl **4.8** was brought through to the next reaction without further purification.

4.1.6 Bis-Pentafluorophenyl Activated Ester 4.9

The bis-pentafluorophenyl activated ester **4.9** was synthesised from 1,3,5-trimesic acid **4.13** as shown in **Scheme 4.6**. Oxalyl chloride was used to form an acid chloride followed by reaction with *n*-pentanol to yield the bis-carboxylic acid pentyl ester **4.14**. The bis-carboxylic acid **4.14** was isolated by selective extraction from aqueous base: quenching the mixed carboxylates in a basic aqueous layer using 5 M HCl, followed by extraction into ethyl acetate at pH range 6-3. Undesired mono-acid di-ester was removed by extracting at higher than pH 6 and any unreacted tri-acid was extracted below pH 3. As expected, a statistical yield of 31 % was obtained.

Conversion of the di-acid to **4.9**, was achieved by reacting **4.14** with DCC and pentafluorophenol. Unwanted DCU by-product was removed by filtration and purification was achieved by flash chromatography giving **4.9** in a yield of 84 %.



4.13 **4.14** **4.9**

Reagents and Conditions
(a) Oxalyl chloride, *N*,*N*-DIPEA, DMF, THF, *n*-pentanol.
(b) pentafluorophenol, DCC.

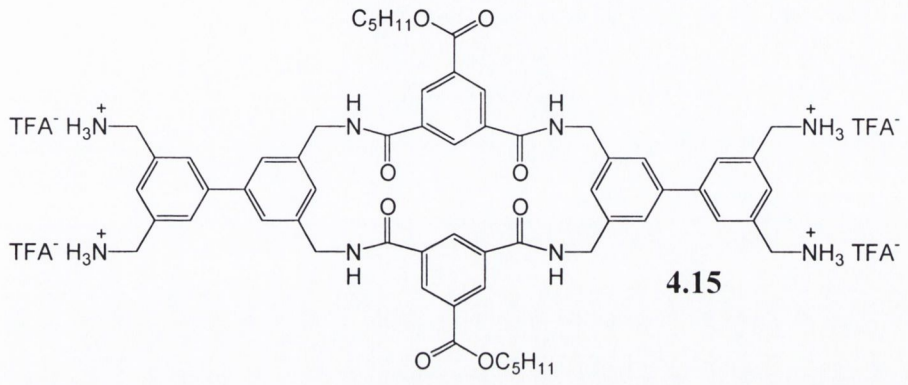
Scheme 4.6 Synthesis of bis-pentafluorophenyl active ester spacer unit **4.9**

4.1.7 First Cyclisation to Synthesise Tetra-Boc Macrocycle 4.10

Cyclisation of the deprotected biaryl **4.8** with the active ester **4.9** was carried out at high dilution to promote the intramolecular final amide bond formation to give the tetra-Boc protected macrocycle **4.10**. The active ester, as a dilute solution in THF (1.5 mM), was added dropwise to the biaryl in a solution of THF:DMF (4.5:1) (3.0 mM) over a period of 6 hours under argon. Dropwise addition over a long period of time, as well as using high dilution conditions helped to promote cyclisation and limit the possibility of higher oligomers forming. DMF was found to be necessary to obtain solubility and prevent aggregation. As mentioned earlier, the order of amine deprotection on the biaryl is important, as the macrocycle **4.10** was found to be more soluble than the tetra-Cbz protected analogue of **4.10**. However, purification of **4.10** was not trivial. Initially, purification by flash column chromatography eluting with MeOH/DCM (0.5:99.5) was attempted. Some of the product was found to be insoluble and remained on the top of the column. The material was flushed through with MeOH/DCM (10:90) and was insoluble in MeOH/DCM (1:99). A recrystallisation from DCM/MeOH was attempted. The product formed a jelly which was filtered off and washed with small portions of MeOH/DCM (10:90). The jelly was concentrated *in vacuo* to a white solid, which was mostly desired product but with some impurities present, thought to be higher oligomers. The best yield for this cyclisation was 67 %, the product being synthesised on a 140 mg scale.

4.1.8 Tetra-Boc Deprotection of 4.10

Removal of the Boc protecting groups in **4.10** was carried out using TFA/DCM (1:1). The reaction mixture was concentrated *in vacuo* to an oil after 2 hours reaction time. The product was triturated from the oil by the addition of diethyl ether and the resulting white solid product **4.15** was reacted in the final cyclisation without further purification.



4.15

4.1.9 Pentyl Macrotricycle 2.57

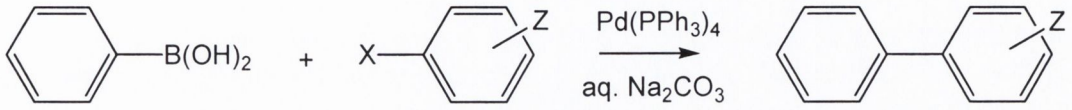
The final cyclisation of **4.15** with 2 equivalents of active ester **4.9** was again carried out at high dilution. The active ester, as a solution in THF (1.0 mM), was added dropwise to a solution of the tetra-ammonium salt **4.15** in THF, DMF and *N*,*N*-DIPEA (12 eq) (0.5 mM) over a period of 6 hours at 40 °C. After an acid/base work-up, purification was initially by flash chromatography eluting with MeOH/AcOH/DCM (3-10:1:96-89) followed by preparative TLC. The desired macrotricycle **2.57** was isolated in a 25 % yield and in a quantity of 38 mg. Testing of **2.57** has been described in Chapter Two.

4.2 First Project Aim- An Alternative Route to an Unsymmetrical Biaryl

The step which had caused the most difficulty in the synthesis of the macrotricycle **2.57** by Wareham, was the formation of unsymmetrical biaryl **4.7**. As explained in section **4.1.4**, both the stannane formation as well as the actual Stille coupling, were low yielding (50 % and 29 % respectively) and biaryl formation was only carried out on a small scale (360 mg of desired material obtained).

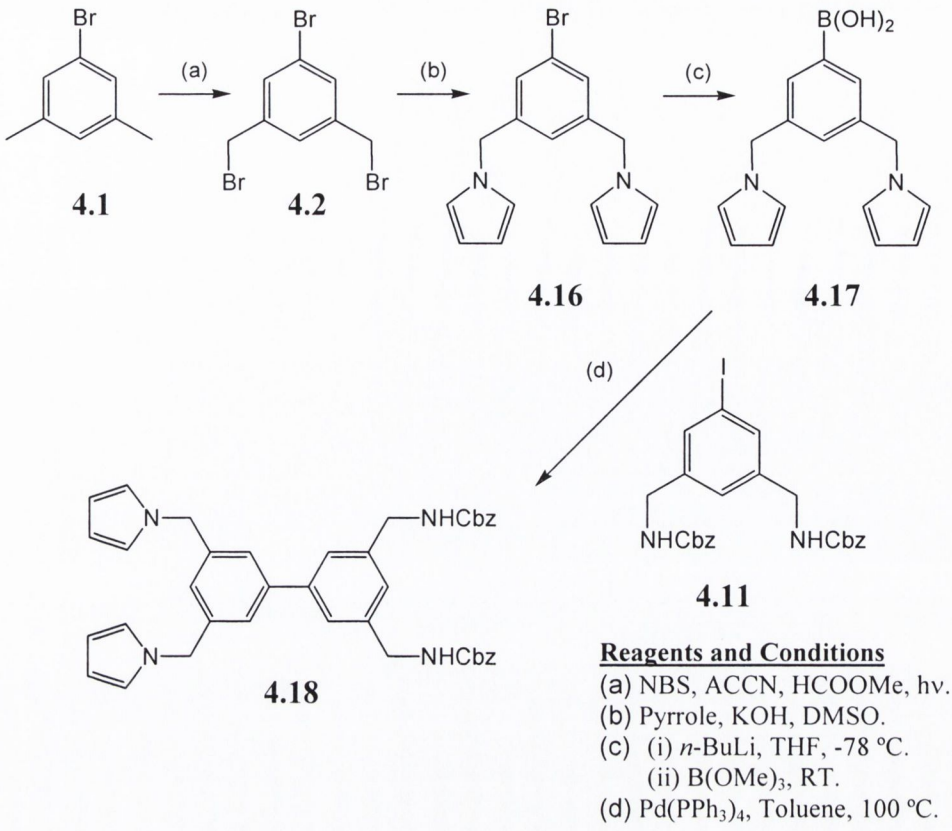
The Suzuki reaction was the alternative choice due to its tolerance of a wide range of functionalities and its use in unsymmetrical biaryl formation was the initial aim of this project. The Suzuki reaction involves the palladium catalysed cross-

coupling of an aryl boronic acid (or ester) with an aryl halide or triflate and was first reported by Suzuki and co-workers in 1981 (**Scheme 4.7**).[57] The boronic acid acts as the nucleophile and the aryl halide acts as the electrophile. Since the 1980s this reaction has become the biaryl coupling procedure of choice for many chemists owing to its tolerance of various functional groups represented as Z in **Scheme 4.7**.



Scheme 4.7 Suzuki coupling of a boronic acid to an aryl halide

The Suzuki reaction presents its own set of difficulties however. The usual route to an aryl boronic acid is the low temperature lithiation of an aryl halide followed by reaction with a trialkyl borate, $B(OR)_3$. The borate then gets hydrolysed under weakly acidic conditions to the boronic acid. The problem with this method of preparation is the use of butyl lithium to lithiate the aryl halide. This is a very strong base and places a limitation on the type of amine protecting group employed on the aryl halide prior to boronic acid formation. One of the few amine protecting groups that is able to withstand these conditions is pyrrole. Although not strictly a protecting group, it can mask the primary amine and has been used in this capacity in peptide synthesis.[58] Deprotection of the pyrrole ring is achieved by ozonolysing the double bonds to the corresponding ozonides followed by reduction to an *N*-formyl group. The *N*-formyl group can then be hydrolysed with mild acid to give the corresponding primary free amine. Pyrrole has also been used as a primary amino protecting group in organometallic reagents in cholaphane synthesis within the Davis group.[59] **Scheme 4.8** illustrates the route taken for the synthesis of bis-Cbz bis-pyrrole protected unsymmetrical biaryl **4.18**.



Scheme 4.8 Alternative route to an unsymmetrical biaryl

4.2.1 1-Bromo-3,5-bis(bromomethyl)benzene 4.2

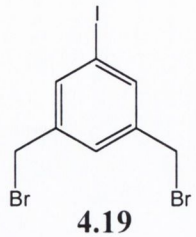
As illustrated in **Scheme 4.1**, 1-bromo-3,5-bis(bromomethyl)benzene **4.2** had already been synthesised by Wareham. However, yields were very poor predominantly between 20 and 30 %. The best yield recorded was 35 %, on a 3.2 g scale.

Following a literature procedure,[60] a number of changes were made to Wareham's synthesis of **4.2**. A reaction time of 18 hours as well as the use of only 2 equivalents of *N*-bromosuccinimide was reported, whereas previously a reaction time of 4 hours using 3.5 equivalents of *N*-bromosuccinimide had been implemented. The work-up was also changed to that of the literature procedure. After concentration of the reaction mixture *in vacuo* to a solid, the desired material was repeatedly extracted into portions of boiling hexane from which it crystallised out upon cooling. This avoided the use of carbon tetrachloride, as had been used previously.

An alternative radical initiator to 1,1′-azobis(isobutyronitrile) (AIBN), as had been used in the literature procedure mentioned, was also tried out, namely 1,1′-azobis(cyclohexanecarbonitrile). This is reported to be a more efficient radical

initiator than the previously used AIBN.[61] The effect of these changes was to increase the yield from 35 % to 60 %. Also **4.2** was synthesised on a larger scale, typically 28 g.

4.2.2 1-Iodo-3,5-bis(bromomethyl)benzene 4.19



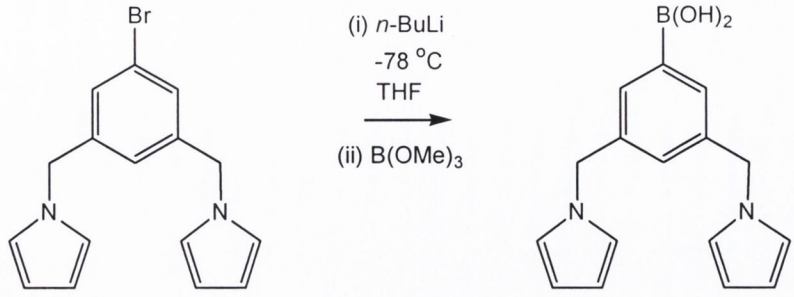
4.19

Bis-Cbz protected aryl iodide **4.11** was used by Wareham as the electrophile in the Stille coupling as opposed to the bromo analogue **4.5**, owing to iodide being a better leaving group than bromide (**Scheme 4.5**). This required the synthesis of 1-iodo-3,5-bis(bromomethyl)benzene **4.19**. The reaction conditions were the same as those for the synthesis of bromo analogue **4.2** and the same changes to Wareham's original synthesis were implemented for this compound. The new procedure improved the yield from *ca.* 30 % to 55 % and the reaction was scaled up to 40 g.

4.2.3 1-Bromo-3,5-bis(pyrrolo-*N*-methyl)benzene 4.16

Pyrrolation of **4.2** was carried out following the procedure by Heaney *et al.*[62] 2 equivalents of potassium hydroxide were used for the deprotonation of each pyrrole nitrogen. The use of a polar solvent, such as DMSO, is essential for the reaction. The desired product was extracted into ethyl acetate and most of the DMSO was removed by washing with water. Recrystallisation from methanol gave the desired bis-pyrrole protected compound **4.16** in a 78 % yield, on a 13 g scale. Analysis by ^{1}H NMR in $CDCl_3$ showed two distinct triplets for the pyrrole protons at δ 6.6 and δ 6.2. These signals are positioned well away from the other resonances in the molecule's ^{1}H NMR spectrum and would therefore be easily followed when deprotection needed to be carried out.

4.2.4 3,5-bis(pyrrolo-*N*-methyl)phenyl-1-boronic acid 4.17



4.16 **4.17**

Preparation of the aryl boronic acid **4.17** was achieved by lithium-halogen exchange of aryl bromide **4.16**, followed by introduction of boron by means of reacting trimethyl borate with the lithiated species. The arylboronate was hydrolysed to the acid by quenching with saturated aqueous ammonium chloride. Lithiation was carried out at low temperature (–78 °C) in THF. Two equivalents of trimethyl borate were added and this was done in one single injection to avoid two lithiated molecules attacking one boron centre.

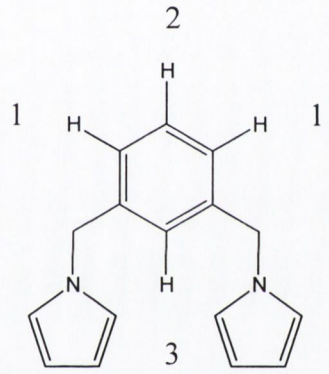
The boronic acid was synthesised in 81 % yield, on a 6 g scale. ^{1}H NMR analysis showed the desired product with characteristic boronic acid protons at δ 8.0 in d_6-DMSO. As was anticipated, the pyrrole rings survived the lithiation step and their proton resonances were unchanged from those of **4.16**.

Although the boronic acid was synthesised in 82 % yield on a small scale and in 81 % yield on a *ca.* 6 g scale, great difficulty was encountered in repeating the reaction. Several repeats were carried out with varying degrees of success.

In one particular reaction, 1.1 equivalents of *n*-BuLi were added to **4.16** at –90 °C in anhydrous THF. Trimethyl borate was added 20 minutes later and the reaction mixture was allowed to warm to room temperature and stirred for a further 2.5 hours. No starting material was present by TLC at this point, which indicated a successful lithiation step. The reaction was quenched by the addition of saturated aqueous ammonium chloride solution and the mixture was left to stir overnight to ensure hydrolysis of the methoxy groups to yield the boronic acid **4.17**. After work-up, the product was concentrated *in vacuo* to give an off-white solid. ^{1}H NMR analysis revealed the presence of two main products, consistent with the desired product and a partially or non-hydrolysed boronic ester. The presence of a peak at δ

3.3 was suggestive of a methyl ester(s). This was surprising considering hydrolysis had been allowed to occur over a 12 hour period. After redissolving the product in ethyl acetate, saturated aqueous ammonium chloride solution was added and the mixture was shaken and heated slightly. After work-up, ^{1}H NMR analysis revealed only one set of peaks consistent with the desired product. The peak at δ 3.3 thought to be a methyl ester had disappeared. This showed that hydrolysis of the boronic ester was not as straightforward as initially believed.

Another repeat reaction revealed the presence of the desired boron ester 2.5 hours after the addition of trimethyl borate but also what appeared to be starting material by TLC analysis. After hydrolysis of the methoxy groups and work-up, ^{1}H NMR analysis showed a mixture of two products. On this occasion the desired product **4.17** was present along with what appeared to be the de-brominated material **4.20**.



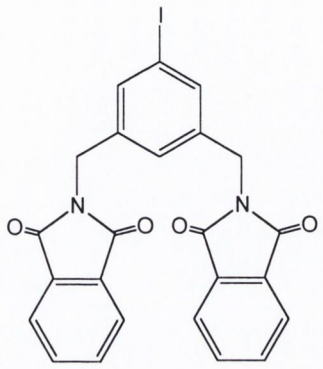
4.20

A triplet for proton 2 (δ 7.3), a doublet for proton 1 (δ 7.2) and a singlet for proton 3 (δ 7.0) were all evident. The R_f of this de-brominated material was the same as that of the starting material. Therefore the lithiation had worked but the boronation had partially failed resulting in the desired product **4.17** as well as **4.20**, which was probably as a result of some water being present in the reaction mixture or due in some part to the borate material being inactive.

The use of triisopropylborate as a means of introducing boron onto the aryl ring was also attempted as an alternative to trimethylborate. This reagent is known to be more reactive and more stable than trimethyl borate. ^{1}H NMR analysis showed that the desired boronic acid was present in greater proportions compared with the majority of the trimethyl borate reactions but the spectrum was still a little unclean, with what appeared to be de-brominated product also evident.

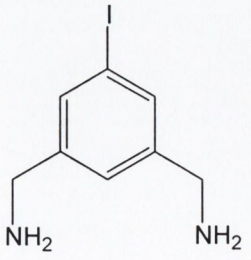
These problems contributed to our decision to adopt the alternative boronate preparation discussed in section **4.3**.

4.2.5 1-Iodo-3,5-bis(phthalimido-*N*-methyl)benzene 4.21[55]



4.21

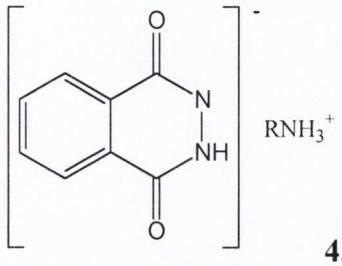
Following Wareham's application of the Gabriel synthesis, the two bromines of **4.19** were substituted for primary amines, in an S_N2 type reaction. Potassium phthalimide is usually the reagent of choice for introduction of the phthalimide group but sodium phthalimide and phthalimide in the presence of a base may also be used. DMF is the most commonly used solvent for the reaction as potassium phthalimide is somewhat soluble in it. Recrystallisation from chloroform/hexane gave **4.21** in yields of *ca.* 70 %, similar to those obtained by Wareham. While this material was clean enough to continue with in the synthesis, it was not sufficiently pure for microanalysis, due to the presence of succinimide, which could be seen in the ^1H NMR spectrum at δ 2.75. Recrystallisation from toluene, following a literature procedure by Manabe[63] for the bromo analogue of **4.21**, gave the product as colourless needles in 68 % and on this occasion the material passed microanalysis.



4.22

Deprotection of the phthalimide groups to give the free amine **4.22** was carried out using the standard procedure of hydrazinolysis. This involved refluxing

the phthalimide material with hydrazine hydrate in MeOH/DCM (4:1) for 3 hours. Intermolecular attack by a hydrazine amino moiety at a phthalimide carbonyl followed by intramolecular attack by the second hydrazine amino group results in the intermediate amine salt of phthalhydrazine **4.23** which, upon treatment with acid, forms the water-soluble amine hydrochloride and the water insoluble phthalazine-1,4-dione by product. This allows for a convenient separation and a basic work-up affords the desired free amine **4.22**.

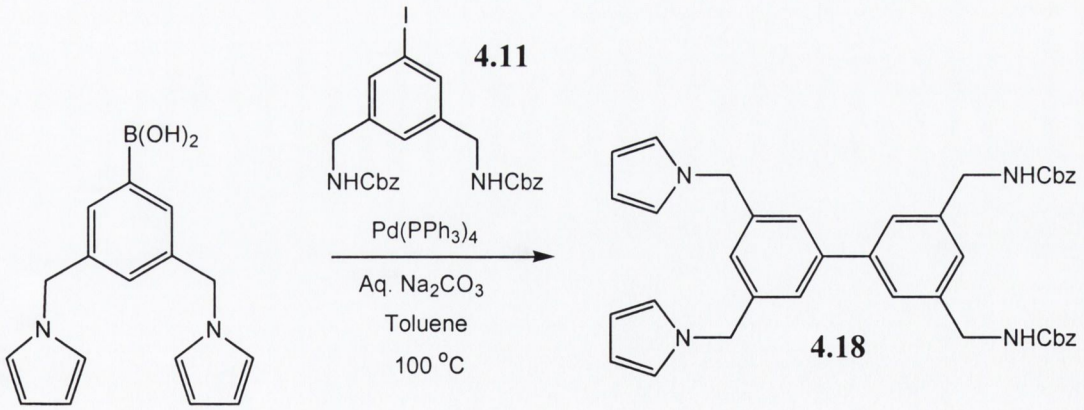


4.23

4.2.6 1-Iodo-3,5-bis[(*N*-benzyloxycarbonyl)methylamino]benzene 4.11

The synthesis for this compound was unchanged from that of Wareham, as was the purification. However, the synthesis was scaled up from 1.5 g of product to 10.5 g and the yield increased from *ca.* 70 % to 86 %. The compound has also been fully characterised since its initial synthesis by Wareham (see Chapter Seven).

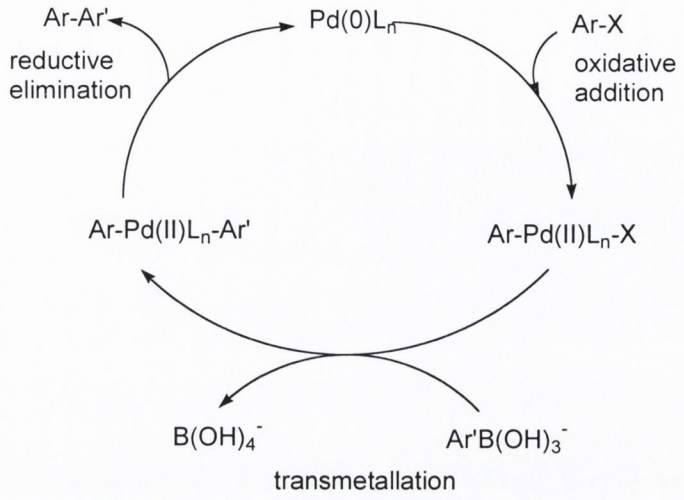
4.2.7 3,5-Bis(pyrrolo-*N*-methyl)-3′,5′-bis[(*N*-benzyloxycarbonyl)methylamino] biphenyl 4.18



4.17

Unsymmetrical biaryl coupling of the bis-pyrrole protected boronic acid **4.17** to the bis-Cbz protected aryl iodide **4.11** was carried out following the procedure of Suzuki *et al.*[64] The reaction is catalysed by a Pd(0) complex, the most commonly used being tetrakis(triphenylphosphine) palladium, $Pd(PPh_3)_4$. The reaction is

usually carried out at elevated temperatures in the presence of a base, with aqueous sodium carbonate giving the best results and using toluene as solvent.[57], [65] The mechanism for the Suzuki coupling is shown in **Scheme 4.9**.



Scheme 4.9 Mechanism for the Suzuki biaryl coupling reaction

Oxidative addition of the palladium catalyst to an aryl or alkyl halide is often the rate-determining step in a catalytic cycle. The relative reactivity of the substituents decreases in the order of I > OTf > Br >> Cl. As regards biaryl coupling, the rate-determining step can be dictated by the type of halide present on the aryl ring. For instance, aryl bromides have been known to have a rate-determining oxidative addition step, yet aryl iodides may have a rate-determining transmetallation step.[66]

Suzuki's first report[57] suggested that both water and base are essential in the coupling reaction. It is proposed that 2 equivalents of base, with respect to the boronic acid, are required. One equivalent of base is required to activate the boronic acid as shown in **Equation 4.2** and a second equivalent is required to neutralise the boric acid side-product of coupling, shown in **Equation 4.3**. Boronic acids form a borate complex with hydroxide ions and these anions have been shown to be 10^6 times more reactive in electrophilic reaction compared with neutral boronic acids. This is evidence that they are the active species in the transmetallation step.[66]



Equation 4.2



Equation 4.3

Studies have shown that when carbonate is used as the base, coupling is observed but not when bicarbonate, a weaker base, is used. These results are consistent with the formation of the borate anion. The pKa of an aryl boronic acid is *ca.* 8.8, carbonate is 10.3 and is basic enough to form the boronate anion, but bicarbonate has a pKa of only 6.35 and is therefore too weak.[66]

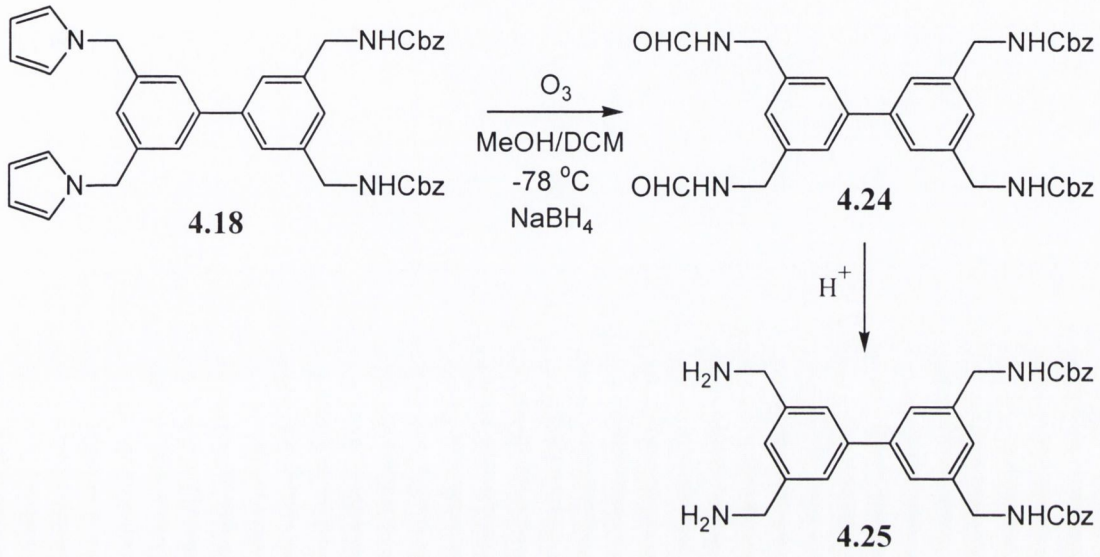
Investigations into room temperature Suzuki coupling by Anderson *et al.* contradict this ideal stoichiometry of base.[67] They suggest that 1.5 equivalents of base with respect to the boronic acid is optimal. One equivalent is necessary to activate the boronic acid but another whole equivalent is not necessary to neutralise the by-product of boric acid.

Bis-pyrrole boronic acid **4.17** was successfully coupled to the aryl iodide **4.11** in a yield of 90 %. In this coupling, 1.3 equivalents of boronic acid **4.17** were used for each equivalent of the aryl iodide **4.11**. Following Anderson's work,[67] it was decided to use only 1.5 equivalents of base for each equivalent of boronic acid present. The reaction was carried out in toluene at 100 °C for 18 hours using 5 mol % tetrakis(triphenylphosphine) palladium as catalyst. Following work-up, the product **4.18** was isolated by flash column chromatography. Efforts made at recrystallising the biaryl failed, but the material was of sufficient purity to pass microanalysis. The yield of 90 % was obtained on a 300 mg scale of product. The reaction was also scaled up to give 1.2 g of desired product **4.18**, this time in a yield approaching 70 %. On a 300 mg scale, when compared with the Stille coupling, the Suzuki coupling shows an increase in the percentage yield from 29 % to 90 %.

This Suzuki coupling as described was also attempted using aryl bromide **4.5** as the electrophile. TLC analysis of the crude product showed mostly desired biaryl **4.18** but also the presence of some starting material **4.5**. These two compounds were close running on TLC and proved difficult to separate by flash column chromatography eluting with MeOH/DCM (0.25:99.75). ^{1}H NMR of the crude product showed the desired biaryl **4.18**, but also aryl bromide starting material **4.5**. The difficulty encountered in separating the aryl bromide **4.5** from the desired biaryl

4.18 by flash chromatography showed the importance of getting all of the aryl halide to react. As aryl iodides are known to be better electrophiles in this type of reaction, they should be the aryl halide of choice.

4.2.8 Ozonolysis of 3,5-bis(pyrrolo-*N*-methyl)-3′,5′-bis[(*N*-benzyloxycarbonyl) methylamino]biphenyl



Treatment of a pyrrole system with ozone at –78 °C gives the corresponding ozonide, which upon reduction with sodium borohydride results in formamide.[59] *N*-formyl groups can be converted to the free amine by acid hydrolysis followed by a base wash.[68] It was decided to unmask the pyrrole groups prior to Cbz removal as any potential problems with the ozonolysis step would be more easily handled on a smaller system.

Biaryl **4.18** was treated with ozone in a MeOH/DCM mixture (1:1). TLC analysis after 1 hour revealed that no starting material was present. The solution was blue at this point indicating ozone saturation and the reaction was left to stir for a further 1.5 hours with ozone bubbling through the solution. Tetrabutylammonium borohydride in methanol was then added to reduce the ozonide to the corresponding *N*-formyl group. ^{1}H NMR analysis showed that neither of the two pyrrole proton resonances at δ 6.2 and δ 6.7 were evident and ozone had therefore reacted with the pyrrole double bonds in the time allowed. After work-up, purification of the crude product by flash column chromatography eluting initially with DCM followed by MeOH/DCM (5:95) was attempted. The crude product streaked on TLC in

MeOH/DCM (7:93) and the desired bis-*N*-formyl bis-Cbz biaryl **4.24** was not isolated.

The reaction was repeated using sodium borohydride as a reducing agent. Again, the TLC of the crude product was very streaky and there appeared to be three compounds present along with baseline material.

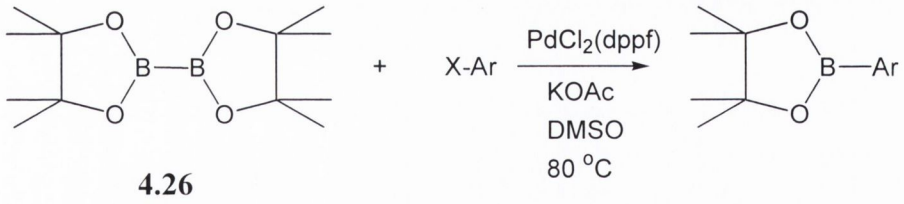
Ozonolysis of the biaryl was repeated once again, this time in DCM. After 5 minutes no starting material was left by TLC analysis. A ^{1}H NMR spectrum showed two new proton resonances downfield at δ 9.10 and δ 8.15. *N*-formyl protons were possibly present by ^{1}H NMR but the material was impure. The pyrrole CH_2 resonance had disappeared but three new peaks were present at δ 4.74, δ 4.72 and δ 4.36.

It was clear that the pyrrole rings were reacting very quickly with ozone, but the problems associated with reducing the ozonide and purifying the bis-formamide bis-Cbz biaryl **4.24** led to the abandonment of this route to the unsymmetrical biaryl.

4.3 Use of an (Alkoxy)diboron Compound in Unsymmetrical bis-Cbz bis-Boc Biaryl 4.7 Formation

From biaryl coupling investigations within the research group, the Suzuki method evidently gave the best results. However, the need to use a strong base such as butyl lithium in the borylation of an aryl halide placed a limitation on the choice of amine protecting groups employed. This was a result of the lack of suitable boron nucleophiles for the direct synthesis of an organoboron compound from an organoelectrophile under mild conditions. For example, organotin and organosilicon compounds may be synthesised directly from distannanes and disilanes respectively *via* transition metal catalysed reactions.

However, with the advent of (alkoxy)diboron **4.26**, known as bis(pinacolato)diboron, as a commercially available material, a direct route to the borylation of organic halides and triflates became possible. The diboron compound acts as a boron nucleophile in the presence of a base in a palladium catalysed cross coupling with organic halides. Miyaura and co-workers first reported the Pd(0) catalysed cross coupling of bis(pinacolato)diboron **4.26** with an aryl halide as a direct route to aryl boronic esters in 1995, as shown in **Scheme 4.10**.[69]



4.26

Scheme 4.10 Bis(pinacolato)diboron as a route to arylboron ester formation

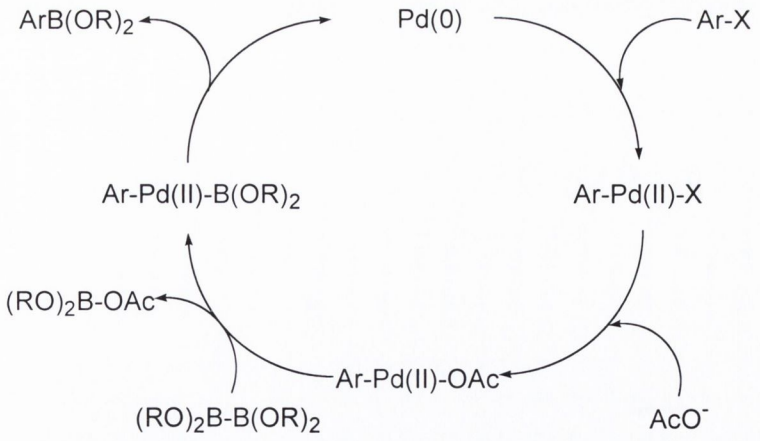
As was seen in the Suzuki reaction, the cross-coupling of organoboron compounds with aryl halides requires the presence of a base. The coupling of diboron **4.26** with an aryl halide also requires base, but a much weaker base than that of the Suzuki reaction. KOAc has been shown to give the best yields, as stronger bases such as K_3PO_4 and K_2CO_3 cause the boron ester product to couple with the aryl halide resulting in significant quantities of undesired symmetrical biaryl (30-60 %).[69]

Solvent choice is important for the rate of the reaction, with polar solvents such as DMSO and DMF proving to be the best.

The reaction is catalysed by Pd(0) but tetrakis(triphenylphosphine)palladium, frequently used for Suzuki couplings, gives only moderate yields of boron ester. Furthermore, contamination by coupling of a phenyl group from a triphenyl phosphine ligand with the diboron has been observed by Miyaura *et al.* The catalyst which gave the best results was palladium dichloride diphenyl phosphinoferrocene, $PdCl_2$(dppf), in 3 mol %. The catalysts $Ni(PPh_3)_4$, $Pt(PPh_3)_4$ and $RhCl(PPh_3)_3$ did not prove to be effective for the reaction at all.

Most importantly from these investigations, the reaction tolerates the presence of a wide range of functionalities such as nitro, cyano, ester, aldehyde and alkoxy groups.[69]

The mechanism for the formation of the arylboron ester is outlined in **Scheme 4.11**.

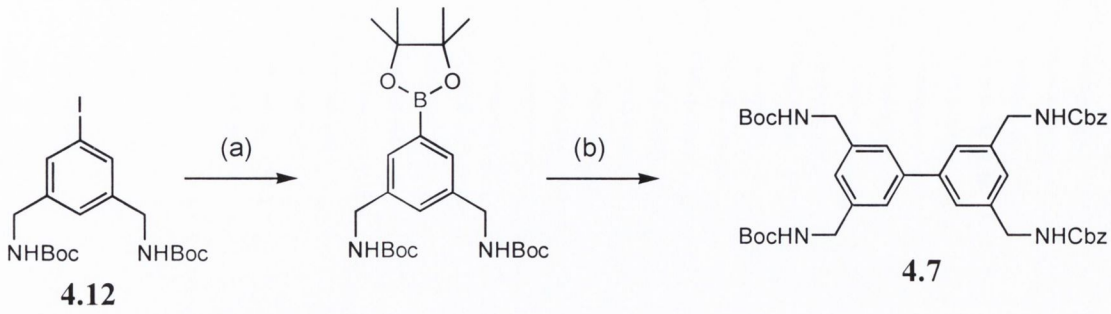


Scheme 4.11 Mechanism for the formation of an arylboron ester

The first step in the mechanism is the oxidative addition of Pd(0) with the aryl halide to give a Pd(II) species. The acetoxy anions do not act as a base to coordinate with boron as is similarly seen in the Suzuki mechanism. This time, the acetoxy anion displaces the halide from the palladium centre forming Ar-Pd(II)-OAc, the palladium species believed to be involved in the transmetallation step. Evidence to support this is the formation of *trans*-PhPd(OAc)(PPh_3)$_2$ when *trans*-PhPdBr(PPh_3)$_2$ is treated with KOAc. The Pd-OAc compound was shown to react with diboron **4.26**, whereas the Pd-Br compound did not. The involvement of Ar-Pd(II)-OAc in the transmetallation step with diboron **4.26** may be attributed to two things. Firstly, the high reactivity of the Pd–O bond which consists of a soft-acid and hard-base and secondly, the high oxophilicity of boron.

Giroux and co-workers used diboron **4.26** for unsymmetrical biaryl formation *via in situ* boron ester formation in a one pot synthesis.[70] Formation of the aryl boron ester followed the procedure of Miyaura as discussed in section **4.3**. Coupling of the boron ester to an aryl halide was attained *in situ* by modifying the Suzuki reaction conditions. A second addition of $PdCl_2$(dppf) was made to the reaction mixture to effect biaryl coupling instead of Pd(PPh_3)$_4$ and the solvent already present from the boron ester formation step was DMF, whereas the Suzuki reaction uses toluene. In keeping with the Suzuki biaryl coupling, aqueous Na_2CO_3 was used as base. Yields were good, typically 60-80 %. It must be noted that most of these reactions were carried out using aryl bromides as the coupling partner, holding out the promise of improved yields with the use of aryl iodide electrophiles.

4.3.1 3,5-bis[(*N*-*tert*-butoxycarbonyl)methylamino]-3′,5′-bis[(*N*-benzyloxy carbonyl)methylamino]biphenyl 4.7



4.12 **4.7**

Reagents and Conditions
(a) Bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, DMSO, 80 °C.
(b) Additional $PdCl_2$(dppf), Na_2CO_3, bis-Cbz **4.11**, 80 °C.

Scheme 4.12 Synthesis of unsymmetrical biaryl **4.7** using bis(pinacolato)diboron

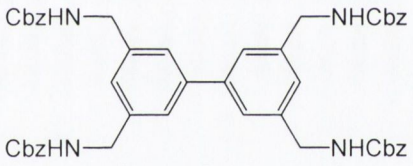
Modifying the procedure of Giroux *et al.*,[70] a one pot Suzuki biaryl coupling was undertaken. It was decided to use anhydrous DMSO as the solvent rather than Giroux's choice of DMF, as Miyaura's original report on aryl boron ester formation suggested that DMSO may be a slightly better solvent.[69] Boron ester formation occurred after 3 hours reaction time at 80 °C had elapsed. No **4.12** was evident by TLC analysis. The reaction mixture was cooled prior to the addition of the bis-Cbz protected aryl iodide **4.11** and Pd catalyst for the second step and the mixture was maintained at 80 °C for 24 hours to effect biaryl formation.

Good separation was achieved between the desired biaryl product **4.7** and unwanted material by flash column chromatography eluting with EtOAc/toluene (25:75). Although the crude product was not fully soluble in this solvent system, loading of the crude product onto the column in chloroform, followed by elution with EtOAc/toluene (25:75), achieved the desired separation. The faster running material was identified by TLC, ^{1}H NMR and FAB MS as unreacted bis-Cbz protected aryl iodide **4.11** added at the second step of the reaction. Of the 2 equivalents of this material which had been added, 36 % was recovered.

The overall yield of isolated unsymmetrical biaryl **4.7** was 93 % for the two steps, on a 2.5 g scale. This yield of 93 % was achieved once and an average yield for the reaction was typically 81 %. As was anticipated, this was an improved yield on that of Giroux and co-workers owing to the use of aryl iodides rather than aryl

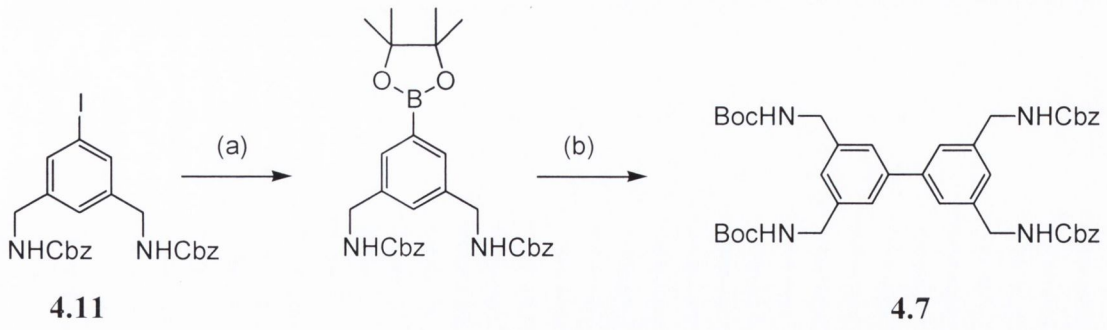
bromides. Both the yield and quantity was a significant improvement on the Stille coupling to produce the same biaryl product (50 % for stannane formation and 29 % for Stille coupling, 315 mg scale). Moreover, the use of bis(pinacolato)diboron as an aryl borylating reagent allowed the use of amine protecting groups of choice, namely Boc and Cbz, which had not been possible with the lithiation-based route to an unsymmetrical biaryl. The successful development of this biaryl coupling, combined with the use of readily removable protecting groups, allowed for the synthesis of significant quantities of biaryl for use in controlled cyclisations towards various macrocyclic receptors.

Whether the synthesis of the biaryl **4.7** started with the borylation of bis-Boc protected aryl iodide **4.12** as in **Scheme 4.12** or with the bis-Cbz protected aryl iodide **4.11** as in **Scheme 4.13**, followed by coupling with the corresponding coupling partner, made little difference to the yield. However, it did affect the side products which were formed. It was observed that borylating bis-Boc **4.12** followed by coupling with bis-Cbz **4.11** resulted in the formation of a small amount of tetra-Cbz protected symmetrical biaryl **4.27**.



4.27

Upon loading of a flash column for the purification of the crude biaryl product, it was observed that some of the crude product was either precipitating or was not soluble in chloroform. This material was filtered off and analysis by ^{1}H NMR and FAB MS revealed it to contain tetra-Cbz protected symmetrical biaryl **4.27**. Therefore, co-running of this unwanted symmetrical biaryl with the desired biaryl **4.7** was not an issue and the yields obtained when starting the synthesis with bis-Boc aryl iodide **4.12** were not affected with impurities and are true yields. Recovery of some of the excess aryl halide coupling partner **4.11** was possible by flash column chromatography and a recovery of *ca.* 70 % was attained.

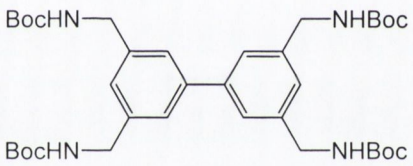


4.11 **4.7**

Reagents and Conditions
(a) Bis(pinacolato)diboron, $PdCl_2(dppf)$, KOAc, DMSO, 80 °C.
(b) Additional $PdCl_2(dppf)$, Na_2CO_3, bis-Boc **4.12**, 80 °C.

Scheme 4.13 Synthesis of unsymmetrical biaryl **4.7** using bis(pinacolato)diboron

Conversely, borylating the bis-Cbz material **4.11** followed by coupling with bis-Boc **4.12** as in **Scheme 4.13** resulted in the formation of a small amount of tetra-Boc protected symmetrical biaryl **4.28**.



4.28

This side-product had the same R_f as the desired biaryl **4.7** and therefore could not be removed by flash column chromatography. It could however be separated from **4.7** once hydrogenolysis of the Cbz groups was carried out as the resulting bis-amino bis-Boc biaryl **4.8** could be retained on a silica plug and the tetra-Boc biaryl **4.28** could be washed off (see section **4.3.2**). Symmetrical biaryl **4.28** was isolated and calculated to be 5.4 % of the bis-Boc aryl iodide **4.12** added at the second step of the synthesis. Therefore, the yield of the biaryl coupling, when starting the reaction with bis-Cbz aryl iodide **4.11**, is actually 74 %, the side-product accounting for *ca.* 10 % by weight of the final biaryl product **4.7**. According to Giroux, an excess (2 eq in total) of the coupling partner needs to be added in the second step of the coupling. Much of the unreacted bis-Boc **4.12** was recovered by flash column chromatography as, in the purification of the desired product **4.7**, it is the fastest running of the

compounds on the column and is easily separated. Typically, *ca.* 70 % of the excess bis-Boc aryl iodide **4.12** was recoverable.

The formation of the symmetrical biaryls **4.27** and **4.28** occurs at the second step in the reactions. This process while not fully understood, requires the presence of a reasonably strong base such as Na_2CO_3. It is noteworthy that no symmetrical biaryl was isolated from the first step owing to KOAc being a much weaker base than Na_2CO_3. While both described routes result in good yields of unsymmetrical biaryl **4.7** on gram scale, starting with bis-Boc **4.12** as in **Scheme 4.12** is marginally better as the yields are higher by approximately 5 % on average.

4.3.2 Cbz Deprotection of Biaryl 4.7

Deprotection of the Cbz groups of **4.7** was achieved by hydrogenolysis using activated 10 % Pd/C, typically half weight equivalent with respect to the bis-Cbz bis-Boc biaryl **4.7**, following the procedure of Wareham. The solvent used was a 1:1 mixture of MeOH/DCM and hydrogenolysis was carried out at atmospheric pressure and room temperature. As had been encountered in the past, reaction times varied which was possibly due to the activity of the catalyst. The catalyst was activated, by heating it at 160-200 °C under vacuum, at *ca.* 0.1 mmHg, for 2 hours. In some instances deprotection was complete in an overnight reaction, while other experiments required extra reaction time and/or extra additions of palladium catalyst.

Throughout these reactions, the catalyst employed was palladium, 10 weight % on activated carbon, *Aldrich* catalogue number 20,569-9.[71] It was noted that a palladium catalyst with 10 weight % on activated carbon on a dry basis was also available, *Aldrich* catalogue number 33,010-8. The latter catalyst has a higher palladium loading and was explicitly recommended for debenzylations and decarboxybenzylations. This material was used for Cbz deprotection, with ethanol as solvent, and consistently gave full deprotection. Using this catalyst, reactions were left to stir for two days at room temperature under atmospheric hydrogen pressure, but this length of time may not be necessary and may be reduced in future deprotections.

As mentioned earlier, purification of the deprotected material **4.8** was not undertaken in the past, as it was deemed that it would be more feasible when carried out on a larger scale. If **4.7** was synthesised according to **Scheme 4.12** then purification of the Cbz deprotected biaryl **4.8** was not necessary as discussed in

section **4.3.1**. If however, **4.7** was synthesised according to **Scheme 4.12**, then purification of the Cbz deprotected biaryl **4.8** was undertaken due to the presence of symmetrical biaryl **4.28**. TLC analysis of the crude product showed two compounds, the desired bis-amino bis-Boc biaryl **4.8** as baseline in ethyl acetate/toluene (1:1) and the tetra-Boc protected symmetrical biaryl **4.28** at R_f = 0.6. By means of a silica plug, the desired material was trapped on the silica and **4.28** was washed off using MeOH/DCM (5:95). The desired product was flushed off the silica using ammonia saturated MeOH/DCM (8:92). This gave **4.8** in an 82 % yield and on a 500 mg scale. ^{1}H NMR and FAB MS confirmed the faster running material to be tetra-Boc symmetrical biaryl **4.28**. Due to the tetra-Boc material co-running with the bis-Cbz bis-Boc biaryl **4.7**, calculation of the weight of **4.28** at this stage allowed for the true percentage yield of the biaryl formation reaction to be obtained, in which **4.28** was the side-product.

4.3.3 Bis-Pentafluorophenyl Active Pentyl Ester 4.9

The synthesis of the bis-pentafluorophenyl active ester **4.9** was performed following the route of Wareham (*cf.* section 4.1.6). This synthesis was already successful and no changes were made to it. However, the reaction to form the di-acid mono-pentyl ester **4.14** was scaled up from *ca.* 2 g, to give 4.83 g of product. Scaling up may have helped in improving the yield from 31 % to 36 %. The formation of **4.9** was also scaled up, giving 5.22 g of product in a 96 % yield, compared with 1.84 g in an 84 % yield. With no changes made to the synthesis the increase in the yield may again be attributed to the change in scale.

4.3.4 Tetra-Boc Protected Macrocycle 4.10

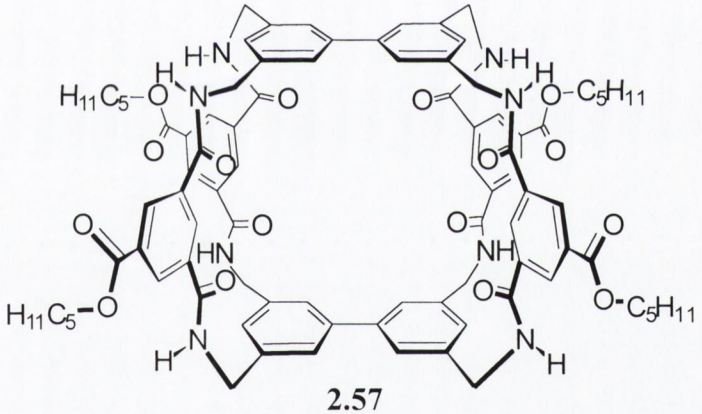
Cyclisation of the bis-amino bis-Boc biaryl **4.8** with the active ester **4.9** was carried out following the procedure of Wareham. Carrying out the cyclisation at 40 °C, rather than room temperature which had previously been used, made no difference to the yield obtained. Previously, cyclisations were undertaken on a scale of *ca.* 100 mg of biaryl **4.8** and the largest quantity of desired product obtained was 140 mg. However, with larger quantities of biaryl **4.8** available, cyclisation was possible on a 500 mg scale of starting material, with the largest quantity of product obtained being 350 mg.

Purification by flash chromatography was hampered in the past, due to the insoluble nature of the crude product of cyclisation. This resulted in the product being precipitated from MeOH/DCM in the form of a jelly. However, MeOH/$CHCl_3$ (1:99) as eluent in flash chromatography, was found to be a suitable solvent system for purification. The yield was only marginally improved upon, the best obtained being 72 % (previously 67 %).

4.3.5 Tetra-Boc Deprotection 4.15

Removal of the four Boc protecting groups of **4.10** to give the tetra-TFA ammonium salt **4.15** was achieved using the standard conditions of TFA/DCM (1:1). The reaction was complete within 2 hours. As expected, this reaction went quantitatively and trituration of the resulting oil with diethyl ether gave the product **4.15** as a white solid. The material was used in the final cyclisation without further purification.

4.3.6 Pentyl Macrotricycle 2.57



2.57

The [2 + 1] cyclisation of the active ester **4.9** with the tetra-TFA salt **4.10** was carried out modifying the procedure of Wareham.[54] It was decided to increase the number of equivalents of *N,N*-DIPEA from 12 to 30, so as to ensure full deprotonation of the amino groups of the tetra-TFA salt. Also, the concentration of the active ester was lowered from 1.0 mM to 0.5 mM and the concentration of the tetra-TFA salt from 0.5 mM to 0.25 mM with the aim of further enhancing intramolecular cyclisation. It is difficult to deduce whether this increase in dilution improved the reaction as the purification of the final compound proved to be difficult. After an acid/base work-up, purification by flash chromatography eluting

with AcOH/MeOH/DCM (1:2.5:96.5-1:4:95) was carried out. The product collected after column chromatography required further purification by preparative TLC, using MeOH/DCM (8:92) as eluent. The best separation was achieved when *ca.* 20 mg of the columned product was loaded onto a TLC plate (20 × 20 cm, 2.0 mm thickness) and the plate eluted twice *i.e.* eluted, dried and eluted again. The cleanest product appeared in the uppermost band on the plate. Typically, a 20 mg plate loading would return *ca.* 10 mg of desired macrotricycle.

A ^{1}H NMR spectrum of the pentyl macrotricycle **2.57** in $CD_3OH/CDCl_3$ (8:92) is shown in **Figure 4.1**. Part of the aliphatic region of the spectrum has been omitted for clarity. For a full range spectrum see Appendix **A**.

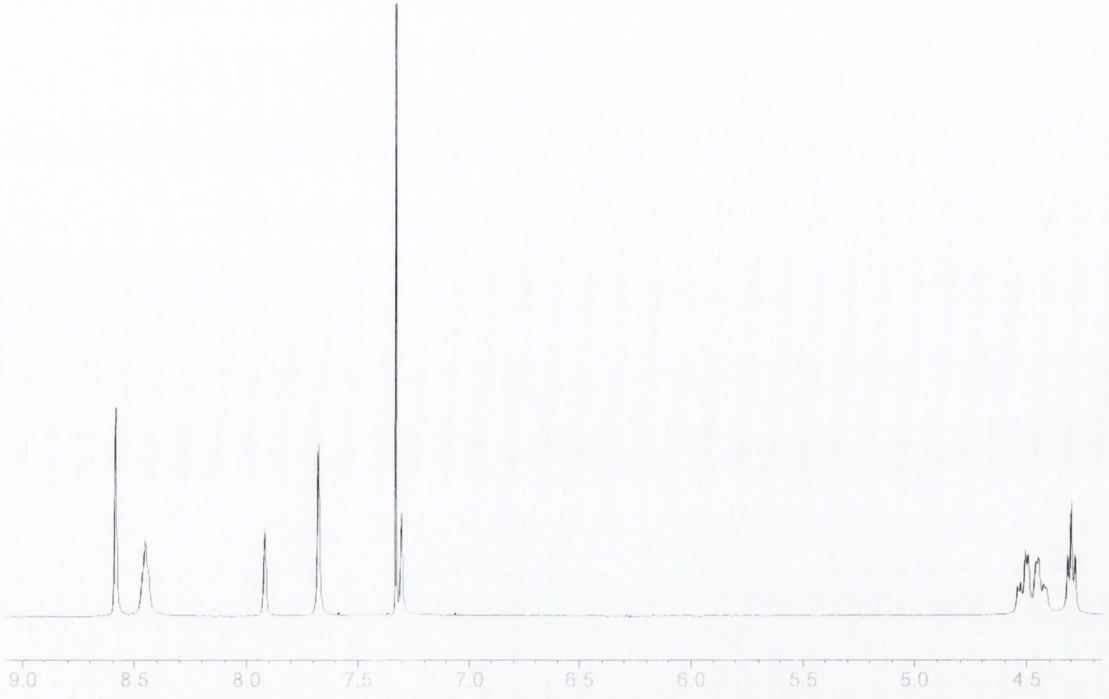


Figure 4.1 ^{1}H NMR of macrotricycle **2.57** in $CD_3OH/CDCl_3$ (8:92)

Given that the molecule's empirical formula is $C_{88}H_{92}N_8O_{16}$, it has a surprisingly simple ^{1}H NMR spectrum, owing to it's symmetry characterisation in the point group D_{2h}. Only four aromatic resonances can be seen; two for the aromatic spacer at δ 8.59 and δ 7.92 and two for the biaryl at δ 7.67 and δ 7.30. In $CD_3OH/CDCl_3$ (8:92) the amide protons can also be seen at δ 8.45. The benzylic protons give a characteristic ABX type splitting. This is due to the two protons on each of the eight benzylic positions being in different environments to each other. Geminal coupling (J = 13.6 Hz) and vicinal coupling with the amide NH (J = 4.9 Hz) affords the ABX

system at δ 4.48 integrating for sixteen protons in total. The pentyl protons are seen in the upfield part of the spectrum as expected.

As pointed out, purification of the crude material is not trivial. ^{1}H NMR analysis of 238 mg of isolated crude product after work-up revealed that the majority of the material was indeed desired macrotricycle **2.57**. This indicates that the final cyclisation is not problematic and the development of an improved purification technique, such as preparative HPLC, would benefit the synthesis greatly.

CHAPTER FIVE

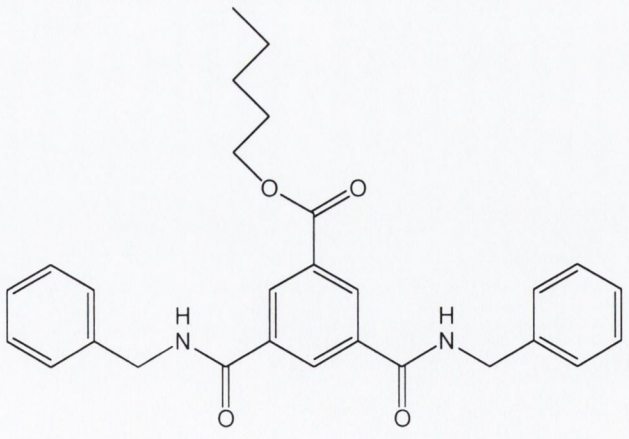
Preamble

Having improved certain aspects of the synthesis of the pentyl macrotricycle **2.57**, particularly the unsymmetrical biaryl step, the next stage was to synthesise a water-soluble receptor, as well as analogues of **2.57** for carbohydrate recognition in organic media. This chapter deals with the attempts made at manipulating **2.57** so as to form a receptor soluble in aqueous media, as well as the synthesis of two new macrocyclic receptors.

5.1 Ester Hydrolysis of Pentyl Macrotricycle 2.57

A number of attempts were made by Wareham to hydrolyse the four pentyl ester chains of macrotricycle **2.57**, in order to yield a water-soluble receptor. While the hydrolysis of the esters was believed to proceed well by stirring the macrotricycle in THF with 2.0-3.0 equivalents of 0.5 M KOH, the purification was not developed and required a reasonable amount of material (> 20 mg) to be produced.

Before hydrolysing the macrotricycle's ester groups it was decided to investigate the reaction conditions and purification technique that would be required, using a model compound. This led to the synthesis of compound **5.1**, which consists of the pentyl ester needed for hydrolysis and the benzyl amide groups, which must withstand the deprotection conditions.



5.1

Synthesis of **5.1** was straightforward, reacting the active ester **4.9** with 2.2 equivalents of benzylamine in dry THF in the presence of *N*,*N*-DIPEA. Purification by flash chromatography resulted in 600 mg of the model compound in an 80 %

yield. ^{19}F NMR showed that no pentafluorophenol side-product remained after chromatography.

Hydrolysis of the pentyl ester of **5.1** was carried out using 2.5 equivalents of cesium hydroxide in DMSO/H_2O (95:5). The starting material had reacted according to TLC analysis (MeOH/DCM 5:95) after being allowed to stir at room temperature overnight and only baseline material was evident. The DMSO and water were removed under high vacuum over a 14 hour period. Purification of the product and protonation of the carboxylates were attempted using the cation-exchange resin, Dowex® 50WX4, NH_4^+ form. Triply distilled water was used to elute the column and the fractions containing the product were identified by means of reverse-phase TLC analysis. This resulted in an off-white solid, which, when analysed by ^1H NMR, showed that no pentyl resonances remained, but did not reveal the presence a carboxylic acid proton peak at the expected value of δ 13 however. It is possible the cesium salt of the carboxylate was still present.

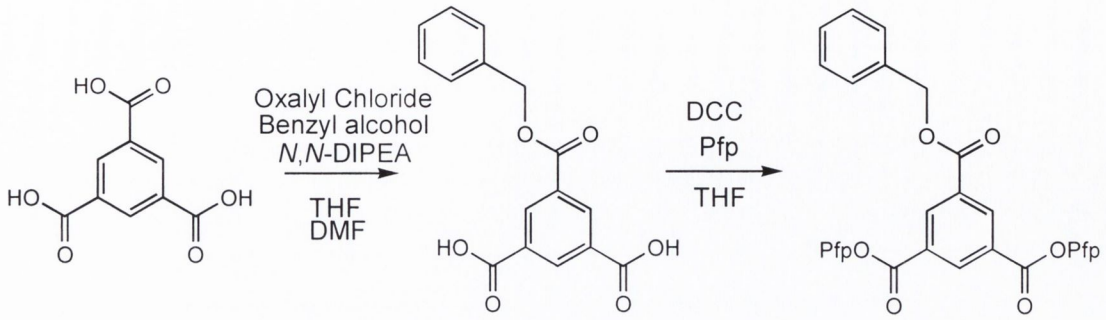
Hydrolysis of the pentyl ester groups of macrotricycle **2.57** was attempted using the same conditions. Again, using 2.5 equivalents of cesium hydroxide for each ester group, no starting material was evident by TLC after allowing the reaction stir at room temperature overnight. Purification by means of a DOWEX® column eluting with triply distilled water was unsuccessful. Elution with 0.1 M aqueous HCl solution was also unsuccessful.

5.2 Benzyl Ester as a Spacer

Owing to the difficulties encountered in the purification of macrotricycle **2.57** after pentyl ester hydrolysis, it was decided to synthesise a spacer molecule, which, once incorporated into the macrotricycle, would be readily manipulated and easily purified to give a water-soluble receptor. The obvious candidate was a benzyl ester, as this would be organic solvent soluble and would allow for cyclisations to take place in THF in the macrotricycle synthesis. Also, the benzyl ester groups would be easily reduced to form a carboxylic acid by means of hydrogenolysis with the only by-product being toluene. This would be a clean reaction to complete the synthesis, as full deprotection would mean that only a filtration followed by removal of the solvent needed to be undertaken.

5.2.1 Synthesis of Benzyl Spacer 5.3

The synthesis of the benzyl spacer **5.3** was based on that of the pentyl spacer **4.9**, using benzyl alcohol instead of *n*-pentanol, as shown in **Scheme 5.1**.



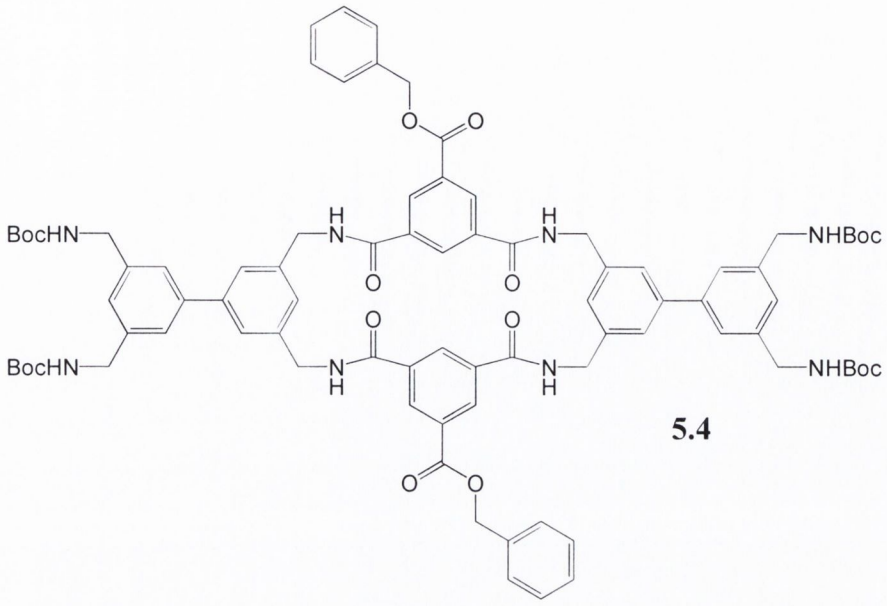
5.2 **5.3**

Scheme 5.1 Synthesis of bis-pentafluorophenyl active benzyl ester **5.3** from 1,3,5-trimesic acid

1,3,5-trimesic acid was reacted with oxalyl chloride in THF and DMF, forming the di-carboxylic acid mono-acid chloride. Reaction with freshly distilled benzyl alcohol in the presence of *N*,*N*-DIPEA gave the di-acid mono-benzyl ester **5.2** which, like the pentyl analogue **4.14**, was isolated by quenching the carboxylates in a basic aqueous layer using 5 M HCl, followed by extraction into ethyl acetate at pH range 6-3. The yield obtained was 43 %, which was higher than the expected statistical yield and the product **5.2** was formed on a 6.2 g scale.

Reacting **5.2** with DCC and pentafluorophenol, again attained conversion of the di-acid to the active ester, this time **5.3**. Unwanted DCU by-product was removed by filtration and purification was by recrystallisation from ethyl acetate/hexane rather than column chromatography as was the case with **4.9**, giving **5.3** in a yield of 76 % and on a 9.8 g scale.

5.2.2 Tetra-Boc macrocycle 5.4

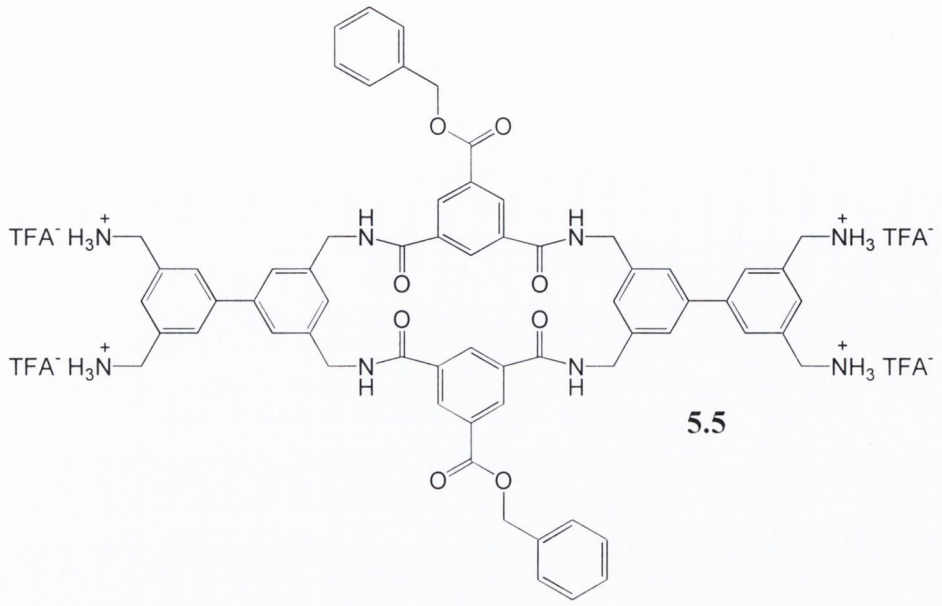


5.4

Cyclisation of the bis-amino bis-Boc biaryl **4.8** with active ester **5.3** was carried out following the same procedure as that of the pentyl analogue **4.10**. A solution of **5.3** in THF (*ca.* 1.66 mM) was added dropwise to a solution of **4.8** in THF and DMF (*ca.* 3.0 mM) over a period of *ca.* 8 hours at room temperature under argon. It was noted that the reaction mixture was cloudy after being allowed to stir at room temperature for 12 hours after addition of the bis-activated ester **5.3**. This prompted future cyclisations for the formation of **5.4** to be carried out at an elevated temperature of 40 °C. Under these conditions, the reaction was still cloudy 3 hours into the addition time of the bis-active ester **5.3**, but 12 hours after the addition was complete the reaction mixture was clear.

Upon work-up of the reaction, it became apparent that **5.4** was less soluble than its pentyl analogue **4.10**. The crude product was not fully soluble in MeOH/$CHCl_3$ even using 3 % MeOH. Recrystallisation from ethyl acetate also failed as only a small portion (*ca.* one fifth) of the product came out of solution. ^1H NMR analysis of the product in d_6-DMSO showed the presence of mostly desired material **5.4** and it was decided to continue with the removal of the Boc groups with the view that trituration of the resultant tetra-ammonium salt would help to clean the material further. Yields for the cyclisation step were calculated by working back after the Boc deprotection stage and were similar for both the room temperature and 40 °C cyclisations, being 76 % and 67 % respectively.

5.2.3 Tetra-Boc Deprotection of 5.4

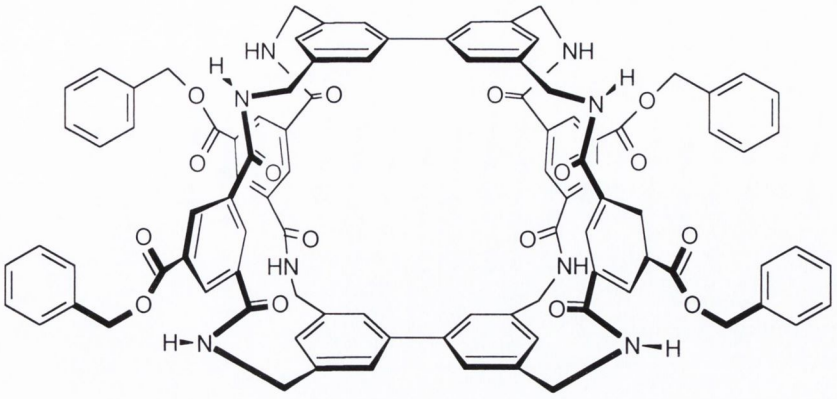


5.5

Removal of the Boc groups of **5.4** to afford the tetra-TFA salt **5.5** was carried out using the standard conditions of TFA/DCM, as undertaken with the tetra-Boc macrocycle **4.10**. Again, reaction time was 2 hours and the reaction mixture was concentrated to yield an oil. Trituration with diethyl ether of the crude oil helped to purify the product, as verified by ^{1}H NMR in d_6-DMSO. The small amount of tetra-Boc **5.4** precipitated from ethyl acetate was deprotected separately from the remaining tetra-Boc material and the yield for deprotection was found to be 98 %, higher than non-precipitated material, typically 80 %, probably owing to higher purity.

The crude product from the deprotection was analysed by LCMS. The product showed only one peak on the UV absorbance trace at retention time of 13.06 minutes. The observed mass peaks were 535 and 1070, corresponding to the doubly protonated and singly protonated tetra-amine, respectively. FAB MS also showed the product as the singly protonated tetra-amine with a mass of 1070.

5.2.4 Benzyl Macrotricycle 5.6



5.6

A [2 + 1] cyclisation of the active ester **5.3** with the tetra-TFA salt **5.5** was carried out using the same procedure as that for the pentyl macrotricycle **2.57**. This involved the dropwise addition of a 0.5 mM solution of **5.3** into a 0.25 mM solution of **5.5** in the presence of 30 equivalents of *N,N*-DIPEA at 40 °C, under argon. It was noted that addition of toluene to the concentrated reaction mixture, for the purpose of azeotroping any remaining DMF, caused the product to precipitate out of solution as an off-white solid.

At the work-up stage, washing of the chloroform layer, containing the product, with 0.5 M aqueous HCl solution, saturated aqueous $NaHCO_3$ solution or brine, caused the formation of a white emulsion. This was observed to a much lesser extent with the work-up for the pentyl macrotricycle **2.57**. This emulsion, when filtered gave a solid and analysis by ^{1}H NMR showed this to consist of unclean benzyl macrotricycle **5.6**. The formation of an emulsion led to the work-up being by-passed and the direct purification, by column chromatography of the crude solid from the concentrated reaction mixture. Elution of the column with AcOH/MeOH/$CHCl_3$ (1:2:97) purified the product somewhat, but co-elution of some side-products was not prevented. Beginning with 200 mg of tetra-TFA **5.5**, 96 mg of column-purified **5.6** was recovered, which represents a 46 % yield. From ^{1}H NMR analysis, most of this was desired material **5.6**.

Further purification of the columned material by preparative TLC resulted in clean macrotricycle **5.6**, as shown by ^{1}H NMR in d_6-DMSO, **Figure 5.1**.

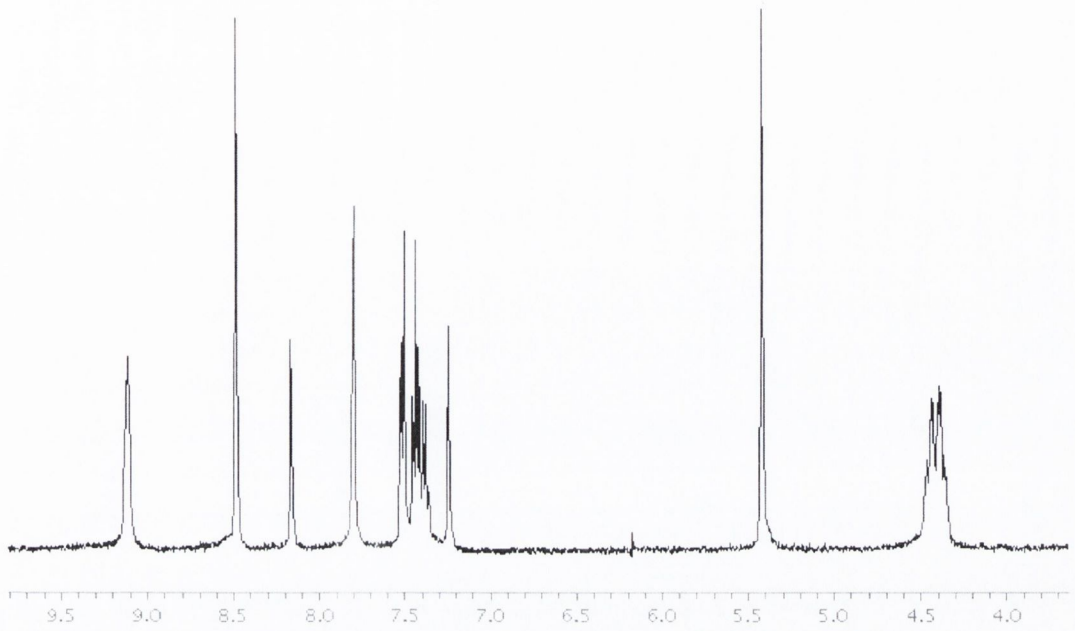


Figure 5.1 ^{1}H NMR of benzyl macrotricycle **5.6** in d_6-DMSO

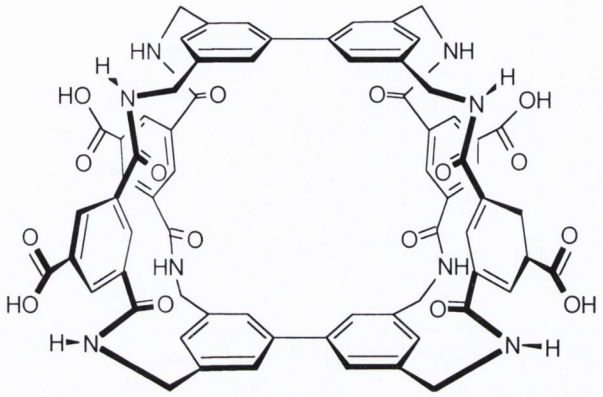
Again, a maximum of 20 mg of material per preparative TLC plate, as well as eluting the plate twice in MeOH/DCM (8:92), gave the best separation. A projected yield for the final cyclisation, based on the preparative TLC purification is 18 %.

As with pentyl macrotricycle **2.57**, **5.6** has a relatively simple ^{1}H NMR spectrum. Two resonances for the aryl spacer unit appear at δ 8.48 and δ 8.15 and two resonances for the biaryl protons appear at δ 7.79 and δ 7.24. The amide protons appear furthest downfield at δ 9.10, as a triplet, owing to vicinal coupling with the biaryl benzylic protons. The aryl protons of the benzyl ester appear as a multiplet as expected for a mono-substituted aryl ring, with the benzyl protons of the spacer appearing as a singlet. An ABX system is again seen for the biaryl benzylic protons, owing to geminal coupling between the spatially different benzylic protons (J = 13.6 Hz) and vicinal coupling between the benzylic protons and the amide proton (J = 5.5 Hz). A full range ^{1}H NMR spectrum is shown in Appendix **B**.

The benzyl macrotricycle **5.6** proved to be less soluble in organic solvents, compared with the pentyl macrotricycle. This was to be expected, as alkyl chains are known to be better organic-solubilising groups than aryl functionalities. This prevented **5.6** being used as an organic-soluble carbohydrate receptor, particularly in

the case of liquid-liquid extractions, as the macrotricycle was seen to precipitate out of solution somewhat, at the work-up stage.

5.2.5 Tetra-Carboxylic Acid Macrotricycle 5.7



5.7

Initially, hydrogenolysis of the four benzyl ester groups was carried out in MeOH/DCM (1:1) with 10 % Pd/C as catalyst and hydrogen at atmospheric pressure. After 24 hours, no starting material was present by TLC analysis. The product obtained after filtration was analysed by ^{1}H NMR in d_6-DMSO, in which it was very soluble. The spectrum showed all the desired product peaks and that the benzyl ester groups had been fully removed. The carboxylic acid peak at δ 13.40 was very encouraging, however the spectrum was not entirely clean and the presence of an unwanted peak at δ 4.07 was cause for concern. It was believed that this peak might have been due to the presence of a methyl ester, generated by reacting a carboxylic acid with methanol co-solvent. This was amplified by the fact that the compound remained very cloudy in D_2O, even in the presence of an excess of CsOH.

These results prompted a solvent change in the reaction, from MeOH/DCM to acetic acid. Using the same conditions, the reaction had completed after 24 hours. Filtration through celite to remove the Pd/C followed by concentration *in vacuo* gave the product **5.7** in a 70 % yield. This time however, the resulting product was not very soluble in d_6-DMSO, but enough material had dissolved to obtain a ^{1}H NMR spectrum, which was much cleaner than the reaction using MeOH/DCM as the reaction solvent system. The desired product peaks were all apparent and no suspicious peak at δ 4.07 was present. All benzyl ester groups were fully removed

and only four peaks were present in the aromatic section, consistent with the desired tetra-carboxylic acid macrotricycle **5.7**, as shown in **Figure 5.2**.

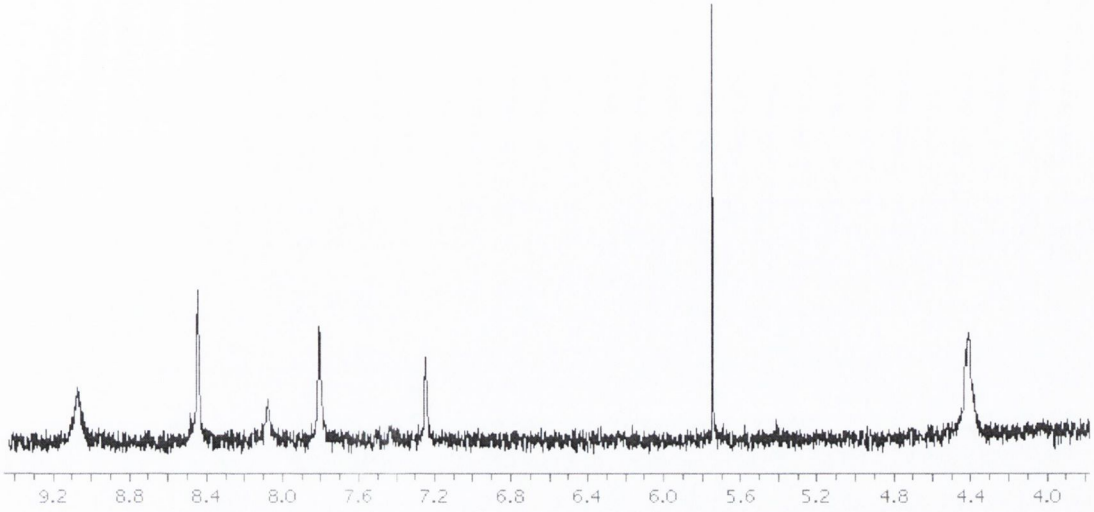


Figure 5.2 Tetra-carboxylic acid macrotricycle **5.7** in d_6-DMSO

5.7 was not soluble in D_2O, however in a small excess of CsOH (2 eq per acid group) most of the material dissolved, although a faint cloud still remained. The product was analysed by ^1H NMR with DSS as internal standard. At 30 °C, four aromatic peaks were present corresponding to the two spacer and two biaryl resonances, which were at δ 8.38, 7.82, 7.78 and 7.45. The resonance at δ 7.78, integrating for four protons, appeared as a shoulder on the peak at δ 7.82. The benzylic protons were hidden under the water peak, as shown in **Figure 5.3**.

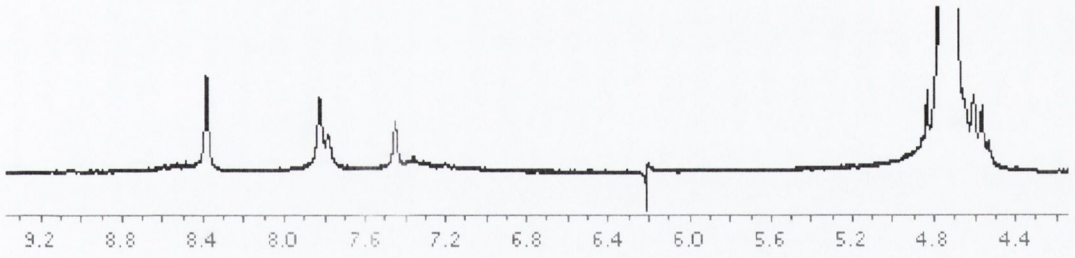


Figure 5.3 Tetra-carboxylate of **5.7** in D_2O at 30 °C

To confirm the presence of the correct peaks as well as integration values, a variable temperature ^1H NMR study was undertaken. Increasing the temperature from 30 °C to 50 °C had the effect of moving the aromatic shoulder peak, previously at δ 7.78, fully under the aromatic peak previously at δ 7.82. However, the water peak shifted

by *ca.* 1.6 ppm and allowed for most of the biaryl benzylic resonances to be seen, **Figure 5.4**.

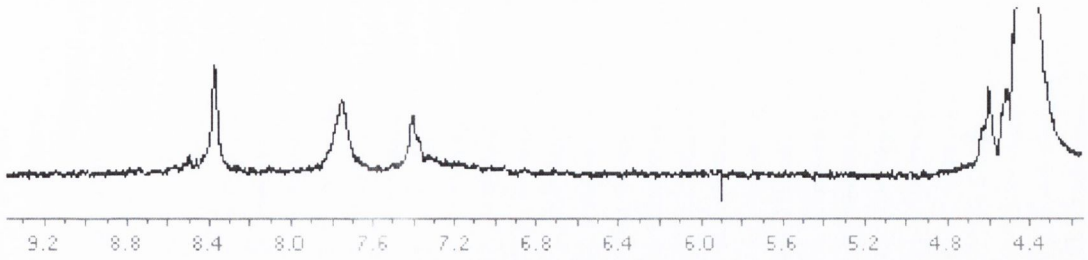


Figure 5.4 Tetra-carboxylate of **5.7** in D_2O at 50 °C

Further increase of the temperature to 80 °C allowed for the two overlapping aromatic peaks to be shifted apart, with the shoulder peak now appearing downfield from the aromatic peak on which it was a shoulder on the upfield side at 30 °C. Also at 80 °C, the biaryl benzylic resonances at δ 4.5 became fully separated from the water peak as shown in **Figure 5.5**, which allowed for accurate integration.

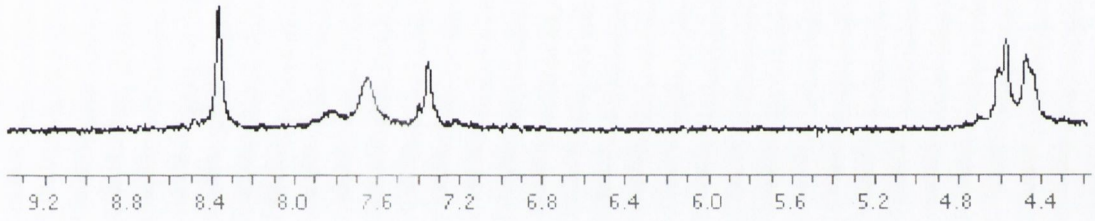
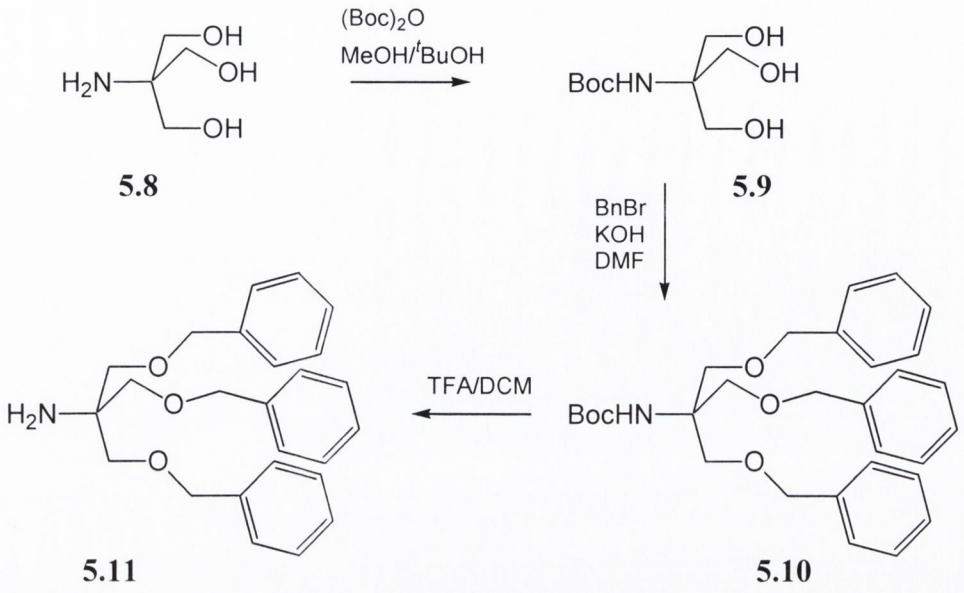


Figure 5.5 Tetra-carboxylate of **5.7** in D_2O at 80 °C

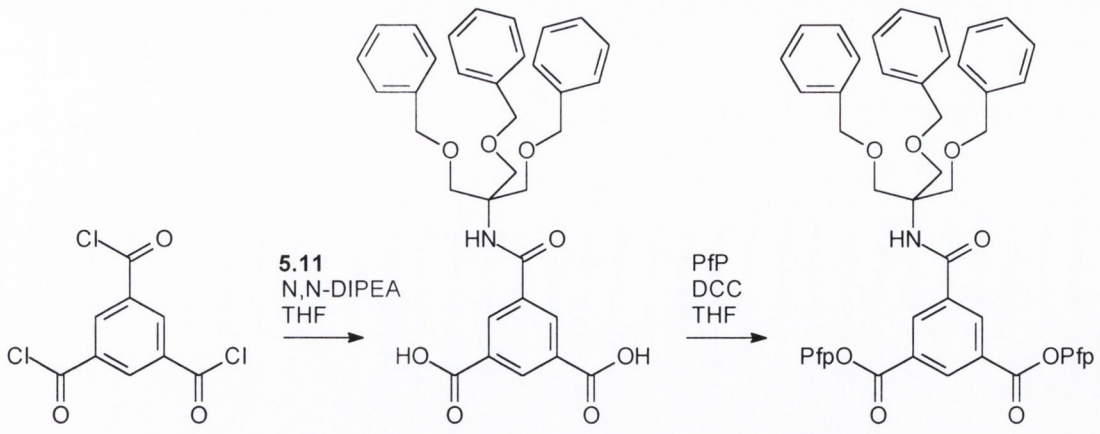
Owing to the tetra-carboxylate not being fully soluble in aqueous solution, it was decided to synthesise a macrotricycle with increased water solubility properties.

5.3 An Improved Organic-Solubilising Group



Scheme 5.2 Synthesis of tris-benzyl ether aminomethane **5.11**

To generate an improved macrocyclic analogue the synthesis of a spacer molecule that would impart both greater organic solubility, which would allow for liquid/liquid extraction studies not previously undertaken, as well as possessing the potential for water solubility, was desirable. Work carried out by the Ungaro[72] group brought the tris-benzyloxy amine **5.11** to our attention, as shown in **Scheme 5.2**. This molecule had been incorporated into the lower rim of various calixarenes with a view to water solubility. This was achieved through hydrogenolysis of the benzyl ethers, resulting in water-solubilising hydroxyl groups. By incorporating **5.11** into an aryl spacer molecule greater organic solubility was envisaged as well as an alternative route towards water solubility. This therefore led to the design and synthesis of the active ester **5.14**, illustrated in **Scheme 5.3**.



5.12 **5.13** **5.14**

Scheme 5.3 Synthesis of tris-benzylether spacer unit **5.14**

5.3.1 Synthesis of Tris-Benzyl Protected Amine 5.11

Conversion of **5.8** to the tris-benzyl ether **5.11** was carried out following the procedures of Ungaro.[72] Boc protection of **5.8** was achieved using di-*tert*-butyl-dicarbonate in MeOH/*t*BuOH (2:3) at room temperature. Concentration of the reaction mixture *in vacuo* gave the desired product on an 8.7 g scale in a 95 % yield, without further purification.

Benzylation of the three hydroxy groups was undertaken using an excess of benzyl bromide (5.6 eq) with potassium hydroxide (5.6 eq) in DMF. The reaction was complete within 2 hours at room temperature. Removal of DMF had to be carried out below 45 °C, using a high vacuum (0.5 mm Hg), as excessive heating would have caused the product to decompose. The crude product was purified by flash column chromatography, gradient eluting with petroleum spirit-ethyl acetate/petroleum spirit (7.5:92.5), giving the desired product as a colourless oil on a 5 g scale and in a yield of 44 %. This was a little lower than the yield reported by Ungaro and co-workers (65 %), but the reaction was scaled up from 1 g of **5.9** to 5 g. It was observed, in an attempt to increase the yield, that prolonged reaction time was detrimental. Increasing the reaction time from 2 hours to 12 hours resulted in a 14 % yield of isolated product. This lower yield is probably due to exposure of the product to strongly basic conditions for a prolonged period.

Removal of the Boc groups was carried out immediately prior to the free amine being used in the synthesis of **5.14**. This was undertaken using standard

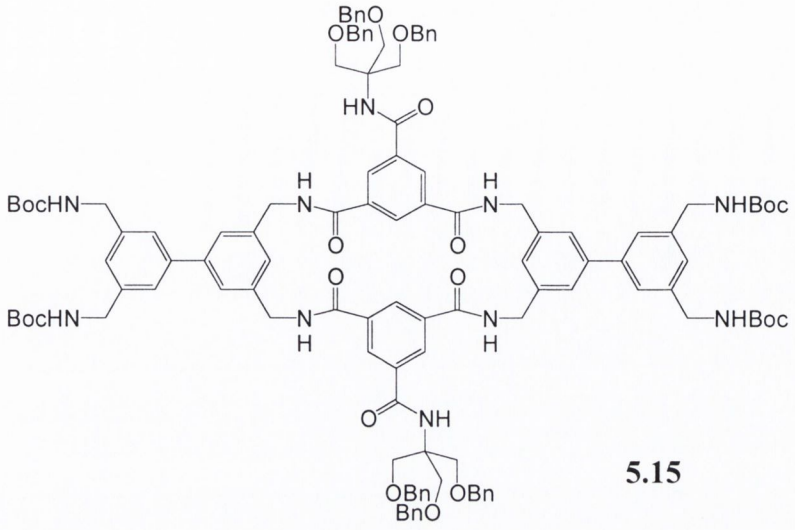
conditions of TFA/DCM initially at 0 °C and being allowed to rise to room temperature. Deprotection was complete within 4 hours and was accomplished in a 95 % yield resulting in 2.6 g of free amine **5.11**.

5.3.2 Synthesis of Bis-Pentafluorophenyl Active Ester 5.14

It was decided to use 1,3,5-benzenetricarbonyl trichloride as the starting material for **5.13**, rather than trying to form the mono-acid chloride from 1,3,5-benzenetricarboxylic acid, as undertaken with the previously described spacer molecule syntheses. Dropwise addition of **5.11**, as a dilute solution in THF (0.01 M), to an excess of the tri-acid chloride **5.12** in THF (0.1 M), was carried out over 3 hours at room temperature. After the reaction mixture was allowed to stir overnight the free unreacted acid chloride groups were converted to carboxylates by the addition of aqueous potassium hydroxide solution. As with the synthesis of bis-pentafluorophenyl pentyl spacer **4.9**, the desired di-carboxylic acid **5.13** was isolated by quenching the carboxylates in the basic aqueous layer using aqueous HCl solution followed by extraction into ethyl acetate at various pH ranges. The di-acid **5.13** was extracted at the pH range 5-6 and was isolated on a 5.8 g scale and in a 78 % yield. A yield of 96 % for the synthesis of the di-acid was obtained once, but only on a 250 mg scale of product. The material extracted at the various pH ranges were analysed by LCMS. The UV absorbance trace of the pH 5 extract exhibited one large peak at retention time 16.7 minutes, with masses in the total ion chromatogram of 584 and 476. These masses correspond to the desired compound and mono-debenzylated desired product, respectively. The UV absorbance trace of the pH 6 extract had one main peak at retention time 16.6 minutes, also corresponding to masses of 584 and 476.

Conversion of the di-acid to the active ester **5.14** was achieved by the same procedure as was used with the pentyl and benzyl spacer molecules. DCC was employed as a coupling agent and after 2 hours reaction time a precipitate of DCU was observed which was removed by filtration. Purification by flash column chromatography resulted in 5.5 g of **5.14** in a yield of 96 %, similar to that of the pentyl analogue **4.9**.

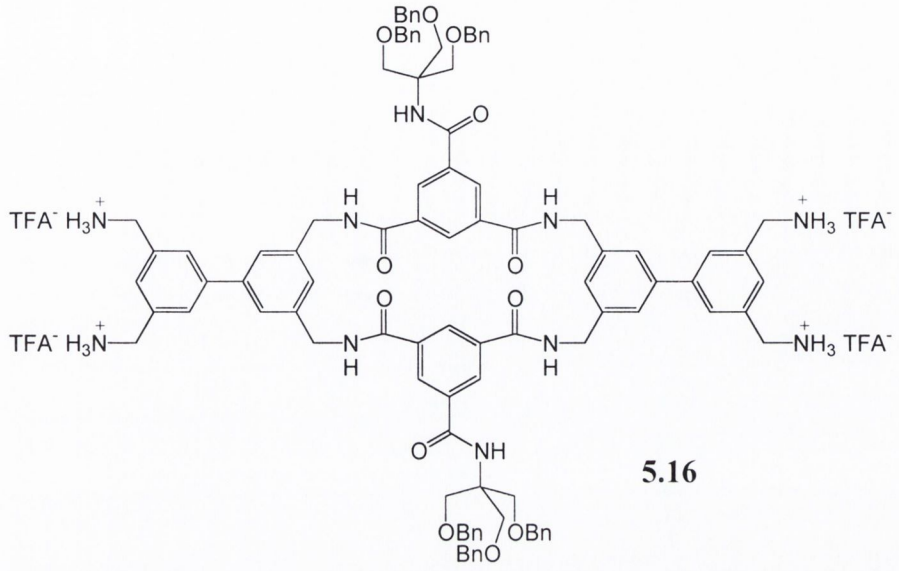
5.3.3 Tetra-Boc Protected Macrocycle 5.15



5.15

Cyclisation of the bis-amino bis-Boc biaryl **4.8** with tris-benzyl ether spacer **5.14** was carried out following the same procedure as outlined previously for the initial cyclisation. A solution of **5.14** in THF (*ca.* 1.66 mM) was added dropwise to a solution of the bis-amino bis-Boc biaryl **4.8** in THF and DMF (*ca.* 3.0 mM) over a period of *ca.* 8 hours at room temperature under nitrogen. The reaction mixture was allowed stir at room temperature for 12 hours after addition of the bis-activated ester. After work-up, the reaction mixture was concentrated *in vacuo* to a yellow oil which was purified by flash column chromatography eluting with ethyl acetate/toluene (40:60-50:50). Unlike the Boc protected benzyl macrocycle **5.4**, this material was readily soluble in organic solvents. However, the compound tended to streak on TLC, which made separation from impurities somewhat difficult. Isolation of the desired product was achieved in a 40 % yield and on a 431 mg scale. The yield for the cyclisation step is higher than this figure suggests, but problems with separation prevented all of the product being isolated. Later fractions from the column were analysed by ^{1}H NMR, which showed the presence of predominantly desired product along with unknown by-product. Also, analysis by FAB MS showed predominantly one peak at 2058 corresponding to the sodium adduct of the desired product **5.15** (2035).

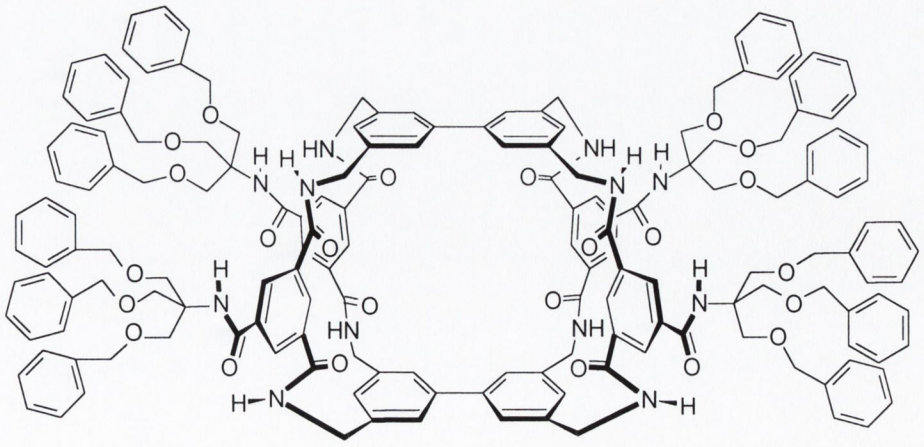
5.3.4 Tetra-Boc Deprotection 5.16



5.16

Removal of the Boc groups was achieved using the standard conditions of TFA/DCM (1:1) and the reaction was complete within 4 hours. Deprotection was quantitative and the largest scale reaction carried out resulted in 401 mg of the tetra-ammonium salt **5.16** being synthesised. Full deprotection was verified by LCMS with the UV absorbance trace showing one peak at retention time 13.75 minutes with a corresponding mass of 818, which represents **5.16** as the doubly charged tetra-amine. The desired material was also confirmed by FAB MS which showed **5.16** as the tetra-amine corresponding to 1636 $[M]^+$, 1658 $[MNa]^+$ and 818 $[M]^{++}$.

5.3.5 "Polybenzyl" Macrotricycle 5.17



5.17

A [2 + 1] cyclisation of the active ester **5.14** with the tetra-TFA salt **5.16** was carried out using the same procedure as that for the previously described final cyclisations. This involved the dropwise addition, over a 10 hour period, of a 0.5 mM solution of active ester **5.14** into a 0.25 mM solution of **5.16** in the presence of 30 equivalents of *N*,*N*-DIPEA at 40 °C under nitrogen. After the addition was completed, the reaction was stirred for a further 5 hours. After an acid/base work-up, the reaction mixture was concentrated to an off-white solid, which was purified by flash column chromatography. The crude material was much more soluble than that from the previous cyclisations and it was possible to elute the material in MeOH/DCM (3:97). Unlike its tetra-Boc precursor, **5.17** was not streaky by TLC which enabled better separation by column chromatography, resulting in polybenzyl macrotricycle **5.17** being obtained in a 62 % yield and on a 309 mg scale.

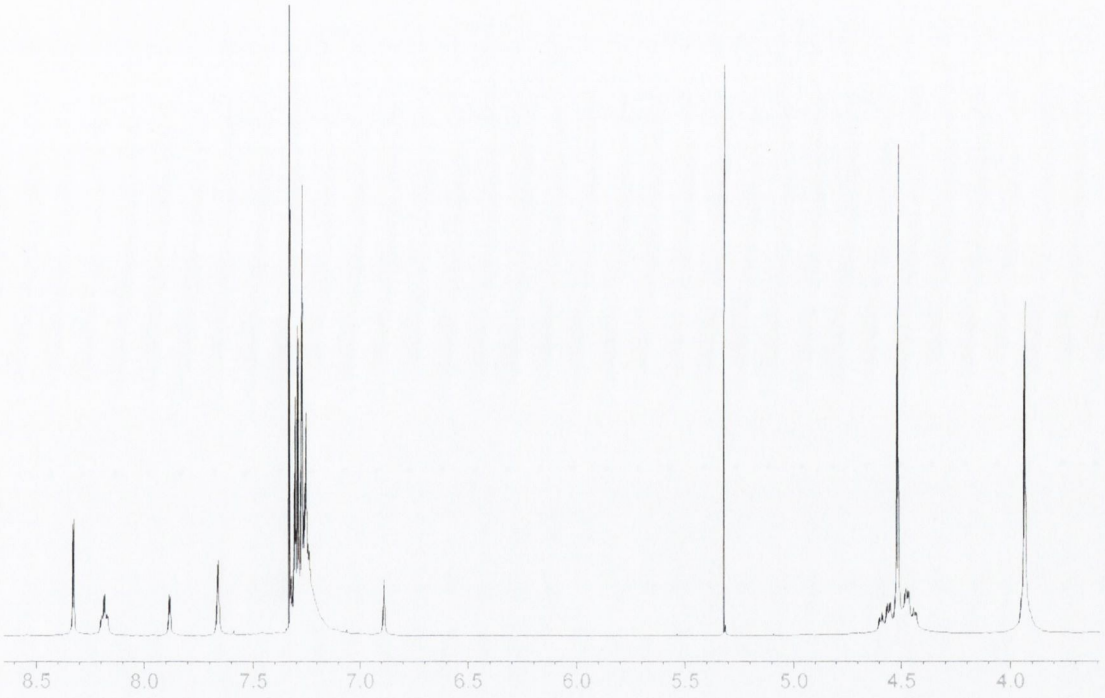


Figure 5.6 ^{1}H NMR of polybenzyl macrotricycle **5.17** in $CD_3OH/CDCl_3$ (8:92)

A ^{1}H NMR spectrum of **5.17** is shown in **Figure 5.6**. The use of d_3-methanol prevented deuterium/proton exchange as is evident with d_4-methanol and allowed for the core amide protons to be seen as a triplet at δ 8.20, due to coupling with the biaryl methylenes. Fine splitting, not observed in a d_6-DMSO spectrum (see Chapter Seven), is evident in the aromatic section. A doublet at δ 8.33 and a triplet at δ 7.88 represent the "external" and "internal" protons of the spacer unit, respectively. The expected two biaryl resonances appear at δ 7.66 and δ 6.89 representing the eight

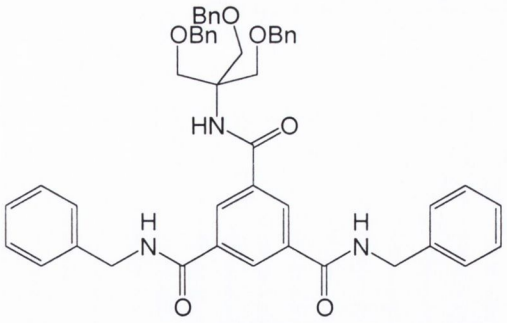
equivalent protons and four equivalent protons respectively. The biaryl methylene protons appear as an ABX system at δ 4.5 and are bisected by the singlet corresponding to the benzyl ether methylene protons. The third and final methylene signal is seen at δ 3.93 as a singlet. A full range ^{1}H NMR spectrum is shown in Appendix **C**.

Unlike the pentyl and benzyl macrotricycles **2.57** and **5.6** respectively, further purification by preparative TLC was not necessary as **5.17** was sufficiently clean after columning to pass microanalysis with the inclusion of six water molecules. It is not uncommon for such receptors to trap water molecules in their cavities as was exhibited by cholaphane **2.35** (Chapter Two), the X-ray crystal structure of which revealed the presence of five water molecules within the cavity.[21]

As was anticipated, polybenzyl macrotricycle **5.17** proved to be much more organic-soluble than its pentyl or benzyl analogues and this enabled liquid/liquid extraction of sugars to be carried out, which was not possible with the previously described macrotricycles. This will be discussed in detail in Chapter Six.

5.4 Tris-Benzyl Ether Model Compound 5.18

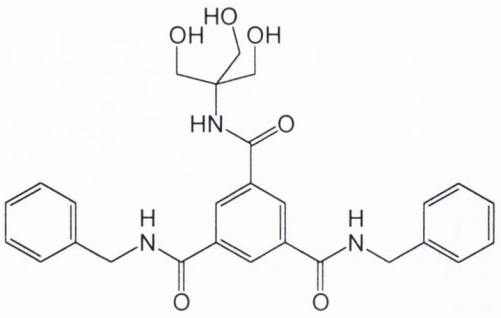
As regards manipulating **5.17** so as to form a water-soluble receptor, it was decided to synthesise a model compound **5.18** on which benzyl ether deprotection could be developed and optimised. As with the pentyl model compound **5.1**, synthesis was straightforward and was achieved by reacting the bis-pentafluorophenyl active ester **5.14** with 2.2 equivalents of benzylamine in dry THF in the presence of *N*,*N*-DIPEA. Purification by flash column chromatography resulted in desired product **5.18**, which was shown to be clean by ^{1}H NMR and ^{19}F NMR. This was synthesised on a 240 mg scale and in a 90 % yield. One particular batch of the model compound was contaminated with pentafluorophenol by-product, as shown by ^{19}F NMR, however, it was possible to remove this material by simply azeotroping several times with toluene giving the desired product as a white solid.



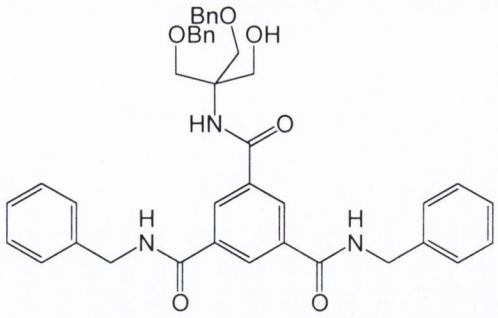
5.18

5.4.1 Benzyl Ether Deprotection by Hydrogenolysis

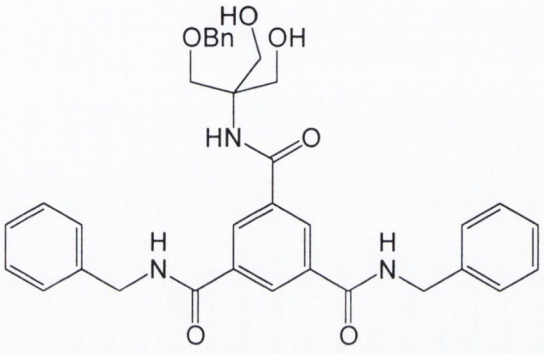
The obvious and desired choice of reaction for debenzylation was hydrogenolysis. If the reaction proved to be successful, all that would be required would be a filtration to remove the catalyst followed by concentration *in vacuo* to remove the solvent and toluene by-product. Hydrogenolysis of the model compound was undertaken at 4 bar pressure of hydrogen with shaking using a Parr hydrogenation apparatus. Greater than a half weight equivalent of 10 % Pd/C was used in ethanol/acetic acid (2:1) and the reaction time was 4.5 days. TLC analysis showed no starting material to be present after this time. LCMS analysis showed a number of peaks in the UV absorbance trace. A peak with a retention time of 12.81 minutes had a mass of 492 corresponding to the fully deprotected tris-alcohol **5.19**. Another peak with retention time of 14.66 minutes had a mass of 583 corresponding to the partially deprotected bis-alcohol **5.21**. FAB MS also confirmed the mixture of compounds. The desired tris-alcohol **5.19** could be seen at 492 with its sodium adduct at 514. The mono-benzyl bis-alcohol **5.21** could be seen at 583 with its sodium adduct at 604 and the di-benzyl mono-alcohol **5.20** could be seen at 672 with its sodium adduct at 694. Therefore, hydrogenolysis at 4 bar pressure for 4.5 days proved to be unsuccessful in the removal of three benzyl ether groups. An alternative method was obviously required if all twelve benzyl ether groups of the polybenzyl macrotricycle were to be successfully removed.



5.19 (M.W. 492)



5.20 (M.W. 672)



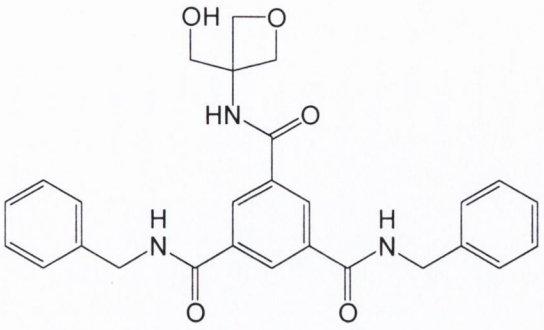
5.21 (M.W. 582)

5.4.2 Benzyl Ether Deprotection by TMSI

According to Greene,[73] the use of trimethylsilyl iodide is a clean and efficient way of cleaving benzyl ethers. TMSI is very reactive and requires the use of anhydrous conditions. Reaction times are short, typically 20 minutes and yields are high if not quantitative.

Initially, the reaction was undertaken in acetonitrile using 1.3 equivalents of TMSI, for each benzyl ether present. **5.18** was not fully soluble in acetonitrile but upon addition of TMSI it became soluble. No starting material was present by TLC analysis after 1.5 hours and the reaction was left to stir for a further 3 hours after which time it was quenched with methanol. Purification by reverse phase flash column chromatography was attempted using H_2O/CH_3CN (40:60) as eluent. This solvent was not polar enough as the product eluted quickly from the column. However, LCMS analysis revealed the presence of mixed products. One peak at retention time 13.87 minutes showed the desired mass of 492. A peak at 14.02 minutes had a mass of 474, which is consistent with the dehydrated product **5.22**

which may have resulted from an intramolecular attack of one hydroxyl group on a neighbouring trimethylsilyl alcohol.



5.22 (M.W. 474)

Also, a peak at 14.65 minutes had a mass of 582 consistent with the mono-benzyl bis-alcohol **5.21**, showing that full deprotection had not been achieved.

It was decided to repeat the reaction increasing the number of equivalents of TMSI from 1.3 to 3 per benzyl ether. The reaction was also carried out at higher concentration, 0.07 M instead of 0.02 M and in DCM which solubilised **5.18**. TLC analysis showed that the starting material was still present 2 hours into the reaction. The reaction time allowed was 5 hours in total and quenching was again with methanol. LCMS analysis of the crude product revealed the presence of four main peaks: a peak at 13.09 minutes had the desired mass of 492; a peak at 13.75 minutes had the masses 474 and 515 possibly corresponding to the dehydrated compound **5.22** and the sodium adduct of **5.19** respectively; a peak at 14.15 minutes corresponded to **5.21** having a mass of 582 and a peak at 16.84 minutes had a similar retention time to benzyl iodide and is most probably benzyl bromide side-product, see Appendices **D-G**. Analysis of the crude product by FAB MS showed the desired mass of 492 as the main product but with a significant peak corresponding to **5.21** having a mass of 583. Although the reaction was known not to have fully succeeded, it was decided to purify the material by reverse-phase flash column chromatography eluting this time with H_2O/CH_3CN (60:40). One of the earlier fractions from the column was analysed by LCMS and was believed to be the desired material having a mass of 492. However, ^1H NMR revealed that something was co-ordinating to one of the three oxygens as the three CH_2 groups were not equivalent, as would be expected if full deprotection had occurred. A doublet at δ 3.67 integrated for 4

protons and was consistent with methylene protons of two CH_2OH groups. A singlet at δ 4.42 integrated for 2 protons and was the third CH_2O group with an unknown species co-ordinated to the oxygen atom. ^{13}C NMR also confirmed this. FAB MS showed a peak of 493, which combined with the mass of 492 detected by LCMS, pointed to the presence of a group which was easily cleaved under the conditions at which these types of mass spectrometry operate.

The increase in the number of equivalents of TMSI did not cleave all of the benzyl ethers as indicated by the presence of **5.21** by LCMS and FAB MS. It is possible that after two trimethyl silyl groups have co-ordinated to two of the benzyl ether oxygen atoms of **5.18** that there is steric crowding preventing a third from attacking.

One option at this stage was to treat the tris-benzyl ether **5.18** as described, quench with methanol and treat with TMSI once again to deprotect any remaining benzyl ether groups. However, the presence of what appears to be **5.22**, as a result of intramolecular cyclisation was cause for concern. This led to the investigation of a third method for benzyl ether deprotection.

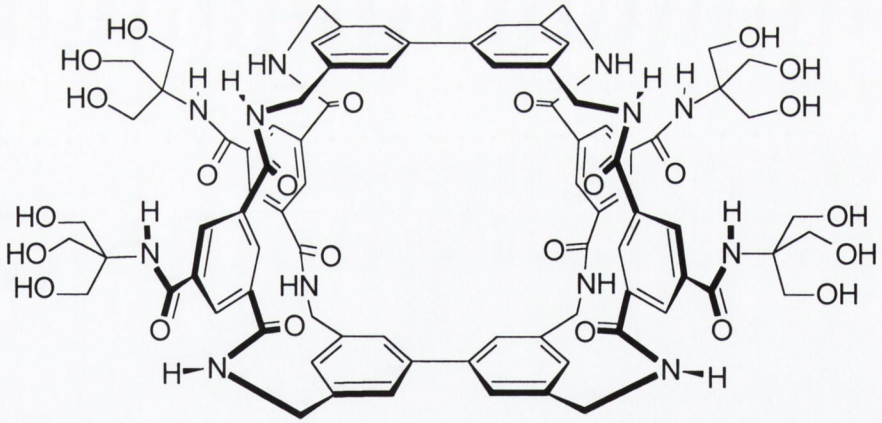
5.4.3 Benzyl Ether Deprotection by BBr_3

BBr_3 is also reported as being a useful reagent for the deprotection of benzyl ethers, as described by Greene[73] and Ward.[74] As with TMSI, this reagent is very reactive and requires the use of anhydrous as well as low temperature conditions. BBr_3 had the potential to be better than TMSI as it is sterically less bulky and therefore more likely to be able to attack all three proximal ether groups. On a 10 mg scale of tris-benzyl ether **5.18**, using 3 equivalents of BBr_3 for each benzyl ether, in dry THF at –78 °C initially and then warming to room temperature, the starting material was not evident after 10 minutes by TLC analysis. Also, LCMS analysis after 10 minutes of reaction time had elapsed showed only two major products, making the crude reaction mixture much cleaner than the TMSI reaction. The first peak appeared at retention time 14.15 minutes and its mass was 492, corresponding to the desired material. The second peak was believed to be benzyl bromide side product, but its mass was not detectable owing to its non-polar nature. A known sample of benzyl bromide was passed through the LCMS and was shown to have the same retention time as this second peak and also a non-detectable mass, confirming it

as the by-product, see Appendices **H-J**. The reaction was quenched with water after 1.5 hours reaction time, which caused a precipitate to appear in the reaction mixture. This was filtered off and analysed by ^{1}H NMR and ^{13}C NMR which showed clean desired product.

The reaction was repeated on a 62 mg scale and this time quenching took place after 20 minutes. The resultant white precipitate was washed with DCM to remove any benzyl bromide side product. LCMS analysis revealed one major peak at retention time 13.15 minutes corresponding to the desired product. A much smaller peak at 13.51 corresponded to **5.21** meaning one benzyl group on this material was still remaining. However, this peak was much smaller compared with the TMSI reaction, and quenching had taken place after only 20 minutes reaction time. ^{1}H NMR showed that the desired product **5.19** was the major compound. Purification by flash column chromatography on normal-phase silica eluting with ammonia-saturated MeOH/$CHCl_3$ (10:90) resulted in clean tri-ol **5.19** in a 60 % yield.

5.5 Benzyl Ether Deprotection- "Polyhydroxy Macrotricycle" 5.23



5.23

Deprotection of the twelve benzyl ethers of **5.17** was initially attempted using hydrogenolysis. This was carried out on a small scale and was prior to any deprotection being investigated on the model compound **5.18**. 20 mg of activated Pd/C was added to 10 mg of **5.17** in ethanol/acetic acid (1:1) and was subjected to hydrogenolysis on a Parr hydrogenation apparatus at 2 bar pressure at room temperature for 3 days. TLC analysis in MeOH/DCM (5:95) revealed baseline

material. Filtration through celite followed by concentration *in vacuo* resulted in a white solid which appeared as a mixture of compounds by ^{1}H NMR analysis. FAB MS was unhelpful showing only baseline noise.

A report by Hawker and co-workers[75] suggested that debenzylations proceed well in THF at 50 °C. 17 mg of polybenzyl macrotricycle **5.17** in dry THF had 13 mg of 10 % Pd/C added to it and was heated to reflux under hydrogen at 50 °C. TLC analysis in MeOH/DCM (5:95) after 15 hours showed the presence of starting material along with a slower running material and baseline material. After 2 days of hydrogenolysis, TLC analysis showed a streak. ^{1}H NMR analysis of the recovered material revealed the presence of a mixture of products. Again, hydrogenolysis proved to be unsuccessful as a multiple debenzylation method.

As explained previously, much work with the model compound **5.18** revealed BBr_3 to be a suitable reagent for multiple benzyl ether deprotections. This method was undertaken on 40 mg of **5.17** in dry DCM. 20 equivalents in total of BBr_3 was used to effect deprotection of the twelve benzyl ethers. Addition of BBr_3 as a 1.0 M solution in DCM was undertaken at –78 °C. **5.17** was not evident by TLC analysis (MeOH/DCM 5:95) after only 5 minutes reaction time. LCMS analysis after 30 minutes reaction time revealed one peak at 11.67 minutes with another small peak at a slightly higher retention time (precise retention time unknown as peak height is below the limit for automatic peak picking) as well as a large peak at 16.67 minutes consistent with benzyl bromide by-product. The range of the mass spectrometer is limited to 150-1600 mass units and it is therefore not possible to see the molecular ion of the desired product. Also, benzyl bromide by-product was not detectable by mass spectrometry owing to its non-polar nature. After 1.5 hours reaction time the mixture was quenched with water and evaporation resulted in a solid which was washed twice with DCM to remove benzyl bromide. ^{1}H NMR in d_6-DMSO showed the desired compound peaks of **5.23** with all benzyl ether groups deprotected. LCMS showed one major peak at 12.64 minutes. The weight of material obtained was 43 mg and still contained boron residues at this point. Water was added and the solid ground up in an attempt to solubilize the polyhydroxy macrotricycle **5.23**. 6 mg of undissolved solid remained which was filtered off and analysed by ^{1}H NMR in d_6-DMSO and may have contained material with some benzyl ethers still attached. LCMS showed one peak at 11.57 minutes but FAB MS showed only baseline noise. The water filtrate was concentrated *in vacuo* to yield an off-white coloured solid, 22

mg. Analysis by ^{1}H NMR in D_2O showed no product peaks but upon addition of 20 % CD_3CN peaks consistent with **5.23** were observed, as shown in **Figure 5.7**. A ^{1}H NMR spectrum in CD_3CN/D_2O showing a wider range is shown in Appendix **M**, where the methylene protons adjacent to the hydroxyl groups can be seen as a doublet at δ 3.88 integrating for 24 protons. The benzylic methylene resonances are possibly hidden under the water peak at δ 4.6. LCMS showed one peak at 11.66 minutes but again FAB MS showed only baseline noise.

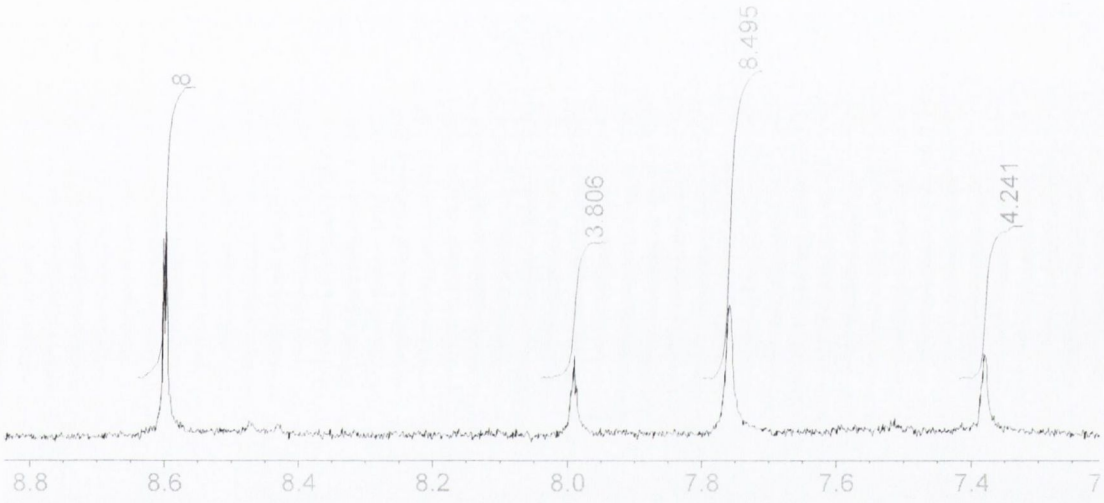


Figure 5.7 ^{1}H NMR of polyhydroxy macrotricycle **5.23** in $CD_3CN:D_2O$ (20:80)

The deprotection was repeated on a 57 mg scale, this time using 24 equivalents of BBr_3. Again, starting material was not evident by TLC after 5 minutes reaction time. After quenching the reaction with water, the mixture was concentrated to an off-white solid which was washed twice with DCM to remove benzyl bromide by-product. The crude product was analysed by ES^+ MS and the doubly charged product peak was evident at 825 mass units, as shown in Appendix **N**. Purification by reverse phase flash chromatography yielded the product **5.23** as an off-white solid which was water-soluble. LCMS analysis showed one main peak at 10.79 minutes, as shown in Appendix **K**. It was decided to run the LCMS in the medium mode which spans the range of 500-950 mass units in the hope of seeing the doubly charged product peak at 825. This was found to be the case, as shown in Appendix **L**. Unlike the tetra-carboxylate of macrotricycle **5.7**, the polyhydroxy

macrotricycle **5.23** was fully soluble in water and no cloudiness was evident. A ^{1}H NMR spectrum was obtained in D_2O at 45 °C as shown in **Figure 5.8**.

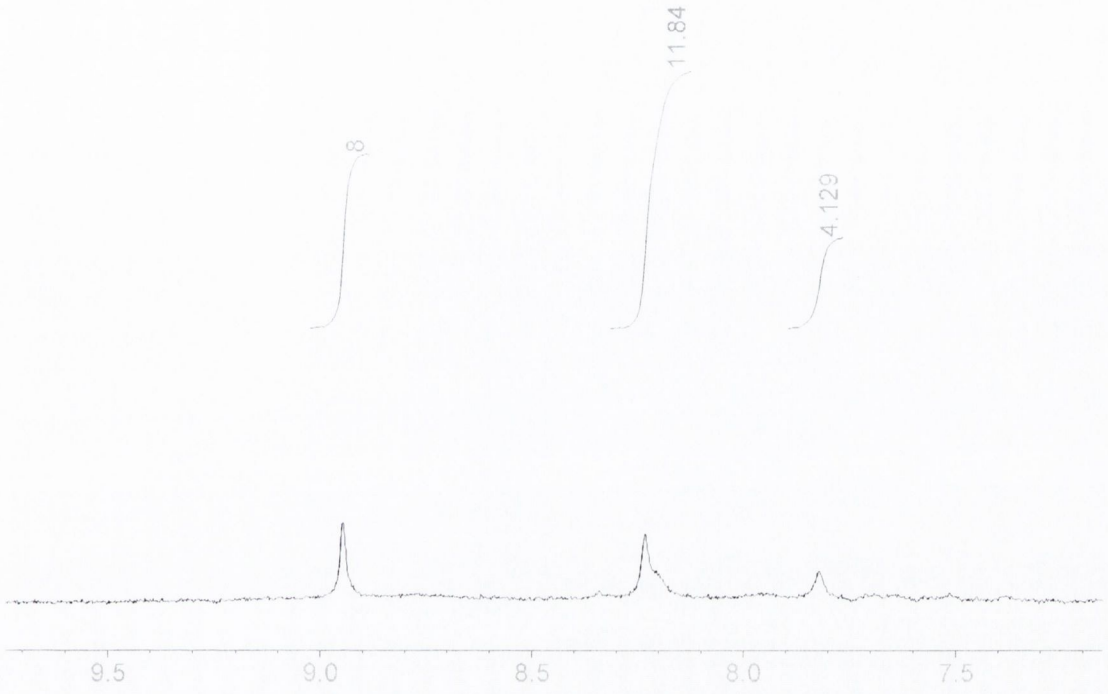


Figure 5.8 ^{1}H NMR of polyhydroxy macrotricycle **5.23** in D_2O

A full range spectrum is shown in Appendix **O**, where the methylene protons adjacent to the hydroxyl groups can be seen at δ 4.39 integrating for 24 protons. The benzylic methylene resonances are again possibly hidden under the water peak at δ 5.0. This same sample in D_2O was analysed by ES^+ MS and the molecular ion at 1650 was evident as shown in Appendix **P**, as well as the doubly, triply and quadruply charged species as shown in Appendix **Q**. Approximately 1 equivalent of D-glucose was added to the macrotricycle in D_2O and ^{1}H NMR at 45 °C showed no shift in the resonances of the receptor. The macrotricycle **5.23** may require purification by preparative HPLC before being analysed further.

CHAPTER SIX

Preamble

This chapter discusses the various results obtained for carbohydrate recognition by the macrotricycles discussed in Chapter Five. In particular, the pentyl macrotricycle **2.57** was investigated for its recognition of resin-bound galactoside by means of magic angle spinning (MAS) NMR and the polybenzyl macrotricycle **5.23** was studied for its ability to extract various sugars from aqueous into organic media as well as its ability to bind organic-soluble sugars as followed by ^{1}H NMR.

6.1 Gel-Phase MAS NMR Spectroscopy of Pentyl Macrotricycle 2.57

Gel-phase MAS NMR spectroscopy had been used recently and successfully by Sanders and co-workers to investigate metal-ligand interactions at the solid liquid interface.[76] ArgoGel® beads were employed which consist of highly flexible polyethylene glycol chains bonded to polystyrene beads. Attachment of a pyridyl or bipyridyl ligand to the ends of the PEG chains allowed for investigation of the interaction between these ligands and metal porphyrins by MAS NMR. This technique gives very good results for such a system as the swelling of the beads in a suitable solvent, together with the PEG chain acting as a flexible tether, produces a solution like environment.

With an improved route developed towards the pentyl macrotricycle **2.57**, more material was synthesised and a collaboration with the group of J. K. M. Sanders (Cambridge) was established so as to investigate the binding properties of **2.57** for resin-bound galactoside.[77] **2.57** had shown good affinities for various pyranosides as discussed earlier and its K_a for octyl-β-D-galactoside in $CD_3OH/CDCl_3$ (8:92), as measured by ^{1}H NMR, was 220 M^{-1}. Although this was not the highest K_a of the sugars tested (octyl-β-D-glucoside had a K_a of 980 M^{-1}), galactoside proved to be the simplest to work with on ArgoGel® beads. Using the same solvent system, the Cambridge group performed titrations of **2.57** against the resin-bound galactoside using ^{1}H MAS NMR to follow the change in resonances. **2.57** is represented as a doughnut in the following illustrations, as in **Figure 6.1**.

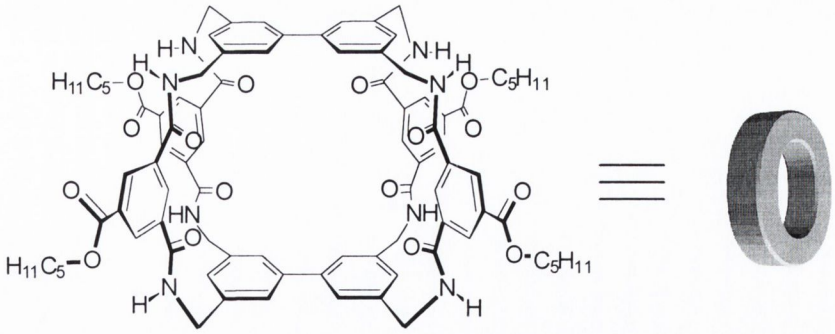


Figure 6.1 Pentyl macrotricycle **2.57** represented as a doughnut

The expected binding of the macrotricycle to the sugar is illustrated in **Figure 6.2** and it was anticipated that study of the interaction would be possible at the solid and liquid interface.

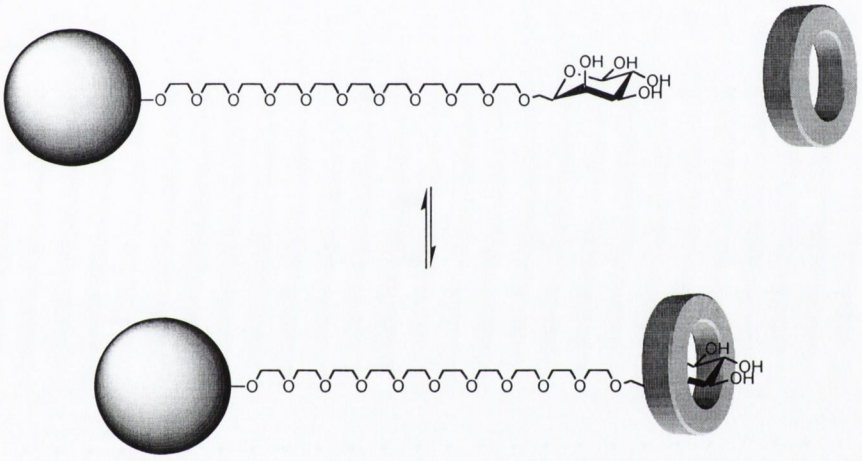


Figure 6.2 Anticipated binding of PEG bound sugar by the macrotricycle **2.57**

Titrations of the macrotricycle revealed unexpected splitting of the PEG resonance into two peaks. As the binding to the galactoside was reversible, it was postulated that this splitting may be due to threading of the macrotricycle along the chain resulting in bound and free PEG, as shown in **Figure 6.3**.

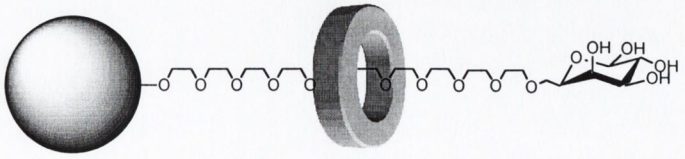


Figure 6.3 Threading of the macrotricycle along the PEG chain

To prove that this was indeed the case, three control experiments were undertaken.

The first of these involved titration of the macrotricycle with unfunctionalised ArgoGel® beads as shown in **Figure 6.4**. As expected, the PEG resonance split into two peaks, the new peak appearing broad and upfield, indicative of an interaction with the macrotricycle, probably due to threading. Upon addition of glucoside, which created competition between the PEG and the sugar for the macrotricycle, the resonance of the PEG chain sharpened and shifted back towards its original unbound chemical shift as shown in **Figure 6.5**.

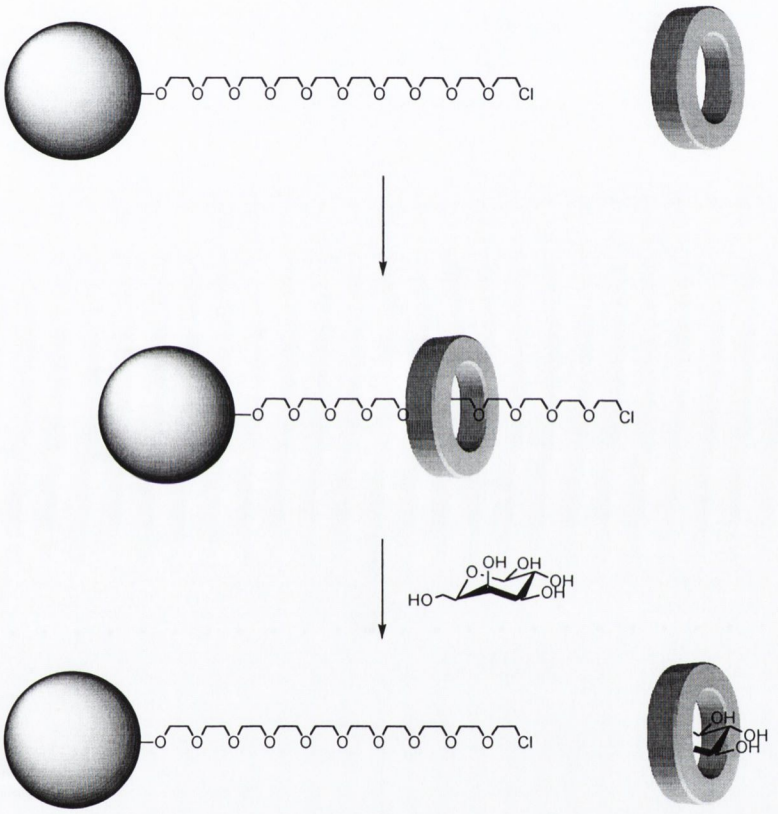


Figure 6.4 First control experiment to prove threading occurs

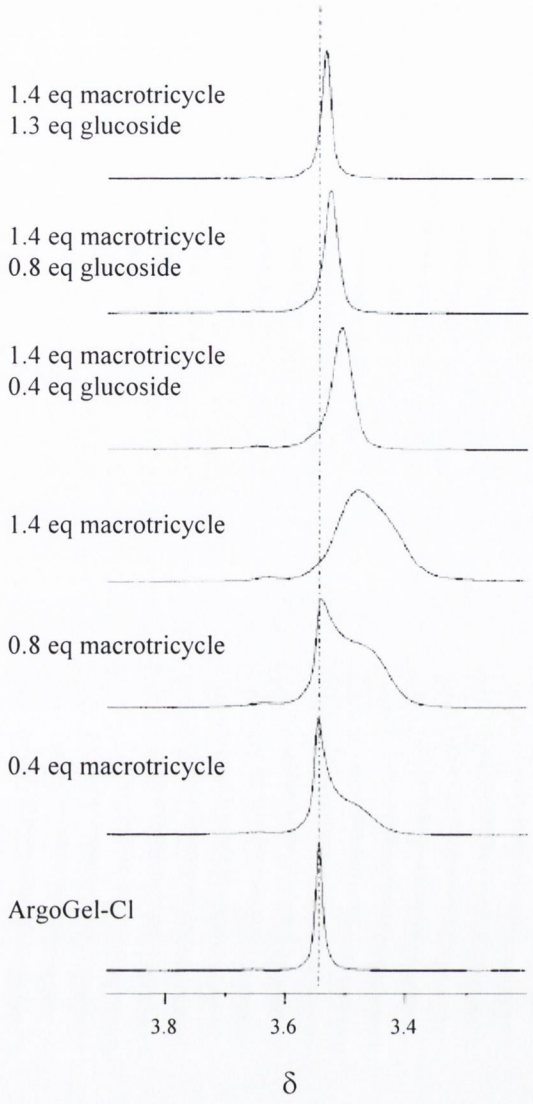


Figure 6.5 Control experiment showing competition between PEG chain and glucoside for macrotricycle **2.57**

The addition of glucoside caused the macrotricycle to de-thread, showing a preference for interaction with the sugar, causing the PEG resonance to sharpen as it became unbound.

A second control experiment involved titrating the macrotricycle against porphyrin-dimer-terminated ArgoGel® beads, which resulted in no change in the PEG resonance (**Figure 6.6**). This demonstrated that the bis-porphyrin unit acted as a stopper preventing the macrotricycle from threading. Also, it shows that splitting of the PEG resonance was not due to any side-on interaction with the macrotricycle.

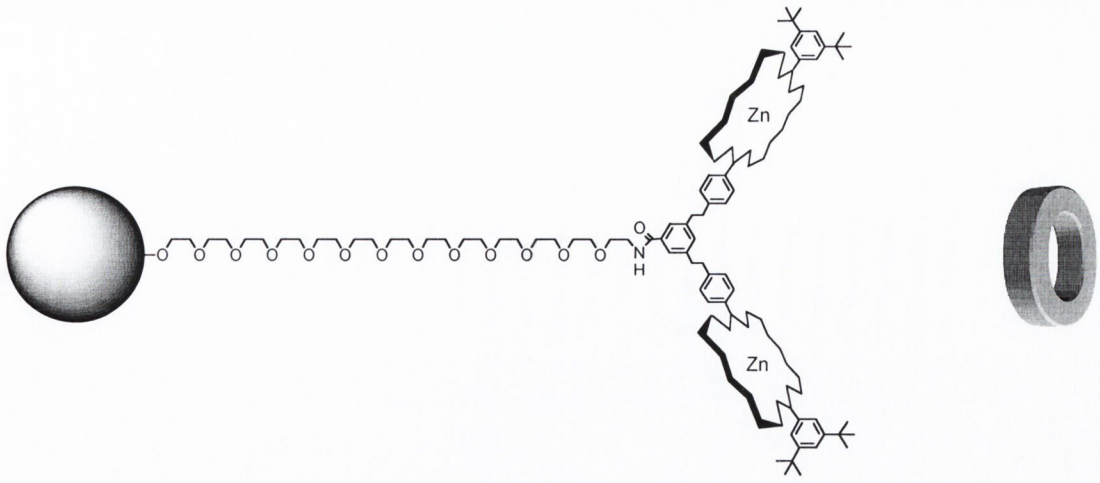


Figure 6.6 Second control experiment to prove that threading occurs and to disprove side-on interaction

The final control experiment, performed in Bristol University, involved a solution phase ^{1}H NMR titration between the macrotricycle and hexaethylene glycol in $CD_3OH/CDCl_3$ (8:92). The internal proton of the aromatic spacer was followed throughout the titration and the data was analysed assuming 1:1 stoichiometry, giving a binding constant of *ca.* 9000 M^{-1} and a limiting $\Delta\delta$ of 0.06 ppm (**Figure 6.7**). However, the binding curve obtained was not a good fit to the theoretical curve probably because of a non-1:1 binding stoichiometry, as might be expected.

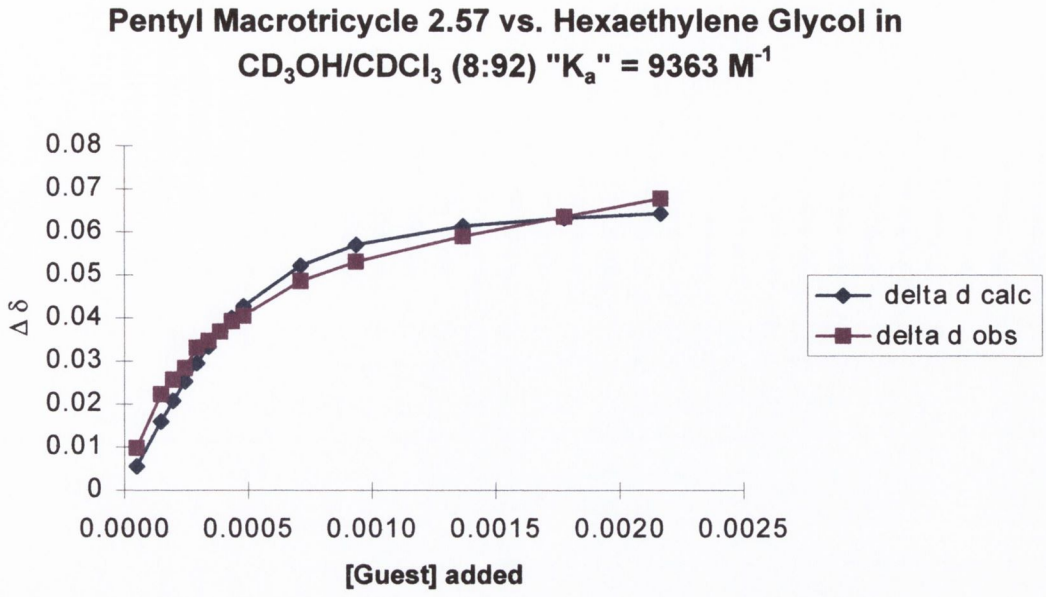


Figure 6.7 Binding study of pentyl macrotricycle **2.57** vs. hexaethylene glycol

These results prompted the synthesis of what is believed to be the first resin-bound rotaxane as illustrated in **Figure 6.8**.

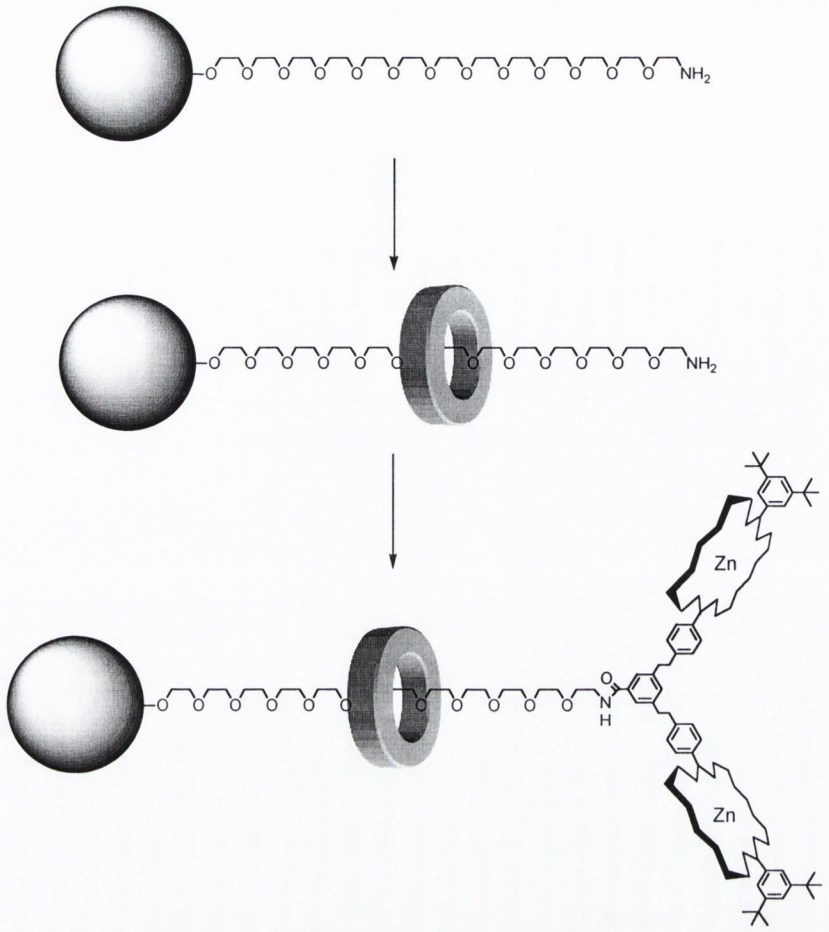


Figure 6.8 Rotaxane formation using a bis-porphyrin stopper

After allowing the macrotricycle to thread, the terminal PEG amine was bonded to a bis-porphyrin and the beads were washed thoroughly to remove any unbound macrotricycle. NOESY MAS NMR revealed interaction between the macrotricycle aromatic protons and the PEG chain and between the macrotricycle pentyl chains and PEG chains. This proved the formation of a solid supported rotaxane. See also Appendix **S**.

6.2 Binding Studies of Carboxylic Acid Macrotricycle 5.7 vs. Me-β-D-glucoside in Water

^{1}H NMR binding studies were carried out to investigate interaction between the tetra-carboxylate of macrotricycle **5.7** and methyl-β-D-glucoside in D_2O. Cesium hydroxide was used to deprotonate the carboxylic acid groups to promote solubility of the receptor. The sample appeared slightly cloudy and no changes were observed in the chemical shifts of the receptor's proton resonances upon addition of the sugar.

A fluorescence study was also carried out in H_2O at a host concentration of 10^{-5} M, in the hope that higher dilution may improve solubility. Fluorescent intensities fluctuated upon additions of methyl-β-D-glucoside possibly due to aggregation of the receptor and therefore no binding was observed.

6.3 Binding Study of Polybenzyl Macrotricycle (5.17) vs. *n*-Octyl-β-D-glucoside in $CD_3OH/CDCl_3$ (8:92)

A binding study of *n*-octyl-β-D-glucoside with polybenzyl macrotricycle **5.17** was undertaken in $CD_3OH/CDCl_3$ (8:92) for a direct comparison with the pentyl macrotricycle **2.57**. **2.57** had shown an affinity for *n*-octyl-β-D-glucoside in this solvent system with a K_a of 920 M^{-1}. **5.17** was expected to bind this sugar with a similar K_a as no changes had been made to the core part of the receptor, which would be involved in binding. This was indeed found to be the case with **5.17** binding *n*-octyl-β-D-glucoside with a K_a of 720 M^{-1}. Good agreement was observed with the theoretical binding curve, showing 1:1 complexation as shown in **Figure 6.9**.

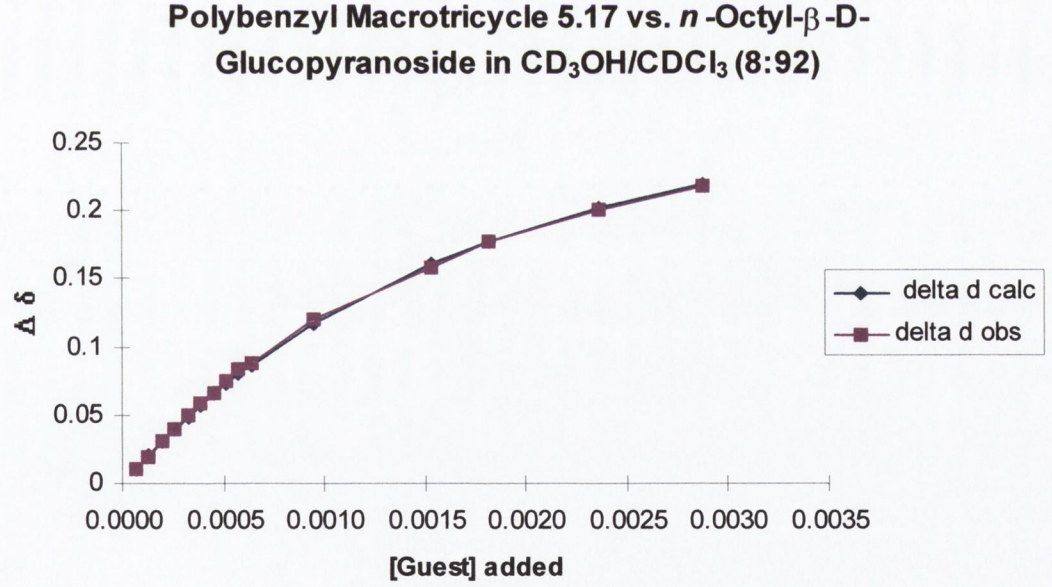


Figure 6.9 Binding study of polybenzyl macrotricycle **5.17** vs. *n*-octyl-β-D-glucoside

A similar shift was observed for the internal aromatic spacer proton of the two receptors, **2.57** and **5.17**, upon complexation. **2.57** exhibited a downfield shift of

0.32 ppm while the **5.17** had a shift of 0.21 ppm in the same direction, indicative of interaction with the glucoside. Splitting of the amide protons into two broad peaks as well as an upfield shift was observed. This is also consistent with the pentyl macrotricycle and the upfield shift observation may be explained by displacement of methanol by the carbohydrate resulting in a structure where not all NH groups are maximally bonded. An alternative explanation may be a change in the NH positions relative to the biaryl rings due to conformational change. On first analysis it may be assumed that fast exchange would result in only one amide resonance. However, the splitting of the NH resonance into two distinct broadened peaks is due to the eight benzyl amide protons becoming inequivalent upon complex formation. The sugar, lacking any symmetry, results in eight possible orientations but the symmetry of the macrotricycle allows for only four such orientations resulting in two sets of four resonances, seen as two broad signals.

Complexation also caused the biaryl benzylic methylene protons to split into two broad bands, as had been seen with the pentyl macrotricycle **2.57**. Again, this may be attributed to the unsymmetrical nature of the sugar.

6.4 Binding Study of Polybenzyl Macrotricycle (5.17) vs. *n*-Octyl-β-D-glucoside in $CDCl_3$

As discussed in Chapter Two, ^1H NMR titrations in $CDCl_3$ involving the pentyl macrotricycle were not possible due to the unresolved nature of the spectra. As the polybenzyl analogue was more soluble in organic solvents, it was decided to attempt a binding study of *n*-octyl-β-D-glucoside in $CDCl_3$. The binding constant of the pentyl macrotricycle for *n*-octyl-β-D-glucoside in $CHCl_3$, as measured by fluorescence, was 3×10^5 M^{-1}. A similar binding constant would be expected for the polybenzyl system which, being of the order of 10^5, would be beyond the scope of NMR measurement, but it was hoped that much structural information could be gained from the resultant chemical shifts of the complex.

Unlike the pentyl macrotricycle, the polybenzyl macrotricycle proved to be resolved in $CDCl_3$ as shown in **Figure 6.10**.

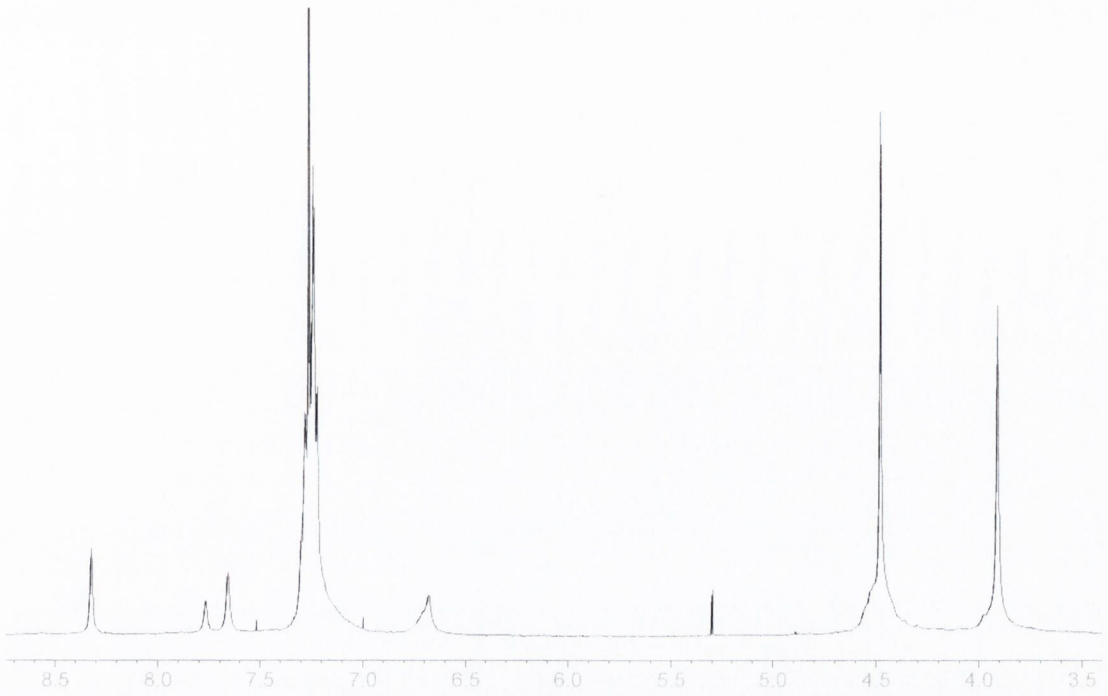


Figure 6.10 ^{1}H NMR of polybenzyl macrotricycle **5.17** in $CDCl_3$

A ^{1}H NMR binding study was undertaken in $CDCl_3$ with host concentration 0.35 mM. After 0.1 equivalents of *n*-octyl-β-D-glucoside were added, the internal aromatic spacer peak at δ 7.78 shifted downfield by *ca.* 0.025 ppm and broadened significantly. The benzylic amide proton peak also shifted downfield by *ca.* 0.042 ppm and became broad upon the first addition. After 0.2 equivalents were added, all of the aromatics broadened a lot and the internal spacer proton resonance, as well as the benzyl amide resonance, broadened to become almost baseline and therefore impossible to follow for binding constant calculations. It is clear that strong interaction was occurring between the macrotricycle and the sugar but quantifying this interaction was not possible by ^{1}H NMR.

6.5 Extraction of Sugars from Aqueous into Organic Phase

With the advent of the more organic-soluble polybenzyl macrotricycle **5.17**, the opportunity for liquid/liquid extractions of various sugars from aqueous into organic phase presented itself. Very few systems have been shown to extract sugars, particularly the fully hydroxylated pyranosides such as glucose, from aqueous into organic solvent through non-covalent interactions (Chapter Two). At the time of writing, pentyl macrotricycle **2.57** had shown the strongest binding of sugars in organic solvent throughout the literature, but due to solubility problems encountered,

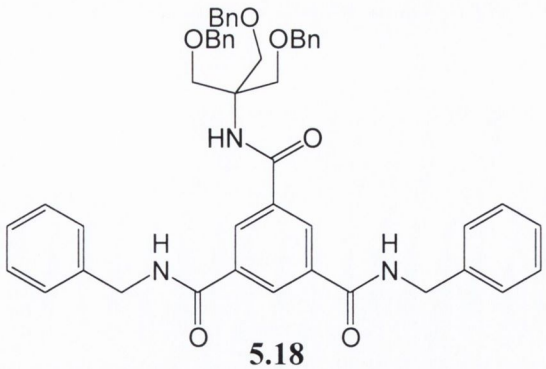
it remained untested in liquid/liquid extraction studies. **5.17** was therefore tested against a range of sugars in liquid/liquid extractions and the results obtained are presented in **Table 6.1**.

	1.0 M	**0.7 M**	**0.5 M**	**0.4 M**	**0.3 M**	**0.2 M**	**0.1 M**
Me-β-D-glucoside	1.0		1.0		0.75*	0.6*	0.6*
Me-α-D-glucoside	1.1*		0.6	0.6*			0.4*
D-xylose	1.1						
L-fucose	1.0						
D-mannose	0.2						
D-ribose	0.7						
D-lyxose	0.77						
D-allose							0.13
D-glucose	1.0	1.0	0.5	0.5			0.2

Table 6.1 Liquid/liquid extraction values (mol eq) obtained for various sugars by polybenzyl macrotricycle **5.17** (*carried out by G. LeCollinet)

The extractions listed in **Table 6.1** were all carried out using the "typical procedure" described in Chapter Seven, with the exception of the 0.1 M D-glucose extraction. In this case, the extraction was scaled up so that 8 mg of **5.17** in 10 mL of chloroform was shaken with 10 mL of a 0.1 M D-glucose solution. Scaling up the quantities allowed for the anomeric peaks of the extracted D-glucose to be visible and integratable on the ^{1}H NMR spectrum as this was not the case when working on the usual smaller scale. The ^{1}H NMR extraction of L-fucose is shown in Appendix **R** as an example of a ^{1}H NMR extraction spectrum.

Control experiments were carried out for each of the above sugars (except for D-allose due to material shortage) at 1.0 M in the absence of **5.17**. In each instance, no sugar was seen to be extracted by either TLC or ^{1}H NMR.



5.18

A control extraction using **5.18** was undertaken so as to eliminate the possibility of carbohydrate binding being attributed to any interaction by the tris-benzyl ether groups of the spacer unit. Using the same concentration solution as the test solutions containing **5.17** and following the same procedure, extraction of a 0.5 M D-glucose solution was attempted. ^{1}H NMR analysis in d_6-DMSO of the concentrated organic layer showed no sign of D-glucose having been extracted.

Not much success had been achieved in the past regarding extraction of carbohydrates by synthetic receptors from aqueous into organic solutions. As discussed in Chapter Two, Aoyama's compound **2.1** showed limited ability in extracting various sugars from aqueous solutions into CCl_4. Better success was attained with the extraction of pentoses rather than hexoses. The substituent present on C-5 was shown to be very important to the extractabilities of the various sugars: the sugars having a lipophilic CH_3 C-5 substituent, such as fucose and rhamnose, were extracted better than those having just hydrogen at C-5, which in turn were extracted with greater efficiency than those having a CH_2OH C-5 substituent.

In contrast to this, **5.17** was capable of extracting both pentoses and hexoses from aqueous solutions with similar affinities. Moreover, the extraction values obtained with receptor **2.1** used sugar concentrations of 2.4 M, whereas **5.17** has been shown to be effective at extracting a range of sugars at 1.0 M, as shown in **Table 6.1**. The most hydrophilic and therefore most challenging carbohydrate for extraction studies, as well as the most biologically important is glucose. Receptor **2.1** showed low levels of affinity for glucose, extracting ≤ 0.03 molar equivalents from a 2.4 M aqueous solution, whereas **5.17** is capable of extracting 0.2 molar equivalents of glucose from a 0.1 M solution.

Also noteworthy is the selectivity obtained by **5.17** for D-glucose over D-mannose. Extraction of a 1.0 M aqueous solution of D-mannose resulted in 0.2

equivalents being extracted whereas for the same D-glucose concentration, one equivalent was extracted, showing a 5-fold selectivity. The only difference between D-glucose and D-mannose is that D-mannose has an axial orientation of the C-2 hydroxyl while D-glucose has an equatorial arrangement.

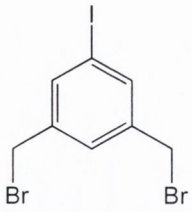
CHAPTER SEVEN

Experimental

General

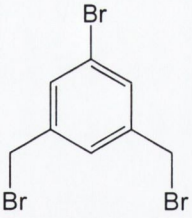
^{1}H, ^{13}C and ^{19}F NMR spectra were recorded using four different instruments: a Bruker DPX 400 MHz spectrometer, a Jeol Delta/GSX 270 MHz spectrometer, a Jeol Lambda 300 MHz spectrometer and a Jeol Eclipse 400 MHz spectrometer. Chemical shifts (δ) are quoted in parts per million downfield from tetramethylsilane. Infrared spectra were recorded on either a Perkin-Elmer Paragon 1000 spectrometer or a Perkin Elmer Spectrum One spectrometer. Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. TLC was carried out using Merck DC Alufolien Kieselgel 60 F_{254} 0.2 mm plates, Merck DC-Fertigplatten SIL G-25 UV_{254} 0.25mm plates and Merck DC Alufolien RP-18 F_{254s} 0.2mm plates. UV chromophoric compounds were visualised under UV light, high molecular weight compounds were visualised by charring or by staining with phosphomolybdic acid (5 % w/v in ethanol) and amines were visualised by staining with ninhydrin (5 % w/v in *n*-butanol). Flash chromatography was carried out using Merck Kieselgel 60 400-230 mesh, Fluorochem Silica Gel 40-63μ 60Å, or Fluka Silica gel 100 C_{18}-Reverse Phase, by the technique of Still.[78] Hydrophobic filter paper used in extraction experiments was Whatman 1PS phase separator paper. LCMS analysis was performed using a Waters 2700 Sample Manager, a Waters 600 Controller, a Waters 996 Photodiode Array Detector and a Micromass Platform LC spectrometer. Solvents were, when appropriate, distilled prior to use and were, if necessary, dried by standard techniques as described by Perrin[79], or collected from an Anhydrous Engineering[80] dry solvent dispenser, based on the procedure by Grubbs *et al.*[81] Mass spectra were obtained using a Micromass Autospec-Trisector Double Focusing mass spectrometer. FAB mass spectra used nitrobenzyl alcohol as matrix and cesium ions of 20 keV; EI mass spectra were obtained at a potential of 70 eV and CI mass spectra using methane as reagent gas. ES mass spectra were obtained using a Micromass Quattro mass spectrometer. Microanalyses were carried out in the Chemistry Department, University College Dublin or in the School of Chemistry, University of Bristol on a Perkin-Elmer 240C analyser.

1,3-Bis-bromomethyl-5-iodo-benzene 4.19



The method was based on that of van Koten for the synthesis of tri-bromide **4.2**.[60] To a suspension of *N*-bromosuccinimide (70.27 g, 0.395 mol, 2.1 eq) and 5-iodo-*m*-xylene (43.66 g, 0.188 mol) in dry methyl formate (200 mL) was added 1,1′-azobis(cyclohexanecarbonitrile) (0.125 g, 0.5 mmol). The resulting mixture was irradiated with a 100W light bulb which induced reflux, under argon, for 16 h. The reaction mixture was evaporated *in vacuo* to yield a red solid and the residue extracted with boiling hexane (7 × 60 mL). The combined extracts were cooled (–23 °C) which resulted in aryl iodide **4.19** as off-white crystals (40.17 g, 0.103 mol, 55 %). R_f = 0.28 (DCM/hexane 3:7); m.p. 107-109 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.67 (d, ^3J(H,H) = 1.6 Hz, 2 H; ArH C-2), 7.37 (t, ^3J = 1.6 Hz, 1 H; ArH C-4), 4.38 (s, 4 H; CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 140.3 (C-3), 137.8 (C-2), 129.0 (C-4), 94.3 (C-1), 31.3 (CH_2); IR (Nujol): ν_{max} (cm^{-1}): 691 (CBr); MS (EI) (70eV): *m/z*: 390 $[M]^+$; elemental analysis calcd (%) for $C_8H_7Br_2I$: C 24.65, H 1.81; found C 24.63, H 1.68.

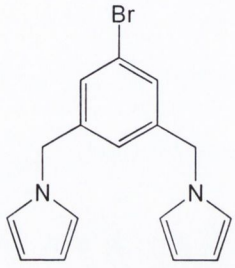
1-Bromo-3,5-bis-bromomethyl-benzene 4.2



The method was based on that of van Koten.[60] To a suspension of *N*-bromosuccinimide (51.0 g, 0.286 mol, 2.1 eq) and 5-bromo-*m*-xylene (25.2 g, 0.1362 mol) in dry methyl formate (150 mL) was added 1,1′-azobis(cyclohexanecarbonitrile) (0.6 g, 2.5 mmol). The resulting mixture was irradiated with a 100W light bulb which induced reflux, under an argon atmosphere, for 16 h. The reaction mixture was evaporated *in vacuo* to yield a white solid and the residue extracted with boiling hexane (6 × 60 mL). The combined extracts were cooled (–23 °C) which resulted in aryl bromide **4.2** as white crystals (27.97 g, 81.5

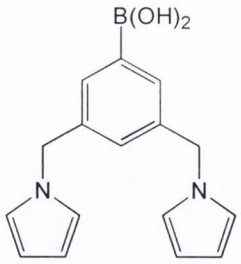
mmol, 60 %). R_f = 0.3 (hexane); m.p. 91-93 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47 (d, ^4J(H,H) = 1.6 Hz, 2 H; ArH C-2), 7.34 (t, ^4J(H,H) = 1.6 Hz, 1 H; ArH C-4), 4.41 (s, 4 H; CH_2) (lit.[60] 200 MHz, C_6D_6, 6.94, 6.62, 3.57); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 140.3 (C-3), 131.9 (C-2), 128.2 (C-4), 122.7 (C-1), 31.4 (CH_2) (lit.[60] 50 MHz, C_6D_6, 140.5, 131.6, 128.1, 122.6, 31.3); IR (Nujol): ν_{max} (cm^{-1}): 692 (CBr); MS (CI) (70eV): *m/z*: 343 $[M]^+$.

1-Bromo-3,5-bis(pyrrolo-*N*-methyl)benzene 4.16



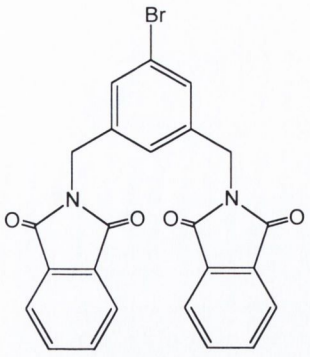
To crushed pellets of potassium hydroxide (23.5 g, 0.42 mol, 2.0 eq) under argon, was added dry DMSO (110 mL) and the mixture was stirred for 5 min. Pyrrole (14.5 mL, 0.21 mol, 4.0 eq) was added and a colour change from white to green was observed. The mixture was stirred for one hour at RT. 1-bromo-3,5-bis(bromomethyl)benzene **4.2** (18.0 g, 52.5 mmol) was added and the flask cooled briefly, using an ice bath. The mixture was stirred for a further 2 h at which point it appeared brown. The reaction was quenched with water (200 mL) and extracted into ethyl acetate (3 × 300 mL). The combined organic phases were washed with water (2 × 500 mL), brine (500 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to yield a brown solid. Recrystallisation from MeOH yielded bis-pyrrole **4.16** as light brown coloured crystals (12.88 g, 40.89 mmol, 78 %). R_f = 0.35 (DCM/hexane 3:7); m.p. 124-125 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.14 (s, 2 H; ArH α to CBr), 6.78 (s, 1 H; ArH γ to CBr), 6.65 (t, ^3J(H,H) = 2.0 Hz, 4 H; pyrrole H), 6.21 (t, ^3J(H,H) = 2.0 Hz, 4 H; pyrrole H), 4.98 (s, 4 H; CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 140.9 (Ar*C*CH_2), 129.2 (ArCH), 124.1 (ArCH), 123.3 (CBr), 121.1 (ArCH), 109.0 (ArCH), 52.5 (CH_2); IR (film from $CHCl_3$): ν_{max} (cm^{-1}): 1569 (pyrrole), 1494 (pyrrole); MS (CI) (70eV): *m/z*: 315 $[M]^+$; elemental analysis calcd (%) for $C_{16}H_{15}BrN_2$: C 60.97, H 4.80, N 8.89; found C 60.92, H 4.71, N 8.72.

3,5-Bis(pyrrolo-*N*-methyl)phenyl-1-boronic acid 4.17



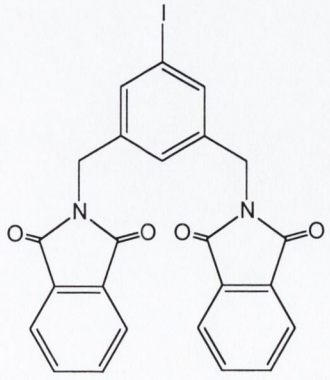
n-BuLi (17.45 mL, 27.9 mmol, 1.1 eq) was added dropwise to a solution of the bis-pyrrole **4.16** (8.0 g, 25.4 mmol) in dry THF (127 mL) at –90 °C under argon. The solution went from colourless to light yellow in appearance. After 20 min trimethyl borate (6.35 mL, 55.9 mmol, 2.2 eq) was added in one single injection and the reaction mixture was allowed to warm to RT and stirred for a further 2.5 h. The solvent was removed under reduced pressure and the reaction mixture quenched by the addition of sat. aq. ammonium chloride solution (250 mL). The mixture was extracted with ethyl acetate (3 × 200 mL) and the combined organic phases were washed with brine (300 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to an off-white solid. The crude product was analysed by ^{1}H NMR and was deemed to be usable without further purification. A portion of the crude product was recrystallised, for characterisation purposes, from ethyl acetate yielding boronic acid **4.17** as a white microcrystalline product (2.1 g, 7.5 mmol). The uncrystallised material was isolated (3.68 g, 13.1 mmol). Total yield 81 %. R_f = 0.3 (MeOH/DCM 1:99); m.p. 193-194 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 7.99 (s, 2 H; $B(OH)_2$), 7.57 (s, 2 H; ArH α to $CB(OH)_2$), 7.12 (s, 1 H; ArH γ to $CB(OH)_2$), 6.76 (t, ^3J(H,H) = 2.0 Hz, 4 H; pyrrole CH), 6.00 (t, ^3J(H,H) = 2.0 Hz, 4 H; pyrrole CH), 5.03 (s, 4 H; CH_2); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 142 (ArCB), 137.82 (Ar*C*CH_2), 132.43 (ArCH), 128.33 (ArCH), 120.68 (pyrrole CH), 107.83 (pyrrole CH), 52.32 (CH_2).

1-Bromo-3,5-bis(phthalimido-*N*-methyl)benzene 4.3



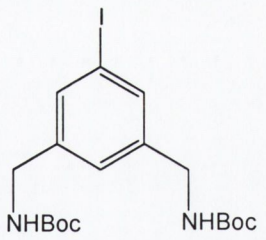
Potassium phthalimide (7.93 g, 42.8 mmol, 2.1 eq) was added to a stirred solution of the bis-benzyl bromide **4.2** in dry DMF (50 mL) at RT under argon. After stirring for *ca.* 5 min a white solid precipitated out of solution. The solid was dissolved in $CHCl_3$ (500 mL) and washed with water (3 × 200 mL) to remove DMF. The organic phase was washed with brine (200mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to yield **4.3** as a white solid (6.0 g, 12.6 mmol, 62 %), which was used without further purification. R_f = 0.61 (DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.86 (dd, ^3J(H,H) = 5.6, 3.0 Hz, 4 H; ArH phthalimide), 7.73 (dd, ^3J(H,H) = 5.6, 3.0 Hz, 4 H; ArH phthalimide), 7.44 (s, 2 H; ArH α to CBr), 7.44 (s, 1 H; ArH γ to CBr), 4.78 (s, 4 H; CH_2) (lit.[63] 270 MHz, $CDCl_3$ 7.86, 7.73, 7.44, 7.44, 4.79); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.8 (CO), 138.8 (ArC), 134.1 (ArCH), 132.0 (ArCH phthalimide), 130.8 (ArC), 127.5 (ArCH), 123.5 (ArCH phthalimide), 40.7 (CH_2) (lit.[63] 67.8 MHz, $CDCl_3$ 167.8, 138.7, 134.1, 132.0, 130.8, 127.5, 123.5, 40.8); IR (solid state): ν_{max} (cm^{-1}): 1768 (NCO), 1703 (NCO) (lit.[63] 1770, 1710); MS (EI) (70eV): *m/z*: 474 $[M]^+$ (lit.[63] 474).

1-Iodo-3,5-bis(phthalimido-*N*-methyl)benzene 4.21



Potassium phthalimide (19.60 g, 0.106 mol, 2.1 eq) was added to a stirred solution of the bis-benzyl bromide **4.2** in dry DMF (175 mL) at RT under nitrogen. After stirring for *ca.* 5 min a white solid precipitated out of solution. The solid was dissolved in $CHCl_3$ (1000 mL) and washed with water (3 × 750 mL) to remove DMF. The organic phase was washed with brine (750mL), dried ($NaSO_4$), filtered and evaporated *in vacuo* to yield a white solid which was recrystallised from toluene (*ca.* 1.75 L) to yield **4.21** as colourless needle like crystals (17.68 g, 33.9 mmol, 67 %). R_f = 0.6 (MeOH/DCM 1:99); m.p. 246-247 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85 (dd, ^3J(H,H) = 5.5, 3.0 Hz, 4 H; ArH phthalimide), 7.72 (dd, ^3J(H,H) = 5.5, 3.0 Hz, 4 H; ArH phthalimide), 7.64 (d, ^4J(H,H) = 1.7 Hz, 2 H; ArH α to CI), 7.46 (t, ^4J(H,H) = 1.7 Hz, 1 H; ArH γ to CI), 4.76 (s, 4 H; CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.8 (CO), 138.7 (ArC), 136.8 (ArCH α to CI), 134.1 (ArCH phthalimide), 132.0 (ArC), 128.3 (ArCH γ to CI), 123.5 (ArCH phthalimide), 94.7 (CI), 40.7 (CH_2); IR (solid state): ν_{max} (cm^{-1}): 1767 (NCO), 1698 (NCO); HRMS (FAB): *m/z* mass calcd for $C_{24}H_{15}N_2O_4INa$ 544.9974, found 544.9967; elemental analysis calcd (%) for $C_{24}H_{15}IN_2O_4$: C 55.19, H 2.89, N 5.36; found C 54.92, H 2.81, N 5.18.

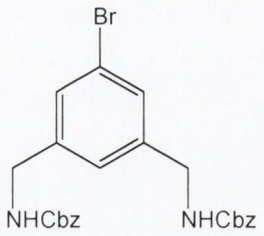
3,5-Bis[(*N-tert*-butyloxycarbonyl)aminomethyl]-1-iodobenzene 4.12



Hydrazine hydrate (98 %) (6.04 mL, 0.125 mol, 5.0 eq) was added to a suspension of the bis-phthalimide **4.21** in MeOH/DCM (4:1, 250 mL). The resultant mixture was refluxed for 3 h at 75 °C. The mixture was allowed to cool to RT and water (150 mL) was added. The volatiles were removed under vacuum to give a white slurry. Concentrated HCl (70 mL) and water (30 mL) were added and the resultant mixture stirred for 1 h, under argon. The insoluble white solid was removed by filtration and washed with 1 M aq. HCl solution (2 × 100 mL). The aqueous layer was brought to pH 14 using 5 M aq. NaOH. The resultant cloudy aqueous layer was extracted with DCM (7 × 150 mL). The combined organic extracts were washed with brine (400 mL), dried ($MgSO_4$), filtered and evaporated *in*

vacuo to yield bis-amine **4.22** as a yellow solid (5.85 g, 22.3 mmol, 90 %). Di-*tert*-butyldicarbonate (10.72 g, 49.1 mmol, 2.2 eq) was added to a solution of the bis-amine intermediate (5.85 g, 22.3 mmol) and *N*,*N*-DIPEA (15.53 mL, 9.2 mmol, 4.0 eq) in dry THF (110 mL) at 0 °C under argon. The reaction mixture was allowed to warm to RT and stirred for 12 h. The reaction mixture was evaporated *in vacuo* to yield an off-white solid and ethyl acetate (300 mL) was added. The organic phase was washed with 1 M aq. HCl solution (3 × 100 mL), sat. $NaHCO_3$ solution (2 × 100 mL) and brine (100 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated *in vacuo* to yield an off white solid which was recrystallised from ethyl acetate and hexane (*ca.* 1:2) to yield **4.12** as white crystals (9.09 g, 19.7 mmol, 88 %). R_f = 0.25 (EtOAc/hexane 1:3); m.p. 144-144 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32 (s, 2 H; ArH), 7.10 (s, 1 H; ArH), 4.90 (br s, 2 H; NH), 4.27 (d, ^3J(H,H) = 6.0 Hz, 4 H; CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.8 (CO), 141.6 (ArC -*C*CH_2), 129.2 (ArCH α to CI), 124.8 (ArCH α to CCH_2), 122.8 (CI), 79.8 (*C*CH_3), 44.0 (CH_2), 28.4 (CH_3); IR (film from DCM): ν_{max} (cm^{-1}): 1693 (CONH), 1517 (CONH); MS (EI) (70eV): *m/z*: 405 $[M - C_4H_9]^+$, 57 $[C_4H_9]^+$; elemental analysis calcd (%) for $C_{18}H_{27}IN_2O_4$: C 46.76, H 5.89, N 6.06; found C 46.66, H 5.81, N 5.95.

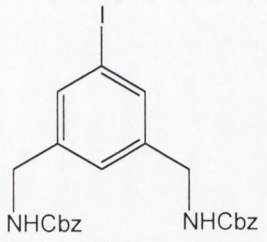
3,5-Bis[(*N*-benzyloxycarbonyl)aminomethyl)]-1-bromobenzene 4.4



Hydrazine hydrate (100 % w/w) (3.06 mL, 63.0 mmol, 5.0 eq) was added to a suspension of the bis-phthalimide **4.3** in MeOH/DCM (4:1, 125 mL). The resultant mixture was refluxed for 2.5 h at 75 °C. The solid had not dissolved at this point, so a further 90 mL of DCM was added which dissolved the material. The mixture was refluxed for a further 2 h at 75 °C. The mixture was then allowed cool to RT and water (100 mL) was added. The volatiles were removed under reduced pressure. Concentrated HCl (30 mL) was added and the mixture was stirred for *ca.* 12 h. The resultant insoluble white solid was removed by filtration and washed with 1 M aq. HCl solution (2 × 100 mL). The aqueous layer was brought to pH 14 using 5 M aq. NaOH. The resultant cloudy aqueous layer was extracted with DCM (8 × 150 mL).

The combined organic extracts were washed with brine (500 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to yield a white solid (1.20 g, 5.6 mmol, 44 %). Benzyl chloroformate (1.75 mL, 12.28 mmol, 2.2 eq) was added to a solution of the bis-amine intermediate (1.20 g, 5.58 mmol) and *N*,*N*-DIPEA (2.14 mL, 12.3 mmol, 2.2 eq) in dry THF (40 mL) at 0 °C under argon. The reaction mixture was stirred at RT for 3 h. The mixture was then evaporated *in vacuo* to an oil to which ethyl acetate (50 mL) was added. The organic phase was washed with 1 M aq. HCl solution (3 × 30 mL), saturated aq. $NaHCO_3$ solution (3 × 30 mL) and brine (30 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated *in vacuo* to a white solid which which was recrystallised from ethyl acetate/hexane to yield **4.4** as white crystals (1.27 g, 2.6 mmol, 47 %). R_f = 0.44 (MeOH/DCM 1:99); m.p. 129-131 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34 (m, 12 H; ArCH), 7.08 (s, 1 H; ArH γ to CBr), 5.12 (s, 6 H; C*H*$_2$C$_6$H$_5$, 2 × NH), 4.30 (s, 4 H; NCH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.4 (CO), 141.1 (ArC *C*Br), 136.3 (ArC C_6H_5), 129.4 (ArC *C*CH_2NH), 128.6 (ArCH Cbz), 128.23 (ArCH Cbz), 128.16 (ArCH Cbz), 125.0 (ArCH γ to CBr), 123.0 (CBr), 67.1 (OCH_2), 44.4 (NCH_2); IR (solid state): ν_{max} (cm^{-1}): 1684 (CONH), 1532 (CONH); MS (FAB): *m/z*: 483 $[MH]^+$; elemental analysis calcd (%) for $C_{24}H_{23}BrN_2O_4$: C 59.64, H 4.80, N 5.80; found C 59.25, H 5.05, N 5.67.

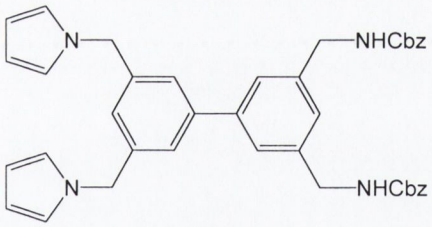
3,5-Bis[(*N*-benzyloxycarbonyl)aminomethyl)]-1-iodobenzene 4.11



Hydrazine hydrate (98 %) (6.57 mL, 0.136 mol, 5.0 eq) was added to a suspension of the bis-phthalimide **4.21** in MeOH/DCM (4:1, 270 mL). The resultant mixture was refluxed for 2.5 h at 70 °C. The mixture was allowed to cool to RT and the volatiles were removed under reduced pressure. Water (150 mL) and concentrated HCl (85 mL) were added and the mixture was stirred for 1 h. The resultant insoluble white solid was removed by filtration and washed with 1 M aq. HCl solution (2 × 100 mL). The aqueous layer was brought to pH 14 using 5 M aq. NaOH. The resultant cloudy aqueous layer was extracted with DCM (7 × 150 mL). The combined organic extracts were washed with brine (500 mL), dried ($MgSO_4$),

filtered and evaporated *in vacuo* to a yellow solid (5.97 g, 22.8 mmol, 84 %). Benzyl chloroformate (7.16 mL, 50.2 mmol, 2.2 eq) was added to a solution of the bis-amine intermediate (5.97 g, 22.79 mmol) and *N*,*N*-DIPEA (8.74 mL, 50.2 mmol, 2.2 eq) in dry THF (150 mL) at 0 °C under argon. The reaction mixture was stirred at RT for 12 h. The mixture was evaporated *in vacuo* to a white solid which was recrystallised from ethyl acetate/hexane to yield **4.11** as white crystals (10.44 g, 19.7 mmol, 86 %). R_f = 0.46 (EtOAc/hexane 1:1); m.p. 141-142 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.89 (t, ^3J(H,H) = 6.1 Hz, 2 H; NH), 7.50 (s, 2 H; ArH α to CI), 7.36 (m, 10 H; ArH Cbz), 7.16 (s, 1 H; ArH α to CCH_2), 5.05 (s, 4 H; Cbz CH_2), 4.15 (d, ^3J(H,H) = 6.1 Hz, 4 H; NCH_2); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 156.2 (CO), 142.3 (Ar *C*CH_2NH), 137.0 (Ar *C*CH_2O), 133.9 (ArCH α to CI), 128.3 (ArCH Cbz α to CCH_2), 127.7 (ArCH Cbz γ to CCH_2), 127.6 (ArCH Cbz β to CCH_2), 125.2 (ArCH γ to CI), 94.6 (CI), 65.3 (OCH_2), 43.0 (NCH_2); IR (film from DCM): ν_{max} (cm^{-1}): 1685 (CONH), 1535 (CONH); MS (EI) (70eV): *m/z*: 439 [M – C_7H_7]$^+$, 91 [C_7H_7]$^+$; elemental analysis calcd (%) for $C_{24}H_{23}IN_2O_4$: C 54.35, H 4.37, N 5.28; found C 54.29, H 4.31, N 5.22.

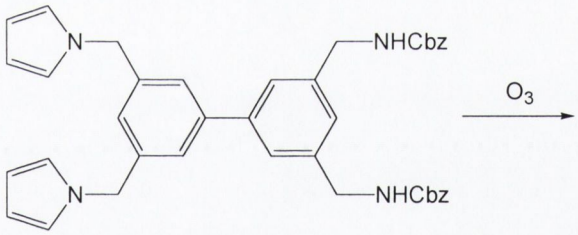
3,5-Bis(pyrrolo-*N*-methyl)-3′,5′-bis[(*N*-benzyloxycarbonyl)aminomethyl] biphenyl 4.18



The reaction flask was charged with 2 M aq. Na_2CO_3 solution (0.54 mL), toluene (4.3 mL), bis-Cbz derivative **4.11** (290 mg, 0.6 mmol) and tetrakistriphenylphosphine palladium catalyst (31.7 mg, 0.03 mmol, 5 mol %). To this vigorously stirred mixture, a solution of the arylboronic acid **4.17** (200 mg, 0.7 mmol) in MeOH (2 mL) was added dropwise at RT under argon. The reaction mixture was refluxed for 18 h at 100 °C. The reaction mixture was allowed cool to RT and was diluted with water (20 mL). The mixture was extracted with DCM (3 × 35 mL) and the combined organic phases were washed with brine (50 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to a brown oil. Purification by flash column chromatography, using basified flash silica (5 % triethylamine wash), eluting

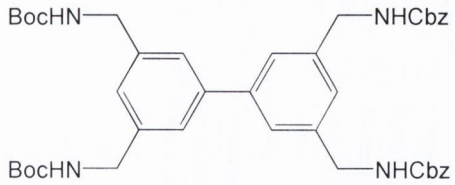
with MeOH/DCM (5:95) yielded biaryl **4.18** as a white solid (316 mg, 0.5 mmol, 90 %). R_f = 0.55 (MeOH/DCM 1:99); m.p. 133-134 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.85 (t, ^3J(H,H) = 6 Hz, 2 H; NH), 7.36 (m, 14 H; ArH), 7.21 (s, 1 H; biaryl H α to CCH_2NH), 7.09 (s, 1 H; biaryl H α to CCH_2pyrrole), 6.83 (t, ^3J(H,H) = 2.1 Hz, 4 H; pyrrole H α to N), 6.04 (t, ^3J(H,H) = 2.1 Hz, 4 H; pyrrole H β to N), 5.14 (s, 4 H; CH_2pyrrole), 5.08 (s, 4 H; OCH_2), 4.27 (d, ^3J(H,H) = 6 Hz, 4 H; CH_2NH); ^{13}C (100 MHz, [D$_6$]DMSO): δ = 156.3 (CO), 140.7 (ArC), 140.4 (ArC), 139.9 (ArC), 139.8 (ArC), 137.0 (ArC C_6H_5), 128.2 (ArCH *meta*C_6H_5), 127.7 (ArCH *para*C_6H_5), 127.6 (ArCH *ortho*C_6H_5), 125.4 (biaryl CH α to CCH_2pyrrole), 125.0 (biaryl CH α to CCH_2NH), 124.8 (biaryl CH), 124.1 (biaryl CH), 120.7 (pyrrole C α to N), 108.0 (pyrrole C β to N), 65.3 (OCH_2), 52.0 (CH_2pyrrole), 43.8 (CH_2NH); IR (film from $CHCl_3$): ν_{max} (cm^{-1}): 1702 (CONH), 1520 (CONH); MS (EI) (70eV): *m/z*: 638 [M]$^+$; elemental analysis calcd (%) for $C_{40}H_{38}N_2O_4$: C 75.21, H 6.00, N 8.77; found C 75.38, H 5.95, N 8.72.

Attempted Ozonolysis of 3,5-bis(pyrrolo-*N*-methyl)-3′,5′-bis[(*N*-benzyloxycarbonyl) aminomethyl] biphenyl



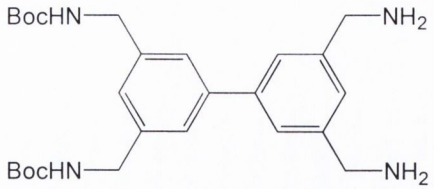
Ozone was bubbled through a solution of the bis-Cbz bis-pyrrole biaryl **4.18** (100 mg, 0.16 mmol) in MeOH/DCM (1:1) (12 mL) at –78 °C for 1 h. The solution appeared light blue at this point and was allowed to warm to RT. Reduction of the resulting ozonide was carried out using tetrabutylammonium borohydride (0.403 g, 1.6 mmol, 10 eq) added dropwise as a solution in MeOH (2 mL). The mixture was washed with saturated ammonium chloride solution (20 mL) and brine (20 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated *in vacuo* to an oil.

3,5-Bis[(*N*-benzyloxycarbonyl)aminomethyl]-3′,5′-bis[(*N*-*tert*-butyloxycarbonyl)aminomethyl]biphenyl 4.7[70]



The reaction flask was charged with bis-Boc derivative **4.12** (1.656 g, 3.58 mmol), bis(pinacolato)diboron (1.0 g, 3.9 mmol, 1.1 eq), potassium acetate (1.06 g, 10.8 mmol, 3.0 eq), $PdCl_2$(dppf) (0.078 g, 0.11 mmol, 3 mol %) and was flushed with argon. To this was added DMSO (21 mL) and the reaction mixture was stirred at 80 °C for 3 h. The reaction mixture was cooled to RT and to this was added the bis-Cbz derivative **4.11** (3.80 g, 7.2 mmol, 2.0 eq), $PdCl_2$(dppf) (0.078 g, 0.11 mmol, 3 mol %) and Na_2CO_3 (8.96 mL of a 2 M aq. solution, 17.9 mmol, 5.0 eq). The mixture was stirred at 80 °C for 24 h after which it appeared red/brown in colour. The mixture was allowed cool to RT and water (100 mL) and chloroform (150 mL) were added. The product was extracted into chloroform (3 × 150 mL) and the combined organic phases were washed with water (3 × 100 mL), brine (100 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to a red/brown oily solid which solidified upon standing. The crude product was purified by flash column chromatography (silica 400 g), eluting with ethyl acetate/toluene (1:3), to yield biaryl **4.7** as a white solid (2.46 g, 3.3 mmol, 93 %). R_f = 0.25 (EtOAc/toluene 1:3); ^{1}H NMR (400 MHz, [D_6]DMSO): 7.89 (t, ^3J(H,H) = 6.0 Hz, 2 H; NHCbz), 7.44 (t, ^3J(H,H) = 6.0 Hz, 2 H; NHBoc), 7.35 (m, 14H; ArH), 7.17 (s, 1 H; biaryl H α to CCH_2NHCbz), 7.13 (s, 1 H; biaryl H α to CCH_2NHBoc), 5.05 (s, 4 H; OCH_2), 4.25 (d, ^3J(H,H) = 6.0 Hz, 4 H; CH_2NHCbz), 4.17 (d, ^3J(H,H) = 6.0 Hz, 4 H; CH_2NHBoc), 1.40 (s, 18 H; CH_3); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 156.3 ($COOCH_2$), 155.7 ($COOC(CH_3)_3$), 140.8 (ArC biaryl), 140.4 (ArC biaryl), 140.3 (ArC biaryl), 139.9 (ArC biaryl), 137.0 (ArC C_6H_5), 128.2 (ArCH *meta*C_6H_5), 127.7 (ArCH *para*C_6H_5), 127.6 (ArCH *ortho*C_6H_5), 124.9 (ArCH), 124.5 (ArCH), 124.1 (2 × ArCH α to CCH_2NHCbz), 123.8 (2 × ArCH α to CCH_2NHBoc), 77.7 (CCH_3), 65.3 (OCH_2), 43.9 (CH_2NHCbz), 43.4 (CH_2NHBoc), 28.2 (CH_3); IR (film from DCM): ν_{max} (cm^{-1}): 1691 (CONH), 1529 (CONH); HRMS (FAB): *m/z* mass calcd for $C_{42}H_{50}N_4O_8Na$ 761.3526, found 761.3552; elemental analysis calcd (%) for $C_{42}H_{50}N_4O_8.1H_2O$: C 66.65, H 6.93, N 7.40; found C 66.88, H 6.77, N 7.39.

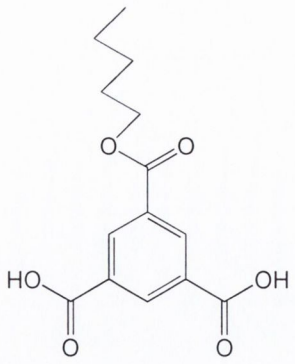
3,5-Bis(aminomethyl)-3′,5′-bis[(*N-tert*-butyloxycarbonyl)aminomethyl]biphenyl 4.8



Palladium on carbon (10 % wt. (*Aldrich* catalogue number 33,010-8), 900 mg before activation) was heated at 200 °C for 2 h under vacuum (0.1 atm). The flask was allowed cool to RT under nitrogen and to this was added EtOH (50 mL) and bis-Cbz bis-Boc biaryl **4.7** (1.0 g, 1.36 mmol). The flask was evacuated and filled with hydrogen (1 atm) and the mixture was stirred for 2 days under hydrogen at RT. The mixture was filtered through pre-washed (EtOH) celite and then evaporated *in vacuo* to a white solid. Purification was carried out by passing the material through a silica plug (60 g), eluting initially with MeOH/DCM (5:95) followed by ammonia saturated MeOH/DCM (12:88). The desired fractions were evaporated *in vacuo* to yield **4.8** as a white solid (517 mg, 1.1 mmol, 81 %). R_f = 0.20 (MeOH.NH_3/DCM 10:90); ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.64 (br s, 4 H; NH_2), 7.84 (s, 2 H; 2 × ArH α to CCH_2NH_2), 7.61 (s, 1 H; 1 × ArH α to CCH_2NH_2), 7.49 (s, 2 H; 2 × ArH α to CCH_2NHBoc), 7.42 (t, ^3J(H,H) = 5.7 Hz, 2 H; NHBoc), 7.18 (s, 1 H; 1 × ArH α to CCH_2NHBoc), 4.19 (d, ^3J(H,H) = 5.7 Hz, 4 H; CH_2NHBoc), 4.06 (s, 4 H; CH_2NH_2), 1.40 (s, 18 H; CH_3); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 155.8 (CO), 140.9 (ArC), 140.6 (ArC), 138.9 (ArC), 134.9 (ArC), 128.7 (1 × ArCH α to CCH_2NH_2), 127.1 (2 × ArCH α to CCH_2NH_2), 124.8 (1 × ArCH α to CCH_2NHBoc), 124.1 (2 × ArCH α to CCH_2NHBoc), 77.8 ($C(CH_3)_3$), 43.5 (CH_2NHBoc), 42.1 (CH_2NH_2), 28.2 (CH_3); IR (solid state): ν_{max} (cm^{-1}): 1687 (CONH), 1514 (CONH); HRMS (FAB): *m/z* mass calcd for $C_{26}H_{38}N_4O_4Na$ 493.2791, found 493.2813; elemental analysis calcd (%) for $C_{26}H_{38}N_4O_4$.1MeOH: C 64.52, H 8.42, N 11.15; found C 64.34, H 8.16, N 11.37.[†]

[†] Although ^{1}H NMR analysis was not carried out on this particular batch that was microanalysed, methanol was evident in previous ^{1}H NMR analyses.

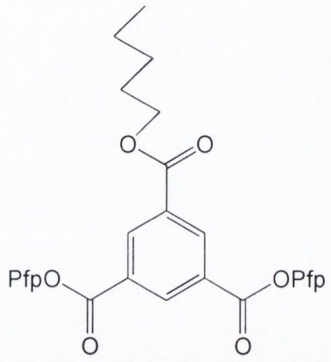
Benzene-1,3,5-tricarboxylic acid 1-pentyl ester 4.14



Oxalyl chloride (26.18 mL of a 2 M solution in DCM, 52.4 mmol, 1.1 eq) was added dropwise to a solution of 1,3,5-benzene tricarboxylic acid (10.0 g, 47.6 mmol) and dry DMF (0.5 mL) in dry THF (60 mL) at –10 °C under argon. HCl gas was liberated from the mixture and was vented from the reaction flask. The reaction mixture was allowed to warm to RT and stirred for a further hour. A solution of *n*-pentanol (5.18 mL, 47.6 mmol, 1.0 eq) and *N*,*N*-DIPEA (24.87 mL, 0.143 mol, 3.0 eq) in dry THF (20 mL) was then added dropwise. The resultant mixture was then allowed to stir at RT for 12 h. The mixture was evaporated *in vacuo* to a white solid to which ethyl acetate (300 mL) was added. The organic phase was washed with 0.5 M aq. HCl solution (3 × 100 mL) and was then extracted with sat. aq. $NaHCO_3$ solution (3 × 100 mL). The combined basic aqueous extracts were washed with ethyl acetate (100 mL). 15 mL portions of 4 M aq. HCl solution were then added until the pH reached 6.5. Extraction with ethyl acetate (2 × 200 mL) was carried out to remove any unwanted di- and tri-ester which had formed. The pH was then lowered to 5 by the addition of 15 mL portions of 4 M aq. HCl solution, at which point the aqueous layer was extracted with ethyl acetate (2 × 200 mL). The pH was then lowered to 4, again by the addition of 4 M aq. HCl solution, at which point the aqueous layer was extracted with ethyl acetate (2 × 200 mL). The ethyl acetate solutions, which had extracted at pH 5 and pH 4, were combined and evaporated *in vacuo* to yield **4.14** as a white solid (4.83 g, 17.3 mmol, 36 %). R_f = 0.48 (AcOH/MeOH/DCM 1:10:89); m.p. 227-229 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 13.65 (br s, 2 H; COOH), 8.65 (t, ^4J(H,H) = 1.7 Hz, 1 H; ArH γ to COOC), 8.63 (d, ^4J(H,H) = 1.7 Hz, 2 H; ArH α to COOC), 4.32 (t, ^3J(H,H) = 6.7 Hz, 2 H; OCH_2), 1.74 (quintet, ^3J(H,H) = 7.0 Hz, 4 H; CH_2CH_2O), 1.37 (m, 4 H; $CH_2CH_2CH_3$), 0.89 (t, ^3J(H,H) = 7.2 Hz, 3 H; CH_3); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 165.7 (CO),

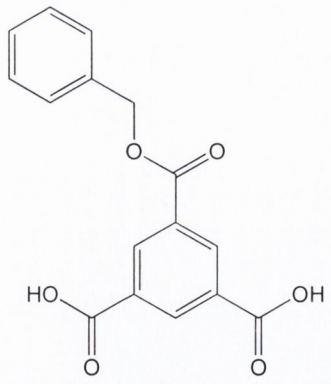
164.4 (CO), 133.8 (ArCH), 133.2 (ArCH), 132.1 (ArC), 130.9 (ArC), 65.3 (CH_2O), 27.7 (CH_2), 27.5 (CH_2), 21.7 (CH_2), 13.7 (CH_3); IR (film from $CHCl_3$): ν_{max} (cm^{-1}): 2900 (COOH), 1728 (COOC); elemental analysis calcd (%) for $C_{14}H_{16}O_6$: C 60.00, H 5.75; found C 60.08, H 5.76.

Benzene-1,3,5-tricarboxylic acid 1,3-dipentafluorophenyl ester 5-pentyl ester 4.9



DCC (4.60 g, 22.3 mmol, 2.5 eq) was added to an ice-cold stirred solution of the di-acid **4.14** (2.5 g, 8.9 mmol) and pentafluorophenol (4.10 g, 22.3 mmol, 2.5 eq) in dry THF (75 mL) under argon. The reaction mixture was allowed to stir at 0 °C for 1 h and was then allowed to warm to RT and stir for a further 1 h. A cloudy white solution resulted. The solid precipitate of DCU was removed by filtration and the filtrate evaporated *in vacuo* to an oil. The crude product was purified by flash column chromatography (silica 400 g), eluting with DCM to yield the active ester **4.9** as a white solid (5.22 g, 8.5 mmol, 96 %). R_f = 0.82 (DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.16 (t, ^4J(H,H) = 1.7 Hz, 1 H; ArH γ to COOC), 9.13 (d, ^4J(H,H) = 1.7 Hz, 2 H; ArH α to COOC), 4.44 (t, ^3J(H,H) = 7.0 Hz, 2 H; OCH_2), 1.85 (quintet, ^3J(H,H) = 7.0 Hz, 2 H; OCH_2CH_2), 1.43 (m, 4 H; $CH_2CH_2CH_3$), 0.94 (t, ^3J(H,H) = 7.0, 3 H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.1 (CO pentyl ester), 160.8 (CO Pfp ester), 136.9 (ArCH α to $COOCH_2$), 136.2 (ArCH γ to COOC), 132.9 (ArC), 128.7 (ArC), 66.5 (OCH_2), 28.3 (CH_2), 28.1 (CH_2), 22.4 (CH_2), 14.0 (CH_3); IR (film from DCM): ν_{max} (cm^{-1}): 1772 (CO), 1732 (CO); MS (CI) (70eV): *m/z*: 613 $[MH]^+$; elemental analysis calcd (%) for $C_{26}H_{14}F_{10}O_6$: C 51.00, H 2.30; found C 50.94, H 2.41.

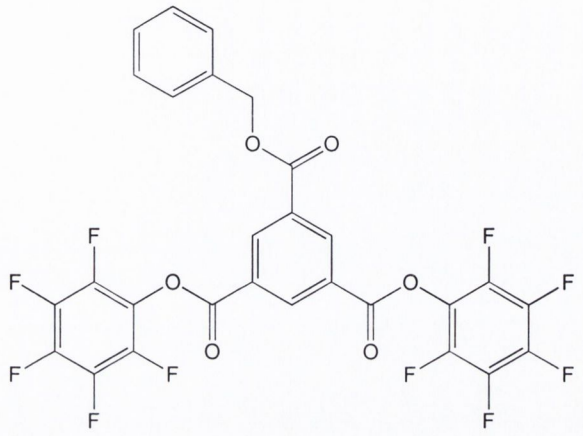
Benzene-1,3,5-tricarboxylic acid 1-benzyl ester 5.2



Oxalyl chloride (26.18 mL of a 2 M solution in DCM, 52.4 mmol, 1.1 eq) was added dropwise to a solution of 1,3,5-benzene tricarboxylic acid (10.0 g, 47.6 mmol) and dry DMF (0.5 mL) in dry THF (60 mL) at –10 °C under argon. HCl gas was liberated from the mixture and was vented from the reaction flask. The reaction mixture was allowed to warm to RT and stirred for a further hour. A solution of benzyl alcohol (4.92 mL, 47.6 mmol, 1.0 eq) and *N*,*N*-DIPEA (24.87 mL, 0.143 mol, 3.0 eq) in dry THF (20 mL) was then added dropwise. The resultant mixture was then allowed to stir at RT for 12 h. The mixture was evaporated *in vacuo* to a white solid to which ethyl acetate (300 mL) was added. The organic phase was washed with 0.5 M aq. HCl solution (3 × 100 mL) and was then extracted with sat. aq. $NaHCO_3$ solution (3 × 100 mL). The combined basic aqueous extracts were washed with ethyl acetate (100 mL). 15 mL portions of 4 M aq. HCl solution were then added until the pH reached 6.5. Extraction with ethyl acetate (2 × 200 mL) was carried out to remove any unwanted di- and tri-ester which had formed. The pH was then lowered to 5 by the addition of 15 mL portions of 4 M aq. HCl solution, at which point the aqueous layer was extracted with ethyl acetate (2 × 200 mL). The pH was then lowered to 4, again by the addition of 4 M aq. HCl solution, at which point the aqueous layer was extracted with ethyl acetate (2 × 200 mL). The ethyl acetate solutions, which had extracted at pH 5 and pH 4, were combined and evaporated *in vacuo* to yield **5.2** as a white solid (6.2 g, 20.7 mmol, 43 %). $R_f = 0.14$ ($MeOH/CHCl_3$ 3:7); m.p. 256-260 °C; ^1H NMR (400 MHz, [D_6]DMSO): δ = 13.55 (br s, 2 H; COOH), 8.66 (m, 3 H; ArH α to CO), 7.44 (m, 5 H; ArH C_6H_5), 5.42 (s, 2 H; CH_2); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 165.6 (COOH), 164.1 (*C*$OOCH_2$), 135.6 (ArC C_6H_5), 133.9 (ArCH γ to COOC), 133.3 (ArCH α to COOC), 132.1 (ArC), 130.6 (ArC), 128.5 (ArCH *ortho*C_6H_5), 128.3 (ArCH *para*C_6H_5), 128.2

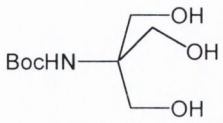
(ArCH *meta*C_6H_5), 66.8 (CH_2); IR (KBr disc): ν_{max} (cm^{-1}): 3036 (COOH), 1727 (COOC); MS (FAB): *m/z*: 323 $[MNa]^+$.

Benzene-1,3,5-tricarboxylic acid 1-benzyl ester 3,5-dipentafluorophenyl ester 5.3



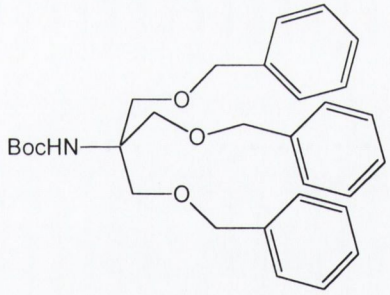
DCC (10.57 g, 51.3 mmol, 2.5 eq) was added to an ice-cold stirred solution of the di-acid **5.2** (6.15 g, 20.5 mmol) and pentafluorophenol (9.43 g, 51.3 mmol, 2.5 eq) in dry THF (172 mL) under argon. The reaction mixture was allowed to stir at 0 °C for 1 h and was then allowed to warm to RT and stir for a further 1 h. A cloudy white solution resulted. The solid precipitate of DCU was removed by filtration and the filtrate evaporated *in vacuo* to a white solid. The crude product was purified by recrystallisation from EtOAc/hexane, yielding the product as a white solid (9.82 g, 15.5 mmol, 76 %). R_f = 0.62 (DCM/hexane 1:3); m.p. 153-154 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.15 (s, 3 H; ArH α to COOC), 7.44 (m, 5 H; ArH C_6H_5), 5.48 (s, 2 H; CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.9 (CO benzyl ester), 160.7 (CO Pfp ester), 137.0 (2 × ArCH α to CO), 136.3 (1 × ArCH α to CO), 135.1 (ArC C_6H_5), 132.6 (ArC), 128.83 (ArC), 128.80 (ArCH C_6H_5), 128.79 (ArCH C_6H_5), 128.7 (ArCH C_6H_5), 68.0 (CH_2); IR (film from $CHCl_3$): ν_{max} (cm^{-1}): 1771 (CO), 1732 (CO); MS (EI) (70eV): *m/z*: 449 $[M - C_6F_5O]^+$, 184 $[C_6F_5O]^+$; elemental analysis calcd (%) for $C_{28}H_{10}F_{10}O_6$: C 53.18, H 1.59; found C 53.38, H 1.84.

***N*-(*tert*-Butyloxycarbonyl)tris(hydroxymethyl)aminomethane 5.9**[82], [72]



A solution of di-*tert*-butyl dicarbonate (11.72 g, 53.7 mmol, 1.3 eq) in *t*-BuOH (75 mL) was added to a suspension of 2-amino-2-(hydroxymethyl)-1,3-propanediol **5.8** (5.0 g, 41.3 mmol) in a mixture of *t*-BuOH/MeOH (1:1) (302 mL) and the mixture was stirred at RT for 18 h. The mixture was evaporated *in vacuo* yielding **5.9** as a white solid (8.68 g, 39.3 mmol, 95 %). R_f = 0.44 (AcOH/MeOH/DCM 1:10:89); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 5.72 (s, 1 H; NH), 4.46 (t, ^3J(H,H) = 5.8 Hz, 3 H; OH), 3.52 (d, ^3J(H,H) = 5.8 Hz, 6 H; CH_2), 1.37 (s, 9 H; CH_3); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 154.9 (CO), 77.7 ($C(CH_3)_3$), 60.2 ($C(CH_2)_3$), 60.1 (CH_2), 28.1 (CH_3); IR (nujol mull): ν_{max} (cm^{-1}): 3300 (OH), 1678 (CONH), 1549 (CONH); MS (FAB): *m/z*: 222 $[MH]^+$, 244 $[MNa]^+$; elemental analysis calcd (%) for $C_9H_{19}NO_5$: C 48.86, H 8.66, N 6.33; found C 48.84, H 8.62, N 6.20.

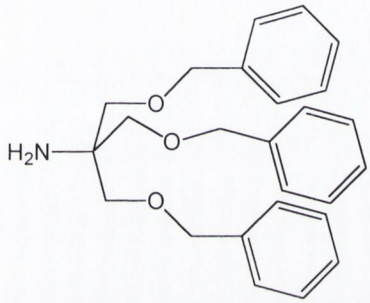
***N*-(*tert*-Butyloxycarbonyl)tris[(benzyloxy)methyl]aminomethane 5.10**[72]



A solution of the Boc protected trishydroxymethyl aminomethane **5.9** (5.0 g, 22.6 mmol) in dry DMF (56 mL) was stirred at RT under nitrogen and to this was added benzyl bromide (15.07 mL, 0.127 mol, 5.6 eq) followed by finely ground potassium hydroxide (7.10 g, 0.127 mol, 5.6 eq) over 10-15 minutes. A cloudy white suspension resulted which was stirred at RT for 2 h. The reaction mixture was evaporated *in vacuo* to a white solid with the temperature not exceeding 45 °C. The crude product was dissolved in DCM (250 mL) and was washed with water (5 × 250 mL), brine (250 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to a pale yellow oil. Purification by flash column chromatography (silica 400 g) eluting with petroleum spirit-EtOAc/petroleum spirit (5:95)-EtOAc/petroleum spirit (7.5:92.5)

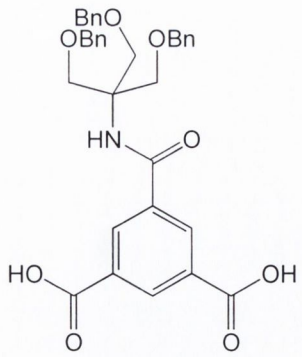
yielded **5.10** as a colourless oil (4.95 g, 10.1 mmol, 44 %). R_f = 0.43 (EtOAc/hexane 15:85); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30 (m, 15 H; ArH), 5.04 (br s, 1 H; NH), 4.49 (s, 6 H; $CH_2C_6H_5$), 3.79 (s, 6 H; $NHCCH_2$), 1.41 (s, 9 H; CH_3) (lit.[72] 100 MHz, $CDCl_3$ 7.27, 4.92, 4.49, 3.781.40); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.8 (CO), 138.3 (ArC C_6H_5), 128.3 (ArCH *para*C_6H_5), 127.54 (ArCH *ortho*C_6H_5), 127.51 (ArCH *meta*C_6H_5), 79.0 ($C(CH_3)_3$), 73.4 ($CH_2C_6H_5$), 69.4 ($C(CH_2)_3$), 58.6 ($NHCCH_2$), 28.4 (CH_3) (lit.[72] 25 MHz, $CDCl_3$ 154.9, 138.4, 128.3, 127.5 (× 2), 79.0, 73.4, 69.5, 58.7, 28.4); IR (oil): ν_{max} (cm^{-1}): 1717 (CONH), 1498 (CONH); MS (FAB): *m/z*: 492 $[M]^+$, 392 $[M+H - Boc]^+$.

Tris[(benzyloxy)methyl]aminomethane 5.11[72]



TFA (11.95 mL, 0.155 mol) was added dropwise to a solution of the Boc protected tris-benzyl ether **5.10** (3.46 g, 7.0 mmol) in DCM (28.2 mL) at 0 °C over 15 min. The reaction mixture was allowed to warm to RT and was stirred for 4 h. The mixture was evaporated *in vacuo* to a brown oil to which EtOAc (100 mL) was added. The organic phase was washed with aq. Na_2CO_3 solution (5 %) (100 mL), brine (100 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to a yellow oil (2.616 g, 6.7 mmol, 95 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.29 (m, 15 H; C_6H_5), 4.50 (s, 6 H; OCH_2Ar), 3.47 (s, 6 H; CCH_2O), 1.72 (br s, 2 H; NH) (lit.[72] 100 MHz, $CDCl_3$ 7.29, 4.50, 3.47); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.5 (ArC), 128.3 (ArCH), 127.5 (2 × ArCH), 73.3 (OCH_2Ar), 72.5 ($C(CH_2O)_3$), 56.1 ($C(CH_2O)_3$) (lit.[72] 25 MHz, $CDCl_3$ 138.5, 128.3, 127.5 (× 2), 73.4, 72.6, 56.2).

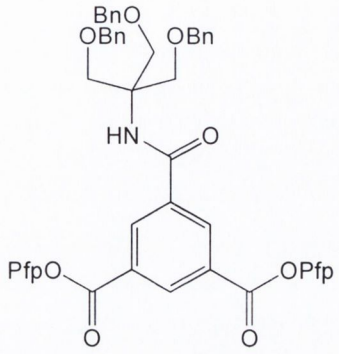
3,5-Bis-(hydroxycarbonyl)-[*N*-tris(benzyloxy)methyl]benzamide 5.13



N,*N*-DIPEA (1.47 mL, 8.4 mmol, 1.0 eq) was added to a solution of 1,3,5-benzenetricarbonyl trichloride (6.72 g, 25.3 mmol, 3.0 eq) in dry THF (253 mL, 0.1 M with respect to the tris-acid chloride) at RT under nitrogen. To this, a solution of the tris-benzyl protected amine **5.11** (3.3 g, 8.4 mmol) in dry THF (750 mL, 0.01 M with respect to the amine) was added dropwise over *ca.* 3 h, under nitrogen, with rapid stirring. The reaction was left to stir at RT for *ca.* 12 h, to which aq. KOH solution (100 mL, 2.3 M, 10 eq with respect to the tris-acid chloride) was then added. The mixture was washed with diethyl ether (2 × 500 mL). To the basic aqueous layer was added aq. HCl (2 M) slowly, to adjust the pH to 6. The aqueous layer was extracted at pH 6 with EtOAc (2 × 300 mL). Further portions of aq. HCl solution (2 M) were added to adjust the pH of the aqueous layer to 5, which was then extracted with EtOAc (2 × 300 mL). Further portions of aq. HCl solution were added to adjust the pH to 2 and extraction into EtOAc (2 × 300 mL) was carried out. The organic phases from each of the three extractions at the three different pH readings were worked up separately. The organic phases were washed with brine (300 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to yield **5.13** as a white solid: pH 6 (0.523g, 0.9 mmol, 11 %); pH 5 (3.292 g, 5.7 mmol, 67 %). Total yield 78 %. R_f = 0.52 (AcOH/MeOH/DCM 1:10:89); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 13.4 (br s, 2 H; COOH), 8.62 (br s, 1 H; ArH α to CCOOH), 8.56 (d, ^4J(H,H) = 1.5 Hz, 2 H; ArH α to CCONH), 8.16 (s, 1 H; NH), 7.31 (m, 15 H; ArH C_6H_5), 4.52 (s, 6 H; $CH_2C_6H_5$), 3.88 (s, 6 H; CH_2CNH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 166.3 (COOH), 165.7 (CONH), 138.4 (ArC C_6H_5), 136.4 (ArC), 132.0 (ArCH α to CCONH), 131.9 (ArCH α to CCOOH), 131.6 (ArC), 128.1 (ArCH C_6H_5), 127.3 (ArCH C_6H_5), 72.4 (CH_2 α to C_6H_5)), 67.6 (CH_2CNH), 60.7 (CNH); IR (film from

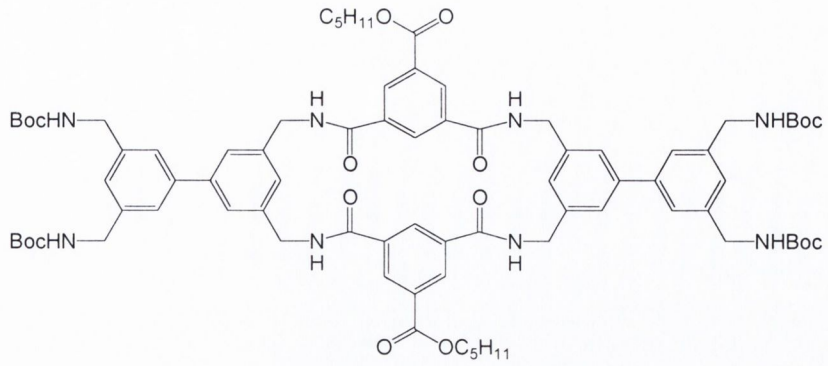
$CHCl_3$): ν_{max} (cm^{-1}): 2928 (COOH), 1705 (COOH), 1652 (CONH), 1527 (CONH); HRMS (FAB): *m/z* mass calcd for $C_{34}H_{33}NO_8Na$ 606.210387, found 606.211517.

3,5-Bis-(pentafluorophenyloxycarbonyl)-[*N*-tris(benzyloxy)methyl]benzamide 5.14



DCC (3.87 g, 18.7 mmol, 3.0 eq) was added to an ice-cold stirred solution of the di-acid **5.13** (3.64 g, 6.2 mmol) and pentafluorophenol (3.44 g, 18.7 mmol, 3.0 eq) in dry THF (104 mL) under nitrogen. The reaction mixture was stirred at 0 °C for 1 h and then allowed to warm to RT and stirred for a further 1 h. A cloudy white mixture was observed. The precipitate of DCU was removed by filtration and the filtrate evaporated *in vacuo* to an off-white solid. The crude product was dissolved in $CHCl_3$ and undissolved byproduct was removed by filtration. The filtrate was evaporated *in vacuo* to a colourless oil which was purified by flash chromatography (silica 400 g) eluting with EtOAc/petroleum spirit (5:95-7:93) to yield active ester **5.14** as a white solid (5.45 g, 6.0 mmol, 96 %). R_f = 0.28 (EtOAc/petroleum spirits 2:8); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.07 (t, ^4J(H,H) = 1.5 Hz, 1 H; ArH γ to CCON), 8.80 (d, ^4J(H,H) = 1.5 Hz, 2 H; ArH α to CCON), 7.27 (m, 15 H; ArH C_6H_5), 6.71 (s, 1 H; NH), 4.53 (s, 6 H; $CH_2C_6H_5$), 3.97 (s, 6 H; CH_2CN); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.2 (CONH), 160.9 (COO), 137.9 (ArC C_6H_5), 137.5 (ArC), 134.8 (ArCH γ to CCON), 134.4 (ArCH α to CCON), 128.6 (ArC), 128.4 (ArCH *meta*C_6H_5), 127.7 (ArCH *para*C_6H_5), 127.6 (ArCH *ortho*C_6H_5), 73.5 ($CH_2C_6H_5$), 68.8 (CH_2CNH), 60.7 (CNH); IR (film from DCM): ν_{max} (cm^{-1}): 1769 (COOC), 1677 (CONH), 1522 (CONH); HRMS (FAB): *m/z* mass calcd for $C_{46}H_{31}F_{10}NO_8Na$ 938.178770, found 938.180359.

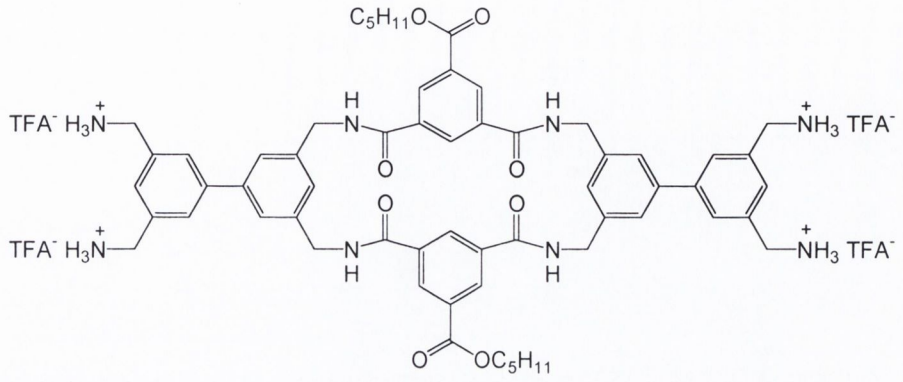
Tetra-Boc Macrocycle 4.10



A solution of the bis-Pfp pentyl spacer **4.9** (0.326 g, 0.53 mmol, 1.0 eq) in dry THF (320 mL) was added dropwise over 6 h to a solution of the bis-amine **4.8** (0.250 g, 0.5 mmol) in dry THF (130 mL), dry DMF (40 mL) and *N*,*N*-DIPEA (0.46 mL, 2.7 mmol, 5.0 eq) under argon with rapid stirring at RT. After addition the reaction mixture was stirred for 12 h. The mixture was evaporated *in vacuo*, azeotroping the DMF using toluene (5 × *ca.* 50 mL). Ethyl acetate (120 mL) was added and the organic phase was washed with 0.5 M aq. HCl solution (3 × 100 mL), sat. aq. $NaHCO_3$ solution (3 × 100 mL) and brine (100 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated *in vacuo* to a brown oil. The crude product was purified by flash column chromatography (silica 90 g), eluting with MeOH/$CHCl_3$ (1:99), to yield **4.10** as a white solid (275 mg, 0.19 mmol, 72 %). R_f = 0.3 (MeOH/DCM 5:95); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.35 (t, ^3J(H,H) = 5.8 Hz, 4 H; NHCOAr), 8.59 (s, 2 H; ArH spacer γ to CCOOC), 8.55 (s, 4 H; ArH spacer α to CCOOC), 7.50 (s, 4 H; ArH biaryl), 7.42 (s, 4 H; ArH biaryl), 7.35 (m, 4 H; NHBoc), 7.23 (s, 2 H; ArH biaryl), 7.13 (s, 2 H; ArH biaryl), 4.54 (d, ^3J(H,H) = 4.5 Hz, 8 H; CH_2NHCOAr), 4.33 (t, ^3J(H,H) = 6.8 Hz, 4 H; OCH_2), 4.20 (d, ^3J(H,H) = 5.0 Hz, 8 H; CH_2NHBoc), 1.76 (m, 4 H; OCH_2CH_2), 1.40 (s, 36 H; CH_3), 1.36 (m, 8 H; $CH_2CH_2CH_3$), 0.90 (t, ^3J(H,H) = 7.3 Hz, 6 H; CH_3); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 164.7 (*C*$OOCH_2$), 164.3 (ArCONH), 155.7 (COBoc), 140.81 (ArC), 140.79 (ArC), 140.3 (ArC), 140.0 (ArC), 139.7 (ArC), 134.6 (ArC), 130.3 (ArCH γ to COOC spacer), 130.2 (ArCH α to COOC spacer), 124.8 (ArCH biaryl), 124.6 (ArCH biaryl), 124.5 (ArCH biaryl), 123.7 (ArCH biaryl), 77.8 (CCH_3), 65.1 (OCH_2), 43.4 (CH_2NHBoc), 42.8 (CH_2NHCOAr), 28.2 (CH_3Boc), 27.8 (CH_2), 27.5 (CH_2), 21.7 (CH_2), 13.7 (CH_3CH_2); IR (film from DCM): ν_{max} (cm^{-1}): 1685 (CONH), 1646 (CONH), 1524 (CONH); HRMS (FAB): *m/z* mass calcd for $C_{80}H_{100}N_8O_{16}Na$

1451.7155, found 1451.7152; elemental analysis calcd (%) for $C_{80}H_{100}N_8O_{16}.½DMF$: C 65.54, H 6.87, N 7.64; found C 65.96, H 7.22, N 7.08.‡

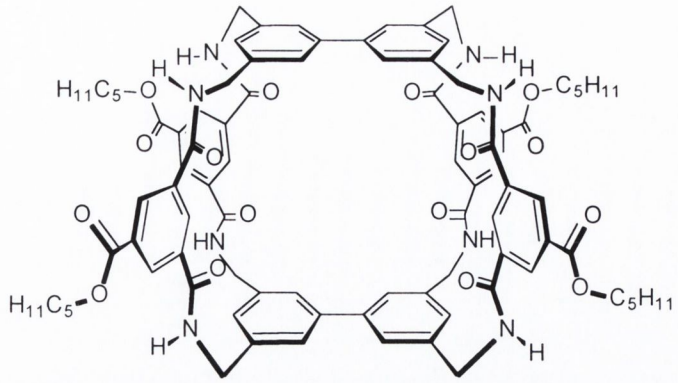
Tetra-TFA salt 4.15



TFA (14 mL) was added dropwise to a suspension of the tetra-Boc protected **4.10** (267 mg, 0.19 mmol) in DCM (14 mL) at 0 °C. All the solid material dissolved upon addition. The mixture was stirred under argon for 2 h after being allowed to warm to RT. The mixture was evaporated *in vacuo* to a pale yellow oil which was triturated with diethyl ether (50 mL). A white solid resulted which was collected by vacuum filtration and washed with diethyl ether (20 mL) yielding the product as a white powder (280 mg, 0.19 mmol, 100 %). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.42 (t, ^3J(H,H) = 5.5 Hz, 4 H; NHCOAr), 8.61 (s, 2 H; ArH spacer γ to CCOOC), 8.54 (s, 4 H; ArH spacer α to CCOOC), 8.34 (br s, 12 H; $NH_3^+TFA^-$), 7.88 (s, 4 H; biaryl H), 7.59 (s, 4 H; biaryl H), 7.48 (s, 2 H; biaryl H), 7.21 (s, 2 H; biaryl H), 4.57 (d, ^3J(H,H) = 4.5 Hz, 8 H; CH_2NHCOAr), 4.32 (t, ^3J(H,H) = 6.6 Hz, 4 H; OCH_2), 4.15 (br s, 8 H; $CH_2NH_3^+$), 1.76 (quintet, ^3J(H,H) = 6.8 Hz, 4 H; OCH_2CH_2), 1.37 (m, 8 H; $CH_2CH_2CH_3$), 0.90 (t, ^3J(H,H) = 7.3 Hz, 6 H; CH_3); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 164.7 ($COOCH_2$), 164.3 (ArCONH), 141.3 (ArC), 140.5 (ArC), 140.4 (ArC), 138.8 (ArC), 135.0 (ArC), 134.4 (ArC), 130. 4 (ArCH), 130.2 (ArCH), 130.1 (ArCH), 127.0 (ArCH), 124.3 (ArCH), 65.1 (OCH_2), 42.14 (CH_2), 42.06 (CH_2), 27.8 (CH_2), 27.4 (CH_2), 21.7 (CH_2), 13.7 (CH_3); IR (solid state): ν_{max} (cm^{-1}): 1645 (CONH), 1545 (CONH); HRMS (FAB): *m/z* mass calcd for $C_{60}H_{68}N_8O_8Na$ 1051.5058, found 1051.5045.

‡ DMF was still evident by ^{1}H NMR even after purification by flash column chromatography.

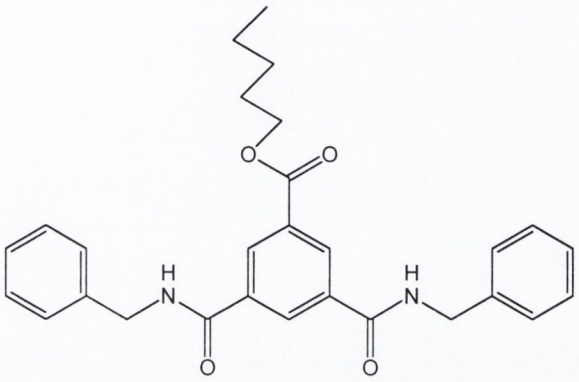
Pentyl Macrotricycle 2.57



A solution of the bis-Pfp pentyl spacer **4.9** (0.219 g, 0.36 mmol, 2.0 eq) in dry THF (730 mL) was added dropwise over *ca.* 8 h to a solution of the tetra-TFA salt **4.15** (0.265 g, 0.18 mmol) in dry THF (700 mL), dry DMF (30 mL) and *N*,*N*-DIPEA (0.93 mL, 5.4 mmol, 30 eq) under nitrogen with rapid stirring at 40 °C. After addition, the reaction mixture was left to stir for *ca.* 12 h at 40 °C. The mixture was evaporated *in vacuo*, azeotroping the DMF using toluene (5 × *ca.* 50 mL) to give the crude product as an off-white solid. $CHCl_3$ (100 mL) was added to the solid and the organic phase was washed with 0.5 M aq. HCl solution (2 × 100 mL), sat. aq. $NaHCO_3$ solution (100 mL) and brine (100 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated *in vacuo* to an off white solid (290 mg). Initial purification by flash column chromatography (silica 80 g) eluting with AcOH/MeOH/DCM (1:2.5:96.5-1:4:95) gave the product as a white solid (100 mg). Further purification was by preparative TLC using MeOH/DCM (8:92) as solvent, which yielded pentyl macrocycle **2.57** as a white solid (46 mg, 0.03 mmol, 17 %). R_f = 0.72 (MeOH/DCM 10:90); ^{1}H NMR (400 MHz, $CD_3OH/CDCl_3$ 8:92): δ = 8.59 (d, ^4J(H,H) = 1.0 Hz, 8 H; ArCH α to CCOOC), 8.45 (t, ^3J(H,H) = 4.9 Hz, 8 H; NH), 7.92 (s, 4 H; ArCH α to CCONH), 7.67 (s, 8 H; ArCH biaryl), 7.30 (s, 4 H; ArCH biaryl), 4.52 (A part of ABX, J(A,B) = 13.6 Hz, J(A,X) = 4.9 Hz, 8 H; CH_2NH), 4.44 (B part of ABX, J(A,B) = 13.6 Hz, J(A,X) = 4.9 Hz, 8 H; CH_2NH), 4.30 (t, ^3J(H,H) = 6.7 Hz, 8 H; OCH_2), 1.75 (quintet, ^3J(H,H) = 6.2Hz, 8 H; OCH_2CH_2), 1.39 (m, 16 H; $CH_2CH_2CH_3$), 0.92 (t, ^3J(H,H) = 7.0 Hz, 12 H; CH_3) (lit.[32] 400 MHz, [D$_6$]DMSO 9.09, 8.46, 8.15, 7.81, 7.26, 4.43, 4.33, 1.76, 1.38, 0.91); ^{13}C NMR (100 MHz, $CD_3OH/CDCl_3$ 8:92): δ = 165.9 (CONH), 164.4 ($COOCH_2$), 138.1 (ArC), 137.8 (ArC), 134.2 (ArC), 130.6 (ArC), 130.4 (ArCH α to CCOOC), 128.4 (4 × ArCH biaryl), 127.9 (ArCH α to CCONH), 124.7 (8 × ArCH biaryl), 65.0 (OCH_2), 43.8

(CH_2NH), 27.3 (CH_2), 27.1 (CH_2), 21.3 (CH_2CH_3), 12.9 (CH_3) (lit.[32] 100.6 MHz, [D_6]DMSO 164.80, 164.77, 139.8, 136.7, 135.3, 130.4, 130.3, 129.1, 124.9, 65.3, 43.7, 27.8, 27.6, 21.8, 13.8); IR (solid state): ν_{max} (cm^{-1}): 1717 (COOC), 1644 (CONH), 1532 (CONH).

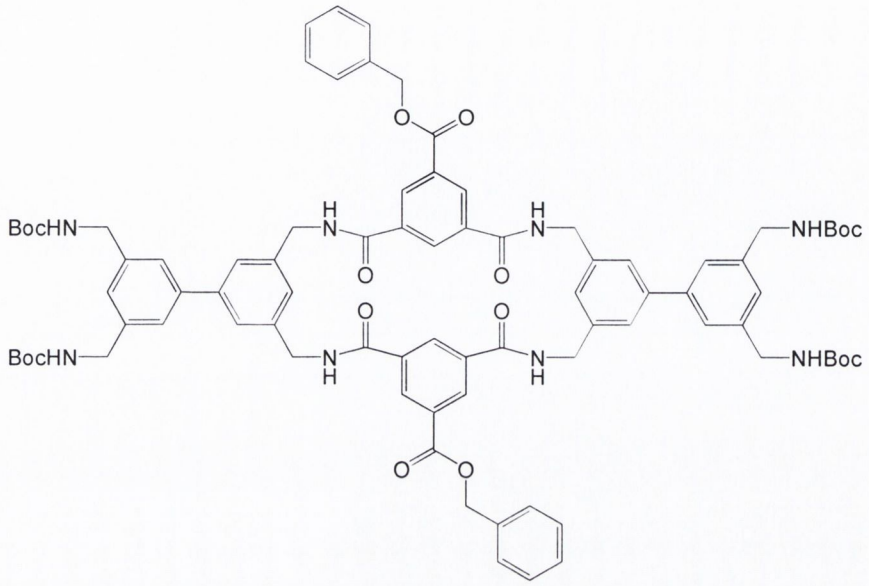
3,5-Bis-benzylcarbamoyl-benzoic acid pentyl ester 5.1



Benzylamine (98 µL, 0.9 mmol, 2.2 eq) was added to a solution of the bis-Pfp pentyl spacer **4.9** (250 mg, 0.41 mmol) in THF (6 mL) and *N*,*N*-DIPEA (0.12 mL, 0.8 mmol, 2.0 eq). The reaction mixture was stirred under argon at RT for 2 h. The reaction mixture was evaporated *in vacuo* to a colourless oil, which was redissolved in DCM (200 mL). The organic phase was washed with 1 M aq. HCl solution (3 × 75 mL), sat. aq. $NaHCO_3$ solution (75 mL) and brine (75 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated *in vacuo* to a sticky colourless solid. Purification by flash column chromatography (silica 90 g) eluting with MeOH/DCM (1:99-2:98) yielded **5.1** as a white solid (0.598 g, 1.3 mmol, 80 %). R_f = 0.7 (MeOH/DCM 5:95); m.p. 135-137 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.35 (t, ^3J(H,H) = 6.0 Hz, 2 H; NH), 8.65 (t, ^4J(H,H) = 1.5 Hz, 1 H; ArH γ to CCOOC), 8.58 (d, ^4J(H,H) = 1.5 Hz, 2 H; ArH α to CCOOC), 7.30 (m, 10 H; ArH C_6H_5), 4.51 (d, ^3J(H,H) = 6.0 Hz, 4 H; CH_2N), 4.34 (t, ^3J(H,H) = 6.8 Hz, 2 H; OCH_2), 1.75 (quintet, ^3J(H,H) = 6.8 Hz, 2 H; OCH_2CH_2), 1.37 (m, 4 H; $CH_2CH_2CH_3$), 0.90 (t, ^3J(H,H) = 7.0 Hz, 3 H; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.9 (CO), 164.8 (CO), 139.2 (ArC), 135.2 (ArC), 130.6 (ArCH), 130.3 (ArC), 130.2 (ArCH), 128.2 (ArCH), 127.3 (ArCH), 126.8 (ArCH), 65.2 (CH_2), 42.8 (CH_2), 27.8 (CH_2), 27.5 (CH_2), 21.7 (CH_2), 13.8 (CH_3); IR (film from DCM): ν_{max} (cm^{-1}): 1722 (COOC), 1645 (CONH), 1540 (CONH); MS (CI) (70eV): *m/z*: 459 $[MH]^+$; HRMS (CI): *m/z* mass calcd for

$C_{28}H_{31}N_2O_4$ 459.228383, found 459.228058; elemental analysis calcd (%) for $C_{48}H_{47}N_3O_6$: C 73.34, H 6.59, N 6.11; found C 73.62, H 7.34, N 6.30.*

Tetra-Boc Macrocycle 5.4

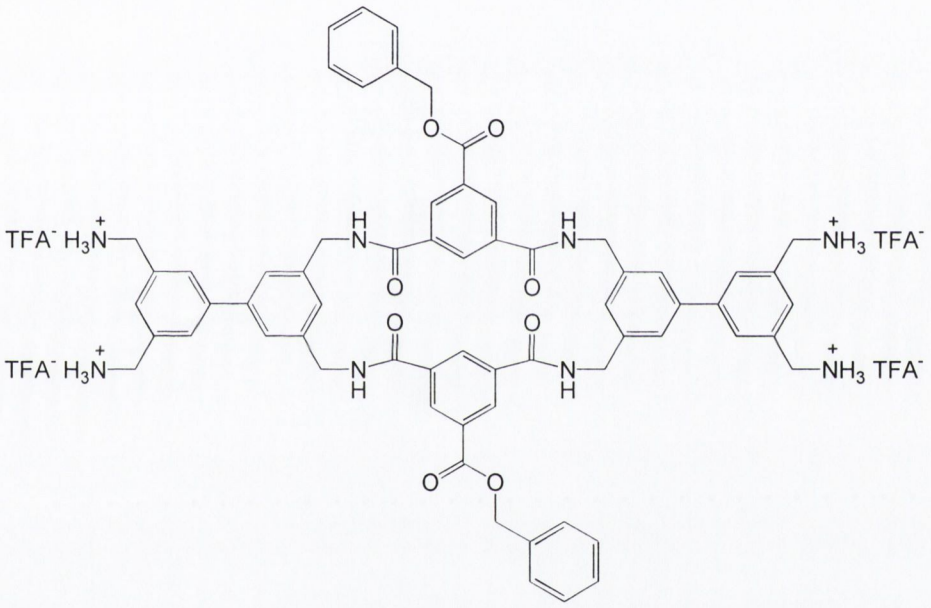


A solution of the bis-Pfp benzyl spacer **5.3** (0.672 g, 1.1 mmol, 1.0 eq) in dry THF (640 mL) was added dropwise over *ca.* 8 h to a solution of the bis-amine **4.8** (0.50 g, 1.1 mmol) in dry THF (260 mL), dry DMF (70 mL) and *N*,*N*-DIPEA (0.93 mL, 5.3 mmol, 5.0 eq) under argon with rapid stirring at RT. After addition the reaction mixture was stirred for 12 h at RT. The mixture was evaporated *in vacuo*, azeotroping the DMF using toluene (5 × *ca.* 50 mL), which resulted in an oily solid. EtOAc (150 mL) was added and the organic phase was washed with 0.5 M aq. HCl solution (3 × 100 mL), sat. aq. $NaHCO_3$ solution (3 × 100 mL) and brine (100 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated *in vacuo* to an off-white solid (830 mg). Purification by flash column chromatography and recrystallisation was unsuccessful, except for a small quantity of material which was precipitated from boiling EtOAc for characterisation purposes. The remaining material was used without further purification. R_f = 0.33 (MeOH/$CHCl_3$ 4:96); ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.37 (t, ^3J(H,H) = 5.5 Hz, 4 H; N*H*CH_2), 8.59 (s, 2 H; ArH spacer γ to CCOOC), 8.58 (s, 4 H; ArH spacer α to CCOOC), 7.40 (m, 22 H; 18 × ArH, 4 × NH), 7.22 (s, 2 H; ArH), 7.12 (s, 2 H; ArH), 5.41 (s, 4 H; OCH_2),

*
We have experimental evidence to suggest that recent microanalyses for hydrogen, performed in the University of Bristol, may be inaccurate. The fact that C and N for this sample are within expected limits is suggestive of adequate purity.

4.52 (d, ^3J(H,H) = 5.0 Hz, 8 H; CH_2), 4.18 (d, ^3J(H,H) = 5.5 Hz, 8 H; CH_2), 1.39 (s, 36 H; CH_3); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 164.6 (COOC), 164.3 (ArCONH), 155.7 (COBoc), 140.8 (ArC α to CH_2NHBoc), 140.2 (ArC), 139.9 (ArC), 139.6 (ArC), 135.8 (ArC C_6H_5), 134.6 (ArC), 130.5 (ArCH α to CCOOC), 130.3 (ArCH γ to CCOOC), 129.9 (ArC), 128.44 (ArCH), 128.40 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 124.8 (ArCH Biaryl), 124.6 (ArCH Biaryl), 123.6 (ArCH Biaryl), 77.7 ($C(CH_3)_3$), 66.5 ($CH_2C_6H_5$), 43.3 (CH_2NHBoc), 42.8 (CH_2NHCOAr), 28.1 (CH_3); IR (solid state): ν_{max} (cm^{-1}): 1722 (COOC), 1685 (CONH), 1516 (CONH); MS (FAB): *m/z*: 1491 $[MNa]^+$.

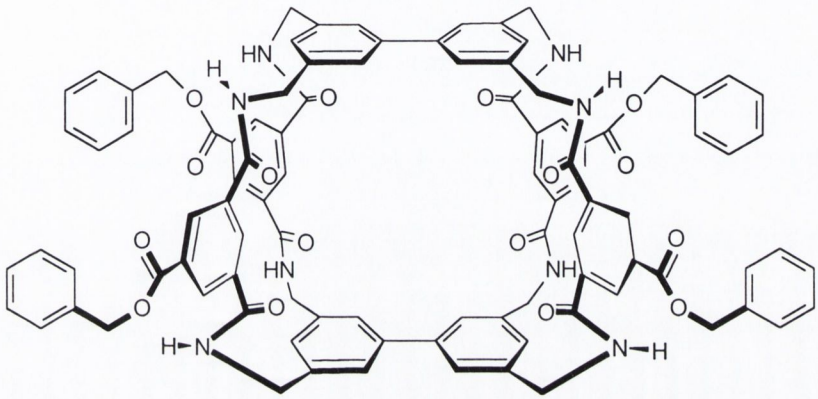
Tetra-TFA salt 5.5



TFA (15 mL) was added dropwise to a suspension of the tetra-Boc macrocycle **5.4** (267 mg, 0.18 mmol) in DCM (15 mL) at 0 °C under argon. All the solid material dissolved upon addition. The mixture was stirred under argon for 2 h after being allowed to warm to RT. The mixture was evaporated *in vacuo* to a brown oil which was triturated with diethyl ether (30 mL). A white solid resulted which was collected by vacuum filtration and washed with diethyl ether (20 mL) yielding the product as a white powder (272 mg, 0.18 mmol, 98 %). ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.44 (t, ^3J(H,H) = 5.5 Hz, 4 H; N*H*CH_2), 8.63 (s, 2 H; ArH spacer γ to CCOOC), 8.59 (s, 4 H; ArH spacer α to CCOOC), 8.34 (br s, 12 H; NH_3^+), 7.87 (s, 4 H; ArH), 7.58 (s, 4 H; ArH), 7.40 (m, 12 H; 2 × C_6H_5, 2 × biaryl H), 7.21 (s, 2 H; Biaryl H), 5.40 (s, 4 H; OCH_2), 4.55 (d, ^3J(H,H) = 5.0 Hz, 8 H; C*H*$_2$NH), 4.14 (br

s, 8 H; CH_2NH$_3^+$); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 164.6 (*C*OOCH$_2$), 164.3 (ArCONH), 140.4 (ArC), 139.5 (ArC), 135.8 (ArC), 135.0 (ArC), 134.5 (ArC), 130.5 (ArCH), 130.3 (ArCH), 130.0 (ArCH), 128.5 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.0 (ArCH), 124.8 (ArCH), 124.4 (ArCH), 66.6 (CH$_2$), 42.8 (CH$_2$), 42.1 (CH$_2$); IR (solid state): ν_{max} (cm^{-1}): 1670 (CONH), 1535 (CONH); MS (FAB): *m/z*: 1070 [MH]$^+$, 1092 [MNa]$^+$.

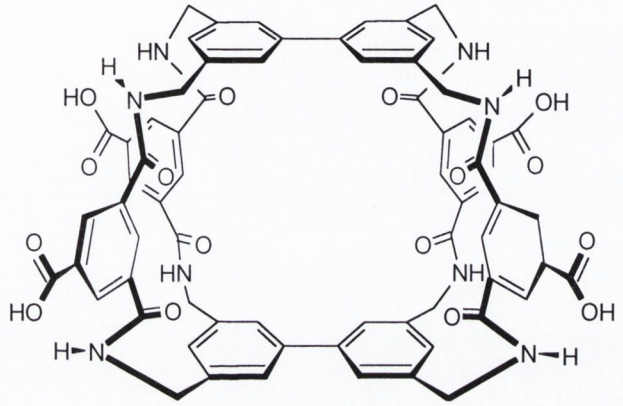
Benzyl Macrotricycle 5.6



A solution of the bis-Pfp benzyl spacer **5.3** (0.166 g, 0.26 mmol, 2.0 eq) in dry THF (500 mL) was added dropwise over *ca.* 8 h to a solution of the tetra-TFA salt **5.5** (0.20 g, 0.13 mmol) in dry THF (460 mL), dry DMF (40 mL) and *N*,*N*-DIPEA (0.69 mL, 3.93 mmol, 30 eq) under argon with rapid stirring at 40 °C. After addition, the reaction mixture was left to stir for *ca.* 8 h at 40 °C. The mixture was evaporated *in vacuo*, azeotroping the DMF using toluene (5 × *ca.* 50 mL) to give the crude product as an off-white solid. Initial purification by flash column chromatography (silica 90 g), eluting with AcOH/MeOH/CHCl$_3$ (1:2:97), yielded mainly **5.6** as a white solid. Further purification by preparative TLC, using MeOH/DCM (8:92) as eluent, yielded **5.6** as a white solid (38 mg, 0.02 mmol, 18 %). R_f = 0.67 (AcOH/MeOH/DCM 1:8:91); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.10 (t, ^3J(H,H) = 5.5 Hz, 8 H; NH), 8.48 (s, 8 H; spacer ArH α to CCOOC), 8.15 (s, 4 H; spacer ArH γ to CCOOC), 7.79 (s, 8 H; biaryl H), 7.43 (m, 20 H; ArH C$_6$H$_5$), 7.24 (s, 4 H; biaryl H), 5.41 (s, 8 H; OCH$_2$), 4.44 (A part of ABX, *J*(A,B) = 13.6 Hz, *J*(A,X) = 5.5 Hz, 8 H; CH_2NH), 4.36 (A part of ABX, *J*(A,B) = 13.6 Hz, *J*(A,X) = 5.5 Hz, 8 H; CH_2NH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 164.59 (CO), 164.56 (CO), 139.8 (ArC biaryl α to CH$_2$), 136.5 (ArC), 135.7 (ArC C$_6$H$_5$), 135.3 (ArC), 130.4 (ArCH γ to CCOOC), 130.2 (ArCH α to CCOOC), 130.0 (ArC), 128.9 (4 ×

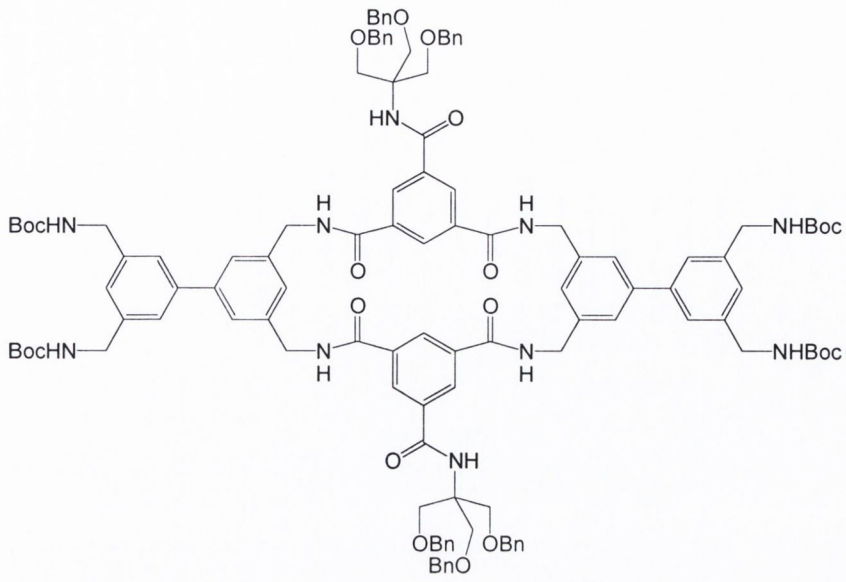
ArCH biaryl), 128.5 (ArCH *meta*C_6H_5), 128.2 (ArCH *para*C_6H_5), 128.1 (ArCH *ortho*C_6H_5), 124.7 (8 × ArCH biaryl), 66.7 (OCH_2), 43.6 ($NHCH_2$); IR (film from $CHCl_3$): ν_{max} (cm^{-1}): 1714 (COOC), 1643 (CONH); 1537 (CONH); HRMS (FAB): *m/z* mass calcd for $C_{96}H_{76}N_8O_{16}Na$ 1619.5277, found 1619.5363.

Tetra-Carboxylic Acid Macrotricycle 5.7 from Benzyl Macrotricycle 5.6



10 % Pd/C (18 mg) was added to a suspension of the benzyl macrotricycle **5.6** (11 mg, 0.007 mmol) in AcOH (6 mL) and the reaction mixture was stirred under hydrogen (1 atm) at RT for 48 h. The reaction mixture was filtered through pre-washed (AcOH) celite, and washed through with MeOH/DCM (1:1) (25 mL). The filtrate was evaporated *in vacuo* to yield the tetra-carboxylic acid macrotricycle **5.7** as a white solid (8 mg, 0.006 mmol, 94 %). ^1H NMR (400 MHz, [D_6]DMSO): δ = 13.40 (br s, 4 H; COOH), 9.08 (br s, 8 H; NH), 8.44 (s, 8 H; spacer ArH α to CCOOC), 8.08 (s, 4 H; spacer ArH γ to CCOOC), 7.81 (s, 8 H; biaryl H), 7.24 (s, 4 H; biaryl H), 4.41 (br s, 16 H; C*H*$_2$NH); insufficient sample for ^{13}C spectrum; ν_{max} (cm^{-1}): 3080 (COOH), 2963 (COOH), 1711 (COOH), 1639 (CONH); 1535 (CONH); MS (FAB): *m/z*: 1237 $[M]^+$.

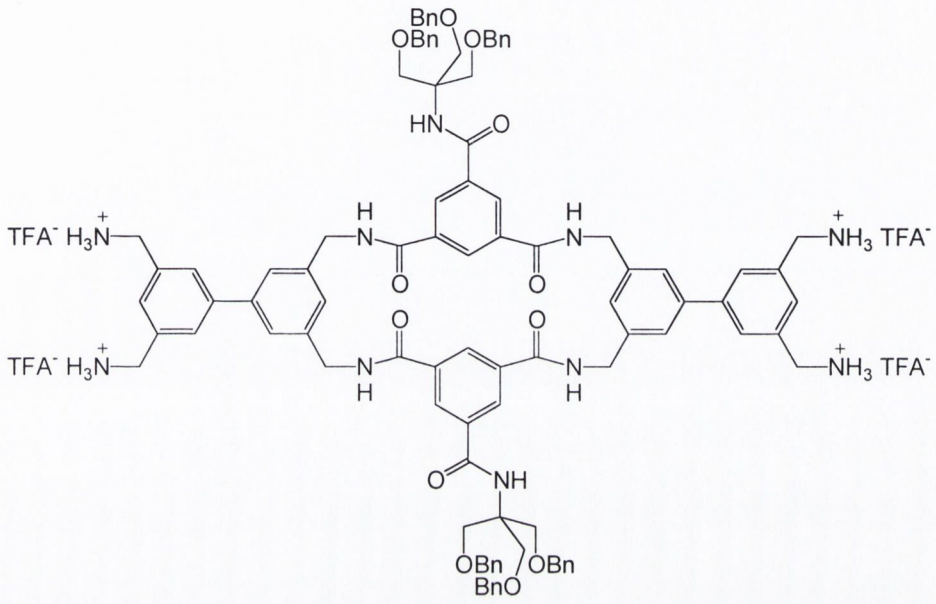
Tetra-Boc Macrocycle 5.15



A solution of the bis-Pfp polybenzyl spacer **5.14** (0.963 g, 1.05 mmol, 1.0 eq) in dry THF (630 mL) was added dropwise over *ca.* 8 h to a solution of the bis-amine **4.8** (0.495 g, 1.05 mmol) in dry THF (320 mL), dry DMF (30 mL) and *N*,*N*-DIPEA (0.92 mL, 5.26 mmol, 5.0 eq) under nitrogen with rapid stirring at RT. After addition, the reaction mixture was stirred for 12 h. The mixture was evaporated *in vacuo*, azeotroping the DMF using toluene (5 × *ca.* 50 mL). $CHCl_3$ (200 mL) was added and the organic phase was washed with 0.5 M aq. HCl solution (3 × 150 mL), sat. aq. $NaHCO_3$ solution (3 × 150 mL) and brine (150 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated *in vacuo* to a yellow oily solid. The crude product was purified by flash column chromatography (silica 95 g), eluting with EtOAc/toluene (40:60-50:50), to yield **5.15** as a white solid (431 mg, 0.21 mmol, 40 %). R_f = 0.36 (MeOH/DCM 5:95); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.30 (t, ^3J(H,H) = 5.3 Hz, 4 H; NH), 8.43 (s, 2 H; ArCH γ to CCONHCCH$_2$), 8.38 (d, ^4J(H,H) = 1.0 Hz, 4 H; ArCH α to CCONHCCH$_2$), 7.94 (s, 2 H; N*H*CCH$_2$), 7.52 (s, 4 H; biaryl ArCH), 7.45 (t, ^3J(H,H) = 6.2 Hz, 4 H; NH), 7.40 (s, 4 H; biaryl ArCH), 7.24 (m, 32 H; 30 × ArCH, 2 × biaryl ArCH), 7.13 (s, 2 H; biaryl ArCH); 4.49 (m, 8 H; biaryl CH$_2$), 4.48 (s, 12 H; ArCH$_2$O); 4.16 (m, 8 H; biaryl CH$_2$), 3.84 (s, 12 H; NHCC*H*$_2$), 1.40 (s, 36 H; CH$_3$); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 166.1 (*C*ONHCCH$_2$), 164.9 (*C*ONHCH$_2$), 155.7 (CO Boc), 140. 8 (ArC), 140.3 (ArC), 139.9 (Biaryl ArC), 139.7 (Biaryl ArC), 138.3 (ArC C$_6$H$_5$), 135.8 (ArC), 134.3 (ArC), 129.0 (ArCH α to CCONHCCH$_2$), 128.2 (ArCH α to CCONHCH$_2$), 128.04 (ArCH *meta*C$_6$H$_5$), 128.02 (ArCH *para*C$_6$H$_5$), 127.2 (ArCH *ortho*C$_6$H$_5$), 125.3

(ArCH biaryl), 124.5 (ArCH biaryl), 123.7 (ArCH biaryl), 77.7 ($C(CH_3)_3$), 72.4 ($ArCH_2O$), 67.6 ($NHCCH_2$), 60.6 ($NHCCH_2$), 43.3 (CH_2 biaryl), 43.0 (CH_2 biaryl), 28.1 (CH_3); IR (film from $CHCl_3$): ν_{max} (cm^{-1}): 1694 (CONH), 1646 (CONH); 1531 (CONH); MS (FAB): *m/z*: 2058 $[MNa]^+$.

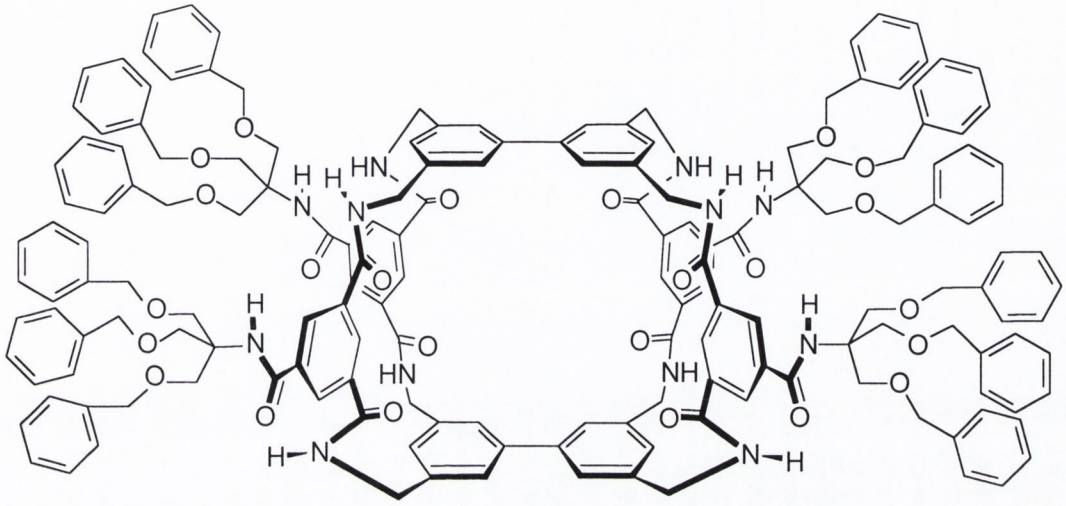
Tetra-TFA salt 5.16



TFA (14.3 mL) was added dropwise over ten minutes to a solution of the tetra-Boc protected **5.15** (390 mg, 0.19 mmol) in DCM (14.3 mL) at 0 °C. The mixture was stirred for 4 h after being allowed to warm to RT. The mixture was evaporated *in vacuo* to a brown oil which was triturated with diethyl ether (30 mL). A white solid resulted which was collected by vacuum filtration and washed with diethyl ether (20 mL) to yield the tetra-TFA salt **5.16** as a white powder (401 mg, 0.19 mmol, 100 %). ^1H NMR (400 MHz, $[D_6]$DMSO): δ = 9.37 (br s, 4H; CH_2NHCO), 8.38 (m, 18 H; 2 × ArCH γ to $CCONHCCH_2$, 4 × ArCH α to $CCONHCCH_2$, 4 × NH_3^+), 7.93 (s, 2 H; $NHCCH_2$), 7.86 (s, 4 H; ArH), 7.62 (s, 2 H; ArH), 7.48 (s, 2 H; ArH), 7.25 (m, 34 H; ArH), 4.54 (br s, 8 H; biaryl CH_2), 4.48 (s, 12 H; $ArCH_2O$), 4.12 (m, 8 H; biaryl CH_2), 3.83 (s, 12 H; $NHCCH_2$); ^{13}C NMR (100 MHz, $[D_6]$DMSO): δ = 166.1 ($CONHCCH_2$), 164.9 ($CONHCH_2$), 140.6 (ArC), 140.4 (ArC), 138.7 (ArC), 138.3 (ArC), 138.2 (ArC), 135.9 (ArC), 135.0 (ArC), 134.4 (ArC), 134.2 (ArC), 129.0 (ArCH α to $CCONHCCH_2$), 128.2 (ArCH α to $CCONHCH_2$), 128.1 (ArCH C_6H_5), 127.24 (ArCH C_6H_5), 127.20 (ArCH C_6H_5), 127.0 (ArCH biaryl), 124.96 (ArCH biaryl), 124.94 (ArCH biaryl), 72.3 ($ArCH_2O$),

67.6 ($NHCCH_2$), 60.6 ($NHCCH_2$), 42.9 (Biaryl CH_2), 42.1 (Biaryl CH_2); IR (solid state): ν_{max} (cm^{-1}): 1662 (CONH), 1521 (CONH); MS (FAB): *m/z*: 1636 $[MH]^+$, 1658 $[MNa]^+$.

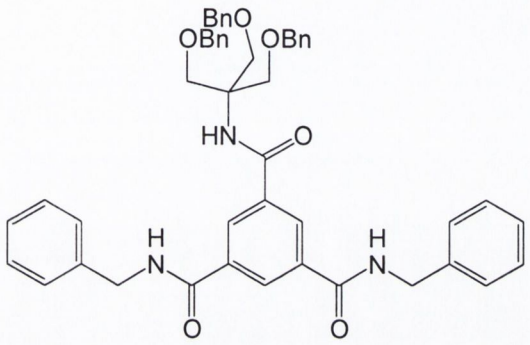
Polybenzyl Macrotricycle 5.17



A solution of the bis-Pfp polybenzyl spacer **5.14** (333 mg, 0.36 mmol, 2.0 eq) in dry THF (725 mL) was added dropwise over *ca.* 10 h to a solution of the tetra-TFA salt **5.16** (380 mg, 0.18 mmol) in dry THF (695 mL), dry DMF (30 mL) and *N*,*N*-DIPEA (0.95 mL, 5.45 mmol, 30 eq) under nitrogen with rapid stirring at 40 °C. After addition, the reaction mixture was left to stir for *ca.* 5 h at 40 °C. The mixture was evaporated *in vacuo*, azeotroping the DMF using toluene (5 × *ca.* 50 mL) to give the crude product as a light brown oily solid. The oil was dissolved in $CHCl_3$ (200 mL) and the organic phase washed with 0.5 M aq. HCl solution (150 mL), sat. aq. $NaHCO_3$ solution (150 mL) and brine (150 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated *in vacuo* to an off-white solid. The crude product was purified by flash column chromatography (silica 95 g), eluting with MeOH/DCM (3:97) to yield polybenzyl macrocycle **5.17** as a white solid (309 mg, 0.11 mmol, 62 %). R_f = 0.40 (MeOH/$CHCl_3$ 5:95); ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.12 (br s, 8 H; $NHCH_2$), 8.31 (s, 8 H; ArH α to $CCONHCCH_2$), 8.03 (s, 4 H; ArH γ to $CCONHCCH_2$), 8.01 (s, 4 H; $NHCCH_2$), 7.80 (s, 8 H; biaryl ArH), 7.24 (m, 64 H; C_6H_5 and 4 × biaryl ArH), 4.49 (s, 24 H; $OCH_2C_6H_5$), 4.43 (m, 16 H; CH_2NH), 3.85 (s, 24 H; $NHCCH_2$); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 165.9 ($CONHCH_2$), 165.2 ($CONHCCH_2$), 139.7 (ArC), 138.3 (ArC C_6H_5), 136.4 (ArC), 135.7 (ArC), 134.7 (ArC), 129.1 (ArCH), 128.8 (ArCH), 128.3 (ArCH), 128.0

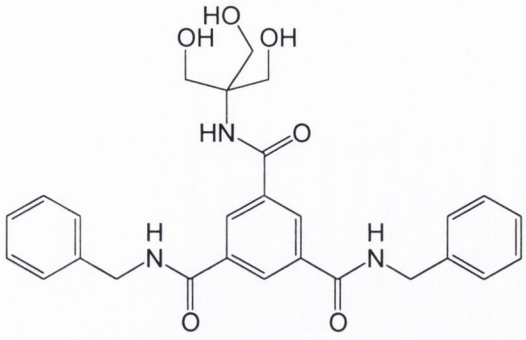
(ArCH), 127.2 (ArCH), 124.7 (ArCH), 72.4 ($CH_2C_6H_5$), 67.6 (CH_2CNH), 60.6 (CH_2CNH), 43.6 (CH_2NH); IR (film from DCM): ν_{max} (cm^{-1}): 1655 (CONH); 1520 (CONH); MS (FAB): *m/z*: 2754 $[MNa]^+$; elemental analysis calcd (%) for $C_{168}H_{160}N_{12}O_{24}.6H_2O$: C 71.07, H 6.11, N 5.92; found C 71.14, H 5.88, N 5.91.

Benzene-1,3,5-tricarboxylic acid bis-benzylamide (2-benzyloxy-1,1-bis-benzyloxymethyl-ethyl)-amide 5.18



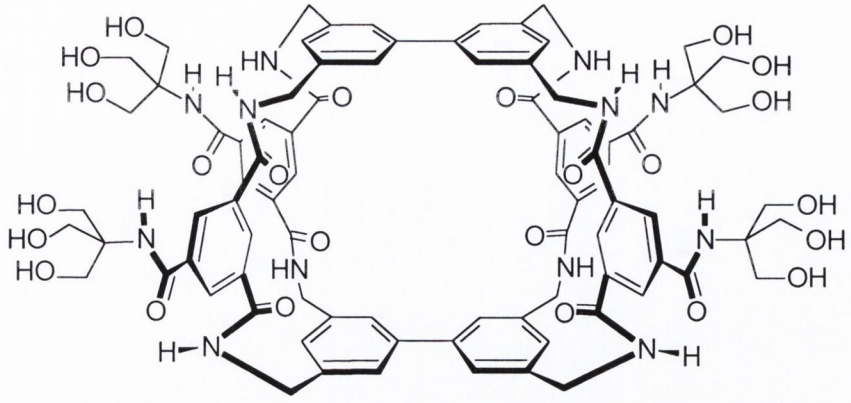
N,*N*-DIPEA (0.122 mL, 0.7 mmol, 2.0 eq) was added to a solution of the bis-Pfp polybenzyl spacer **5.14** (320 mg, 0.35 mmol) in dry THF (10 mL) under nitrogen. Benzylamine (0.084 mL, 0.8 mmol, 2.2 eq) was added and the reaction mixture was stirred under nitrogen at RT for 12 h. The reaction mixture was evaporated *in vacuo* to a colourless oil. Purification by flash column chromatography (silica 60 g) eluting with MeOH/DCM (1.5:98.5) yielded **5.18** as a white solid (240 mg, 0.31 mmol, 90 %). R_f = 0.48 (EtOAc/Petroleum spirit 1:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 9.27 (t, ^3J(H,H) = 6.0 Hz, 2 H; N*H*CH$_2$), 8.49 (t, ^4J(H,H) = 1.5 Hz, 1 H; ArH γ to CONHCCH$_2$), 8.39 (d, ^4J(H,H) = 1.5 Hz, 2 H; ArH α to CONHCCH$_2$), 7.92 (s, 1 H; NHCCH$_2$), 7.29 (m, 25 H; ArH C_6H_5), 4.50 (m, 10 H; 2 × NHC*H*$_2$, 3 × ArCH$_2$O), 3.85 (s, 6 H; NHCC*H*$_2$); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 166.0 (*C*ONHCH$_2$), 165.4 (*C*ONHCCH$_2$), 139.3 (ArC $C_6H_5CH_2O$), 138.3 (ArC), 135.7 (ArC), 134.6 (ArC), 128.8 (ArCH α to CONHCCH$_2$), 128.4 (ArCH γ to CONHCCH$_2$), 128.2 (ArCH), 128.1 (ArCH), 127.3 (ArCH), 126.7 (ArCH), 72.4 (ArCH$_2$O), 67.6 (*C*H$_2$CNH), 60.6 (NH*C*CH$_2$), 42.7 (CH$_2$NH); IR (film from DCM): ν_{max} (cm^{-1}): 1642 (CONH), 1557 (CONH); MS (FAB): *m/z*: 762 $[M]^+$; elemental analysis calcd (%) for $C_{48}H_{47}N_3O_6.0.5H_2O$: C 74.79, H 6.28, N 5.45; found C 74.80, H 6.10, N 5.46.

Benzene-1,3,5-tricarboxylic acid bis-benzylamide (2-hydroxymethyl-ethyl)-amide 5.19



BBr_3 (0.326 mL of a 1.0 M solution in dry DCM, 0.33 mmol, 4.0 eq) was added to a solution of the tris-benzyl protected model compound **5.18** (62 mg, 0.08 mmol) in dry DCM (4 mL) at –78 °C under nitrogen. The reaction mixture was allowed to warm to RT and stirred for 20 min. The mixture was analysed by TLC and LCMS after 20 min. The mixture was quenched with water (4 mL) and DCM (5 mL) was then added. The resultant white precipitate was filtered off and washed with DCM yielding a white powder (47 mg). Purification of 26 mg of this material by flash column chromatography (silica 9 g), eluting with $MeOH.NH_3/CHCl_3$ (10:90), yielded the tris-alcohol **5.19** as a white solid (13 mg, 0.03 mmol, 59 %). R_f = 0.24 ($MeOH.NH_3/CHCl_3$ 10:90); ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.31 (t, ^3J(H,H) = 6.0 Hz, 2 H; N*H*CH_2), 8.50 (t, ^4J(H,H) =1.7 Hz, 1 H; ArH γ to $CONHCCH_2$), 8.42 (d, ^4J(H,H) =1.7 Hz, 2 H; ArH α to $CONHCCH_2$), 7.55 (s, 1 H; N*H*CCH_2), 7.29 (m, 10H; ArH), 4.74 (t, ^3J(H,H) =6.0 Hz, 3 H; OH), 4.51 (d, ^3J(H,H) =6.0 Hz, 4 H; C*H*$_2$NH), 3.73 (d, ^3J(H,H) =6.0 Hz, 6 H; C*H*$_2$OH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 166.3 (CO), 165.3 (CO), 139.3 (ArC C_6H_5), 135.7 (ArC), 134.5 (ArC), 128.7 (ArCH α to $CONHCCH_2$), 128.5 (ArCH γ to $CONHCCH_2$), 128.2 (ArCH), 127.2 (ArCH), 126.7 (ArCH), 62.9 ($NHCCH_2$), 60.1 (CH_2OH), 42.7 (CH_2NH); IR (solid state): ν_{max} (cm^{-1}): 3286 (OH), 1643 (CONH), 1526 (CONH); HRMS (FAB): *m/z* mass calcd for $C_{27}H_{29}N_3O_6Na$ 514.1954, found 514.1972.

Polyhydroxy Macrotricycle 5.23



BBr_3 (500 mL of a 1.0 M solution in dry DCM, 0.50 mmol) was added to a solution of polybenzyl macrotricycle **5.17** (57 mg, 0.021 mmol) in dry DCM (4 mL) at –78 °C under nitrogen. After *ca.* 5 min the reaction mixture was allowed to warm to RT and was stirred for another 1.5 h. The reaction mixture was quenched with water (1.0 mL) and was evaporated *in vacuo* to an off-white solid. The solid was then washed with DCM (3 × *ca.* 5 mL). Purification by reverse phase flash column chromatography (C-18 silica 50 g) eluting with TFA/CH_3CN/H_2O (1:30:69) yielded **5.23** as an off-white solid (61 mg). R_f = 0.39 (TFA/CH_3CN/H_2O 1:30:69); ^{1}H NMR (400 MHz, CD_3CN/D_2O 20:80): δ = 8.62 (d, ^4J(H,H) = 1.7 Hz, 8 H; ArH α to $CONHCCH_2$), 8.01 (t, ^4J(H,H) = 1.7 Hz, 4 H; ArH γ to $CONHCCH_2$), 7.78 (s, 8 H; biaryl ArH), 7.40 (s, 4 H; biaryl ArH), 3.90 (d, ^3J(H,H) = 1.7 Hz, 24 H; OH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 164.8 (CO), 164.5 (CO), 139.6 (ArC), 136.5 (ArC), 135.27 (ArC), 130.8 (ArCH), 129.6 (ArCH), 124.84 (ArCH), 124.79 (ArCH), 59.5 (CH_2OH), 43.7 (CH_2 biaryl); IR (solid state): ν_{max} (cm^{-1}): 3190 (OH), 1637 (CONH), 1400 (OH); MS (ES): *m/z*: 1651 $[MH]^+$.

Typical Procedure for Extraction of Sugars from Aqueous into Organic Media

A solution of polybenzyl macrotricycle **5.17** (4.0 mg, 1.46 μmol) in chloroform (5.0 mL, thus [Host] = 0.00029 M) and a solution of D-glucose (1.0 M, 5.0 mL) were placed in a 50 mL round-bottomed flask and allowed 15 min to warm to 30 °C by suspension in a water bath. The flask was shaken vigorously for 1 min and the mixture was allowed 15 min to separate into two phases, at 30 °C. The organic phase was isolated by pouring the two phases onto hydrophobic (silicone treated) filter paper and allowing the organic phase to pass through. The chloroform layer was analysed by TLC (MeOH/DCM 5:95) and was evaporated *in vacuo* to a white

solid which was then dissolved in 700 μL of [D_6]DMSO. A ^{1}H NMR spectrum (128 scans) was obtained. Integration of the anomeric protons of D-glucose with respect to the biaryl signals of the host at δ 7.80 showed the guest:host ratio to be 1.0:1.

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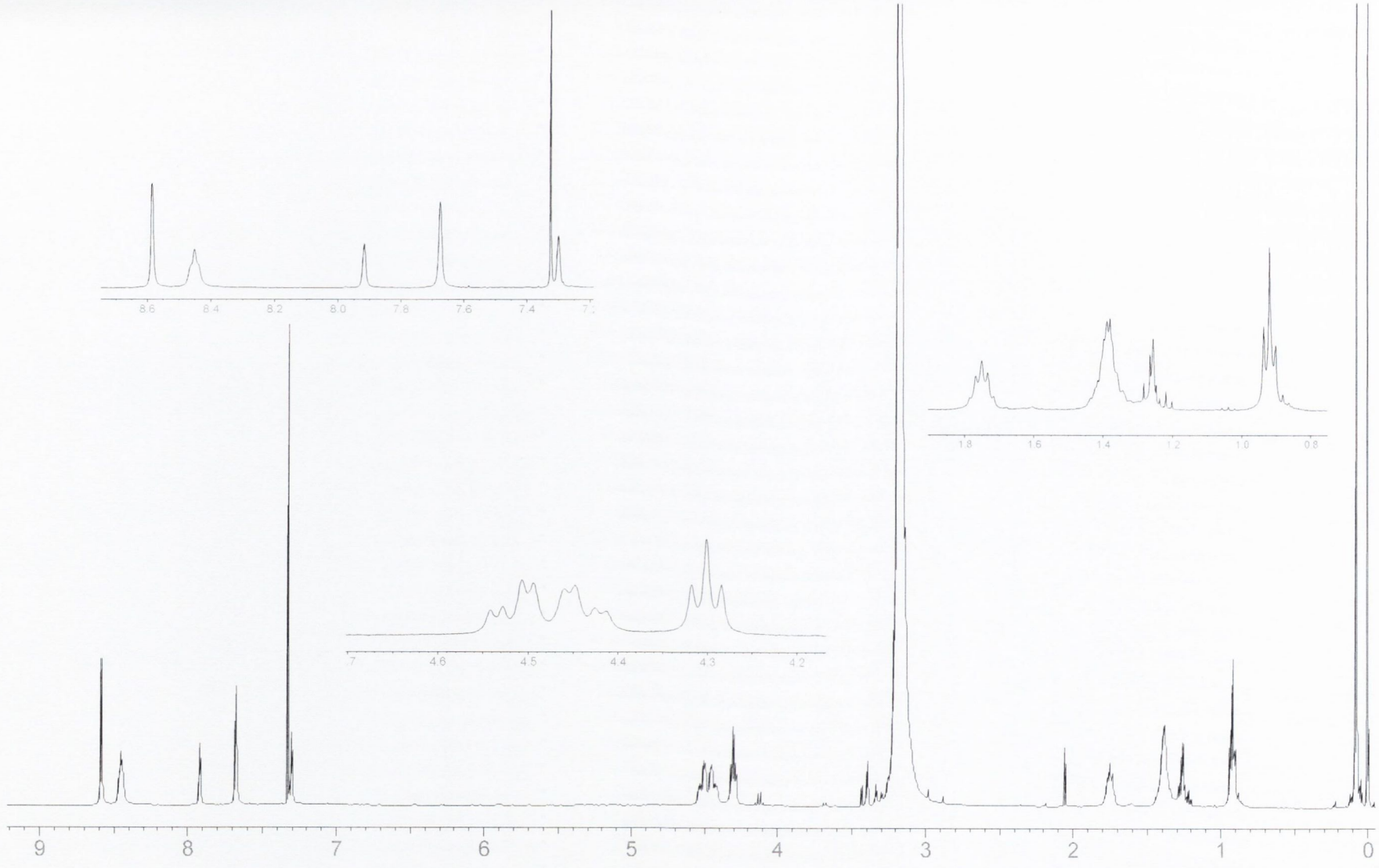
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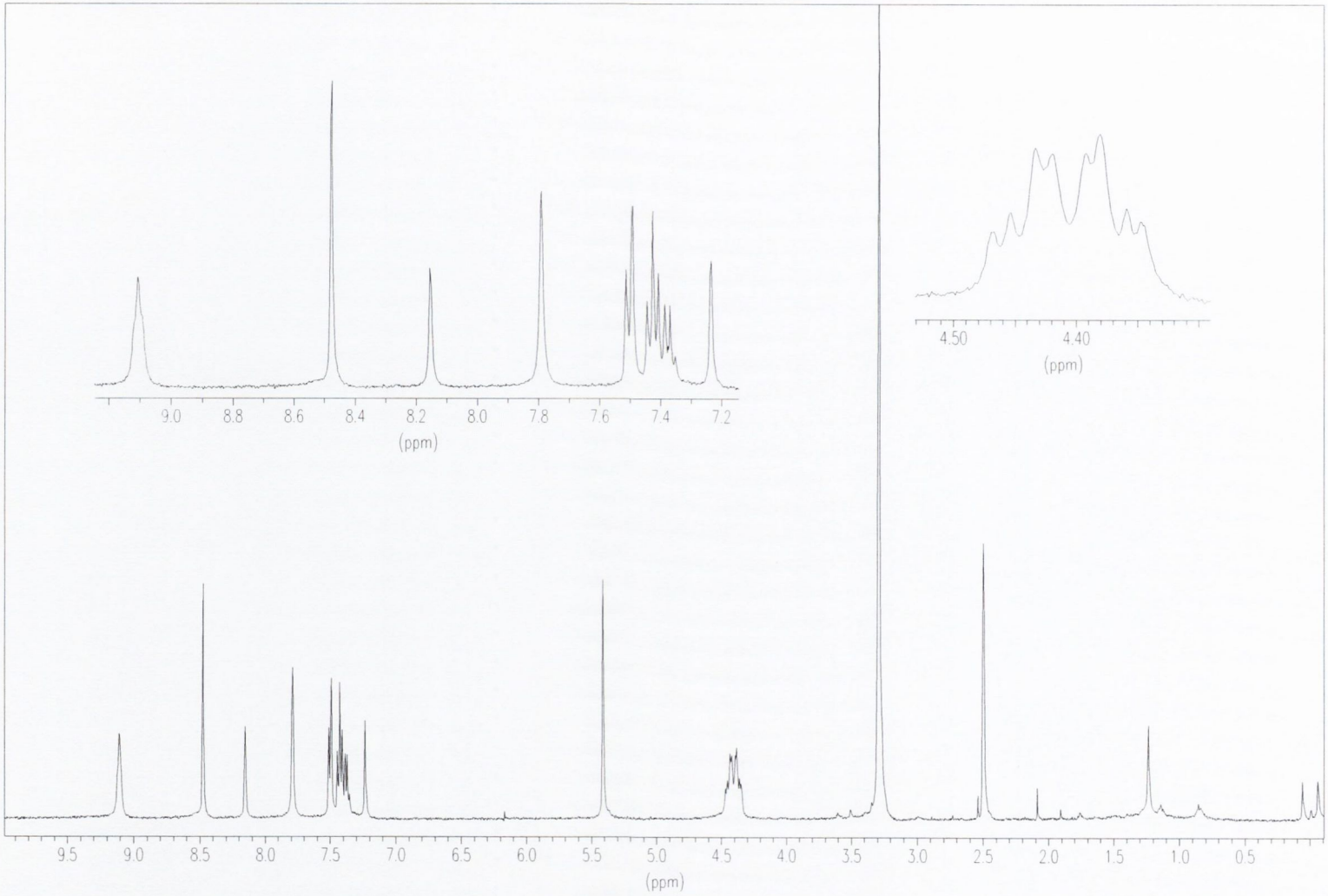
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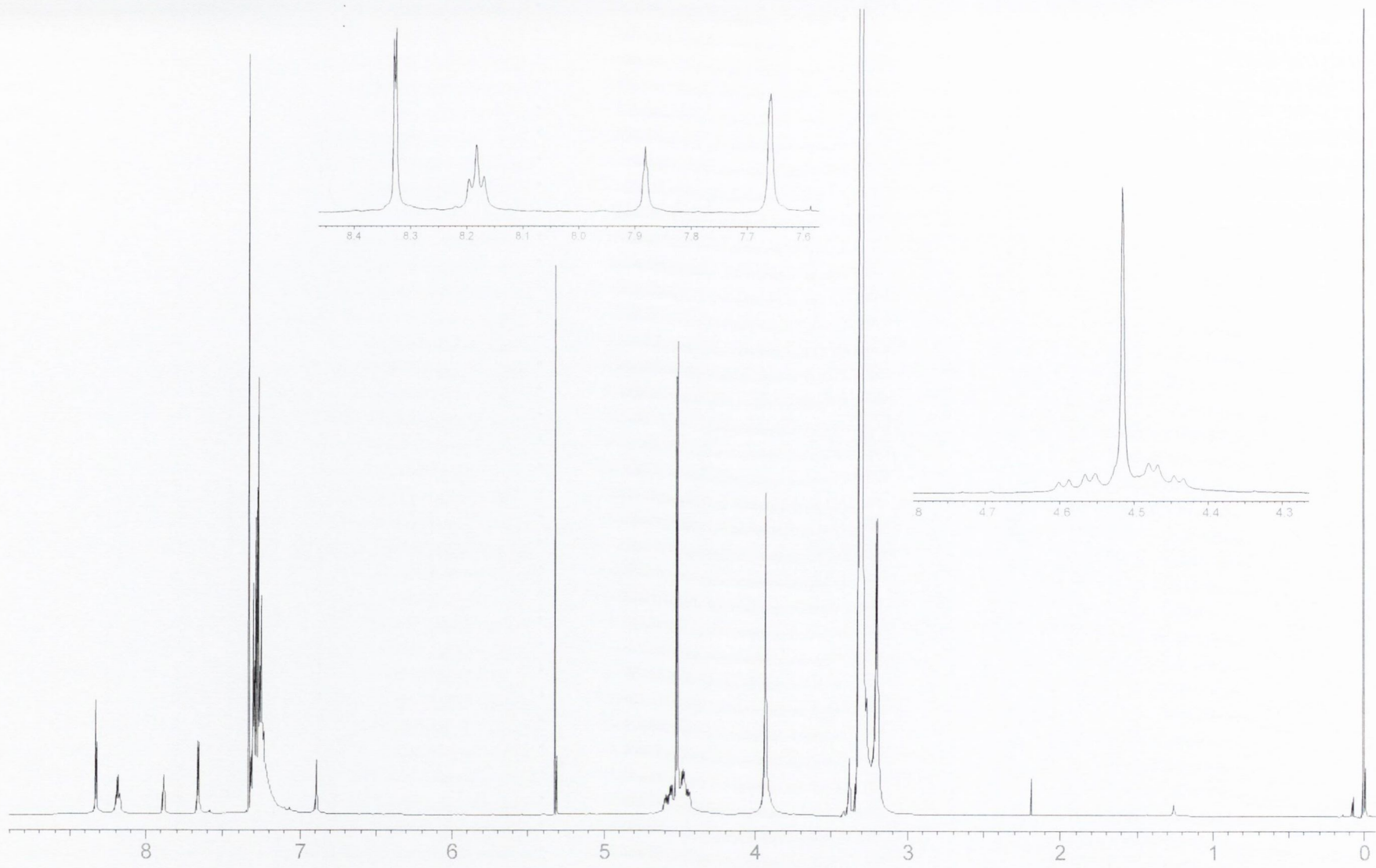
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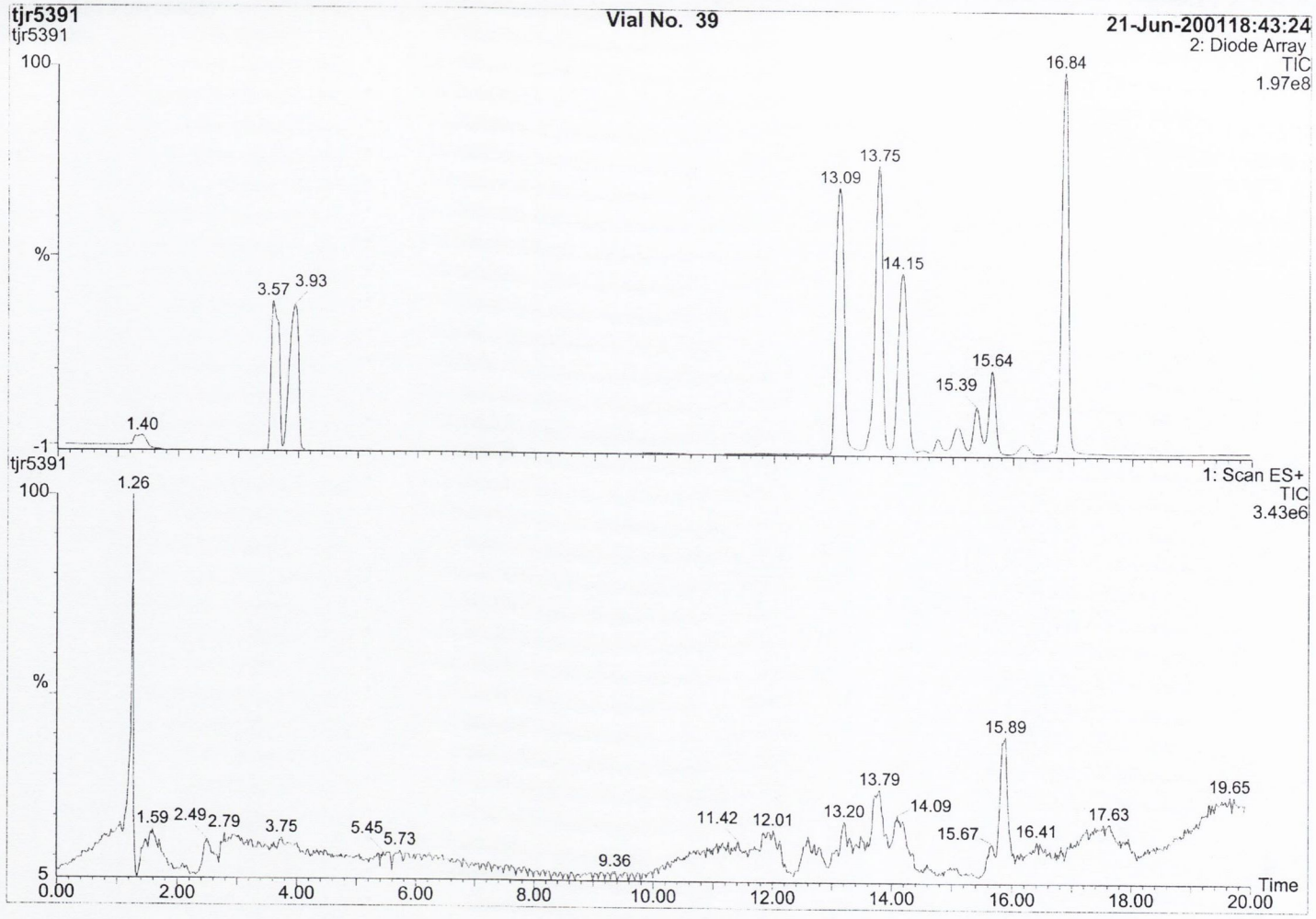
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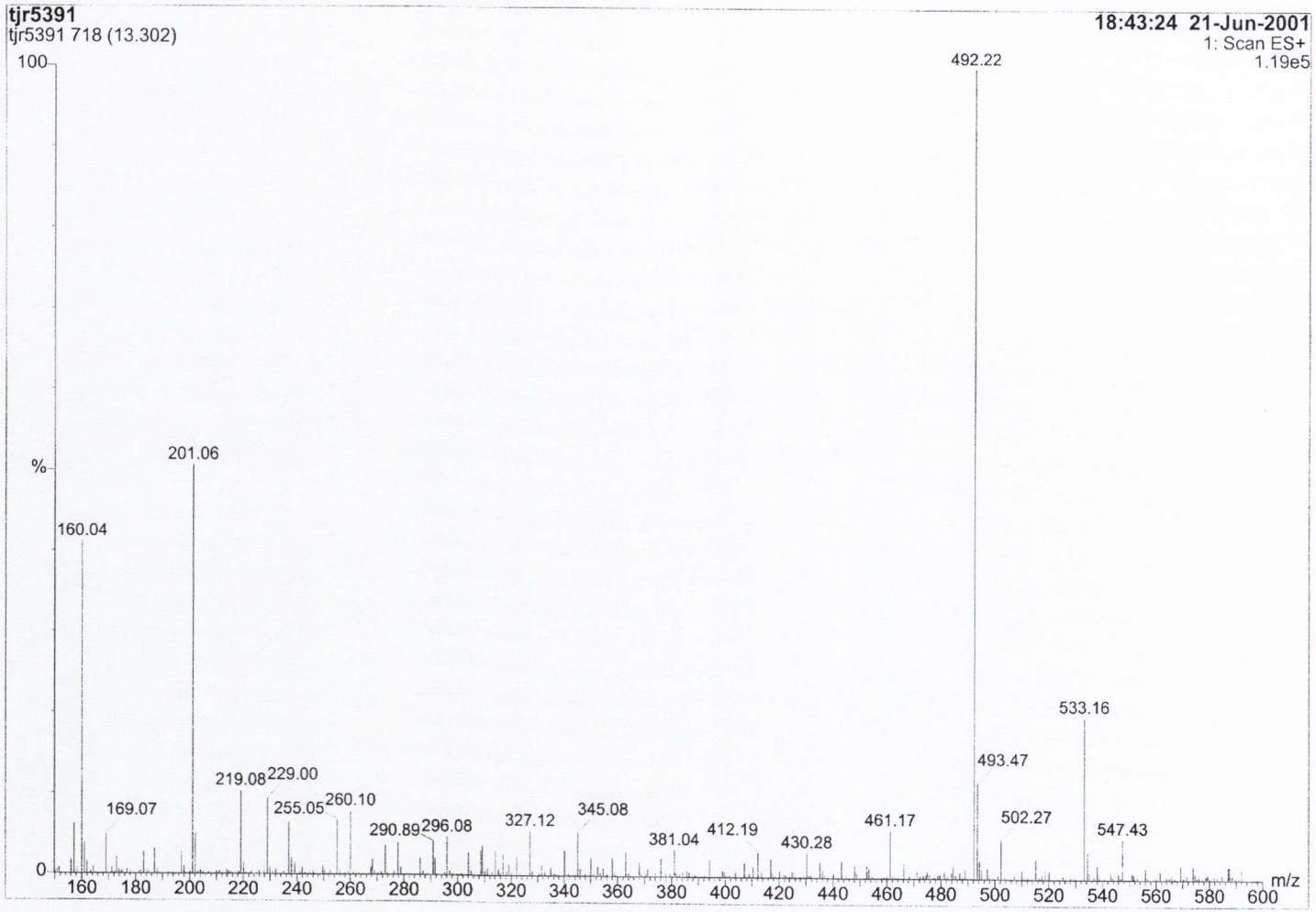
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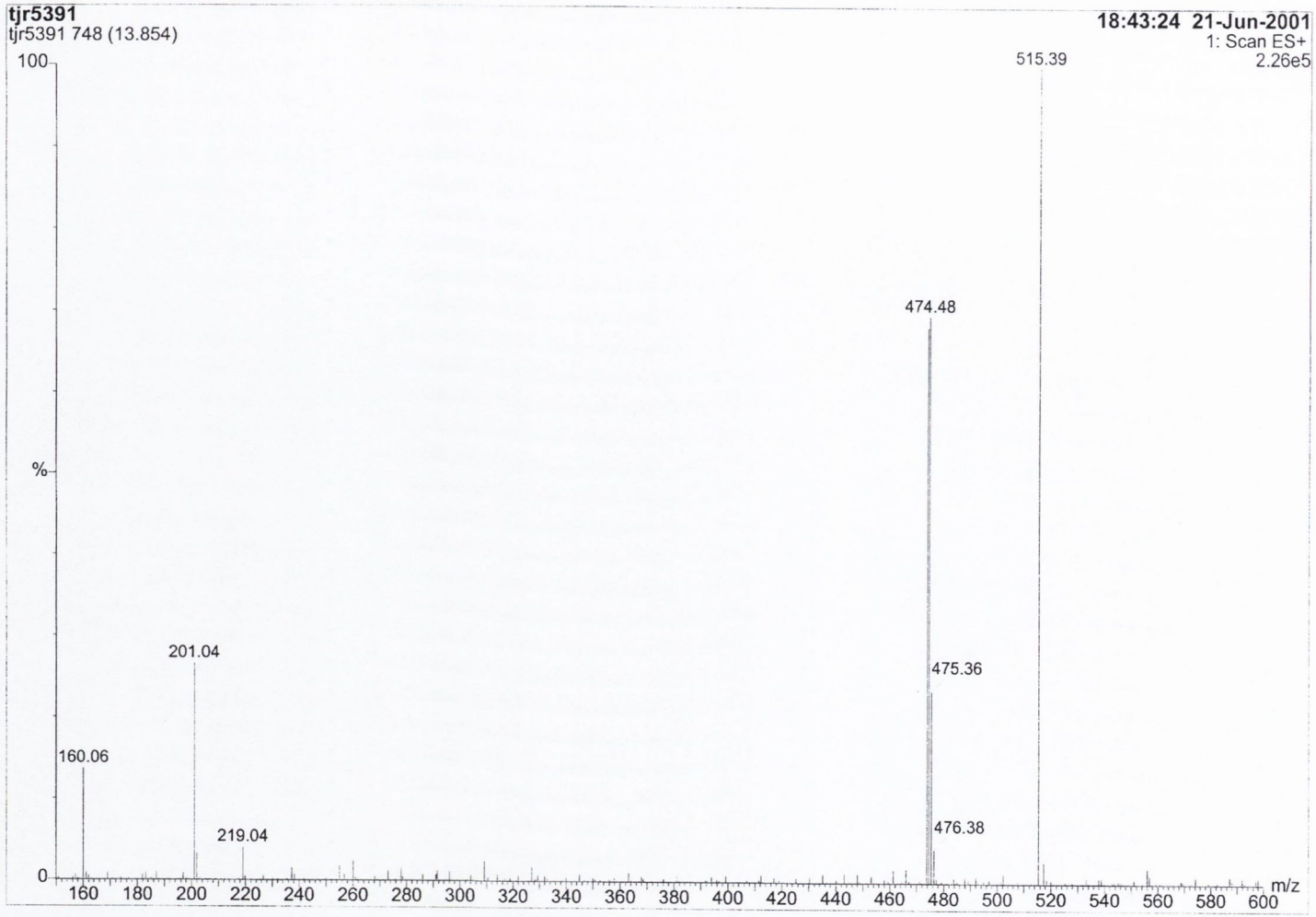

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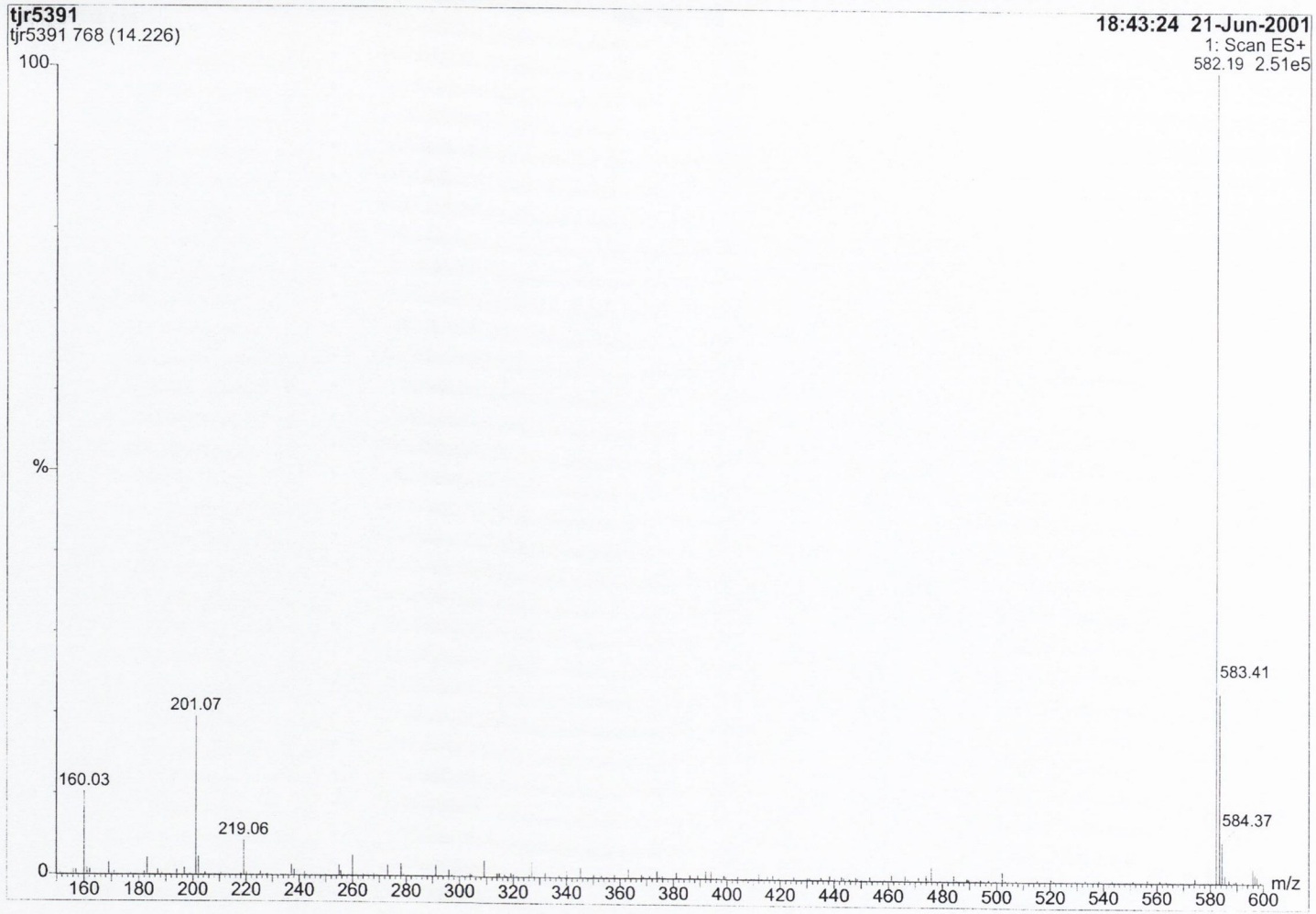

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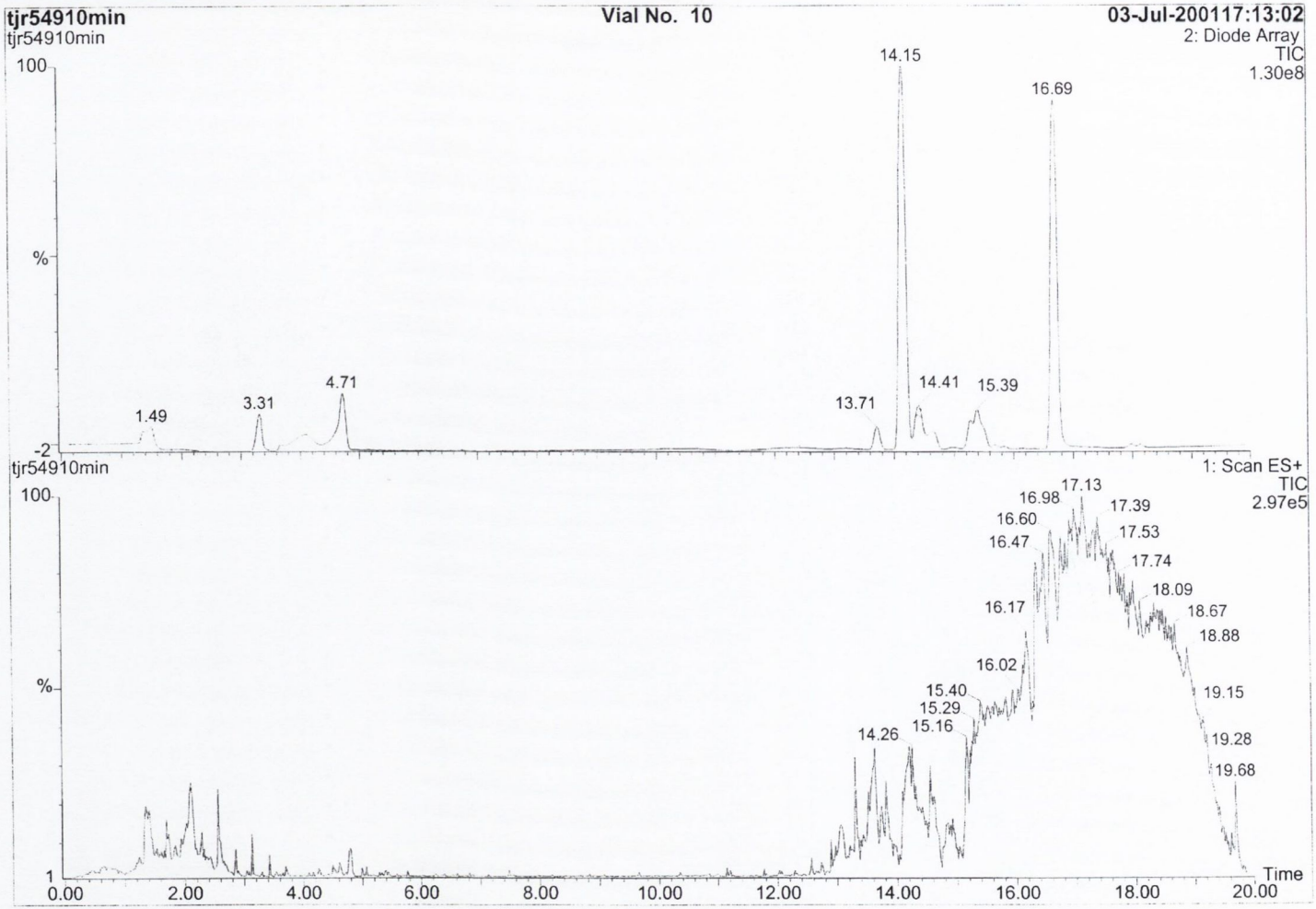

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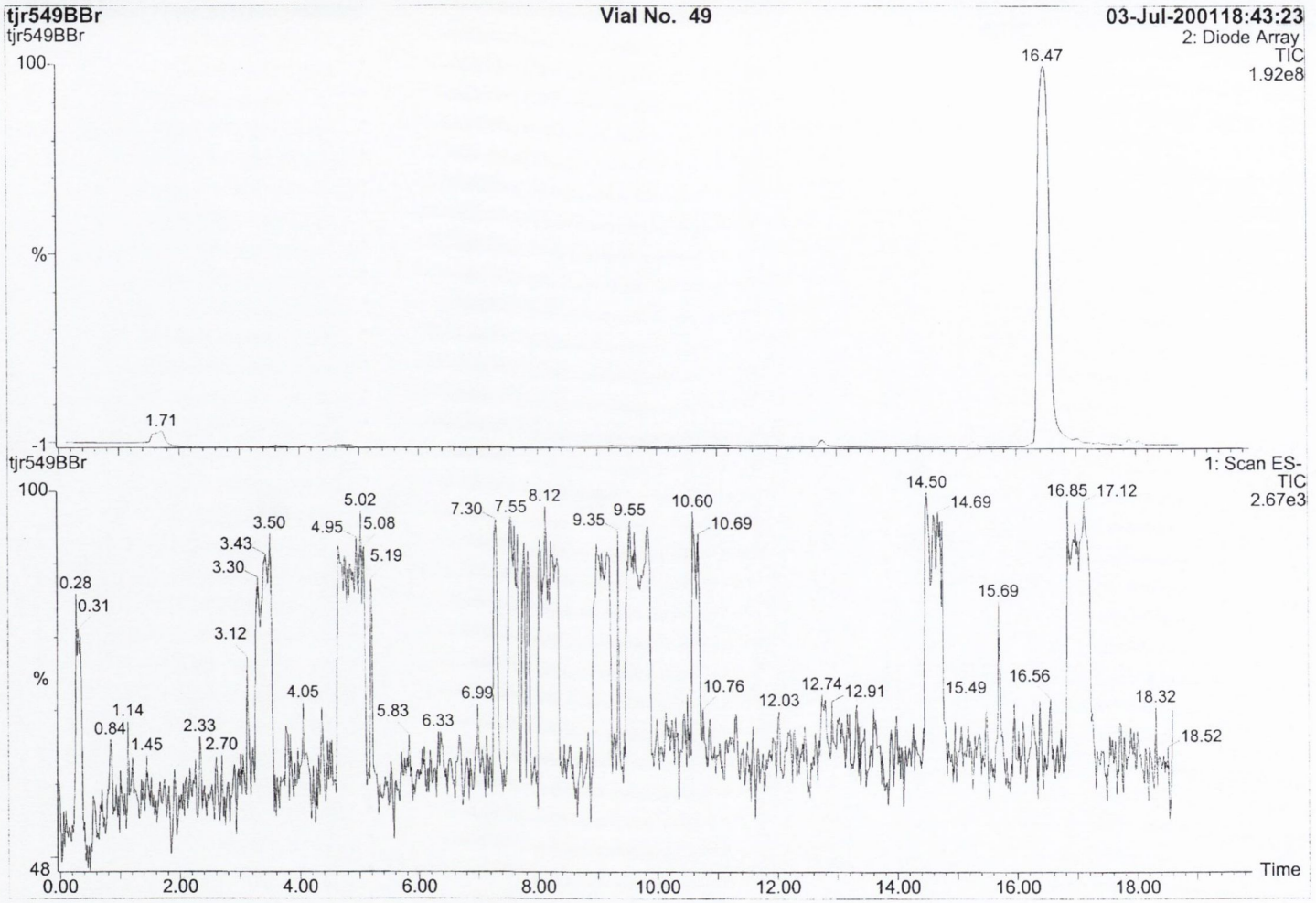

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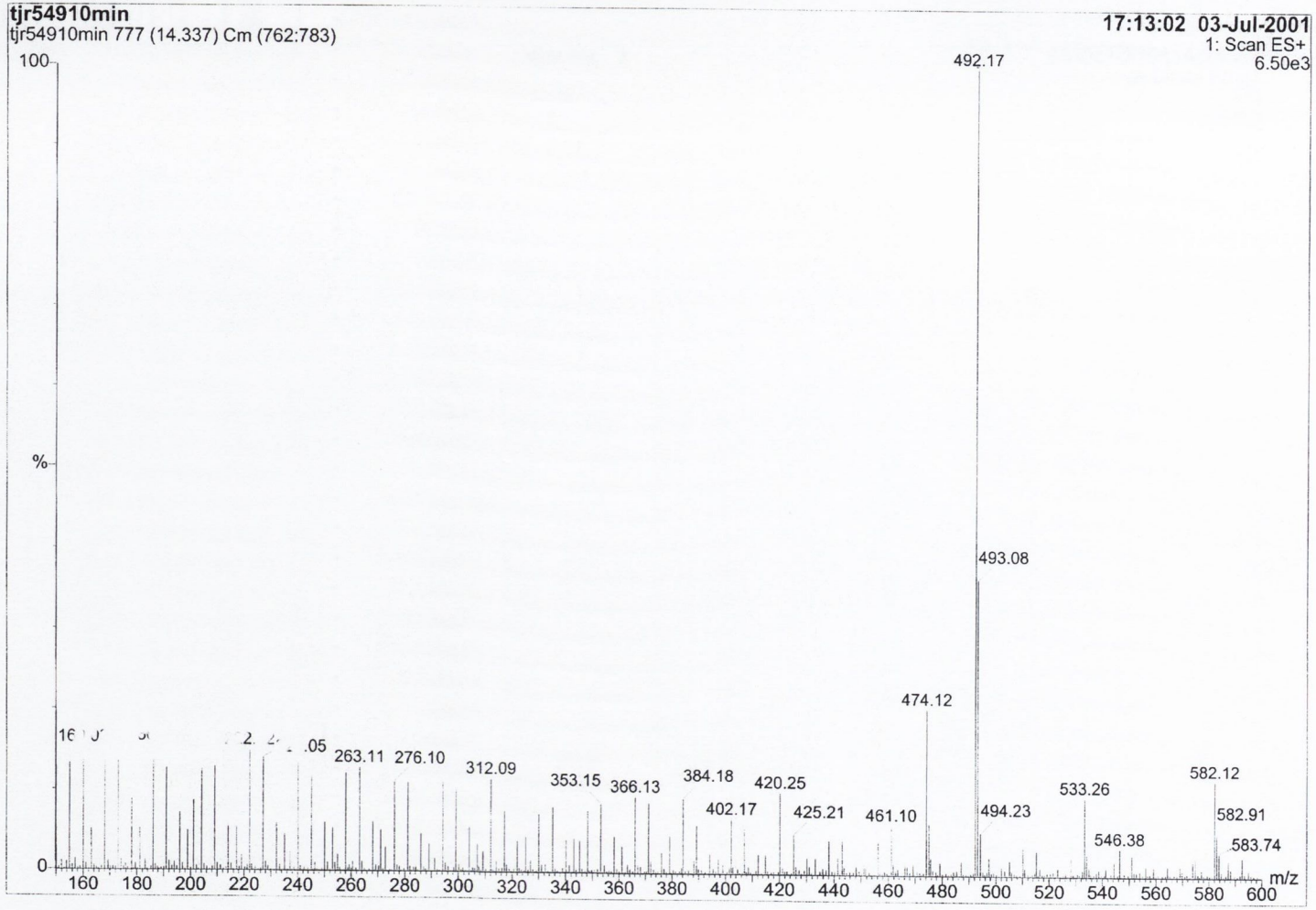

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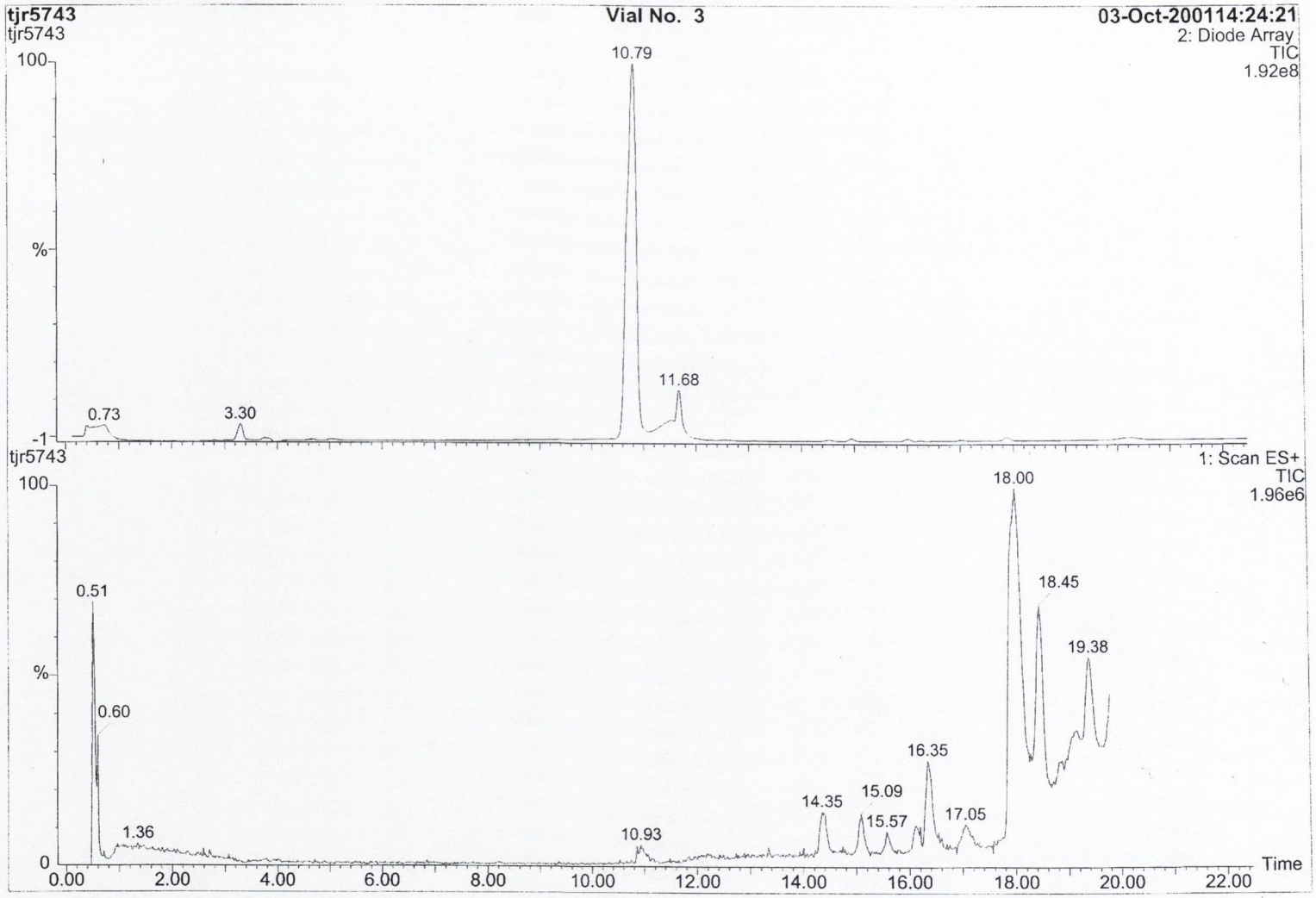

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476.38
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160.06
219.04
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m/z

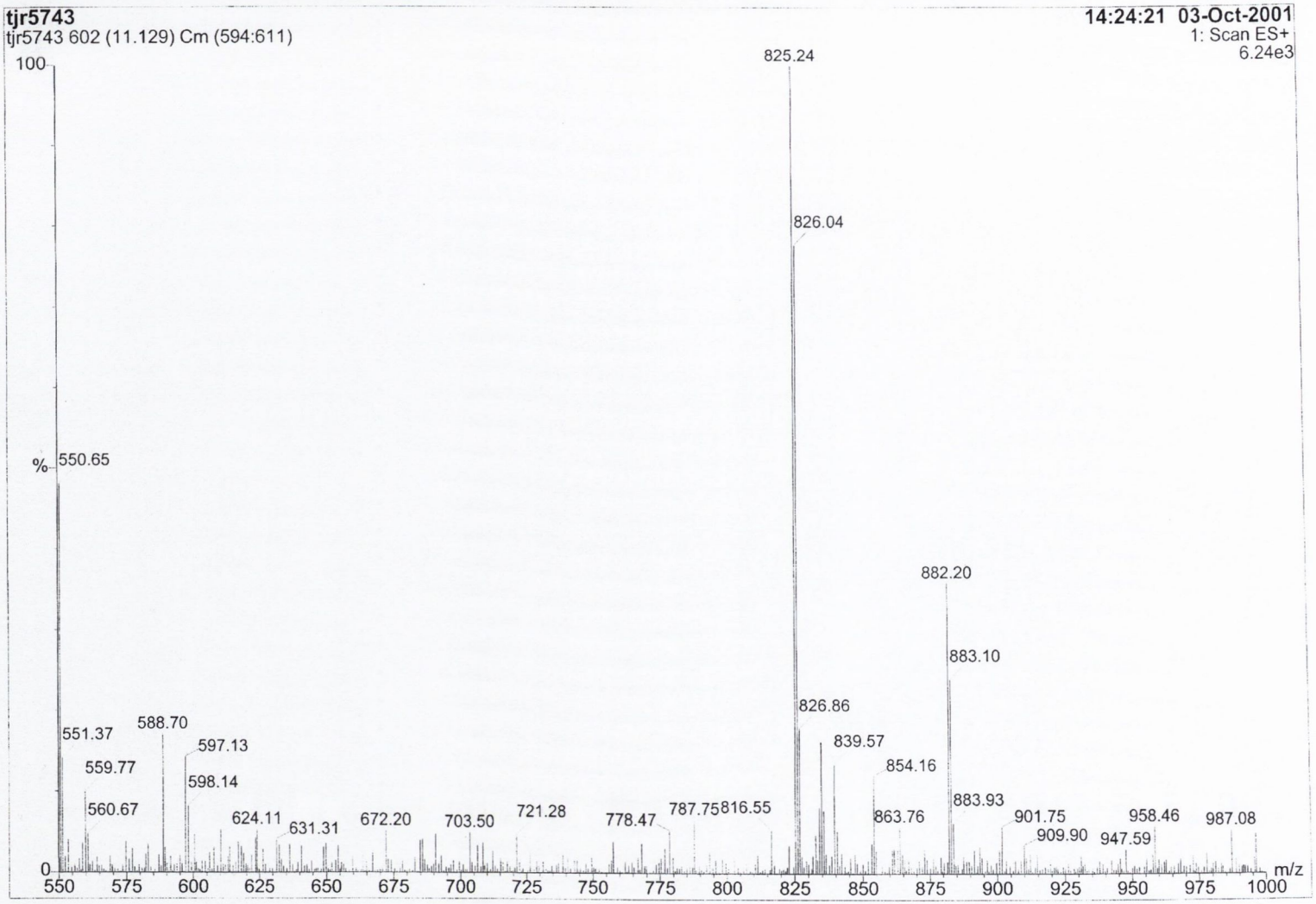

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%
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583.41
201.07
160.03
219.06
584.37
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m/z


tjr54910min
Vial No. 10
03-Jul-200117:13:02
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2: Diode Array
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%
-2
14.15
16.69
4.71
3.31
1.49
13.71
14.41
15.39
tjr54910min
1: Scan ES+
TIC
2.97e5
100
%
1
17.13
16.98
17.39
16.60
16.47
17.53
17.74
18.09
16.17
18.67
18.88
16.02
15.40
19.15
15.29
15.16
14.26
19.28
19.68
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4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00

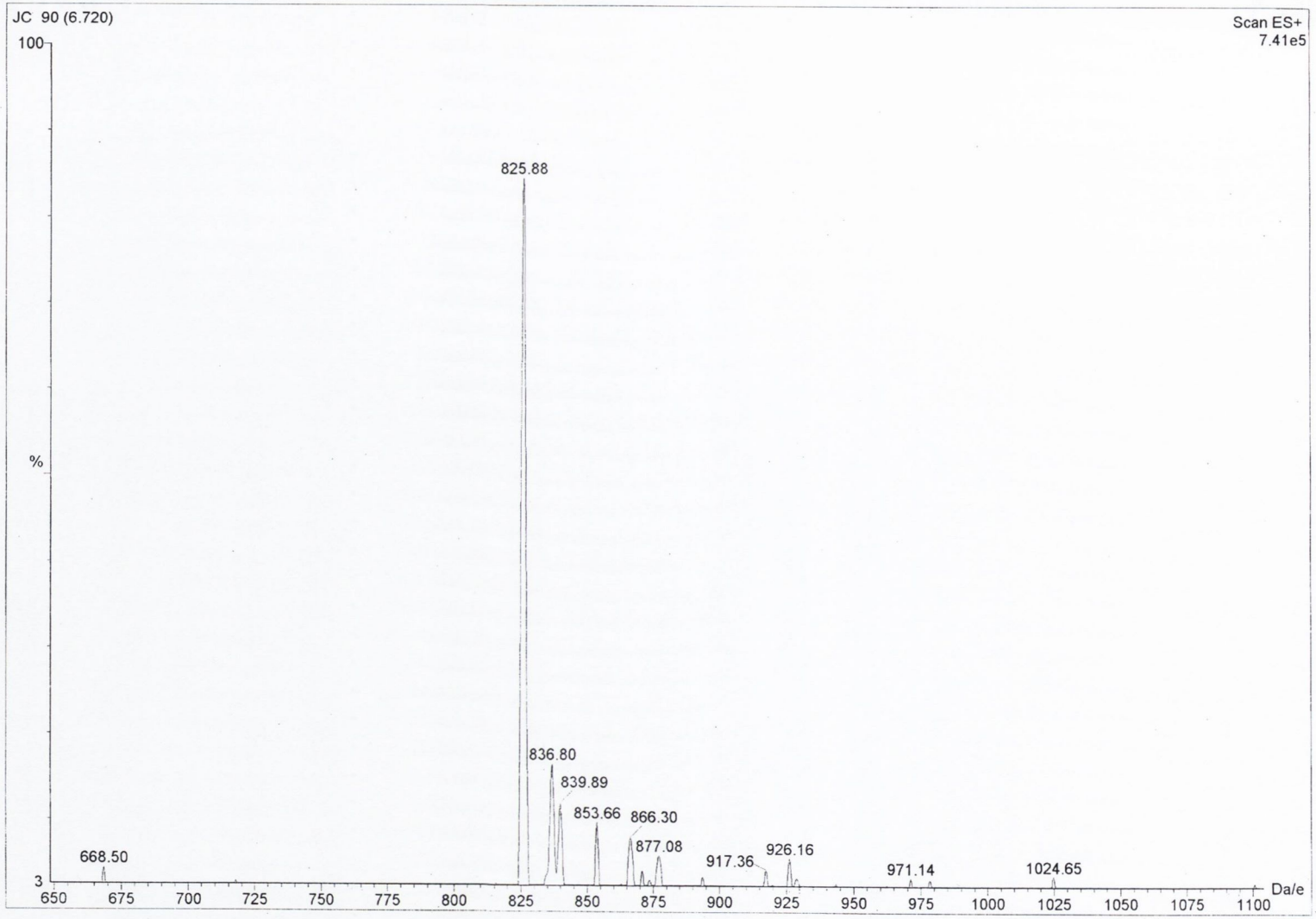

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1.71
tjr549BBr
1: Scan ES-
TIC
2.67e3
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0.31
0.84
1.14
1.45
2.33
2.70
3.12
3.30
3.43
3.50
4.05
4.95
5.02
5.08
5.19
5.83
6.33
6.99
7.30
7.55
8.12
9.35
9.55
10.60
10.69
10.76
12.03
12.74
12.91
14.50
14.69
15.49
15.69
16.56
16.85
17.12
18.32
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%
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16.00
18.00

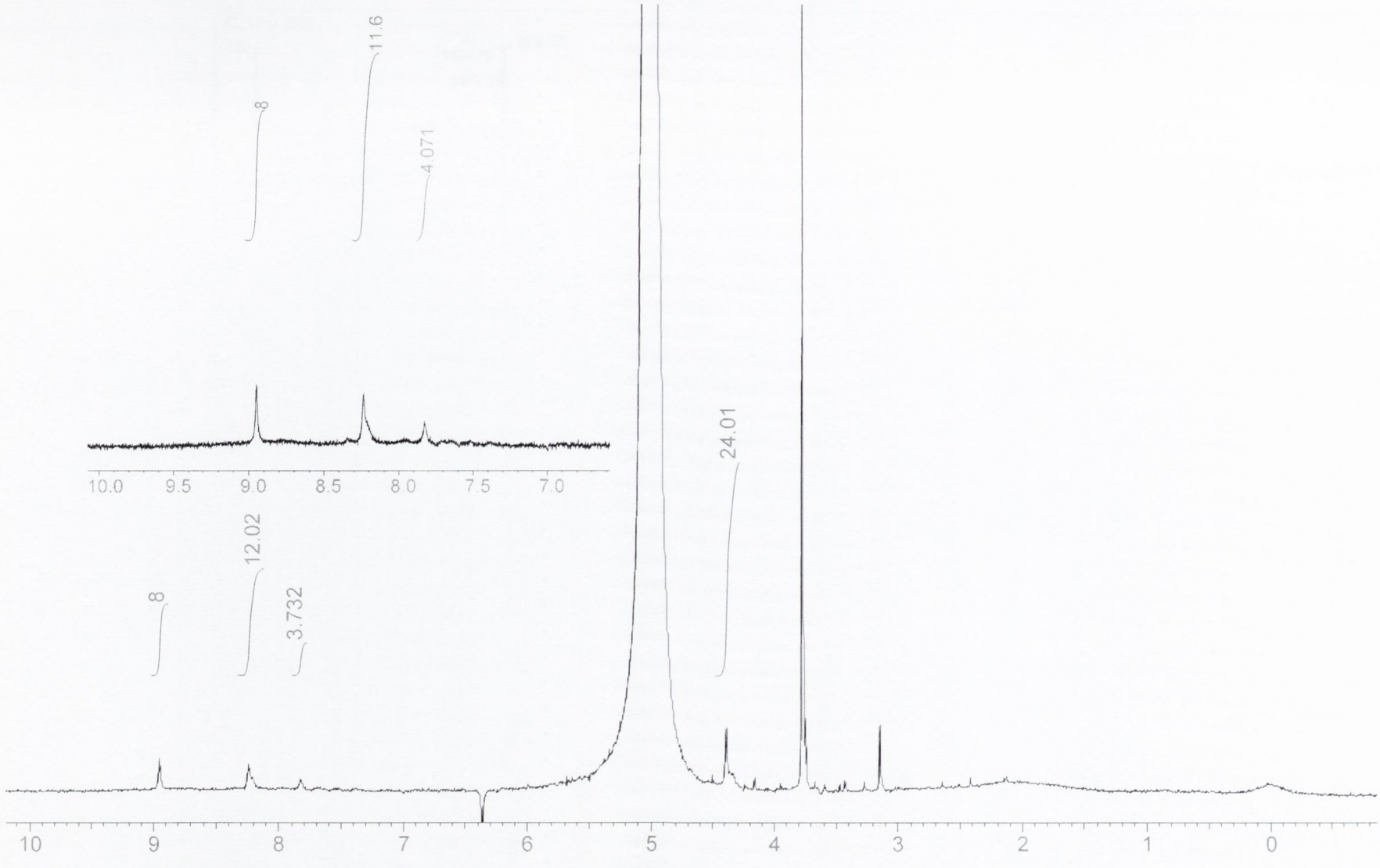

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%
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366.13
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420.25
402.17
425.21
461.10
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m/z

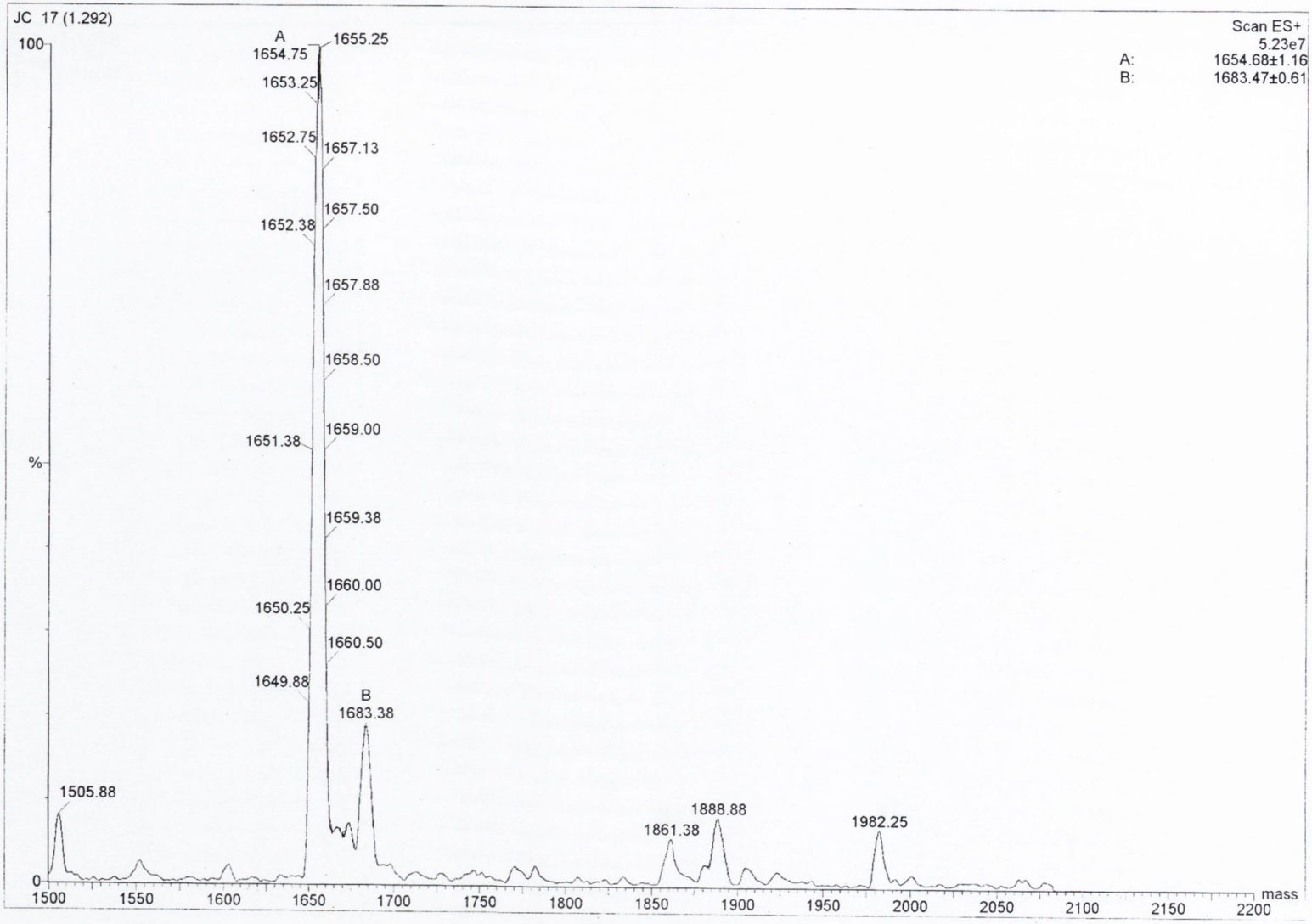

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%
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10.79
11.68
0.73
3.30
tjr5743
1: Scan ES+
TIC
1.96e6
100
%
0
18.00
18.45
19.38
0.51
0.60
1.36
10.93
14.35
15.09
15.57
16.35
17.05
Time
0.00
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4.00
6.00
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10.00
12.00
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16.00
18.00
20.00
22.00

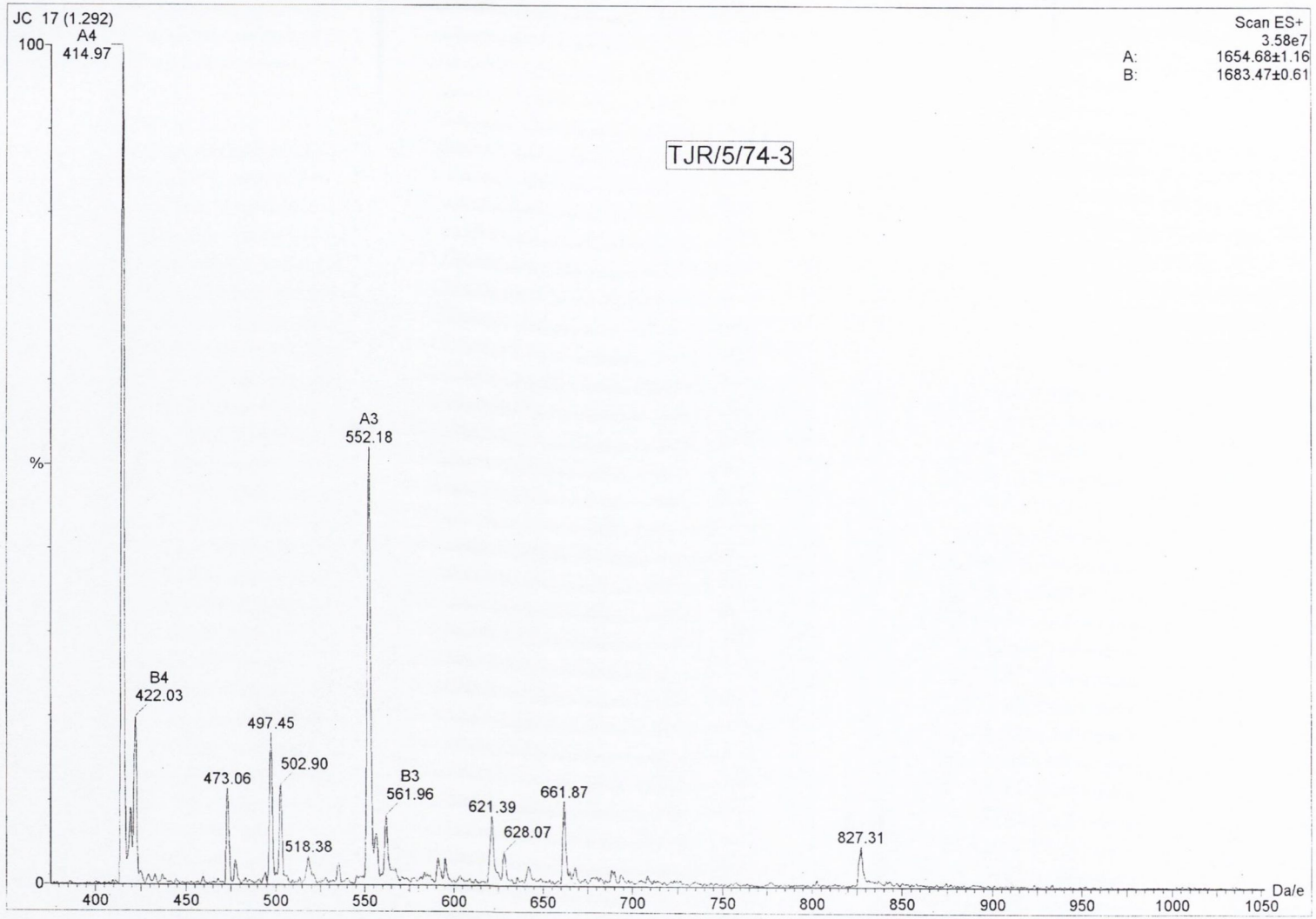

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%
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826.04
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882.20
883.10
826.86
588.70
551.37
597.13
839.57
559.77
854.16
598.14
883.93
560.67
816.55
787.75
901.75
624.11
631.31
672.20
703.50
721.28
778.47
863.76
958.46
987.08
909.90
947.59
m/z
550
575
600
625
650
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900
925
950
975
1000

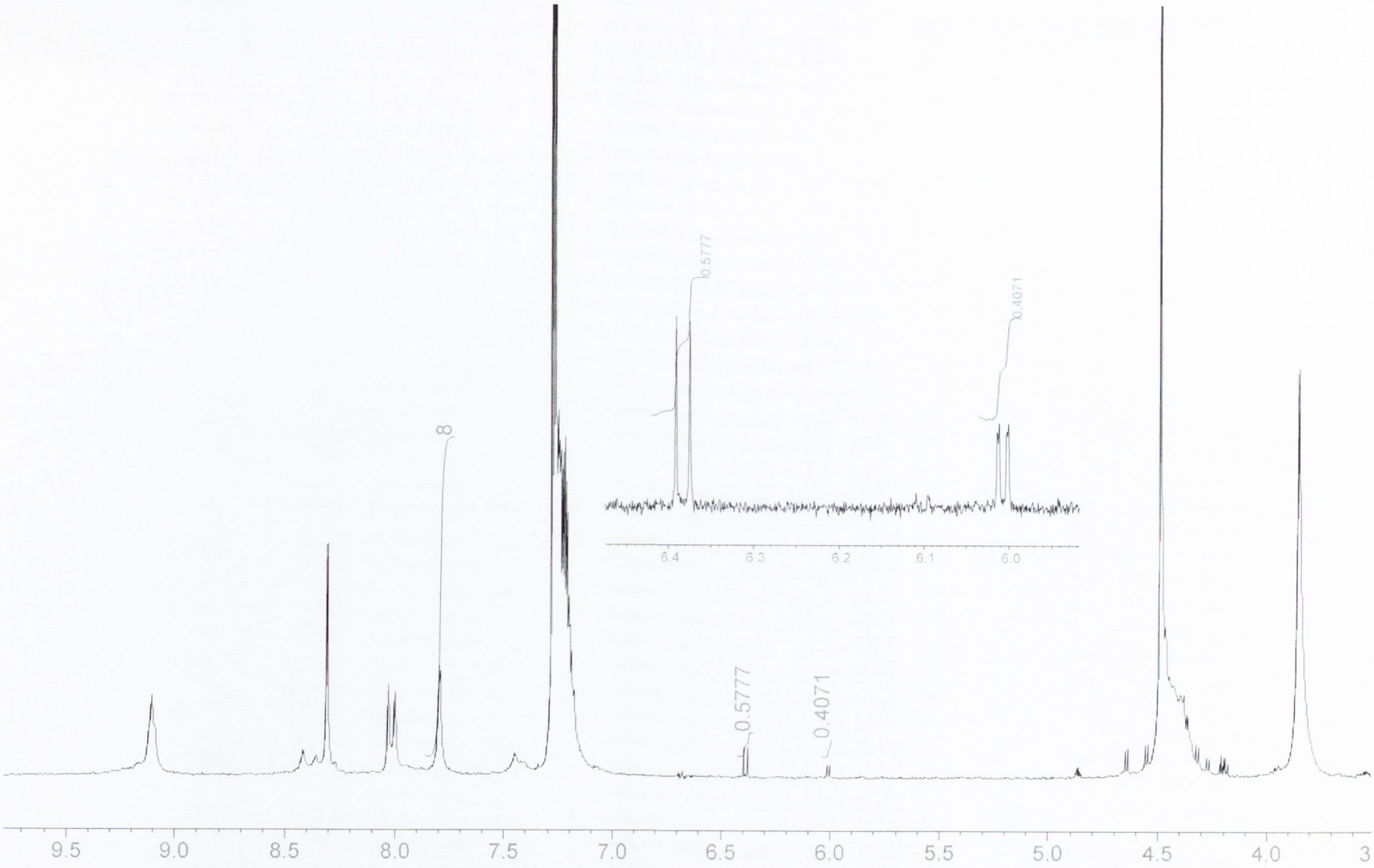
9
8
7
6
5
4
8
3.806
8.495
4.241
24.24


JC 90 (6.720)
Scan ES+
7.41e5
100
%
3
825.88
836.80
839.89
853.66
866.30
877.08
668.50
917.36
926.16
971.14
1024.65
650
675
700
725
750
775
800
825
850
875
900
925
950
975
1000
1025
1050
1075
1100
Da/e


8
11.6
4.071
24.01
12.02
3.732
8
10.0
9.5
9.0
8.5
8.0
7.5
7.0
10
9
8
7
6
5
4
3
2
1
0


JC 17 (1.292)
Scan ES+
5.23e7
A: 1654.68±1.16
B: 1683.47±0.61
100
%
0
A
1654.75
1655.25
1653.25
1652.75
1657.13
1657.50
1652.38
1657.88
1658.50
1651.38
1659.00
1659.38
1660.00
1650.25
1660.50
1649.88
B
1683.38
1505.88
1888.88
1861.38
1982.25
1500
1550
1600
1650
1700
1750
1800
1850
1900
1950
2000
2050
2100
2150
2200
mass


JC 17 (1.292)
Scan ES+
3.58e7
A: 1654.68±1.16
B: 1683.47±0.61
TJR/5/74-3
100
%
0
A4
414.97
A3
552.18
B4
422.03
497.45
502.90
473.06
B3
561.96
621.39
661.87
628.07
518.38
827.31
400
450
500
550
600
650
700
750
800
850
900
950
1000
1050
Da/e


8
0.5777
0.4071
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3
6.4
6.3
6.2
6.1
6.0

Experimental Section

3: All reactions were carried out in the presence of a small amount of BHT and, except for the lipoxygenase-catalyzed hydroperoxidation, under a nitrogen atmosphere. Methyl peracetal acid **7** was prepared from DHA as previously described for linoleic acid,[2a] and isolated by flash column chromatography, followed by drying over $MgSO_4$. After filtration, the solvent was evaporated at below 30 °C, and the resulting **7** (ca. 0.15 g, 0.4 mmol) dissolved in ethanol-free chloroform, followed by the addition of DCC (0.2 g, 0.9 mmol), dimethylaminopyridine (0.012 g, 0.09 mmol) and lyso-PC **8** (0.15 g, 0.3 mmol). The reaction mixture was stirred at room temperature for 48 h, and the product purified on a silica gel column ($CHCl_3$/MeOH/NH_3 (aq.) 60:30:1 to 50:30:3) to give phospholipid **9** (0.09 g, 30 % from **8**), which was then dissolved in THF/AcOH/H_2O (4:2:1) and stirred for 24 h, which achieved complete deprotection of the hydroperoxy group. The product thus obtained was purified by reversed-phase chromatography (ODS, $CHCl_3$/MeOH/H_2O, 4.5:100:5) to give high-purity **3** as a resinous solid (0.065 g, 7 % from **5**). ^1H NMR (500 MHz, $CDCl_3$): $\delta = 0.88$ (t, 3H; ω-CH_3), 0.95 (t, 3H; ω'-CH_3), 1.26 (m, 32H; $OCO(CH_2)_{16}CH_3$), 2.03 (m, 2H; H21'), 2.28 (m, 1H; one proton of C18'), 2.39 (m, 4H; H2' and H3'), 2.46 (m, 1H; one proton of C18'), 2.87 (m, 4H; H6' and H9'), 2.98 (m, 2H; H12'), 3.28 (s, 9H; $N(CH_3)_3$), 3.72 (m, 2H; OCH_2CH_2N), 3.97 (m, 2H; CH_3OP), 4.18 (m, 1H; one proton of $OCH_2CH(OR)CH_2OP$), 4.30 (m, 2H; OCH_2CH_2N), 4.38 (m, 2H; H17', one proton of $OCH_2CH(OR)CH_2OP$), 5.24 (brs, 1H; $OCH_2CH(OR)CH_2$-OP), 5.42 (m, 9H; H4', H5', H7', H8', H10', H11', H13', H19', H20'), 5.64 (dd, $J = 15$, 8 Hz, 1H; H16'), 6.10 (t, $J = 11$ Hz, 1H; H14'), 6.58 (dd, $J = 11$, 15 Hz, 1H; H15'); ES-MS: found: *m/z*: 866.6; calcd for $[C_{48}H_{84}NO_{10}P+H^+]$: *m/z*: 866.6.

Received: December 18, 2000 [Z16285]

[1] a) M. J. Gonzalez, R. A. Schemmel, J. I. Gray, L. Dugan, Jr., L. G. Sheffield, C. W. Welsch, *Carcinogenesis* **1991**, *12*, 1231–1235; b) O. A. Levander, A. L. Ager, Jr., V. C. Morris, R. G. May, *Am. J. Clin. Nutr.* **1989**, *50*, 1237–1239; c) E. Germain, V. Chajes, S. Cougnault, C. Lhuillery, P. Bougnoux, *Int. J. Cancer* **1998**, *75*, 578–583, and references therein.

[2] a) N. Baba, K. Yoneda, S. Tahara, J. Iwasa, T. Kaneko, M. Matsuo, *J. Chem. Soc. Chem. Commun.* **1990**, *18*, 1281; b) N. Baba, Y. Shigeta, Y. Kishida, S. Nakajima, S. Tahara, T. Kaneko, M. Matsuo, S. Shimizu, *Biosci. Biotechnol. Biochem.* **1993**, *57*, 1615.

[3] The corresponding methyl ester from EPA had $[\alpha]_D^{20} = -5$ ($c = 1.43$ in $CHCl_3$).

[4] M. Hamberg, B. Samuelsson, *J. Biol. Chem.* **1967**, *242*, 5320–5335.

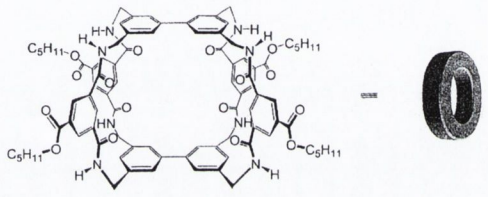
[5] By column chromatography on silica gel 60, eluted with MeOH/$CHCl_3$/aq. NH_3 (60:30:1).

[6] C. Zhu, T. Ohashi, T. Morimoto, A. N. Onyango, T. Kaneko, S. Shimizu, S. Nakajima, N. Baba, *J. Chem. Res. Synop.* **1999**, 500–501.

Gel-Phase MAS NMR Spectroscopy of a Polymer-Supported Pseudorotaxane and Rotaxane: Receptor Binding to an "Inert" Polyethylene Glycol Spacer**

Yiu-Fai Ng, Jean-Christophe Meillon, Theo Ryan, Andrew P. Dominey, Anthony P. Davis, and Jeremy K. M. Sanders*

The combination of magic-angle spinning (MAS) and high-resolution probe technology has provided chemists with a powerful technique for obtaining solutionlike ^1H NMR spectra of resin-bound samples.[1] As previously described, we have been successful in applying gel-phase MAS spectroscopy to the study of metal–ligand interactions at the solid–liquid interface.[2] Following this success, we set out to extend our understanding of other interactions, such as those between the tricyclic polyamide receptor **1** and carbohydrates. However, during the course of this study, we discovered an unexpected phenomenon which led to the synthesis of a resin-bound rotaxane as described below.



1

Macrocycle **1** is a good receptor for octyl pyranosides.[3] Therefore, a galactose residue was attached onto ArgoGel resin through the primary hydroxide group, so that **1** would bind to the sugar unit and allow us to study the binding between the solid and liquid interfaces. ArgoGel resins contain highly flexible polyethylene glycol (PEG) chains (30–40 units) appended to a 1 % cross-linked polystyrene bead; the flexibility of these PEG chains allows us to obtain solutionlike NMR spectra using MAS techniques. An NMR titration was performed to investigate for possible binding between **1** and the solid-supported galactoside. However, this titration unexpectedly showed a splitting of the PEG reso-

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School of Chemistry, University of Bristol
Bristol BS8 1TS (UK)

[**] We thank the EPSRC and Enterprise Ireland as well as the EU TMR programme for financial support.

nance (Figure 1). Since the binding between the macrocycle and the sugar is reversible, there might be a possibility that the macrocycle could dissociate from the sugar unit into the PEG

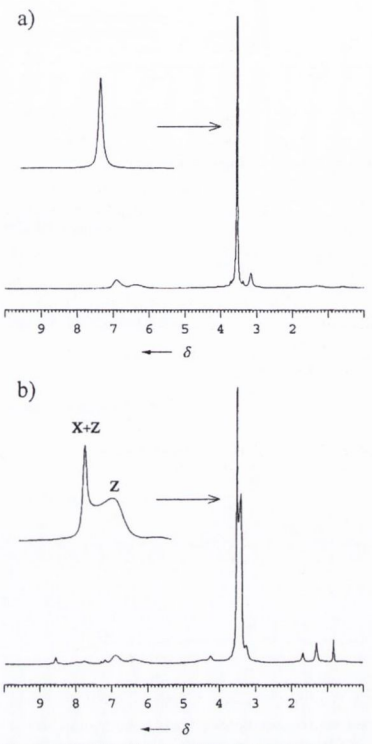
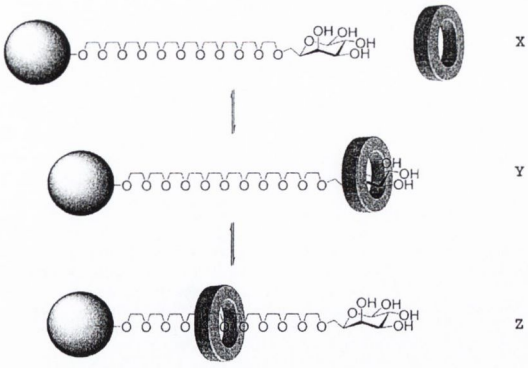


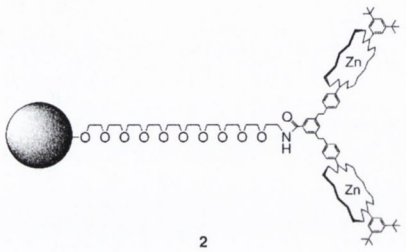
Figure 1. 400 MHz ^{1}H MAS NMR spectra of galactoside terminated beads (a) the and after addition of one equivalent of **1** (b). A splitting of the PEG resonance is observed. **X** and **Y** correspond to the combination of free PEG chain and sugar-bound macrocycle, and **Z** corresponds to the threaded macrocycle.

chain of the ArgoGel, thus resulting in threading (Scheme 1). Harada and co-workers[4] and Wenz and Keller[5] have observed a similar threading of α-cyclodextrin onto PEG chains. However, another possibility was that **1** might bind to the side of the PEG chain, with the ring current from **1** thus causing an upfield shift of the PEG resonance.

Three control experiments were carried out to explore the possibility of threading: 1) Porphyrin-terminated ArgoGel beads **2** were titrated with **1**, and showed no significant changes in the PEG resonance. This result suggests both that the porphyrin dimer attached to the end of the PEG chain acts as a stopper, thus preventing **1** from threading, and that **1** does not bind to the PEG chain on the side. 2) Unfunctionalized ArgoGel-Cl beads were titrated with **1**, and gave a broadened and upfield-shifted ($\Delta\delta = 0.6$ ppm) PEG resonance. Upon addition of glucoside, the resonance for the PEG chain sharpened and shifted towards the original chemical shift.



Scheme 1. Proposed binding modes of receptor **1** to galactosyl beads.



2

These observations indicate that **1** was threaded around the PEG chain in the first instance which caused a broadening and a change in the chemical shift. This effect was then reversed by the addition of octyl glucopyranoside. Since **1** is known to be a good receptor for sugars, the presence of glucoside causes **1** to dethread from the PEG chain, so that the broadened and upfield-shifted PEG resonance moved downfield and sharpened (Figure 2). 3) An NMR titration between hexaethylene glycol and **1** was carried out in solution, and a binding constant of approximately 9000 M^{-1} was observed. This value indicates that polyethylene glycol does bind to **1**. The NOESY spectrum of the 1:1 mixture of **1** and galactosyl beads showed NOE cross-peaks between the galactosyl proton and the aromatic protons of **1**. These experiments suggested that the three proposed modes **X**, **Y**, and **Z** in Scheme 1 do co-exist. The splitting of the PEG resonance shown in Figure 1b might arise from slow exchange between the bound, unbound, and threaded macrocycle, or from some of the PEG chains being inaccessible as a result of cross-linking, a process that might occur during chemical modification.

To further support our interpretation and demonstrate the analytical power of gel-phase MAS NMR we prepared the porphyrin-stoppered rotaxane **3** as shown in Scheme 2. The ^{1}H MAS NMR spectrum showed resonances of porphyrin dimer and **1**, but most importantly the splitting of the PEG resonance was once again observed. The outer aromatic protons of **1** were now inequivalent as a result of the large ring current of the porphyrin stopper making one side of the

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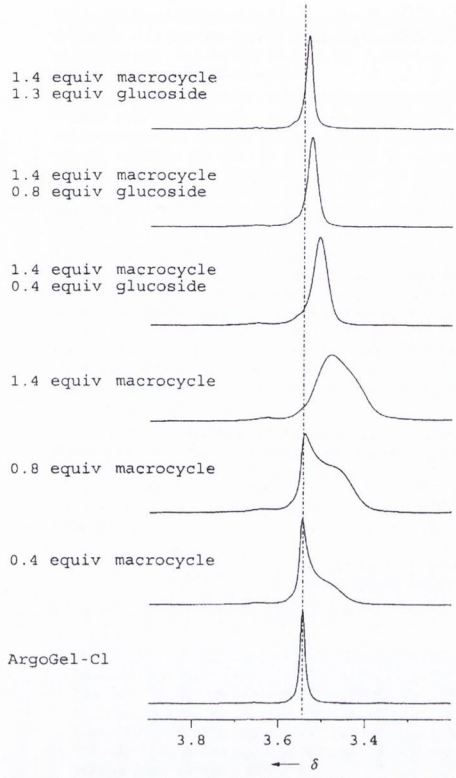
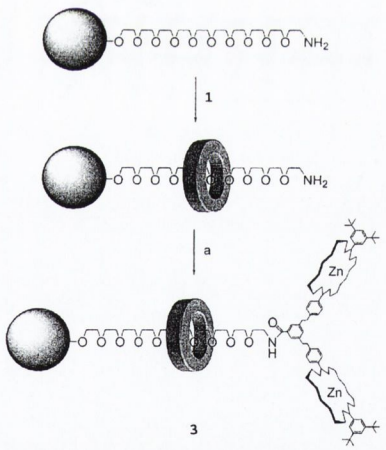


Figure 2. Control experiment showing the competition between the PEG chain of ArgoGel and octyl glucopyranoside for macrocycle **1**.



Scheme 2. Synthesis of porphyrin-stoppered rotaxane. a) Porphyrin dimer, TBTU, and $(i\text{Pr})_2\text{NH}$ in chloroform.

macrocycle different from the other. The COSY MAS NMR spectrum allowed assignment of the pentyl side chains of **1** and the NOESY spectrum gave NOE cross-peaks between the PEG chain and the aromatic protons of **1** and between the PEG chains and the pentyl groups of **1** (Figure 3). Since the beads were extensively washed with chloroform and methanol, no free macrocycle should be trapped inside the beads. In any case, we would not expect to detect NOEs between the PEG chains and the free macrocycle. We conclude that **1** is held on the PEG chain as a rotaxane and stoppered by the porphyrin dimer.

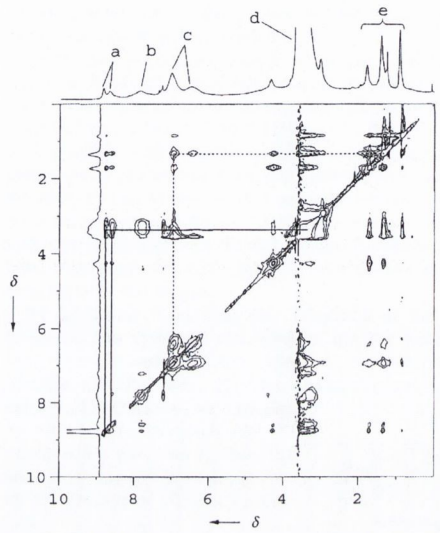


Figure 3. NOESY MAS NMR spectrum of porphyrin-stoppered rotaxane **3** showing NOE cross-peaks between the PEG chain and the outer aromatic protons of **1** (solid line), and between the polystyrene backbone of the ArgoGel and the pentyl side chain of **1** (dotted line). The 1D spectrum shown on the F2 axis is the ^{1}H MAS NMR spectrum of **3**, and the 1D spectrum shown inside the box is a cross-section taken from the NOESY experiment at $\delta = 8.87$. Resonances a correspond to the inequivalent outer aromatic protons of **1** (see text), and resonances b, c, d, and e correspond to the other aromatic protons of **1**, the polystyrene backbone of ArgoGel, PEG, and the pentyl side chains of **1**, respectively.

In conclusion, we have successfully used MAS NMR spectroscopy to confirm the possibility of a tricyclic polyamide receptor threading around the PEG chain of ArgoGel. This observation has led us to the synthesis of a solid-supported rotaxane. Further study, using different macrocycles and stoppers, is currently underway.

Experimental Section

NMR spectra were acquired at room temperature on a Bruker DRX400 spectrometer using a Bruker HR MAS probe. Rotors containing a suspension of the beads in a mixture of $CDCl_3$ and CD_3OD (92:8) were spun at 4 kHz. One-dimensional spectra were obtained using 64 scans and titrations were carried out using 10 μL injections of **1** (ca. 0.2 equiv). NOESY MAS NMR spectra were obtained at room temperature with

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suppression at the PEG resonance (70 dB), a 200 ms mixing time, 32 scans, and 512 increments.

The solution-state NMR titration between hexaethylene glycol and **1** was carried out at room temperature by monitoring the inner aromatic proton of the spacer unit. The results showed that the stoichiometry corresponds approximately to a 1:1 binding model.

Galactosyl beads: 1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose (ca. 50 equiv with respect to the loading of Argogel-Cl) was treated with sodium hydride (ca. 100 equiv) in tetrahydrofuran. After filtration of the mixture, the filtrate was added to ArgoGel-Cl (0.41 – 0.45 mmol g^{-1}) and shaken for 2 days. The resulting beads were washed and deprotected with a mixture of trifluoroacetic acid and water (9:1, 2 mL). Washing the beads with tetrahydrofuran, methanol, and chloroform afforded the galactosyl beads.

2: A solution of a rigid porphyrin dimer[6] (50 mg) and palladium on carbon (50 mg) in tetrahydrofuran (25 mL) was stirred under hydrogen for 24 h. Filtration of the mixture through celite and removal of the solvent afforded a flexible porphyrin dimer. A mixture of ArgoGel-NH_2 (13 mg, 0.41 – 0.45 mmol g^{-1}), flexible porphyrin dimer (30 mg), TBTU (4 mg), and diisopropylamine (3 mg) in a mixture of *N,N*-dimethylformamide (1 mL) and dichloromethane (0.5 mL) was shaken for 4 days. The solvent was then removed by filtration and the beads were washed extensively with dichloromethane and methanol.

3: A mixture of the flexible porphyrin dimer (22 mg), TBTU (5 mg), and diisopropylamine (3 mg) in chloroform (1 mL) was added to a mixture of **1** (12 mg) and ArgoGel-NH_2 (15 mg) in chloroform (1 mL). The resulting mixture was shaken for 1 day. The solvent was then removed by filtration and the beads were washed extensively with dichloromethane and methanol.

Received: September 22, 2000
Revised: January 30, 2001 [Z15846]

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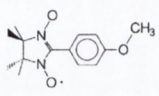
Cobalt(II)-Nitronyl Nitroxide Chains as Molecular Magnetic Nanowires**

Andrea Caneschi, Dante Gatteschi,* Nikolia Lalioti, Claudio Sangregorio, Roberta Sessoli, Giovanni Venturi, Alessandro Vindigni, Angelo Rettori, Maria G. Pini, and Miguel A. Novak

The observation of slow magnetic relaxation in molecular clusters is now considered as one of the most important achievements of molecular magnetism of the last few years.[1] In fact systems like Mn12, Fe8 have provided unique opportunities for the investigation of molecular magnetic hysteresis,[2] quantum tunneling of the magnetization,[3, 4] and of phase interference (Berry phase).[5] All these features depend on slow magnetic relaxation.

While clusters can be considered as zero-dimensional materials, in principle slow relaxation of the magnetization can also be expected in one-dimensional (1D) materials, as suggested by Glauber in 1963.[6] However, to date, it has not been possible to observe this behavior because no suitable experimental system has been produced. In fact the conditions to be met to observe slow magnetic relaxation in 1D materials are rather stringent: 1) the ratio of the interaction within the chain, J, and that between chains, J', must be rather high, larger than 10^4; 2) the material must behave as a 1D Ising ferro- or ferrimagnet.

We have now found that slow relaxation of the magnetization and hysteresis effects which are not associated with three-dimensional (3D) order can be observed in $[Co(hfac)_2(NITPhOMe)]$ (**1**; hfac = hexafluoroacetylacetonate, NITPhOMe = 4′-methoxy-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide). We want to show that these findings provide an experimental confirmation of Glauber's prediction.



NITPhOMe

Complex **1** consists of alternating $Co(hfac)_2$ and radical moieties arranged in 1D arrays with a helical structure arising from the trigonal crystallographic

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[**] The financial support of Italian MURST and CNR and of Brazilian CNPq and FUJB is acknowledged. The support from the European Community through the TMR program 3MD (contract no ERB4061PL97-0197) is also acknowledged.

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