



Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

Synthetic Approaches Towards Thioester-Mediated Bioconjugation and Lanthanide-Based Glycoconjugate Probes



**Helen M. Burke, B.A. (Mod.)
October 2016**

**Trinity College Dublin
The University of Dublin**

**Based on research carried out under the direction of Prof. Eoin
M. Scanlan and Prof. Thorfinnur Gunnlaugsson**

*A thesis submitted to the School of Chemistry, Trinity College
Dublin, The University of Dublin, for the degree of Doctor of
Philosophy*



Thesis 11361

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

I agree to deposit this thesis in the University's open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.



Declaration

This thesis is submitted for the degree of Doctor of Philosophy to the University of Dublin, Trinity College and has not been submitted before for any degree or examination to this or any other university. Other than where acknowledged, all work described herein is original and carried out by the author alone. Permission is granted so that the Library may lend or copy this thesis upon request. This permission covers only single copies made for study purposes, subject to normal conditions of acknowledgment.



Abstract

This thesis, entitled “Synthetic Approaches Towards Thioester-Mediated Bioconjugation and Lanthanide-Based Glycoconjugate Probes” is divided into six chapters. Chapter 1, the introduction, firstly introduces the concept of bioconjugation and the use of conjugation strategies to improve current therapeutics. A brief overview of two protein post-translational modifications (PTMs), ubiquitination and glycosylation is then given with the implications of such bioconjugation discussed. Following this, the importance of access to sufficient quantities of homogeneous, post-translationally modified glycopeptides is described with the formation of *O*- and *N*-linked glycopeptides discussed, both biosynthetically and chemically. The remainder of this chapter is divided into two sections; the first of which serves to introduce peptide synthesis. The significant advances in peptide synthesis and chemical ligation strategies are outlined with their use for the incorporation of post-translational modifications included. Particular emphasis is placed on native chemical ligation (NCL) with some of the strategies introduced to address NCLs main limitations reviewed. The last section of this chapter delivers an overview of the carbohydrate-active glycosidase enzymes with their synthetic applicability briefly mentioned. The chapter concludes by outlining examples of the current glycoconjugate probes used for the detection of the biologically relevant glycosidase enzymes.

Chapter 2 begins with a discussion of the prevalent PTM *N*-glycosylation and summarises some of the most common methods currently used for the preparation of homogenous *N*-linked glycopeptides. This chapter goes on to present a novel ligation strategy toward the synthesis of *N*-linked glycopeptides. This synthetic approach combines thiol-ene click chemistry and an *S,N*-acyl transfer reaction to ligate a carbohydrate and non-glycan moiety to form a native amide bond at the carbohydrate anomeric centre. The first examples of complex thioacids being employed in thiol-ene reactions are presented. Various thioacids varying in steric hindrance (e.g. alkyl, phenyl, and peptidyl) were prepared to investigate the substrate scope of the thiol-ene reaction when incorporating thioacids. In successfully ligated products, a high

degree of product conversion was observed with complete stereoselectivity achieved.

Chapter 3 covers ubiquitination, another common PTM, with some of the current synthetic methods toward non-native ubiquitin (Ub) conjugates initially presented. This chapter then focuses on the application of the ligation methodology developed in Chapter 2 for its use in the chemical ligation of peptide units. A biologically relevant tripeptide sequence was prepared and its ability to undergo thiol-ene ligations with a Ub related thioacid evaluated. A subsequent *S,N*-acyl transfer reaction was conducted with its efficiency over a large transition state (18-membered) determined. This method incorporates thiol-ene coupling chemistry to overcome the requirement for cysteine residues in conventional NCL and as a speedy alternative to the slow transthioesterification reactions employed in other peptide ligation strategies.

Chapter 4 begins with a brief introduction to the lanthanide metal ions and their photophysical properties with some of the current lanthanide-based probes used for the detection of enzymes discussed. The chapter then presents the design, synthesis, and photophysical evaluation of an array of lanthanide-based complexes and their ability to function as luminescent probes for observing glycosidase enzyme activity in real time. All of the lanthanide complexes are based on the macrocyclic framework cyclen with six of those being *O*-glycosylated, each bearing different carbohydrate moieties. The effect of the concentration on the emissive properties of the glycosylated lanthanide-based probes was investigated. The chapter concludes with preliminary work into the use of the mannose-appended lanthanide complex for the detection of lectin ConA.

Chapter 5 concludes the work described in this thesis.

Lastly, Chapter 6 outlines the experimental procedures and presents the synthesis and characterisation of the compounds discussed within this thesis.

Abbreviations

μL	Microlitre
μM	Micromolar
4-NP	4-nitrophenol
ABPs	Activity-based probes
ACE	Aggregation-caused emission
ACQ	Aggregation-caused quenching
AIBN	2,2'-azobisisobutyronitrile
AIE	Aggregation-induced emission
Ala	Alanine
AML	Auxiliary-mediated ligation
APCI	Atmospheric pressure chemical ionisation
app	Apparent
Asn	Asparagine
Asp	Aspartic acid
ATP	Adenosine triphosphate
br	Broad
BSA	Bovine serum albumin
CAZy	Carbohydrate-active enzymes
Cbz-OSu	<i>N</i> -(benzyloxycarbonyloxy)succinimide
CDI	1,1'-carbonyldiimidazole
ConA	Concanavalin A
COSY	Correlation spectroscopy
cyclen	1,4,7,10-tetraazacyclododecane
Cys	Cysteine
d	Doublet
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DIC	Diisopropyl carbodiimide
DIEA	<i>N,N</i> -diisopropylethylamine
DM-2	Diabetes mellitus-2
DMAP	4-Dimethylamino pyridine
DMF	<i>N,N</i> -dimethylformamide
DMS	Dimethyl sulfide

DMSO	Dimethylsulfoxide
DPAP	2,2-dimethoxy-2-phenylacetophenone
DUB	Deubiquitinating enzyme
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EPL	Expressed protein ligation
EPO	Erythropoietin
ER	Endoplasmic Reticulum
ESI	Electrospray ionisation
ET	Energy transfer
EtOAc	Ethyl acetate
exSAL	Extended sugar-assisted ligation
FDG	Fluorescein di- β -D-galactopyranoside
Fmoc	9-fluorenylmethyloxycarbonyl
FRET	Fluorescence resonance energy transfer
Fuc	Fucose
Gal	Galactose
GlcNAc	<i>N</i> -acetylglucosamine
Glu	Glutamic acid
Gly	Glycine
GOPAL	Genetically encoded orthogonal protection and ligation
GSH	Gluthathione
h	Hour
HCC	Hepatocellular carcinoma
HER2	Human epidermal growth factor receptor 2
Hex	Hexane
HMBC	Heteronuclear multiple bond correlation
HOBt	1-hydroxybenzotriazole
HOSu	<i>N</i> -(hydroxy)succinimide
HRMS	High resolution mass spectrometry
Ile	Isoleucine
IR	Infrared
ISC	Intersystem crossing
<i>J</i>	Coupling constant

K	Kelvin
KCL	Kinetically controlled ligation
LAP	Lithium acyl phosphinate
LBs	Lewy Bodies
LEAPT	Lectin-directed enzyme-activated prodrug therapy
Leu	Leucine
LLO	Lipid-linked oligosaccharide
Ln(III)	Trivalent lanthanide metal ion
LRET	Luminescence resonance energy transfer
LSD	Lysosomal storage disease
Lys	Lysine
m	Multiplet
<i>m/z</i>	Mass to charge ratio
MALDI	Matrix-assisted laser desorption/ionisation
MAP	4-methoxyacetophenone
MCL-1	Myeloid cell leukemia-1
Met	Methionine
MHz	Megahertz
mL	Millilitre
Mp	Melting point
MRI	Magnetic resonance imaging
ms	Millisecond
MUC1	Mucin-1
NBS	<i>N</i> -bromosuccinimide
NCL	Native chemical ligation
NGP	Neighbouring group participation
NMR	Nuclear magnetic resonance
<i>N</i> -Phth	<i>N</i> -phthalimido
OSTase	Oligosaccharyltransferase
PD	Parkinson's disease
ppm	Parts per million
Pro	Proline
ps	Picosecond

PTM	Post-translational modification
q	number of metal bound water molecules
RITC	Rhodamine-B-isothiocyanate
RNase	Ribonuclease
rt	Room temperature
S_1	Singlet excited state
SAL	Sugar-assisted Ligation
SAR	Structure-activity relationship
SCAL	Side-chain assisted ligation
Ser	Serine
SPPS	Solid phase peptide synthesis
t	Time or triplet (as relevant)
T_1	Triplet excited state
TBAF	Tetrabutylammonium fluoride
TBDMS	<i>t</i> -butyldimethylsilyl
TBDPS	<i>t</i> -butyldiphenylsilyl
TCA	Trichloroacetimidate
TES	Triethylsilane
TFA	Trifluoroacetic acid
TFA	Trifluoroacetic acid
TFMSA	Trifluoromethanesulfonic acid
Thr	Threonine
TLC	Thin layer chromatography
TOCSY	Total correlation spectroscopy
TPE	Tetraphenylethene
Trp	Tryptophan
U	Units of enzyme
Ub	Ubiquitin
UCH	Ubiquitin C-terminal hydrolase
UDP	Uridine diphosphate
UV	Ultraviolet
Val	Valine
Xyl	Xylose

α -syn	α -synuclein
β Gal	β -galactosidase
β Glc	β -glucosidase
β Glu	β -glucuronidase
τ	Luminescent lifetime
τ_{D_2O}	Lifetime in deuterated water
τ_{H_2O}	Lifetime in non-deuterated water

Acknowledgements

Firstly, I would like to thank my two supervisors Prof. Eoin Scanlan and Prof. Thorfinnur Gunnlaugsson for giving me the opportunity to their work within their research groups and for the support they have provided me, both personally and professionally, throughout the past 4 years.

I would like to thank the Irish Research Council and the School of Chemistry, Trinity College Dublin for providing financial support for this research.

My deepest gratitude to the technical staff at Trinity College who donated so much of their time to help me along the way! Thanks to Dr. John O'Brien and Dr. Manuel Reuther for NMR assistance and to Dr. Martin Feeney and Dr. Gary Hessman for HRMS measurements.

I feel very fortunate to have been part of two research groups which gave me the chance to form many more friendships. Within the Scanlan lab, many thanks to the past members: Jean, Claire, Robin, Lyn, Aoife, Stefan and Vincent for welcoming me into the lab when I first began. A massive thank you to the current members of the Scanlan group with whom I have shared most of my PhD experience with; to Danielle, Moss, Lauren, Adam, Mimmi, Daniella, and Leticia. Thanks for keeping the lab fun and providing much needed support and friendship over the past 4 years! Also a special thanks to Danielle and Lauren for also donating their time in proof reading sections of this document!

Within the Gunnlaugsson group – both past and present: Ana, Oxana, Chris, Emma, Dawn, Esther, Joe, Jenn, Eoin, Bjorn, Amy, Dermot, Sandra, Hannah, Isabel, and Bruno. A particular thanks to Oxana who assisted me with alot of problems along the way and to Esther and Chris for reading sections of this document.

A special thanks to Sam, Sachi, and Fergus with whom I shared the thesis writing experience with! Thank you for all the support, laughs, and tea/tear breaks over the past 3 months!

Also thanks to the other groups in the Chemistry department especially Michelle, Judith, and Emiliano for the breaks away from the writing and assuring me there was more to life than another failed reaction.

A massive thanks to Michela, Andy, and Paddy. There have been so many ups and downs over the past few years but thanks for your support, humour, and love! Please never stop telling me to stop being unreasonable!!

Lastly, the biggest gratitude to my Dad, Conor, John, and Sinead and all my Wicklow girls for their unconditional support, understanding, and for spurring me on to keep going over the past 4 years! Im sure you'll be just as happy to know its almost over ☺

Publications

H. M. Burke, T. Gunnlaugsson and E. M. Scanlan. "Recent advances in the development of synthetic chemical probes for glycosidase enzymes". *Chem. Commun.*, 2015, **51**, 10576.

H. M. Burke, T. Gunnlaugsson and E. M. Scanlan. "Glycosylated lanthanide cyclen complexes as luminescent probes for monitoring glycosidase enzyme activity". *Org. Biomol. Chem.*, 2016, **14**, 9133.

Contents

Abstract	i
Abbreviations.....	iii
Acknowledgements.....	viii
Publications	ix

Chapter 1 - Introduction

1.0 Introduction.....	1
1.1 The importance of conjugation in chemical biology.....	1
1.1.1 Protein post-translational modifications	4
1.1.1.1 Ubiquitination.....	5
1.1.1.2 Glycosylation	6
1.2 Synthesis of glycopeptides	8
1.2.1 Glycoprotein diversity and the need for homogeneity.....	8
1.2.2 <i>O</i> -linked glycans.....	9
1.2.2.1 Biosynthesis	9
1.2.2.2 Chemical synthesis	10
1.2.3 <i>N</i> -linked glycans	12
1.2.3.1 Biosynthesis	12
1.2.3.2 Chemical synthesis	13
1.2.4 Synthesis of non-native glycoconjugates	17
1.2.4.1 Radical chemistry used in the synthesis of non-native glycopeptides	17
1.2.4.1.1 Thiol-ene click	18
1.3 Peptide synthesis	21
1.3.1 Formation of the amide bond	21
1.3.2 Solid phase peptide synthesis vs. solution phase synthesis.....	21
1.3.2.1 Linear vs. convergent	23
1.3.3 Ligation strategies commonly used for the synthesis of peptides and glycopeptides	24
1.3.3.1 Native chemical ligation	24
1.3.3.1.1 Auxiliary mediated ligation.....	26
1.3.3.1.2 Desulfurisation.....	27
1.3.3.1.3 Kinetically controlled ligation	29

1.3.3.2 Native chemical ligation for the synthesis of glycopeptides	30
1.3.3.2.1 Synthesis of thioesters	30
1.3.3.2.2 Examples of glycopeptides prepared by native chemical ligation	32
1.3.3.3 Sugar-assisted ligation	33
1.3.3.4 Expressed Protein Ligation	35
1.3.4 Formation of the ubiquitin isopeptide bond	37
1.3.4.1 Enzymatic	37
1.3.4.2 Chemical synthesis	38
1.4 Enzymatic processing of glycopeptides	39
1.4.1. The glycosidase enzymes	39
1.4.2 Glycosidase enzymes used in the chemoenzymatic synthesis	41
1.4.3 Probes used for the detection of glycosidases	43
1.4.3.1 Chromogenic substrates	44
1.4.3.2 Fluorogenic substrates	45
1.4.3.3 Lanthanide ions	46
1.4.3.3.1 Probes based on lanthanide luminescence	48
1.4.3.3.2 Probes based on lanthanide relaxivity	48
1.5 Work described within this thesis	51

Chapter 2 - Thiol-ene mediated synthesis of *N*-linked glycosyl amides

2.0 Introduction	54
2.1 Chemical synthesis of <i>N</i> -linked glycopeptides	54
2.2 Aims of this work	56
2.3 Synthesis of glycosyl alkene acceptor	58
2.3.1 Reduction of the anomeric azide	60
2.3.2 Initial thiol-ene ligation with thioacetic acid	63
2.4 Synthesis of peptidyl thioacid donors	66
2.4.1 Synthesis of <i>S</i> -trityl protected thioacid	68
2.4.2 Synthesis of EPO tripeptide thioacid sequence	69
2.5 Attempted one-pot procedure using thioacids and glycosylamine	72
2.5.1 Glycosylamine hydrolysis	74
2.6 Thioacid derivatives to probe scope of the methodology	75

2.6.1 CSO elimination	77
2.6.2 Synthesis of glutamic acid thioacid	78
2.7 Reduction of azido after thiol-ene ligation	80
2.7.2 Introduction of a bulky <i>N</i> -phthalimido protecting group	82
2.8 Conclusion	85

Chapter 3 - Thiol-ene mediated approach towards ubiquitinated peptides

3.0 Introduction.....	87
3.1 Chemical synthesis of native Ub and Ub-conjugates.....	87
3.2 Chemical synthesis of Ub polymers.....	89
3.3 Aims of this work.....	92
3.4 Synthesis of α -synuclein associated tripeptide unit	94
3.4.1 Azides and the diazo transfer reaction	94
3.5 Route towards tripeptide incorporating a silyl protecting group	98
3.6 Esterification of Ser using 4-pentenoyl chloride	99
3.7 Initial thiol-ene ligation using thioacetic acid and dipeptide.....	101
3.8 Reduction of the azide group	102
3.9 One-pot: azide reduction, <i>S,N</i> -acyl shift and base hydrolysis	105
3.9.1 Possible <i>O,N</i> -acyl transfer.....	108
3.10 Application to α -synuclein tripeptide and Gly thioacid	109
3.11 Conclusion	111

Chapter 4 - *O*-Glycosylated lanthanide-based cyclen complexes

4.0 Introduction.....	114
4.1 Biological importance of the glycosidase enzymes	114
4.2 General introduction to the lanthanides.....	115
4.2.1 Photophysical properties of the lanthanides	116
4.2.2 Lanthanide luminescent quenching	118
4.2.3 Examples of lanthanide-based luminescent bioprobes	119
4.3 Aims of this work.....	121
4.4 Synthesis of β -D-glucose appended ligand 192	122
4.4.1 Unexpected alkylation reaction	127

4.5 Synthesis and characterisation of β -D-glucose Ln(III) complexes ...	128
4.6 Glycosylated Tb(III) complexes prepared	130
4.7 Synthesis of control complex	132
4.8 Synthesis of phenolic complex	132
4.9 Photophysical evaluation of Ln(III) complexes	134
4.9.1 Determination of metal bound water molecules	136
4.9.2 Response of 195.Tb as a function of pH.....	138
4.10 Response of 195.Tb in the presence of β Glc	139
4.10.1 Enzymatic experiments conducted at high concentrations.....	140
4.10.2 Unusual behaviour observed in solution	142
4.10.3 Determination of metal bound water molecules at high substrate concentrations.....	142
4.10.4 Hypothesis for the observed behaviour	143
4.11 Probes used for the detection of lectins	144
4.12 The use of 210.Tb as a probe for lectin, ConA.....	147
4.12.1 Hypothesis for the observed behaviour	149
4.15 Conclusion	150

Chapter 5 - Overall conclusions

5.0 Overall conclusions	152
-------------------------------	-----

Chapter 6 - Experimental

6.0 General experimental details.....	155
6.1 UV-Visible absorption and luminescence spectroscopy	156
6.2 Luminescence lifetime measurements.....	156
6.3 General nomenclature of D- and L-sugars for characterisation.....	156
6.4 General experimental procedures.....	156
6.5 Experimental details for Chapter 2.....	161
6.6 Experimental details for Chapter 3.....	200
6.7 Experimental details for Chapter 4.....	224

Chapter 7 - References

7.0 References.....	266
---------------------	-----

1.0 Introduction

The chemical synthesis and *in vitro* detection of biologically-important proteins are two areas of intense research. The chemical synthesis of proteins and their bioconjugates allows precise control over molecular structure, with the added advantage of introducing unnatural chemical modifications. Furthermore, enzymes are known to play a key role in the pathology of several diseases, with enzyme dysregulation known to occur in many disease states.¹ Therefore, the ability to accurately detect the activity of biologically-relevant enzymes has been a focus of many research groups.

This chapter begins with an overview of protein glycosylation and ubiquitination, two common PTMs. Next, various chemical methods to obtain homogeneously modified peptides are presented, with a focus on both chemical ligation strategies and radical chemistry. In the last section of this chapter, the glycosidase enzymes which are involved in the post-translational modification of proteins are discussed, with their application in chemo-enzymatic synthesis followed by a short review of the various probes used for the detection of these enzymes detailed.

1.1 The importance of conjugation in chemical biology

Bioconjugation refers to the covalent derivatisation of molecules to form biomolecules. This covalent coupling of different types of molecules leads to novel biomolecules of different properties.² Nature introduces functionality through various protein PTMs. For example, protein proteolysis is regulated by the addition of ubiquitin (Ub) units to protein targets; the attachment of lipids results in membrane anchoring; and protein phosphorylation is frequently involved in signal transduction.³ In order to study the effect these modifications have on the function of a protein, access to homogeneously modified proteins is required and the ability to modify biomolecules with a high degree of selectivity and specificity is needed. The concept of conjugation with biomolecules to relay information, or as a strategy to improve the bioavailability of therapeutics, has been widely employed.⁴ It was originally reported in 1929 that immunogenicity of carbohydrates could be enhanced by conjugation to a protein and this is now well established.⁵ Conversely, a peptides biological activity can also be enhanced by conjugation to

carbohydrates. For example, a study which separated different ribonuclease (RNase) B glycoforms demonstrated that the glycosylation caused decreased flexibility and greater resistance toward proteases compared with the non-glycosylated forms, possibly due to a glycan “steric shield” formed through glycosylation.⁶

During the past decade there has been a surge of interest in biologic-based therapeutics with protein-based drugs representing approximately a quarter of new drug approvals with many being glycopeptides.^{7,8} Insulin, a protein hormone, used primarily in the treatment of diabetes suffers from being broken down within hours in the body, thus treatment requires frequent injections. Nishimura and co-workers incorporated sialic acid residues into mutant insulins resulting in prolonged activity through the prolongation of serum lifetimes.^{9,10} Furthermore, it was shown that the activity of the peptide-based anti-diuretic arginine-Vasopressin is almost doubled by a simple galactosylation.¹¹

A bioconjugation approach to drug delivery known as lectin-directed enzyme-activated prodrug therapy (LEAPT) was reported by Davis and co-workers, the mechanism of which is depicted in Figure 1.1.¹² This approach allowed for the targeted delivery of the anti-cancer agent, doxorubin, to hepatocytes in a mouse liver model. The “glycotargeting” bipartite approach takes advantage of the lectins which are proteins containing specific carbohydrate recognising domains. Firstly, a non-mammalian glycosidase enzyme, rhamnosidase, is conjugated to a mammalian targeting carbohydrate. This is then taken into the cell by sugar-mediated endocytosis by a hepatocyte membrane bound lectin. The second step involves the administration of doxorubin capped with L-rhamnose. The doxorubin drug can then be released by the removal of the L-rhamnose cap by the previously administered rhamnosidase enzyme, allowing for targeted release of the pro-drug.

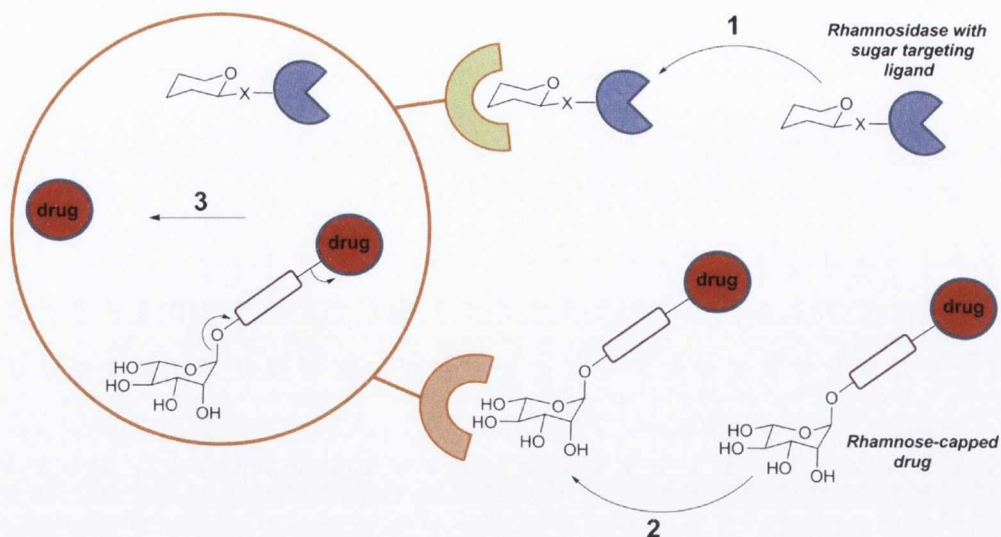


Figure 1.1: LEAPT strategy (Image adapted from Davis *et. al.*).¹³ (Step 1) Site-selective delivery of a glycosylated rhamnosidase enzyme; (Step 2) Delivery of a rhamnose-capped prodrug; (Step 3) Site-selective activation of the prodrug by the previously administered rhamnosidase enzyme.

Bioconjugates encompassing Ub, a common PTM, have been explored as a delivery vehicle for peptide-based anti-cancer drugs. Ub is a natural ligand for CXCR4 which is a chemokine receptor overexpressed in many tumours, with the expression level of CXCR4 found to correlate with the aggressiveness of the cancer. Lin and co-workers reported the conjugation of Ub to NoxaBH3 peptide, a potent inhibitor of MCL-1 (Myeloid Cell Leukemia-1), a key gene involved in cancer cell survival.^{14,15} The NoxaBH3 peptide-Ub conjugate showed increased inhibitory activity and selective ablation of CXCR4-expressing cancer cells suggesting that the Ub/CXCR4 axis may serve as a good route for the targeted delivery of anti-cancer drugs. Furthermore, the development of bioconjugate probes has contributed to the current understanding of enzymes in biological processes, as they provide insight into enzyme activity and substrate recognition. For example, synthetic probes which consist of a carbohydrate or Ub appended to a reporter molecule can be used to detect the action of glycosidase or deubiquitinating enzymes (DUBs), respectively, through the release of a reporter molecule upon enzyme action.^{16,17} The use of glycosidase activated chemical probes will be discussed in more detail in Section 1.4.3.

1.1.1 Protein post-translational modifications

It is estimated that the human genome consists of 20,000-25,000 genes while the human proteome is comprised of over 1 million proteins.¹⁸ The increased complexity observed on going from a gene to a protein is furthered by a plethora of PTMs. As the name implies, PTMs are the chemical modifications of proteins occurring after their translation. Broadly speaking, PTMs can be classified into two types: the covalent addition of chemical groups and the cleavage of the protein backbone, known as proteolysis.¹⁹ PTMs can reversibly or irreversibly alter the structure and function of proteins depending on the nature of the modification and they encompass a wide range of reactions, ranging from simple acetylation modifications to ubiquitination, all of which are depicted in Figure 1.2.

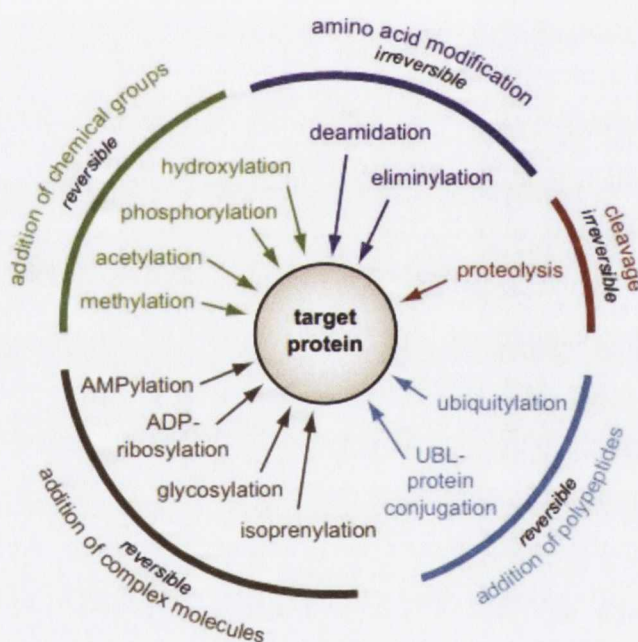


Figure 1.2: Diversity of PTMs. (Image taken from Cossart *et al.*)²⁰

PTMs are essential in maintaining many cellular processes including protein degradation, protein-protein interactions, and cellular signalling.²¹ Accordingly, defects in PTMs can signal disease states with many commonly observed PTMs being used as disease markers or as molecular targets for developing target specific treatments.^{21,22} A general goal of PTM-related research is to understand how the formation of the resulting bioconjugates

affects protein function. A wide range of enzymes are involved in PTMs with it being estimated that 5% of the genome is dedicated to the enzymes which carry out these modifications.¹⁹ To consider the large number of PTMs would require a lengthy review and is outside the scope of this thesis, therefore, this thesis will focus only on two common PTMs: ubiquitination and glycosylation.

1.1.1.1 Ubiquitination

Ubiquitination is an important PTM which involves the covalent addition of Ub, a small protein comprised of 76 amino acids, to a protein target. Interestingly, Ub was given its name as it was thought to be ubiquitous in prokaryotes and eukaryotes, however, it is now known to only exist in eukaryotes.²³ Ub can be added as a monomer or as polymers, to a protein target where it acts as a molecular signal to determine the fate of the target protein. In this process, Ub is attached *via* its C-terminal glycine (Gly) residue to the ϵ -NH₂ group of a substrate lysine (Lys) residue forming an isopeptide bond, as illustrated in Figure 1.3.^{24,25}



Figure 1.3: Formation of isopeptide bond between Ub C-Terminal Gly residue and Lys residue of the substrate.

The enzymatic process of ubiquitination involves three groups of enzymes and will be discussed in more detail in Section 1.3.4. The ubiquitination of Lys residues has emerged as an important modification for the regulation of many biological processes, such as DNA damage repair, cellular signalling, and protein degradation.^{26,27} Ub is most widely known as a signal for protein degradation, with the degradation of proteins now being known to be involved in a wide range of cellular processes and is heavily involved in the quality control of cells. The 2004 Nobel Prize in Chemistry was jointly awarded to Aaron Ciechanover, Avram Hershko and Irwin Rose for their research concerning the role of Ub in protein degradation.^{19,27} Defects in the components of the ubiquitination system can contribute to disease with aberrations now known to underlie the mechanism of many diseases including

neurodegenerations and certain malignancies.²⁸ For example, Parkinson's disease (PD), the most common neurodegenerative movement disorder, is characterized by the loss of dopaminergic neurons and the presence of intracellular inclusions, known as Lewy bodies (LBs). These LBs are primarily composed of α -synuclein (α -syn) which is a 140-amino acid residue protein.²⁹ The majority of α -syn species isolated from patients with PD displayed mono- or diubiquitination at multiple Lys residues. It has been suggested that aberrations in the Ub degradation pathway could lead to the toxic accumulation of LBs, as seen in the case of PD. Several studies have pointed to a link between PD pathogenesis and dysfunction in the Ub PTM system; therefore, understanding the role of ubiquitination in α -syn aggregation and LB formation is crucial for the understanding of PD pathogenesis.^{30,31}

The process of ubiquitination is reversible with DUBs functioning to remove Ub monomers or polymers.^{24,32,33} Interestingly, a pair of German siblings with inherited PD displayed markedly reduced DUB hydrolase activity suggesting that impaired DUB hydrolysis could also attribute to PD.³¹ Due to the critical importance that Ub bioconjugation plays in a range of diseases, focus has been placed on obtaining sufficient quantities of Ub and its bioconjugates to allow for biological interrogation to realise its precise role. There are a number of synthetic methodologies employed for preparation of Ub and its bioconjugates, some of which will be discussed later in this chapter and in Chapter 3.

1.1.1.2 Glycosylation

Protein glycosylation, the attachment of a sugar moiety to a protein, is one of the most important PTMs. It is estimated that over 50% of human proteins undergo glycosylation.^{34,35} It provides the greatest proteomic diversity, compared with other PTMs, and protein modification in this way is essential for many key biological processes including cell growth, cell-cell adhesion, fertilisation, immune defence, and inflammation.^{13,36,37,38} This PTM can stabilise the tertiary protein structure, aid subsequent folding and transport, and protect proteins from proteolysis.^{13,39,40}

Protein glycosylation can be categorised into different groups based on the nature of the sugar-peptide bond: *N*-, *O*- and *C*-linked glycosylation (Figure

1.4). In *N*-linked glycoproteins, *N*-acetylglucosamine (GlcNAc) is β -linked to the amide side chain of asparagine (Asn) in the conserved sequence Asn-Xaa-serine/threonine (Ser/Thr), where Xaa represents any amino acid, other than proline (Pro). *O*-linked glycoproteins are linked to the hydroxyl group of Ser or Thr, usually *via* *N*-acetylgalactosamine (GalNAc), and there is no consensus sequence required.⁴¹ The most common *O*-linked sugar is GalNAc, however other sugars implicated in *O*-glycosylation include fucose (Fuc), xylose (Xyl), and GlcNAc.³⁵ *N*- and *O*-glycosylation are very commonplace whereas *C*-glycosylation, the mannosylation of tryptophan (Trp) at the C-2 position of the indole ring, is relatively rare.¹⁹ This thesis focuses on the synthesis of *N*- and *O*-glycosides, therefore further discussion will be limited to these two types of glycosylation. The removal of carbohydrate moieties is similarly essential and is catalysed by a group of enzymes, called the glycosidases. The glycosidases act by hydrolysing the glycosidic bond of glycoconjugates and will be discussed in Section 1.4.

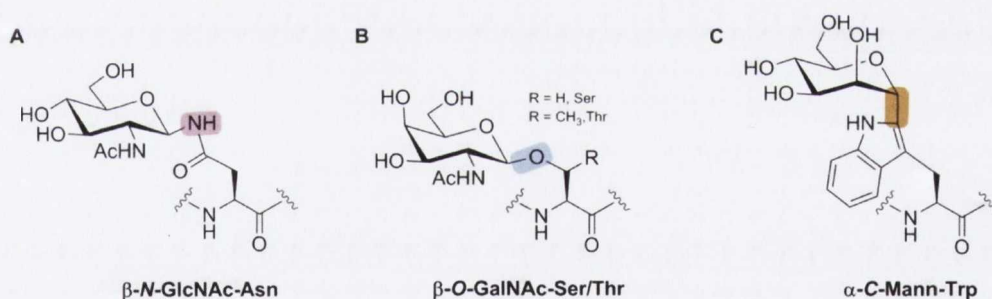


Figure 1.4: Glycopeptide structures (A) *N*-linked glycopeptide; (B) *O*-linked glycopeptide; (C) *C*-linked glycopeptide.

One of the most notable glycoproteins in drug discovery is erythropoietin (EPO) which is a blockbuster drug used for the treatment of anaemia. EPO is synthesised in the kidneys and circulated in the blood to stimulate red blood cell production and bone marrow differentiation.⁴² EPO is a 30kDa glycoprotein with a highly conserved polypeptide domain and four conserved sites of glycosylation, three of which are *N*-linked. However, the carbohydrate arrays themselves, namely the *N*-glycans, are highly variable.⁴³ There have been many investigations into the role glycosylation has on the biological function of EPO. In an early study, Fukada and co-workers showed

the importance of glycosylation in modulating the circulating half-life of proteins.⁴⁴ They demonstrated that variation in the *N*-glycans altered the serum half-life of EPO with terminal sialic acid residues required to accomplish action *in vivo*. Furthermore, Egrie and Browne found that increasing the carbohydrate content led to enhanced biological activity.¹⁰ A second generation therapeutic Aranesp containing five *N*-linked carbohydrate chains, rather than three, exhibited a slower clearance rate and, therefore, a longer half-life resulting in increased overall effectiveness and clinical benefit. Interestingly, in marked contrast, it was found that EPO entirely lacking terminal sialic acid residues, known as asialoEPO, resulted in an entirely different *in vivo* effect, where it was reported that asialoEPO displayed broad neuroprotective effects.^{45,46,47} This example of EPO and the impact of varying degrees of glycosylation serves to highlight the important role that glycosylation plays in the overall biological function of proteins.

1.2 Synthesis of glycopeptides

1.2.1 Glycoprotein diversity and the need for homogeneity

Carbohydrates play a fundamental role in many biological processes with significant efforts focused on understanding the precise role of attached carbohydrates on glycoprotein function. The carbohydrates along with proteins and nucleic acids constitute the three major biological macromolecules and of these three, it is carbohydrates that are the least studied and understood. This can be attributed to the sheer complexity of carbohydrates due to the range of constructs they can form. For example, each monosaccharide has several hydroxyl groups, with varying stereochemistry, all of which can be conjugated to different sugars (pyranose or furanose) along with the possibility of various modifications (e.g. sulfation, phosphorylation, acylation). Therefore vast amounts of biological information can be encoded in a short carbohydrate array, known as the glycode.^{48,49} This has led to the term “glycomics”, being introduced in recent years to accompany the existing fields of genomics and proteomics.⁵⁰

Despite the advances in the field of glycobiology, investigating glycoprotein function is considerably hampered by the fact that high purity glycoproteins are extremely difficult to access. The biosynthesis of the glycan

portion of glycoproteins is neither template-driven nor under direct transcriptional control.⁵¹ Their synthesis is subject to various glycosidases, glycosyltransferases, and their substrate specificity - the result of which is “glycoforms” which possess the same peptide backbone but differ in the site and nature of glycosylation.^{52,53} Studies which have provided access to single glycoforms have required extremely laborious separation techniques and therefore the prospect is far from routine.⁶ Without access to homogeneous samples, accurate structure-activity relationships (SAR) become difficult to unravel and biological roles cannot be accurately determined. It is now accepted that synthetic chemistry, or a combination between synthetic chemistry and enzymatic-mediated transformations, can be used to access homologous glycoproteins.⁵⁴

The need for homogeneity to interrogate the carbohydrates’ precise role in glycoproteins has resulted in a surge of general and efficient methodologies for their preparation; examples of which will be discussed throughout this thesis. As discussed previously, the two most common types of glycopeptides are *N*- and *O*-linked. In *N*-linked glycopeptides, the carbohydrate moiety is linked to the peptide *via* the amide side chain of Asn (Figure 1.4). In *O*-linked glycopeptides, the carbohydrate is attached to a Ser or Thr residue *via* an ether linkage. The synthesis of glycopeptides requires a combination of carbohydrate and peptide chemistry with the formation of the glycan-amino acid bond being central to the synthetic strategy. Several reviews have emerged covering the chemical formation of glycosidic linkages in glycoproteins.^{13,55,56} The following sections focus on the biosynthesis of *O*- and *N*-linked glycopeptides along with various synthetic methods used for the formation of *O*- and *N*-linked glycosidic bonds.

1.2.2 *O*-linked glycans

1.2.2.1 Biosynthesis

The process of *O*-glycosylation occurs post-translationally, beginning in the Golgi complex, where single sugar residues are added to an amino acid residue sequentially resulting in glycoproteins which are significantly less complex than *N*-glycoproteins, which will be discussed in the next section.⁵⁷ The process of *O*-glycosylation is orchestrated by the glycosyltransferase enzymes

which display incredible specificity. They are known to act as “one enzyme-one linkage” whereby they add sugar residues incrementally with each glycosidic linkage being the product of a single enzyme. They act by catalysing the transfer of a sugar from a glycosyl donor (e.g. sugar-nucleotide) onto a suitable acceptor (e.g. peptide, glycan, or lipid).⁵⁸ As depicted in Figure 1.5, a Ser/Thr residue is modified by the addition of a GalNAc residue from a uridine diphosphate (UDP)-GalNAc donor **1** by a glycosyltransferase enzyme, GalNAc transferase, to form **2**. This glycan can then be extended by another specific nucleoside phosphate sugar donor (UDP-Gal) and specific glycosyltransferase enzyme (Gal transferase) to give **3**. In this way, the carbohydrate array is elaborated sequentially by specific enzymes.⁵⁹

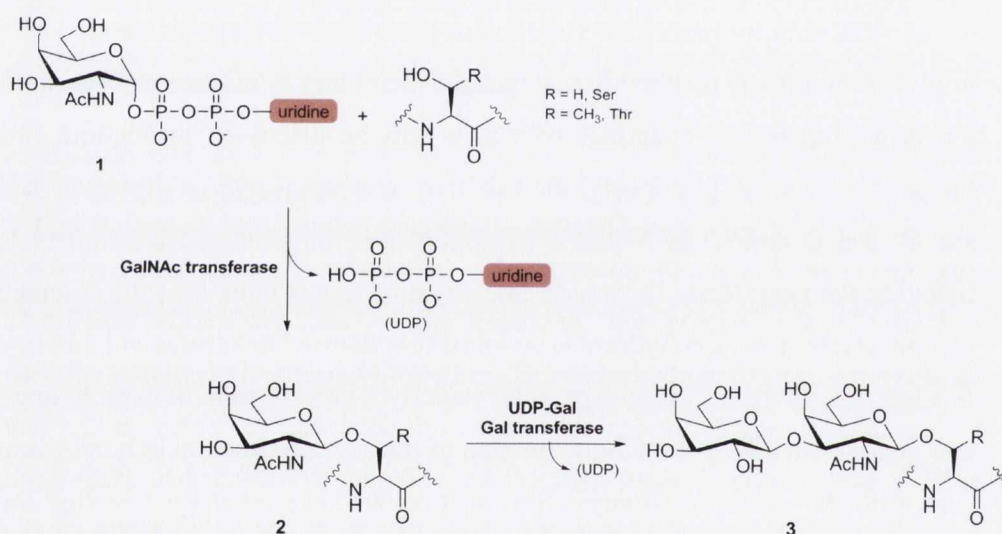


Figure 1.5: Biosynthesis of *O*-linked glycopeptides.⁵⁹ The transfer of a GalNAc and Gal residue from a sugar nucleotide donor to a Ser/Thr residue catalysed by GalNAc and Gal transferase, respectively.

1.2.2.2 Chemical synthesis

O-Glycoside synthesis is based around glycosylation reactions which involve a glycosyl donor with a leaving group at the anomeric centre of an appropriately protected glycosyl moiety, a suitable promoter, and an acceptor which can react with the donor through a free hydroxyl group. Here, there is the possibility of two linkages being formed: 1,2-*cis* and 1,2-*trans* glycosides (Figure 1.6).

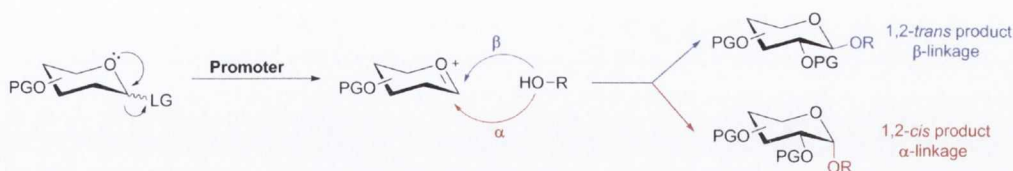


Figure 1.6: General glycosylation mechanism for the synthesis of *O*-glycosidic bonds to form 1,2-*cis* or 1,2-*trans* linkages, where LG denotes leaving group.⁶⁰

The nature of the protecting group at the C-2 position of the glycosyl donor is a major determinant of the resulting anomeric stereochemistry. A participating group at C-2 can direct the stereochemical outcome of a glycosylation reaction through anchimeric assistance, known as neighbouring group participation (NGP), to form 1,2-*trans* glycosides. As shown in Figure 1.7, a promoter activates the anomeric leaving group forming an oxocarbenium ion with subsequent NGP of the 2-*O*-acyl group to form a *cis*-acyloxonium ion which is attacked by an alcohol acceptor, from only one face in this case, providing the 1,2-*trans* glycoside.⁶¹ Here, protecting groups act as an asset allowing stereocontrol which can compensate for the general lack of “atom efficiency” associated with protecting groups. Conversely, 1,2-*cis* glycosides can be obtained when a non-participating group is present at C-2 however, in this case the stereoselectivity of the glycosylation reaction is more difficult to control and is determined by various conditions such as solvent, temperature, and promoter used.^{62,63}

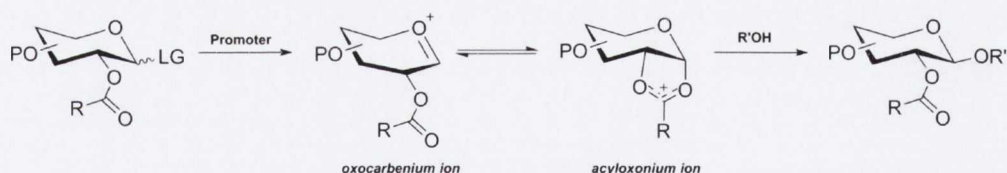


Figure 1.7: Neighbouring group participation in the synthesis of 1,2-*trans* glycosides.⁶¹ Promoter-assisted departure of the leaving group (LG) results in the formation of the oxocarbenium ion, which subsequently forms the acyloxonium ion due to NGP of the C-2 acyl group. Addition of acceptor ($R'OH$) results in nucleophilic attack from only one face to form the 1,2-*trans* glycoside.

In the chemical synthesis of an *O*-linked glycopeptide, the *O*-glycosidic link is installed through a glycosylation reaction between a glycosyl donor and the hydroxyl group of a suitably protected Ser or Thr residue. A broad range of glycosyl donors can be used, including trichloroacetimidates (TCA),

thioglycosides, halides, and *n*-pentenyl glycosides, which are illustrated in Figure 1.8.^{64,65,66,67}

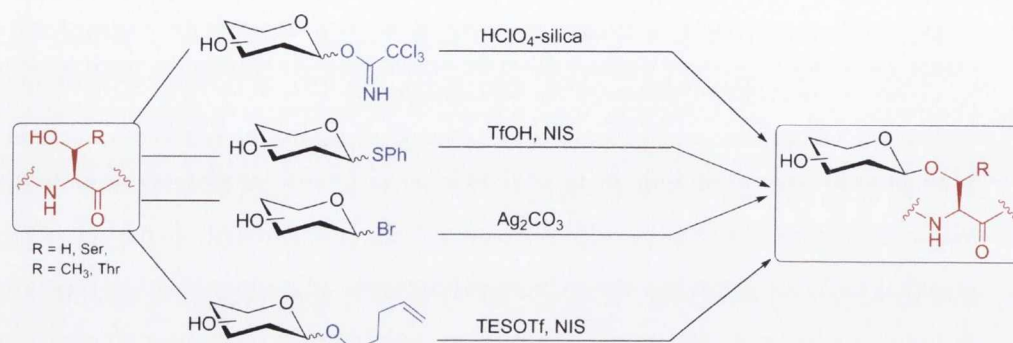


Figure 1.8: Common glycosyl donors and the corresponding activation conditions used for *O*-glycan installation to a Ser/Thr residue.^{64,65,66,67} TfOH: trifluoromethane sulfonic acid, NIS: *N*-iodosuccinimide, TESOTf: triethyl trifluoromethanesulfonate.

1.2.3 *N*-linked glycans

1.2.3.1 Biosynthesis

N-glycosylation is the most prevalent form of protein glycosylation with 90% of all glycoproteins being *N*-glycosylated. The process of *N*-glycosylation typically involves more complex structures, when compared with *O*-glycosylation, and this difference is reflected in the biosynthesis of *N*-glycosides.^{68,69} The process of *N*-glycosylation actually occurs co-translationally meaning the glycan moiety is attached to the target protein as it is being translated in the Endoplasmic Reticulum (ER). All *N*-linked glycans are derived from a lipid-linked oligosaccharide (LLO) precursor composed of a 14-mer oligosaccharide, $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$, linked to a dolichol through a diphosphate bond. As depicted in Figure 1.9, the first step involves the *en bloc* delivery of this 14-mer oligosaccharide to an Asn residue of the Asn-Xaa-Ser/Thr recognition sequence as it enters the ER lumen, catalysed by oligosaccharyltransferase (OSTase).

The second step involves the release of the nascent glycopeptide from the ribosome where it is then subject to enzymatic processing. Initial processing involves the removal of three glucose (Glc) and a terminal mannose (Man) residue by a series of glycosidase enzymes. In the third step, folded *N*-linked glycoproteins are exported from the ER to the Golgi where the glycan array is subjected to a battery of trimming and remodelling steps conducted by the glycosidases and glycosyltransferases.^{19,59,70} The resulting *N*-linked

glycoproteins can be categorised into three subtypes: high mannose, hybrid, and complex, as shown in Figure 1.9. They all share a common pentasaccharide core comprising of $\text{Man}_3\text{GlcNAc}_2$ and variable distal composition.

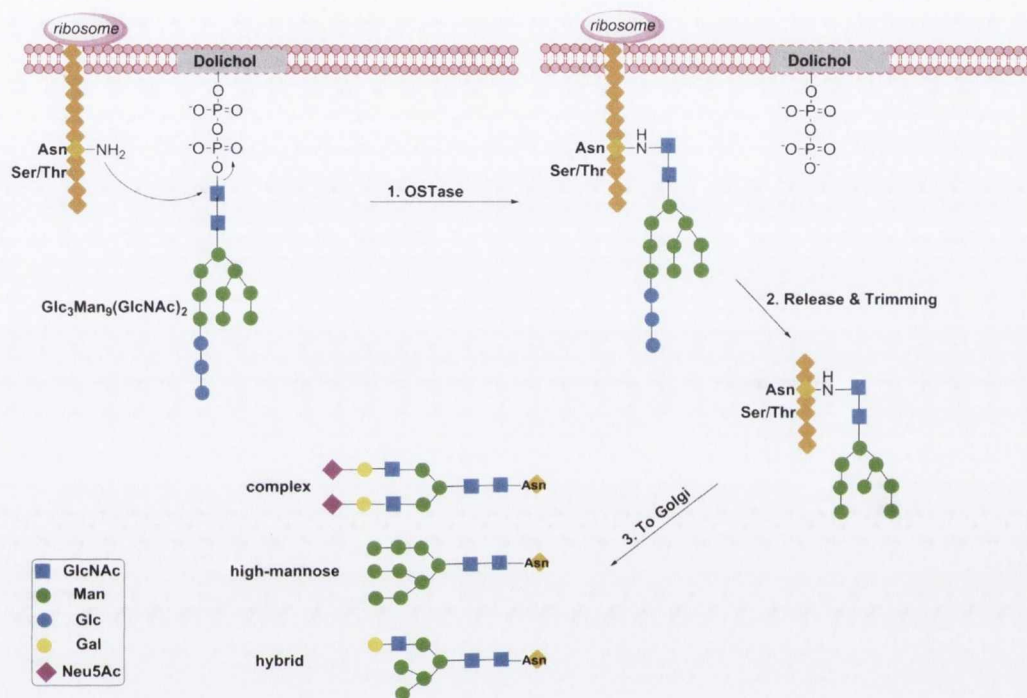


Figure 1.9: Schematic representation of the biosynthesis of *N*-linked glycoproteins and the resulting three main *N*-glycans found on *N*-glycoproteins.⁵⁹ (Step 1) Addition of 14-mer $\text{Glc}_3\text{Man}_9(\text{GlcNAc})_2$ to Asn residue catalysed by OSTase; (Step 2) The release of the nascent glycopeptide and trimming of distal residues by glycosidase enzymes; (Step 3) Enzymatic elaboration in the Golgi to give the three different *N*-linked glycopeptides.

1.2.3.2 Chemical synthesis

The conventional method for the synthesis of *N*-linked glycopeptides involves the amidation of an activated aspartic acid (Asp) residue, although this has been extended to include other approaches, many of which are summarised in Figure 1.10 and will be discussed in the following sections.

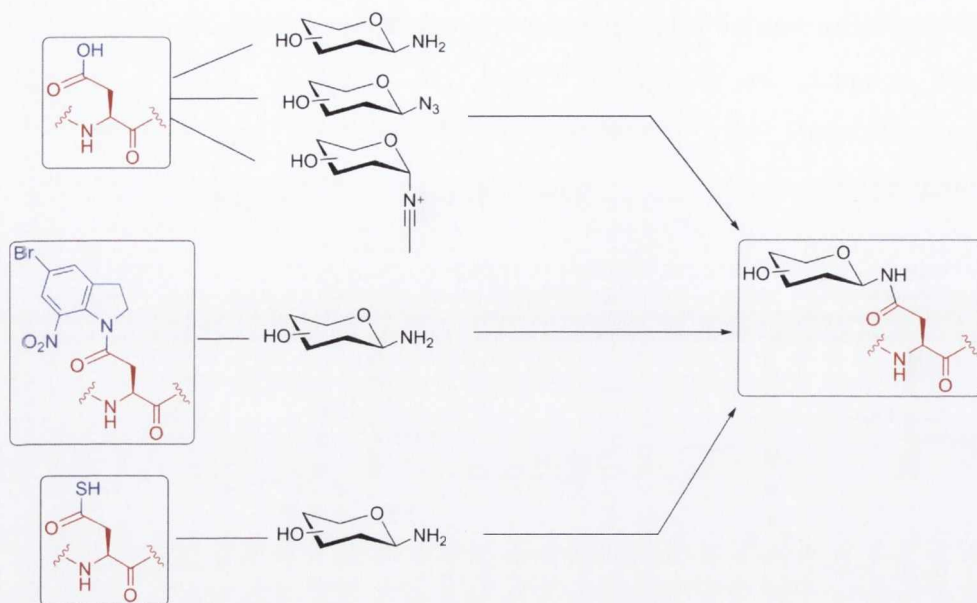


Figure 1.10: Common methods for *N*-glycopeptide formation using glycosyl azides, glycosylamines, and glycosyl nitrilium ion.

A method known as Lansbury aspartylation is the most commonly employed method for the preparation of *N*-linked glycopeptides (Figure 1.11A).^{71,72} In this protocol, a partially protected peptide containing an activated Asp residue is coupled with a glycosylamine **4** in the presence of coupling reagents and a suitable base. Glycosylamines are usually accessed through reduction of the corresponding azides or through the Kochetkov reaction whereby a hemiacetal is treated with a large excess of ammonium bicarbonate.⁷³

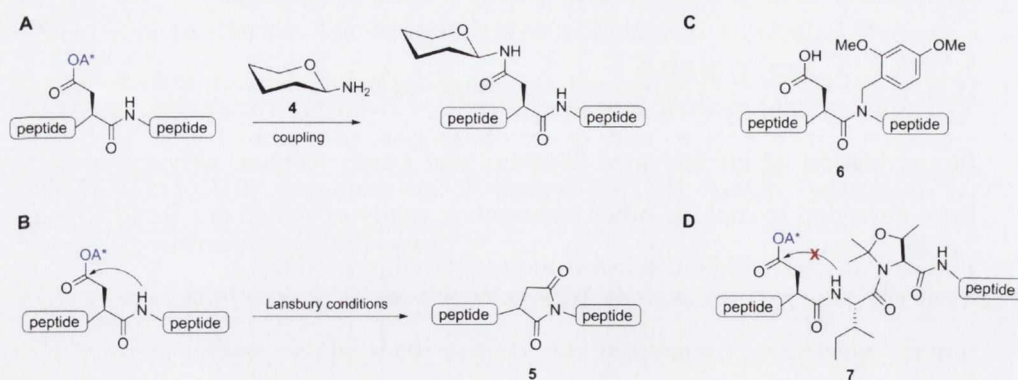
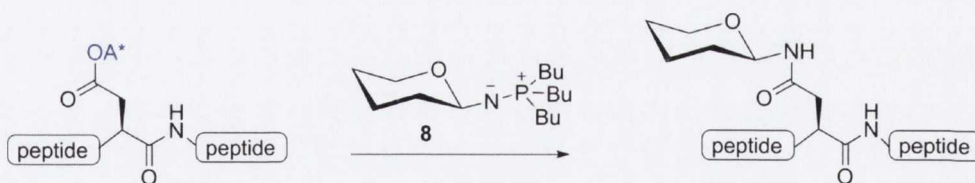


Figure 1.11: Strategies for chemical aspartylation with glycosylamines, where OA* denotes activated ester. (A) Lansbury aspartylation between activated Asp ester and glycosylamine **4**.⁷¹ (B) Aspartimide **5** formation, a major side product formed under Lansbury aspartylation conditions; (C) Dmb amide protecting group employed to prevent aspartimide formation;⁴¹ (D) Pseudoproline approach used to minimise aspartimide formation.⁷⁴

The major disadvantage of this method is the formation of aspartimide side-products **5** (Figure 1.11B). This side-product forms by reaction of the adjacent amino acid amide NH with the activated Asp residue to produce **5** which significantly diminishes yields. Different methods have been reported to avoid aspartimide formation, which usually involves the introduction of a protecting group on the amide directly preceding the glycosylation site.⁷⁵ For example, Payne and co-workers reported that incorporation of a 2,4-dimethoxy benzyl (Dmb) group **6** completely eliminated aspartimide formation during Lansbury aspartylation carried out during SPPS (Figure 1.11C).⁴¹ Furthermore, as the consensus sequence for *N*-glycosylation is Asn-Xaa-Ser/Tyr, Danishefsky addressed the problem of aspartimide formation by the conversion of the Ser/Tyr residue to a temporary pseudoproline motif **7** to mitigate aspartimide formation with this approach being successfully applied to the preparation of EPO fragments (Figure 1.11D).⁷⁴

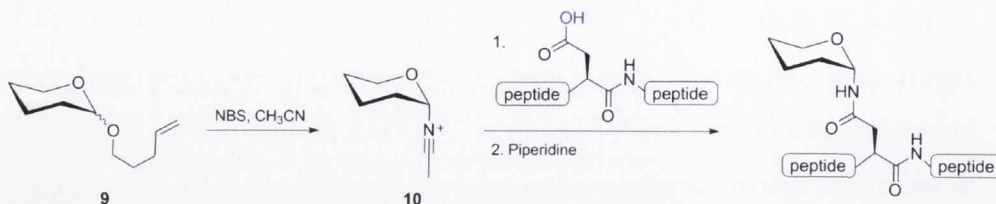
Davis *et al.* reported the synthesis of *N*-linked glycosyl amino acids from deprotected glycosyl azides using the Staudinger reaction (Scheme 1.1).⁷⁶ In this approach, the Asp side chain is activated followed by addition of a glycosyl azide and tributylphosphine (Bu_3P) to form aza-ylid **8** which can react with the activated ester to form the desired amide in good yields with complete stereocontrol.



Scheme 1.1: Synthesis of *N*-glycosyl amino acids using the Staudinger reaction, where OA^* denotes activated ester.⁷⁶ This involves acylation of an activated Asp residue with aza-ylid **8**.

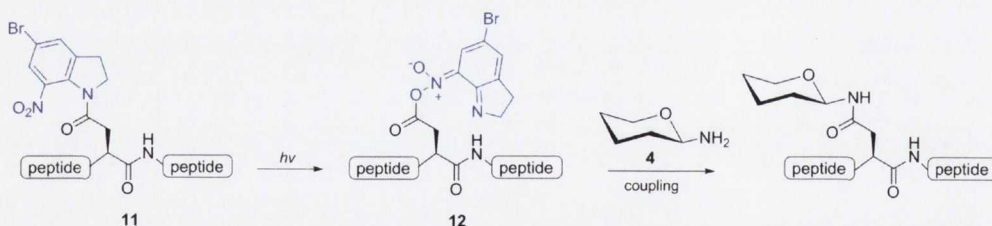
The Ritter reaction can be used for the preparation of α -linked *N*-glycopeptides from *n*-pentenyl glycosides (Scheme 1.2).⁷⁷ The glycosyl donor **9** is activated by the addition of *N*-bromosuccinimide (NBS) in acetonitrile to form α -acetonitrilium ion **10**. This can then be trapped by the addition of a carboxylic acid forming an amide bond with the nitrogen acetylated. Deacetylation using piperidine results in the natural peptide bond. The

stereoselectivity of this reaction can be tuned to prepare β -linked glycopeptides through the incorporation of a bulky C-2 phthalimido group.⁷⁸



Scheme 1.2: Synthesis of α -linked *N*-glycosyl amides using the Ritter reaction.⁷⁷ The activation of *n*-pentenyl glycoside with NBS yields the nitrilium ion **10**. Addition of a peptide with a free carboxylic group followed by piperidine gives the desired *N*-glycosyl amide.

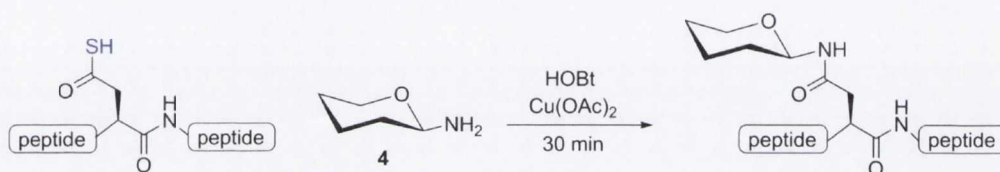
Lansbury aspartylation has been extended to allow the use of photolabile protecting groups (Scheme 1.3). Michael and co-workers used this approach for the convergent synthesis of model sequences of human EPO.⁷⁹ The Asp side chain was protected with a nitroindoline moiety **11** which is converted to a powerful acylating agent, nitronic anhydride motif **12**, upon exposure to UV light ($\lambda_{\text{ex}} \approx 350\text{nm}$). Subsequent addition of a glycosylamine **4** furnished the desired glycosyl asparagine linkage. This photolabile group acts as an orthogonal protecting group for the Asp residue as it can then be triggered (by $h\nu$) to form an activated ester and has the added advantage of operating under base free conditions, reducing the formation of aspartimide side products.



Scheme 1.3: Use of photolabile protecting group for *N*-glycopeptide synthesis.⁷⁹ The nitroindoline moiety in **11** can be triggered by $h\nu$ to form activated ester **12**. Addition of glycosylamine **4** then furnishes the *N*-linked glycopeptide.

Garner and co-workers reported the use of peptidyl thioacids for ligation with glycosylamines to give *N*-glycosylated peptides (Scheme 1.4).⁸⁰ The reaction is promoted 1-hydroxybenzotriazole (HOBt) and Cu(OAc)₂ with reaction times of 30 min making it a remarkably fast ligation method. Although the mechanism of the reaction remains to be determined, control experiments

carried out demonstrated the necessity for both Cu(II) and HOBt. This is in conflict with a similar method reported by Danishefsky *et al.* for the synthesis of *N*-linked glycopeptides using peptidyl thioacids and glycosylamines promoted by HOBt alone.⁸¹ This coupling, reported by Danishefsky, is suggested to proceed *via* an oxidative coupling mechanism, however, in the hands of Garner and co-workers, this reaction proved unsuccessful. Nevertheless, approaches towards amide bond formation which are thioacid-mediated have been gaining momentum due to their unique reactivity and selectivity, when compared with carboxylic acids.⁸²



Scheme 1.4: Coupling of peptidyl thioacids and glycosylamines, as reported by Garner *et al.*⁸⁰ The peptidyl thioacid is activated by HOBt and Cu(OAc)₂ followed by acylation with glycosylamine **4**.

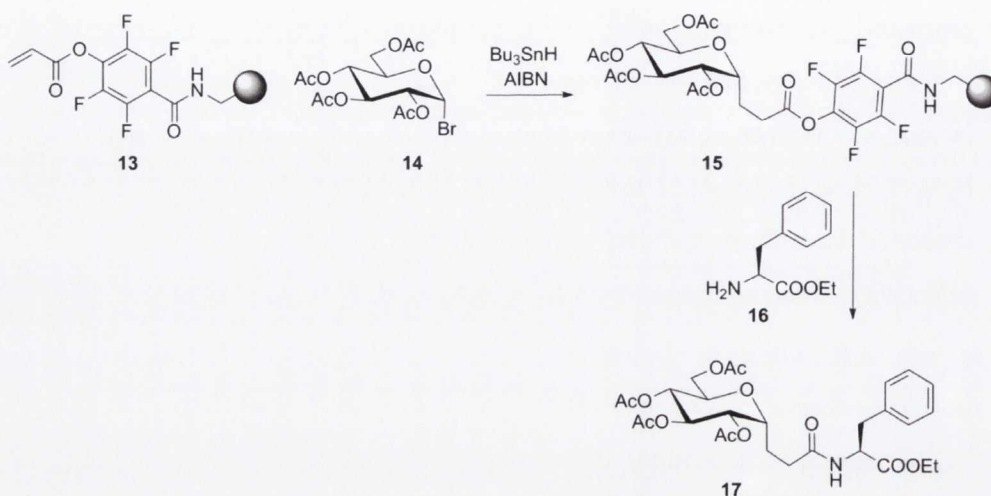
1.2.4 Synthesis of non-native glycoconjugates

Glycoconjugates containing non-native linkages can be tremendously valuable in therapeutics where the unnatural linkages can show enhanced chemical and enzymatic stability. For example, natural glycopeptides can be substrates for glycosidase enzymes where the glycan portion can suffer from hydrolysis by these hydrolase enzymes. Accordingly, much research has focused on the formation of non-native glycoconjugates which are inherently more stable while aiming to retain biological activity.^{83,84,85}

1.2.4.1 Radical chemistry used in the synthesis of non-native glycopeptides

Wilden and co-workers reported the synthesis of *C*-glycopeptide mimetics using radical chemistry conducted on the solid phase.⁸⁶ As depicted in Scheme 1.5, they used a resin appended with tetrafluorophenol acrylate **13** which could react as a radical acceptor with a glycosyl bromide **14** in the presence of 2,2'-azobisisobutyronitrile (AIBN) and tributyl tin hydride (Bu₃SnH) to form activated intermediate **15**. This could then be released from the solid phase resin by the addition of phenylalanine ethyl ester **16** to furnish the desired *C*-

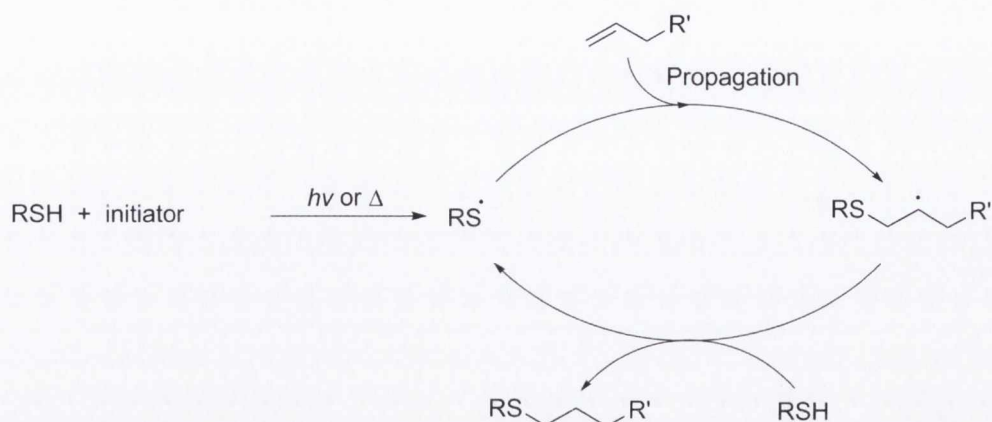
linked glycopeptide **17**. Various C-linked glycopeptides were reported in moderate yields (16-57 %).



Scheme 1.5: Synthesis of C-linked glycomimetics using an on-resin radical chemistry approach.⁸⁶ The radical acceptor **13** was reacted with glycosyl donor **14** in the presence of AIBN and Bu_3SnH to give activated intermediate **15**. Cleavage from the resin and formation of the C-linked mimetic occurred by acylation with phenylalanine **16** to give the C-linked glycomimetic **17**.

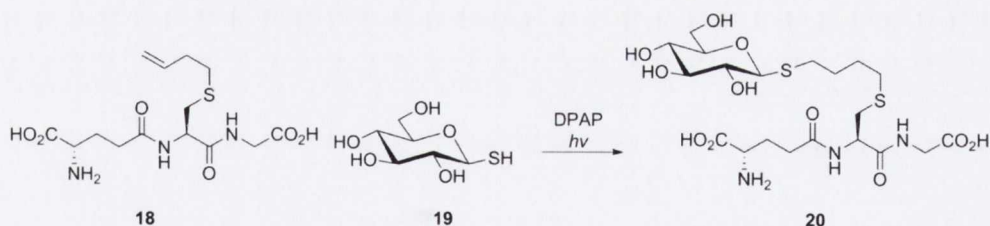
1.2.4.1.1 Thiol-ene click

An emerging reaction used for glycoconjugation and for the synthesis of non-native glycopeptides is the thiol-ene “click” reaction. It was first reported by Posner in 1905 and involves the coupling of a thiyl radical and an alkene to form a thioether bond, the mechanism of which is depicted in Scheme 1.6.⁸⁷ Upon thermal or UV activation in the presence of an initiator, a thiyl radical is generated which adds to an alkene in an anti-Markovnikov manner to give a carbon centred radical which then abstracts a thiol proton to propagate the reaction. The click status of the thiol-ene reaction is supported by it being high yielding, orthogonal to a wide range of functional groups, as well as it being compatible with aqueous and oxygenated conditions.⁸⁸ Thiol-ene chemistry has been extensively used in polymer and materials chemistry, while its application in glycoconjugation has only recently been explored despite the beneficial attributes it has to offer; the reaction enables the formation of a robust thioether linkage which is stable to a multitude of reaction conditions.⁸⁹ The thiol-ene ligation will be core to the ligation methodology discussed in Chapters 2 and 3 of this thesis.



Scheme 1.6: Mechanism of the thiol-ene reaction.⁹⁰ Hydrothiolation of the alkene by photochemical or thermal activation in the presence of an initiator.

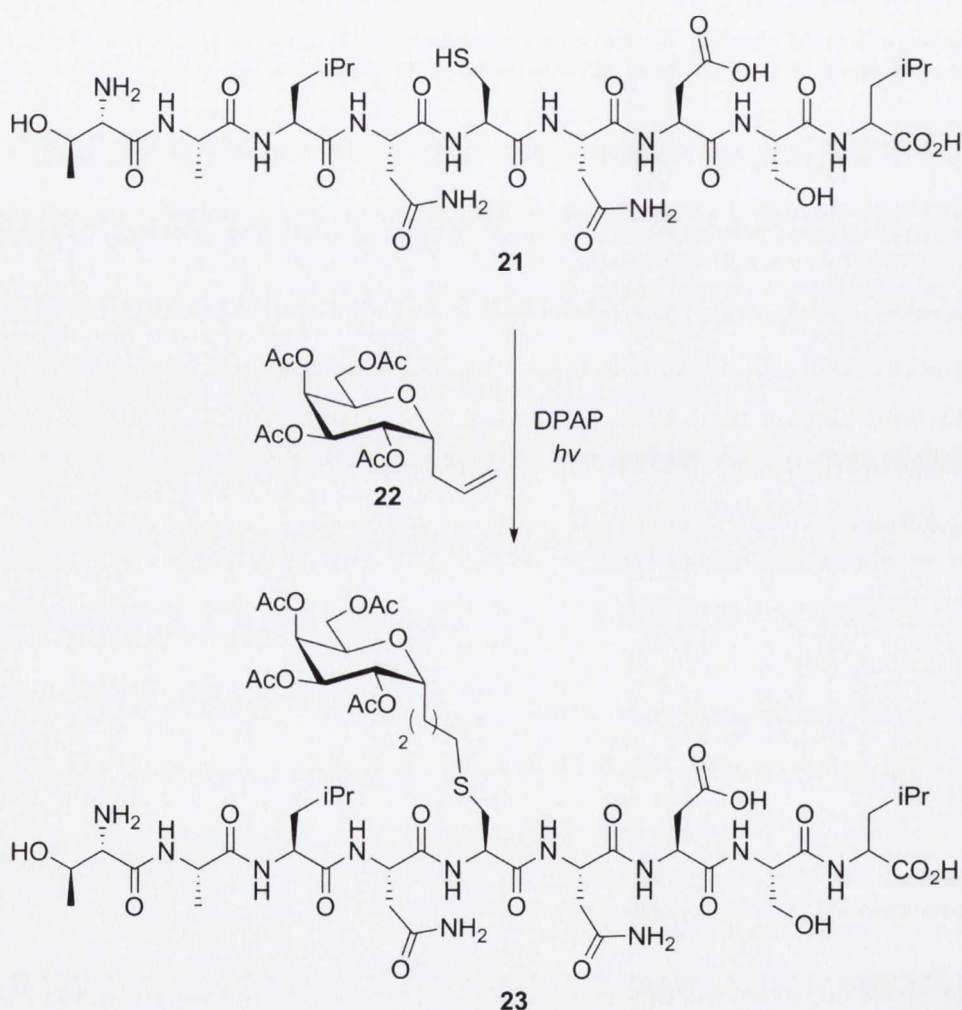
The groups of Dondoni and Davis have both demonstrated the utility of thiol-ene chemistry for the preparation of *S*-linked glycopeptides. Dondoni and co-workers reported the synthesis of *S*-glycosyl amino acids by reaction of peptidic alkenes with glycosyl thiols.⁹¹ For example, they demonstrated the *S*-glycosylation of glutathione (GSH) by hydrothiolation of *S*-homoallyl GSH **18** with glucose thiol **19** in the presence of photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DPAP) to yield the target *S*-glycopeptide **20** in near quantitative yield (Scheme 1.7).



Scheme 1.7: *S*-glycosylation of alkenyl glutathione **18** with glucose thiol **19** under photochemical thiol-ene conditions to give **20**.⁹¹

Davis *et al.* extended this methodology to allow for the *S*-glycosylation of various proteins.⁹² This was done by site specifically introducing an L-homoallylglycine into a number of proteins to generate an olefinic “tag”, which was subsequently reacted with glycosyl thiols allowing for protein glycoconjugation.

The Dondoni group later prepared a library of glycopeptides with non-native linkages using alkenyl *C*-glycosides and the free sulfhydryl Cys residue of various peptides.⁹³ They demonstrated the utility of this approach by reacting nonapeptide **21** in a coupling reaction with alkenyl *C*-galactoside **22** which furnished the glycosylated product **23** in high yields after just one hour (Scheme 1.8). They also successfully applied this approach to the galactosylation of the globular protein bovine serum albumin (BSA), which is known to contain one free Cys residue.



Scheme 1.8: Preparation of non-native glycopeptide using thiol-ene chemistry.⁹³ Alkenyl *C*-glycoside **22** reacts with the free Cys residue of nonapeptide **21** under photochemical conditions to furnish an *S*-glycopeptide **23**.

1.3 Peptide synthesis

1.3.1 Formation of the amide bond

The amide bond is the key chemical connection in peptide and proteins and in fact, acylation of amines accounts for 16% of all chemical reactions carried out in the modern pharmaceutical industry, making it the most common chemical reaction.^{82,94} Conventional approaches are still widely used which involve the condensation of an amine with a carboxylic acid, *via* an activated ester, in the presence of coupling reagents and suitable base (Figure 1.12). Nonclassical routes toward amide bond formation, a multitude of coupling reagents, protecting groups strategies, and comprehensive reviews have emerged facilitating progress in this field.^{94,95,96,97}

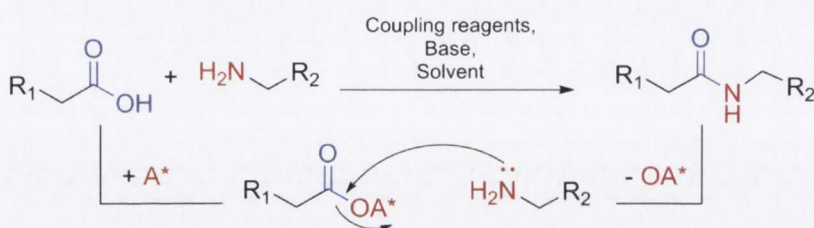


Figure 1.12: Conventional formation of an amide bond *via* an activated ester, where OA* denotes activated ester.

1.3.2 Solid phase peptide synthesis vs. solution phase synthesis

Solution phase synthesis is often referred to as the classical approach to protein synthesis and involves the preparation and coupling of orthogonally protected peptide segments in organic solvents. Although still widely used for the synthesis of short peptides, this approach presents numerous problems such as: (i) extensive purification, (ii) it is technically demanding, and (iii) fully protected polypeptides have limited solubility. The field of peptide chemistry was made synthetically feasible through the development of solid phase peptide synthesis (SPPS) by Merrifield in 1963 when he reported the synthesis of a tetrapeptide using SPPS.⁹⁸ This broadened the range of accessible peptides through simple, repetitive steps also allowing for automation of peptide synthesis to be introduced.

The process of SPPS is depicted in Figure 1.13A and involves: the covalent linking of an *N*-protected *C*-terminal amino acid, *via* its carboxylate group, to a reactive group bound to an insoluble polymeric resin. This nascent

amino acid can then be extended in a linear fashion through a series of repetitive cycles involving deprotection of the *N*-terminus and subsequent coupling reactions. These cycles are repeated, extending the anchored peptide in a *C*-terminal to *N*-terminal fashion, until the desired sequence is obtained. In the final step, the peptide is released from the resin and all protecting groups concomitantly removed. Reaction conditions in SPPS usually involve a large excess of reagents at high concentrations, which drives the coupling reactions to completion resulting in high yields usually being achieved. All soluble reagents can simply be removed through filtration and washing at the end of each coupling step, obviating the need for tedious purification of intermediates.⁹⁹ However, SPPS can generate a complex impurity profile due to the lack of purification. Whereas solution phase synthesis allows intermediates to be isolated and characterised at every step, if necessary.^{100,101}

Two main protecting group protocols are used for the *N*-amino functionality (Figure 1.13B):

- i. The Boc protecting group which is typically removed by trifluoroacetic acid (TFA), therefore, when using this approach side chains need to stable to repeated cycles of Boc removal. Unfortunately, the strong acidic conditions (HF) employed to cleave the peptide from the resin precludes the use of Boc-SPPS for *O*-glycopeptide synthesis due to the acid lability of *O*-glycosidic linkages.
- ii. The second strategy uses the 9-fluorenylmethyloxycarbonyl (Fmoc) protecting group, which is usually removed using a secondary amine such as piperidine in *N,N*-dimethylformamide (DMF). The milder conditions of the Fmoc protocol has led to it being the more preferred method.¹⁰²

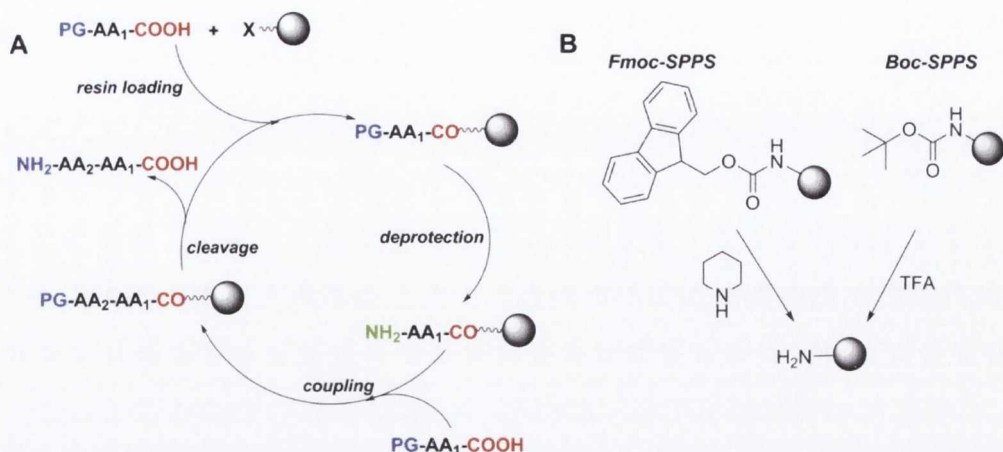


Figure 1.13: (A) Schematic representation of SPPS, where PG and AA denote protecting group and amino acid, respectively; (B) Two common *N*-terminal PG strategies (Fmoc and Boc) used in SPPS and their respective removal conditions.

SPPS represents the most efficient way to synthesise peptides and coupled with the continuous improvements in protecting group strategies, coupling reagents, and resins has made the synthesis of small amounts of moderately sized (30-50 residues) peptides routine.⁸² SPPS is increasingly used in the large-scale production of peptide therapeutics with the synthesis of anti-HIV agent, enfurvitide (Fuzeon) by Roche being the most noteworthy example.¹⁰³ The peptide, comprising of 36-amino acid residues, was produced in an overall yield of 30% over 106 steps, with 99 of those being synthesised by Fmoc-SPPS. This example represents an efficient, cost effective production of a macromolecule at multi-tonne scale by chemical syntheses.

1.3.2.1 Linear vs. convergent

Two main assembly strategies can be employed for the synthesis of peptides or glycopeptides: linear and convergent, with both illustrated in Figure 1.14.

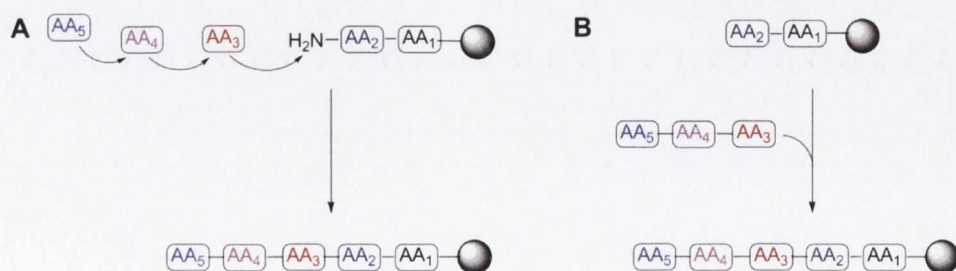


Figure 1.14: Strategies for peptide synthesis: (A) Linear; (B) Convergent.

In the linear approach, the amino acid residues are stitched together sequentially in a stepwise manner while a convergent approach involves the coupling together of peptide fragments. This linear approach is still widely used for the preparation of *O*-linked glycopeptides, as a convergent synthesis of a significant length *O*-linked glycopeptide has not been achieved to date.¹⁰⁴ However, the linear approach can result in low yields as the yield drops with each coupling step, therefore, convergent syntheses are generally preferred. The convergent strategy is favoured as it overcomes the issue of poor quality peptides which are attained by a stepwise synthesis.¹⁰⁵ Furthermore, the final full-length peptide obtained at the end of a convergent synthesis usually has different chemical properties to the starting material which makes isolation of the product easier. Another useful attribute of a convergent synthesis is that it allows the synthesis of various analogues from a single fragment.

One major drawback of SPPS is its length limitation. Realistically, SPPS imposes a size limitation as it can only produce peptides ≤ 50 amino acids in length and so, there is a need to develop new methods to synthetically access proteins and peptides of larger size, many of which will be discussed in the next section.

1.3.3 Ligation strategies commonly used for the synthesis of peptides and glycopeptides

As previously discussed, convergent syntheses are generally preferred, as they seek to divide large targets into synthetically accessible fragments which can be joined in a convergent fashion at a later stage. To access larger structures of biological interest, chemoselective ligation strategies are required which can overcome the size limitation associated with SPPS. These ligations should be compatible with aqueous media and be conducted in the presence of all protein functionality.¹⁰⁶

1.3.3.1 Native chemical ligation

The breakthrough in the chemical synthesis of proteins came in 1994 with the emergence of native chemical ligation (NCL), a thioester-mediated native amide bond forming ligation method, reported by Kent and co-workers.¹⁰⁷ The concept of NCL actually dates back to the 1950s with the work of Wieland

however, it did not gain widespread attention until the synthesis of human interleukin-8 (IL-8) by Kent *et al.* using NCL.¹⁰⁸ The reaction involves an *N*-terminal cysteine (Cys) and a *C*-terminal thioester reacting in a reversible transthioesterification step to yield a thioester intermediate (Figure 1.15). This intermediate can subsequently rearrange *via* an *S,N*-acyl transfer, through a 5-membered transition state, to give a native peptide bond at the ligation site.

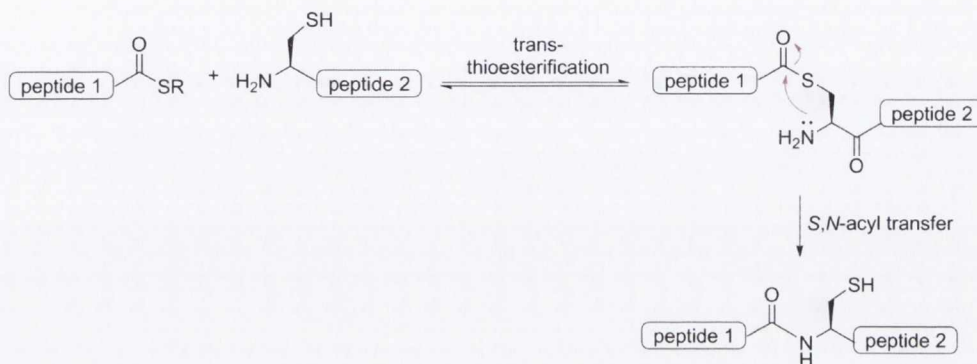


Figure 1.15: Native chemical ligation.¹⁰⁸ Chemoselective ligation method between a *C*-terminal thioester and *N*-terminal Cys residue. The first step involves a transthioesterification reaction to give a thioester intermediate. The second step consists of an intramolecular *S,N*-acyl transfer which forges a native amide bond between the two peptide units.

Alkyl thioesters can be easily prepared by SPPS but are relatively unreactive in NCL, with time periods of 24-48 h required for the initial transthioesterification to reach completion.¹⁰⁷ This rate-determining transthioesterification step can be accelerated by the addition of an exogenous aryl thiol, which can participate in thiol exchange with the alkyl thioester. Generally, aryl thioesters are more reactive than alkyl thioesters due to their lower pKa values which make them better leaving groups; therefore, addition of an aryl thiol can promote the *in situ* formation of a more reactive thioester, through thiol exchange, thus accelerating the rate of ligation.^{109,110}

The steric nature of the *C*-terminal thioester (peptide 1 in Figure 1.16) was found to be the most influential factor in NCL; ligations occurred faster at less hindered amino acids, such as Gly or alanine (Ala). Dawson and co-workers demonstrated that ligations were possible using all 20 amino acids, however, ligation junctions involving valine (Val), isoleucine (Ile), and proline (Pro) resulted in slower ligation rates.¹¹¹ NCL has revolutionised the total chemical synthesis of peptides and proteins as it allows the chemoselective ligation of two mutually reactive peptide entities in aqueous solution at neutral

pH to form a single product in near-quantitative yields. Accordingly, NCL has become one of the most commonly employed methods of peptide ligation. Using SPPS to assemble the two peptide entities, NCL can be used in a convergent manner to allow access to peptides and proteins that were previously inaccessible, with the synthesis of a HIV-1 protease covalent dimer being the most impressive.¹¹² This example, reported by Kent and co-workers, is a 203-residue polypeptide and represents the largest linear polypeptide prepared by chemical synthesis to date.

However, despite its utility, certain limitations of NCL have persisted. An ongoing criticism of NCL is its requirement for an *N*-terminal Cys at the ligation site. This limitation is coupled with the fact that Cys is not a very abundant amino acid (~1.7% in human proteins) and is seldom suitably distributed throughout a peptide sequence so as to facilitate NCL.^{113,114} Furthermore, NCL, in its original form, is not fully convergent as multiple peptide fragments are joined in a linear fashion from the *C*-terminus to the *N*-terminus. However, many interesting approaches have been reported to address these limitations, three of which are discussed below: (i) auxiliary mediated ligation; (ii) desulfurisation; and (iii) kinetically controlled ligation.

1.3.3.1.1 Auxiliary mediated ligation

One approach to overcome the requirement of Cys for ligation is auxiliary mediated ligation (AML) which has been relatively successful. AML involves the use of a removable auxiliary group which contains a free thiol at the *N*-terminus to mimic the Cys residue at the ligation site. Examples of auxiliaries developed include 2-mercaptoethyl and 2-mercaptobenzyl, as shown in Figure 1.16.^{115,116} As illustrated, the thiol of the auxiliary group reacts with the *C*-terminal thioester in a transthioesterification step which is followed by an S,N-acyl transfer to give a native amide bond between the two peptide fragments. The auxiliary group can then be removed after ligation to yield the desired peptide. However, AML itself comes with its own limitations

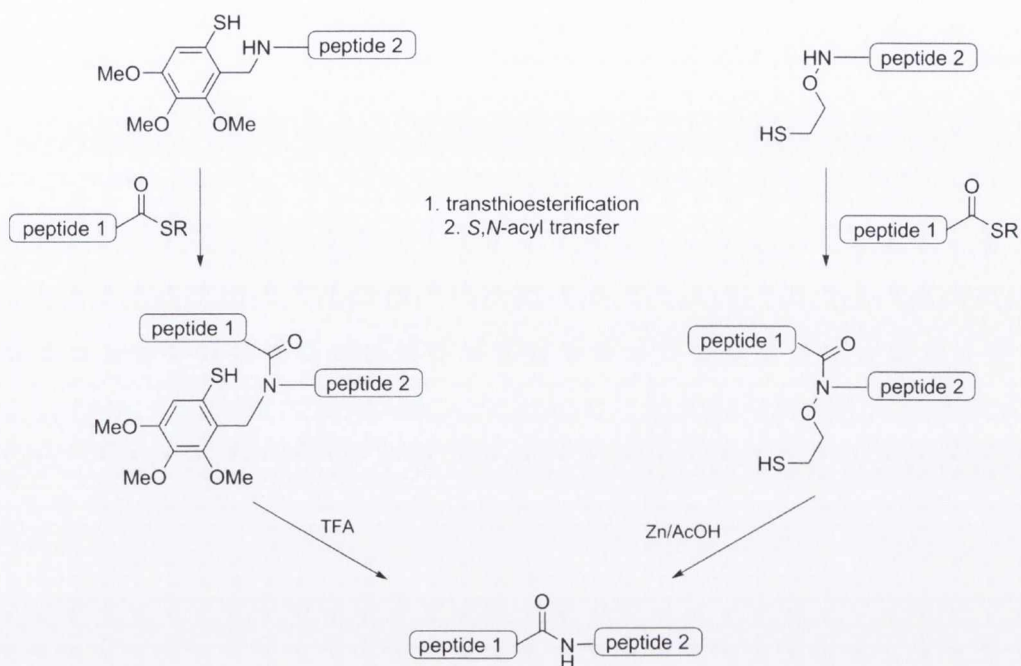


Figure 1.16: Common groups used in AML.^{115,116} An auxiliary group containing a thiol acts as a Cys mimic and reacts with a peptide thioester to form an amide bond. The auxiliary group can then be removed to give the unmodified peptide (*Left*) 2-mercaptobenzyl auxiliary for use at non-Cys ligation sites. This auxiliary can be removed using TFA after ligation; (*Right*) Ethanethiol auxiliary which facilitates peptide ligation and can be subsequently removed using Zn in an acidic medium.

The scope of AML is restricted by the sterically demanding nature of the auxiliaries and the generation of a secondary amine, rather than a primary in the case of conventional NCL. Therefore, ligations have only been found to proceed efficiently at non-sterically demanding ligation sites such as GlyGly or GlyAla.¹¹⁷ Hence, a requirement of AML is the presence of Gly at one of the ligation sites due to the steric hindrance conferred by the auxiliary group.¹⁰⁰ Wong and co-workers reported a thiol auxiliary approach, known as sugar-assisted ligation (SAL), for the synthesis of glycopeptides, which will be discussed in Section 1.3.3.3.

1.3.3.1.2 Desulfurisation

One of the most important advancements to overcome the requirement for Cys as a ligation partner in NCL was reported by Dawson and co-workers and involves the use of NCL coupled with desulfurisation.¹¹⁸ This concept was initially proved using Ala where an Ala residue was mutated to Cys and after NCL, the Cys residue was desulfurised to Ala using Raney nickel, as depicted

in Figure 1.17. This allowed for ligation at Ala sites, which are naturally more abundant than Cys.

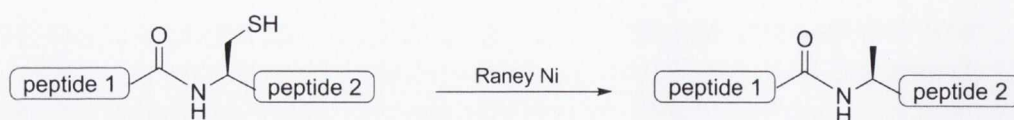
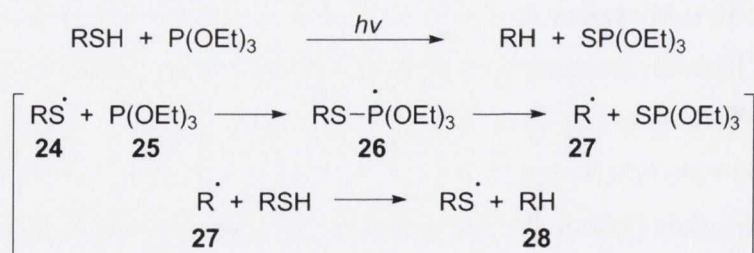


Figure 1.17: Desulfurisation of Cys to Ala using Raney Ni to afford an Xaa-Ala ligated junction.¹¹⁸

Although the desulfurisation strategy reported by Dawson represented a major milestone in the use of NCL at non-Cys ligation sites, problems such as low product recoveries and non-selective reduction of thioesters and thioethers led to the development of free-radical desulfurisation methods.

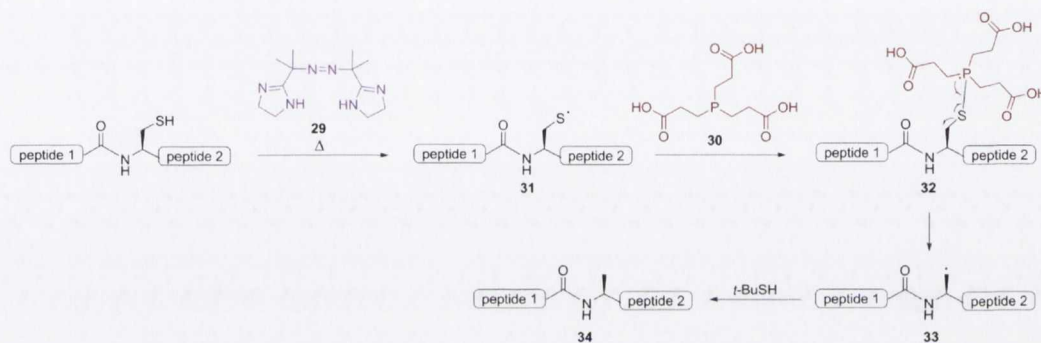
Hoffman *et al.* were the first to disclose a desulfurisation reaction between mercaptan and trialkylphosphite derivatives under thermal and photochemical conditions.¹¹⁹ Shortly thereafter, Walling and Rabinowitz proposed the mechanism of the desulfurisation, which is depicted in Scheme 1.9.¹²⁰ It was proposed that the generated alkylthiyl radical **24** adds to the phosphite **25** generating a phosphoranyl radical intermediate **26** which can subsequently furnish an alkyl radical. Hydrogen abstraction from the parent thiol can furnish the alkane product **28** while also propagating the chain reaction.



Scheme 1.9: Proposed mechanism of phosphite-mediated radical desulfurisation proposed by Walling and Rabinowitz.¹²⁰

This radical desulfurisation was modified by Danishefsky and co-workers to give a more environmentally friendly dethylation method for use in NCL, the process of which is illustrated in Scheme 1.10.¹²¹ They used radical initiator 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) **29** and tris(2-carboxyethyl)phosphine (TCEP) **30**, a reducing agent commonly

used in NCL, as the phosphine source. Upon heating in aqueous solution, **29** decomposes to give 2-isopropyl-4,5-dihydro-1H-imidazole radical initiator, which on reacting with Cys generates a thiol radical **31**. This thiol radical can then react with **30** to form a phosphine radical intermediate **32**. Intermediate **32** then undergoes C-S bond scission to generate alkyl radical **33** which can be quenched by the addition of a thiol additive, such as *t*-butyl mercaptan (*t*-BuSH), allowing for hydrogen abstraction to give **34**.¹¹³ This approach was found to produce the desulfurised products with yields in excess of 80% and the method was compatible with a variety of functionalities such as methionine (Met), thioesters, and thiazolidine protected Cys residues.



Scheme 1.10: Danishefsky's metal free dethylation method.¹²¹ The radical desulfurisation is thermally initiated using VA-044 **29** to give a thiol radical **31** which reacts with phosphine source **30** to allow for desulfurisation of Cys to Ala.

Inspired by this initial work, many groups extended this strategy to other Cys mimics, in the form of mercapto amino acids, which can be desulfurised in the last step. In mercapto amino acids, a thiol group is incorporated into the amino acid and acts as a Cys mimic making ligation at non-Cys sites possible, without the need for bulky auxiliary groups.^{122,123,124} Brik and co-workers used this strategy for the preparation of Ub bioconjugates, which will be discussed later in Section 1.3.4.2.

1.3.3.1.3 Kinetically controlled ligation

The issue of the lack of convergence in NCL was tackled by Kent and co-workers who reported kinetically controlled ligation (KCL).¹²⁵ KCL exploits the difference in reactivity between alkyl and aryl thioesters, allowing the pre-organisation of the order of NCL reactions (Figure 1.18). By conducting a one-

pot experiment, they demonstrated that the more reactive aryl peptide thioester is ligated with a Cys peptide bearing a less reactive alkyl thioester with the alkyl thioesters remaining intact until further activation.¹²⁶ This approach allows for selective peptide elongation in both the *N*- and *C*-terminal direction. They showcased the utility of KCL through the preparation of crambin, a 46-amino acid protein, by a fully convergent synthesis involving six peptide segments and only two protecting groups.¹²⁷



Figure 1.18: KCL with Ar and Alk denoting aryl and alkyl, respectively.¹²⁵ Ligation should occur between the Cys and the more reactive aryl thioester, rather than the alkyl thioester.

1.3.3.2 Native chemical ligation for the synthesis of glycopeptides

NCL has revolutionised the field of peptide synthesis and this has been extended to the synthesis of glycopeptides. However, the inclusion of a glycan can complicate the ligation process through functional group incompatibilities between the glycan and peptide moieties. For example, the thioester functionality is incompatible with Fmoc-SPPS due to its hydrolysis under Fmoc-removal conditions; therefore, this forced an initial reliance on Boc-SPPS for the preparation of the thioesters for use in NCL. However, when incorporating chemically fragile PTM modifications such as acid sensitive glycosidic linkages, the use of Boc-SPPS and strongly acidic conditions are not ideal. Therefore, several methods for the production of peptide thioesters using Fmoc-SPPS were required, two of which will be described in the next section.

1.3.3.2.1 Synthesis of thioesters

A method developed to combat the incompatibility of thioesters with Fmoc-SPPS is the use of a “safety-catch” linker strategy. In this strategy, a sulfonamide-based linker is selectively activated by a discrete chemical modification, a concept originally introduced by Kenner.^{128,129} This was later modified by Ellman *et. al* for the preparation of peptide thioesters, as depicted in Figure 1.19A.¹³⁰

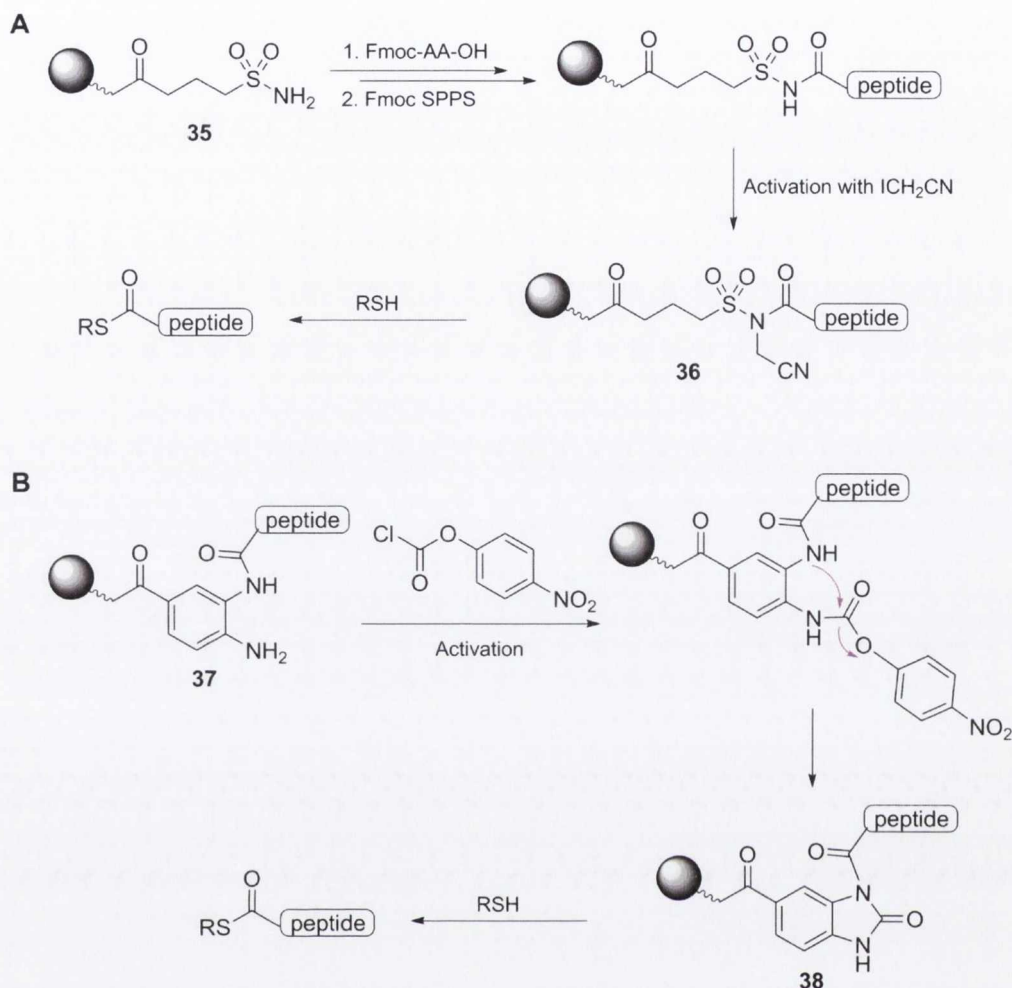


Figure 1.19: Methods developed for the Fmoc-SPPS of peptide thioesters.^{130,131} (A) Ellmans “safety-catch” linker whereby a sulfonamide linker is activated by cyanomethylation, followed by thiolysis to give the desired thioester; (B) Dawson’s Dbz linker **37** whereby the linker is activated by acylation, followed by cyclisation to Nbz **38**. Addition of an exogenous thiol allows release of the thioester.

A resin bearing a sulfonamide group **35** is linked to the C-terminus of a peptide and can be elongated using standard Fmoc-SPPS. After peptide synthesis, the sulfonamide is activated by cyanomethylation to give **36**. Subsequent addition of a thiol allows release of the peptide thioester from the solid support. This strategy was used to successfully prepare diptericin, a glycosylated antimicrobial, which was the first glycopeptide prepared by NCL (discussed in more detail in the next section).¹³² Later, Dawson *et al.* reported a strategy for the synthesis of thioesters using Fmoc-SPPS (Figure 1.19B). This approach involves a C-terminal modification comprised of a 3,4-diaminobenzoic acid (Dbz) moiety **37** linked to the peptide.¹³¹ This group can be activated by amine

acylation to yield *N*-acyl-benzimidazolinone (Nbz) **38**. When an exogenous thiol is added, **38** can undergo thiolysis to yield the desired thioester.

1.3.3.2.2 Examples of glycopeptides prepared by native chemical ligation

The first major example of a glycopeptide prepared through NCL was diptericin, an 82-residue *O*-linked antimicrobial, reported by Bertozzi and co-workers.¹³² Native diptericin exists as a mixture of *O*-linked glycoforms, with the simplest glycoform containing a single *O*-linked GalNAc residue at two sites: Thr11 and Thr54. The assembly of this single glycoform was achieved through the convergent assembly of two glycopeptide fragments, each prepared through SPPS, by NCL as depicted in Figure 1.20. The major challenge was the preparation of the 24-residue glycopeptide *C*-terminal thioester. This obstacle was overcome using Ellmans “safety-catch” linker (Figure 1.19A) which allowed SPPS of the glycopeptide thioester using standard Fmoc-SPPS. This thioester was then ligated with a 58-residue *N*-terminal Cys fragment, also prepared using Fmoc-SPPS, and followed by deacetylation with aqueous hydrazine to furnish homogenous diptericin containing two glycosylation sites.

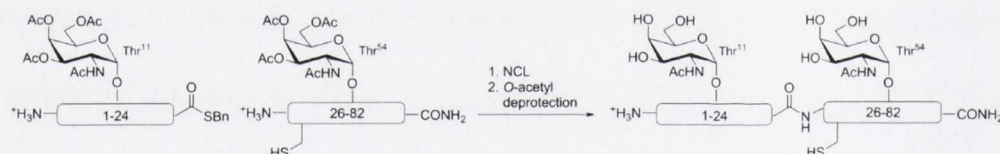


Figure 1.20: The first glycopeptide, diptericin, prepared using NCL, reported by Bertozzi and co-workers.¹³² The thioester was prepared using Ellmans “safety-catch” linker and ligated with an *N*-terminal Cys fragment. Deacetylation of the carbohydrate protecting groups furnished the native diptericin.

The first example of NCL being used to construct a complex *N*-linked glycopeptide was RNase.¹³³ Since then, the construction of impressive glycopeptides using NCL has continued, with the synthesis of EPO being the most impressive example to date (Figure 1.21). EPO represents a significant synthetic challenge, with it comprised of 166 amino acids and bearing four glycosylation sites: three *N*-linked to Asn residues and one *O*-linked to a Ser residue. To further complicate the synthesis, EPO only contains four Cys residues, all of which are at unfortunate positions (7, 29, 33, 161), rendering them unsuitable for NCL. Nevertheless, this glycoprotein was chemically

synthesised as a single glycoform by Danishefsky and co-workers using NCL and the desulfurisation of Cys to Ala as the key strategy.¹³⁴

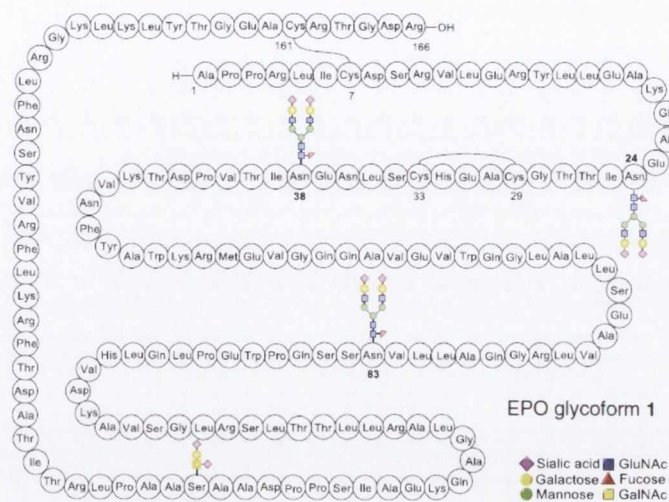
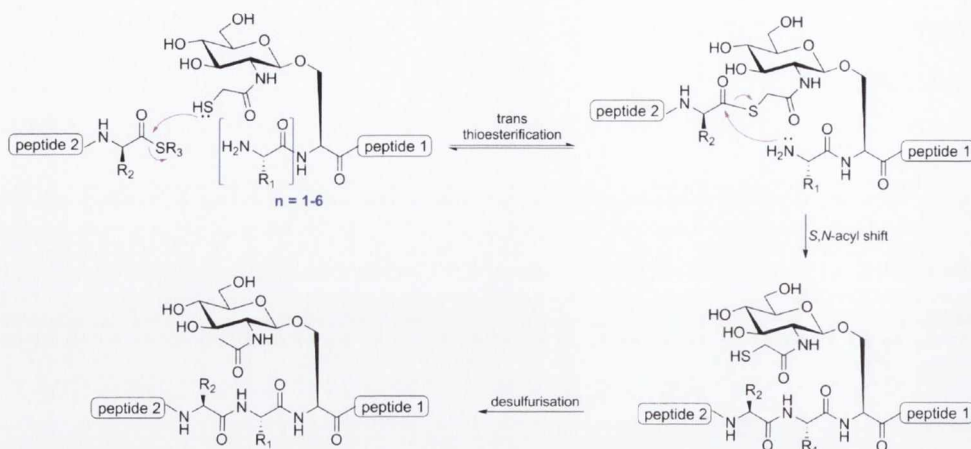


Figure 1.21: Schematic representation of homogeneous EPO glycoform 1. (Image taken from Danishefsky *et al.*)¹³⁴

1.3.3.3 Sugar-assisted ligation

Of particular interest for the synthesis of glycopeptides is SAL, which is a form of AML introduced and developed by the Wong group.¹³⁵ As depicted in Scheme 1.11, the thiol auxiliary is placed on the *N*-acetyl moiety of a 2-acetamido-2-deoxy sugar that is linked to a Ser residue. The thiosugar auxiliary is prepared as a glycosyl amino acid building block and installed during SPSS near the ligation site. The first step involves transthioesterification between the thiol auxiliary and a peptidyl thioester, which is followed by an *S,N*-acyl transfer to produce a native peptide bond. The thiol auxiliary is then removed by desulfurisation.¹¹⁸

The key difference between SAL and conventional NCL is the size of the cyclic transition state with SAL proceeding *via* a 14-membered transition state. Consequently, SAL reactions tend to be slower when compared to NCL, which proceeds through a 5-membered transition state.^{136,137} However, ligations proceeding through these large transition states are suggested to be facilitated by the sugar moiety, which acts as a rigid scaffold bringing the reacting groups into close proximity thus facilitating ligation.



Scheme 1.11: Proposed mechanism of SAL.¹³⁵ The first step involves transthioesterification, which is then followed by an *S,N*-acyl transfer to form an amide bond. The thiol handle is subsequently removed by desulfurisation.

As with NCL, the most influential factor in SAL is the steric nature of the thioester component (R_2 in Scheme 1.11), with thioesters known to be difficult to ligate include Val, Pro, and Ile.^{111,136,138} To achieve ligation efficiency, it was found that the ligation site must include at least one Gly residue (R_1 or R_2 in Scheme 1.11), which could limit the general applicability of SAL in glycopeptide synthesis.¹³⁵ However, in contrast with the previously described AMLs, SAL can tolerate a wider range of amino acids at the *N*-terminus (R_1) and is not limited to GlyGly or GlyAla junctions, as with the examples outlined in Figure 1.16. This increased tolerance is attributed to the amine participating in the *S,N*-acyl transfer, in the case of SAL, being a primary amine, whereas in previous auxiliaries, the participating amine is secondary.¹³⁶

Later studies were carried out to gauge the effect of increasing the number of amino acids between the glycosylated residue and the *N*-terminal amino acid ($n = 1-6$ in Scheme 1.11). It was found that up to five amino acids can be inserted, which Wong termed extended SAL (exSAL).^{137,138} However, these extensions resulted in a significant decrease in the reaction rate as the proposed transition state got larger (from 14-29 membered transition state for the 1-5 amino acid extensions). Nevertheless, exSAL was successfully implemented to synthesise the tandem repeating unit of a cancer-associated mucin1 (MUC1) glycoprotein, composed of 20-amino acids and two glycosylation sites. An obvious limitation of SAL is the requirement for desulfurisation to remove the “thiol handle” which would be incompatible with

native Cys residues elsewhere in the glycopeptides. It has been suggested to use a glycosidase enzyme to remove the templating sugar moiety rendering the process a traceless peptide ligation strategy.¹¹⁴

Shortly after the emergence of SAL, Brik and co-workers reported an AML strategy, known as side-chain assisted ligation (SCAL) which relies on principles similar to SAL.^{139,140} SCAL uses a cyclohexane-bearing auxiliary attached to the side chain of Asp, to allow for peptide ligation at non-Cys sites. The rigid nature of the side-chain is proposed to assist the *S,N*-acyl transfer through large transition states, with the auxiliary removed by base hydrolysis in the final step.

1.3.3.4 Expressed Protein Ligation

The desire to combine recombinant proteins with established NCL methods led to the development of expressed protein ligation (EPL) by Muir and co-workers in 1998. EPL is based on the natural occurring protein splicing mediated by inteins, as depicted in Figure 1.22A.¹⁴¹

An intein is a natural protein splicing element which consists of an internal protein domain that is flanked by two polypeptide units, termed the *N*-extein and *C*-extein. An intein mediates its own excision from a peptide sequence through a series of acyl transfer reactions.¹⁴³ The first step involves an *N,S*-acyl shift between the intein Cys residue and *N*-extein producing a thioester intermediate. A subsequent transthioesterification transfers *N*-extein to *C*-extein, linked through a Cys residue. The last step involves cyclisation of a conserved Asn residue to form a succinimide intermediate and an *S,N*-acyl transfer to form a peptide bond, completing the process of protein splicing, a common PTM.

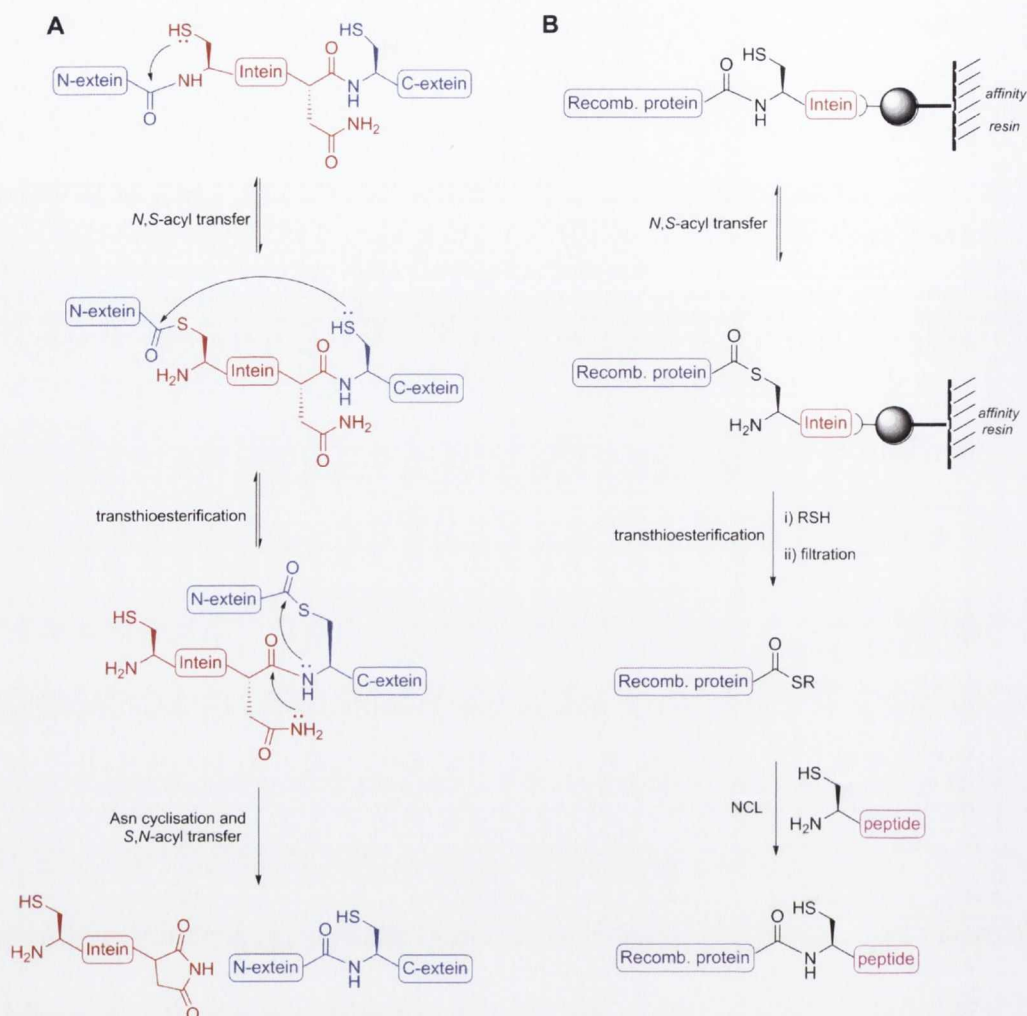


Figure 1.22: (A) Scheme representing the proposed mechanism for protein splicing. The process involves a series of acyl transfer reactions which results in the linking of two polypeptides, the *N*- and *C*-exteins, through an amide bond; (B) EPL using a recombinant thioester. Mutation of the *C*-terminal Asn to an Ala residue blocks the final step in protein splicing (see Figure 1.23A). Recombinant proteins can therefore be converted to the corresponding thioester for participation in NCL with a cysteinyl peptide.¹⁴²

EPL vastly expands the size limitation and abilities of protein synthesis by combining NCL with recombinant protein production. This can be accomplished by ligation of a recombinant protein thioester with a synthetic peptide containing an *N*-terminal Cys, the process which is described in Figure 1.22B. The protein of interest is expressed *N*-terminally to an intein domain which is bound through its *C*-terminus to a solid support. The conserved Asn residue in the intein is mutated to an Ala residue, thereby halting *C*-terminal cleavage and splicing. An initial *N,S*-acyl shift generates a thioester intermediate which then undergoes transthioesterification with an exogenous thiol. Subsequent filtration releases the desired recombinant protein thioester

from the solid support, where it can subsequently participate in NCL with synthetic cysteinyl peptides.^{143,144} The ability to access recombinant products of hybrid biological and chemical origin has caused EPL to become a powerful tool in protein engineering through the introduction of unnatural amino acid sequences, various PTMs, fluorophores, or reactive synthetic handles allowing for further modification or surface immobilisation.^{145,146,147,148} The applicability of EPL is well illustrated in the production of fluorescent enzyme biosensors. An example reported by Muir demonstrated the careful incorporation of two fluorophores within Crk-II, a cAbl protein tyrosine kinase substrate which is involved in many signalling pathways, using EPL. This allowed for the monitoring of protein phosphorylation in real time, hence kinase activity, using fluorescence resonance energy transfer (FRET) measurements.¹⁴⁹

1.3.4 Formation of the ubiquitin isopeptide bond

As discussed in section 1.1.1.1, ubiquitination is an important PTM implicated in several cellular pathways such as protein degradation, inflammatory responses, and DNA repair.¹⁵⁰ Ubiquitination involves the formation of an isopeptide bond, a non-canonical amide linkage, between the C-terminal Gly of Ub and the ϵ -NH₂ of the substrate Lys. The enzymatic process of ubiquitination and an example of ubiquitination by chemical means is discussed in the next section. Further discussion regarding ubiquitin its bioconjugates is found in Chapter 3.

1.3.4.1 Enzymatic

Ubiquitination is a multi-step process orchestrated by three key enzymes: activating enzymes (E1), conjugating enzymes (E2), and ligases (E3), with the overall process depicted in Figure 1.23. The first step is an ATP-dependent process which involves E1 forming a thioester linkage between its catalytic Cys residue and the Ub C-terminal Gly residue. In the second step, the activated Ub molecule is transferred to an E2 enzyme *via* a transthioesterification reaction with an E2 Cys residue. In the third step, the E3 ligase catalyses the formation of the isopeptide bond between the Ub C-terminal Gly and the ϵ -NH₂ of the substrate Lys residue.^{24,32,33} The conjugation of Ub to a protein may involve the addition of a monomer (monoubiquitination) or as a chain of Ubs

of various types (polyubiquitination) with these different modifications producing a variety of molecular signals depending on the nature of the ubiquitination.²⁷

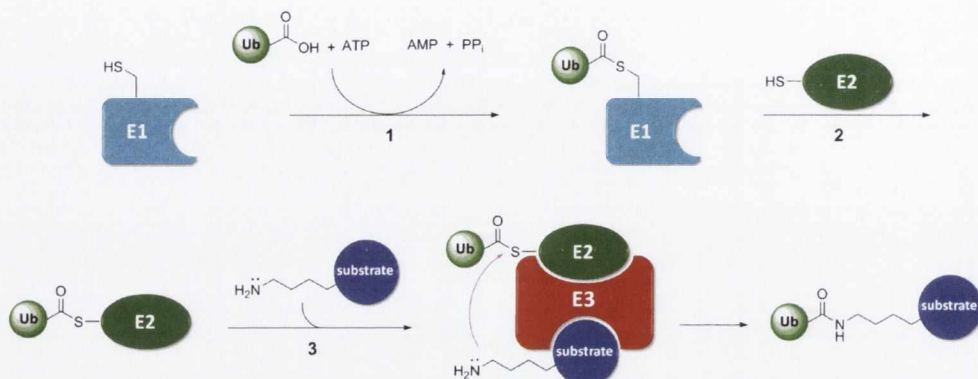


Figure 1.23: Enzymatic pathway for ubiquitination.²⁶ The E1, E2, and E3 enzymes work together to attach Ub to a substrate protein *via* a Lys residue.

1.3.4.2 Chemical synthesis

The implications of ubiquitination are still poorly understood therefore, the development of chemical strategies toward obtaining Ub and its bioconjugates in adequate quantities are essential for subsequent biological study. Peptide ubiquitination has been achieved through the use of removable auxiliaries, however ligation rates are slow.¹⁵¹ The desulfurisation approach introduced by Dawson and co-workers (discussed in Section 1.3.3.1.2) is one of the most influential modifications of NCL, as it triggered many groups to prepare mercapto amino acids, that contain a thiol handle for use in NCL which can be later desulfurised, allowing for NCL at non-Cys containing sites.

Brik and co-workers used this approach to allow for peptide ubiquitination.¹²⁴ As illustrated in Figure 1.24, a thiol was installed at the Lys δ -carbon generating a Cys mimic which can undergo transthioesterification with a Ub thioester. This was followed by an *S,N*-acyl transfer to form the desired isopeptide bond with subsequent desulfurisation using Raney nickel. Although mercapto amino acids have proved to be useful, their use is hampered by the lengthy synthesis required to obtain the thiol-modified amino acid.

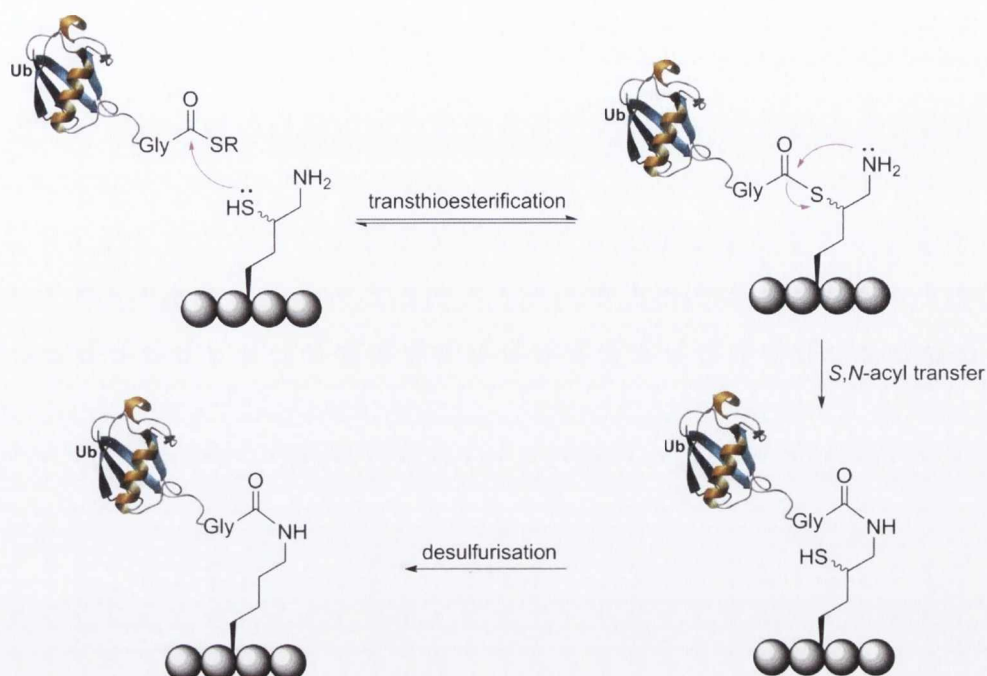


Figure 1.24: Peptide ubiquitination using a δ -mercapto Lys. (Image modified from Brik *et al.*)¹²⁴ The first step involves transthioesterification between the δ -thiol and Ub thioester. Subsequent S,N -acyl transfer forms the isopeptide bond which is then desulfurised using Raney nickel.

1.4 Enzymatic processing of glycopeptides

As previously mentioned, the biosynthesis of *O*- and *N*-linked glycoproteins is conducted by two main groups of enzymes, the glycosidases and the glycosyltransferases. The glycosidases are hydrolase enzymes which hydrolyse glycoconjugates whereas glycosyltransferases construct glycosidic bonds. An example of these two groups of enzymes working in tandem was discussed in the Section 1.2.3.1 during discussion of the biosynthesis of *N*-linked glycopeptides (Figure 1.9). The action of the glycosyltransferases was discussed briefly for the synthesis of *O*-linked glycoproteins (Section 1.2.2.1); however, due to the glycosidase enzymes being a core topic in Chapter 4, further discussion will be limited to the glycosidases, which will be described in the following sections.

1.4.1. The glycosidase enzymes

The hydrolysis of glycosidic linkages is catalysed by the glycosidase enzymes. These enzymes increase the rate of hydrolysis rate by 10^{17} fold when compared to spontaneous hydrolysis events.¹⁵² This remarkable acceleration in rate identifies glycosidases as highly efficient catalysts for this process with

approximately 3% of the human genome being dedicated to the encoding of these enzymes. The large number of glycosidases found in nature has led to their classification into various categories based on criteria such as catalytic mechanism, substrate specificity, and site of glycosidic bond cleavage.¹⁵³ They have been ordered into 130 different families which has led to the generation of the carbohydrate-active enzymes (CAZy) database (available online at www.cazy.org).^{154,155,156} Generally, the glycosidases are described on the basis of the sugar they process and the type of glycosidic bond they cleave; for example, β -glucosidase cleaves glucose linked through a β -glycosidic bond. Glycosidases are referred to as *exo*, if they specifically act on the termini of polysaccharides, while *endo*-glycosidases promote internal hydrolysis of glycosidic linkages within a chain.¹⁵⁷ The hydrolysis of the glycosidic bond can occur *via* one of two widely accepted mechanisms; proceeding with either retention or inversion of stereochemistry at the anomeric position (Figure 1.25), as outlined by Koshland in 1953.¹⁵⁸ In both cases, an oxacarbenium ion intermediate and two catalytic carboxylate amino acids [Asp or glutamic acid (Glu)] are involved in the mechanism.^{152,159}

Retaining glycosidases act by a double-displacement mechanism involving a glycosylation and deglycosylation step, as outlined in Figure 1.25A. In the first glycosylation step, the carboxylate residue acts as a general acid protonating the aglycon with concomitant bond cleavage; the other carboxylate functions as a nucleophile to form a glycosyl-enzyme intermediate with the opposite stereochemistry to the starting material. In the second deglycosylation step, a proton is abstracted from an incoming water molecule followed by hydrolysis of the covalent intermediate to give the hydrolysed product with overall net retention of anomeric configuration.¹⁵²

Inverting glycosidases function through a single step mechanism, do not generate a covalent intermediate and cause inversion of the anomeric configuration (Figure 1.25B). This mechanism involves protonation of the aglycon by a general acid; the other carboxylate acts as a general base activating an incoming water molecule which displaces the aglycon to give the product with inverted configuration.¹⁵²

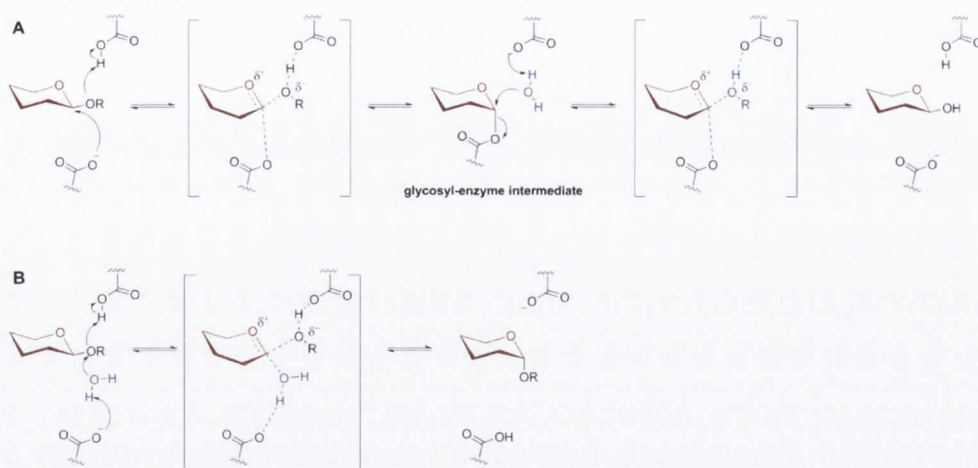


Figure 1.25: Glycosidase mechanisms.¹⁵² (A) Retaining glycosidases hydrolyse the glycosidic bond through a two-step mechanism resulting in retention of the anomeric stereochemistry; (B) Inverting glycosidases hydrolyse the glycosidic linkage *via* a single-displacement step with inversion of the stereochemistry at the anomeric centre.

1.4.2 Glycosidase enzymes used in the chemoenzymatic synthesis

Enzymes involved in glycan biosynthesis can lend their useful attributes to synthetic carbohydrate chemistry where chemoenzymatic approaches combine the flexibility of chemical synthesis with the high regio- and stereoselectivity of enzymatic transformations, to allow for the synthesis of complex glycopeptides in a protecting group free manner.¹⁶⁰ As discussed, the glycosidases are hydrolase enzymes responsible for the hydrolysis of glycosidic bonds. However, glycosidases can be used to form glycosidic linkages under “unnatural” conditions, where a carbohydrate hydroxyl group competes with water as an efficient nucleophile. Most of the glycosidases used for synthetic purposes are retaining enzymes; therefore, mechanistically, incubation of an activated glycoside with a glycosidase enzyme gives a glycosyl-enzyme intermediate which can be intercepted by water or an acceptor resulting in hydrolysis or the formation of a new glycoside, respectively.¹⁶¹

This chemoenzymatic approach can be used to remodel glycoproteins from natural sources. Glycosidases can be used in a concept known as “glycoprotein remodelling” (GPR), which involves the combined trimming and subsequent elaboration of glycan structures.¹³ Endo- β -*N*-acetylglucosaminidases (ENGases) are the most commonly used glycosidases enzymes in this approach. For example, endoglycosidase-H (Endo-H) can be

used to degrade a complex heterogeneous carbohydrate array to a single GlcNAc residue; this residue then acts as a “tag” for the transglycosylation activity of ENGases which enzymatically extend the structure as desired. Endo-A (from *Arthrobacter*) and Endo-M (from *Mucor hiemalis*) are commonly used ENGases which transfer high-mannose and complex *N*-glycans, respectively.⁴ A key example of GPR was reported by the Wang group (Figure 1.26), where heterogeneous RNase B was enzymatically degraded to a single GlcNAc residue and using a glycosyl donor activated as an oxazoline, the truncated glycoprotein was enzymatically extended to produce a single RNase B glycoform.¹⁶²

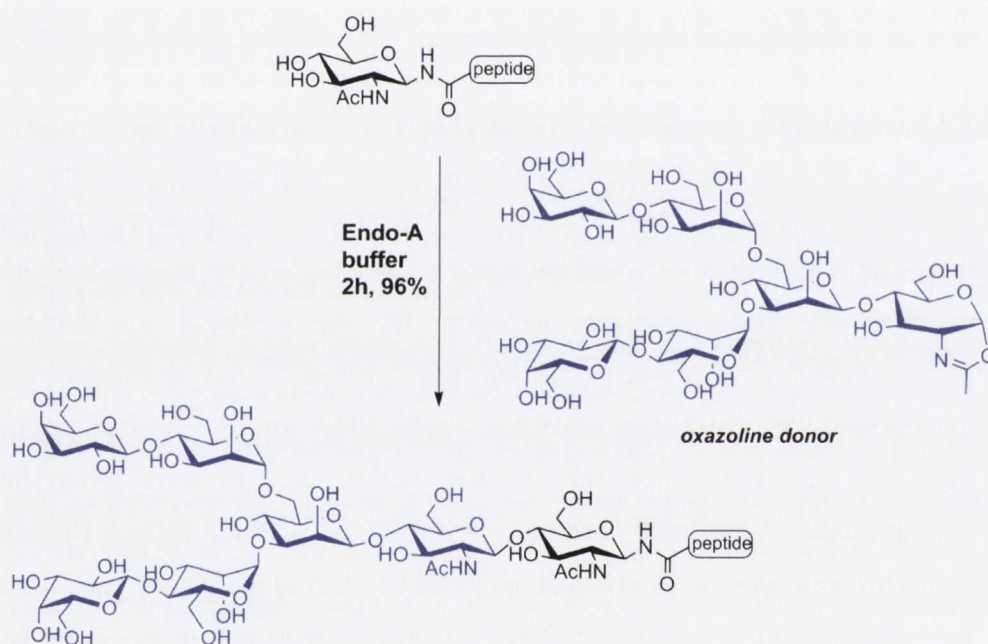


Figure 1.26: Endoglycosidase-catalysed synthesis of RNase B using glycosyl donor activated as an oxazoline and Endo-A.¹⁶²

Although the use of the glycosidases represents a speedy approach to access complex glycopeptides, their use is hampered by their inherent hydrolytic behaviour; when the product itself is a good substrate for the enzyme, it can undergo hydrolysis, resulting in low yields.^{163,164,165} To address this shortcoming, Withers and co-workers reported a class of specifically mutated glycosidases called the glycosynthases, which are devoid of hydrolytic activity.

This new class of enzymes represents a significant advancement in the field of chemoenzymatic synthesis but will not be discussed here.^{166,167,168,169}

1.4.3 Probes used for the detection of glycosidases

The glycosidases have been associated with a number of important biological processes with the dysfunction of these enzymes forming the basis of many disease states, such as lysosomal storage diseases (LSDs).^{170,171} Furthermore, glycosidase inhibitors are an important class of compounds routinely used to treat a diverse range of disorders. For example, Tamiflu (Roche) is approved for the treatment of influenza and functions by inhibiting the action of a viral neuraminidase. Inhibition of this viral glycosidase enzyme prevents the release of progeny viruses during infection.⁶⁰ Glyset (Pfizer) is used for the treatment of Diabetes Mellitus type 2 (DM-2) and acts by inhibiting specific glycosidases in the small intestine. Inhibition of these glycosidases prevents the degradation of complex carbohydrates and the subsequent absorption of glucose into the bloodstream thereby preventing dangerously high blood glucose levels, the hallmark of DM-2.¹⁷² The realisation of glycosidases' dominant role in many biological processes has boosted research into the development of techniques to accurately detect and profile these enzymes so as to improve understanding of their function. Also, the ability to quickly and accurately screen emerging compounds for glycosidase inhibition is of great importance and has intensified research into the development of new sensors for a variety of glycosidase enzymes.

Many of the probes reported in the literature, and those presented in this thesis, function through the release of a reporter molecule upon hydrolysis of a glycosidic bond by an appropriate glycosidase enzyme, as depicted in Figure 1.27. The earliest probes reported were chromogenic probes which are still widely used today. Following on from these, methods based on fluorescence and the lanthanide ions have been reported, with other approaches including the incorporation of glycosidase substrates into gels or nanoparticle systems.¹⁶

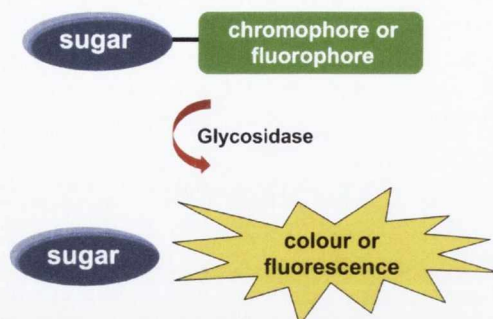


Figure 1.27: Schematic illustration of the mode of action of many chromogenic or fluorogenic glycosidase probes. Hydrolysis of the glycosidic bond in the substrate releases a reporter molecule enabling the detection of the glycosidase enzyme.

The following sections will discuss some of the different probes used for the detection of the glycosidase enzymes. Activity-based probes (ABPs) function by the covalent attachment of a fluorophore to an enzyme target in an irreversible manner. ABPs contain a reactive group, usually an electrophile, which can specifically react with a nucleophilic residue in the active site of the target enzyme, allowing for enzyme detection. These enzymatic probes have been comprehensively reviewed and will not be discussed any further.¹⁷³

1.4.3.1 Chromogenic substrates

The simplest method for the detection of glycosidases is based on the liberation of a coloured product upon cleavage of the glycosidic linkage in a suitable glycoconjugate, with many being commercially available. This approach does not require the use of specialised equipment, can be visualised with the naked eye, and can be applied in glycosidase inhibitor high-throughput screening (HTS).

Glycoconjugates of 4-nitrophenol (4-NP) **39** were amongst the earliest chromogenic glycosidase substrates (Figure 1.28), with the released nitrophenolate **40** displaying an intense yellow colour and absorbance at 405 nm upon cleavage by β -glucosidase (β Glc). The yellow colour and absorbance of **40** is only visible at alkaline pH thus this probes utility can be hampered when the absorbance of the inhibitor being screened overlaps with that of the nitrophenolate **40**. However, this method is still widely used for the screening of glycosidase inhibitors and as a reference for the determination of absolute activities of glycosidases.¹⁷⁴ Another class of chromogenic probes are the indoxyl glycosides, with the most commonly used β -galactosidase (β Gal)

substrate being X-Gal **41**. Hydrolysis of the glycosidic bond in **41** releases a reactive indoxyl which is oxidised, under aerobic conditions, to produce an insoluble dimeric blue indigoid dye **42** (Figure 1.28). However, indigoid dyes tend to be insoluble in water thus can require the addition of organic solvents which may partly inhibit enzyme activity.¹⁷⁵

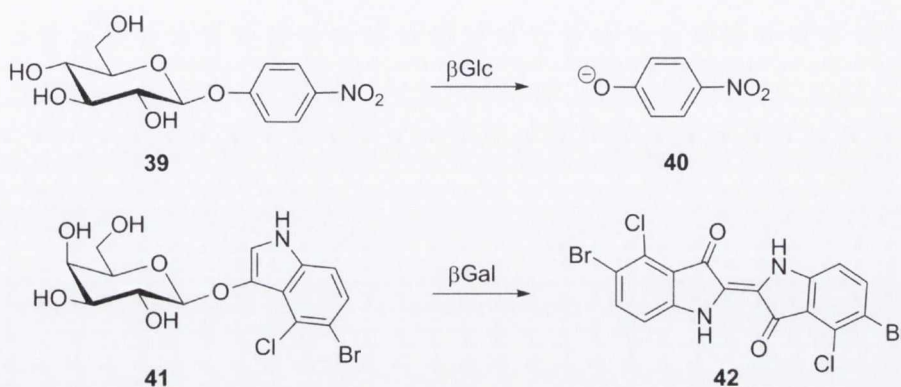


Figure 1.28: Examples of chromogenic glycosidase substrates. (Top) Glucosidase substrate **39** based on the 4-NP structure; (Bottom) X-Gal **41**, a galactosidase substrate based on the indoxyl structure.

1.4.3.2 Fluorogenic substrates

Fluorescence based measurements are several orders of magnitude more sensitive and tend to have lower limits of detection than corresponding chromogenic methods. Therefore, they can be used to detect enzymes at lower concentrations which is crucial for measurement in complex mediums. If carefully designed, they should exhibit negligible/no signal until they react with the target enzyme allowing “on-off” switchability.

Since its introduction in 1975, compound **43**, which is based on the umbelliferone structure, has been widely used as a fluorogenic sialidase substrate (Figure 1.29). Glycoconjugate **43** is practically non-fluorescent whereas the corresponding hydrolysis product is highly fluorescent at pH 10–11. One major disadvantage of **43** is the requirement to basify the mixture to pH 10 to observe a signal at 450 nm. This results in termination of any sialidase activity in the sample.^{176,177} Later, Schauer and co-workers reported **44**, which is a slight modification of **43**, incorporating a trifluoromethyl functionality, which reports on sialidase hydrolytic activity at 500 nm without the need for pH adjustment to observe a signal.¹⁷⁸

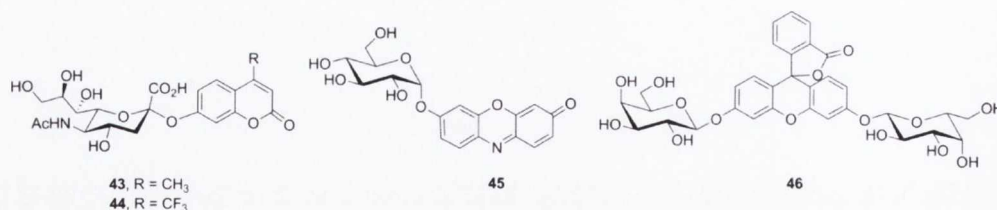


Figure 1.29: Fluorogenic glycosidase substrates based on the umbelliferone, resorufin, and fluorescein structures.

Zheng and co-workers reported resorufin- α -D-glucopyranoside **45** as a substrate for α - (α Glc).¹⁷⁹ This was superior to previous probes based on the umbelliferone structure.¹⁸⁰ The emission of the hydrolysis product is bathochromically shifted to 590 nm resulting in reduced interference from background fluorescent compounds, as seen with umbelliferone substrates **43** and **44**.¹⁸¹

Another commonly employed fluorophore is fluorescein which belongs to the superfamily of xanthene dyes. A widely exploited feature of fluorescein is its ability to switch between the non-fluorescent spirolactone and ring-opened forms.¹⁸² Thus, fluorescein probes can be utilised which are non-fluorescent (spirolactone) until reaction with a target molecule occurs which generates the fluorescent form (ring-opened). For example, fluorescein di- β -D-galactopyranoside (FDG) **46** is a good substrate for β Gal and is commercially available (Figure 1.29).^{183,184} Substrate **46** is hydrolysed in a sequential manner to release fluorescein whose fluorescence can be monitored at 514 nm. MacDonald reported the use of **46** in a multiwell plate system and demonstrated the higher sensitivity of **46** when compared with 4-NP and umbelliferone substrates.¹⁸⁵ Although it was noted in this case of **45** that the turnover rate is slower due to the requirement for sequential glycosidic bond hydrolysis. Many fluorescent glycosidase probes have been reported based on the coumarin, rhodamine, squarine, BODIPY, and cyanine structures, however, they have been discussed in a review we recently published and therefore will not be discussed any further here.¹⁶

1.4.3.3 Lanthanide ions

The intricate optical and spectroscopic properties of the lanthanides ions make them useful spectroscopic tools for probing biological processes. The

lanthanides exhibit sharp, narrow bands in their emission spectra, which correspond to the f - f transitions of the lanthanide ion (Ln(III)), and a large *pseudo* Stokes shift.¹⁸⁶ Perhaps the most useful property of the Ln(III) ions is their long-lived excited states owing to the Laporte forbidden f - f transitions.¹⁸⁷ These long-lived excited states allow for the implementation of time-gating procedures (Figure 1.30A). In this process, there is a delay between the excitation and measurement of emission which allows for any background fluorescence from organic molecules (typically in the nanosecond range) to decay. Afterwards, the longer-lived luminescence from the Ln(III) ion can be selectively recorded.^{188,189} Implementation of time-gated procedures avoids the detection of interfering short-lived autofluorescence and light scattering from biological materials, making the probing of biological environments possible.¹⁹⁰ However, the Laporte forbidden nature of the f - f transitions results in weak absorption, with the Ln(III) ions having inherently low extinction coefficients of less than $10 \text{ M}^{-1} \text{ cm}^{-1}$, as opposed to 10^4 - $10^5 \text{ M}^{-1} \text{ cm}^{-1}$ for typical organic fluorophores.¹⁹¹ As a result, direct excitation of the Ln(III) ions is difficult; therefore, to circumvent this limitation, the design of the lanthanide-based probes must incorporate an appropriate chromophore which is capable of “indirectly sensitising” the Ln(III) ion, in a process known as “the antenna effect”.¹⁹² As depicted in Figure 1.30B, an absorbing chromophore (antenna) is excited allowing for energy transfer to the Ln(III) excited state and subsequent Ln(III) emission. The “antenna effect” and the photophysical properties of the lanthanides will be discussed in more detail in Chapter 4.

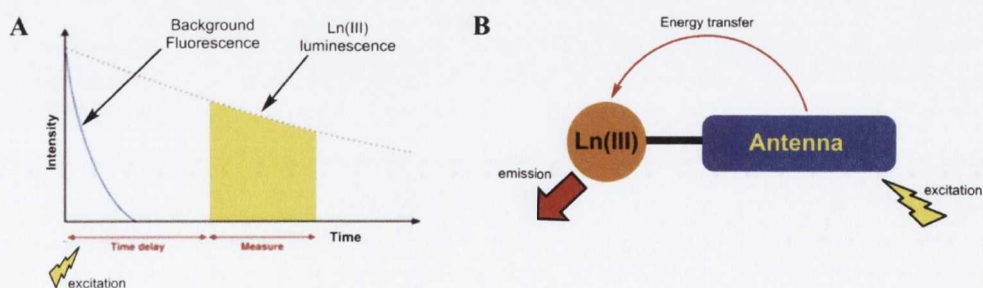


Figure 1.30: Graphical representation of: (A) time-gating procedures, where there is a time delay between excitation and Ln(III) luminescence allowing for background fluorescence to decay and selective detection of Ln(III) luminescence; (B) The “antenna effect”, where an absorbing antenna is used to indirectly sensitise the Ln(III) ion resulting in emission.

1.4.3.3.1 Probes based on lanthanide luminescence

Borbas and co-workers reported an analyte-responsive Ln(III) probe capable of detecting H_2O_2 , $\text{Pd}^{0/2+}$, F^- and βGal by alternating the analyte-responsive moiety.¹⁹³ They previously demonstrated that coumarins could serve as efficient antennae for the sensitisation of Tb(III) and Eu(III).¹⁹⁴ They used this concept to develop a “caged-coumarin” approach for the detection of various analyte, the process of which is depicted in Figure 1.31.

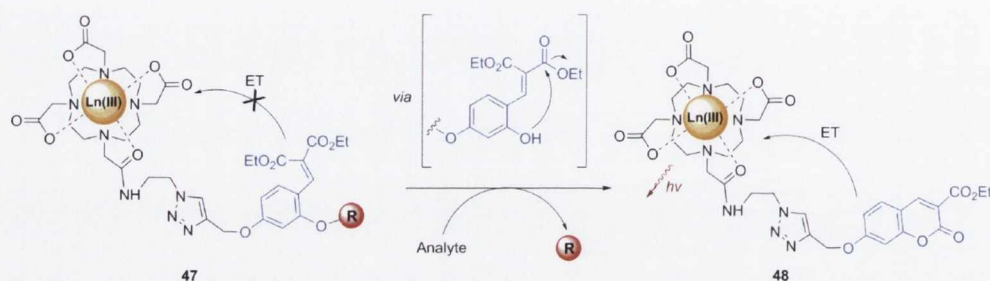


Figure 1.31: Analyte-responsive lanthanide-based probe, reported by Borbas and co-workers, where ET denotes energy transfer.¹⁹³ Complex **47** is triggered to form the coumarin sensitised complex **48** upon analyte-mediated cleavage of the R group. This antenna transformation approach was used to detect βGal activity, H_2O_2 , F^- , and $\text{Pd}^{0/2+}$ by altering the R group.

In complex **47**, the Ln(III) ion is not effectively sensitised, as there is no appropriate antenna to indirectly sensitise the Ln(III) ion (*via* the “antenna effect”), therefore, there is no emission. When the R group is a galactose moiety, exposure to the βGal enzyme causes the cleavage of the glycosidic bond and triggers the formation of the coumarin complex **48**. Formation of the coumarin antenna allows for the indirect sensitisation of the Ln(III) ion resulting in Ln(III) emission thus reporting on βGal activity. Altering the R group to a benzylboronic acid, allyl, or triisopropylsilyl (TIPS) functionality allowed for H_2O_2 , $\text{Pd}^{0/2+}$ or F^- to be detected, respectively. Currently, this example represents the only reported glycosidase probe based on Ln(III) luminescence. Some other enzyme responsive Ln(III)-based probes will be discussed in Chapter 4.

1.4.3.3.2 Probes based on lanthanide relaxivity

Magnetic resonance imaging (MRI) is one of the most powerful techniques in clinical medicine. The process of MRI involves inserting a specimen within an external magnetic field and applying a short radio frequency pulse in order to

excite water protons. These water protons subsequently relax at different rates within different types of environments in the body; these relaxation rates are recorded and spatially encoded to generate an image of internal anatomy. Tissues displaying faster relaxivity times produce a signal of greater intensity resulting in a brighter image.^{195,196,197} To improve the sensitivity of MRI, contrast agents based on the paramagnetic Gd(III) ion are frequently employed to give enhanced image contrast and to shorten the image acquisition times. These agents cause an increase in relaxivity through the binding of water molecules to the Gd(III) ion; the protons of these inner sphere water molecules then exchange with the bulk solvent protons resulting in the paramagnetic influence of the Gd(III) being propagated into the bulk tissue. Overall, this process results in a stronger signal giving enhanced contrast with background tissue and an overall better quality image. In fact, approximately 30% of all MRI scans used a contrast agent, most of which were based on the Gd(III) ion.^{195,198}

The work of enzyme-responsive probes based on the relaxivity properties of the Ln(III) was pioneered by the Meade lab, with them reporting the first “smart” MRI contrast agent, EGad, which is modulated by the action of β Gal.¹⁹⁹ This contrast agent is an example of a probe where the response is governed by changes in the coordination environment or the hydration state (q value) of the Ln(III) ion. The q value is the number of water molecules coordinated to the Ln(III) metal centre and will be discussed in more detail in Chapter 4. In the case of EGad (Figure 1.32), in complex **49**, the sugar is positioned in such a way that restricts water access to the Gd(III) centre resulting in a low relaxivity state. Upon hydrolysis of the glycosidic linkage to form **50**, water accessibility to the Gd(III) inner coordination sphere is increased resulting in a higher relaxivity state which is visualised as a positive contrast in an MR image.

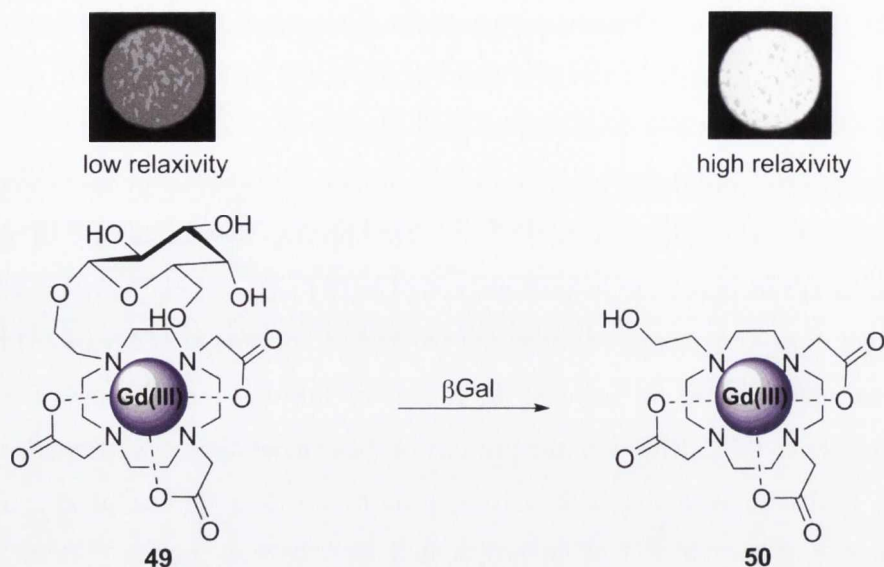


Figure 1.32: “Smart” enzyme-responsive MRI contrast agent EGad, developed by Meade *et al.*¹⁹⁹ The galactopyranose group restricts water access to the Gd(III) metal centre in complex **49**. Addition of β Gal results in cleavage of the glycosidic bond to form **50**, resulting in increased water accessibility to the Gd(III) centre and the transition from a low to high relaxivity state, which is visualised as a brighter MR image.

Despite EGad displaying changes in relaxivity in response to β Gal, the enzyme kinetics of **49** were not optimal. To address this issue, Meade and co-workers later reported another enzyme-activated probe for the detection of β -glucuronidase (β Glu) (Figure 1.33).²⁰⁰ In complex **51**, the sugar group is extended from the Gd(III) centre and the probe operates by a self-immolative mechanism where cleavage of the glycosidic bond releases 4-hydroxy-3-nitrobenzyl alcohol **52**, carbon dioxide, and **53**.

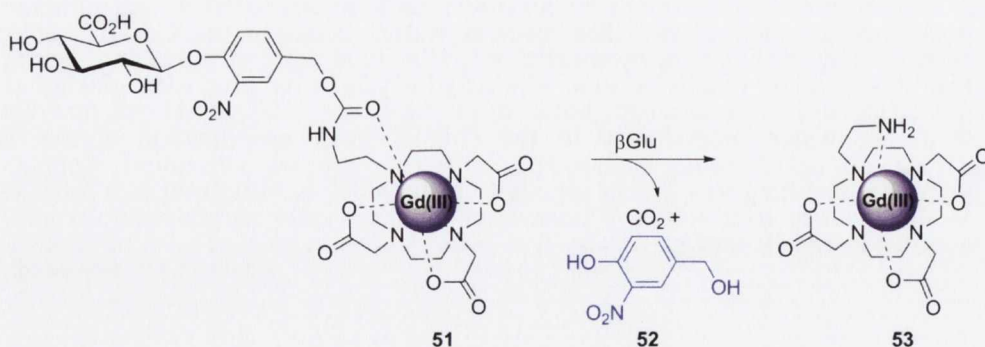


Figure 1.33: Self-immolative MRI contrast agent for the detection of β Glu.²⁰⁰ The cleavage of the glycosidic bond releases glucuronic acid, CO_2 , **52** and complex **53**. Formation of complex **52** resulted in a decrease in the measured relaxivity. Measuring the absorbance of compound **51** allowed the enzyme kinetics of β Glu to be determined.

Unfortunately, the transition from **51** to **53** resulted in a decrease in relaxivity.

However, the release of **52** enabled enzyme kinetics to be measured by recording the absorbance at 422 nm with **51** being found to be a better substrate for β Glu, when compared with 4-NP probes. Therefore, activation of probe **51** generates two distinct outputs, **51** and **52**, both of which report on β Glu activity.

1.5 Work described within this thesis

As discussed throughout this chapter, bioconjugates are desirable synthetic targets. The ability to prepare sufficient quantities of homogeneously modified peptides is vital to allow for subsequent biological interrogation. Significant developments have been made in the field of peptide ligation strategies and glycoconjugation, many of which have been discussed throughout this chapter. Furthermore, the preparation of bioconjugates for the profiling of biologically relevant enzymes is also an area of great interest with the development of such probes providing insight into enzymatic activity and allowing for the identification of enzymatic substrates.

Chapter 2 details *N*-glycosylation, a prevalent PTM, and the development of a new thioester-mediated ligation strategy for the synthesis of *N*-linked glycopeptides. Currently, the main method used to access *N*-linked glycopeptides is Lansbury aspartylation, however this approach suffers from the formation of aspartimide side-products which significantly diminishes yields. Our approach combines thiol-ene coupling chemistry and *S,N*-acyl transfer reaction to ligate a carbohydrate and non-glycan moiety to form a native amide bond at the carbohydrate anomeric centre in a stereoselective manner. As discussed in this chapter, thiol-ene chemistry involves the hydrothiolation of an alkene with a thiol. However, in this work, we investigated the use of thioacids, rather than thiols, as thiol donors in the thiol-ene reaction. An initial thiol-ene reaction between an alkene appended to a glycosylamine and a thioacid results in the ligation of the two entities to form a thioester intermediate in a fast, high yielding step which is compatible with aqueous conditions. Subsequently, an *S,N*-acyl transfer occurs between the anomeric amine and the thioester, proceeding through a large transition-state (13-membered) which was expected to be aided by the templating effect of the sugar moiety, as seen with SAL which was discussed in Section 1.3.3.3. The

final step involves base hydrolysis to give an amide bond at the anomeric centre. Thiol-ene chemistry has the advantage of being fast (reaction times of 1 h), high yielding (yields > 80 %) and reactions are compatible with aqueous media. This approach avoids the use of an activated ester for aspartylation and operates under base-free conditions in an effort to mitigate the formation of aspartimide side products. Furthermore, this methodology outlines the first examples of peptidyl thioacids being employed in the thiol-ene click reaction.

Chapter 3 is based on protein ubiquitination, another common PTM. This chapter uses the thioester-mediated methodology developed in Chapter 2 for application in the chemical ligation of peptide fragments, with a focus on Ub related substrates. This methodology involved the preparation of a peptide fragment consisting of a modified Ser residue bearing an alkene moiety and the ϵ -NH₂ of Lys masked as an azide. This alkene can participate in a thiol-ene reaction with a peptidyl thioacid. Subsequent azide reduction and *S,N*-acyl transfer then furnishes a native isopeptide bond. This is followed by base hydrolysis of the appended ester to give the unmodified peptide product. The initial transthioesterification step of NCL can be quite slow and requires the addition of exogenous thiols to accelerate the reaction. However, our approach exploits the rapid nature of the thiol-ene reaction for the initial ligation step. Furthermore, this ligation methodology requires a Ser or Thr residue for ligation, rather than a Cys residue in NCL, which are naturally more abundant in proteins.

In Chapter 4, the photophysical properties of the lanthanides and their use in enzymatic probes are initially discussed. An array of lanthanide-based complexes were synthesised and their photophysical properties evaluated for their ability to detect the glycosidases enzymes. All of the complexes are based on the macrocyclic cyclen framework and bear an antenna to allow for the indirect sensitisation of the Ln(III) ion. Various *O*-linked glycans were introduced onto the cyclen framework to allow for the generation of a small library of glycosylated lanthanide complexes. It was anticipated that upon addition of a relevant glycosidase enzyme, cleavage of the *O*-glycosidic linkage would occur resulting in concomitant modulation in the lanthanide emission, allowing for glycosidase activity to be detected. In the final section

of this chapter, a mannose-appended lanthanide complex was investigated for its ability to serve as a sensor for the mannose-binding lectin, ConA.

Chapter 6 concludes the work described in this thesis.

Lastly, Chapter 5 details the experimental procedures and characterisation of all compounds prepared within preceding chapters of this thesis.

2.0 Introduction

The majority of proteins in the human body are known to be post-translationally modified with protein glycosylation being one of the most widely observed PTMs. It is estimated that over 50% of proteins are glycosylated with 90% of these glycoproteins involving *N*-linked glycans.^{34,35} Glycosylation is a non-template driven modification with many enzymes involved in the process resulting in the production of heterogeneous glycopeptides. This heterogeneous glycosylation makes the study of the effect of a single glycoform, i.e. one protein modified with one type of glycan, very difficult. Preparation of glycopeptides by chemical means offers a powerful solution to obtaining homogeneous samples, free from undesired glycoforms, for biological study. In this chapter, the development of a new method towards *N*-linked glycopeptides is detailed. Prior to that, a short summary of the most common chemical methods for the preparation of *N*-linked glycopeptides is given.

2.1 Chemical synthesis of *N*-linked glycopeptides

All *N*-linked glycopeptides consist of large, branched oligosaccharide structures, which are divided into 3 types: complex, hybrid, and high mannose, the structures of which are illustrated in Figure 1.4. However, all *N*-glycosides share one common feature; they contain an Asn linked through a β -GlcNAc bridgehead within an Asn-Xaa-Ser/Thr consensus sequence.²⁰¹ Consequently, the vast majority of research in this area is concerned with the formation of this particular linkage in a regio- and stereo-controlled fashion. The construction of the *N*-glycosidic bond proceeds through amide bond forming reactions, rather than glycosylation reactions, with many of the synthetic strategies toward the formation of the *N*-glycosidic bond outlined in Chapter 1. The most popular approaches are summarised in Figure 2.1. One popular method is the stepwise approach whereby the key *N*-glycan linkage is established early in the synthetic strategy with the glycosylamino acid building block being incorporated into a growing peptide chain during SPPS. The major drawback of this approach is the reduced efficiency of subsequent peptide coupling reactions due to the hindered nature of amino acids bearing glycans.²⁰² Furthermore, the

incorporation of a glycan into SPPS typically forces a reliance on Fmoc-SPPS due to the acid lability of glycosidic linkages. An alternative, more convergent approach is Lansbury aspartylation, which is one of the most commonly used methods for the preparation of *N*-linked glycopeptides.^{72,71} This reaction involves the coupling of a glycosylamine with an activated Asp residue on a fully furnished peptide chain in the presence of a suitable base. The main disadvantage of this approach is the requirement for extensive protection of the peptide backbone to prevent unwanted side reactions, such as aspartimide formation, which can significantly diminish yields.⁴¹ The third method involves a chemoenzymatic approach. This can involve the incorporation of a simple glycosyl amino acid early in the synthetic strategy which can be enzymatically elaborated later, therefore combining early stage regioselectivity with the convergence of introducing larger carbohydrate arrays at a later stage in the synthesis. Alternatively, this chemoenzymatic approach can be used to remodel glycoproteins from natural sources. Here, a mixture of glycoforms can be enzymatically trimmed to a truncated *N*-glycan by endoglycosidases. This single sugar residue can then be enzymatically elaborated using endoglycosidases when the sugar donor to be transferred is activated as an oxazoline.²⁰³ This approach allows rapid access to complex structures however, the inherent hydrolytic behaviour of these glycosidase enzymes is a major disadvantage when the glycans attached to the peptide are good enzyme substrates.¹⁶⁵

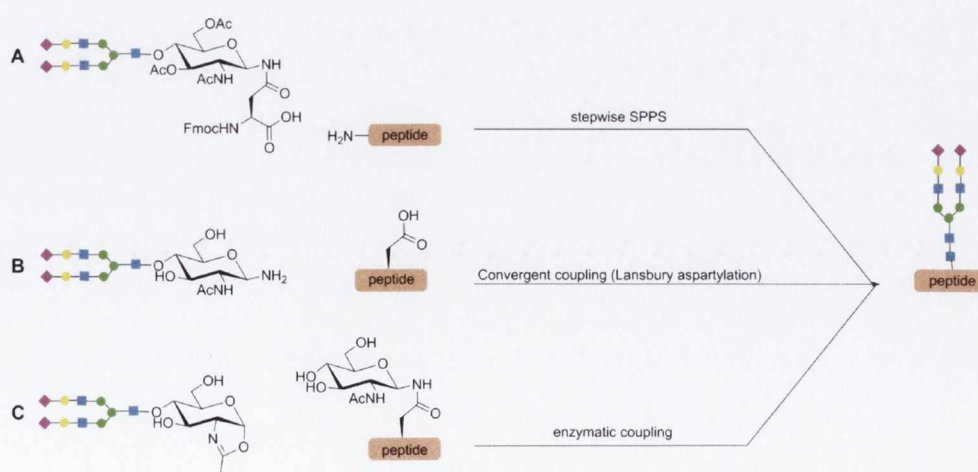


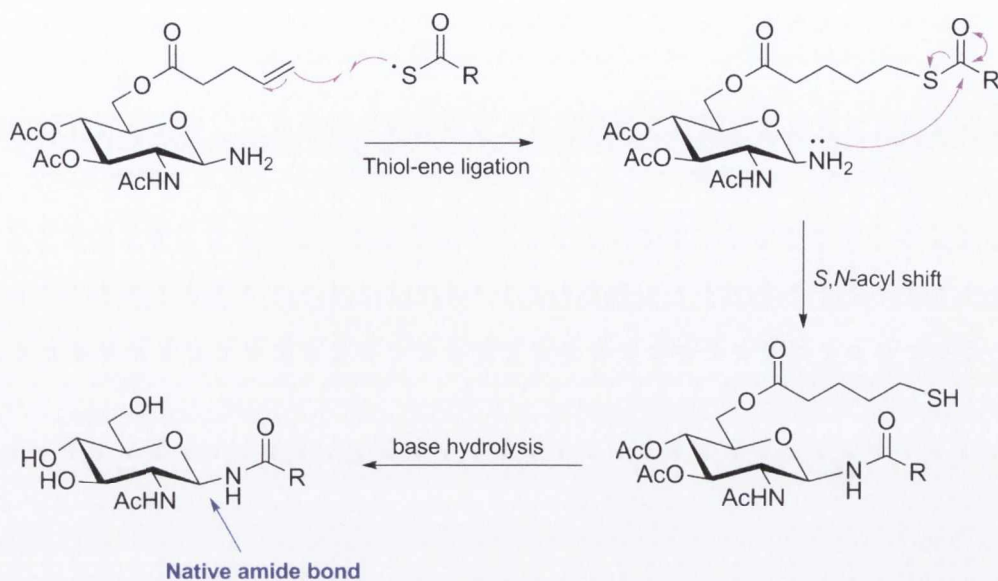
Figure 2.1: Common strategies for the preparation of *N*-linked glycopeptides *via*: (A) coupling of preformed glycosylamino acid into a growing peptide chain in SPPS; (B) Convergent

Lansbury aspartylation of an activated Asp residue with a glycosylamine; (C) Chemoenzymatic synthesis using a glycosyl oxazoline and endoglycosidase enzymes.

2.2 Aims of this work

As discussed, a number of approaches toward *N*-linked glycopeptides have been reported however, these are not without limitations. Therefore, the development of a new method towards the synthesis of *N*-linked glycopeptides is envisaged in this chapter.

One reaction that is emerging in the field of bioconjugation is the thiol-ene click reaction, discussed in Chapter 1, Section 1.2.4.1.1, which involves the hydrothiolation of an alkene *via* a two-step radical process under photochemically/thermally-induced conditions. The “click” status of this reaction is supported by it being fast, highly efficient, orthogonal to a wide range of functional groups, and its compatibility with aqueous and oxygenated conditions.⁸⁸ Thiol-ene coupling has been employed as an efficient tool in bioconjugation however, its application in glycoconjugate synthesis has been somewhat limited to the formation of sulphide-spacer glycosides and *neo*-glycopeptides.^{204,205,206} Therefore, we wanted to exploit the “click” aspects of this reaction for the formation of native *N*-linked glycopeptides. Our approach combines thiol-ene coupling and an *S,N*-acyl transfer reaction to ligate a carbohydrate and non-glycan motif to form a native amide bond at the carbohydrate anomeric centre, the mechanism of which is depicted in Scheme 2.1. The proposed methodology aims to allow for the convergent assembly of *N*-linked glycopeptides under base-free conditions.



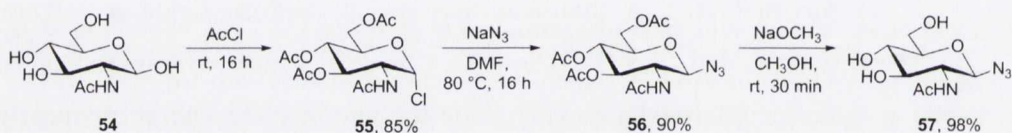
Scheme 2.1: The formation of a native anomeric amide bond using thiol-ene mediated ligation and an *S,N*-acyl transfer reaction. Step one involves a thiol-ene reaction between a thioacid generated thiol radical and an alkene functionality to give a thioester intermediate. A subsequent *S,N*-acyl transfer reaction between the anomeric amine and thioester yields a native amide bond. The last step involves the base hydrolysis of the ester groups to give the desired deprotected *N*-glycan.

In the first step, a thiol-ene reaction is used to ligate an alkene functionality appended on a GlcNAc with a thioacid generated thiol radical to yield a thioester intermediate. This thioester intermediate can subsequently undergo an *S,N*-acyl transfer reaction with the anomeric amine through a 13-membered transition state. This transition state is rather large, when compared with NCL, however, the templating effect of the carbohydrate is expected to assist the acyl transfer. In the case of SAL, Wong and co-workers postulate that the restricted conformation of the carbohydrate moiety brings the amino group and the thioester into close proximity facilitating the rearrangement *via* a 14- or 15-membered ring.²⁰⁷ This templating effect of the carbohydrate unit was later employed by Brik and co-workers when they used a cyclohexane auxiliary to mimic the carbohydrate unit in SAL to facilitate acyl transfer over a 11-membered transition state for use in peptide ligation.¹³⁹ Therefore, precedence for *S,N*-acyl shifts proceeding through comparable ring sizes encouraged the pursuit of this approach. The final step in the reaction sequence involves base hydrolysis of all the ester moieties to deliver the unprotected glycan with a native amide bond at the anomeric position. As discussed in Chapter 1, Section 1.2.4.1.1, thiol-ene ligations typically involve the addition of a thiol onto an

alkene. Therefore, the use of a thioacid as a thiol source is a novel approach to forging the useful thioester linkage which is heavily implicated in current chemical ligation methodologies. GlcNAc is the sole carbohydrate employed in this methodology due to the fact that all *N*-linked glycopeptides comprise of a GlcNAc residue forming the initial sugar-peptide linkage. This approach involves the synthesis of the alkene-containing GlcNAc compound and an array of thioacids, the synthesis of which will be described in the following sections.

2.3 Synthesis of glycosyl alkene acceptor

As depicted in Scheme 2.1, a GlcNAc glycosylamine with an alkene appended at the C-6 position was required for the initial ligation reaction; therefore, an azide functionality was envisaged at the anomeric position which could be subsequently reduced to the desired amine. The synthetic scheme toward the glycosyl azide **57** is depicted in Scheme 2.2.



Scheme 2.2: Synthesis of glycosyl azide **4** from GlcNAc.

Firstly, commercially available 2-acetamido-2-deoxy-D-glucopyranose **54** was treated with acetyl chloride (AcCl) and left to stir at rt for 16 h. After an aqueous work up and subsequent recrystallisation from diethyl ether (Et₂O), **55** was isolated as colourless crystals in 85% yield. The presence of the chloride donor **55** was confirmed by the downfield shift of the anomeric H-1 proton at δ 6.21 ppm, due to the deshielding effect of the chloride group. The glycosyl halide **55** was expected to adopt an α -configuration due to the anomeric effect and this configuration was confirmed by the small H1-H2 vicinal coupling constant of 3.6 Hz, which is indicative of the α -anomer.

The anomeric effect is a general phenomenon in carbohydrate chemistry which refers to the preference of electron withdrawing substituents at the anomeric carbon to adopt an axial orientation (α -anomer), rather than an equatorial orientation (β -anomer). This phenomenon was first observed by Edward in 1955 here in Trinity College Dublin and later coined as the

anomeric effect by Lemieux in 1958.^{208,209} A popular rationalisation for the anomeric effect is based on the electron delocalisation from the endocyclic oxygen, which is illustrated in Figure 2.2. In the case of the α -anomer, it is stabilised by delocalisation from the endocyclic oxygen lone pair of electrons to the C-X antibonding orbital ($n \rightarrow \sigma^*$). Investigations into compounds which experience the anomeric effect show a lengthened C-X bond while the adjacent C-O bond is shortened, which supports the theory of hyperconjugation. This stabilising effect does not occur in the case of the β -anomer hence this configuration is less favoured.²¹⁰

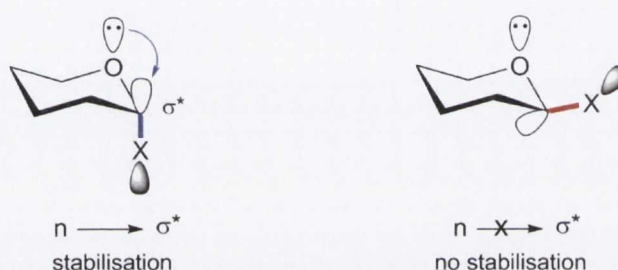
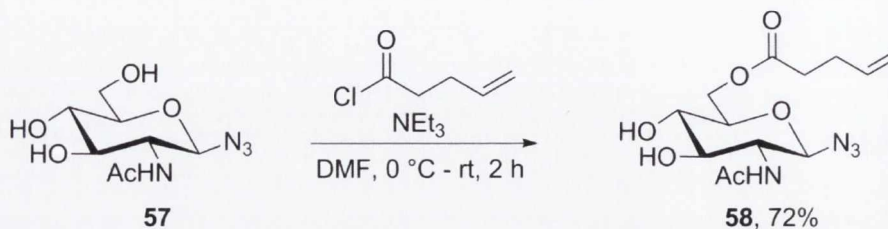


Figure 2.2: The anomeric effect, where X is an electron withdrawing substituent.^{208,211} (Left) Electron donation from the oxygen lone pair to the C-X antibonding orbital stabilises the α -anomer. (Right) This effect is not observed with the β -anomer and therefore there is no stabilisation effect.

The chloride of **55** was subsequently displaced in an S_N2 reaction using sodium azide in DMF at 80 °C to give **56** in 90% yield. The installation of the azide at the anomeric position was confirmed by the upfield shift from δ 6.21 ppm to 4.78 ppm and the larger H1-H2 vicinal coupling constant of 9.0 Hz, indicating stereochemical inversion. The presence of the azido group was confirmed by infrared spectroscopy (IR) which showed a characteristic sharp peak at 2112 cm^{-1} . The *O*-acetyl groups were subsequently removed under Zémpfen conditions using freshly prepared NaOCH_3 in CH_3OH to furnish **57**. The next step involved the regioselective introduction of the alkene moiety at the C-6 position. An ester group bearing the required alkene moiety was chosen due to the ease of removal of the ester functionality which would be required later in the synthetic strategy. Treatment of **57** with 1.2 eq. of 4-pentenoyl chloride and triethylamine (NEt_3) in anhydrous DMF gave **58** in 72% yield after column chromatography (Scheme 2.3). The regioselective esterification at the C-6 position was confirmed by ^1H NMR as the H-6 protons shifted downfield from 3.73 and 3.92 ppm to 4.26 and 4.49 ppm, respectively.

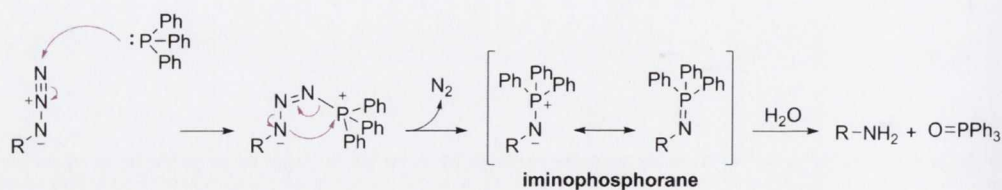
This selective esterification was further confirmed by heteronuclear multiple bond correlation (HMBC) from H-6, δ_{H} 4.26 and 4.49 ppm to C=O, δ_{C} 172.3 ppm.



Scheme 2.3: Regioselective esterification of the **57** C-6 hydroxyl using 4-pentenoyl chloride and NEt_3 .

2.3.1 Reduction of the anomeric azide

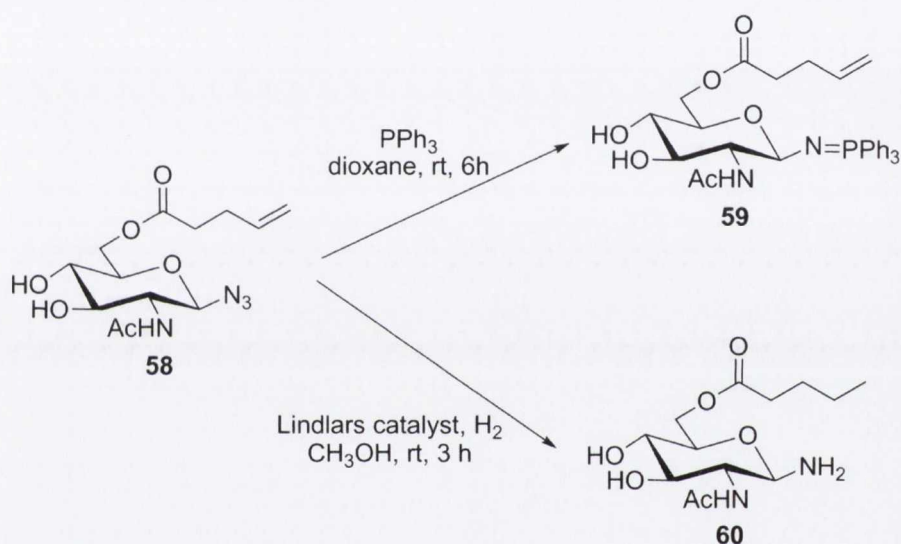
The final step in the planned synthesis involved the reduction of the azide to obtain the required amine. Due to the presence of the double bond, a method for azide reduction which did not reduce the alkene was required. The Staudinger reduction was the first choice for the reduction of the azide in compound **58**. This reaction was first described in 1919 and has since been widely employed in organic chemistry due to its mild reaction conditions, namely the reduction of azides in the presence of unsaturated bonds.^{212,213} The reaction involves an azide reacting with a tertiary phosphine, such as triphenylphosphine (PPh_3), and H_2O to yield an amine, the mechanism of which is depicted in Scheme 2.4. The PPh_3 reacts with the azide to form a phosphazide intermediate which loses nitrogen to yield an iminophosphorane. The addition of H_2O leads to the hydrolysis of the iminophosphorane to furnish an amine and triphenylphosphine oxide.



Scheme 2.4: The Staudinger reduction mechanism.^{212,213} The azide is reduced to a primary amine using PPh_3 and H_2O *via* an iminophosphorane intermediate.

Accordingly, compound **58** was dissolved in dioxane followed by the addition of PPh_3 and was left to stir for 6 h, followed by the addition of H_2O to hydrolyse the iminophosphorane intermediate (Scheme 2.5). However, after

column chromatography, the major product isolated was the glycosyl iminophosphorane **59**, despite H₂O being added to the reaction mixture. The presence of the iminophosphorane was indicated by ¹H NMR with the presence of the 15 phenyl protons of the PPh₃ being observed. This was further confirmed through HRMS displaying a *m/z* peak at 563.2310, corresponding to the [59+H]⁺ species. Hydrolysis of the iminophosphorane **59** was attempted by refluxing in ethanol, as reported by Pinter and co-workers, however, in our hands this was unsuccessful and repeatedly furnished the stable iminophosphorane.²¹⁴ Therefore, an alternative reduction approach to the desired glycosylamine was sought.

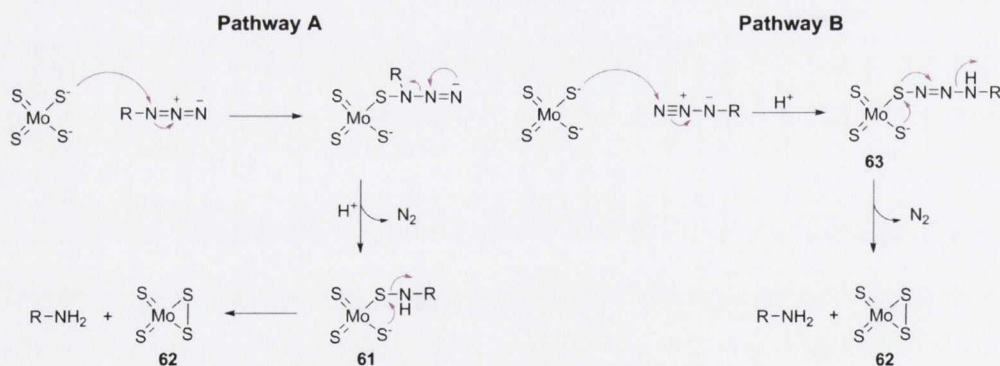


Scheme 2.5: Attempted reduction of the anomeric azide using the Staudinger reduction (*top*) and Lindlar catalyst (*bottom*).

Catalytic hydrogenation is operationally simple and is widely used for the reduction of azides, however, it results in the additional reduction of any unsaturated bonds present in the molecule. A significant advance in achieving chemoselectivity in hydrogenation was achieved through the introduction of Lindlar catalyst. Lindlar catalyst is an alternative catalyst used in hydrogenation which consists of Pd deposited on calcium carbonate and poisoned with traces of lead resulting in a catalyst which is slightly deactivated.²¹⁵ This deactivation has found use in hydrogenation reactions, whereby alkynes are reduced to alkenes, without further reduction to the alkane. Furthermore, Lindlar catalyst has been employed for the reduction of azides to amines in the presence of sensitive groups, such as alkenes and

benzyl protecting groups.²¹⁶ Considering this, Lindlar catalyst was applied for the reduction of **58** with the aim of achieving selective reduction of the azide moiety. Following a literature procedure reported by Baskaran and co-workers, azide **58** was successfully reduced to the amine however, reduction of the alkene to the alkane was also observed yielding **60**.²¹⁷

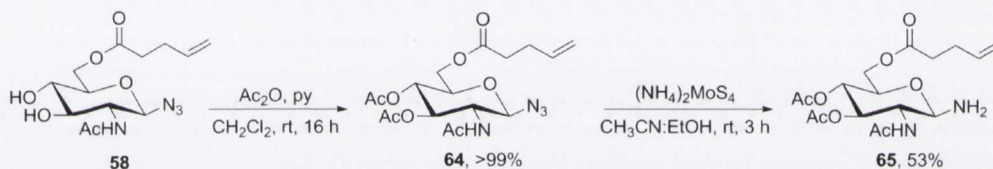
An interesting method for the reduction of glycosyl azides was reported by Chandrasekaran and co-workers which involves the treatment of a protected azide with ammonium tetrathiomolybdate ((NH₄)₂MoS₄).²¹⁸ They reduced a variety of glycosyl azides using this reagent with reaction times reduced from 16 h to just 3 h when using sonochemical conditions. The authors proposed two plausible mechanisms for the azide reduction, both of which are depicted in Scheme 2.6. In pathway A, the MoS₄²⁻ attacks the α-nitrogen of the azide to give **61** following the loss of nitrogen. Intermediate **61** can subsequently give rise to mononuclear persulfido molybdenum **62** and the desired free amine. The intermediate **62** is subsequently converted to the Mo(V) dimer, Mo₂S₈²⁻. Alternatively, in pathway B, MoS₄²⁻ attacks the γ-nitrogen of the azide to give intermediate **63** which can decompose to liberate the free amine, nitrogen, and **62**. The mononuclear complex **62** is subsequently oxidised to the dinuclear Mo₂S₈²⁻.



Scheme 2.6: Proposed mechanism for the reduction of glycosyl azides to glycosylamines using (NH₄)₂MoS₄.²¹⁸ Pathway A involves attack at the α-nitrogen of the azide; Pathway B involves attack at the γ-nitrogen. The mononuclear persulfido molybdenum **62** is subsequently oxidised to give the dinuclear complex Mo₂S₈²⁻.

Due to the literature procedure reported by Chandrasekaran *et al.* using protected glycosyl azides, compound **58** was acetylated using acetic anhydride (Ac₂O) and pyridine (py) in CH₂Cl₂ to give **64** in quantitative yield (Scheme

2.7). Compound **64** was then treated with $(\text{NH}_4)_2\text{MoS}_4$ in a $\text{CH}_3\text{CN}:\text{EtOH}$ (1:1) mixture under sonochemical conditions for 3 h to give **65** in 53% yield after filtration. Gratifyingly, the alkene moiety remained intact, as observed by ^1H NMR, and the characteristic IR azide peak at 2217 cm^{-1} was no longer observed indicating successful reduction of the azido group. Furthermore, the desired stereochemistry of the glycosylamine remained intact with **65** displaying a H1-H2 vicinal coupling constant of 9.9 Hz, indicative of the desired β -anomer confirming no anomerisation of the glycosylamine had occurred. Although the TLC indicated complete consumption of the starting material, the moderate yield of 53% is most likely due to the workup involving repeated filtering of the reaction mixture to remove all traces of the persulfido **62** and dinuclear species $\text{Mo}_2\text{S}_8^{2-}$.



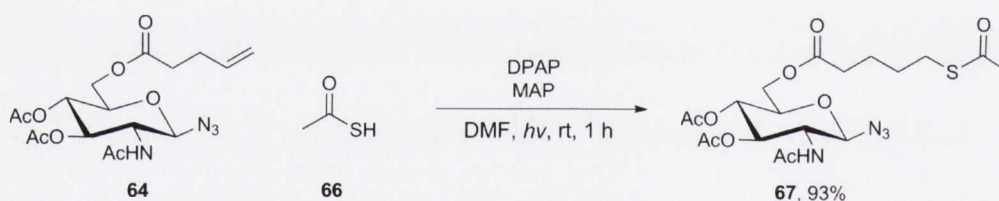
Scheme 2.7: Acetylation of glycosyl azide **58** followed by reduction of the *O*-acetyl protected glycosyl azide **64** using $(\text{NH}_4)_2\text{MoS}_4$ to give **65**.

2.3.2 Initial thiol-ene ligation with thioacetic acid

With the glycosylamine in hand, it was decided to investigate the ability of thioacids to serve as thiyl radical donors using a simple thioacid initially. Therefore, commercially available thioacetic acid **66** was used to optimise the reaction conditions. As discussed in Chapter 1, thiol-ene reactions have been used for the site specific modification of peptides, however to date, thiol-ene chemistry has not been employed for the formation of *N*-linked glycopeptides, or indeed any amide bond. Although thiol-ene ligations are normally conducted with an alkene and a thiol, rather than a thioacid, Fraser-Reid reported the elongation of *n*-pentenyl glycosides using thiol-ene chemistry with thioacetic acid being one of the thiols utilised.²⁰⁵ These reactions were thermally initiated using AIBN with reactions being conducted at high temperatures of $75\text{ }^\circ\text{C}$. To be generally applicable in glycopeptide synthesis, milder conditions for the thiol-ene reaction were required. Previous members of the Scanlan group have used thiol-ene ligations for the functionalisation of peptides. For example, Dr.

Lyn Markey used thiol-ene chemistry for the functionalisation of short Cys-containing peptides with different alkenyl moieties.²¹⁹ The optimal reaction conditions involved the use of radical initiator DPAP and photosensitiser 4-methoxyacetophenone (MAP) in DMF. Furthermore, Yagci and co-workers compared the efficiency of various radical initiators for use in thiol-ene ligations and found that the initiator DPAP led to the highest efficiencies.²²⁰

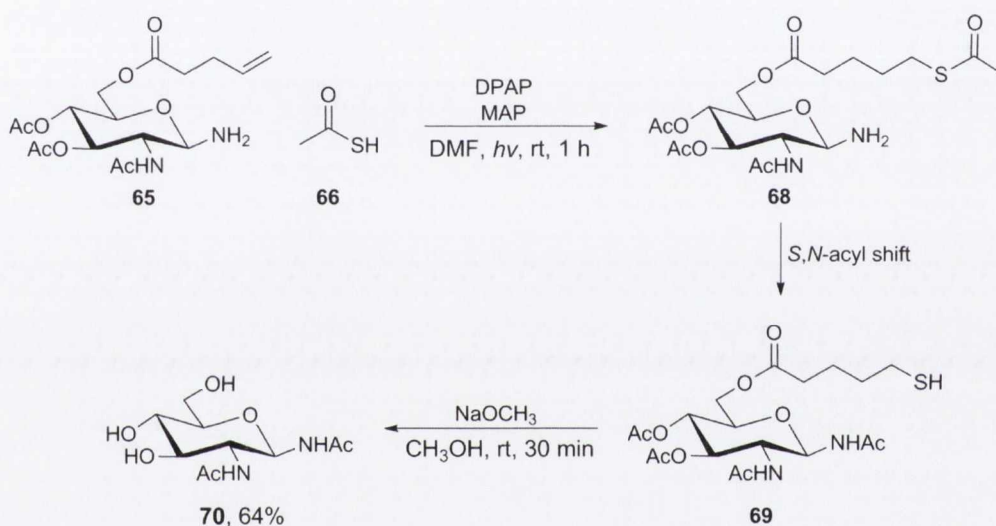
Therefore, it was decided to apply these optimised reactions conditions to investigate whether thioacetic acid donor **66** could participate in a thiol-ene ligation with glycosyl azide **64** and glycosylamine **65**. In order to optimise the reaction, the first thiol-ene reaction was conducted using the glycosyl azide **64** to allow for the radical reaction to proceed without the subsequent *S,N*-acyl shift, as depicted in Scheme 2.8. Glycosyl azide **64** was added to a solution of DPAP (0.1 eq.) and MAP (0.1 eq.) in anhydrous DMF followed by the addition of **66** (1.1 eq.) and the mixture was irradiated in a UV oven for 1 h at rt. Gratifyingly, the desired product **67** was obtained in 93% yield after column chromatography.



Scheme 2.8: Thiol-ene ligation between glycosyl azide **64** and **66**.

After verification that **66** was a suitable donor for the thiol-ene reaction, the same reaction conditions were applied to glycosylamine **65**. The proposed reaction pathway is outlined in Scheme 2.9. In this case, the initial thiol-ene reaction yields intermediate **68** which was then left to stir for 16 h to allow for the *S,N*-acyl transfer reaction to proceed. Reports of *S,N*-acyl transfer reactions occurring over comparable ring sizes, reported by Wong and Brik, at non-hindered ligation junctions required 8 and 5 h, respectively.^{139,135} Removal of the solvent followed by crude NMR analysis implied that the *S,N*-acyl shift had indeed occurred due to a HMBC correlation from H-1 to a carbonyl peak at 173.5 ppm, indicating the formation of an amide **69** at the anomeric position. To confirm this, the mixture was subjected to Zémlen conditions to hydrolyse

all of the ester groups. Gratifyingly, after column chromatography, compound **70** was produced in an overall yield of 64% over the three steps: thiol-ene ligation, *S,N*-acyl transfer, and ester hydrolysis. The presence of **70** was confirmed by the presence of two acetyl groups in the ^1H NMR accompanied by two signals in the NH correlation spectroscopy (COSY) confirming that the obtained product contained two amide functionalities. Furthermore, the desired anomeric β -configuration was confirmed by the large H1-H2 vicinal coupling of 9.9 Hz. No α -linked glycosylamine was recovered indicating that no anomerisation of the glycosylamine had occurred.

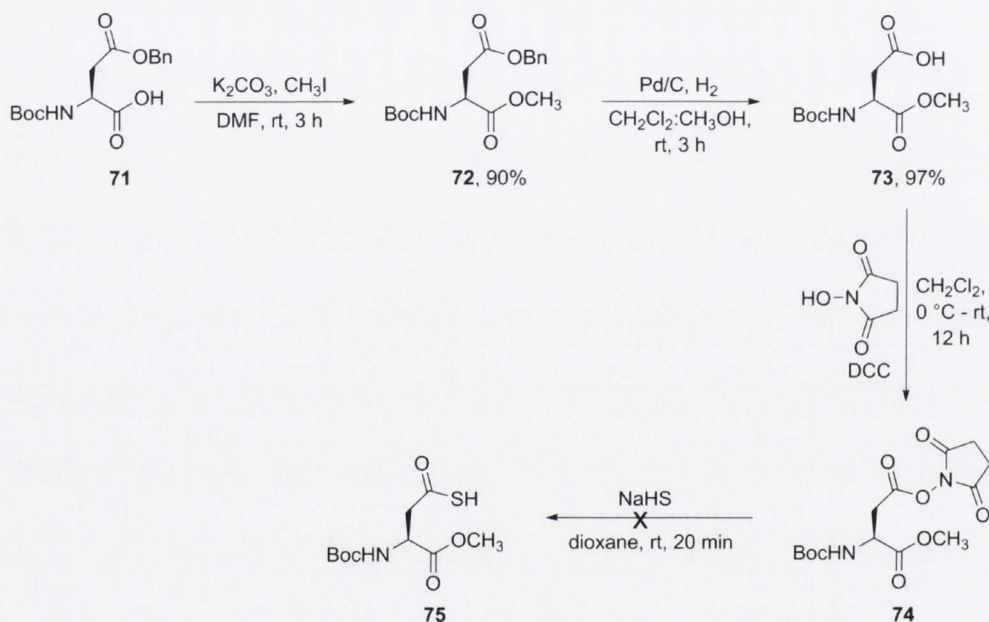


Scheme 2.9: Proposed reaction pathway between glycosylamine **65** and thioacetic acid **66**. The first step involves a thiol-ene reaction between the glycosyl alkene **65** and **66** to give a thioester intermediate **68**. This is followed by an *S,N*-acyl shift to give anomeric amide **69**. The last step involves Zemplén conditions to furnish the deprotected glycosyl amide **70**.

The above result indicates that **66** can be successfully employed in thiol-ene ligations and glycosylamine **65** can participate in the subsequent *S,N*-acyl transfer reaction. Furthermore, the large 13-membered transition state appears to not be an issue with the acyl migration most likely aided by the templating effect of the carbohydrate, as reported by Wong and Brik.^{139,117} This finding, demonstrated for the first time that a thiol-ene, *S,N*-acyl shift cascade process could be employed for the formation of amide bonds in high yield, therefore paving the way toward a novel ligation methodology for bioconjugation.

2.4 Synthesis of peptidyl thioacid donors

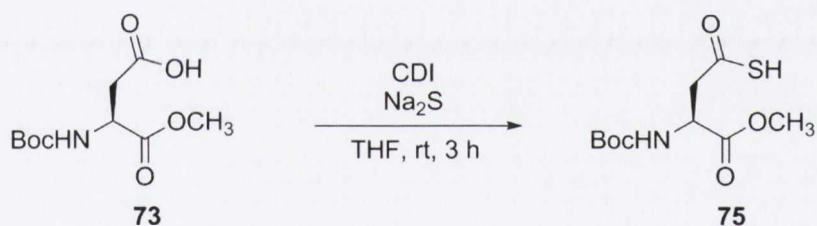
Encouraged by the initial results, we next investigated the methodology for the formation of an *N*-linked glycopeptide. As *N*-glycosylation contains a consensus sequence of Asn-Xaa-Ser/Thr, with the Asn amide side chain being the site of glycosylation, it was important for us to apply this methodology to form this key amide linkage. Accordingly, the preparation of an Asp thioacid was required. The first attempt at the synthesis of the Asp thioacid is outlined in Scheme 2.10.



Scheme 2.10: Attempted synthesis of Asp thioacid **75** via an activated *N*-hydroxysuccinimide ester, as reported by Crich and co-workers.²²¹

The first step involved the methylation of commercially available Boc-Asp(OBzl)-OH **71** using iodomethane and K_2CO_3 in DMF to give **72** in 90% yield. The benzyl group was then removed using catalytic hydrogenation conditions of Pd/C (0.1 eq.) under an atmosphere of hydrogen to furnish **73** in 97% yield after filtration. The preparation of thioacid **75** proved to be more challenging. Initial attempts to form thioacid **75** utilised a method reported by Crich and co-workers.²²¹ The carboxylic acid **73** was treated with *N,N'*-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide to give the activated ester **74** which was subsequently reacted with sodium hydrogen sulphide (NaHS). This procedure requires an aqueous workup involving an

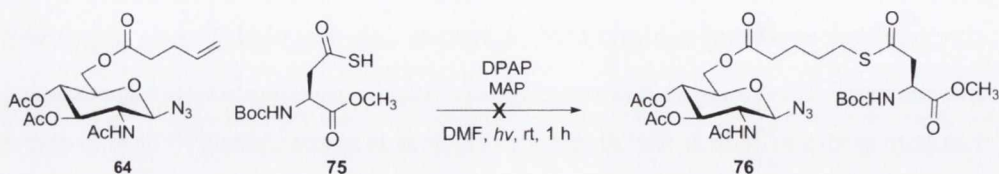
acid wash (1M aq. HCl solution) to remove excess NaHS. As the hydrolysis of thioacids is reported to be acid catalysed, the workup was conducted quickly in an attempt to minimise undesired hydrolysis of the thioacid.²²² Furthermore, due to the susceptibility of the thioacid to hydrolysis, the reaction mixture was immediately analysed using HRMS, however only the acid **73** was found with no thioacid **75** being detected whatsoever, despite multiple reaction attempts. A second method reported by Yudin and co-workers was tested.²²³ This involved treatment of **73** with 1,1'-carbonyldiimidazole (CDI) and sodium sulphide (Na_2S), as shown in Scheme 2.11. The workup also involved an acid wash, however they reported prior cooling of the reaction mixture and the aq. HCl solution to 0 °C before the quenching of the reaction, with the cooled HCl also added slowly. This step appeared to be crucial to avoid hydrolysis of the formed thioacid **75**. Initial HRMS analysis showed the formation of the desired thioacid **75** displaying a m/z peak at 262.0749, corresponding to a $[\text{75-H}]^+$ species with some of the corresponding carboxylic acid **73** also being detected.



Scheme 2.11: Formation of thioacid **75** using CDI and Na_2S , as reported by Yudin and co-workers.²²³

Despite the presence of the carboxylic acid **73**, it was decided to attempt the thiol-ene ligation using the crude thioacid **75** with glycosyl azide **64**, as shown in Scheme 2.12. Unfortunately, using the same thiol-ene conditions previously described, the desired product **76** was not formed with the alkene functionality still clearly visible by ^1H NMR. Attempts to elicit a reaction between **64** and **75** by applying excess of the crude thioacid (up to 6.0 eq.), increasing the amount of DPAP (up to 1.0 eq.) and prolonging the irradiation time (up to 5 h) were all unsuccessful in furnishing **76** in appreciable yields. The lack of reaction was attributed to the hydrolysis of the thioacid **75** to the corresponding carboxylic acid **73**. Using the procedure depicted in Scheme 2.11, the amount of thioacid **75** being formed and subsequently subjected to thiol-ene reaction with glycosyl

azide **64** is unknown, due to the consistent detection of carboxylic acid **73** by HRMS. Therefore, an alternative method that would allow for more effective preparation of the thioacid **75** was required.

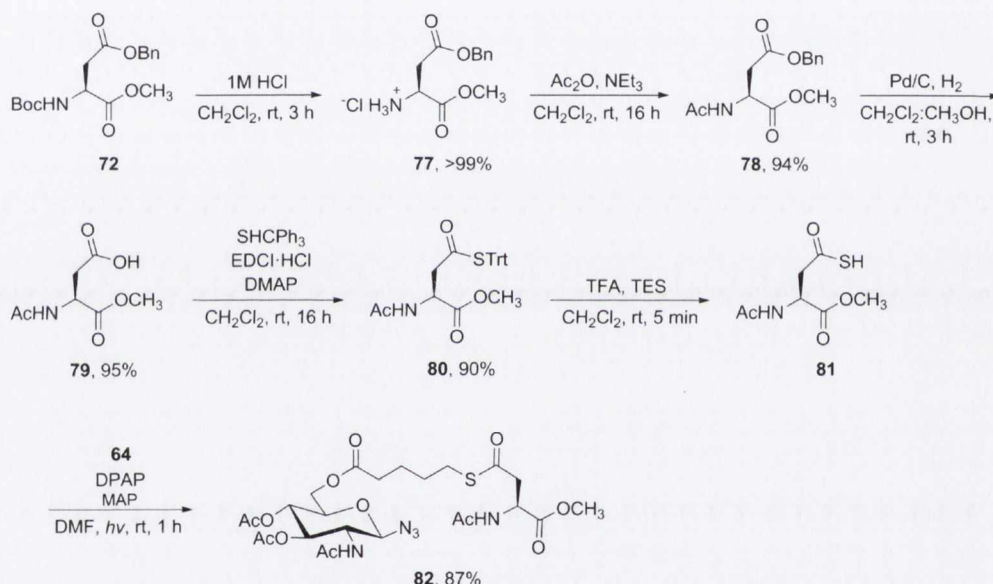


Scheme 2.12: Attempted thiol-ene ligation using glycosyl azide **64** and Asp thioacid **75**.

2.4.1 Synthesis of *S*-trityl protected thioacid

Crich and co-workers reported the use of the trityl group as a temporary protecting group for peptidyl thioacids, as it can be removed rapidly and the generated thioacid reacted *in situ*.²²⁴ This approach would obviate the need for an aqueous workup, therefore attenuating the problem of thioacid hydrolysis. The deprotection of the trityl group requires treatment with TFA; therefore, using this strategy the previously employed Asp amino group in **72** was protected with an *N*-acetyl (Ac) group, rather than the acid-labile Boc protecting group. As depicted in Scheme 2.13, the Boc group in compound **72** was removed by treatment with HCl in CH₂Cl₂ to afford **77** quantitatively as the hydrochloride salt. The amine was subsequently acetylated using Ac₂O and NEt₃ to give the *N*-acetylated **78**. The benzyl group was removed using catalytic hydrogenation, as described previously, to furnish **79** with the free carboxylic acid side chain. The *S*-trityl group was then introduced by reaction of **79** with the coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI·HCl) and triphenylmethanethiol (SHCPh₃) to give **80** in 90% yield after column chromatography. With the protected thioacid in hand, the *S*-trityl group was subsequently removed to give **81** using a 25% solution of TFA in CH₂Cl₂ together with triethylsilane (TES), a reducing agent capable of trapping the released trityl cation. A colour change from colourless to yellow was observed upon formation of the trityl cation which serves as a useful indicator for the reaction progress, along with the yellow colour disappearing upon scavenging of the cation by TES. The reaction was monitored by TLC analysis with full consumption of **80** occurring after 5 min. As mentioned previously, the thioacids are susceptible to

hydrolysis hence once the reaction was complete, the solvent was rapidly removed *in vacuo* and the crude thioacid was immediately used in the next step. The crude thioacid **81** was added to a mixture of glycosyl azide **64**, DPAP, and MAP in DMF and irradiated in the UV oven for 1 h. Encouragingly, the thiol-ene reaction was successful in furnishing the glycoconjugate **82** in 87% after column chromatography. This reaction required the use of a slight excess of the thioacid (1.5 eq.) as previous conditions using just 1.1 eq. of the thioacid furnished the ligated product **82** in a lower yield of 56%. Reduction of the glycosyl azide and investigation of the *S,N*-acyl shift will be discussed later in this chapter.

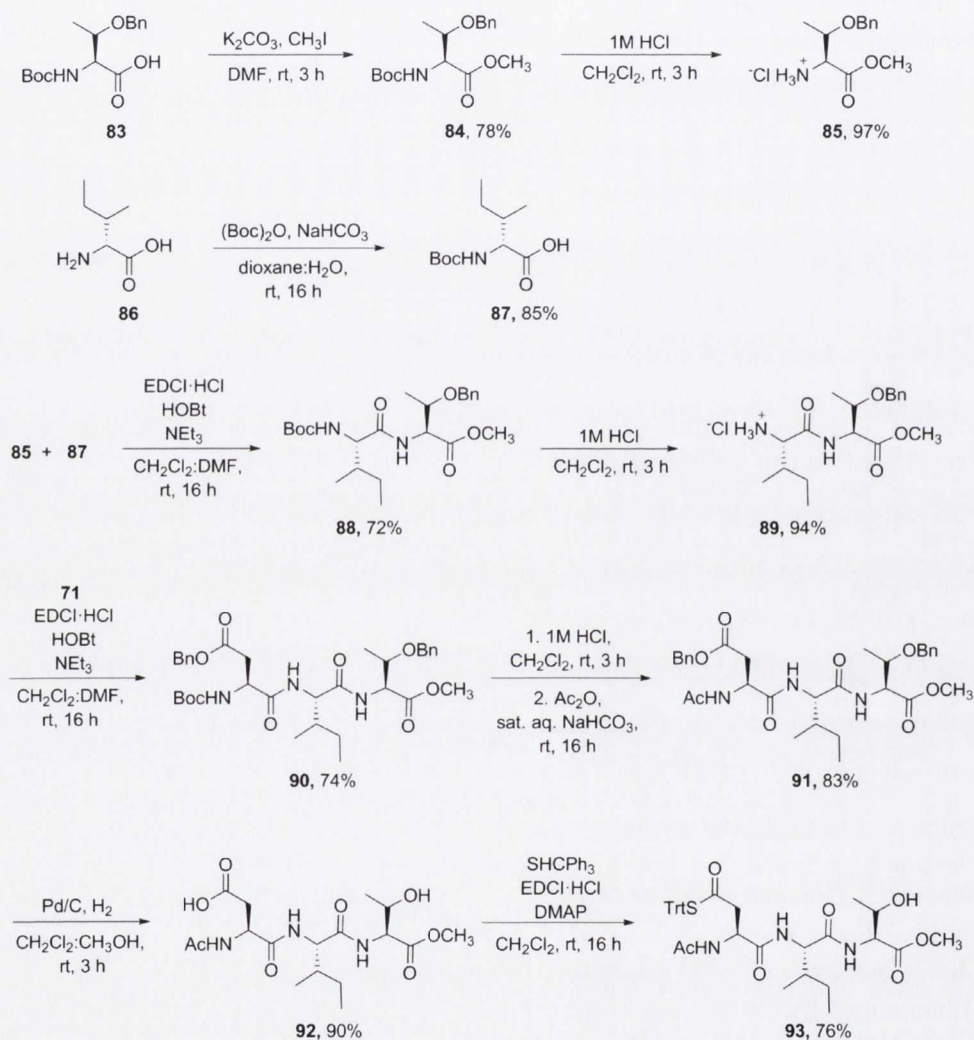


Scheme 2.13: Synthesis of *S*-trityl protected thioacid **80** and its subsequent deprotection and thiol-ene ligation with glycosylazide **64**.

2.4.2 Synthesis of EPO tripeptide thioacid sequence

Encouraged by the success of the thiol-ene reaction with the Asp thioacid **81**, it was decided to prepare a more complex peptidyl thioacid with the aim of generating a small library of *N*-linked glycopeptides. As discussed in Chapter 1, EPO is a glycoprotein hormone which is commonly used as a synthetic target for the preparation of *N*-linked glycopeptides, as it contains 4 glycosylation sites, 3 of which are *N*-linked. Considering this, a tripeptide unit from EPO was chosen for the preparation of a peptidyl thioacid. The tripeptide chosen consists of an Asn-Ile-Thr sequence, the synthesis of which is depicted

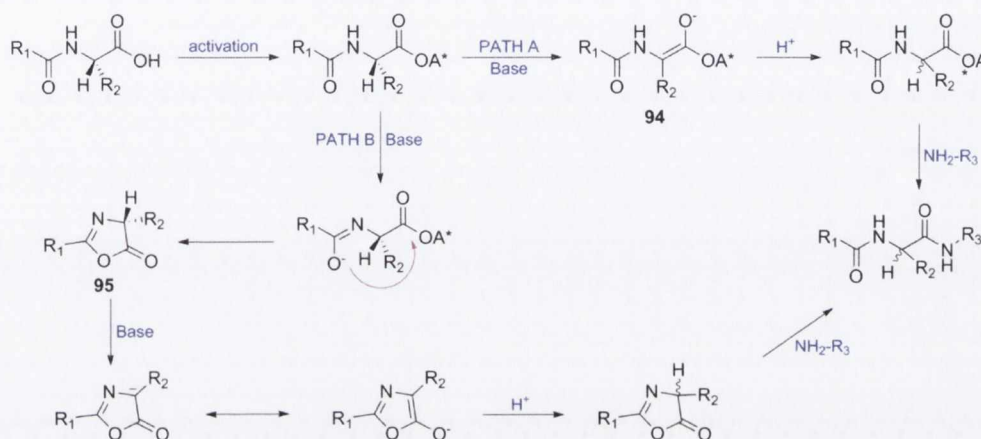
in Scheme 2.14. The first step in the synthesis involved the methylation of the carboxylic acid group of commercially available Boc-Thr(Bzl)-OH **83** using iodomethane and K₂CO₃, as described previously, to give **84** in 78% yield. The Boc protecting group was subsequently removed to furnish **85** as the hydrochloride salt in 97% yield. The amino group of Ile **86** was protected using Boc anhydride ((Boc)₂O) and NaHCO₃ to give **87** in 85% yield.



Scheme 2.14: Synthesis of *S*-trityl protected tripeptidyl thioester **93**.

The next step involved the first peptide coupling reaction to form the desired dipeptide **88**. Activation of the carboxylic group can lead to racemisation, which can proceed through one of two pathways, both of which are depicted in Scheme 2.15. Path A involves direct abstraction to form an enolic intermediate **94** or path B which proceeds *via* cyclisation to an

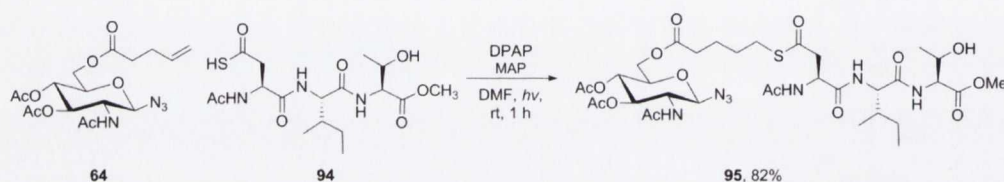
oxazolone intermediate **95** which can subsequently racemise.^{94,225} Both pathways compromise the chiral integrity of the resulting peptide product and therefore need to be minimised. Accordingly, additives such as HOBt are routinely employed in peptide coupling reactions, with HOBt being one of the most effective suppressors of racemisation by the formation of a less reactive HOBt ester.



Scheme 2.15: Racemisation pathways, where OA* denotes activated ester.⁹⁴ (Path A) Direct enolisation: Abstraction of the α -proton generates oxyanion **94** which can be reprotonated on either face leading to racemised products; (Path B) Oxazolone formation: Racemisation by this route is promoted by activation of the α -carboxyl group which facilitates cyclisation to give an oxazolone **95**. Deprotonation of the α -proton followed by reprotonation from either face results in a racemic mixture.

Considering this possible side reaction, peptide coupling between **85** and **87** was conducted in the presence of coupling reagent EDCI·HCl, NEt₃ and HOBt to give **88** in 72% yield with no racemisation observed. The Boc group of **88** was subsequently removed using acidic conditions of HCl in CH₂Cl₂ to afford **89**. Compound **89** was then coupled with commercially available **71**, using the same coupling conditions previously employed, to give the desired tripeptide **90** in 74% yield. Due to the planned introduction of the *S*-trityl group later in the synthetic strategy, the acid labile Boc group of **90** was removed using the previously described acidic conditions and then acetylated by stirring with Ac₂O in sat. aq. NaHCO₃ solution to furnish **91** in 83% yield over the two steps. The benzyl group was then removed using catalytic hydrogenation conditions to afford **92** in 90% yield. Lastly, the free carboxylic group of **92** was converted to the *S*-trityl thioester, using the conditions previously employed, to furnish the final protected thioacid **93** in 76% yield.

With the desired tripeptide **93** in hand, the *S*-trityl group was subsequently removed using TFA and TES, to give the free thioacid **94** (Scheme 2.16). This thioacid **94** was immediately added to a solution of glycosyl azide **64**, DPAP, and MAP in DMF and irradiated in the UV oven for 1 h. This thiol-ene reaction successfully furnished the glycoconjugate **95** in 82% yield after column chromatography. In order to test the compatibility of the reaction with aqueous conditions, the reaction depicted in Scheme 2.16 was repeated in a mixture of DMF:phosphate buffer (50:50), with the ligated product **95** being obtained in comparable yield of 80% after column chromatography.

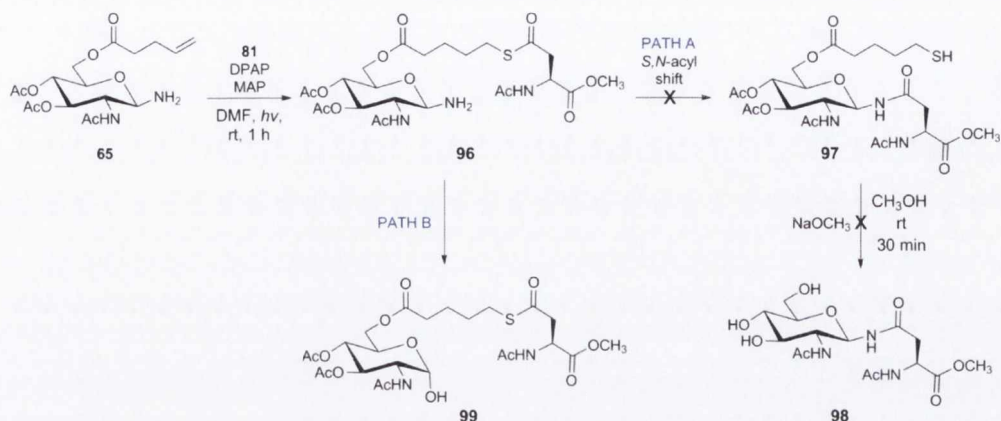


Scheme 2.16: Thiol-ene reaction between glycosyl azide **64** and tripeptide thioacid **94** to give conjugate **95**.

2.5 Attempted one-pot procedure using thioacids and glycosylamine

Following the success of the thiol-ene reactions with the peptidyl thioacids **81** and **94**, it was decided to try the one-pot procedure of thiol-ene, *S,N*-acyl shift, and ester hydrolysis using the free glycosylamine with the aim of generating the corresponding *N*-linked glycopeptides. Firstly, glycosylamine **65** and Asp thioacid **81** were subjected to the thiol-ene reaction conditions and then the reaction mixture was left to stir for 16 h at rt to allow the *S,N*-acyl shift to occur (Scheme 2.17, Path A). The mixture was then subjected to Zémlen conditions to hydrolyse the ester functionalities. Unfortunately, none of the desired product **98** was recovered from the reaction mixture, suggesting that the reaction had not proceeded as expected. However, there was no evidence of the alkene functionality in ^1H NMR, indicating that the initial thiol-ene reaction was successful. This implies that the *S,N*-acyl migration had not occurred and that perhaps the reaction had not proceeded past intermediate **96**. Katritzky and co-workers have demonstrated *S,N*-acyl migrations occurring through 5- to 19-membered transition states for peptide systems when carried out under microwave conditions at 50 °C.^{226,227} Therefore, the reaction was repeated as

before followed by microwave irradiation at 50 °C for 6 h in an attempt to assist the acyl migration.



Scheme 2.17: The attempted preparation of Asn *N*-linked glycopeptide **98**. (Path A) The ligated intermediate **96** undergoes an *S,N*-acyl shift to give **97** with a native amide bond at the anomeric centre. Zemplén conditions furnish the desired deprotected *N*-linked glycopeptide **98**; (Path B) Glycosylamine hydrolysis of intermediate **96** to give the α -hemiacetal **99**.

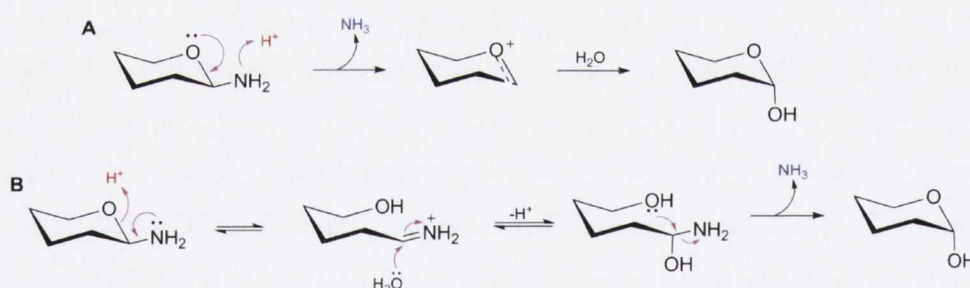
Unfortunately, this attempt to enhance the migration reaction was not fruitful and none of the desired product **98** was recovered. In an attempt to understand this result, the thiol-ene reaction between **65** and **81** was repeated followed by stirring in DMF for 16 h. The solvent was then removed, prior to base hydrolysis, to allow for crude NMR analysis. The crude ^1H NMR showed that the thiol-ene reaction had proceeded due to the disappearance of the alkene functionality; however, the thioester was still intact as deduced from the carbonyl signal at 198 ppm in the ^{13}C NMR. This indicated that the reaction had not progressed past intermediate **96**. Notably, it was observed that the anomeric position was now displaying a smaller H1-H2 vicinal coupling constant of 2.7 Hz, indicative of an α -anomer, rather than the initial β -anomer.

AML, which was discussed in Chapter 1, is very sensitive to the nature of the amino acids at the ligation site with those outlined in Section 1.3.4.1.1, Figure 1.16 being limited to Gly-containing junctions.^{115,116} However in the case of AML, a hindered secondary amine is engaged in the *S,N*-acyl transfer whereas in this system a primary amine, albeit a weakly nucleophilic glycosylamine, is involved instead. The use of a primary amine was expected to impart increased sequence tolerance at the ligation junction, as seen in the case of SAL where a primary amine participates in the *S,N*-acyl migration.¹³⁶

However, in the case of glycosylamine **65** and aspartyl thioacid **81**, it was thought that the *S,N*-acyl migration was sluggish when compared with the previously employed thioacetic acid due to the increased steric hindrance at the thioester intermediate. This slow acyl transfer resulted in the hydrolysis of the glycosylamine to the corresponding α -hemiacetal **99** (Scheme 2.17, Path B). The hydrolysis of the glycosylamine was confirmed by HRMS with *m/z* peak at 615.1808, corresponding to the $[99+\text{Na}]^+$ product.

2.5.1 Glycosylamine hydrolysis

Hydrolysis of glycosylamines can proceed *via* one of two mechanisms, which are depicted in Scheme 2.18, both of which are acid-catalysed. The first mechanism (A) involves the release of ammonia and proceeds through an oxacarbenium ion whereas the stepwise mechanism (B) involves the hydrolysis of an imine intermediate to give ammonia and the resulting hemiacetal. The latter mechanism involving an acyclic imine intermediate is the most widely accepted mechanism of glycosylamine hydrolysis.^{228,229,230}



Scheme 2.18: Mechanism of acid catalysed glycosylamine hydrolysis.²²⁸ (A) Concerted mechanism which proceeds *via* an oxacarbenium ion intermediate; (B) Stepwise hydrolysis proceeding *via* an acyclic intermediate.

It is widely reported that glycosylamine hydrolysis displays strong dependence on the pH of the solution with it being at its maximum in weakly acidic solutions.^{228,231,232} Therefore, it was thought that residual TFA from the removal of the *S*-trityl group could be catalysing the hydrolysis of the glycosylamine. Accordingly, the reaction of **65** with **81** was repeated in the presence of NEt_3 (2.0 eq.) to neutralise any residual TFA and in an attempt to curtail the hydrolytic conversion to the free reducing sugar. Unfortunately, this approach was not successful. Other attempts included conducting the reaction in aqueous buffered conditions (pH 7.4 phosphate buffer) however the α -

anomer at H-1 was consistently observed. Unfortunately, reaction of glycosylamine **65** and tripeptide thioacid **94** yielded similar results, whereby the *S,N*-acyl shift was not occurring with glycosylamine hydrolysis being the prominent reaction.

2.6 Thioacid derivatives to probe scope of the methodology

As the screened reaction conditions were unsuccessful in delivering the desired *N*-linked glycopeptides, it was decided to probe the scope of the reaction in an attempt to gain insight into why the *S,N*-acyl shift was not occurring in the case of the Asn thioacids **81** and tripeptide **94**. Various *S*-trityl protected thioacids were prepared firstly to investigate the scope of the thiol-ene reaction when thioacids are employed. Secondly, these thioacids would be tested for their ability to undergo subsequent *S,N*-acyl transfer reactions with glycosylamine **65**. The *S*-trityl protected thioacids prepared are illustrated in Figure 2.3 and were synthesised from the corresponding commercially available carboxylic acids to furnish the protected thioacids in good yields. As discussed in Chapter 1, the ligation efficiency of NCL and SAL is heavily dependent on the steric nature of the thioester entity with sterically demanding thioesters being more difficult to ligate.^{207,233,110} Accordingly, the prepared thioacids vary in steric hindrance (alkyl, phenyl, peptidyl) to probe the ability of the glycosylamine to undergo the *S,N*-acyl shift with the thioester intermediate.

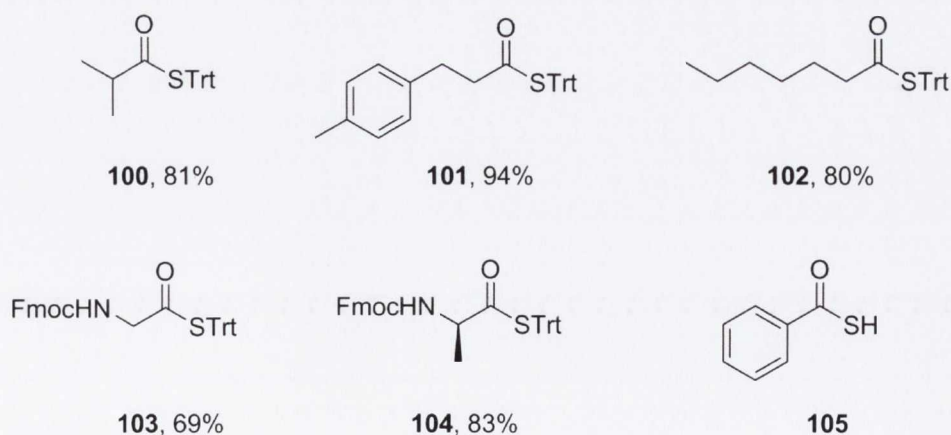
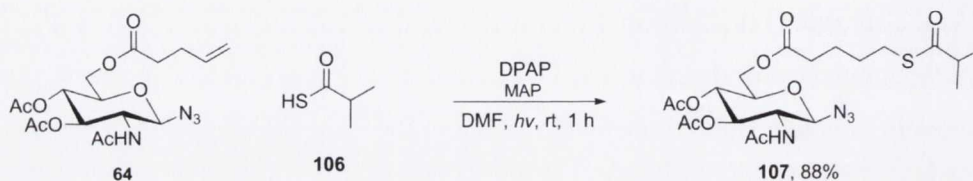


Figure 2.3: Prepared *S*-trityl protected thioacids and the corresponding yields. The thioesters were prepared from the corresponding carboxylic acids. Thiobenzoic acid **105** is commercially available.

Firstly, the *S*-trityl isobutyric acid derivative **100** was deprotected using the previously described conditions of TFA and TES to afford **106** (Scheme 2.19), which was then reacted with the glycosyl azide **64** to give the desired glycoconjugate **107** in 88% yield. This result further illustrates that various thioacid derivatives can be employed as thiyl donors in the thiol-ene reaction.



Scheme 2.19: Thiol-ene ligation with glycosyl azide **64** and thioacid **106** to give **107**.

The *S*-trityl protected thioacids, depicted in Figure 2.3, were then subjected to the previously used reaction conditions with glycosylamine **65**. This procedure involved an initial thiol-ene reaction with the *in situ* generated thioacid (1.5 eq.), DPAP, MAP, and glycosylamine **65** in DMF, all of which were irradiated in a UV oven for 1 h. The reaction mixture was then allowed to stir for 16 h at rt to allow the *S,N*-acyl shift to occur. The final step involved the removal of the ester groups using basic conditions of NEt₃ (25.0 eq.) in a mixture of H₂O and CH₃OH for 4 h. These milder hydrolysis conditions were employed, rather than the previously employed Zémplen conditions, to avoid hydrolysis of the base labile Fmoc functionality in **103** and **104**. The reactions of protected thioacids **100**, **101**, **102**, and **103** with glycosylamine **65** all produced the desired products after column chromatography, the structures of which are depicted in Figure 2.4. The ligated products were furnished in good yields with only β -linked products being formed.

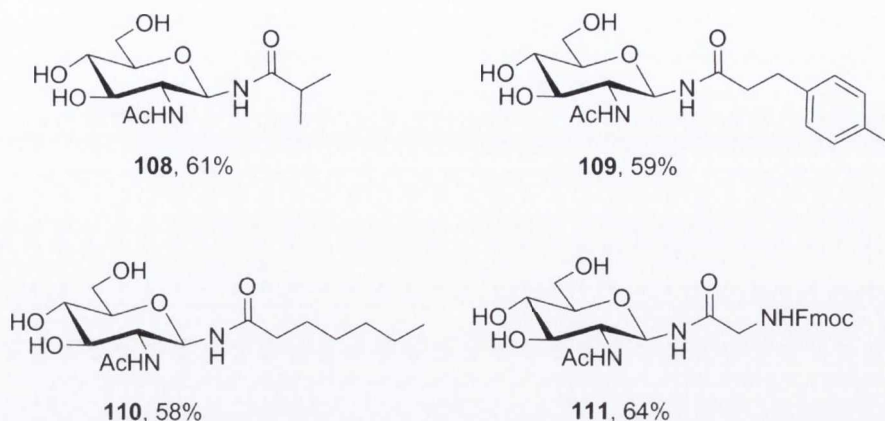
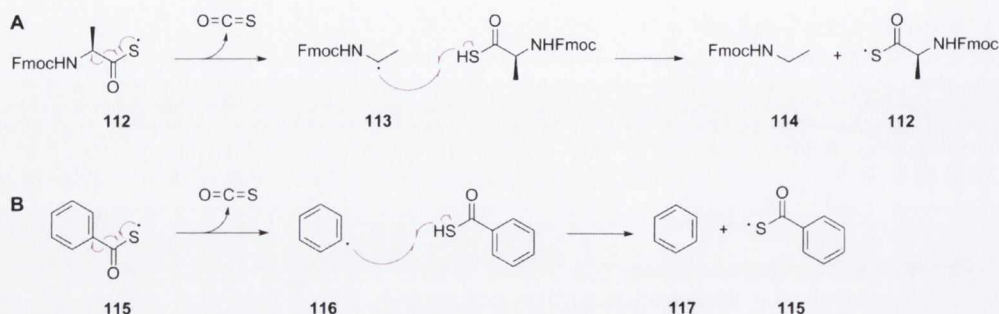


Figure 2.4: Compounds successfully made using the sequential ligation methodology. Reactions involved a thiol-ene ligation between glycosylamine **65** and the corresponding *S*-trityl protected thioacid in DMF for 1 h in a UV oven. This was followed by stirring in DMF for 16 h at rt. The final step involved base hydrolysis using NEt_3 in a $\text{H}_2\text{O}:\text{CH}_3\text{OH}$ mixture. Yields over the three synthetic steps are reported.

2.6.1 CSO elimination

Intriguingly, the reactions of glycosylamine **65** and *S*-trityl protected alanine **104** and commercially available thiobenzoic acid **105** did not furnish any of the desired products. In fact, it was found that the initial thiol-ene reaction was not proceeding despite excess thioacid (up to 5.0 eq.), excess DPAP (up to 1.0 eq.) and longer reaction times (up to 5 h) being employed. The use of thioacids in thiol-ene ligations was simultaneously being investigated by another member of the Scanlan group (Lauren McSweeney) with similar results being obtained with thioalanine **104**. A side reaction was discovered to be depleting the thiyl radical which was in turn reducing the efficiency of the thiol-ene reaction, as depicted in Scheme 2.20A. It was found that CSO elimination of the thioalanine thiyl radical **112** to form **113** was competing with the thiol-ene ligation, with **114** being confirmed by HRMS. This CSO elimination was also likely to be a viable side reaction in the case of thiobenzoic acid (Scheme 2.20B). Thiyl radical **115** degrades to form a stable phenyl radical **116** which can subsequently lead to the formation of benzene **117**. Considering this, it is surprising that the isobutyric thioacid **106** participated so well as a thiyl radical donor in the thiol-ene reaction with glycosyl azide **64** (Scheme 2.17), as CSO elimination would lead to a reasonably stable secondary radical species. In the case of **101**, **102**, and **103**, CSO elimination would lead to the formation of a high energy primary radical species. Therefore, it is likely that the thiol-ene reaction is favoured over CSO elimination due to the formation of a less stable

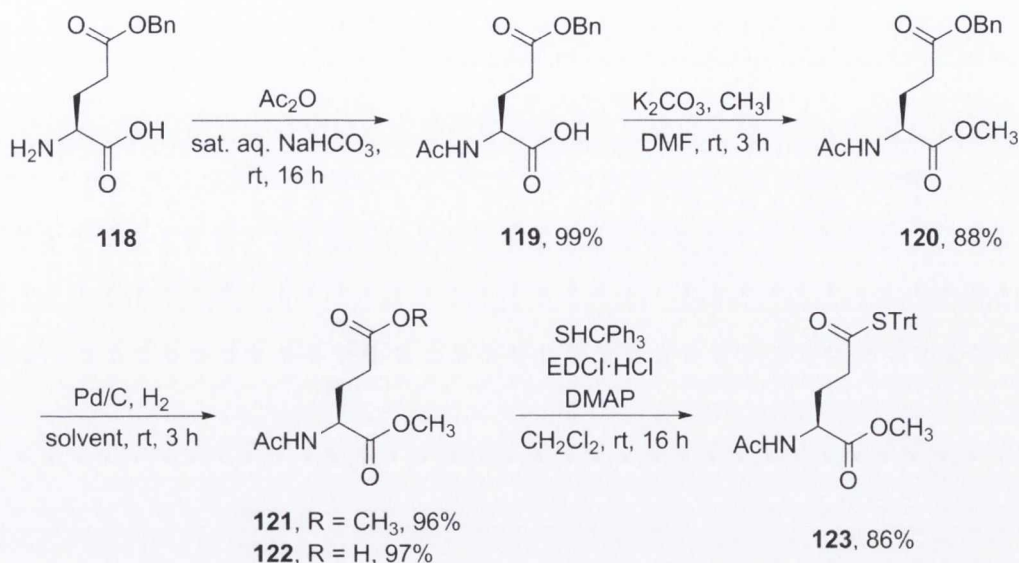
primary radical species. It is possible that the CSO elimination could be avoided or reduced through the use of lower reaction temperatures; however, due to time constraints in the project, this was not fully investigated.



Scheme 2.20: CSO elimination of thioacid generated thiyl radicals. (A) CSO elimination of the thioalanine thiyl radical **112** gives secondary radical **113** which can be quenched by parent thioacid to give **114**; (B) CSO elimination of **115** gives benzyl radical **116** which abstracts a proton from thiobenzoic acid to give benzene **117**.

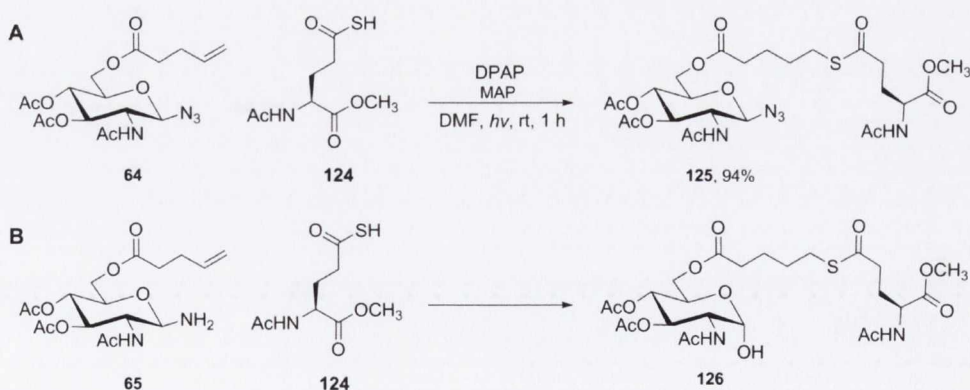
2.6.2 Synthesis of glutamic acid thioacid

In an attempt to further probe the scope of the methodology, namely the *S,N*-acyl shift, a glutamic acid (Glu) thioacid was prepared. Glu bears a strong resemblance to Asp but contains an extra CH₂ on its side group; therefore, it was thought that the Glu thioacid would be less sterically crowded at the thioester intermediate, and may participate in the *S,N*-acyl transfer with glycosylamine **65**. Accordingly, the Glu *S*-trityl protected thioacid was prepared, with the synthesis outlined in Scheme 2.21. Firstly, the amino functionality of commercially available **118** was acetylated using Ac₂O in sat. aq. NaHCO₃ solution to give **119** in quantitative yield. The carboxylic acid of **119** was then methylated, as previously described, to yield **120**. Interestingly, catalytic hydrogenation of **120** with Pd/C carried out in the mixed solvent of CH₂Cl₂:CH₃OH led to an unexpected product **121** whereby the liberated carboxylic acid was methylated, resulting in a dimethylated product **121**, rather than the desired **122**. This methylation was obviated by switching the solvent to EtOAc which furnished the desired product **122**. The *S*-trityl group was then installed, using the conditions described previously, to give the protected thioacid **123** in 86% yield after column chromatography.



Scheme 2.21: Synthetic scheme to Glu *S*-trityl protected thioacid **123**.

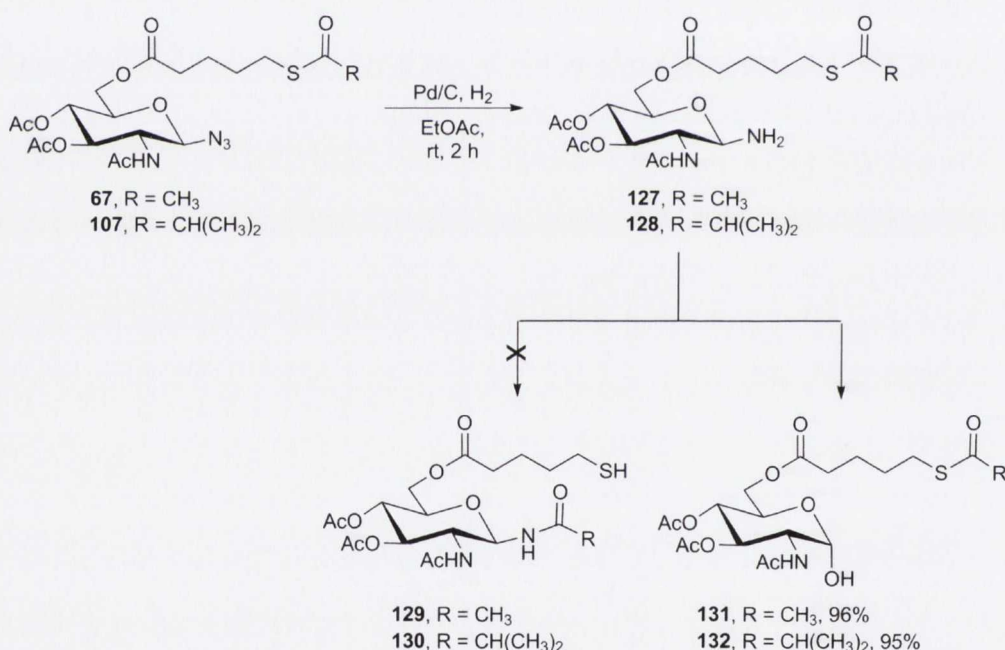
With **123** in hand, it was deprotected using TFA and TES in CH₂Cl₂ to give **124** (Scheme 2.22A). This crude thioacid was then subjected to thiol-ene conditions with glycosyl azide **64** to give the glycoconjugate **125** in 94% yield. Subsequently, thioacid **124** was reacted with the glycosylamine **65** in order to determine whether the *S,N*-acyl shift could proceed using the less sterically demanding thioacid **124** (Scheme 2.22B). Unfortunately, multiple attempts yielded no success with no ligated product being formed and the formation of an α -species being observed at the anomeric centre which was found to be the α -hemiacetal **126**.



Scheme 2.22: Thiol-ene reactions of Glu thioacid **124**. (A) Successful thiol-ene ligation of glycosyl azide **64** with Glu thioacid **124**; (B) Unsuccessful *S,N*-acyl migration of glycosylamine **65** with Glu thioacid **124** forming α -hemiacetal **126**.

2.7 Reduction of azido after thiol-ene ligation

Due to the glycosylamine hydrolysis occurring in the case of the Asp and Glu thioacids, it was considered that reduction of the azido group after installation of the thioester functionality could reduce the timeframe for glycosylamine hydrolysis, allowing for the *S,N*-acyl shift to proceed. This hypothesis was tested on the previously prepared acetyl and isobutyric azido compounds **67** and **107**, as shown in Scheme 2.23. Initially, the reduction of the azide in compounds **67** and **107** was attempted using the previously employed reduction conditions of $(\text{NH}_4)_2\text{MoS}_4$.²¹⁸ However, this method was unsuccessful with the azide group not undergoing any reduction whatsoever. It was thought that the unsuccessful reduction was due to the presence of the thioester functionality; Stiefel and co-workers reported the oxidation of the tetrathiomolybdate ion, MoS_4^{2-} , to the dimer, $\text{Mo}_2\text{S}_8^{2-}$, by organic disulfides.²³⁴



Scheme 2.23: Reduction of the glycosyl azide functionality in **67** and **107**, using hydrogenation conditions. The desired ligated products **129** and **130** were not formed. Instead, both reactions resulted in glycosylamine hydrolysis to give the α -hemiacetals **131** and **132**.

However, now that the alkene bond was no longer present, hydrogenation could be used for the reduction of the azide group. In the first instance, catalytic hydrogenation using Pd/C (0.1 eq.) was applied to reduce the azide group in **67**. However, no reaction was observed, presumably due to the

sulfur moiety poisoning the Pd.^{235,236} Considering this poisoning effect, the reaction was repeated using equimolar quantities of the Pd catalyst in an attempt to curtail the deactivation of the catalyst by sulfur. After 2 h under a hydrogen atmosphere, complete consumption of the starting material azide **67** was observed by TLC and the characteristic IR azide peak at 2117 cm^{-1} was no longer observed, indicating the reduction of the azide group had occurred. However, analysis of the crude ^1H NMR showed quantitative conversion to an α -anomer, as shown in Figure 2.5. In the azido compound **67**, the anomeric H-1 proton appears as a doublet (d) with a H1-H2 vicinal coupling of 9.4 Hz, indicative of a β -anomer (Figure 2.5, *top*). However, after the hydrogenation conditions, the anomeric H-1 proton appeared as a broad singlet (br s), signifying a change in the stereochemistry at the anomeric centre. Furthermore, the C-1 displayed a downfield shift from δ_{C} 88.4 to 91.8 ppm upon conversion to the α -anomer.

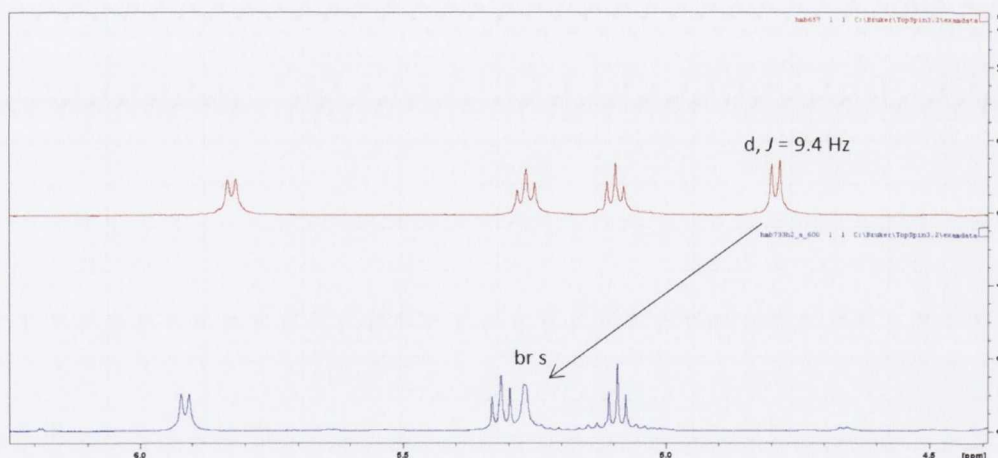


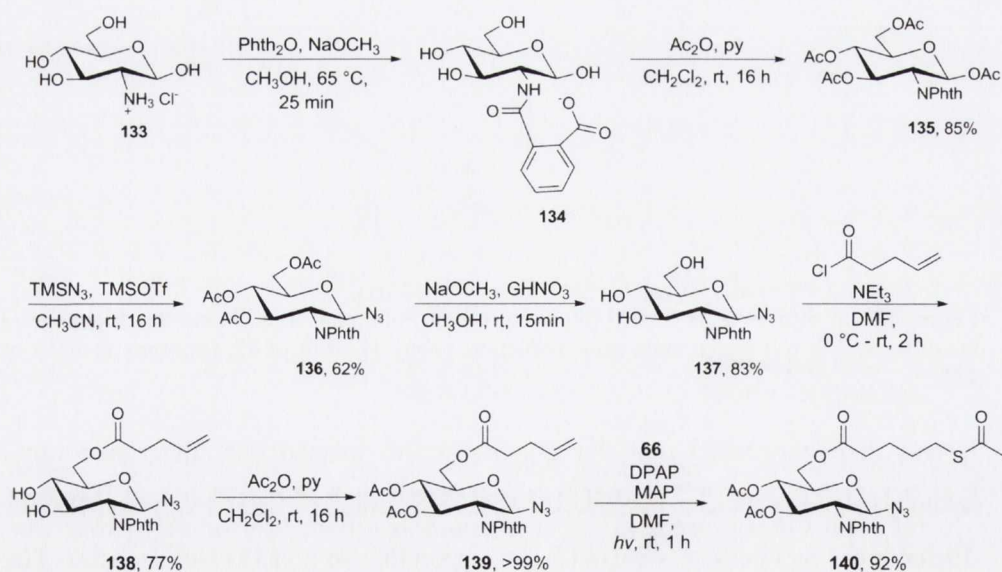
Figure 2.5: Comparison ^1H NMR (400 MHz, CDCl_3) of compounds **67** and **131** highlighting the difference in H-1 signal upon azide reduction. (*Top*) ^1H NMR of **67**; (*Bottom*) ^1H NMR of **131**.

It was then concluded that the glycosylamine formed had undergone rapid hydrolysis to give the α -hemiacetal **131**. This was confirmed through HRMS displaying a m/z peak at 486.1415, corresponding to a $[\mathbf{131}+\text{Na}]^+$ species. The formation of the α -hemiacetal **132** was also observed in the case of the compound **107**. Considering the azido moiety in **67** is reduced to the amine and subsequently hydrolysed within the time period of 2 h with none of the desired ligated product **129** or **130** being formed, it is thought that the hydrogenation

conditions provide an environment which is slightly acidic facilitating the glycosylamine hydrolysis or the equimolar catalyst loading is somehow catalysing the hydrolysis of the glycosylamine. The reduction conditions were repeated under basic conditions of NEt_3 in EtOAc and in aqueous buffered solution, however all tried conditions resulted in the rapid hydrolysis of the glycosylamine.

2.7.2 Introduction of a bulky *N*-phthalimido protecting group

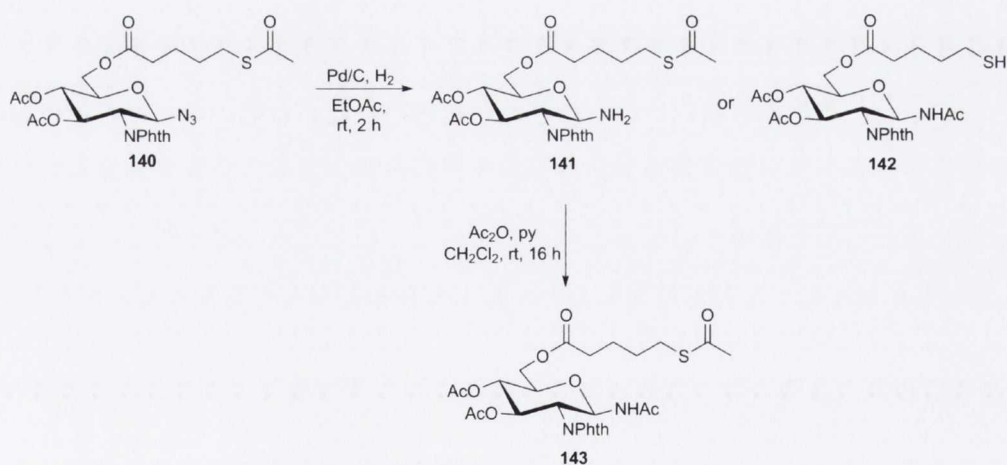
It was then decided to investigate the effect of a bulky protecting group at the C-2 position on the formation of the hydrolysis product. It was speculated that the introduction of a bulky phthalimido group at the C-2 position would exert a strong steric effect on the anomeric position, due to its large size, and sterically perturb the formation of the α -hemiacetal. Furthermore, Murphy and co-workers reported the production of anomeric mixtures of anomeric amides when proceeding *via* glycosylamines. These mixtures were not observed in the glucosamine derivative when a bulky phthalimido group was present at the C-2 position.²³⁷ The synthesis of a phthalimido protected glycosyl azide is illustrated in Scheme 2.24.



Scheme 2.24: Synthesis of C-2 protected phthalimido glycosyl azide **139** and its subsequent thiol-ene reaction with **66**.

The *N*-phthalimido (*N*-Phth) moiety was introduced by reacting **133** with phthalic anhydride in the presence of NaOCH_3 to give **134** which was then globally acetylated to give **135**. The azide group was then introduced

using the lewis acid trimethylsilyl triflate (TMSOTf) and TMS azide (TMSN₃) to furnish **136** in 62% yield, according to a literature procedure reported by Ito and co-workers.²³⁸ Exposure of **136** to previously employed Zémlen conditions resulted in the undesired opening of the phthalimido ring. An alternative approach was employed which involved *O*-deacetylation with a methanolic solution of guanidine/guanidinium nitrate (G/GHNO₃).²³⁹ This allowed for the removal of the *O*-acetates without affecting the phthaloyl group, affording **137** in 83% after column chromatography. The alkene was then regioselectively introduced at the C-6 position using 4-pentenoyl chloride (1.2 eq.) and NEt₃ in DMF to furnish **138** in 77% yield. As before, the regioselective esterification at the 6-position was confirmed through HMBC NMR analysis. The two free hydroxyl groups in **138** were then acetylated with Ac₂O and py in CH₂Cl₂ to give **139** in quantitative yield. For direct comparison of the role of the C-2 protecting group on the glycosylamine hydrolysis under hydrogenation conditions, compound **139** was subjected to thiol-ene conditions with DPAP, MAP, and **66** to furnish **140** in 92% yield. Compound **140** was then subjected to the same hydrogenation conditions employed for compounds **67** and **107**, which involved stirring in EtOAc under an atmosphere of hydrogen with equimolar quantities of Pd/C (Scheme 2.25).



Scheme 2.25: Reduction of azide functionality in the *N*-Phth protected **140** to give two possible products **141** or **142**. The formation of **141** was confirmed through an acetylation reaction to furnish **143**.

The reduction of the azide of **140** was confirmed by the disappearance of the characteristic peak at 2120 cm⁻¹ in the IR. As shown in Figure 2.6, the β-azide

140 displays a H1-H2 vicinal coupling of 9.4 Hz. Upon hydrogenation of **140**, the H-1 anomeric proton of the product species **141** shows an upfield shift with the signal still appearing as a doublet displaying the same H1-H2 vicinal coupling of 9.4 Hz, indicative of a β -anomer. This implies that the *N*-Phth was successful in curtailing the formation of an anomeric α -species upon hydrogenation of the azide. Analysis of the reaction mixture by HRMS showed a m/z peak at 573.1514, which could correspond to both $[\mathbf{141}+\text{Na}]^+$ and $[\mathbf{142}+\text{Na}]^+$ with no hydrolysis product being detected. The reaction mixture was then subject to microwave irradiation in DMF for 5 h at 50 °C to encourage the *S,N*-acyl migration to form **142**. Disappointingly, after microwave irradiation there was no change in the ^1H NMR spectra (Figure 2.6, *bottom*) and HMBC experiments also showed no correlation between the H-1 proton and a carbonyl functionality. Furthermore, the presence of a thioester signal at 195.8 ppm in the ^{13}C NMR confirmed that the *S,N*-acyl migration had not occurred. Unexpectedly, the β -orientation at the anomeric centre remained with no anomerisation or hydrolysis being observed after the microwave irradiation. To further confirm that the anomeric glycosylamine was still intact and had not been hydrolysed, the reaction mixture was subjected to acetylation conditions which furnished compound **143**, confirmed by a m/z peak at 615.1624 corresponding to $[\mathbf{143}+\text{Na}]^+$. The formation of an amide at the anomeric centre was further confirmed by the appearance of a signal in the NH COSY corresponding to the newly formed anomeric amide.

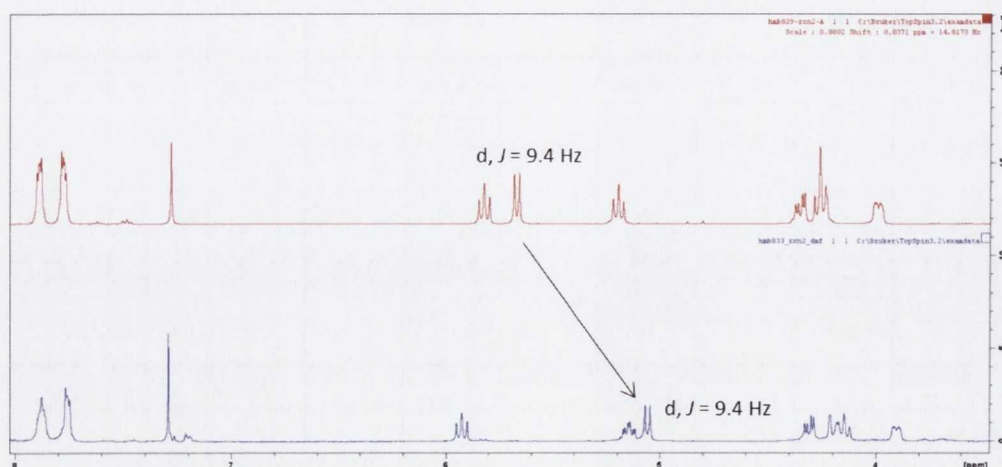


Figure 2.6: ^1H NMR (400 MHz, CDCl_3) of compounds **140** and **141** highlighting no change in the H1-H2 vicinal coupling upon azide reduction. (*Top*) ^1H NMR of **140**; (*Bottom*) ^1H NMR of **141**.

Although the introduction of the *N*-Phth group at the C-2 position successfully prevented glycosylamine hydrolysis, it appears to have had a negative impact on the *S,N*-acyl migration reaction. The *N*-Phth is a very bulky protecting group and the introduction of such a sterically demanding group could be causing the ligation site to be too sterically hindered thus preventing any acyl transfer. Furthermore, the bulky *N*-Phth may have a negative effect on the nucleophilicity of the glycosylamine. The *N*-Phth is more electron withdrawing than the previously employed *N*-acetyl group therefore, this change may cause the glycosylamine to be deactivated toward acyl transfer.

Interestingly, Bernardi and co-workers reported the stereoselective synthesis of glycosyl amides from α -glycosyl azides using the traceless Staudinger ligation.²⁴⁰ It was found that the stereoselectivity of the reaction depended critically on the sugar protecting groups. The *O*-benzyl protected α -glucosyl azides furnished the desired α -amides whereas the *O*-acetyl protected α -azide afforded β -glucosyl amides. It was postulated that the electron withdrawing nature of the *O*-acetyl protecting groups may reduce the rate of acylation and favour anomerisation. Therefore, future work could focus on the use of the deprotected glycosylamine or the use of electron-donating protecting groups, such as *O*-benzyl or *O*-benzoates to enhance the nucleophilicity of the anomeric amine.

2.8 Conclusion

In summary, a route to the desired glycosylamine with an appended alkene chain for use in thiol-ene ligations is described. Reduction conditions involving $(\text{NH}_4)_2\text{MoS}_4$ proved efficient for the reduction of the anomeric azide in the presence of the alkene functionality. A variety of *S*-trityl thioacids with varying steric hindrance (alkyl, phenyl, peptidyl) were synthesised to test the substrate scope of the proposed methodology. The first examples of peptidyl thioacids being employed in thiol-ene coupling reactions are described, with ligations being demonstrated with 4 peptidyl thioacids: Gly, Asp, Glu, and a Leu-Asp-Thr tripeptide unit. The thiol-ene reactions resulted in the ligated glycoconjugate products being furnished in high yields (82-94%) and the reactions were compatible with aqueous conditions. When using thioacids in the thiol-ene reaction, it was found that CSO elimination of the generated thiyl

radical was a competing side reaction. This elimination reduced the efficiency of the thiol-ene ligation through depletion of the thioacid donor. This limitation appears to operate in thioacids which generate a stable radical as this side reaction was not observed in compounds which would generate an unstable primary radical species.

The desired one-pot cascade reaction involving (i) thiol-ene ligation, (ii) *S,N*-acyl shift, and (iii) ester hydrolysis was successfully demonstrated using 5 different thioacid donors with one example furnishing an *N*-linked glycopeptide (GlcNAc-Gly). In these examples, a high degree of product conversion was observed with complete stereoselectivity being achieved. The aim of this work was to use thiol-ene chemistry and an *S,N*-acyl transfer reactions to furnish an *N*-linked glycopeptide containing a GlcNAc-Asn linkage. Unfortunately, this ligation was unsuccessful with glycosylamine hydrolysis being the prominent reaction. The *S,N*-acyl migration was also unsuccessful when a Glu residue was employed, which suggests that these 2 amino acids give rise to sterically hindered ligation junctions. Future work could include exploring a wider array of thioacids and the resulting structures optimised by means of quantum chemical calculations. These calculations could prove very interesting and may shed some light on the functionalities which give rise to a favourable *S,N*-acyl transfer.

It was also found that glycosylamine hydrolysis was prominent under hydrogenation conditions when equimolar Pd/C catalyst loadings were employed. This glycosylamine hydrolysis was prevented through the installation of a bulky phthalimido protecting group at the C-2 position. However, the introduction of this bulky electron withdrawing moiety appears to disfavour *S,N*-acyl transfer, sterically or through inductive deactivation of the anomeric amine. Future work could involve the screening of various glucosamine *N*-amino protecting groups in an attempt to prevent glycosylamine hydrolysis while retaining the nucleophilicity of the glycosylamine. A careful balance of steric bulk and electron donating ability should be considered to allow for the *S,N*-acyl migration.

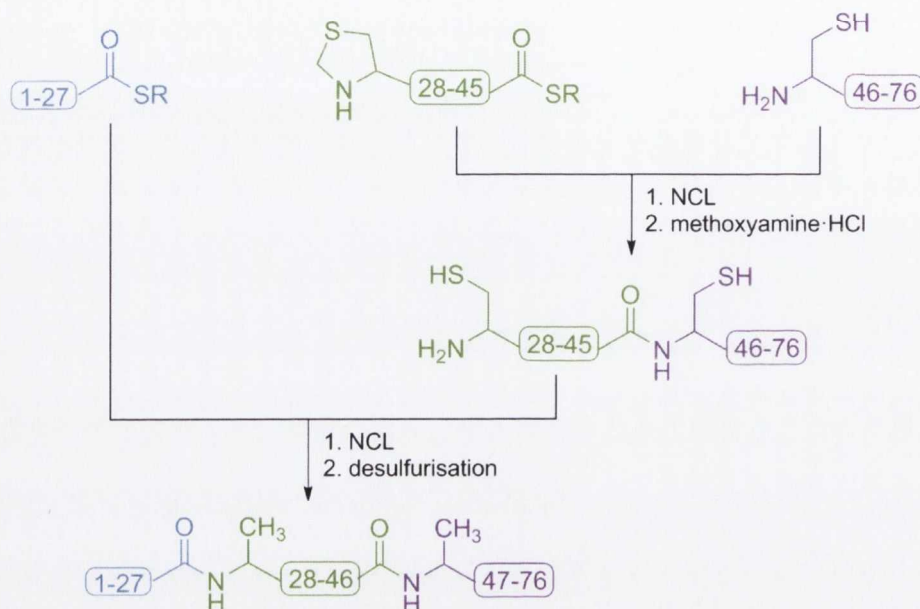
3.0 Introduction

As discussed in Chapter 1, protein ubiquitination is a prevalent PTM which was discovered in the early 1980s and involves the covalent attachment of Ub *via* its C-terminal Gly residue to the ϵ -NH₂ group of a target Lys residue through an isopeptide bond, the process of which was detailed in Chapter 1, Section 1.3.4.²⁴¹ Defects in the ubiquitination system can contribute to disease, namely cancer and neurodegeneration, making the ubiquitination system a potential therapeutic target.²⁴² In fact, the Nobel Prize in Chemistry was awarded in 2004 for the “discovery of ubiquitin-mediated protein degradation”.²⁴¹ Ever since the realisation of the importance of ubiquitination, suitable methodologies to prepare practical quantities of ubiquitinated proteins has been a major driving force for several research groups. Furthermore, the conjugation of Ub to reporter molecules has been widely used to study the action of DUBs.¹⁷ The total chemical synthesis of proteins offers the opportunity to obtain homogeneous post-translationally modified proteins, as well as proteins with non-native linkages, due to the high level of control chemical synthesis has to offer. In this chapter, a chemical ligation method towards the synthesis of ubiquitinated peptides is described. This ligation method is based on a sequential thiol-ene and *S,N*-acyl migration reaction, which was developed in Chapter 2. Prior to that, a brief review of some of the chemical methods to prepare native and non-native Ub conjugates is presented.

3.1 Chemical synthesis of native Ub and Ub-conjugates

The relatively small size of Ub (76 amino acids) allows its chemical synthesis to be performed by SPPS, with the first chemical synthesis of Ub being reported in 1989 by Briand and Ramage.^{243,244} Both the preparation of Ub and its conjugates have been accomplished using ligation strategies, such as NCL. Since there are no Cys residues in Ub, the use of conventional NCL to prepare Ub is not possible; however, Ub contains 2 Ala residues which are suitably located for use as ligation sites. Kent and co-workers prepared Ub using NCL by mutating 2 of the Ala residues (Ala28 and Ala46) to Cys to enable ligation.²⁴⁵ For synthetic convenience, to avoid demethylthiolation, they replaced the Met residue in Ub with Leu, as Leu1 has been shown to retain full

biological activity.²⁴⁶ As depicted in Scheme 3.1, they conducted a one-pot three-segment convergent assembly of 3 peptide fragments followed by desulfurisation of the mutated Cys residues to the native Ala.



Scheme 3.1: Synthesis of Ub using a one-pot three-segment ligation strategy.²⁴⁵ Ala28 and Ala46 were converted to Cys28 and Cys46 to allow ligation by NCL. After the first ligation, the thiazolidine protecting group of Cys28 is removed with methoxyamine·HCl. This allows for the second NCL ligation with the third peptide segment. The final step involves desulfurisation to give the native Ala residues and the completed Ub peptide.

One chemical method used for the preparation of native Ub protein conjugates was reported by Brik and co-workers and involves the use of mercapto Lys derivatives, which was discussed in Section 1.3.4.2, Scheme 1.24.¹²⁴ In this approach, a δ -mercapto Lys residue is prepared to act as a thiol source in the initial transthioesterification reaction with a Ub thioester. This is followed by an *S,N*-acyl shift through a 5-membered transition state to form a native isopeptide bond. The thiol handle can be subsequently removed by chemical desulfurisation. In a joint effort by the Brik and Lashual research groups, the first semi-synthesis of monoubiquitinated α -syn was achieved using this Cys surrogate approach as the key ligation strategy.²⁴⁷ As discussed in Chapter 1, Section 1.1.1.1, α -syn is a 140-amino acid presynaptic protein which is believed to be involved in the pathogenesis of PD, with it being found that the implicated α -syn species are often ubiquitinated.²⁴⁸ Therefore, in order

to determine the role ubiquitination plays in disease progression, α -syn and its Ub conjugates represent a key synthetic target for many research groups.

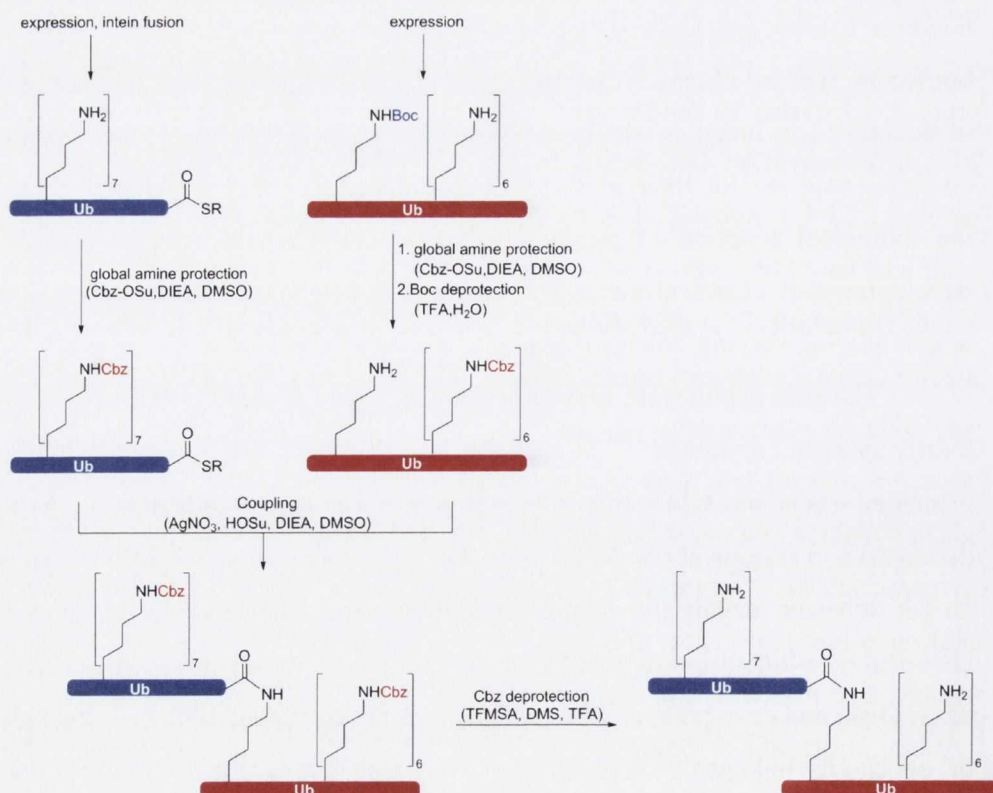
3.2 Chemical synthesis of Ub polymers

The most well-known function of ubiquitination is its targeting of proteins for degradation.²⁶ While some proteins undergo proteasomal degradation with monoubiquitination, for others, it is known that a Ub chain made of four units is necessary; however, there are numerous cellular activities for which the preferred Ub chain length is unknown.^{249,150} The process of ubiquitination is still poorly understood, with an added layer of complexity arising from the ability of Ub to form polymers. The Ub protein itself has seven internal Lys residues (Lys6, Lys11, Lys27, Lys29, Lys48, and Lys63) hence polyubiquitination is a complex modification where the outcome of the resulting signal depends on which Lys residue in Ub is linked to consecutive Ub molecules.²⁵⁰ Methods for the preparation of ubiquitinated peptides and Ub polymers exist which employ enzymes.^{251,252,253} However, challenges exist such as the identification of the relevant enzymes for a specific conjugate and obtaining a high level of control in the *in vitro* preparation of Ub chain of particular linkages and length.²⁷ All chain types have been found *in vivo* however Lys48- and Lys63-linked chains are the most studied chains and are known as 'typical' chains.²⁵ Conversely, very little is known about the function of the other Lys linkages which are known as 'atypical' chains, as the specific enzymes required for their biosynthesis are currently unknown. Insights into the biological function of polyubiquitination can be achieved through the development of chemical methods to prepare Ub polymer chains, of varying length bearing varying linkage types.

The Brik group were first to prepare all seven possible di-Ub chains by a fully synthetic approach.²⁵⁴ As with the ubiquitination of peptides previously discussed (Scheme 1.24), this approach relies on the introduction of a δ -mercapto Lys residue at the desired position (e.g., Lys6) to allow for NCL with an Ub thioester forging the desired isopeptide bond. The final step requires a chemical desulfurisation to give the desired unmodified native linkage. Shortly after, Ovaa and co-workers reported a semisynthetic approach for the synthesis of all di-Ub linkages.²⁵⁵ This method involved the synthesis of both Ub monomers using Fmoc-SPPS. The proximal Ub contained a δ -mercapto Lys

residue at the desired position to mediate isopeptide bond formation. Interestingly, the distal Ub thioester was prepared by the thioesterification of the carboxy terminus using an E1 enzyme. However, this approach required the mutation of the proximal Ub Gly76 to Val to prevent undesired processing by the E1 enzyme.

Chin and Komander reported an approach towards ‘atypical’ di-Ub chains, which they termed genetically encoded orthogonal protection and ligation (GOPAL).²⁵⁶ GOPAL combines genetic engineering with chemoselective chemical synthesis and is illustrated in Scheme 3.2. A genetically encoded Lys(Boc) was introduced at the desired position in Ub while the remaining Lys residues were protected with *N*-(benzyloxycarbonyloxy)succinimide (Cbz-OSu). The Boc group was subsequently removed to allow for isopeptide bond formation with an Ub thioester, which was obtained using intein expression. The last step involved global deprotection to give the target native di-Ub product. One noticeable drawback of this method is the need for protecting group chemistry to allow for the site-selective ligation.



Scheme 3.2: The semi-synthetic GOPAL approach towards di-Ub chains.²⁵⁶ Cbz-OSu: *N*-(benzyloxycarbonyloxy)succinimide, DIEA: *N,N*-diisopropylethylamine; DMSO: dimethylsulfoxide, TFA: trifluoroacetic acid, HOSu: *N*-(hydroxy)succinimide, TFMSA: trifluoromethanesulfonic acid, DMS: dimethyl sulfide.

Nevertheless, Chin and Komander used this approach to prepare both Lys6- and Lys29-linked di-Ub. They then solved the crystal structure of the Lys6-linked di-Ub which displayed a new compact conformation, distinct from that of 'typical' di-Ub chains. Moreover, the synthesis of these 'atypical' linkages allowed the profiling of 11 DUBs which revealed that DUB TRABID, thought to be specific for Lys63-linkages, exhibited 40-fold higher proteolytic efficiency toward K29-linked di-Ub. Fushman and co-workers modified the GOPAL strategy to incorporate Alloc protecting groups, rather than Cbz, which can be removed under mild conditions.²⁵⁷ They also prepared the Ub thioester using an E1 enzyme, as reported previously by Ovaia and co-workers.²⁵⁵ In this way, they prepared an assortment of Ub polymers such as K11-linked di-, tri-, and tetra-Ub.

As discussed throughout this thesis thus far, thiol-ene chemistry is an attractive option for site-selective biomolecule modification.^{258,259} Strieter and co-workers used thiol-ene chemistry to construct di-Ub polymers containing non-native linkages.²⁶⁰ They did this by replacing the desired site of ubiquitination with a Cys residue and an allyl amine functionality was introduced at the C-terminus of another Ub monomer, using the Ub C-terminal hydrolase (UCH) enzyme (Figure 3.1A).

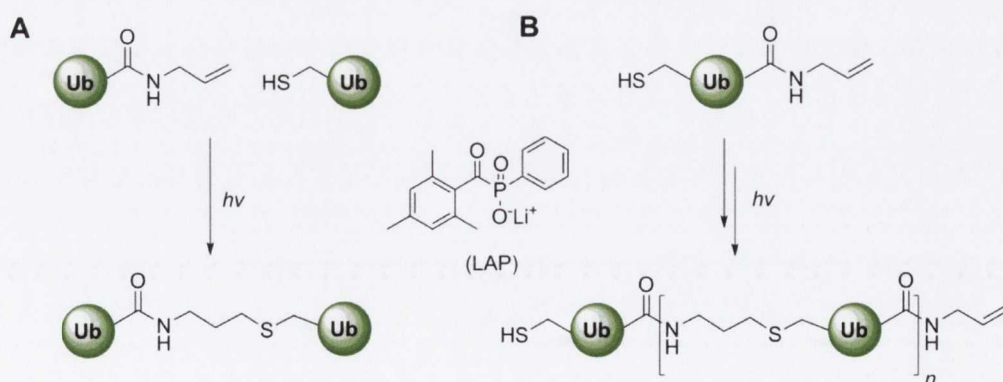


Figure 3.1: Synthesis of non-native Ub chains using thiol-ene chemistry initiated using LAP.^{261,262} (A) Formation of Ub dimers. A Lys to Cys mutation is introduced to one Ub monomer while an allylamine functionality is introduced to another Ub monomer using the UCH enzyme. These two monomers can react under thiol-ene conditions, initiated by LAP, to give a non-native Ub dimer; (B) Polymerisation of a dual functionalised Ub monomer to yield non-native Ub oligomers of varying lengths.

This simple alkene was then reacted with the Cys-containing Ub monomer in the presence of the free-radical initiator lithium acyl phosphinate (LAP). This thiol-ene ligation afforded a Ub dimer which contained a thioether linkage that is 1.5 Å longer than the native isopeptide bond.²⁶¹ They subsequently demonstrated that DUBs could still selectively cleave this non-native linkage in hydrolysis assays, thereby showcasing the utility of thiol-ene coupling in the generation of Ub-Ub linkages. The same group later reported a dual functionalised Ub monomer bearing both the Cys and allyl reactive groups (Figure 3.1B).²⁶² Initiated using LAP and conducted in aqueous media, this monomer was capable of undergoing multiple thiol-ene polymerisations allowing access to a series of oligomers, from tetramers to heptamers, linked through thioether linkages. This approach is useful in that the chemistry is straightforward; coupling reactions are efficient and minimal synthetic manipulations are required to produce these polymers.

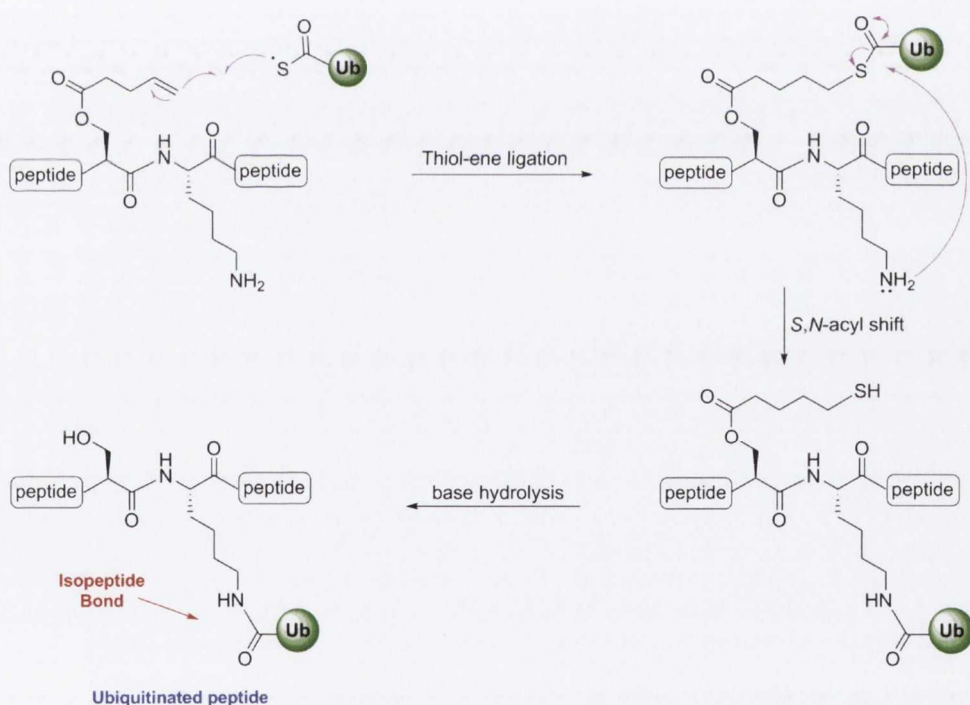
3.3 Aims of this work

Although the Cys surrogate approach reported by Brik *et al.* represents a popular method for the preparation of Ub protein conjugates, the synthesis of the required δ -mercapto Lys residue is lengthy. For instance, the preparation of the mercapto residue depicted in Figure 1.24 requires over 10 synthetic steps from L-glutamic acid before incorporation into SPPS. Therefore, there is a need for a new method for the site-specific incorporation of Ub or poly-Ub chains to tackle mechanistic questions related to Ub function. Considering the demonstrated applicability of thiol-ene ligations in Ub bioconjugation reported by Strieter and co-workers, we were interested in using thiol-ene chemistry to form native Ub conjugates. Herein, we report a chemical ligation method for the preparation of Ub related conjugates based on sequential thiol-ene coupling chemistry and an *S,N*-acyl transfer reaction, the process of which is depicted in Scheme 3.3.

Firstly, a thiol-ene ligation occurs between a C-terminal Ub thioester and a modified Ser residue located adjacent to the Lys that is to be modified. As we have demonstrated in Chapter 2, thiol-ene ligations can be successfully extended to the use of thioacids using the previously developed experimental

conditions. The initial transthioesterification step of NCL reactions can be quite slow and often requires the addition of exogenous thiols to accelerate the reaction; however, this method replaces this sluggish step with a speedy, high yielding “click” alternative.

The second step involves an *S,N*-acyl shift over an 18-membered transition state to furnish a native isopeptide bond. This is analogous to the acyl migration observed in NCL and EPL, although, this approach involves a much larger transition state. However, precedence exists with Katritzky and co-workers having reported successful *S,N*-acyl transfer reactions *via* 5- to 19-membered transition states.²⁶³ Lastly, the final step involves the base hydrolysis of the appended Ser ester to furnish the desired ligated product. This mild base hydrolysis represents a milder alternative to chemical desulfurisation methods.



Scheme 3.3: Outline of the proposed thiol-ene mediated ubiquitination methodology. Step one involves a thiol-ene ligation between a Ub derived thioester and a Ser modified alkene functionality to give a thioester intermediate. A subsequent *S,N*-acyl shift gives the native isopeptide bond. Final base hydrolysis of the ester group yields the unmodified ubiquitinated peptide.

This ligation methodology does not require a Cys residue or Cys surrogate and it operates at Ser residues, which are naturally more abundant.

Ploegh and co-workers have analysed the structure and sequence of 135 known ubiquitination sites in 95 proteins in *Saccharomyces cerevisiae* in an attempt to identify a consensus sequence for ubiquitination.²⁶⁴ Interestingly, it was observed that Ser and Thr sites often flank the Lys residue to be ubiquitinated. Our methodology has been designed to take advantage of this feature found in nature and this offers an additional unique advantage.

As discussed, α -syn is a protein implicated in PD with its Ub conjugates being a synthetic target of many. Accordingly, the aim of this project was to synthesise a fragment of α -syn for ligation with a Ub derived thioacid, using the methodology proposed in Scheme 3.3. The target unit **144** is comprised of a Leu-Ser-Lys tripeptide sequence, as depicted in Figure 3.2. The Ser unit is modified to incorporate an alkene moiety which can participate in a thiol-ene ligation with a Gly thioacid. Furthermore, the Lys ϵ -NH₂ functionality is masked as an azide which can be subsequently reduced to the amine to allow for the *S,N*-acyl transfer reaction. The synthesis of the azido peptide **144** and its subsequent use in thiol-ene ligations and acyl migrations reactions will be discussed in the following sections.

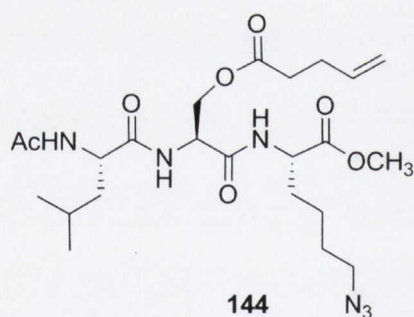


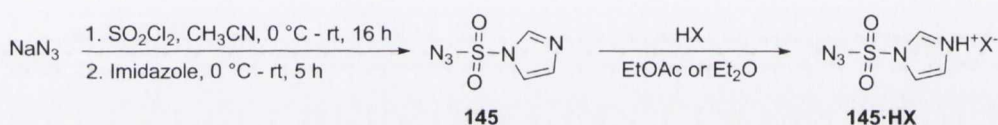
Figure 3.2: Target tripeptide for use in the thiol-ene mediated ligation methodology. The chosen peptide (Leu-Ser-Lys) is a tripeptide sequence from the biologically relevant α -syn and contains a Ser residue modified with an alkene for participation in thiol-ene ligation. The Lys ϵ -NH₂ group is masked as an azide and will be later reduced to the amine to allow for *S,N*-acyl migration with a Ub thioester.

3.4 Synthesis of α -synuclein associated tripeptide unit

3.4.1 Azides and the diazo transfer reaction

As depicted in Scheme 3.3, the Lys ϵ -NH₂ group is involved in the *S,N*-acyl transfer reaction to form the native isopeptide bond. However, this amino functionality requires protection to allow for the synthesis of the desired tripeptide unit. The azide functionality plays an important role as a protecting group for primary amines and circumvents some of the orthogonality problems

encountered when using conventional amine protecting groups since azides are resistant to both acidic and basic conditions.^{265,266} Accordingly, it was decided to mask the Lys ϵ -NH₂ as an azide. Amines may be converted to their corresponding azides using a diazo transfer reaction with a diazo donor, most commonly a sulfonyl azide. The process of diazo transfer is extremely mild, high yielding and most importantly, allows the preservation of any existing stereochemistry.²⁶⁷ The initial diazo donor of choice was trifluoromethanesulfonyl azide (TfN₃), which transforms amines to azides in a single, high yielding step. However, TfN₃ is unstable and inherently explosive when dry, thus requiring *in situ* preparation. Goddard-Borger reported an alternative diazo donor in the form of imidazole-1-sulfonyl azide (ImSO₂N₃) hydrochloride, a crystalline and shelf-stable alternative to TfN₃.²⁶⁸ ImSO₂N₃ can be easily prepared by a two-step procedure, as depicted in Scheme 3.4.

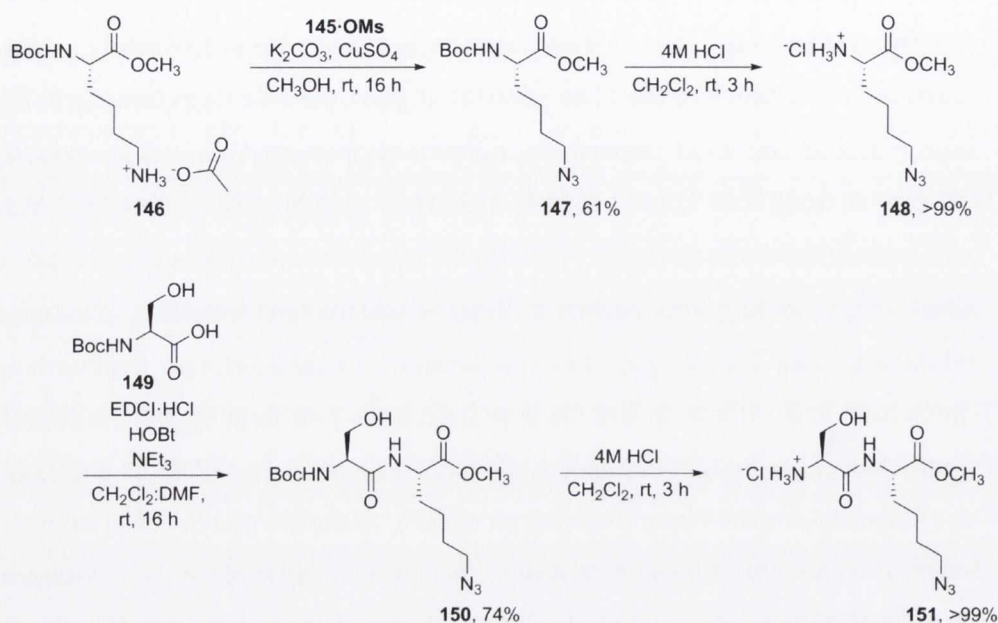


Scheme 3.4: Preparation of imidazole-1-sulfonyl azide salts for use in diazo transfer reactions.²⁶⁹

Firstly, sulfonyl chloride (SO₂Cl₂) is reacted with NaN₃ in CH₃CN to give chlorosulfonyl azide (ClSO₂N₃), which is then reacted with imidazole to give **145**. Compound **145** can then be converted to the corresponding salt by the addition of an appropriate acid in EtOAc or Et₂O. Interestingly, Goddard-Borger and co-workers reported that **145·HCl** was less stable than originally thought, as it was found to be impact sensitive.²⁶⁹ In order to make **145·H₂SO₄**, a solution of sulfuric acid in EtOAc was added slowly to **145** whilst stirring at 0 °C. Unfortunately, each attempt resulted in the formation of an oily residue, rather than the desired crystalline salt. Partitioning **145·H₂SO₄** between EtOAc and sat. aq. NaHCO₃ solution provided pure **145**. Subsequent treatment of **145** in Et₂O with methanesulfonic acid (MsOH) provided the corresponding mesylate salt **145·MsOH** as a crystalline white solid in 68% yield.

With the diazo donor **145·MsOH** in hand, the diazo transfer reaction was conducted on commercially available Boc-L-Lys methyl ester acetate salt **146**, as depicted in Scheme 3.5. The previously prepared **145·MsOH** was

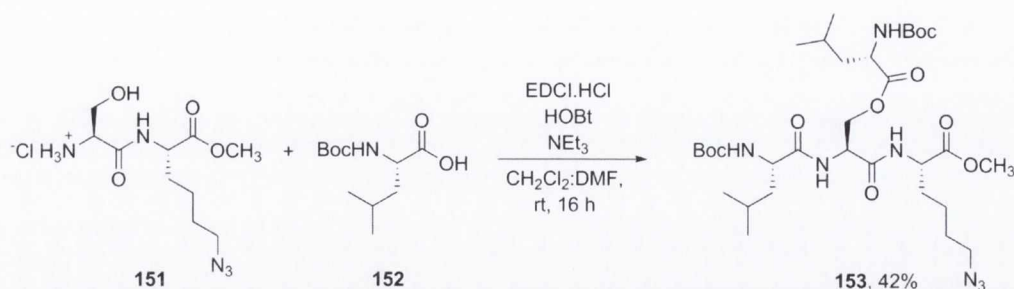
added to a solution of **146**, K_2CO_3 , and $CuSO_4 \cdot H_2O$ in CH_3OH and stirred for 16 h at rt to furnish **147** in 61% yield after column chromatography.



Scheme 3.5: Synthesis of Ser-Lys azido dipeptide **151** using diazo transfer reaction and **145·MsOH**.

The successful introduction of the azide moiety was confirmed by the characteristic IR peak appearing at 2095 cm^{-1} . The Boc group was subsequently removed by stirring **147** in acidic conditions of HCl in CH_2Cl_2 to give **148** as the hydrochloride salt in quantitative yield. The Lys compound **148** was then coupled with Boc-L-Ser **149** in the presence of the coupling reagent EDCI·HCl, NEt_3 , and HOBt to yield dipeptide **150**. The Boc-L-Ser **149** was previously prepared by reaction of L-Ser with $(Boc)_2O$ and NEt_3 in a mixture of dioxane and H_2O to give **149** in 72% yield. The Boc group was then removed by stirring **150** in CH_2Cl_2 with HCl to give **151** in quantitative yield.

The next step involved the coupling of the Leu residue to complete the desired tripeptide sequence. This involved the coupling of dipeptide **151** with commercially available Boc-L-Leu **152**, as shown in Scheme 3.6. Interestingly, the main product obtained was **153** whereby the Ser primary alcohol underwent coupling with the activated Leu residue **152**. This undesired esterification of the Ser alcohol was surprising as only stoichiometric quantities of **152** were employed.



Scheme 3.6: Unexpected formation of product **153** under peptide coupling conditions using **151** and **152**.

The presence of **153** was observed by ¹H NMR (Figure 3.3) whereby 4 NH signals were observed, rather than the 3 expected signals. Additionally, the Boc peak which resonates at 1.45 ppm integrated to double that of the expected product indicating that the product formed contained two Boc functionalities. Furthermore, the Leu side chain methyl groups resonating at 0.94 ppm integrated to 12H, rather than 6H, further confirming the presence of 2 Leu moieties in the obtained product. The presence of **153** was further confirmed by the HRMS with *m/z* of 722.4072 corresponding to the [**153**+Na]⁺ species.

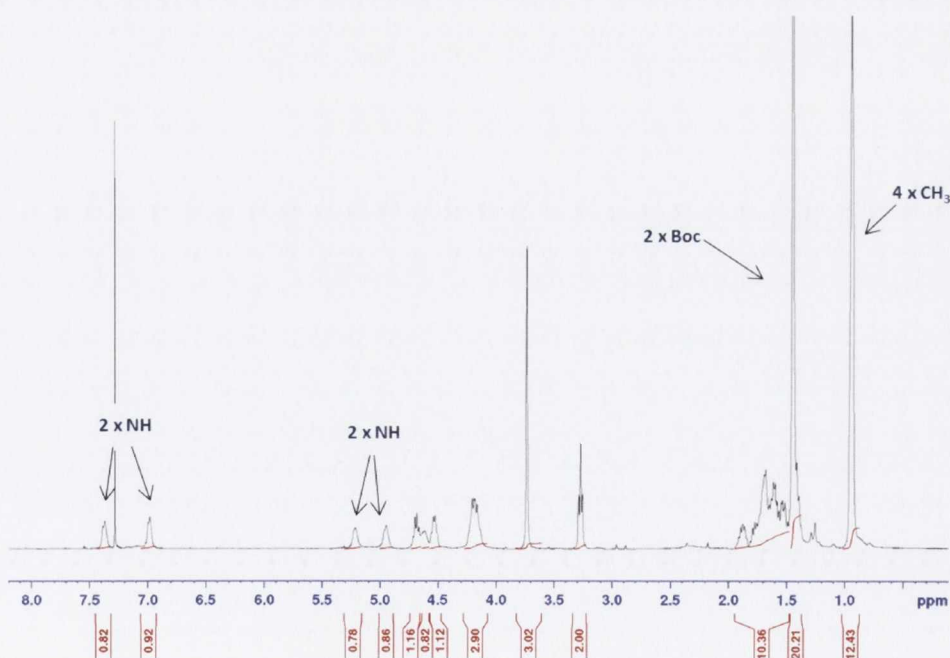
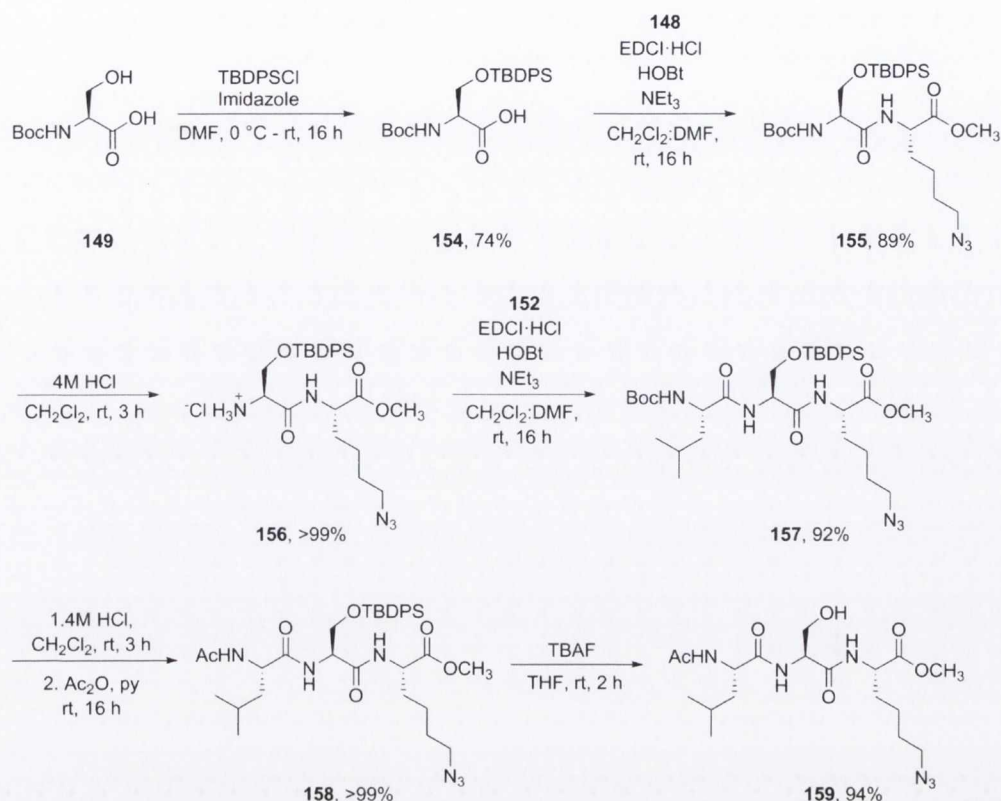


Figure 3.3: ¹H NMR (400 MHz, CDCl₃) spectrum of **153** indicating the presence of 4 NH signals, 2 Boc and 4 methyl functionalities.

3.5 Route towards tripeptide incorporating a silyl protecting group

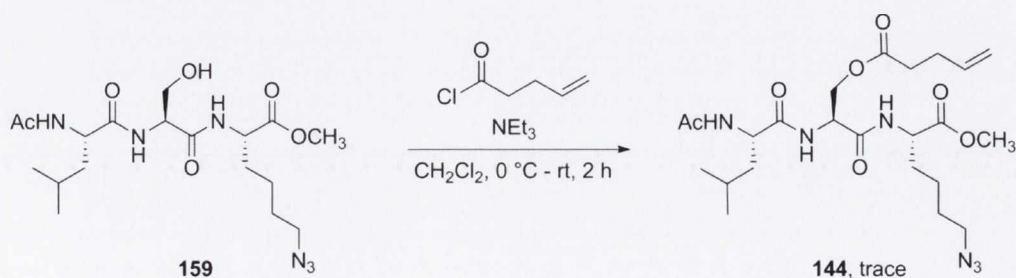
To circumvent the problem of the Ser alcohol competing with the amine for acylation, it was decided to introduce a protecting group to the synthetic strategy. Silyl ethers were the first to be considered as they are easily introduced and are relatively stable.²⁷⁰ Due to the requirement for repeated Boc removal, the silyl ether employed was required to be stable enough to withstand acidic conditions. It is well known that by altering the steric and electronic properties of the substituents on the silicon atom, stability of the silyl protecting group can be varied. For example, the bulkier *t*-butyldiphenylsilyl ether (TBDPS) is more stable than *t*-butyldimethylsilyl (TBDMS) ethers to acid hydrolysis. Therefore, as outlined in Scheme 3.7, the Ser alcohol was masked with a TBDPS group by treatment of **149** with *t*-butyldiphenylchlorosilane (TBDPSCI) and imidazole in DMF to furnish **154** in 74% yield. The azido functionalised Lys residue **148** was then coupled with **154**, using the standard coupling conditions previously employed, to give the dipeptide **155** in 89% yield. The Boc group was then removed under acidic conditions of HCl in CH₂Cl₂ to furnish **156** with the silyl protecting group remaining intact. The Leu residue was then introduced by reaction of dipeptide **156** with Boc-L-Leu **152** to give tripeptide **157** in 92% after column chromatography. The Boc group in tripeptide **157** was subsequently removed under acidic conditions and an *N*-acetyl group was introduced using Ac₂O and py to furnish **158** in quantitative yield over the 2 steps. Lastly, the TBDPS protecting group was removed to furnish the free Ser alcohol to allow for subsequent introduction of the alkene functionality. Silyl ethers can be quickly and orthogonally removed using fluoride reagents. The speedy removal of silyl protecting groups using a fluoride source is driven by the formation of a strong Si-F bond, which is about 30 kcal/mol stronger than the Si-O bond.²⁷⁰ Tetrabutylammonium fluoride (TBAF) is one of the most commonly used sources of fluoride; therefore, the TBDPS group in **158** was removed using 1M TBAF solution in THF to give **159** in 94% yield after column chromatography.



Scheme 3.7: Synthetic route to tripeptide **159** incorporating a silyl protecting group.

3.6 Esterification of Ser using 4-pentenoyl chloride

The last step toward the desired tripeptide **144** involved the esterification of the Ser hydroxyl to introduce the alkene functionality. This esterification proved to be more challenging than expected. Initial conditions involved the addition of 4-pentenoyl chloride (1.2 eq.) and NEt₃ (1.2 eq.) to **159** in anhydrous CH₂Cl₂, as depicted in Scheme 3.8, however, this only gave rise to trace amounts of the desired product **144**.



Scheme 3.8: Introduction of alkene functionality to tripeptide **159** using 4-pentenoyl chloride.

An optimisation strategy was then carried out in order to obtain **144** in appreciable quantities (Table 3.1). Repeating the reaction employing a 2-fold

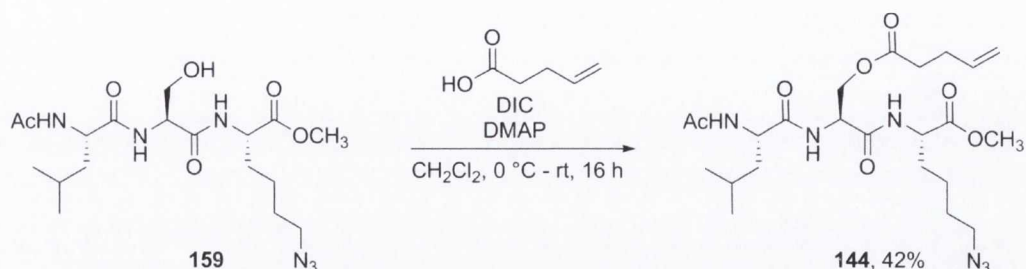
excess of the acid chloride with NEt_3 (2.0 eq.) in CH_2Cl_2 gave rise to the product with an unsatisfactory yield of 14% (Entry 2). Increasing the amount of acid chloride (4.0 eq, added in 2 aliquots) and prolonging the reaction time from 2 h to 3 h, only resulted in a slight increase in the yield with **144** being obtained in 20% yield (Entry 3).

Table 3.1: Optimisation of the esterification of the Ser residue in tripeptide **159** depicted in Scheme 3.7.

Entry	Acid chloride eq.	NEt_3 eq.	DMAP eq.	Time (h)	Solvent	Yield
1	1.2	1.2	–	2	CH_2Cl_2	Trace
2	2.0	2.0	–	2	CH_2Cl_2	14%
3	4.0	4.0	–	3	CH_2Cl_2	20%
4	4.0	4.0	–	3	DMF	34%
5	3.0	3.0	0.1	2	DMF	58%
6	1.2	1.2	1	2	DMF	71%

Reaction conditions: All reaction conditions were performed at tripeptide **159** concentration of 0.05 M with the reaction conducted in anhydrous solvent under an argon atmosphere. All equivalents are based on the tripeptide **159** (1.0 eq.).

At this point, an alternative to the acid chloride was investigated in an attempt to increase the yield of the product. Arsianti and co-workers reported the esterification of Thr using acrylic acid under peptide coupling conditions of diisopropyl carbodiimide (DIC) and 4-dimethylamino pyridine (DMAP).²⁷¹ Therefore, as outlined in Scheme 3.9, 4-pentenoic acid was activated by the addition of DIC and DMAP followed by the addition of tripeptide **159**. This approach produced **144** in a disappointing yield of 42%, although it did prove to be an improvement on the previously screened conditions.

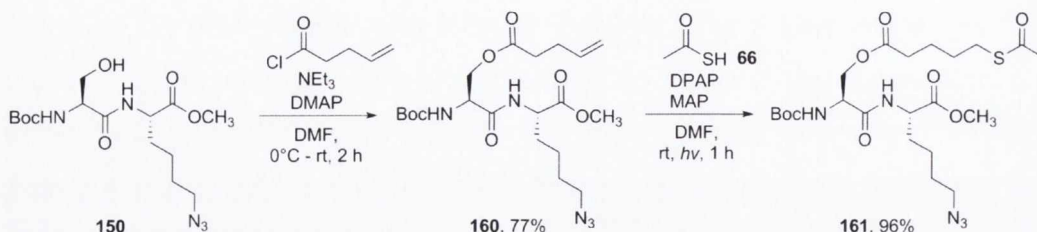


Scheme 3.9: Introduction of alkene functionality to tripeptide **159** using peptide coupling conditions of DIC, DMAP, and 4-pentenoic acid.

Due to the observation that the formation of a DMAP activated ester appeared to increase the yield of the desired product, it was thought that the addition of DMAP to serve as a nucleophilic catalyst would assist the reaction of **159** with acid chloride, 4-pentenoyl chloride. Gratifyingly, addition of catalytic DMAP to the reaction of **159** with 4-pentenoyl chloride (3.0 eq.) and NEt₃ (3.0 eq.) furnished the product in 58% yield (Entry 5). The quantity of acid chloride was subsequently reduced to 1.2 eq. along with the use of stoichiometric amounts of DMAP which furnished the desired product **144** in a satisfactory yield of 71% (Entry 6).

3.7 Initial thiol-ene ligation using thioacetic acid and dipeptide

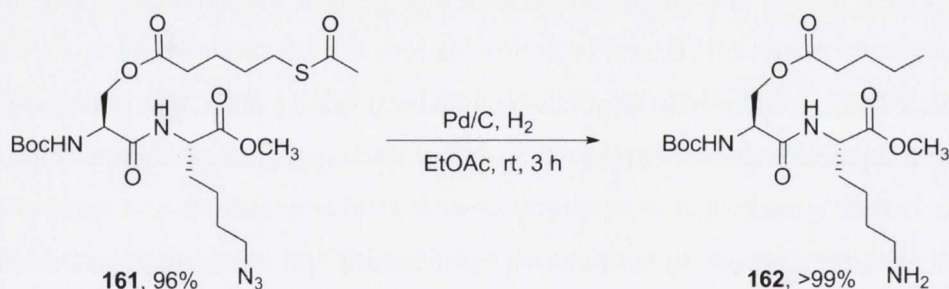
Initially, it was decided to investigate the ligation methodology using the simple thioacetic acid donor **66** as a test substrate. This thiol radical donor was successfully employed in thiol-ene ligations and subsequent *S,N*-acyl migration reactions in Chapter 2. Furthermore, due to the tripeptide **144** being produced in a small quantity and representing a precious intermediate, it was decided to conduct the preliminary reactions on the dipeptide **150**. As outlined in Scheme 3.10, the alkene functionality was introduced to the dipeptide **150** using the previous optimal conditions (Table 3.1, Entry 6) to give **160** in 77% yield. Using the thiol-ene reaction conditions employed in Chapter 2, dipeptide **160** was added to a solution of the photoinitiator DPAP (0.1 eq.), photosensitiser MAP (0.1 eq.) and **66** (1.1 eq.) in DMF and the mixture was irradiated in a UV oven for 1 h; ligated **161** was successfully obtained in 96% yield after column chromatography.



Scheme 3.10: Esterification of dipeptide **150** followed by thiol-ene ligation with **66**.

3.8 Reduction of the azide group

With compound **161** in hand, the azide needed to be reduced to liberate the free amine which would subsequently participate in the *S_N*-acyl shift. As discussed in Chapter 2, Section 2.7, the reduction of the glycosyl azide under hydrogenation conditions required the use of equimolar quantities of Pd/C, due to the poisoning of the catalyst by the sulfur functionality.²³⁶ Accordingly, compound **161** was dissolved in EtOAc followed by the addition of Pd/C (1.0 eq.) and stirred under an atmosphere of hydrogen for 3 h (Scheme 3.11). The reduction of the azide functionality was confirmed by the disappearance of the characteristic azide IR peak at 2097 cm⁻¹. However, during NMR analysis, it was found that these reductions conditions caused desulfurisation of **161** furnishing **162** in quantitative yield.



Scheme 3.11: Desulfurisation of **161** under hydrogenation conditions employing Pd/C.

The desulfurisation to form **162** was indicated by the absence of the thioacetate group in the ¹H NMR and the presence of a butane type alkyl chain. The presence of the methyl terminated alkyl chain (CH₃CH₂CH₂CH₂) was confirmed by a selective total correlation spectroscopy (TOCSY) experiment. TOCSY experiments allow for ¹H-¹H connectivity to be established by extracting out proton signals of a selected spin system.²⁷² This experiment can become very useful when a ¹H NMR spectrum is hampered by overlapping

signals. As depicted in Figure 3.4, bond correlations can be observed by the selective excitation of one peak in the ^1H NMR spectrum, the terminal CH_3 at 0.95 ppm in this case. Magnetization can then be transferred throughout this spin system to provide a spectrum which contains only the peaks in the same spin system, allowing the presence of the desulfurised alkyl chain to be confirmed.²⁷² This desulfurisation was further verified by HRMS with a m/z peak at 432.2706 corresponding to the $[\mathbf{162}+\text{H}]^+$ species.

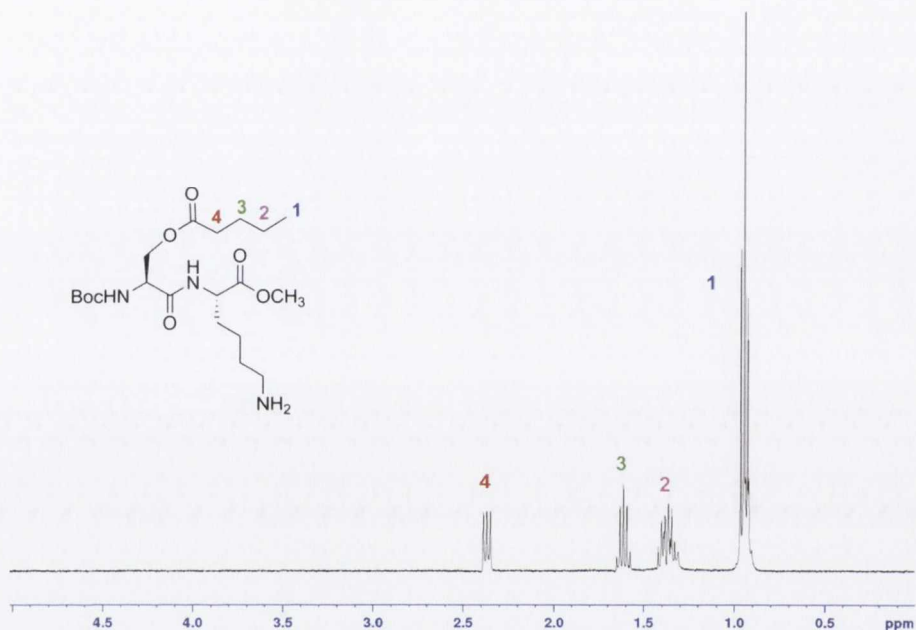
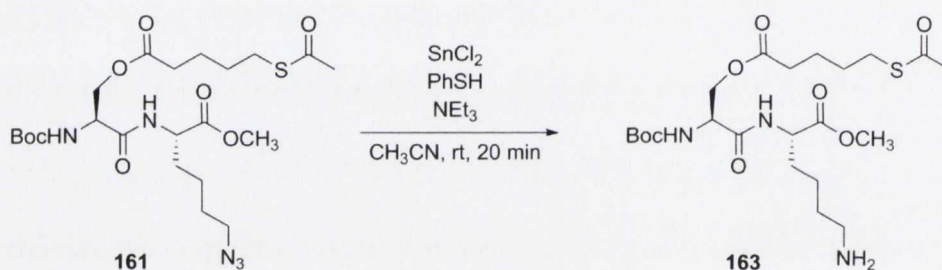


Figure 3.4: Selective TOCSY experiment (400 MHz, CDCl_3) of **162** confirming the desulfurisation of **161**. Selective irradiation was applied at CH_3 (labelled 1) at 0.95 ppm.

Whilst conducting a literature search, it was found that Dawson reported hydrogenolytic desulfurisation of Cys residues using various Pd catalysts, including Pd/C, with it being found to be comparable to Raney nickel.¹¹⁸ Therefore, an orthogonal reduction method was needed which would quickly and cleanly transform the azide to the amine, whilst leaving the labile thioester moiety intact.

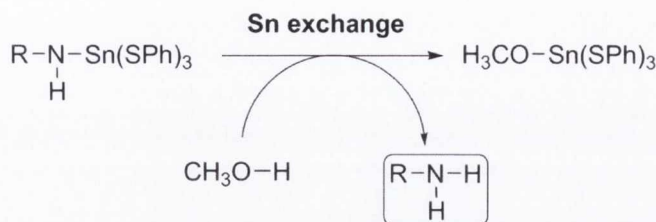
Encouragingly, a method reported by Vilarrasa and co-workers using a $\text{SnCl}_2/\text{PhSH}/\text{NEt}_3$ mixture appeared to be very useful.^{273,274} This approach involves the addition of PhSH (6.0 eq.) and NEt_3 (4.5 eq.) to anhydrous SnCl_2 (1.5 eq.) in CH_3CN to form $[\text{Sn}(\text{SPh})_3]$, which is believed to be the active reduction species. This is then added to a solution of the azide (1.0 eq.) in CH_3CN , to allow for azide reduction in very short reaction times of 20 min.

This procedure was reportedly used for the reduction of an azide in the presence of a labile anhydride, therefore this approach appeared to be a promising reduction reagent for compound **161**.²⁷⁵ Accordingly, $[\text{Sn}(\text{SPh})_3]^-$ was prepared as per the literature procedure followed by the addition of **161** (Scheme 3.12), with the reduction of the azide being complete within 20 min, as determined by TLC analysis. It is notable that only 3 eq. PhSH was employed for the preparation of $[\text{Sn}(\text{SPh})_3]^-$, rather than the excess used in the literature (see experimental), to avoid the presence of excess PhSH in the reaction mixture and any unwanted transthioesterification with the installed thioester. The successful reduction of the azide was confirmed by the loss of the characteristic IR peak at 2097 cm^{-1} and through HMRS displaying a m/z peak at 540.2149, corresponding to the $[\text{163}+\text{Cl}]^-$ species.



Scheme 3.12: Reduction of azide using tin/mercaptan complex.

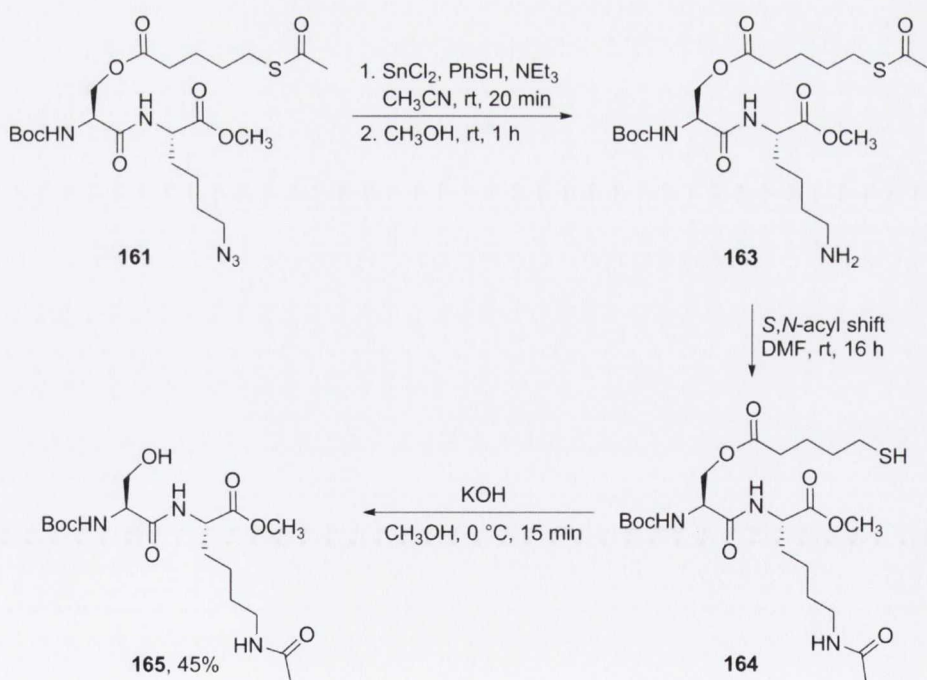
This reduction protocol rapidly reduced the azide functionality in compound **161** but resulted in the problem of effective product isolation after the reaction was complete. The recommended protocol employs a strongly basic workup consisting of 2N NaOH in order to destroy the tin complex. But this procedure is not applicable to substrate **163** due to the labile thioester functionality; therefore, the work up procedure needed to be modified. Instead, a weakly basic workup involving sat. aq. NaHCO_3 solution was used followed by stirring of the combined extracts in CH_3OH for 1 h. Stirring in CH_3OH serves as a tin exchange method, reported by Fu, which transfers the tin from nitrogen to the oxygen of the CH_3OH solvent, as illustrated in Scheme 3.13.²⁷⁶



Scheme 3.13: Tin exchange method using CH_3OH .²⁷⁶ After the reduction of the azide group, the reaction mixture is transferred to CH_3OH and stirred for 1 h at rt. This serves as a Sn exchange method where the Sn is transferred from the amine nitrogen to the oxygen of CH_3OH .

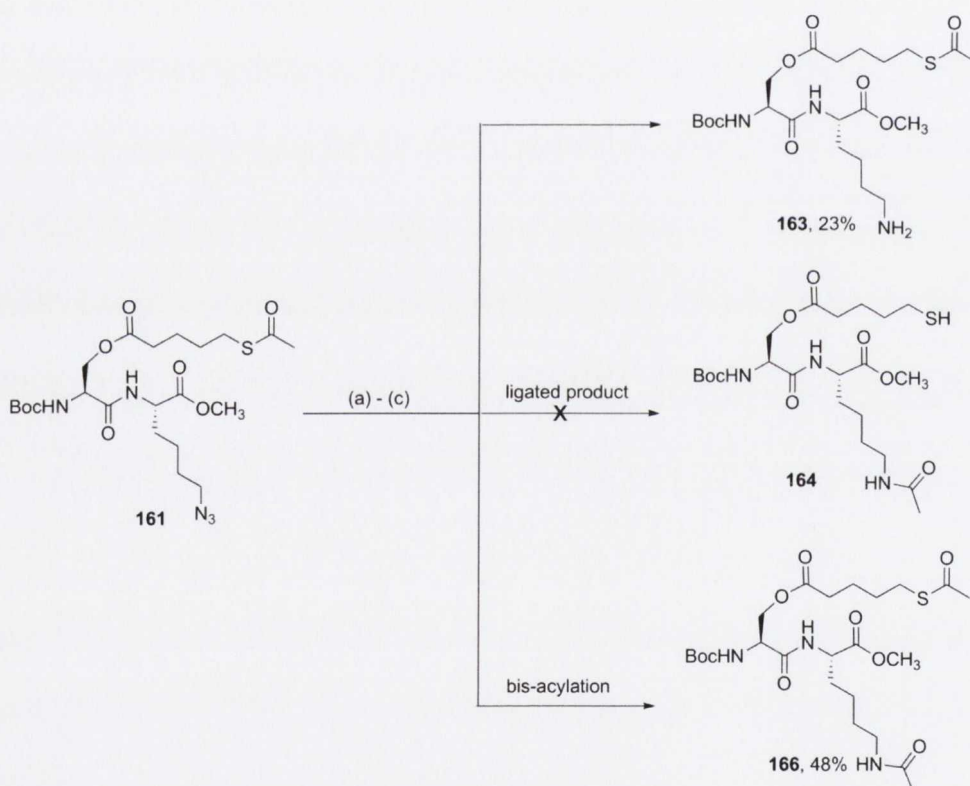
3.9 One-pot: azide reduction, *S,N*-acyl shift and base hydrolysis

Satisfied that the reduction of the azide was successful using the tin/mercaptan method, the reaction was repeated followed by stirring the reaction mixture in DMF for 16 h to allow for the *S,N*-acyl shift to proceed to give **164**, as depicted in Scheme 3.14. This was then followed by treatment with KOH in CH_3OH at 0°C to hydrolyse the pentanoyl ester moiety. The base hydrolysis was neutralised by the addition of DOWEX- H^+ resin which was then filtered. After column chromatography, the desired compound **165** was recovered, albeit in a disappointing yield of 45% calculated over 3 steps.



Scheme 3.14: Proposed one pot reaction for the formation of **165**. The methodology was tested using dipeptide **161**. The first step involved the reduction of the Lys azide using a tin/mercaptan complex, followed by stirring in CH_3OH to allow for Sn exchange. The reaction was then left to stir in DMF to allow the *S,N*-acyl shift to occur to give **164**. The last step involved the hydrolysis of the Ser ester group using KOH to give **165**.

In an attempt to understand why the isolated yield was quite low, the reaction was repeated followed by removal of the solvent after the *S,N*-acyl shift, and prior to the base ester hydrolysis. Crude NMR analysis did not yield a conclusive answer; therefore the mixture was subjected to chromatographic purification. Interestingly, two compounds were isolated after column chromatography, as shown in Scheme 3.15: the unreacted amine **163** and bis-acylated **166** in 23% and 48% yield, respectively. Unfortunately, none of the desired ligated product **164** was obtained. It is well known that thiol containing peptide species can be oxidatively dimerised in solution when exposed to air in the absence of a reducing agent, however, no dimerised species was recovered either.²⁷⁷



Scheme 3.15: Products isolated from reduction of the azido group. (Top) Compound **161** with the free amine was recovered in 23% yield; (Middle) None of the desired ligated product **164** was formed; (Bottom) The bis-acylated compound **166** was recovered in 48% yield whereby intermolecular transacylation occurred. *Reagents and conditions:* (a) SnCl₂, PhSH, NEt₃, CH₃CN, rt, 20 min; (b) CH₃OH, rt, 1h; (c) DMF, rt, 16 h.

As illustrated in Figure 3.5, the isolated compounds were analysed using ¹H NMR whereby the δ -CH₂ functionality was analysed (highlighted in orange)

along with the acetyl moiety. Firstly, the starting azide **161** (*top*, green) displays a triplet (t) at 3.29 ppm corresponding to the δ -CH₂ and the thioester moiety resonates at 2.34 ppm. Upon azide reduction, the δ -CH₂ of **163** (*middle*, red) displayed a slight downfield shift from 2.34 ppm to 3.16 ppm with the signal now appearing as a broad singlet (br s). The thioacetate moiety remained intact signifying that the *S,N*-acyl migration reaction had not occurred. The intermolecular transacylation product **166** (*bottom*, blue) displays a slight downfield shift with the δ -CH₂ appearing as a multiplet (m) at 3.26 ppm. Interestingly, in this case, the bis-acylation was noticed by the presence of the thioacetate moiety along with the appearance of an extra acetyl moiety at 2.02 ppm, corresponding to the *N*-acetyl functionality. As well as being confirmed through NMR analysis, the products **163** and **166** were further verified by HRMS with *m/z* peaks at 540.2149 and 791.3046 corresponding to the [**163**+Cl]⁻ and [**166**+Na]⁺ species, respectively.

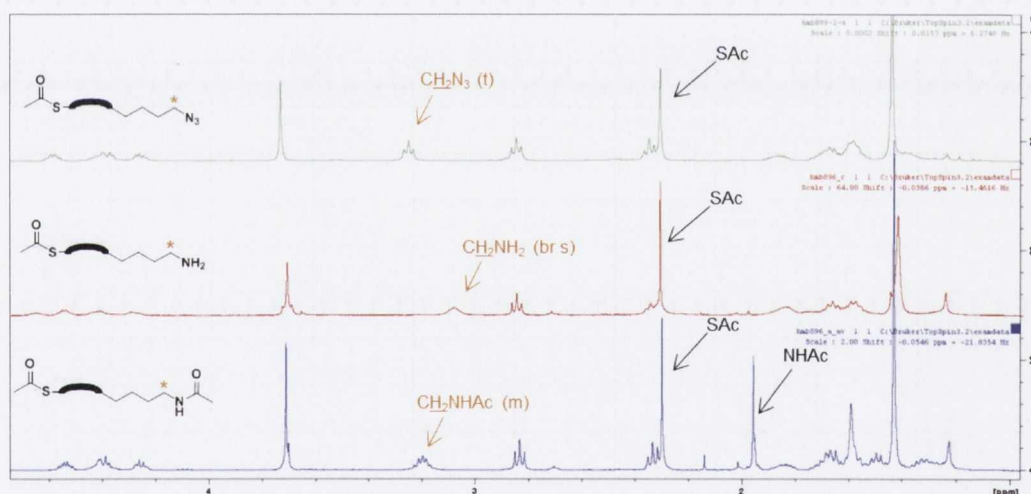


Figure 3.5: ¹H spectra (400 MHz, CDCl₃) of compound **161**, **163**, and **166** with associated structures depicted. (*Top*) Compound **161** with CH₂N₃ and SAc moieties highlighted; (*Middle*) Compound **163** with CH₂NH₂ and SAc moieties highlighted; (*Bottom*) Compound **166** with CH₂NHAc, SAc and new NHAc moieties highlighted.

Katritzky and co-workers have demonstrated *S,N*-acyl migrations occurring over large cyclic transition states assisted by microwave irradiation in DMF and phosphate buffered solutions.²²⁶ Therefore, in an attempt to assist the acyl migration, the amine **163** was dissolved in a 50:50 mixture of DMF:phosphate buffer (pH 7.4) and subjected to microwave irradiation at 50 °C for 5 h in an

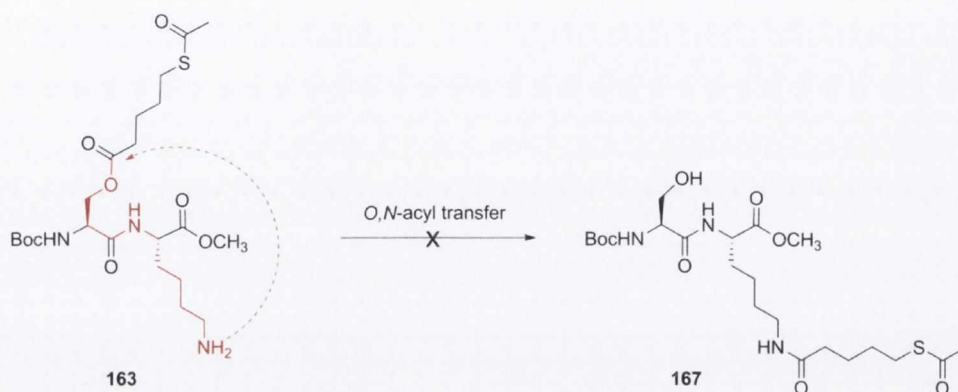
attempt to assist the *S,N*-acyl transfer. Unfortunately, this was not fruitful, with the amine compound **163** being recovered.

Interestingly, Katritzky conducted studies on *S,N*-acyl transfer ligations operating *via* 17-, 18- and 19-membered transition states, both experimentally and computationally.²²⁷ It was deduced that the ratio of the desired ligated and transacylated product obtained depended dramatically on the transition state ring size. It was found that intramolecular *S,N*-acyl migration was favoured for the 17-membered transition state, with the desired product being delivered in 60% after microwave irradiation. However, in the case of the 18-membered cyclic transition state, the intermolecular *S,N*-acyl transfer was found to be the prominent process. With the 18-membered cyclic transition state, Katritzky reported the transacylated product in 82% yield, suggesting that intramolecular acyl migration through an 18-membered transition state is less favoured than through a 17-membered transition state. Therefore, considering the formation of the desired ligation product **164** would proceed through an unfavourable 18-membered transition state, the results obtained with the substrate **163** are consistent with Katritzky's observations of transacylation. However, the fact that no transacylated product **166** was detected after heating **163** under microwave conditions is surprising. This suggests that the formation of the intermolecular transacylated product is dependent on the size of the cyclic transition state but also on the experimental conditions, such as concentration (concn). Accordingly, microwave irradiation of **163** was repeated under various concns in different solvents: (i) DMF, concn = 1.5 M; (ii) DMF, concn = 3 M; (iii) DMF:phosphate buffer (50:50), concn = 1.5 M; (iv) DMF:phosphate buffer (50:50), concn = 3 M. Unfortunately, the desired ligated **164** or the bis-acylated product **166** were not obtained under any of these reaction conditions.

3.9.1 Possible *O,N*-acyl transfer

As no inter- or intramolecular *S,N*-acyl transfer was occurring with amine **163**, it seemed plausible that an *O,N*-acyl migration *via* a 12-membered cyclic transition state could occur, as depicted in Scheme 3.16. Katritzky and co-workers reported *O,N*-acyl migrations *via* 12-19 membered cyclic transition states under microwave heating in DMF-piperidine mixtures.²⁷⁸ In the case of

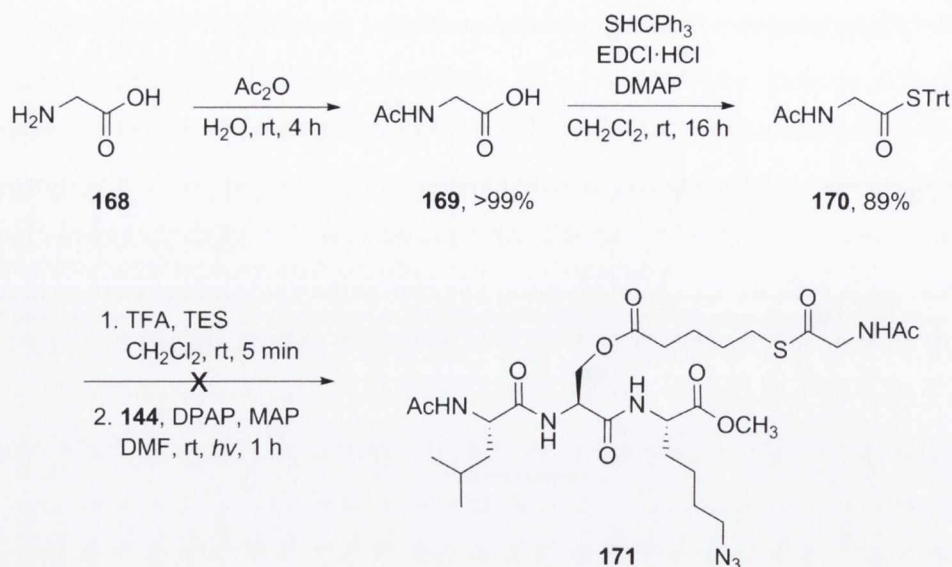
the 12-membered cyclic transition state, they found that only the desired ligated product was formed, with no bis-acylated product being detected. However, through HMBC analysis, it was deduced that *O,N*-acyl migration was not occurring in the case of **163** either.



Scheme 3.16: Possible *O,N*-acyl transfer via a 12-membered transition state.

3.10 Application to α -synuclein tripeptide and Gly thioacid

Although the previous result was disappointing due to the intermolecular bis-acylated species being the main product of the *S,N*-acyl transfer reaction, it was decided to investigate the methodology using the previously prepared tripeptide **144**. To mimic the ubiquitination process, the α -syn tripeptide **144** was to be reacted with a Gly thioacid, due to ubiquitination involving the formation of an isopeptide bond between the substrate Lys residue and the C-terminal Gly of Ub. As detailed in Chapter 2, access to peptidyl thioacids was achieved through the use of *S*-trityl protected thioesters which could be subsequently deprotected using TFA and TES with the generated thioacid reacted *in situ*. It was decided to prepare *N*-acetyl Gly due to the *N*-acetyl group being stable to both acidic and basic conditions. As illustrated in Scheme 3.17, this involved the acetylation of **168** in H₂O using Ac₂O which furnished *N*-acetyl Gly **169** in quantitative yield after removal of the solvent. The *S*-trityl group was then introduced using the same conditions employed in Chapter 2; this involved reaction of **169** with EDCI·HCl, DMAP and SHCPh₃ to give **170** in 89% yield after column chromatography.

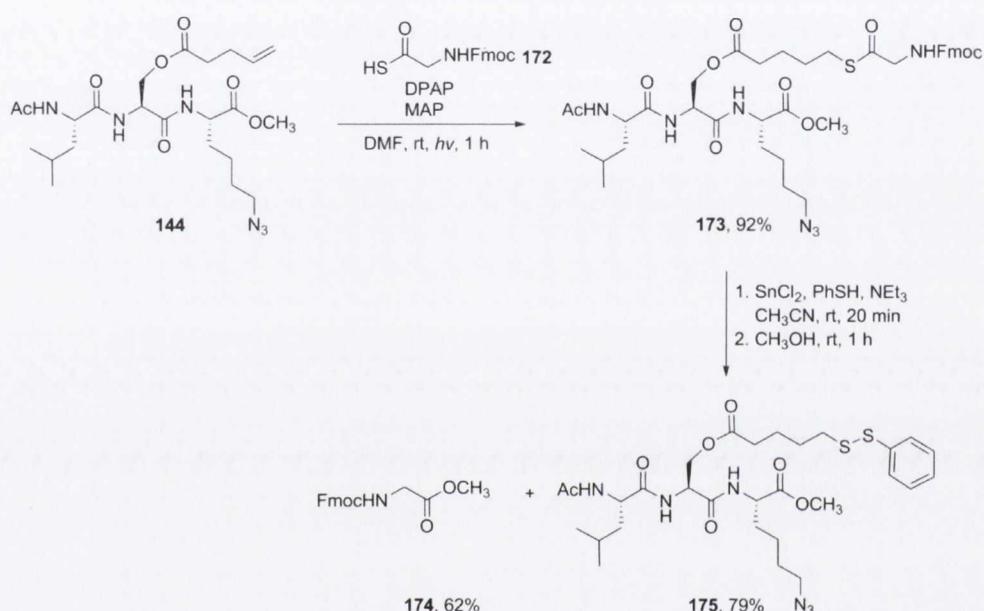


Scheme 3.17: Synthesis of *S*-trityl protected Gly thioacid **170** and its subsequent unsuccessful thiol-ene ligation with tripeptide **144**.

With the Gly protected thioacid **170** in hand, the *S*-trityl group was subsequently removed in 5 min using a 25% solution of TFA in CH_2Cl_2 together with TES. The generated thioacid was then immediately added to a solution of the tripeptide **144**, DPAP, and MAP in DMF and irradiated in a UV oven for 1 h. Unfortunately, this thiol-ene reaction was not successful with only trace amount of product **171** being formed. Attempts to elicit a reaction between **144** and the Gly thioacid **171** by employing excess thioacid (up to 6.0 eq.), increasing the amount of DPAP (up to 1.0 eq.) and prolonging the irradiation time (up to 5 h) were all unsuccessful in furnishing **171** in appreciable yields.

The *S*-trityl protected Gly thioester **103** which was successfully applied to thiol-ene ligations in Chapter 2 was next investigated (Scheme 3.18). The *S*-trityl group was removed using the previously employed conditions to give the crude thioacid **172** which was added to a solution of tripeptide **144** in DMF along with DPAP and MAP. Gratifyingly, the desired ligated tetrapeptide **173** was furnished in 92% yield after column chromatography. Next, **173** was subjected to the previously employed reduction conditions of $\text{SnCl}_2/\text{PhSH}/\text{NEt}_3$ in CH_3CN . After 20 min, the reaction was transferred to CH_3OH to allow for the tin exchange. The crude product was then analysed using IR spectroscopy to verify that the characteristic azide peak was no longer present. However, a peak at 2097 cm^{-1} was still present which indicated that the reduction of the

azide group had not proceeded as expected. It was confirmed by NMR analysis that the reduction of the azide moiety did not proceed. Instead, the thioester functionality was hydrolysed to yield the *N*-Fmoc Gly methyl ester **174** and the disulfide species **175**, both of which were confirmed through NMR analysis and HRMS. The azido moiety was not reduced to the amine with this being attributed to the unsuccessful formation of the active reducing species $[\text{Sn}(\text{SPh})_3]^-$ with the labile thioester functionality instead being hydrolysed.



Scheme 3.17: Thiol-ene ligation of tripeptide **144** and Gly thioacid **172** followed by the attempted reduction of the azide group in **173**. Using the tin/mercaptan reduction conditions, reduction of the azide group in **173** did not occur with the labile thioester being hydrolysed instead.

3.11 Conclusion

In conclusion, routes to the α -syn dipeptide **160** and tripeptide **144** are described. These peptide units, **160** and **144**, are modified with an alkenyl ester which is capable of successfully participating in thiol-ene ligations with thioacetic acid **66** and Gly thioacid **172**, respectively. The reduction of the azido functionality after thiol-ene ligations proved problematic. When hydrogenation conditions of Pd/C were employed, hydrogenolytic desulfurisation occurred. Reduction of the azido moiety utilising a tin/mercaptan complex proved successful in the reduction of the azido group in dipeptide **161**. However, in the case of the ligated tetrapeptide **173**, these reduction conditions resulted in thioester hydrolysis.

The subsequent *S,N*-acyl transfer reaction of the azide reduced dipeptide species **163** was found to favour the intermolecular transacylation reaction over the desired intramolecular *S,N*-acyl transfer. These results are in line with the observations of Katritzky and co-workers with the same sized cyclic transition states. Katritzky and co-workers have studied chemical ligations over a large range of cyclic transition states (5-19) which show variable yields and reactivity trends which are difficult to rationalise.²⁶³ With structurally similar peptide structures differing only in the size of the generated cyclic transition state, intra- vs. intermolecular acylation can vary from almost quantitative yield to almost zero. However, future work should involve the use of 4-butenoyl chloride, in place of 4-pentenoyl chloride, which would generate a 17-membered cyclic transition state. As reported by Katritzky and co-workers, peptide substrates have successfully undergone intramolecular *S,N*-acyl migrations *via* 17-membered cyclic transition states when subjected to microwave heating. Work by Haase and Seitz further suggests the use of a 17-membered cyclic transition state, as they also reported peptide ligations readily occurring through a 17-membered ring.²⁷⁹ With regards to the reduction of the azido moiety, mild reduction conditions are needed which avoid desulfurisation whilst leaving the thioester intact. The Lys ϵ -NH₂ functionality could also be Boc protected followed by removal of this protecting group under acidic conditions. However, this would require the use of Fmoc protecting groups for the amino acids during peptide coupling reactions. Alternatively, the tin/mercaptan complex proved successful in reducing the azide functionality in the presence of the alkene to give **176** (Figure 3.6). Therefore, rather than conducting the azide reduction in the presence of the thioester, future work could involve carrying out thiol-ene ligations on compounds whereby the Lys ϵ -NH₂ is present.

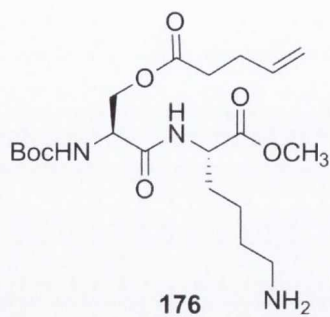


Figure 3.6: Successful reduction of the azide functionality using the tin/mercaptan procedure.

4.0 Introduction

As discussed in Chapter 1, glycosylation is orchestrated in cells by two main families of enzymes; the glycosyltransferases and glycosidases, in the ER and Golgi apparatus.²⁸⁰ Glycosidases are catabolic enzymes which cleave glycosidic linkages of glycoconjugates, whereas glycosyltransferases are enzymes which construct glycosidic linkages. It is imperative that these two families work in harmony in tailoring glycans to convey information about the internal state of the cell.¹⁵⁷ Glycosyltransferases represent a distinct family to the glycosidases and will not be discussed here.²⁸¹ In this chapter, the design, synthesis, and photophysical evaluation of novel lanthanide-based probes for the monitoring of glycosidase activity will be discussed. The probes are based on the macrocyclic framework cyclen, a structural unit commonly used in lanthanide coordination chemistry. Prior to that, a short introduction to the glycosidase enzymes, lanthanide luminescence and some enzymatic probes based on lanthanide luminescence ions will be presented.

4.1 Biological importance of the glycosidase enzymes

Diseases stemming from dysfunction of the glycosidase enzymes are diverse ranging from LSDs, cancer, diabetes, to influenza.⁶⁰ Deficient glycosidase activity is characteristic of LSDs. For example, Gaucher disease which is the most prevalent LSD is characterised by the defective activity of a lysosomal β Glc enzyme. This leads to accumulation of glucosylceramide, a glycolipid, to toxic levels which can result in devastating neurological defects.²⁸² Conversely, elevated glycosidase activity has been observed in many cancers. For example, elevated levels of α -L-fucosidase has been reported in patients with hepatocellular carcinoma (HCC).^{283,284} Ishizuka and co-workers reported that the serial determination of α -L-fucosidase activity was extremely useful in predicting the development of HCC and identifying high-risk groups.²⁸³ Furthermore, Matsumoto *et al.* correlated plasma α -L-fucosidase levels with patient sensitivity to trastuzumab, a potent anticancer agent used in chemotherapy against human epidermal growth factor receptor 2 (HER2)-overexpressing breast cancer.²⁸⁵ Interestingly, trastuzumab does not elicit an anticancer effect in all patients therefore, a biomarker to predict the clinical outcome would be very beneficial.

Efforts have been made to identify such glycobiological biomarkers to allow for the development of sensitive analytical techniques to aid early detection of such diseases, impacting prognosis. We have recently reviewed the current developments in synthetic chemical probes for the detection of the glycosidase enzymes, many of which were discussed in Chapter 1.¹⁶ However, many of the probes reviewed were based on fluorogenic molecules which can be problematic in biological media, due to problems such as autofluorescence and light scattering.²⁸⁶ This chapter will focus on the use of the Ln(III) ions as promising tools for the detection of enzyme activity and our efforts towards developing a luminescent probe to investigate glycosidase activity.

4.2 General introduction to the lanthanides

The lanthanide metals are located in the 5d block of the periodic table and comprise elements 57-71, as shown in Figure 4.1. They are often termed “rare earths” implying they are in low abundance; however, many lanthanides are as abundant as cobalt and lead, for example, in the earths crust. The name actually arose as these metals were among the last to be discovered.²⁸⁷

57 138.91	58 140.12	59 140.91	60 144.24	61 (145)	62 150.36	63 151.96	64 157.25	65 158.93	66 162.50	67 164.93	68 167.26	69 168.93	70 173.05	71 174.97
La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
LANTHANUM	CERUM	PRASEODYMIUM	NEODYMIUM	PROMETHIUM	SAMARIUM	EUROPIUM	GADOLINIUM	TERBIUM	DYSPROSIUM	HOLMIUM	ERBIUM	THULIUM	YTTTERBIUM	LUTETIUM

Figure 4.1: The lanthanide series.²⁸⁸

The lanthanides are characterised by the gradual filling of their 4f orbitals which are well shielded by the outer 5s² and 5p⁶ orbitals.²⁸⁹ The lanthanides usually exist as trivalent cations (Ln(III)) where they bear a ground state configuration of [Xe]4fⁿ with *n* varying from 1 (Ce(III)) to 14 (Lu(III)).²⁸⁹ The bonding in Ln(III) complexes is largely electrostatic and they possess high and varied coordination numbers ranging anywhere from 6 to 12, with 8 and 9 being the most common. These ions are “hard” Lewis acids therefore, they show preferential binding to ligands of a “hard” Lewis base nature, such as oxygen, nitrogen, and fluoride.²⁸⁶ The high coordination requirements of the Ln(III) ions must be fulfilled due to their inherent toxicity while chelation also serves to protect the metal centre from luminescence quenching (discussed in more detail in Section 4.2.2). There are many different ligands used to fulfil

these high coordination requirements, examples being pre-organised macrocyclic ligands or acyclic self-assembled systems.¹⁸⁷ One of the most commonly used macrocyclic ligand frameworks for Ln(III) complexation is tetra-functionalised 1,4,7,10-tetraazacyclododecane (cyclen) and to meet the aforementioned requirements, cyclen is protean in this regard. Cyclen was selected as a suitable ligand in our approach towards a Ln(III)-based probe for the glycosidase enzymes.

4.2.1 Photophysical properties of the lanthanides

Lanthanide luminescence arises from the decay of electronically excited states with emission of a photon. Many of the useful Ln(III) photophysical properties originate from their *f-f* transitions: they exhibit easily recognisable sharp, line-like bands in their emission spectra which are specific for each Ln(III) ion and a large *pseudo* Stokes shift.¹⁸⁷ As discussed in Chapter 1, the Laporte forbidden nature of these *f-f* transitions results in the slow relaxation from the Ln(III) emitting state and thus long luminescent lifetimes are observed when compared with organic fluorophores (micro-millisecond vs nanosecond). Such long lifetimes make the Ln(III) ions very attractive from an analytical perspective, allowing the implementation of time-gated procedures (Figure 1.30A).²⁸⁶ Also discussed in Chapter 1 was the “antenna effect” which is employed to circumvent the low molar absorptivity of the Ln(III) ions. In 1942, Weissman observed that the luminescence from Eu(III) cations was largely enhanced in the presence of organic aromatic ligands.²⁹⁰ Interestingly, the excitation of Eu(III) was observed at wavelengths where it did absorb; therefore, it was concluded that Eu(III) excitation was a multi-step process, now known as the “antenna effect”. In this excitation process, a light harvesting antenna group is incorporated into the Ln(III) ligand design to indirectly sensitise the Ln(III) ion allowing for subsequent emission, the process of which can be summarised in a Jablonski diagram (Figure 4.2, *left*).²⁹¹ This process firstly involves excitation of the organic fluorophore by irradiation from a source, such as a fluorometer, making a singlet excited state ($S_0 \rightarrow S_1$). This singlet excited state may give up a photon and fluoresce or undergo intersystem crossing (ISC) to the longer-lived antenna triplet state ($S_1 \rightarrow T_1$). ISC is a spin forbidden process (both electrons are now in parallel), however, due to the presence of the lanthanide, a

heavy atom, a significant amount of spin-orbit coupling can occur which promotes ISC.²⁹² This excited triplet (T_1) can then decay to the ground state *via* phosphorescence or populate the lowest excited state of the nearby Tb(III) ion (5D_4) by intramolecular energy transfer. This energy can be dissipated from the 5D_4 excited state to the 7F_6 ground state giving rise to the characteristic line-like emission of the Tb(III) ion, with each transition corresponding to an emission band in their spectra (Figure 4.2, *right*).¹⁹⁶

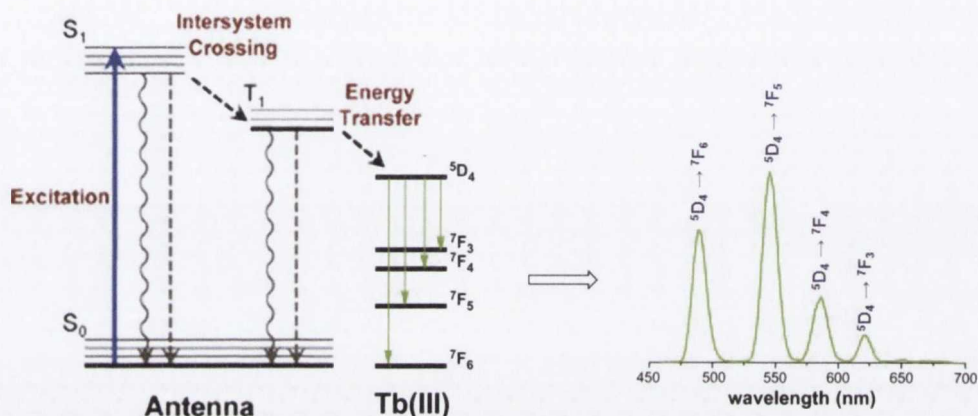


Figure 4.2: Jablonski diagram (*left*) illustrating the sensitisation of a Tb(III) ion by an organic fluorophore, known as the “antenna effect”, with the resulting characteristic Tb(III) emission spectra (*right*) (Image modified from Meade *et. al*).¹⁹⁶ The antenna effect involves light absorption and excitation of the lowest-lying singlet excited state of the antenna (S_1) followed by intersystem crossing (ISC) to the antenna triplet state (T_1). Sensitisation of the Ln(III) occurs by energy transfer to the excited 5D_4 state of the Tb(III) ion. The radiative transition of electrons from the excited 5D_4 state to the 7F_6 ground state results in the Tb(III) emission spectra (*right*), with each transition corresponding to a band in the emission spectra.

Provided that the energy of the T_1 lies at least $1,700\text{ cm}^{-1}$ above that of the accepting level of the Ln(III), the intramolecular energy transfer should occur efficiently. If the energy gap is too small, back-energy transfer to the antenna T_1 can occur. Conversely, if the distance between the two states is too large, successful population of the Ln(III) excited state may be inefficient.^{196,293} The energy transfer process from the antenna T_1 excited state to the Ln(III) excited state can occur *via* one of two mechanisms: Dexter’s (exchange) mechanism or Förster’s (dipole-dipole) mechanism.^{294,295} Dexter’s mechanism is an electron exchange process which involves physical overlap between the orbitals of the antenna and Ln(III) and therefore limits this mechanism to short distances (usually 30 – 50 pm). Whereas Förster’s mechanism involves the coupling of the dipole moment of the antenna T_1 with the dipole moment of the

lanthanide 4f orbitals which allows the Förster mechanism to extend over longer distances (up to 1,000 pm).^{296,297}

The indirect sensitisation process accounts for two of the major advantages of lanthanide luminescence over conventional fluorescence systems. Firstly, the large *pseudo* Stokes shift which allows light absorbed in the near-UV to be emitted in the visible region. Secondly, the long-lived luminescence allows for time-gated measurements to be implemented, the principles of which are depicted in Figure 1.30A.¹⁹⁶ The most commonly employed Ln(III) ions are Eu(III) and Tb(III) due to several factors. Firstly, they display luminescent lifetimes in the millisecond range, compared with the microsecond lifetimes of Sm(III) and Dy(III). Secondly, when compared with the near-IR emitting lanthanides, Eu(III) and Tb(III) show less sensitivity to singlet oxygen quenching with them displaying higher emission intensities and at biologically relevant emission wavelengths in the visible region.^{196,186}

4.2.2 Lanthanide luminescent quenching

The high coordination requirements of the Ln(III) ions must be fulfilled to attenuate the toxicity of the free Ln(III) ions and also to prevent luminescence quenching due to solvent coordination to the metal centre. The coordination of water molecules to the metal centre shortens the Ln(III) excited state lifetime through non-radiative vibrational energy transfer to high frequency O-H oscillators, resulting in reduced emission.^{286,188} In the late 1970s, Horrocks and co-workers showed that the lifetimes of Eu(III) and Tb(III) ions were directly related to the number of directly bound water molecules, known as the *q* value. This parameter can be quantified by comparing the luminescence lifetimes (τ) in H₂O and D₂O. A mathematical equation to determine the number of metal bound water molecules was originally proposed by Horrocks *et al.* and was subsequently modified by Parker and co-workers.^{298,299} Equation 4.1 and 4.2 are now commonly used to calculate the *q* values for Eu(III) and Tb(III) complexes, respectively.

$$q^{\text{Eu(III)}} = A [(1/\tau_{\text{H}_2\text{O}} - 1/\tau_{\text{D}_2\text{O}}) - 0.25 - 0.075x] \pm 0.5 \quad \text{Equation 4.1}$$

$$q^{\text{Tb(III)}} = A [(1/\tau_{\text{H}_2\text{O}} - 1/\tau_{\text{D}_2\text{O}}) - 0.06] \pm 0.5 \quad \text{Equation 4.2}$$

In these equations, A is referred to as the proportionality constant and is specific for a given Ln(III) ion. It is a measure of the degree of quenching from metal bound water molecule with it being 1.2 ms and 5 ms for Eu(III) and Tb(III), respectively. The correction terms, -0.025 and -0.06, represent quenching by second-sphere water molecules, while $-0.075x$ represents quenching by N-H oscillators.

4.2.3 Examples of lanthanide-based luminescent bioprobes

The detection of biologically relevant species such as cations, anions, and pH changes using the Ln(III) ions is an active area of research, with the recent developments and progress comprehensively reviewed.^{192,300} Various “smart” MR contrast agents have been developed which are activated by enzymes such as glycosidases, esterases, proteases, and peroxidases, some of which were discussed in Chapter 1, Section 1.4.3.3.2.^{196,301,302,303,304} However, compared with reported probes for anion recognition, for example, enzyme-activated probes based on the luminescent properties of the Ln(III) ions have been somewhat scarce in comparison.

Kikuchi and co-workers reported the Tb(III)-based complex **177** as a luminescent probe for the detection of calpain activity (Figure 4.3). This protease is involved in many cellular processes, with its activity related to diseases such as Alzheimer’s and PD.³⁰⁵ The complex is based on the cyclen framework with a simple phenyl group serving as the sensitising antenna and an appended peptide sequence which is recognised by calpain. The peptide conjugate **177** was scarcely luminescent, however, addition of calpain enzyme resulted the hydrolysis of the amide moiety (indicated in red) to form **178** causing enhanced Tb(III) emission, hence reporting on calpain enzyme activity. To demonstrate the usefulness of the probe, it was incubated with fluorescent compounds, such as umbelliferone, and its emission was not perturbed, showing its compatibility with organic fluorophores

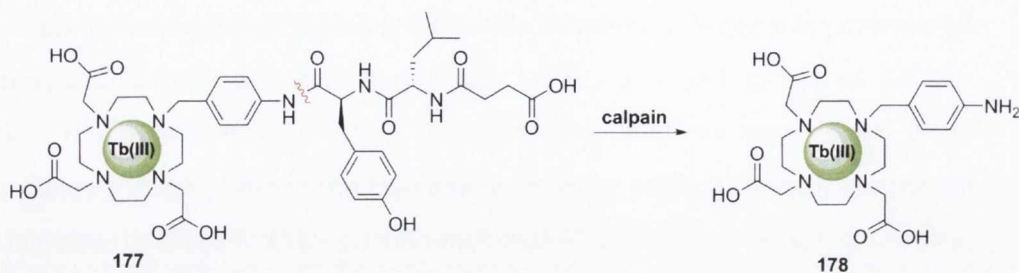


Figure 4.3: Tb(III)-based probe for the detection of calpain protease activity.³⁰⁵ Hydrolysis of the peptidyl bond in **177** by calpain to give an aniline antenna in **178** resulting in an increase in the Tb(III) centred emission.

One enzyme whose detection is being widely pursued is glutathione reductase, which catalyses the reduction of glutathione disulfide (GSSG) to glutathione (GSH). GSH is a predominant tripeptide thiol **179** found within the human cellular system. GSH exists mainly as its reduced form, however it can be oxidised to its dimer GSSG in response to oxidative stress; therefore, changes in the GSH/GSSG ratio can be used as an indicator of oxidative stress.³⁰⁶ The Gunnlaugsson group developed the Tb(III)-based probe **180** for the selective detection of GSH and the real-time monitoring of glutathione reductase activity (Figure 4.4).³⁰⁷ Sensor **180** contains a maleimide moiety which can undergo 1,4-Michael addition with GSH **179** to form **181**. The formation of **181** is accompanied by a large enhancement in the Tb(III) centred emission, thus reporting on the presence of GSH. Furthermore, the real-time monitoring of glutathione reductase activity was achieved (GSSG reduction to GSH) by following the Tb(III) emission of **181**, which directly related to the formation of GSH and its subsequent addition to the maleimide moiety of **180**.

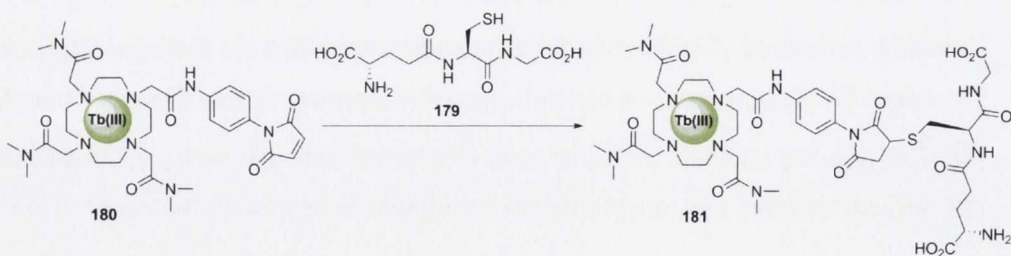


Figure 4.4: Tb(III)-based probe **180** used for the detection of GSH **179** and glutathione reductase activity.³⁰⁷ GSH **179** can undergo 1,4-Michael addition with the maleimide moiety of **180** to give conjugate **181**, which is accompanied by an increase in Tb(III) emission.

4.3 Aims of this work

As outlined in Chapter 1, Section 1.4.3, the development of probes for the detection of the glycosidase enzymes is an active area of research. However, currently the only reported probe for the detection of glycosidase enzymes based on Ln(III) luminescence is **47**, which was reported by Borbas *et al.* (see Section 1.4.3.3.1, Figure 1.40).¹⁹³ Encouraged by the success of the previously described Tb(III) cyclen complexes, **177** and **180**, reported by Kikuchi and Gunnlaugsson, respectively, we set out to develop a generic probe for the detection of various glycosidase enzymes. The design of the probe is depicted in Figure 4.5, with its mode of detection based on Ln(III) luminescence.

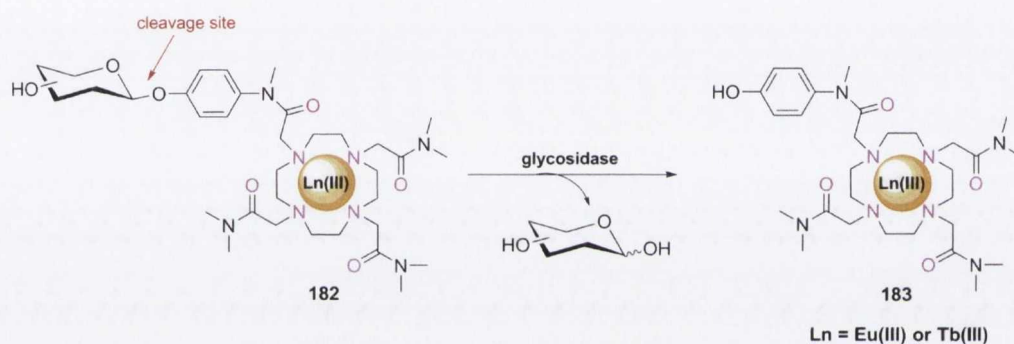


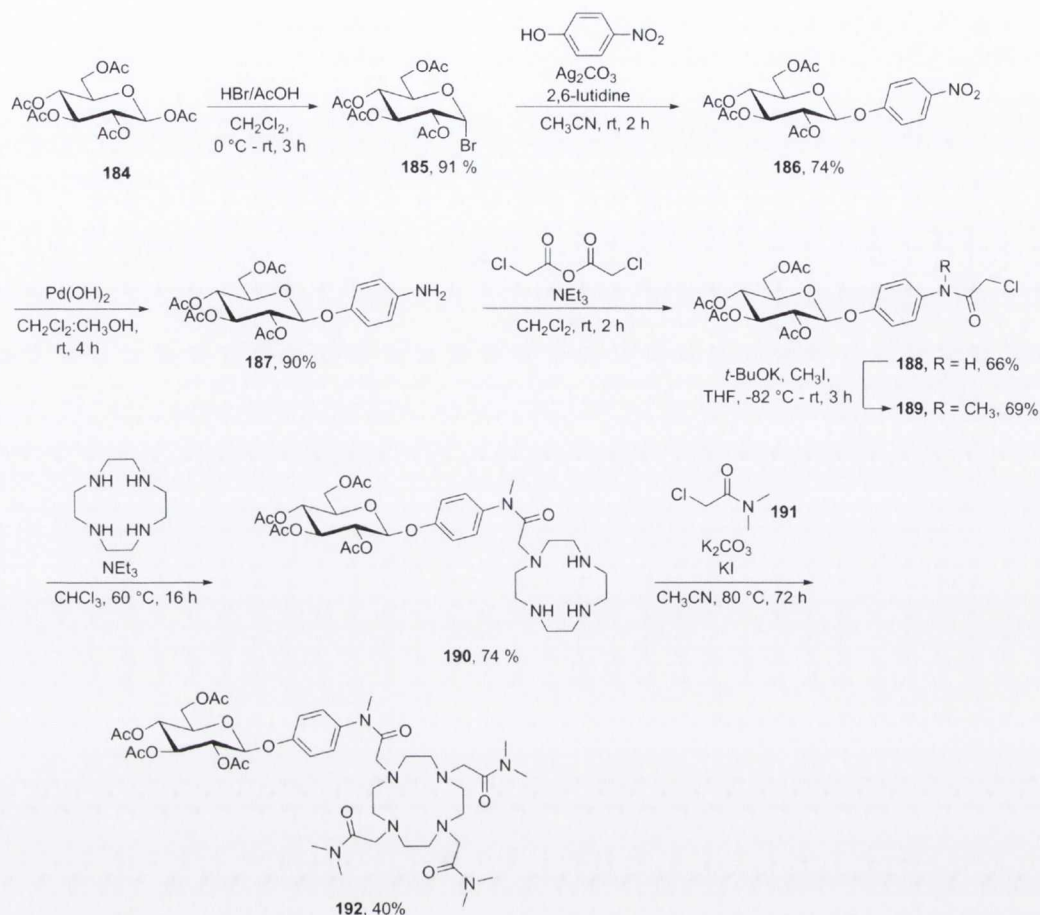
Figure 4.5: Ln(III)-based probe for the detection of glycosidase enzymes. Addition of the relevant glycosidase enzymes results in the hydrolysis of the O-glycosidic linkage in **182** to give **183**. The formation of the phenol antenna in **183** was expected to result in modulation of Ln(III) emission, hence allowing for the detection of glycosidase activity.

The use of cyclen allows for the introduction of a variety of pendant arms to fulfil the high coordination requirements of the Ln(III) ion, with the possibility for the introduction of biomolecules for biological application.^{308,309} The chosen Ln(III) ions, Eu(III) and Tb(III), are among the most emissive Ln(III) ions and both have a coordination number of 9 with 8 of those being fulfilled by the ligand design.³¹⁰ The coordinating sites are highlighted in pink with 4 of those being the nitrogen atoms of the cyclen macrocycle. The other 4 coordinating groups are the carbonyl groups of the attached *N,N*-dimethylamide pendant arms. With this ligand design, it is expected that the remaining coordination site of the Ln(III) ion would be occupied by a single water molecule. The phenyl group serves as the antenna for the Ln(III) sensitisation process, the mechanism of which is depicted in Figure 1.30B and Figure 4.2. It was anticipated that this simple antenna could sensitise the

Ln(III) emission since the phenyl moiety of tyrosine has been reported to efficiently sensitise Eu(III) and Tb(III) ions.³¹¹ A sugar moiety is *O*-linked to the phenyl antenna, which acts as the substrate site for the glycosidase enzyme. It was anticipated that cleavage of the *O*-glycosidic bond by a glycosidase enzyme would result in the formation of a phenol-based antenna and concomitant modulation in the Ln(III) emission allowing for the action of glycosidase enzymes to be detected.

4.4 Synthesis of β -D-glucose appended ligand **192**

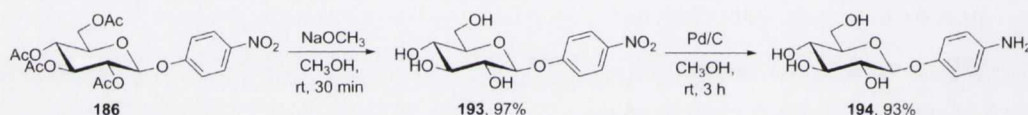
The synthesis of ligand **192** incorporating a β -D-glucose moiety is shown in Scheme 4.1. As detailed in Section 1.2.2.2, carbohydrate chemistry is centred around glycosylation reactions which involve a glycosyl donor reacting with an acceptor in the presence of a suitable promoter. Initially, glycosyl bromides were the donor of choice due to their ease of synthesis without the need to revert to column chromatography. Glycosyl halides were the first type of glycosyl donors used in carbohydrate synthesis and still play an active role in carbohydrate chemistry today.³¹² Accordingly, treatment of commercially available peracetylated glucose **184** with 33% HBr/AcOH solution in CH₂Cl₂ at 0 °C gave the glycosyl bromide **185** in high yield of 91% after recrystallisation from EtOAc/Hex. The presence of bromide donor **185** was confirmed through the loss of an acetyl signal in the ¹H NMR and the downfield shift of the anomeric H-1 proton (from δ 5.72 ppm to δ 6.61 ppm). The bromide at C-1 in **185** was expected to possess an α -configuration due to the anomeric effect (as discussed in Chapter 2, Section 2.3). This configuration was confirmed by the small H1-H2 vicinal coupling constant of 3.8 Hz, which is indicative of the α -anomer.



Scheme 4.1: Initial synthetic strategy toward β -D-glucose appended ligand **192**.

Glycosylation of halides in the presence of heavy metal salts is one of the oldest glycosylation methods, known as the Koenigs-Knorr reaction, with this reaction first being described in 1901.³¹² Activation of the bromide donor **185** was carried out using halophilic Ag_2CO_3 and 4-nitrophenol donor (4-NP) in anhydrous CH_3CN in the presence of 4 Å molecular sieves to produce the expected 1,2-*trans* glycoside **186** in 74% yield. The conversion to β -linked **186** was confirmed by the larger H1-H2 vicinal coupling constant of 7.4 Hz. This desired stereoselectivity is achieved through the NGP of the C-2 O-acyl group, as illustrated in Chapter 1, Figure 1.7. An extremely common side reaction observed in glycosylation reactions is the hydrolysis of the glycosyl donor to form the corresponding hemiacetal.³¹³ This can be due to the presence of water in the solvent or from water generated during the reaction. This side product can be significantly diminished through the use of flame-dried reaction vessels, anhydrous solvents, and water scavenging agents such as molecular sieves.

The next step in this synthetic strategy was the reduction of the nitro group in **186** to the corresponding amine, which proved to be more difficult than expected. Initially, standard catalytic hydrogenation conditions of Pd/C (0.1 eq.) under an atmosphere of hydrogen in a CH₂Cl₂:CH₃OH (6:4) mixture was employed. However, these reduction conditions did not produce **187** at all. Attempts to reduce the nitro group of **186** included performing the hydrogenation in different solvents (THF, EtOAc, and EtOH) and increasing the pressure of hydrogen. However, these approaches did not produce the desired product **187** in appreciable yields. Interestingly, removal of the *O*-acetyl groups from **186**, using NaOCH₃ in CH₃OH, prior to hydrogenation, according to a reported literature procedure, gave the reduced amine **194** in 93%, as depicted in Scheme 4.2.³¹⁴ However, this process was not useful as the carbohydrate hydroxyl groups required protection to continue with the planned synthesis.



Scheme 4.2: Successful reduction of the nitro group on the deprotected glucosyl compound.

Gratifyingly, the use of Pearlman's catalyst, Pd(OH)₂, circumvented the problem. The addition of Pd(OH)₂ (0.1 eq.) to **186** in a mixture of CH₂Cl₂:CH₃OH (6:4) under an atmosphere of hydrogen for 4 h gave with the desired amine **187** in a good yield of 90% after filtration of the catalyst. Pd(OH)₂ has the added advantage of being a non-pyrophoric catalyst, compared to Pd/C. It is noteworthy that removal of the solvent *in vacuo* had to be carried out at the low rotary evaporator bath temperature of 25 °C, as removal of the solvent at higher temperatures led to a complex mixture of products which could not be separated. The desired amine **187** appears to be unstable at rt and should be used straight away or stored under argon in the freezer to prevent degradation. With the desired amine in hand, the amide functionality was introduced through the use of chloroacetic anhydride and NEt₃ in anhydrous CH₂Cl₂ to give **188** in 66%, according to a procedure reported by Roy *et. al.*³¹⁵ This α -chloroamide moiety provides a link for the

attachment to the cyclen macrocycle. The amide NH was subsequently methylated using potassium *t*-butoxide (*t*-BuOK) and iodomethane (CH₃I) in anhydrous THF for 3 h to furnish **189**.³¹⁶ The requirement for amide NH methylation will be discussed in Section 4.4.1.

The selective one step mono-alkylation of cyclen using a range of alkyl halides has been reported by Gunnlaugsson and co-workers.³¹⁷ Therefore, using the same procedure, the selective mono-alkylation of cyclen was achieved using **189** and NEt₃ in refluxing CHCl₃ for 16 h to give **190** in good yield without the need for chromatographic purification. The ¹H NMR spectrum of **190** (Figure 4.6) shows the product of the mono-alkylation step with only one carbohydrate moiety attached to the cyclen macrocycle. This was further confirmed through HRMS displaying a *m/z* peak at 666.3356, corresponding to the [190+H]⁺ species. The reaction required a four-fold excess of the cyclen however, any unreacted cyclen can be recovered using an aqueous extraction and re-used which is convenient considering the commercial cyclen is expensive.

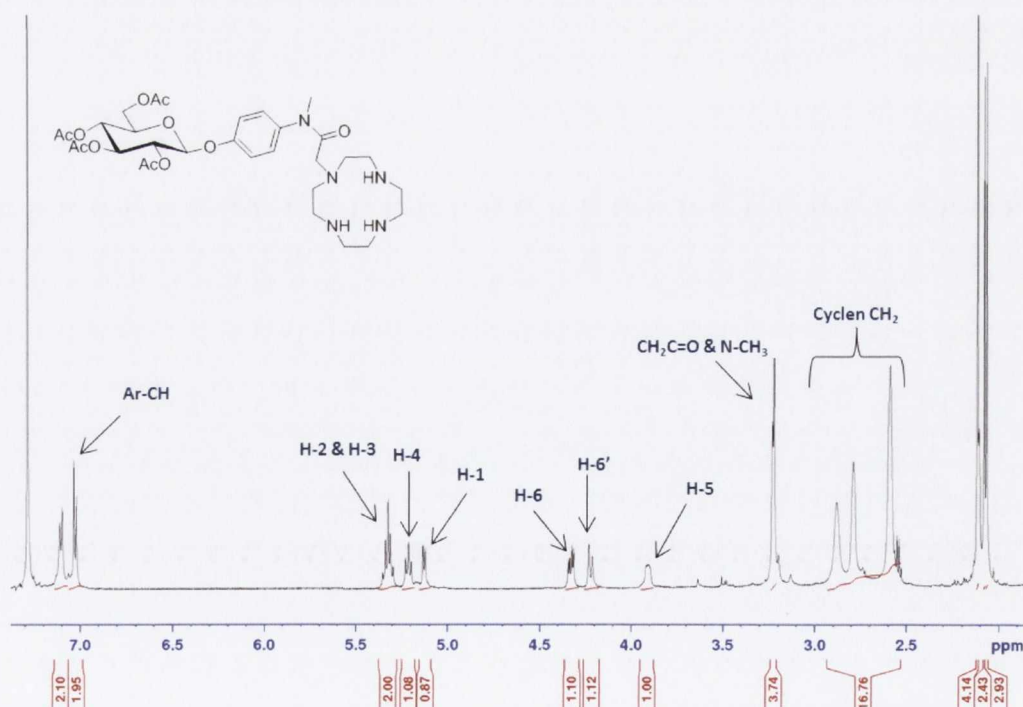
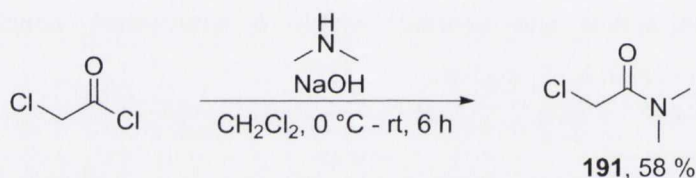


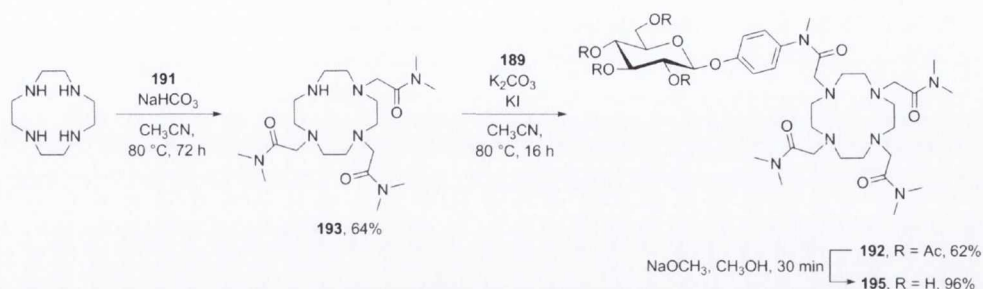
Figure 4.6: The ¹H NMR spectrum (600 MHz, CDCl₃) of the monoalkylated species **190**. The presence of only one glycan functionality on the cyclen macrocycle is evident from the integrals of the highlighted peaks. For example, the anomeric proton H-1:cyclen CH₂ ratio is 1:16.

Having obtained the monoalkylated **190**, the remaining amine sites of cyclen required alkylation using the *N,N*-dimethylamide pendant arms. Accordingly, α -chloroamide **191** was prepared, using Schotten-Baumann conditions, by the addition of chloroacetyl chloride to dimethylamine in CH_2Cl_2 in the presence of excess base at 0 °C (Scheme 4.3).³¹⁸ The use of excess base was used to drive the reaction to the formation of the amide as acylation of amines with acid chlorides produces 1.0 eq. of acid, which can form a salt with the unreacted amine and diminish the yield. Such α -chloroamides are valuable synthons widely used in the synthesis of octa-coordinated Ln(III) complexes, where they serve as stable pendant arms coordinated to the Ln(III) ion. With **191** in hand, the monoalkylated cyclen **190** was functionalised by refluxing it in CH_3CN with 3.2 eq. of **191**, K_2CO_3 and KI to give **192**, albeit in a disappointing yield of 40%.



Scheme 4.3: Schotten-Baumann conditions for the preparation of pendant arm **191**.

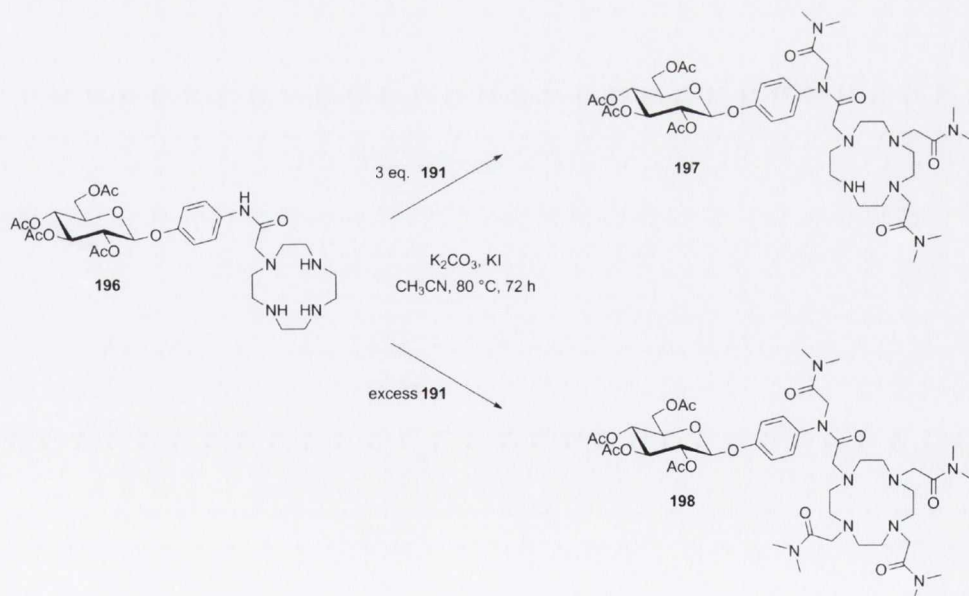
In an attempt to increase the yield of **192**, an alternative route was employed whereby *tris N*-alkylated cyclen **193** was used (Scheme 4.4). Regioselective *tris N*-alkylation of cyclen was achieved by reaction of cyclen with 3.0 eq. of **191** in the presence of NaHCO_3 to produce **193** in a good yield of 64% after alumina column chromatography. Fortunately, the reaction of **189** with **193** gave the desired *O*-acetyl protected ligand **192** in a more satisfactory yield of 62%. The final step involved the removal of the carbohydrate *O*-acetyl protecting groups from **192** under Zémlen conditions to give the final ligand **196** in close to quantitative yield.



Scheme 4.4: Regioselective *tris* *N*-alkylation of cyclen to give **193** followed by reaction with **189** to give the final β-D-glucose ligand **192** which was then deprotected using Zémlen conditions to yield **195**.

4.4.1 Unexpected alkylation reaction

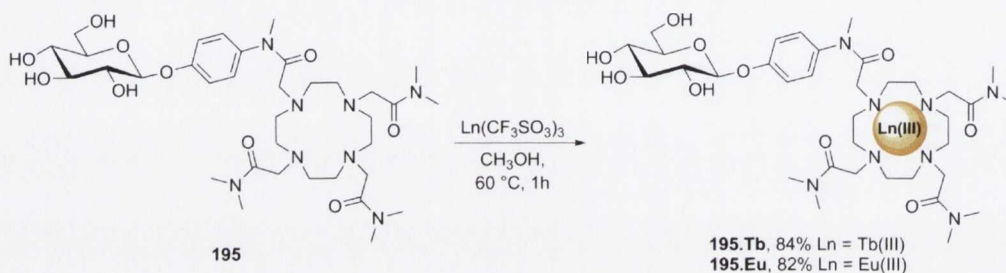
As shown in Scheme 4.5, the methylation of the amide NH in compound **188** proved necessary as reaction of **196** with α-chloroamide **191** produced **197**, where the glycosyl amide was alkylated rather than cyclen amine. Due to the HRMS of **197** and the desired product **198** being the same, confirmation of the NH alkylation was required. This was verified by the reaction of **196** with excess **191** (4.0 eq.) which produced the overalkylated product **198** (Scheme 4.5, bottom). This was confirmed through HRMS displaying a m/z peak at 992.5329, corresponding to the $[\mathbf{198}+\text{H}]^+$ product. Methylation of the NH amide using previously described conditions of CH_3I and *t*-BuOK in THF circumvented the problem.



Scheme 4.5: (Top) Unexpected alkylation product **197** observed upon reaction of **196** with **191**; (Bottom) Overalkylation of **196** using excess **191** to give **198**, which confirmed alkylation at the amide NH.

4.5 Synthesis and characterisation of β -D-glucose Ln(III) complexes

The synthesis of the glucose appended Ln(III) complexes was achieved by heating **195** with 1.0 eq. of $\text{Tb}(\text{CF}_3\text{SO}_3)_3$ or $\text{Eu}(\text{CF}_3\text{SO}_3)_3$ in CH_3OH under microwave conditions at 60 °C for 1 h to give **195.Tb** or **195.Eu**, respectively (Scheme 4.6). Upon completion of the reaction, the solvent was removed *in vacuo* and the residue re-dissolved in minimal CH_3OH (*ca.* 2 mL) and dropped into a large volume of diethyl ether (Et_2O) to give the complexes as fine powders after centrifugation.



Scheme 4.6: Synthesis of **195.Tb** and **195.Eu** by complexation of ligand **195** with $\text{Tb}(\text{CF}_3\text{SO}_3)_3$ and $\text{Eu}(\text{CF}_3\text{SO}_3)_3$, respectively, under microwave conditions.

Verification of the successful Ln(III) complexation was provided by ^1H NMR, HRMS, and IR spectroscopy. The Tb(III) and Eu(III) ions are paramagnetic thus the protons in close proximity to the metal centre are influenced by the metals unpaired f electrons, accelerating the relaxation of these protons. This relaxation causes line broadening, as observed in the case of **195.Tb**, Figure 4.7.³¹⁹ The ^1H NMR spectrum of **195.Tb** displays the characteristic broadening and shifting of signals over a large ppm range, verifying the successful complexation of the Tb(III) ion within the cyclen macrocyclic cavity.

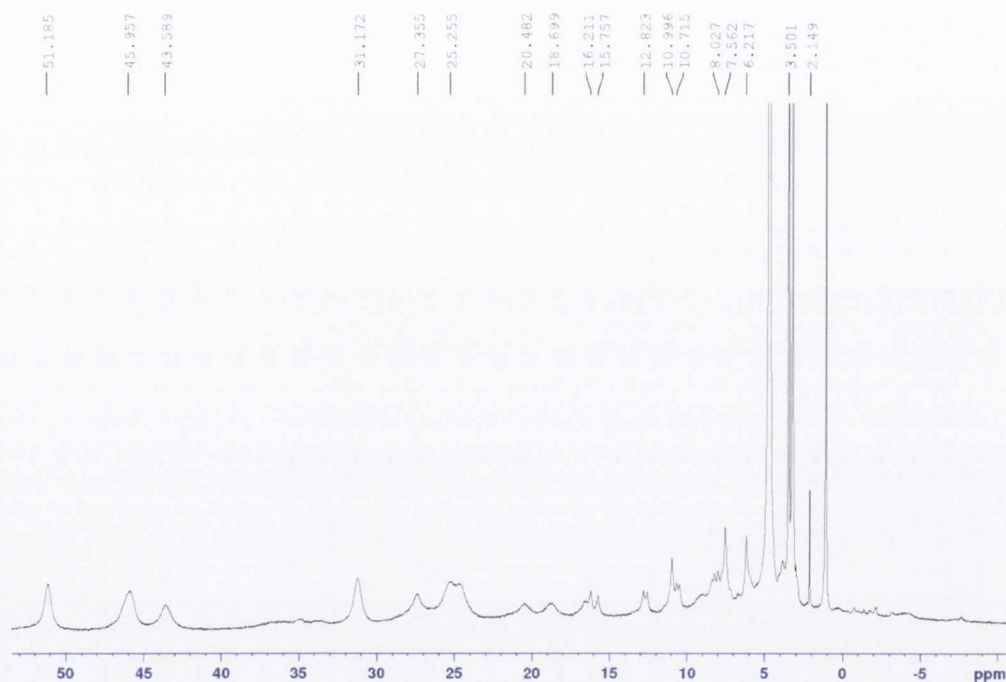


Figure 4.7: The ^1H NMR spectrum (400 MHz, D_2O) of complex **195.Tb**.

IR spectroscopy is another useful tool which can be used to confirm the successful metal complexation. For example, in the case of **195.Tb**, the IR stretching frequency of the carbonyl bands shifted from 1751 cm^{-1} to 1734 cm^{-1} upon Tb(III) complexation. This change to a lower wavenumber can be attributed to the lengthening of the carbonyl bonds, which is induced by coordination to the Tb(III) ion.³²⁰ Further confirmation for the formation of the desired Ln(III) complexes was provided by HRMS analysis (MALDI+). As depicted in Figure 4.8, the experimental isotopic distribution pattern for **195.Tb** and **195.Eu** were found to match that of the calculated formula $[\text{M}+2\text{CF}_3\text{SO}_3]^+$, where $\text{M} = \text{195.Tb}$ and $\text{M} = \text{195.Eu}$. Having successfully synthesised and characterised **195.Tb** and **195.Eu**, it was found that **195.Tb** was significantly more luminescent than **195.Eu**, the details of which will be discussed in more detail in Section 4.9. Accordingly, it was decided that future prepared complexes would only incorporate Tb(III).

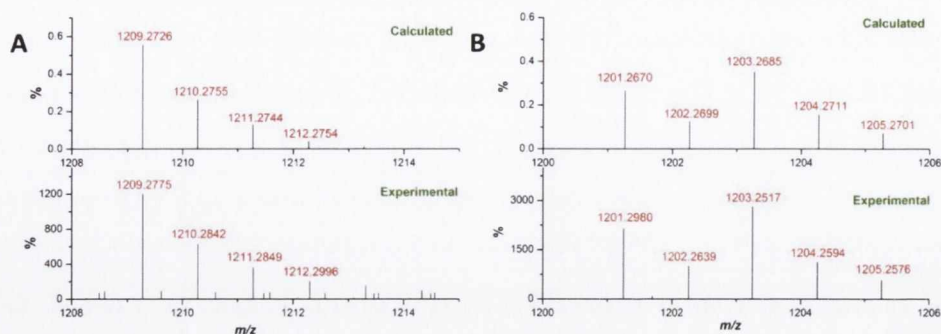
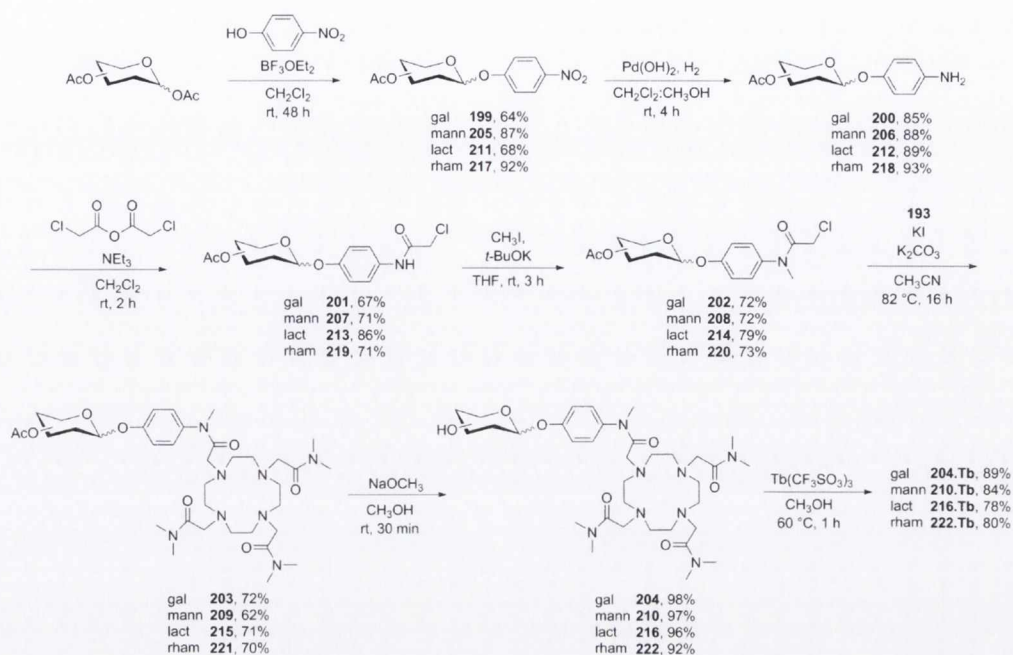


Figure 4.8: (A) Experimental and calculated HRMS (MALDI+) spectra of $[195.\text{Tb}][2\text{CF}_3\text{SO}_3]^+$. Calculated for $\text{C}_{37}\text{H}_{60}\text{N}_8\text{O}_{16}\text{F}_6\text{S}_2\text{Tb}$ $m/z = 1209.2726$ $[\text{M}+2\text{CF}_3\text{SO}_3]^+$. Found $m/z = 1209.2775$; (B) Experimental and calculated HRMS (MALDI+) spectra of $[195.\text{Eu}][2\text{CF}_3\text{SO}_3]^+$. Calculated for $\text{C}_{37}\text{H}_{60}\text{N}_8\text{O}_{16}\text{F}_6\text{S}_2\text{Eu}$ $m/z = 1203.2685$ $[\text{M}+2\text{CF}_3\text{SO}_3]^+$. Found $m/z = 1203.2698$.

4.6 Glycosylated Tb(III) complexes prepared

A small library of glycosylated Tb(III) complexes were prepared, the synthesis of which is depicted in Scheme 4.7. This is the final route used to prepare **195.Tb**, discussed in detail previously, apart from one deviation which was the use of a different donor for the initial glycosylation reaction. In the case of the glucose appended **195.Tb**, the bromide donor was used due to its ease of synthesis and facile activation. However, to avoid the use of heavy metal salts and the expense of the Ag_2CO_3 activator, a different approach was used in the preparation of **204.Tb** – **222.Tb**. This involved an initial glycosylation reaction employing a peracetylated glycosyl donor, rather than a glycosyl bromide. This *O*-acetyl donor can be activated using an excess of the lewis acid $\text{BF}_3 \cdot \text{OEt}_2$ and 4-NP in anhydrous CH_2Cl_2 . The excess 4-NP was removed using basic aqueous washes (3 x 1M NaOH) to give the crude glycosides **199** – **217**, which were recrystallised from EtOAc/Hex and used without further purification. This alternative donor strategy was successfully applied for the synthesis of all 4-NP glycoside intermediates, with the remaining steps identical to those used for the synthesis of **195.Tb**.



Scheme 4.7: General synthetic scheme for the synthesis of *O*-glycosylated complexes **204.Tb** – **222.Tb**.

Glycoconjugates of the D-sugars: glucose **195.Tb**, galactose **204.Tb**, mannose **210.Tb**, and disaccharide lactose **216.Tb** were synthesised. A single example of an L-sugar, rhamnose, **222.Tb** was also prepared. The complex **192.Tb** bearing *O*-acetyl carbohydrate protecting groups was also synthesised to allow for the effect of the free hydroxyl groups of the carbohydrate moiety on the Tb(III) luminescence to be investigated.

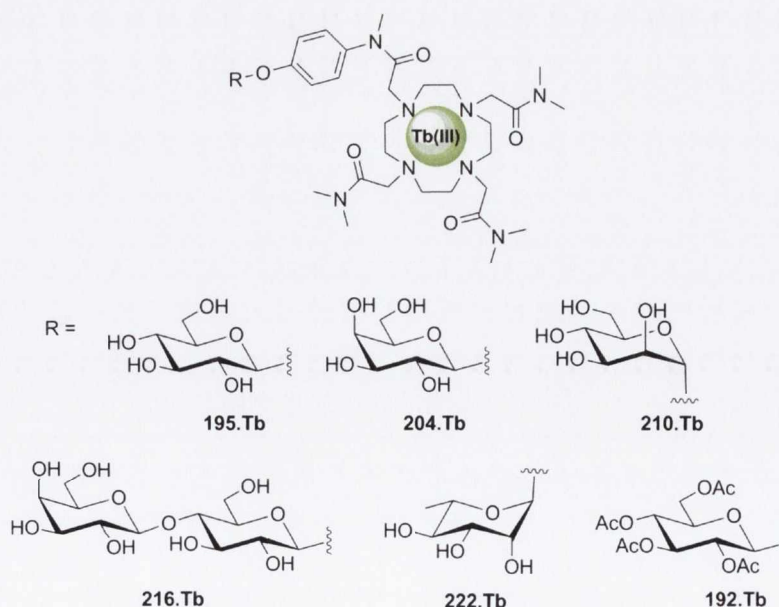
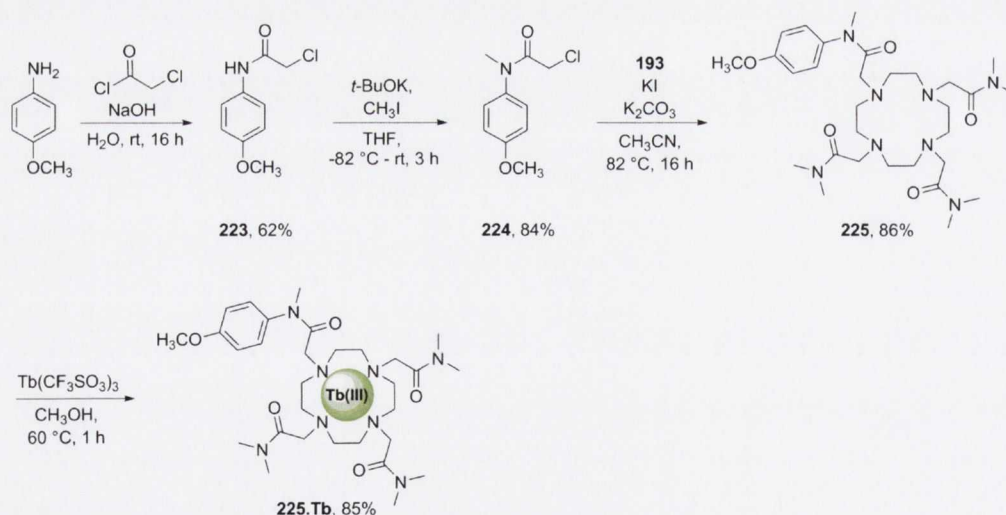


Figure 4.9: The small library of *O*-glycosylated Tb(III) complexes prepared.

Next, the synthesis of a methyl ether complex and phenolic Tb(III) complex was undertaken, which serve as the control and the product of the enzymatic reaction, respectively. Both of these complexes were prepared through related synthetic pathways and therefore will only be discussed briefly.

4.7 Synthesis of control complex

The methyl ether complex was prepared to serve as the control complex for any enzymatic experiments. This complex contains an ether linkage but does not contain a carbohydrate moiety therefore should not be recognised by any glycosidase enzyme. The synthesis is depicted in Scheme 4.8. The α -chloroamide **223** was formed from the reaction of *p*-anisidine with chloroacetyl chloride and NaOH. This was subsequently methylated with CH₃I and *t*-BuOK, using the same procedure as before, to give **224** in 84% yield. This was then introduced onto the cyclen framework by reaction of **224** with *tris* *N*-alkylated cyclen **193** to furnish **225** in a good yield of 86%. The Tb(III) metal was then introduced by reacting **225** with Tb(CF₃SO₃)₃ in CH₃OH under microwave heating at 60 °C for 1 h to furnish **225.Tb**.

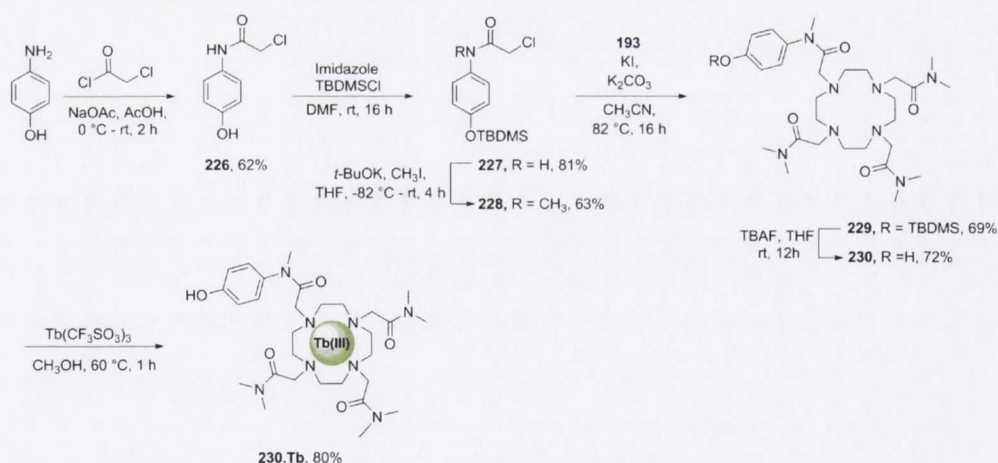


Scheme 4.8: Synthesis of control methyl ether complex **225.Tb**.

4.8 Synthesis of phenolic complex

The phenolic complex is identical to the product of the enzymatic reaction between the *O*-glycosylated Tb(III) complexes and the corresponding glycosidase enzyme. Therefore, it was prepared in order to compare its photophysical properties to that of the hydrolysis products of subsequent

enzymatic experiments. Initially, the phenolic ligand (shown in Scheme 4.9) was to be obtained from ligand **225** by demethylation of the methoxy group upon treatment with bromotribromide (BBr_3), as reported in the literature.³²¹ However, multiple attempts yielded no success with the methoxy functionality remaining unaffected, even with a large excess of BBr_3 . Alternative conditions for the demethylation using trimethylsilyl iodide (TMSI) were also unsuccessful.³²² Both approaches were most likely unsuccessful due to the presence of many oxygen atoms to which the lewis acids (BBr_3 and TMSI) can coordinate, hindering dealkylation.³²³ Therefore, an alternative route to the phenolic ligand was undertaken, which is depicted in Scheme 4.9. Starting from *p*-amino phenol, this was reacted with chloroacetyl chloride to give **226**, according to a literature procedure.³²⁴ The phenol moiety was then protected with a silyl protecting group (TBDMS) using TBDMSCl and imidazole in DMF to give **227** in 81% yield. Compound **227** was subsequently methylated, as before, to give **228**. Compound **228** was then reacted with *tris* *N*-alkylated cyclen **193** to furnish **229** in of 69% yield.



Scheme 4.9: Synthesis of phenolic Tb(III) complex **230.Tb**.

The TBDMS protecting group was subsequently removed using 1M TBAF solution in THF to give **230** in 72% after alumina column chromatography. The use of TBAF is not ideal for products with water solubility, as is the case with **230**. The removal of the excess TBAF and the HF generated in the reaction requires an aqueous work up, therefore an alternative work up was required, due to the water solubility of compound **230**. A convenient solution

to this problem was reported by Kishi and co-workers which eliminates the need for an aqueous work up.³²⁵ Their approach is depicted in Figure 4.10 and was applied in the work up of **230** after TBDMS removal. When the removal of the silyl protecting group was completed, as indicated by TLC, calcium carbonate (CaCO_3), a sulfonic acid resin (DOWEX-H^+) and CH_3OH were added to the THF solution and the suspension was stirred for 1 h at rt. The excess TBAF reacts with the DOWEX-H^+ resin while the CaCO_3 serves to sequester the liberated HF forming CaF_2 . The insoluble material (resin and CaF_2) can then be removed by simple filtration and the *t*-butyldimethylsilyl fluoride removed by evaporation. This allowed **230** to be obtained in good yield and obviated the need for an aqueous work up. With **230** in hand, it was then complexed with $\text{Tb}(\text{CF}_3\text{SO}_3)_3$ to give **230.Tb** in 80% yield.

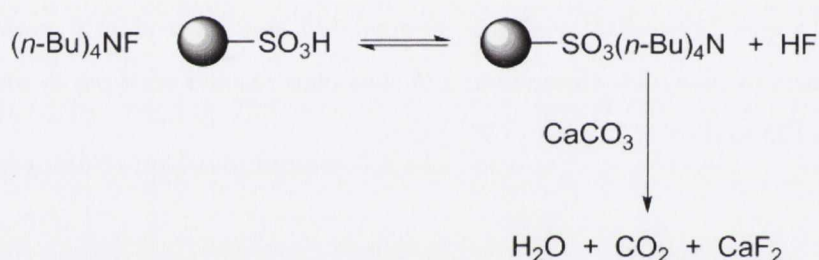


Figure 4.10: Reaction of TBAF with sulfonic acid resin (DOWEX-H^+) in the presence of CaCO_3 , reported by Kishi and co-workers.³²⁵ The excess TBAF reacts with the DOWEX-H^+ to form tetra-*n*-butylammonium sulfonic resin and HF. The equilibrium can be shifted to the right side by the addition of CaCO_3 which reacts with HF to form H_2O , CO_2 and insoluble CaF_2 . The resin and CaF_2 can be removed by filtration.

4.9 Photophysical evaluation of Ln(III) complexes

Having successfully synthesised and characterised the desired *O*-glycosylated, phenol, and methoxy complexes, the photophysical properties of these complexes were next evaluated.

The remainder of this chapter will focus on the photophysics of the prepared complexes and will therefore present the UV-vis spectra, phosphorescence spectra and excited state lifetimes of the Ln(III) complexes. All the following experiments were carried out at 37 °C (310 K) using a thermostat since this is the normal human body temperature, and thus provides insight into the bioapplicability of such probes. However, initial measurements were conducted in duplicate at 37 °C and the lower temperature of 22 °C and no modulation was observed between the two, indicating that such temperature variation had

no effect on the emission of the Ln(III) complexes in solution. Furthermore, all measurements were conducted in aqueous buffered solution (100 mM Tris-HCl, pH = 7.4), unless otherwise stated. For clarity, discussion will focus primarily on the glucose-appended complex as all the O-glycosylated Ln(III) complexes displayed similar photophysical characteristics. As shown in Figure 4.11, the UV-vis spectrum of **195.Tb** at a concentration of 50 μM showed one broad $\pi-\pi^*$ absorption band centred at 273 nm.

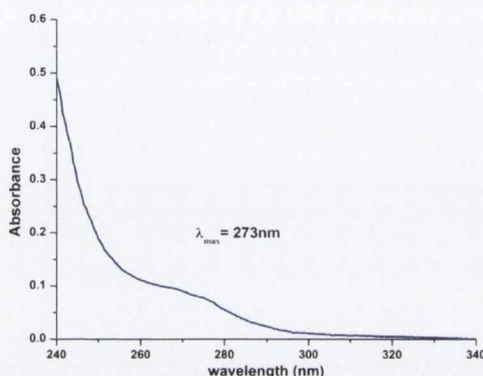


Figure 4.11: The UV-vis absorption spectrum of **195.Tb** (50 μM) recorded in aqueous buffered solution (100 mM Tris-HCl, pH = 7.4) at 37 °C. The λ_{max} at 273 nm is highlighted.

The design of the Ln(III) complexes anticipated the indirect excitation of the Ln(III) ion by the covalently attached phenyl antenna. It was expected that successful energy transfer from the antennas triplet excited state to the $^5\text{D}_4$ and $^5\text{D}_0$ excited states of the Tb(III) and Eu(III) ions, respectively, would occur. The phosphorescence emission spectra of **195.Tb** and **195.Eu** were recorded by exciting at 273 nm (λ_{max} of phenyl antenna). The resulting characteristic line-like emission bands are shown in Figure 4.12. These involve transitions from the $^5\text{D}_0 \rightarrow ^7\text{F}_J$ ($J = 0, 1, 2, 3$, and 4) for Eu(III) and $^5\text{D}_4 \rightarrow ^7\text{F}_J$ ($J = 6, 5, 4$, and 3) for Tb(III), verifying successful energy transfer from the antenna to the lanthanide excited state.

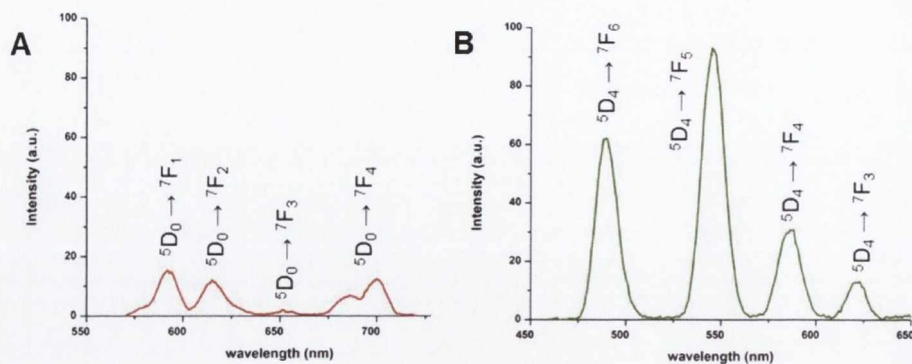


Figure 4.12: Lanthanide luminescence spectra of (A) **195.Eu** (red) (50 μ M) and (B) **195.Tb** (green) (50 μ M) recorded in aqueous buffered solution (100 mM Tris-HCl, pH = 7.4) using indirect excitation at 273 nm at 37 $^{\circ}$ C. The deactivation from the 5D_0 excited state and the 5D_4 excited state to the 7F_J state for **195.Eu** and **195.Tb**, respectively, is depicted.

As illustrated in Figure 4.12, the Ln(III) centred emission is much more efficient in the case of **195.Tb**, compared with **195.Eu**. As discussed in Section 4.2.1, efficient energy transfer occurs when the triplet excited state of the antenna is close to the excited state of the Ln(III) ion. However, when the energy of the feeding state is too close to that of the emitting state, back energy transfer can occur, reducing the efficiency of the intramolecular energy transfer process. The 5D_4 excited state of Tb(III) lies at 20,430 cm^{-1} , while the 5D_0 excited state of Eu(III) lies at a lower level of 17,250 cm^{-1} .²⁹³ Therefore, the weak Eu(III) emission of **195.Eu** could be a result of the larger energy gap between the Eu(III) excited state and the triplet state of the antenna. This larger energy gap would result in less efficient sensitisation of the Eu(III) ion resulting in reduced emission as seen in the case of **195.Eu**. Due to the sensitisation of the Tb(III) analogue **195.Tb** being more efficient, it was decided that further studies with **195.Eu** be discontinued and to perform the remainder measurements on **195.Tb**. Therefore, all measurements and discussion for the remainder of this chapter will be limited to Tb(III) complexes exclusively.

4.9.1 Determination of metal bound water molecules

As discussed, the Ln(III) ions possess high coordination numbers, with the most common coordination number of the Tb(III) ion being 9. Therefore, as described in the design of the glycosidase probe (Section 4.3), 8 of the coordination sites are fulfilled through the ligand design, 4 from the cyclen

nitrogen atoms and 4 from the carbonyl groups of the amides. It was therefore expected that the ninth coordination site would be occupied by a single metal coordinated water molecule, as illustrated in Figure 4.13. The technique employed to determine the hydration state of the Tb(III) ion was to measure the excited state lifetimes of the Tb(III) ion in H₂O ($\tau_{\text{O-H}}$) and D₂O ($\tau_{\text{O-D}}$) by indirect excitation of the Tb(III) ion at 273 nm. As discussed in Section 4.2.2, the main quenching mechanism of Ln(III) luminescence involves vibrational energy transfer to coordinated or diffusing O-H oscillators. Therefore, replacement of the O-H oscillators with O-D oscillators gives rise to longer excited state lifetimes in D₂O.¹⁹² Lifetimes of **195.Tb** were obtained by recording the exponential decay of Tb(III)-centred luminescence with respect to time. The data was best fit to a mono-exponential decay, giving lifetime values of $\tau_{\text{O-H}} = 1.54$ ms and $\tau_{\text{O-D}} = 2.19$ ms and using Equation 4.2 (Section 4.2.2), a q value of 0.66 ± 0.5 was calculated, as shown in Table 4.1. The hydration of **195.Tb** was therefore ascertained to be 1 and thus verified that **195.Tb** was 9 coordinate.

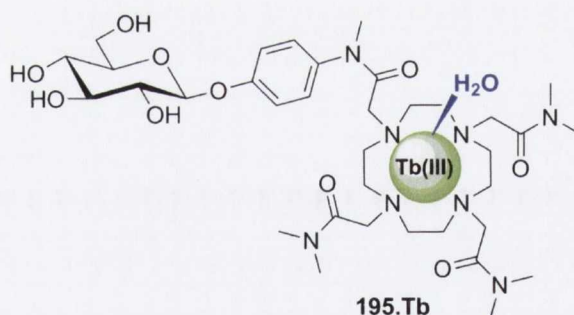


Figure 4.13: Glucose Tb(III) complex **195.Tb** with a single bound water molecule.

Table 4.1: The measured lifetimes and corresponding hydration states (q) of the Tb(III) complexes **195.Tb**, **225.Tb**, and **230.Tb** at concentrations of 50 μM . Luminescence decay was measured at 545 nm. Each value is an average of 3 measurements. *10% CH₃OH and CD₃OD was added to the H₂O and D₂O solutions, respectively, to aid solubility.

Complex	$\tau_{\text{(O-H)}}$ /ms	$\tau_{\text{(O-D)}}$ /ms	q (± 0.5)
195.Tb (Glucose)	1.54 ± 0.01	2.19 ± 0.01	0.66
225.Tb* (Methoxy)	1.10 ± 0.01	1.42 ± 0.01	0.72
230.Tb (Phenol)	1.58 ± 0.04	2.24 ± 0.01	0.62

The hydration states (q) were also determined for the methoxy complex **225.Tb** and the phenolic complex **230.Tb**. The q values were found to be 0.72 ± 0.5 and 0.62 ± 0.5 for the methoxy **225.Tb** and phenolic **230.Tb**, respectively. It was therefore concluded that all these complexes had a hydration state of 1 and thus a total coordination number of 9. Due to the q value being 1 for both the glucose-appended **195.Tb** and the phenolic product **230.Tb**, no change in the hydration state of the Tb(III) metal centre was expected during the cleavage of the glycosidic bond in subsequent enzymatic experiments. Having established that excitation of the phenyl chromophore of **195.Tb** resulted in sufficient energy transfer to the 5D_4 excited state of the Tb(III) ion and that the high coordination requirements of the Tb(III) ion were fulfilled (eight sites from the ligand donor atoms and one water molecule), the next step was to investigate the response of **195.Tb** as a function of pH and in the presence of β Glc. Therefore, the following sections will firstly discuss the response of **195.Tb** as a function of pH and then its response on addition of β Glc.

4.9.2 Response of **195.Tb** as a function of pH

The glycosidase enzymes have been shown to be stable over pH range 5-8.^{326,327} Therefore, it was important that **195.Tb** remains intact and unaltered by pH changes. For this reason, the effect of pH on the response of **195.Tb** was explored to determine whether it could function unperturbed over this pH range. The effect of pH on the ground state properties of **195.Tb** was firstly investigated with the UV-vis absorption spectra of **195.Tb** (50 μ M) recorded in water as a function of pH. As depicted in Figure 4.14A, no real changes were observed in the absorption spectra over the pH range 2-12. The luminescence response of **195.Tb** as a function of pH was performed in parallel with the above UV-vis absorption measurements. Similarly, as shown in Figure 4.14B, no significant changes were observed in the luminescence properties of **195.Tb**. However, a slight decrease in the emission intensity was observed at the more alkaline pH of 8. Overall, the absorption and luminescence response of **195.Tb** remains the same, indicating that **195.Tb** is stable over this pH range.

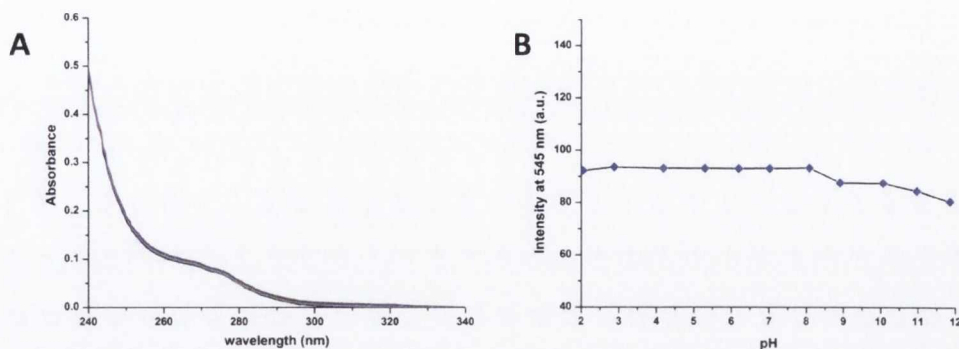


Figure 4.14: Effect of pH on the response of **195.Tb** (50 μ M) in water at 37 $^{\circ}$ C. (A) The UV-vis spectra of **195.Tb**; (B) Luminescence response of **195.Tb** at 545 nm.

4.10 Response of **195.Tb** in the presence of β Glc

We next moved on to investigate the Tb(III) luminescence response in the presence of commercially available β Glc enzyme. Unfortunately, the addition of varying amounts of β Glc (0.1 U to 2 U) to **195.Tb** (50 μ M) resulted in no significant changes in the Tb(III) emission being evidenced. The glycosidic bond was successfully cleaved by the glycosidase enzyme as judged by HRMS analysis which showed the presence of a peak at $m/z = 1047.2245$ corresponding to $[\mathbf{230.Tb} + 2\text{CF}_3\text{SO}_3]^+$ species. However, as illustrated in Figure 4.15, comparing the emission of **195.Tb** (left) and **230.Tb** (right), it is clear that the emission of the substrate and the hydrolysis product are of equal intensity. This equal intensity in the Tb(III) metal centred emission therefore indicates that these conditions were not suitable for monitoring the action of the β Glc enzyme. Despite the glycosidic bond being cleaved, this lack of change in Tb(III) emission is most likely due to the energy transfer from the phenyl antenna to the Tb(III) excited state not being perturbed by the cleavage of the glycosidic bond in the manner anticipated. Because of this setback, the next step was to study the effect of varying the concentration of the Tb(III) complex to determine whether **195.Tb** could be used to monitor glycosidase activity. The following sections will deal with the luminescence response of **195.Tb** at higher concentrations.

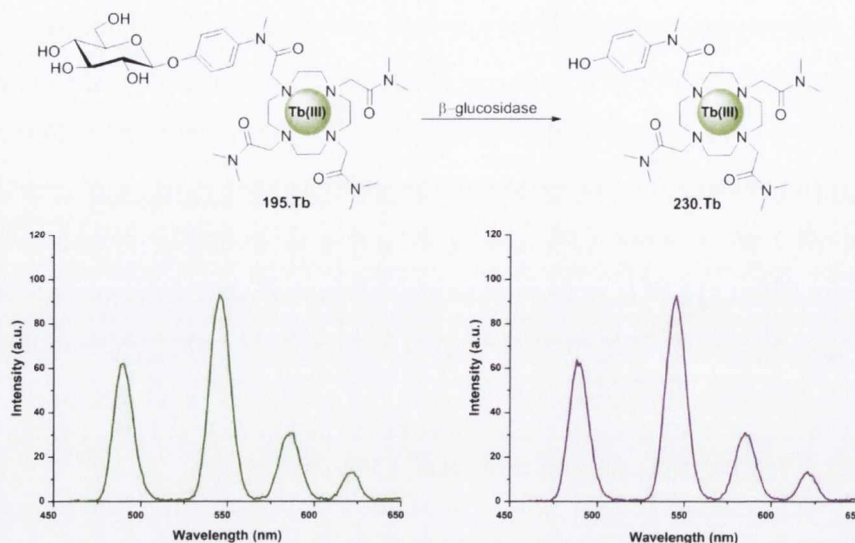


Figure 4.15: Lanthanide luminescence spectra of **195.Tb** (green) and **230.Tb** (purple) and associated structures at a concentration of 50 μ M in aqueous buffered solution (100 mM Tris-HCl, pH 7.4) using indirect excitation at 273 nm at 37 $^{\circ}$ C.

4.10.1 Enzymatic experiments conducted at high concentrations

Different concentrations of **195.Tb** (from 50 μ M to 700 μ M) were screened to investigate whether enzyme activity could be detected in some way using **195.Tb**. No change in the Tb(III) emission was observed over this substrate concentration range. Interestingly, at the higher substrate concentration of 1 mM, it was observed that the emission of **195.Tb** was significantly quenched, when compared to the phenolic **230.Tb**, as depicted in Figure 4.16. It was thought that at the higher concentration of 1 mM, the free hydroxyl groups (O-H oscillators) of the α -D-glucose residue quench the Tb(III) luminescence, although the glycan did not appear to coordinate to the Tb(III) ion as the initial hydration state, q , remained at 1. To confirm that the quenching of the Tb(III) emission was due to the free hydroxyl groups of the carbohydrate, the luminescence of the *O*-acetyl protected derivative **192.Tb** was measured (Figure 4.16, black). Indeed, the luminescence of **195.Tb** was quenched compared to the protected derivative **192.Tb**, which implied that the hydroxyl groups of the carbohydrate quenched the Tb(III) luminescence. Similar quenching effects were observed by Plush and co-workers.³²⁸ They prepared various cyclen-based Eu(III) complexes with an α -D-mannose motif and observed that the complexes containing deprotected carbohydrate groups exhibited reduced emission intensity when compared with *O*-acetyl protected

derivatives. They attributed this reduced emission to the quenching effects of the free hydroxyl groups of the deprotected mannose motif.

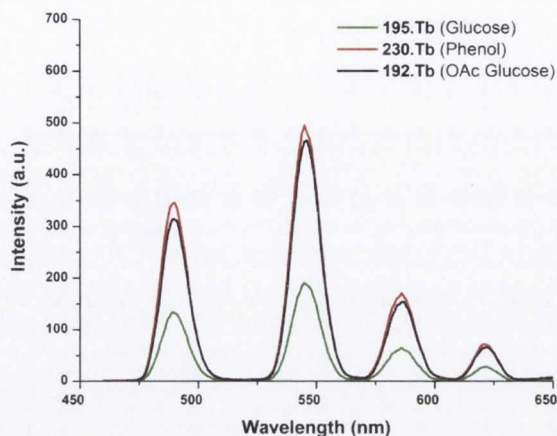


Figure 4.16: Lanthanide luminescence spectra of **195.Tb** (green), **230.Tb** (red), and ***192.Tb** (black) at a concentration of 1 mM in aqueous buffered solution (100 mM Tris-HCl, pH 7.4) using indirect excitation at 273 nm. *10% EtOH was added to **192.Tb** to aid solubility.

With this quenching of luminescence observed at the higher concentration of 1 mM, it was expected that upon addition of β Glc and the concomitant formation of the phenolic product **230.Tb**, there would be an increase in the Tb(III) emission, resulting in a “switch on” effect. Indeed, upon the addition of β Glc (0.1 U) to **195.Tb** at a concentration of 1 mM, a steady increase in the Tb(III)-centred luminescence was observed, with the Tb(III) emission being enhanced by *ca.* 700% (as shown in Figure 4.17A).

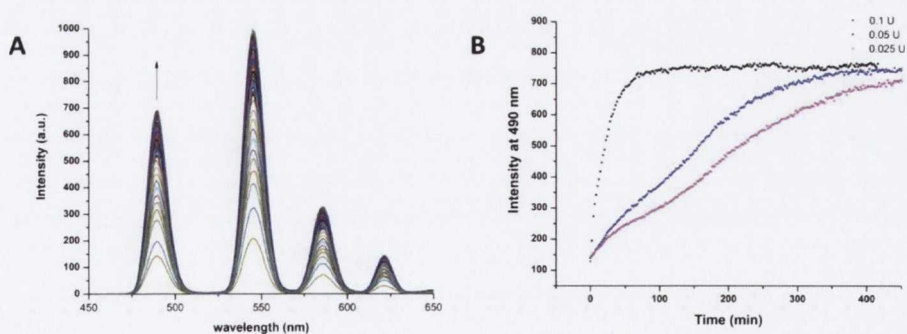


Figure 4.17: (A) Changes in the Tb(III) luminescence of **195.Tb** (1 mM) in the presence of 0.1 U β Glc; (B) Changes in the Tb(III) luminescence of **195.Tb** (1 mM) in the presence of varying amounts of β Glc (0.025 U - 0.1 U). All experiments were carried out in aqueous buffered solution (100 mM Tris-HCl, pH = 7.4) at 37 °C using indirect excitation at 273 nm.

In a control experiment, the same amount of β Glc (0.1 U) was added to the control methoxy complex **225.Tb** and no change in the Tb(III) emission was observed. Next, varying amounts of β Glc enzyme (0.025 U – 0.1 U) were

added to **195.Tb** to probe the effect of enzyme concentration on the luminescence enhancement. As indicated in Figure 4.17B, an increase in the Tb(III) emission was observed, with the rate increasing as the amount of enzyme was increased from 0.025 U to 0.1 U.

4.10.2 Unusual behaviour observed in solution

Although these initial results were promising, unusual behaviour was observed when conducting additional experiments to confirm and further assess the previously obtained results. When monitoring the metal-centred emission of **195.Tb** in the absence of any enzyme, it was observed that the Tb(III) emission gradually increased. This behaviour was only observed for the glycosylated Tb(III) complexes, depicted in Figure 4.9. In the case of the *O*-acetyl protected **192.Tb**, this phenomenon was not observed with the emission unperturbed over time. Furthermore, this steady increase in the Tb(III) emission did not occur with the control and phenol complexes, **225.Tb** and **230.Tb**, respectively. This trend suggests that the observed behaviour in solution was due to the presence of the free hydroxyl groups of the carbohydrate moiety. Interestingly, this increase in the luminescence with time was only observed at the higher concentration of 1 mM and was not displayed by any of the complexes at the lower concentration range of 50 μ M-700 μ M. As this behaviour was only observed at the higher concentration of 1 mM, this suggests that the enhancement in Tb(III) emission is a result of an intermolecular or aggregation process that occurs in solution.

4.10.3 Determination of metal bound water molecules at high substrate concentrations

In an attempt to understand this behaviour in solution, lifetime measurements were conducted to determine the hydration state (q) of the Tb(III) ion at the higher concentration of 1 mM. As mentioned previously, the Tb(III) emission of the glycosylated complexes gradually increased when left in solution in the absence of any enzyme. Therefore, the Tb(III) complexes were left to stand in the H₂O and D₂O solutions for 1 h prior to lifetime measurements to allow for the Tb(III) emission to increase. The lifetimes and resulting q values are presented in Table 4.2.

Table 4.2: The excited state lifetimes and the corresponding hydration states (q) of the Tb(III) complexes at concentrations of 1 mM. Luminescence decay measured at 545 nm and after 1 h standing in solution, unless otherwise stated. Each value is an average of 3 measurements. ^aMeasurements taken immediately, ^b10% CH₃OH and CD₃OD added to aid solubility.

Entry	Complex	$\tau_{(O-H)}/ms$	$\tau_{(O-D)}/ms$	$q (\pm 0.5)$
1	195.Tb^a (Glucose)	0.93 ± 0.01	1.19 ± 0.04	0.89
2	195.Tb (Glucose)	1.86 ± 0.02	2.33 ± 0.01	0.24
3	204.Tb (Galactose)	1.77 ± 0.01	2.34 ± 0.01	0.39
4	210.Tb (Mannose)	1.82 ± 0.01	2.21 ± 0.02	0.18
5	216.Tb (Lactose)	1.27 ± 0.04	1.56 ± 0.01	0.44
6	222.Tb (Rhamose)	1.66 ± 0.01	2.17 ± 0.01	0.41
7	192.Tb^b (GlucoseOAc)	1.64 ± 0.01	2.03 ± 0.01	0.62

Firstly, for complex **195.Tb**, lifetimes were measured immediately with a resulting hydration state of 1 being recorded (Entry 1). However, after 1 h it is clear that the lifetimes significantly increase (Entry 2). In fact, in H₂O, the lifetime increases two-fold from 0.93 ms to 1.86 ms with the resulting q value decreasing for 1 to 0, indicating displacement of the Tb(III) bound water molecule. This increase in the recorded lifetimes was observed for all of the glycoconjugates with free hydroxyl groups, with each of them displaying a q value of 0, as shown in Table 4.2 (Entry 2-6). This change in the lifetime and Tb(III) hydration state was not observed for the *O*-acetyl protected complex **192.Tb** (Entry 7) with a q value of 1 being calculated, further suggesting that the hydroxyl groups of the carbohydrate play a key role in this process.

4.10.4 Hypothesis for the observed behaviour

Considering that the glycosylated complexes show a steady increase in the metal-centred emission over time accompanied by a decrease in the q value from 1 to 0, it is postulated that at the higher concentration of 1 mM, there is a self-assembly process in solution involving the hydroxyl groups of the carbohydrate moiety and the Tb(III) metal centre, the process of which is schematically illustrated in Figure 4.18. Initially, at the concentration of 1 mM, **195.Tb** has one metal bound water molecule and the Tb(III) centred emission is quenched by the O-H oscillators of the carbohydrate moiety. However, upon standing in solution, the free hydroxyl groups of the carbohydrate self-assemble and coordinate to the Tb(III) centre in an intermolecular fashion,

reducing the hydration state of the metal from 1 to 0 thus resulting in enhanced emission. This process was not observed with the *O*-acetyl protected **192.Tb**, methoxy **225.Tb**, or phenol **230.Tb** further illustrating the role of the free hydroxyl groups of the carbohydrate in this process. Due to this self-assembly/aggregation observed at high concentrations, it was decided to explore the use of the Tb(III) complexes for another sensing purpose, which will be discussed in the following section.

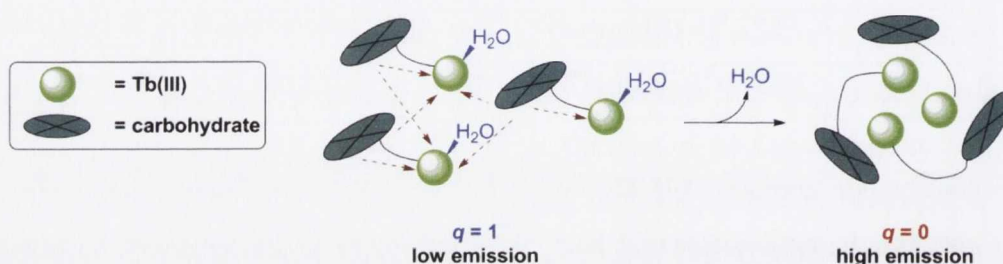


Figure 4.18: Schematic illustration of the self-assembly process that occurs in solution with the deprotected carbohydrate Tb(III) complexes at concentration of 1 mM. (*Left*) Initially, one water molecule is coordinated to the Tb(III) metal centre ($q = 1$) and the Tb(III) emission is quenched by the O-H oscillators of the carbohydrate hydroxyl groups. (*Right*) Overtime, the metal coordinated water molecule is displaced by a neighbouring carbohydrate hydroxyl group to give q value of 0 and enhanced metal centred emission.

4.11 Probes used for the detection of lectins

The detection of biological targets is of paramount importance, with the study of lectins being an active area of research. Lectins are a group of non-enzymatic proteins which contain specific carbohydrate recognising domains. Selective carbohydrate-lectin interactions are involved in a myriad of physiological processes, with lectins serving as invaluable tools for assessing cell surface changes during pathological events.^{329,52} For example, the nature and density of glycans can change depending on the cell type and critically, whether it is cancerous or healthy.³³⁰ Concanavalin A (ConA) is a well-known lectin from *Canavalia ensiformis* (jack bean) which has been extensively employed to study carbohydrate-lectin interactions. ConA exists as a tetramer in solution and is well known to bind to α -mannopyranoside at its 4 binding sites.³³¹

One phenomenon used in the study of cell biology and biological sensing is FRET. FRET is the distance-dependent energy transfer from an excited donor molecule to an acceptor molecule.³³² The donor molecule is

typically a dye which absorbs energy and subsequently transfers this energy to an acceptor; this energy transfer results in a reduction in the donor emission and excited state lifetime and an increase in the acceptors emission. Due to its sensitivity to distance, FRET has been used to investigate molecular interactions.^{333,334} Quici and co-workers reported a method of lectin sensing using the mannose-appended Tb(III) complex **230** and luminescence resonance energy transfer (LRET).³³⁵ LRET is analogous to FRET except a long-lived probe, typically a lanthanide chelate, is used as the donor. In this example, as depicted in Figure 4.19, upon binding of the mannose residue in **230** to rhodamine-B-isothiocyanate labelled ConA (RITC-ConA), there was energy transfer from the Tb(III) complex (donor) to the RITC-ConA (acceptor). This energy transfer resulted in reduced Tb(III) emission accompanied by a reduction in the excited state lifetimes from 1.75 ms to 0.46 ms, thus reporting on lectin binding.

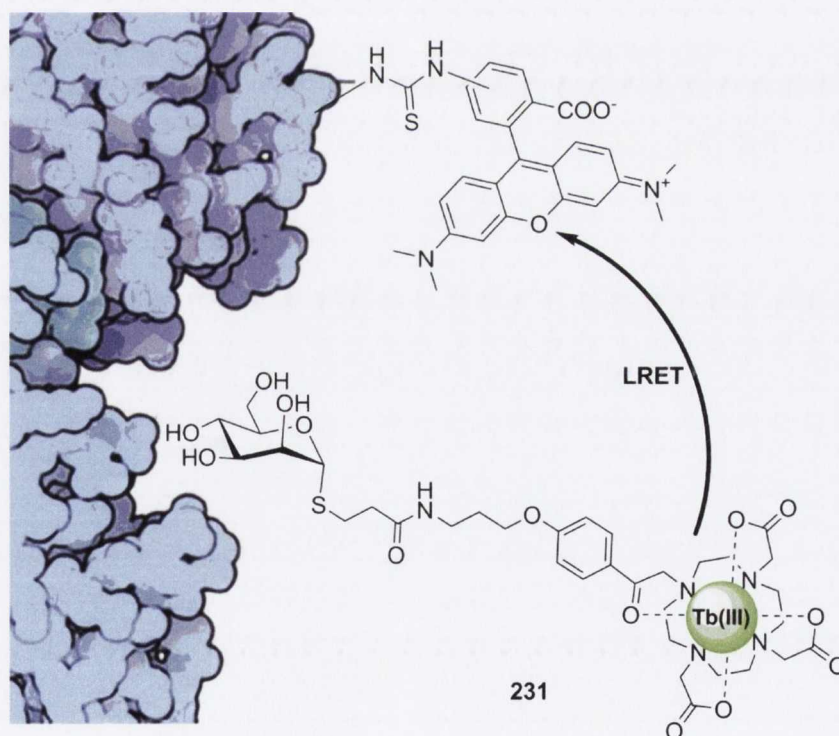


Figure 4.19: Schematic representation of LRET from Tb(III) complex **230** to RITC-ConA. (Image modified from Quici *et al.*)³³⁵ Upon the binding of the mannose residue of **230** to RITC-ConA, there is LRET from the Tb(III) (donor) to the RITC (acceptor). This results in a decrease of the Tb(III) donor emission and excited state lifetimes and an increased acceptor emission.

Many glycoconjugate probes have been reported for the detection of lectins, based on aggregation or de-aggregation events.^{336,337,338,339} These glycoconjugate probes contain specific carbohydrate moieties which can bind to lectins, and hence can be used to report such interactions through a change in their photophysical properties. Kral and co-workers reported the detection of ConA using glycosylated porphyrin structures.³³⁶ It is widely known that these porphyrins tend to form aggregates in aqueous solution through π - π stacking interactions, resulting in aggregation-caused quenching (ACQ) of fluorescence, as depicted in Figure 4.20.^{337,340} However, upon addition of ConA, disaggregation occurs due to the formation of a non-covalent complex between the carbohydrate appended to the porphyrin and the lectin. This specific interaction is accompanied by a strong increase in fluorescence, hence reporting on the lectin interaction.

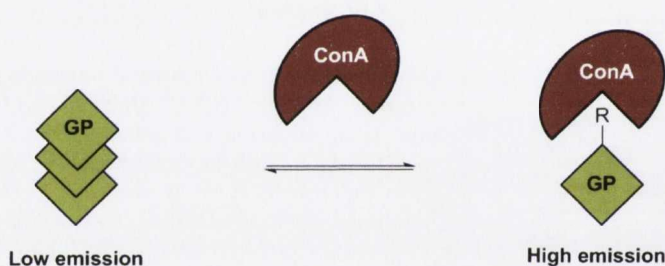


Figure 4.20: Schematic representation of de-aggregation of glycoporphyrins through ConA binding reported by Kral and co-workers, where GP denotes glycoporphyrin and R denotes the carbohydrate group.³³⁶ In aqueous solution, the GP units aggregate resulting in ACQ and low fluorescence. Upon addition of ConA, the carbohydrate appended to the porphyrin binds with the ConA causing de-aggregation of the GP units resulting in increased fluorescence.

An opposite phenomenon to ACQ was reported by Tang and co-workers, which they termed aggregation-induced emission (AIE).³⁴¹ AIE-active molecules display intense emission when in an aggregated state, with restricted intramolecular rotation thought to be the main cause of the phenomenon. The principles of AIE and AIE-active type molecules have been well reviewed and will not be discussed here in detail.^{341,342} However, this phenomenon has been used as a convenient tool for sensing purposes whereby the binding of an AIE-active compound with a biomolecule results in aggregation and subsequent emission. For example, Tanaka *et al.* reported mannose substituted tetraphenylethene (TPE) **232** as an optical probe to monitor ConA binding (Figure 4.21A).³⁴³

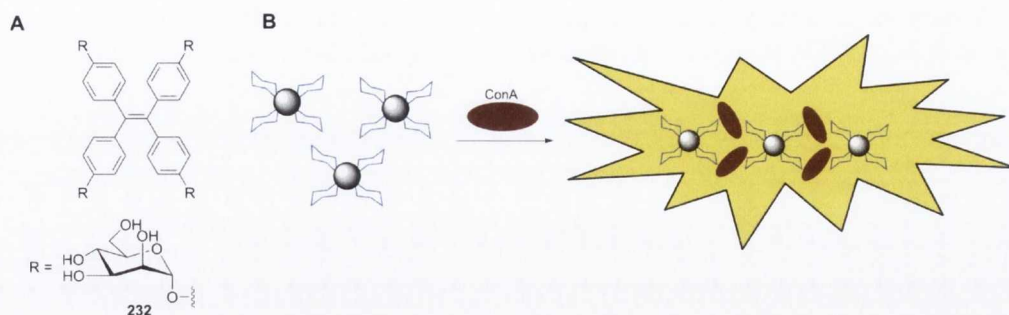


Figure 4.21: (A) TPE-based AIE probe **232** used for the detection of ConA, reported by Tanaka *et al.*,³⁴³ (B) Schematic illustration of the AIE process resulting in enhanced emission upon binding to ConA. In solution, the glycosylated TPE unit displays negligible fluorescence however, upon the addition of ConA and formation of the carbohydrate-protein ensemble, TPE rotation is restricted resulting in enhanced fluorescence.

Initially, TPE emission is “turned off” in solution as it displays negligible fluorescence. However, upon interaction with ConA, the system is “turned on” due to binding of **232** to the lectin, as depicted in Figure 4.21B. This interaction results in restricted intramolecular rotation of the TPE moiety, with the TPE fluorescence being enhanced by 150% upon lectin binding.

4.12 The use of **210.Tb** as a probe for lectin, ConA

Encouraged by the work of Kral and Tanaka and their ability to detect lectins based on de-aggregation and aggregation events, respectively, we decided to investigate whether the glycosylated Tb(III) complexes could be used to detect the presence of a lectin in solution. It was decided to investigate the response of the mannose appended complex **210.Tb** with lectin, ConA. Considering the intermolecular self-assembly/aggregation behaviour observed with the glycosylated Tb(III) complexes at 1 mM, we postulated that the addition of ConA to a solution of **210.Tb** would result in ConA specifically binding with the mannose moiety. This binding could disrupt the coordination of the carbohydrate moiety to the Tb(III) metal centre resulting in the hydration state increasing from 0 to 1 and therefore causing a “switching off” effect to be observed. To test this hypothesis and investigate the effect of ConA on the aggregation behaviour of **210.Tb** in solution, the emission and excited state lifetimes of **210.Tb** were measured after the addition of ConA, the outcome of which will be discussed in the following section. All measurements were carried out at a concentration of 1 mM.

As illustrated in Figure 4.22A, **210.Tb** (black) is highly emissive in aqueous buffered solution. Upon addition of 1.0 eq. of ConA, a drastic decrease in emission (red) was observed. To confirm this decrease in emission was due to specific binding between the mannose moiety and ConA, a sample of lectin solution was denatured by heating to 100 °C for 20 min. As shown in Figure 4.22 (blue), no drastic decrease in emission was observed upon addition of denatured lectin to a solution of **210.Tb**. To further test the specificity of the ConA lectin for mannose appended **210.Tb**, a solution of ConA was added the rhamnose complex **222.Tb**. This resulted in no changes in the Tb(III) emission of **222.Tb**, as ConA has no affinity for the rhamnose moiety.

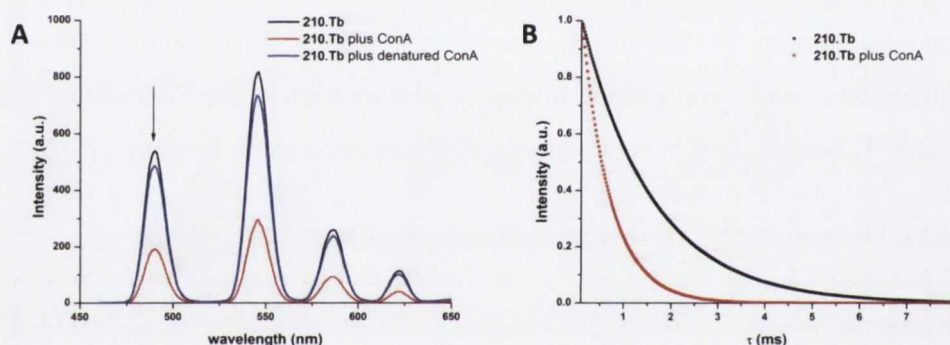


Figure 4.22: (A) Lanthanide luminescence spectra of **210.Tb** (black), **210.Tb** in the presence of 1.0 eq. ConA (red) and **210.Tb** in the presence of denatured ConA (blue); (B) Luminescence decay of the **210.Tb** measured at 545 nm in the absence (black) and presence of ConA (red). All measurements were carried out at **210.Tb** concentration of 1 mM in aqueous buffered solution (100 mM Tris-HCl, 150 mM NaCl, 1 mM MgCl₂, 1 mM CaCl₂, pH = 7.4) using indirect excitation at 273 nm at 37 °C.

In an attempt to understand the effect of the carbohydrate-protein interaction on the Tb(III) metal centre, luminescence lifetimes were measured, as shown in Figure 4.22B. In the absence of ConA, **210.Tb** displays a relatively long lifetime of 1.68 ms, however, upon addition of 1.0 eq. ConA, the lifetime decreases to 0.78 ms. This decrease in the Tb(III) excited state lifetime indicates that the metal centre is subject to more quenching due to the presence of ConA in solution. This increased quenching is thought to be due to the ConA binding to the mannose moiety of **210.Tb** thereby preventing the coordination of the mannose to the Tb(III) centre. This ConA-mannose interaction disrupts the carbohydrate-Tb(III) interactions which were acting as a “glycan shield” for the metal centre. To assess any changes in the hydration

state of the Tb(III) metal centre upon addition of ConA, luminescent lifetime measurements were recorded in H₂O and D₂O and best fit to a mono-exponential decay. The lifetimes and corresponding q values are shown in Table 4.3. As evident, initially the Tb(III) complex **210.Tb** displays a q value of 0 with no metal coordinated water molecules. After the addition of ConA, there was over a two-fold decrease in the recorded lifetimes in H₂O from 1.82 ms to 0.81, resulting in a recorded q value of 1.15 indicating that the Tb(III) ion now had one coordinated water molecule.

Table 4.3: The excited state lifetimes and corresponding hydration state (q) of the **210.Tb** and **210.Tb** plus ConA at concentrations of 1 mM. Luminescence decay measured at 545 nm with each value being an average of 3 measurements.

Complex	$\tau_{(O-H)}/\text{ms}$	$\tau_{(O-D)}/\text{ms}$	$q (\pm 0.5)$
210.Tb	1.82 ± 0.01	2.21 ± 0.02	0.18
210.Tb+ConA	0.81 ± 0.04	1.11 ± 0.01	1.15

4.12.1 Hypothesis for the observed behaviour

As evident from Figure 4.19, **210.Tb** (black) is highly emissive and displays a q value of 0 (Table 4.2). We postulate that at the high concentration of 1 mM, the free hydroxyl groups of the mannose moiety bind to the Tb(III) centre leading to the displacement of the metal bound water molecule and a resulting q value of 0, the process of which is schematically illustrated in Figure 4.23. By addition of ConA, we believe the mannose moieties interact with the lectin instead, disrupting the mannose-Tb(III) association and the “glycan shield” around the Tb(III) centre. Disruption of this “glycan shield” leads to a reduction in the Tb(III)-centred emission and excited state lifetimes and allows a water molecule to now bind the metal centre. This hypothesis was supported by the decrease in the Tb(III) emission and the recorded excited state lifetimes from 1.68 ms to 0.78 ms upon addition of ConA to **210.Tb** in aqueous buffered solution. Moreover, the increase in the q value from 0 to 1 further suggests the above hypothesis.

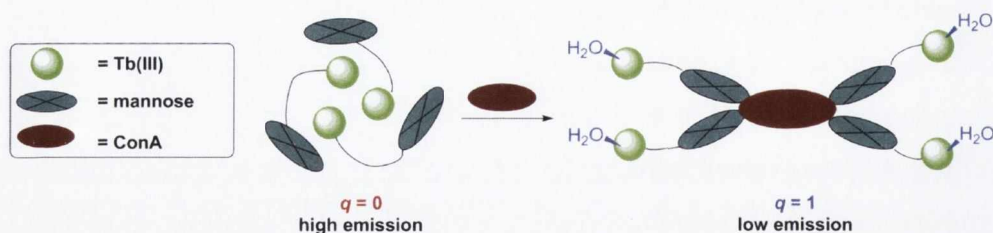


Figure 4.23: Schematic illustration of the process of **201.Tb** (1 mM) binding with lectin, ConA. Initially, the solution of **201.Tb** is highly emissive with a q value of 0 with the Tb(III) complexes thought to be aggregated in solution. Upon addition of ConA, selective binding to the α -mannopyranoside moieties occurs which disrupts the aggregation/association. This de-aggregation results in the coordination of a water molecule to the Tb(III) metal centre ($q = 1$), a reduction in the recorded excited state lifetimes, and concomitant reduction in the Tb(III) emission.

4.15 Conclusion

This chapter initially focused on the synthesis of Tb(III) complexes for use as probes for the biologically relevant glycosidase enzymes, with the primary focus of the synthetic discussion being the β -D-glucose appended complex **195.Tb**. Eight Tb(III) complexes were prepared; 6 of which are glycosylated. Complex **195.Tb** was photophysically characterised and its ability to act as a sensor for β Glc evaluated.

It was found that at the concentration range of 50 μ M to 700 μ M, **195.Tb** had a hydration state of 1 and no change in Tb(III) centred emission was exhibited upon cleavage of the glycosidic bond by β Glc. This was attributed to the intramolecular energy transfer from the phenyl antenna to the Tb(III) excited state not being perturbed by the removal of the carbohydrate moiety.

At the higher substrate concentration of 1 mM, **195.Tb** appeared to function as a sensor for β Glc with rapid enhancements in the Tb(III) emission being observed upon addition of the enzyme. However, in the absence of any glycosidase enzyme, at this high concentration of 1 mM, **195.Tb** exhibited changes in its hydration state with the q value being found to decrease from 1 to 0. This change in the hydration state was accompanied by an increase in Tb(III) emission. It is thought that the free hydroxyl groups of the carbohydrate groups interact with the Tb(III) metal centre displacing the metal bound water molecule. This behaviour was not observed with non-glycosylated Tb(III) complexes or the *O*-acetyl protected carbohydrate derivative. This behaviour

therefore limits the use of the Tb(III) complexes as probes for the glycosidase enzymes.

In order to utilise the self-assembly process for a sensing application, the ability to detect lectin, ConA, was subsequently investigated. Using the mannose-appended complex **210.Tb**, it was found that the addition of ConA disrupted the mannose-Tb(III) interaction in solution leading to a “turn off” response upon lectin binding. The binding of **210.Tb** to ConA also resulted in a decrease in the recorded excited state lifetimes of **210.Tb** accompanied by an increase in the q value from 0 to 1, indicating the coordination of a water molecule to the Tb(III) metal centre. The “turn off” effect was found to be selective for the interaction of mannose appended **210.Tb** with ConA as no modulation in Tb(III) emission was evidenced when ConA was added to a solution of the rhamnose complex **222.Tb**.

As previously mentioned, ConA exists as a tetramer in solution and contains 4 binding sites specific for α -mannopyranoside. Considering this, future work using these Tb(III) complexes could involve the preparation of **215.Tb**. This complex could be prepared using established procedures discussed in this chapter. It is well known that multivalent displays of carbohydrates impart enhanced carbohydrate-lectin binding therefore, experiments using **215.Tb** could lead to enhanced binding with ConA yielding interesting results.

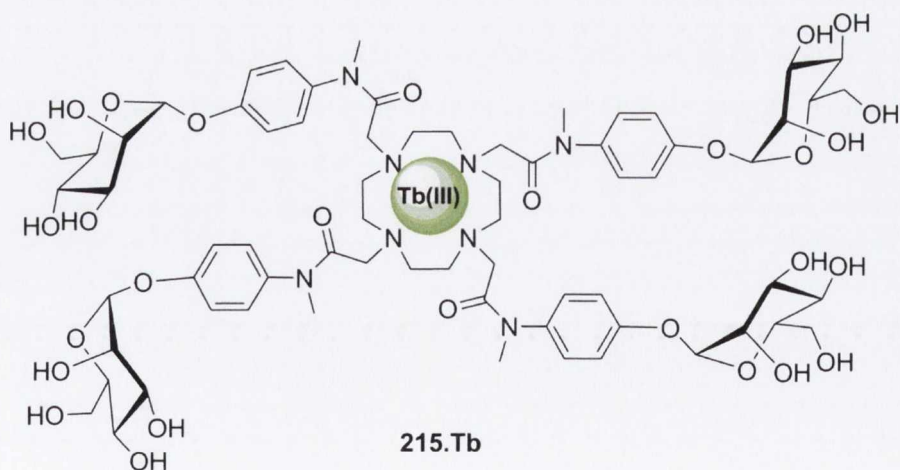


Figure 4.20: Tb(III) complex **215.Tb** for multivalent ConA binding. The 4 mannose residues are expected to interact with the 4 binding sites of ConA resulting in modulation of the Tb(III) emission.

5.0 Overall conclusions

The work in this thesis describes the chemical synthesis of various biologically relevant bioconjugates. The first part describes the development of new ligation strategies towards the synthesis of homogeneous post-translationally modified peptides. The second part describes the synthesis of bioconjugates for the detection of enzymes.

In Chapter 2, a new method for accessing *N*-glycosyl amides is described. This method combines thiol-ene chemistry and an *S,N*-acyl transfer reaction to furnish an amide bond at the carbohydrate anomeric centre, with complete stereoselectivity achieved. This chapter demonstrated, for the first time, the use of peptidyl thioacids being used in the thiol-ene reaction. This was successfully applied to 4 peptidyl thioacids and 4 additional thioacids of varying structure. However, when employing thioacids in the thiol-ene reaction, it was found that CSO elimination of the thiyl donor was a competing reaction. This restricted the use of thioacids to those that, upon CSO elimination, would form unstable radical species. The scope of this modified thiol-ene reaction and experimental conditions which may attenuate CSO elimination are currently being explored further in the Scanlan group. A one-pot cascade reaction (thiol-ene, *S,N*-acyl shift, base hydrolysis) was successful in furnishing 5 different *N*-glycosyl amide products, one of which is an *N*-linked glycopeptide (GlcNAc-Gly). This approach avoids the use of active esters and operates under base-free conditions, which could prove useful in preventing the formation of aspartimide side products, often encountered in current strategies toward *N*-linked glycopeptides. The overall aim of this chapter was to use this methodology for the formation of the GlcNAc-Asn linkage found in *N*-linked glycopeptides. Unfortunately, this methodology was unsuccessful in delivering the desired GlcNAc-Asn linkage due to hydrolysis of the glycosylamine. This hydrolysis was prevented through the incorporation of a bulky *N*-Phth group at the C-2 position of the carbohydrate; however, this bulky protecting group prevents the *S,N*-acyl shift, either sterically or through deactivation of the anomeric amine. Future work could focus on manipulating C-2 to incorporate a group which sterically curtails the formation of the α -hydrolysed product whilst retaining the nucleophilic character of the anomeric

amine. Moreover, the C-2 group needs to remain small enough to ensure the ligation junction is not too sterically demanding. Optimisation of structures by means of quantum chemical calculations could assist the identification of suitable functional groups to allow ligation between the anomeric amine and thioester intermediate.

Continuing with the sequential thiol-ene, acyl transfer methodology developed in Chapter 2, Chapter 3 details the application of this methodology to the chemical ligation of peptide fragments, as in this case, the problem of glycosylamine hydrolysis would not be encountered. The overall aim of this chapter was the formation of a native isopeptide bond, with a focus on Ub related substrates. It was anticipated that the rapid, high yielding nature of the thiol-ene reaction could replace the sluggish transthioesterification reaction used in current peptide ligation strategies. This synthesis of a dipeptide and tripeptide peptide fragments from α -syn, a protein implicated in PD, were prepared. These fragments contained a modified Ser residue with an alkenyl ester and a Lys ϵ -NH₂ masked as an azide for subsequent thiol-ene and reduction reactions, respectively. The reduction of the azide proved problematic with hydrogenation conditions resulting in desulfurisation of the installed thioester functionality. Azide reduction using a tin/mercaptan complex resulted in the quick reduction of the azide to the corresponding amine, in the case of dipeptide substrate. Subsequent *S,N*-acyl transfer forged a native isopeptide bond with the final step involving the hydrolysis of the appended ester group. However, with the proposed *S,N*-acyl transfer occurring through a 18-membered ring, intermolecular acyl transfer was found to be the sole acyl transfer pathway, with none of the desired intramolecular ligated product formed. This observation is in line with the observations of Katritzky and co-workers who reported that the transacylated product was the major product when proceeding through an 18-membered cyclic transition state.²²⁷ A thiol-ene ligation between the α -syn tripeptide fragment and a Gly thioacid was successful in forming a tetrapeptide thioester intermediate; however, subsequent reduction conditions (previously employed tin/mercaptan complex) resulted in thioester hydrolysis, rather than azide reduction. Going forward, 4-butenoyl chloride, rather than 4-pentenoyl chloride, should be used as the Ser esterifying agent as this would generate a 17-membered transition state in a

subsequent *S,N*-acyl transfer reaction. The work of Katritzky and Haase suggests the use of a 17-membered cyclic transition state to favour intramolecular acyl transfer.^{263,279} Regarding the azide functionality, reduction of the azide to the corresponding amine, prior to the thiol-ene reaction, would circumvent the problems encountered with the thioester functionality under azide reduction conditions.

Chapter 4 described the synthesis of various *O*-glycosylated lanthanide-based cyclen complexes for use as luminescent probes for the detection of glycosidase enzymes. Six glycosylated lanthanide-based complexes were synthesised, each bearing a different carbohydrate group. This chapter focused on the β -D-glucose appended Tb(III) complex **195.Tb** and its response to β Glc. It was found that at concentrations of 50 μ M-700 μ M, no change in luminescence properties of **195.Tb** were observed.

At the higher concentration of 1 mM, **195.Tb** initially appeared to function as a probe for β Glc. However, in the absence of any β Glc, a self-assembly/aggregation process was observed whereby the free hydroxyl groups of the carbohydrate coordinated to the Tb(III) ion displacing the metal bound water molecule, resulting in enhanced Tb(III) emission and reduction of the *q* value from 1 to 0. This behaviour was only observed with deprotected carbohydrate complexes at the high concentration of 1 mM. Therefore, it was deduced that **195.Tb** could not be used as a reliable β Glc probe (no change observed at low concentrations and self-assembly behaviour observed at higher concentrations). However, analysis of the probes' behaviour in solution led to the use of the mannose appended **210.Tb** as a sensor for lectin, ConA. In this case, the self-assembly/aggregation observed at 1 mM was disrupted by the addition of ConA which selectively interacted with the mannose moieties. This interaction disrupted the carbohydrate-Tb(III) coordination thereby increasing the Tb(III) *q* value to from 0 to 1 which was accompanied by a reduction in the recorded lifetimes and Tb(III) emission. Future work could focus on the preparation of Ln(III)-based complexes which bear multivalent carbohydrate arrays, as this could impart more enhanced binding between the lectin and carbohydrate motifs and perhaps a more pronounced response upon aggregation/de-aggregation events.

6.0 General experimental details

All commercial chemicals used were supplied by Sigma Aldrich, Fluorochem and Tokyo Chemical Industry and used without further purification unless otherwise stated. Anhydrous CH_2Cl_2 was obtained *via* drying with CaH_2 followed by distillation; anhydrous THF was obtained by drying over sodium/benzophenone, followed by distillation. Silica gel 60 (Merck, 230-400 mesh) or aluminium oxide (neutral, activated with 6.5 % H_2O , Brockmann Grade III) were used for flash column chromatography. All compounds were subject to purification using silica gel, unless otherwise stated. Analytical thin layer chromatography was carried out with silica gel 60 (fluorescence indicator F254; Merck) or aluminium oxide 60 (neutral, F254; Merck) and visualized by UV irradiation, iodine, or molybdenum staining [ammonium molybdate (5.0 g) and concentrated H_2SO_4 (5.3 mL) in 100 mL H_2O]. Melting points are uncorrected and were measured with a Stuart SP-10 melting point apparatus. NMR spectra were recorded using Bruker DPX 400 (400.13 MHz for ^1H NMR and 100.61 MHz for ^{13}C NMR), Bruker AV 600 (600.13 MHz for ^1H NMR and 150.90 MHz for ^{13}C NMR), Bruker AV 400 (400.13 MHz for ^1H NMR and 100.61 MHz for ^{13}C NMR) or Agilent MR400 (400.13 MHz for ^1H NMR and 100.61 MHz for ^{13}C NMR) instruments. Chemical shifts are given in ppm and referenced to the internal solvent signals.³⁴⁴ The assignment of the signals was confirmed by 2D spectra (COSY, HMBC, HSQC). MALDI ToF spectra were acquired using a Waters MALDI Q-ToF Premier in positive or negative mode with DCTB (trans-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile) as the MALDI matrix. ESI mass spectra were acquired in positive and negative modes as required, using a Micromass time of flight mass spectrometer (TOF), interfaced to a Waters 2690 HPLC or a Bruker micrOTOF-Q III spectrometer interfaced to a Dionex UltiMate 3000 LC. APCI experiments were carried out on a Bruker micrOTOF-Q III spectrometer interfaced to a Dionex UltiMate 3000 LC or direct insertion probe in positive or negative modes. EI mass spectra were acquired using a GCT Premier Micromass time of flight mass spectrometer (TOF) in positive mode at 70eV. Specific rotation was recorded in a Rudolph research autopol IV polarimeter with a D-line sodium lamp (589 nm) at 20 °C and are quoted as

$\text{deg cm}^3 \text{ g}^{-1} \text{ dm}^{-1}$. IR spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer.

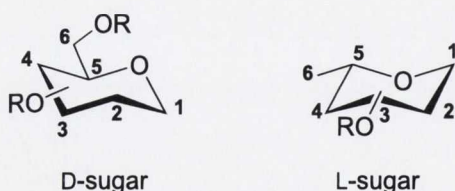
6.1 UV-Visible absorption and luminescence spectroscopy

Using a 1.0 cm path length quartz cell, UV-vis absorption and luminescence spectra were recorded at 37 °C (310 K) using a Varian CARY 50 spectrophotometer. The solvents employed were HPLC or spectrophotometric grade. For UV-Vis absorption measurements, the wavelength range was set from 200 nm to 450 nm with a scan rate of 600 nm min⁻¹. The blank used was a sample of the solvent system in which the titration was undertaken. The luminescence data was collected between 470 and 700 nm for Tb(III) emission and 570 and 720 nm for Eu(III).

6.2 Luminescence lifetime measurements

The luminescence lifetime measurements of the Tb(III) complexes were measured in both H₂O and D₂O and in CH₃OH and CD₃OD and were conducted on a Varian Cary Eclipse Fluorimeter at 37 °C. The measurements were made by measuring the decay at 545 nm, which corresponds to the maxima of the Tb(III) ⁵D₄ → ⁷F₅ transition. All lifetime measurements were obtained from an average of 3 independent measurements, each recorded with a different gate time in the range of 0.020 – 0.045 ms.

6.3 General nomenclature of D- and L-sugars for characterisation



6.4 General experimental procedures

Procedure 1: Carboxylic acid methylation

To a solution of the amino acid (1.0 eq.) in anhydrous DMF was added K₂CO₃ (1.5 eq.) and iodomethane (2.0 eq.) and the solution was stirred at rt for 3 h. H₂O (20 mL) was added and the product was extracted with EtOAc (2 x 15

mL). The combined organic layers were washed with sat. aq. NaHCO_3 solution (20 mL), brine (20 mL), dried over MgSO_4 , filtered, and solvent was removed *in vacuo* and the product was used without further purification.

Procedure 2: Removal of benzyl group

The benzyl protected amino acid (1.0 eq.) was dissolved in an appropriate solvent and Pd/C (0.1 eq.) was added and the suspension deoxygenated with a N_2 bubbler for 10 min. The solution was then placed under a H_2 atmosphere using a balloon and stirred at rt for 3 h. The mixture was filtered through a plug of celite and the solvent was removed *in vacuo* and used without further purification.

Procedure 3: Removal of Boc protecting group

To a solution of the Boc protected amino acid (1.0 eq.) in CH_2Cl_2 was added 4M HCl in dioxane (3.0 eq.) and the solution was stirred at rt for 3 h. The solvent was removed *in vacuo* to yield the product as the hydrochloride salt which was used without further purification.

Procedure 4: Preparation of *S*-trityl thioesters

To a stirred solution of the starting carboxylic acid (1.0 eq.), triphenylmethanethiol (1.1 eq.) and DMAP (0.1 eq.) in anhydrous CH_2Cl_2 was added EDCI·HCl (1.1 eq.) and the solution was stirred at rt for 16 h. The solvent was removed *in vacuo* and the mixture was subjected to chromatographic purification.

Procedure 5: Removal of the *S*-trityl group

To a solution of the *S*-trityl thioester (1.0 eq.) in anhydrous CH_2Cl_2 was added TFA (25%) and triethylsilane (20.0 eq.) and the mixture was stirred for 5 min. Toluene was added and the solvent was removed *in vacuo* to give the crude thioacid which was applied immediately to the next step.

Procedure 6: Thiol-ene ligation

To a mixture of the alkene (1.0 eq.), DPAP (0.1 eq.) and MAP (0.1 eq.) in anhydrous DMF was added the crude thioacid (1.1 eq. or 1.5 eq.) from General

Procedure 5 and the reaction mixture was irradiated in an UV oven at rt without agitation for 1 h. The solvent was removed *in vacuo* and the mixture was subject to chromatographic purification.

Procedure 7: Peptide coupling

To the amino acid-COOH (1.0 eq.) in anhydrous CH₂Cl₂:DMF (9:1) was added EDCI·HCl (1.1 eq.) and HOBT (1.1 eq.) and the solution was stirred under an argon atmosphere at 0 °C for 30 min. To this was added a solution of the amino acid-NH₂ (1.0 eq.) and NEt₃ (2.0 eq.) in anhydrous CH₂Cl₂:DMF (9:1) and the solution was stirred at rt for 16 h. The reaction mixture was diluted with H₂O (20 mL), extracted with CH₂Cl₂ (2 x 15 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over MgSO₄, filtered, the solvent was removed *in vacuo* and the mixture subjected to chromatographic purification.

Procedure 8: Thiol-ene ligation, S,N-acyl shift, base hydrolysis

To a mixture of the alkene (1.0 eq.), DPAP (0.1 eq.) and MAP (0.1 eq.) in anhydrous DMF was added the crude thioacid (1.1 or 1.5 eq.) from General Procedure 5 and the reaction mixture was irradiated in an UV oven at rt without agitation for 1 h followed by stirring at rt for 16 h outside of the UV oven. The solvent was removed *in vacuo* and the mixture was re-dissolved in CH₃OH:H₂O (10 mL, 8:2) and to this was added NEt₃ (25.0 eq.) and the solution was stirred at rt for 4 h. The mixture was neutralised by the addition of Dowex-H⁺ resin and then filtered. The solvent was removed *in vacuo* and the reaction mixture was subjected to chromatographic purification.

Procedure 9: N-Acetylation:

To a stirred solution of the amino acid (1.0 eq.) in sat. aq. NaHCO₃ solution was added Ac₂O (5.0 eq.) and the mixture was stirred at rt for 16 h. The mixture was acidified to pH2 with aq. HCl solution, extracted with EtOAc (2 x 15 mL), dried over MgSO₄, filtered, and solvent was removed *in vacuo* and the product was used without further purification.

Procedure 10: Esterification of Ser

To a solution of the starting amino acid (1.0 eq.) in anhydrous DMF at 0 °C was added NEt_3 (1.2 eq.), DMAP (1.0 eq.) and 4-pentenoyl chloride (1.2 eq.) and the reaction was stirred at rt for 2 h. The solvent was removed *in vacuo* and the mixture was subjected to chromatographic purification.

Procedure 11: Reduction of the Lys azido group

To a solution of anhydrous SnCl_2 (1.5 eq.) in CH_3CN (5 mL) was added thiophenol (4.5 eq.) and NEt_3 (5.0 eq.). To this was added a solution of the azide (1.0 eq.) in CH_3CN and the mixture was stirred at rt for 20 min. The mixture was diluted with CH_2Cl_2 (15 mL), washed with sat. aq. NaHCO_3 solution (2 x 10 mL) and the solvent was removed *in vacuo*. To the product was added CH_3OH (10 mL) and the solution was stirred for 1 h at rt followed by removal of the solvent *in vacuo*.

Procedure 12: Glycosylation of peracetylated carbohydrate with 4-nitrophenol

To peracetylated carbohydrate (1.0 eq.), 4-nitrophenol (3.0 eq.) and activated 4 Å molecular sieves in a flame dried flask under N_2 was added anhydrous CH_2Cl_2 (10 mL/g). To this was added $\text{BF}_3 \cdot \text{OEt}_2$ (5.0 eq.) and the mixture was stirred at rt for 48 h. The reaction was quenched with sat. aq. NaHCO_3 solution (20 mL) and the organic layer was extracted with CH_2Cl_2 (20 mL). The organic layer was washed with 1M NaOH solution (3 x 20 mL), H_2O (20mL), dried over MgSO_4 and filtered. The solvent was removed *in vacuo* and the product recrystallised from EtOAc/Hex and used without further purification.

Procedure 13: Reduction of 4-nitrophenol glycoside

To the peracetylated 4-nitrophenyl glycoside (1.0 eq.) from Procedure 12 in degassed $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ (6:4) was added $\text{Pd}(\text{OH})_2$ (0.1 eq.) and the mixture was stirred under an atmosphere of H_2 for 4 h using a balloon. The mixture was filtered through a plug of celite and the solvent was removed *in vacuo* at 25 °C to yield the product.

Procedure 14: Amidation of 4-aminophenyl glycosides

To the 4-aminophenyl glycoside (1.0 eq.) from Procedure 13 in anhydrous CH_2Cl_2 (10 mL/g) under N_2 was added chloroacetic anhydride (2.0 eq.) and NEt_3 (1.2 eq.) and the mixture was stirred at rt for 2 h. The reaction was diluted with CH_2Cl_2 (10 mL), washed with H_2O (2 x 20 mL), dried over MgSO_4 , filtered and solvents was removed *in vacuo*. The product was purified by flash chromatography (EtOAc:Hex, 60:40) to yield the product.

Procedure 15: Amide methylation

To the amide (1.0 eq.) from Procedure 14 in anhydrous THF was added *t*-BuOK (1.5 eq., 1M solution in THF) slowly and the mixture was stirred at -78°C for 20 min under N_2 atmosphere. Excess iodomethane (25.0 eq.) was added and the solution was stirred for 3 h at -78°C . The reaction was warmed slowly to rt and quenched with sat. aq. NH_4Cl solution (20 mL) and stirred for 30 min at rt. The product was extracted with EtOAc (2 x 20 mL) and the combined organic layers washed with 1M HCl solution (20 mL), brine (15 mL), dried over MgSO_4 , filtered and the solvent was removed *in vacuo* to yield the product.

Procedure 16: Conjugation of the α -chloroamide to cyclen

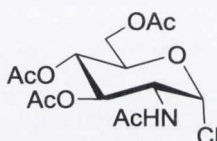
To *tris N*-alkylated cyclen **193** (1.0 eq.) in CH_3CN was added the methylated amide from Procedure 15 (1.2 eq.), K_2CO_3 (1.2 eq.), and KI (1.2 eq.) and resulting mixture was refluxed for 16 h. After cooling to rt, the solution was diluted with CH_3CN and filtered. The product was purified by alumina column chromatography to yield the product.

Procedure 17: Deprotection of *O*-acetyl carbohydrate protecting groups

To a stirred solution of the acetylated carbohydrate (1.0 eq.) in CH_3OH was added a catalytic amount of freshly prepared NaOCH_3 . The mixture was stirred at rt for 30 min. The mixture was neutralised by the addition of DOWEX- H^+ resin, filtered, and the solvent was removed *in vacuo* to yield the product which was used without further purification.

Procedure 18: Ln(III) complexation

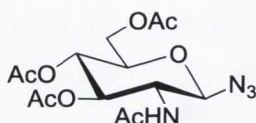
To the ligand (1.0 eq.) in CH₃OH was added Tb(CF₃SO₃)₃ or Eu(CF₃SO₃)₃ (1.0 eq.) and the solution was heated in a microwave at 60 °C for 1 h. The solvent was removed *in vacuo* and the product was dissolved in minimal volume CH₃OH and added dropwise to a large volume of swirling Et₂O. The Et₂O was decanted and product centrifuged (7220 rpm, 10 min) to yield a fine solid.

6.5 Experimental details for Chapter 2**2-Deoxy-2-acetamido-3,4,6-tri-*O*-acetyl- α -D-glucopyranosyl chloride (55)**

Acetyl chloride (15 mL, 196.20 mmol) was added to *N*-acetyl glucosamine (7.0 g, 31.64 mmol) and the solution was stirred at rt for 16 h. The solution was poured onto iced H₂O (100 mL) and the product was immediately extracted with CHCl₃ (3 x 30 mL). The combined extracts were poured into sat. aq. NaHCO₃ solution (50 mL) and immediately separated and the organic layer was dried over MgSO₄, filtered, and solvent was removed *in vacuo*. The product was recrystallised from CH₂Cl₂/Et₂O to yield the product as colourless crystals (9.8 g, 85%). M.p. 124-126 °C, Lit., 124-126 °C.³⁴⁵ The isolated compound was in good agreement with the literature.³⁴⁵

δ_{H} (400 MHz, CDCl₃): 6.21 (1H, d, J = 3.6 Hz, H-1), 5.89 (1H, d, J = 8.4 Hz, NH), 5.35 (1H, app t, J = 10.1 Hz, H-3), 5.24 (1H, app t, J = 10.1 Hz, H-4), 4.56 (1H, m, H-2), 4.31 (1H, m, H-6), 4.29 (1H, m, H-5), 4.16 (1H, m, H-6'), 2.13 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.01 (3H, s, CH₃)

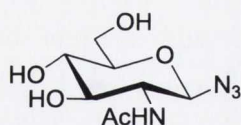
HRMS (m/z ESI⁺): Found: 388.0770 ([M + Na]⁺, C₁₄H₂₀ClNO₈ Requires: 388.0770)

2-Deoxy-2-acetamido-3,4,6-tri-*O*-acetyl- β -D-glucopyranosyl azide (56)

To a solution of **55** (9.0 g, 25.61 mmol) in DMF (30 mL) was added NaN₃ (4.8 g, 73.83 mmol) and the resulting solution was heated at 80 °C for 16 h. The solution was cooled and diluted with EtOAc (20 mL), washed with brine (3 x 15 mL), dried over MgSO₄, filtered, and solvent was removed *in vacuo* to yield the product as an orange oil (8.20 g, 90%). The isolated compound was in good agreement with the literature.³⁴⁶

δ_{H} (400 MHz, CDCl₃): 5.55 (1H, d, J = 8.8 Hz, NH), 5.26 (1H, app t, J = 10.4 Hz, H-3), 5.13 (1H, app t, J = 9.7 Hz, H-4), 4.78 (1H, d, J = 9.0 Hz, H-1), 4.30 (1H, dd, J = 4.8, J = 12.5 Hz, H-6), 4.19 (1H, dd, J = 2.3, J = 12.5 Hz, H-6'), 3.93 (1H, q, J = 9.0 Hz, H-2), 3.81 (1H, m, H-5) 2.13 (3H, s, CH₃), 2.07 (3H, s, CH₃), 2.06 (3H, s, CH₃), 2.01 (3H, s, CH₃)

HRMS (m/z ESI⁺): Found: 395.1175 ([M + Na]⁺, C₁₄H₂₀N₄O₈)
Requires: 395.1173)

2-Deoxy-2-acetamido- β -D-glucopyranosyl azide (57)

To a solution of **56** (7.5 g, 20.14 mmol) in CH₃OH (25 mL) was added a catalytic amount of freshly prepared NaOCH₃ and the solution was stirred at rt for 30 min. The mixture was neutralised by the addition of DOWEX-H⁺ resin and then filtered. The solvent was removed *in vacuo* to yield the product as a yellow oil (4.85 g, 98%). The isolated compound was in good agreement with the literature.³⁴⁷

δ_{H} (400 MHz, CD₃OD): 4.53 (1H, d, J = 9.3 Hz, H-1), 3.92 (1H, dd, J = 1.6, J = 11.9 Hz, H-6), 3.73 (1H, m, H-6'), 3.69

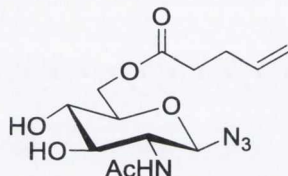
(1H, app t, $J = 9.5$ Hz, H-2), 3.48 (1H, app t, $J = 10.2$ Hz, H-3), 3.38 (1H, m, H-4), 3.38 (1H, m, H-5), 2.01 (3H, s, CH₃)

HRMS (m/z ESI⁺):

Found: 269.0863 ([M + Na]⁺, C₈H₁₄N₄O₅)

Requires: 269.0856)

2-Deoxy-2-acetamido-6-*O*-pent-4-enoate- β -D-glucopyranosyl azide (58**)**



To a solution of **57** (0.49 g, 2.0 mmol) in anhydrous DMF (8 mL) was added NEt₃ (0.33 mL, 2.40 mmol) and the solution was cooled to 0 °C. To this was added 4-pentenoyl chloride (0.26 mL, 2.40 mmol) and the solution was allowed to warm to rt and was stirred for 2 h under an argon atmosphere. The solvent was removed *in vacuo* and the product was purified by flash chromatography (CH₂Cl₂ to CH₂Cl₂:CH₃OH - 88:12) to yield the product as a yellow oil (0.47 g, 72%).

δ_H (600 MHz, CD₃OD): 5.88 (1H, m, CH=CH₂), 5.10 (1H, m, CH=CH₂), 5.02 (1H, m, CH=CH₂), 4.60 (1H, d, $J = 8.6$ Hz, H-1), 4.49 (1H, dd, $J = 2.0$, $J = 12.1$ Hz, H-6), 4.26 (1H, dd, $J = 5.8$, $J = 12.1$ Hz, H-6'), 3.71 (1H, app t, $J = 9.7$ Hz, H-2), 3.63 (1H, m, H-5), 3.55 (1H, app t, $J = 10.2$ Hz, H-3), 3.38 (1H, m, H-4), 2.49 (1H, m, CH₂), 2.39 (1H, m, CH₂), 2.03 (3H, s, CH₃)

δ_C (150 MHz, CD₃OD): 173.0 (C=O), 172.3 (C=O), 136.6 (CH=CH₂), 114.5 (CH=CH₂), 88.4 (C-1), 75.9 (C-5), 74.2 (C-3), 70.5 (C-4), 62.9 (C-6), 55.2 (C-2), 33.0 (CH₂), 28.5 (CH₂), 21.4 (CH₃)

$[\alpha]_D^{20}$ (c 0.01, CH₃OH): -48

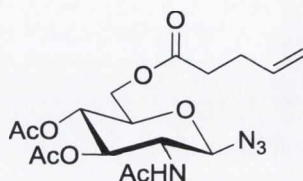
HRMS (m/z ESI⁺):

Found: 351.1281 ([M + Na]⁺, C₁₃H₂₀N₄O₆)

Requires: 351.1275)

$\nu_{\max}$ (film)/ cm^{-1} : 3658 (NH), 3268 (OH), 2981 (CH), 2114 (N_3),
1733 (C=O)

2-Deoxy-2-acetamido-3,4-di-*O*-acetyl-6-*O*-pent-4-enoate- β -D-glucopyranosyl azide (64)



To a solution of **58** (0.18 g, 0.55 mmol) in CH_2Cl_2 (5 mL) was added pyridine (0.3 mL, 3.30 mmol) and Ac_2O (0.3 mL, 3.30 mmol) and the resulting solution was stirred at rt for 16 h. The solvent was removed *in vacuo* to yield the product as a yellow oil (0.23 g, >99%).

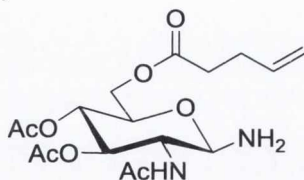
δ_{H} (600 MHz, CDCl_3): 7.02 (1H, d, $J = 9.0$ Hz, NH), 5.80 (1H, m, $\text{CH}=\text{CH}_2$), 5.33 (1H, app t, $J = 9.9$ Hz, H-3), 5.04 (2H, m, $\text{CH}=\text{CH}_2$), 5.03 (1H, m, H-4), 5.02 (1H, m, H-1), 4.22 (1H, dd, $J = 4.6$, $J = 12.4$ Hz, H-6), 4.16 (1H, dd, $J = 1.1$, $J = 12.4$ Hz, H-6'), 3.94 (1H, q, $J = 9.4$ Hz, H-2), 3.82 (1H, m, H-5), 2.45 (1H, m, CH_2), 2.36 (1H, m, CH_2), 1.99 (3H, s, CH_3), 1.99 (3H, s, CH_3), 1.96 (3H, s, CH_3)

δ_{C} (150 MHz, CDCl_3): 172.5 (C=O), 170.5 (C=O), 170.4 (C=O), 169.1 (C=O), 136.6 ($\text{CH}=\text{CH}_2$), 115.7 ($\text{CH}=\text{CH}_2$), 88.4 (C-1), 73.9 (C-5), 72.6 (C-3), 68.6 (C-4), 61.8 (C-6), 54.0 (C-2), 33.1 (CH_2), 28.7 (CH_2), 23.0 (CH_3), 20.5 (CH_3), 20.4 (CH_3)

$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl_3): -53

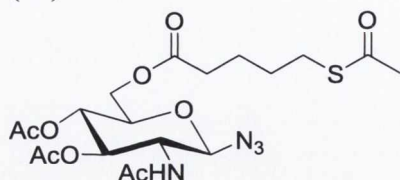
HRMS (m/z ESI $^{+}$): Found: 435.1482 ($[\text{M} + \text{Na}]^{+}$, $\text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_8$)
Requires: 435.1486)

$\nu_{\max}$ (film)/ cm^{-1} : 3285 (NH), 2602, 2497 (CH), 2117 (N_3), 1747 (C=O)

2-Deoxy-2-acetamido-3,4-di-*O*-acetyl-6-*O*-pent-4-enoate- β -D-glucopyranosyl amine (65)

To a solution of **64** (0.35 g, 0.85 mmol) in CH₃CN:EtOH (1:1, 15 mL) was added ammonium tetrathiomolybdate (0.22 g, 0.85 mmol) and the reaction flask was placed in a sonication bath at rt for 3 h. The solvent was removed *in vacuo* and the black residue was dissolved in CH₂Cl₂:Et₂O (1:10, 50 mL) and the mixture filtered. The solvent was removed *in vacuo* and the black residue was re-dissolved in CH₂Cl₂:Et₂O (1:10, 50 mL) and the filtering was repeated (x 4) to yield the product as a colourless oil (0.17 g, 53%).

δ_{H} (600 MHz, CDCl ₃):	5.83 (1H, m, CH=CH ₂), 5.07 (1H, m, H-4), 5.06 (2H, m, CH=CH ₂), 5.06 (1H, m, H-3), 5.02 (1H, d, J = 9.9 Hz, H-1), 4.22 (1H, dd, J = 4.7, J = 12.4 Hz, H-6), 4.16 (1H, dd, J = 1.2, J = 12.4 Hz, H-6'), 4.01 (1H, q, J = 9.6 Hz, H-2), 3.65 (1H, m, H-5), 2.48 (1H, m, CH ₂), 2.40 (1H, m, CH ₂), 2.04 (3H, s, CH ₃), 2.03 (3H, s, CH ₃), 1.97 (3H, s, CH ₃)
δ_{C} (150 MHz, CDCl ₃):	172.7 (C=O), 171.5 (C=O), 170.8 (C=O), 169.3 (C=O), 136.6 (CH=CH ₂), 116.0 (CH=CH ₂), 86.4 (C-1), 73.9 (C-5), 72.6 (C-3), 68.6 (C-4), 62.4 (C-6), 55.0 (C-2), 33.5 (CH ₂), 28.7 (CH ₂), 23.3 (CH ₃), 20.7 (CH ₃), 20.6 (CH ₃)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl ₃):	-65
HRMS (m/z ESI ⁺):	Found: 387.1766 ([M + H] ⁺ , C ₁₇ H ₂₆ N ₂ O ₈) Requires: 387.1767
ν_{max} (film)/cm ⁻¹ :	3628, 3398 (NH), 2968 (NH ₂), 2968 (CH), 1971, 1730 (C=O)

2-Deoxy-2-acetamido-3,4-di-*O*-acetyl-6-*O*-acetylthiopentanoate- β -D-glucopyranosyl amine (67)

Prepared as per Procedure 6 using **64** (50 mg, 0.13 mmol) and **66** (15 μ L, 0.14 mmol) and following flash chromatography (EtOAc:Hex - 70:30), the product was obtained as a colourless oil (56 mg, 93%).

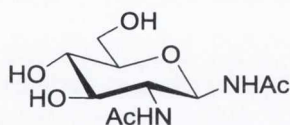
δ_{H} (600 MHz, CDCl_3): 5.83 (1H, d, $J = 9.4$ Hz, NH), 5.27 (1H, app t, $J = 9.8$ Hz, H-3), 5.10 (1H, app t, $J = 9.8$ Hz, H-4), 4.79 (1H, d, $J = 9.4$ Hz, H-1), 4.27 (1H, dd, $J = 4.9$, $J = 12.2$ Hz, H-6), 4.20 (1H, dd, $J = 1.5$, $J = 12.2$ Hz, H-6'), 3.94 (1H, q, $J = 9.7$ Hz, H-2), 3.82 (1H, m, H-5), 2.90 (2H, t, $J = 7.5$ Hz, CH_2), 2.40 (2H, t, $J = 7.5$ Hz, CH_2), 2.34 (3H, s, CH_3), 2.05 (3H, s, CH_3), 2.05 (3H, s, CH_3), 1.99 (3H, s, CH_3), 1.73 (2H, m, CH_2), 1.64 (2H, m, CH_2)

δ_{C} (150 MHz, CDCl_3): 195.8 (C=O), 172.8 (C=O), 170.9 (C=O), 170.4 (C=O), 169.2, (C=O), 88.4 (C-1), 74.0 (C-5), 72.1 (C-3), 68.2 (C-4), 61.9 (C-6), 54.2 (C-2), 33.4 (CH_2), 28.9 (CH_2), 28.6 (CH_2), 23.8 (CH_2), 30.6 (CH_3), 23.2 (CH_3), 20.6 (CH_3), 20.5 (CH_3)

$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl_3): -46

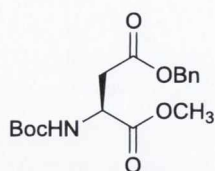
HRMS (m/z ESI^+): Found: 511.1478 ($[\text{M} + \text{Na}]^+$, $\text{C}_{19}\text{H}_{28}\text{N}_4\text{O}_9\text{S}$ Requires: 511.1475)

ν_{max} (film)/ cm^{-1} : 3289 (NH), 2117 (N_3), 1748, 1689 (C=O), 1233 (CH)

2-Deoxy-2-acetamido- β -D-glucopyranosylacetamide (70)

To a solution of **65** (50 mg, 0.13 mmol), DPAP (10 mg, 0.01 mmol) and MAP (10 mg, 0.01 mmol) in anhydrous DMF (3 mL) was added **66** (15 μ L, 0.14 mmol) and the reaction mixture was irradiated in a UV oven at rt without agitation for 1 h followed by stirring at rt for 16 h. The solvent was removed *in vacuo* and the mixture was re-dissolved in CH₃OH (5 mL) and to this was added a catalytic amount of freshly prepared NaOCH₃ and the solution was stirred at rt for 20 min. The mixture was neutralised by the addition of DOWEX-H⁺ resin and then filtered. The solvent was removed *in vacuo* and the product was purified by flash chromatography (CH₂Cl₂ to CH₂Cl₂:CH₃OH - 90:10) to yield the product as a colourless oil (22 mg, 64%). The isolated compound was in good agreement with the literature.³⁴⁸

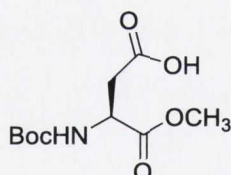
δ_{H} (600 MHz, CD ₃ OD):	4.98 (1H, d, J = 9.9 Hz, H-1), 3.86 (1H, dd, J = 0.9, J = 12.3 Hz, H-6), 3.77 (1H, app t, J = 9.6 Hz, H-2), 3.69 (1H, dd, J = 4.0, J = 11.9 Hz, H-6'), 3.50 (1H, m, H-3), 3.36 (1H, m, H-4), 3.36 (1H, m, H-5), 1.99, 1.96 (3H, s, CH ₃)
δ_{C} (150 MHz, CD ₃ OD):	173.1 (C=O), 172.5 (C=O), 79.0 (C-1), 78.4 (C-5), 74.9 (C-3), 70.5 (C-4), 61.4 (C-6), 54.9 (C-2), 21.4 (CH ₃), 21.4 (CH ₃)
$[\alpha]_{\text{D}}^{20}$ (c 0.04, CH ₃ OH):	-75
HRMS (m/z ESI ⁻):	Found: 261.1091 ([M - H] ⁻ , C ₁₀ H ₁₈ N ₂ O ₆) Requires: 261.1087
ν_{max} (film)/cm ⁻¹ :	3269 (NH), 2924 (OH), 1646 (C=O), 1314 (CH)

Boc-Asp(OBzl)-OMe (72)

Prepared as per Procedure A using Boc-Asp(OBzl)-OH (1.5 g, 4.64 mmol) in DMF (15 mL) to give the product as a colourless oil (1.4 g, 90%). The isolated compound was in good agreement with the literature.³⁴⁹

δ_{H} (400 MHz, CDCl_3): 7.38-7.28 (5H, m, Ar-CH), 5.47 (1H, d, $J = 7.9$ Hz, NH), 5.11 (2H, dd, $J = 12.2$, $J = 15.5$ Hz, CH_2OBn), 4.57 (1H, m, αCH), 3.68 (3H, s, CH_3), 3.02 (1H, dd, $J = 4.6$, $J = 17.2$ Hz, CHCH_2), 2.85 (1H, dd, $J = 5.7$, $J = 17.2$ Hz, CHCH_2), 1.43 (9H, s, $\text{C}(\text{CH}_3)_3$)

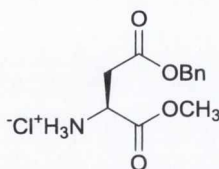
HRMS (m/z ESI^+): Found: 360.1422 ($[\text{M} + \text{Na}]^+$, $\text{C}_{17}\text{H}_{23}\text{NO}_6$)
Requires: 360.1418)

Boc-Asp(OH)-OMe (73)

Prepared as per Procedure 2 using **72** (1.0 g, 4.64 mmol) in $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ (1:1, 25 mL) to give the product as a colourless oil (0.71 g, 97%). The isolated compound was in good agreement with the literature.³⁵⁰

δ_{H} (400 MHz, CDCl_3): 5.48 (1H, d, $J = 6.6$ Hz, NH), 4.57 (1H, m, αCH), 3.75 (3H, s, CH_3), 3.05 (1H, dd, $J = 3.7$, $J = 17.3$ Hz, CH_2), 2.86 (1H, dd, $J = 3.8$, $J = 17.4$ Hz, CH_2), 1.45 (9H, s, $\text{C}(\text{CH}_3)_3$)

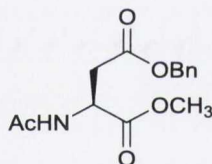
HRMS (m/z ESI^+): Found: 270.0950 ($[\text{M} + \text{Na}]^+$, $\text{C}_{10}\text{H}_{17}\text{NO}_6$)
Requires: 270.0948)

H-Asp(OBzl)-OMe (77)

Prepared as per Procedure 3 using **72** (1.0 g, 2.92 mmol) in CH₂Cl₂ (20 mL) to give the product as a white solid (0.8 g, >99%). M.p. 68-70 °C, Lit., 67-68 °C.³⁵¹ The isolated compound was in good agreement with the literature.³⁵²

δ_{H} (400 MHz, CDCl₃): 8.65 (3H, s, NH₃⁺), 7.27-7.06 (5H, m, Ar-CH), 5.03 (2H, m, CH₂OBn), 4.60 (1H, m, CH), 3.54 (3H, s, OCH₃), 3.23 (2H, m, CHCH₂)

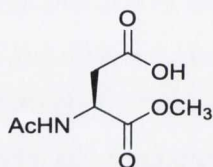
HRMS (m/z ESI⁺): Found: 238.1078 ([M + H]⁺, C₁₂H₁₅NO₄)
Requires: 238.1079)

Ac-Asp(OBzl)-OMe (78)

To a solution of **77** (0.7 g, 2.56 mmol) in CH₂Cl₂ (15 mL) was added NEt₃ (0.71 mL, 5.12 mmol) followed by Ac₂O (0.48 mL, 5.12 mmol) and the solution was stirred at rt for 16 h. The mixture was diluted with H₂O (20 mL), extracted with CH₂Cl₂ (2 x 15 mL) and the combined organic layers were washed with aq. HCl solution (15 mL), brine (15 mL), dried over MgSO₄, filtered, and the solvent was removed *in vacuo* to yield the product as a yellow oil (0.67 g, 94%).

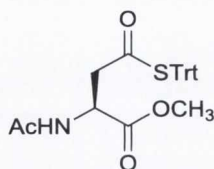
δ_{H} (600 MHz, CDCl₃): 7.35-7.20 (5H, m, Ar-CH), 6.92 (1H, br s, NH), 5.05 (2H, s, CH₂OBn), 4.84 (1H, m, α CH), 3.61 (3H, s, OCH₃), 3.96 (1H, dd, J = 4.7, J = 17.2 Hz, CHCH₂), 2.86 (1H, dd, J = 4.9, J = 16.9 Hz, CHCH₂), 1.93 (3H, s, CH₃)

δ_C (150 MHz, $CDCl_3$):	171.2 (C=O), 170.7 (C=O), 170.0 (C=O), 135.3 (qC), 128.5 (Ar-CH), 128.3 (Ar-CH), 128.2 (Ar-CH), 66.8 ($\underline{CH_2Ar}$), 52.8 (OCH_3), 48.7 (CH), 36.3 ($CH\underline{CH_2}$), 22.9 (CH_3)
$[\alpha]_D^{20}$ (c 0.04, $CHCl_3$):	+10
HRMS (m/z ESI ⁺):	Found: 302.1009 ($[M + Na]^+$, $C_{14}H_{17}NO_5$) Requires: 302.1004)
ν_{max} (film)/ cm^{-1} :	3365 (NH), 1734, 1661 (C=O), 1214 (CH)

Ac-Asp(OH)-OMe (79)

Prepared as per Procedure 2 using **78** (0.60 g, 2.17 mmol) in $CH_2Cl_2:CH_3OH$ (15 mL, 1:1) to give the product as a yellow oil (0.39 g, 95%).

δ_H (400 MHz, CD_3OD):	4.78 (1H, t, $J = 6$ Hz, αCH), 3.75 (3H, s, OCH_3), 2.85 (2H, m, $CHCH_2$), 2.01 (3H, s, CH_3)
δ_C (100 MHz, CD_3OD):	172.3 (C=O), 171.8 (C=O), 171.5 (C=O), 51.6 (OCH_3), 53.4 (CH), 35.3 ($CH\underline{CH_2}$), 20.9 (CH_3)
$[\alpha]_D^{20}$ (c 0.02, $CHCl_3$):	+21
HRMS (m/z ESI ⁻):	Found: 188.0569 ($[M - H]^-$, $C_7H_{11}NO_5$) Requires: 188.0559)
ν_{max} (film)/ cm^{-1} :	3342 (NH), 2941 (OH), 2941 (CH), 1733 (C=O), 1212 (CH)

Ac-Asp(STrt)-OMe (80)

Prepared as per Procedure 4 using **79** (0.35 g, 1.83 mmol) in CH₂Cl₂ (15 mL) and following flash chromatography (EtOAc:Hex - 20:80), the product was obtained as a yellow oil (0.75 g, 90%).

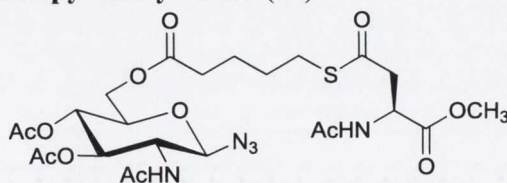
δ_{H} (400 MHz, CDCl₃): 7.36-7.21 (15H, m, Ar-CH), 6.48 (1H, d, J = 8.3 Hz, NH), 4.79 (1H, m, α CH), 3.68 (3H, s, OCH₃), 3.24 (1H, dd, J = 4.9, J = 16.5 Hz, CHCH₂), 3.08 (1H, dd, J = 4.6, J = 16.5 Hz, CHCH₂), 1.97 (3H, s, CH₃)

δ_{C} (100 MHz, CDCl₃): 195.1 (C=O), 170.8 (C=O), 169.8 (C=O), 143.4 (qC), 129.8 (Ar-CH), 127.9 (Ar-CH), 127.3 (Ar-CH), 52.8 (OCH₃), 49.3 (CH), 44.9 (CHCH₂), 23.1 (CH₃)

$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl₃): +9

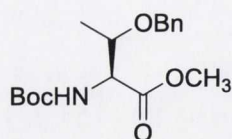
HRMS (m/z ESI⁺): Found: 470.1404 ([M + Na]⁺, C₂₆H₂₅NO₄S)
Requires: 470.1402

ν_{max} (film)/cm⁻¹: 3386 (NH), 1747, 1686 (C=O), 1184 (CH)

2-Deoxy-2-acetamido-3,4-di-O-acetyl-6-O-Ac-L-Asp(OMe)thio-pentanoate- β -D-glucopyranosyl azide (82)

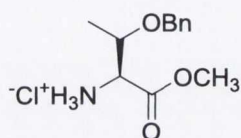
Prepared as per Procedure 5 using **80** (0.54 g, 1.21 mmol) in CH₂Cl₂ (15 mL) to give the crude thioacid **81** which was then immediately subjected to Procedure 6 using **64** (0.35 g, 0.86 mmol) in DMF (3 mL) and following flash chromatography (CH₂Cl₂:CH₃OH - 98:2), the product was obtained as a yellow oil (0.46 g, 87%).

δ_{H} (600 MHz, CDCl_3):	6.70 (1H, d, $J = 7.8$ Hz, NH), 6.51 (1H, d, $J = 8.5$ Hz, NH), 5.29 (1H, app t, $J = 9.9$ Hz, H-3), 5.06 (1H, app t, $J = 9.7$ Hz, H-4), 4.86 (1H, m, H-1), 4.86 (1H, m, CHCH_2), 4.28 (1H, dd, $J = 1.5$, $J = 12.5$ Hz, H-6), 4.20 (1H, dd, $J = 5.3$, $J = 12.5$ Hz, H-6'), 3.99 (1H, q, $J = 9.7$ Hz, H-2), 3.86 (1H, m, H-5), 3.77 (3H, s, OCH_3), 3.23 (1H, dd, $J = 4.6$, $J = 16.5$ Hz, CHCH_2), 3.16 (1H, dd, $J = 4.6$, $J = 16.5$ Hz, CHCH_2), 2.97-2.81 (2H, m, CH_2), 2.40 (2H, m, CH_2), 2.05 (3H, s, CH_3), 2.04 (3H, s, CH_3), 2.04 (3H, s, CH_3), 1.99 (3H, s, CH_3), 1.72 (2H, m, CH_2), 1.63 (2H, m, CH_2)
δ_{C} (150 MHz, CDCl_3):	197.2 (C=O), 172.3 (C=O), 171.1 (C=O), 170.8 (C=O), 170.6 (C=O), 170.2 (C=O), 169.4, (C=O), 88.4 (C-1), 74.3 (C-5), 72.3 (C-3), 68.5 (C-4), 62.0 (C-6), 53.9 (C-2), 49.0 (CHCH_2), 45.1 (CHCH_2), 33.3 (CH_2), 28.5 (CH_2), 28.8 (CH_2), 23.7 (CH_2), 23.1 (CH_3), 23.0 (CH_3), 20.6 (CH_3), 20.6 (CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl_3):	-81
HRMS (m/z ESI $^+$):	Found: 640.1899 ($[\text{M} + \text{Na}]^+$, $\text{C}_{24}\text{H}_{35}\text{N}_2\text{O}_{12}\text{S}$ Requires: 640.1901)
ν_{max} (film)/ cm^{-1} :	3288 (NH), 2117 (N_3), 1743, 1660 (C=O), 1217 (CH)

Boc-Thr(OBzl)-OH (84)

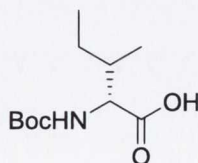
Prepared as per Procedure 1 using Boc-Thr(OBzl)-OH **83** (5.0 g, 16.16 mmol) in DMF (15 mL), the product was obtained as a yellow oil (4.85 g, 93%). The isolated compound was in good agreement with the literature.³⁵³

δ_{H} (400 MHz, CDCl_3):	7.40-7.25 (5H, m, Ar-CH), 5.33 (1H, d, $J = 9.6$ Hz, NH), 4.59 (1H, d, $J = 11.7$ Hz, CH_2OBn), 4.39 (1H, d, $J = 11.7$ Hz, CH_2OBn), 4.34 (1H, dd, $J = 9.7$, $J = 2.3$ Hz, αCH), 4.14 (1H, dddd, $J = 6.2$, $J = 2$ Hz, βCH), 3.71 (3H, s, OCH_3), 1.48 (9H, s, $(\text{CH}_3)_3$), 1.29 (3H, d, $J = 6.3$ Hz, CH_3)
HRMS (m/z ESI $^+$):	Found: 346.1635 ($[\text{M} + \text{Na}]^+$, $\text{C}_{17}\text{H}_{25}\text{NO}_5$) Requires: 346.1630

H-Thr(Obzl)-OH (85)

Prepared as per Procedure 3 using **84** (4.47 g, 13.82 mmol) in CH_2Cl_2 (15 mL), the product was obtained as a yellow oil (3.59 g, >99%). The isolated compound was in good agreement with the literature.³⁵⁴

δ_{H} (400 MHz, D_2O):	7.36-7.21 (5H, m, Ar-CH), 4.56 (1H, d, $J = 11.7$ Hz, CH_2OBn), 4.35 (1H, d, $J = 11.7$ Hz, CH_2OBn), 4.14 (1H, m, βCH), 4.06 (1H, d, $J = 3.3$, αCH), 3.62 (3H, s, OCH_3), 1.25 (3H, d, $J = 6.5$ Hz, CH_3)
HRMS (m/z ESI $^+$):	Found: 224.1286 ($[\text{M} + \text{H}]^+$, $\text{C}_{17}\text{H}_{17}\text{NO}_3$) Requires: 224.1287

Boc-Iso-OH (87)

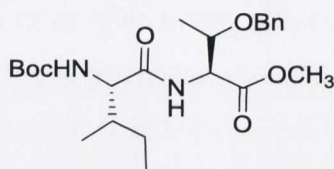
To H-Ile-OH **86** (2.0 g, 15.25 mmol) and NaHCO_3 (0.01 g, 0.15 mmol) in H_2O :dioxane (1:1, 50 mL) was added Boc_2O (13.0 g, 61 mmol) and stirred at rt for 16 h. The mixture was diluted with H_2O (20 mL), washed with CH_2Cl_2 (2 x 20 mL), the aqueous layer acidified to pH2 with sat. aq. HCl solution and

extracted with CH₂Cl₂ (2 x 20 mL). The combined organic layers were washed with H₂O (20 mL), dried over MgSO₄, filtered, and solvent was removed *in vacuo* to give the product as a colourless oil (2.99 g, 85%). The isolated compound was in good agreement with the literature.³⁵⁵

δ_{H} (400 MHz, CDCl₃): 5.06 (1H, d, J = 8.9 Hz, NH), 4.33 (1H, m, α CH), 1.96 (1H, m, β CH), 1.48 (9H, s, C(CH₃)₃), 1.25 (2H, m, CH₂), 1.00 (3H, d, J = 6.8 Hz, CH₃), 0.96 (3H, t, J = 7.4 Hz, CH₂CH₃)

HRMS (m/z ESI): Found: 230.1398 ([M - H]⁻, C₁₁H₂₁NO₄ Requires: 230.1392)

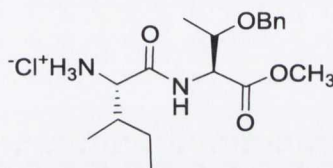
Boc-Iso-Thr(OBzl)-OMe (88)



Prepared as per Procedure 7 using **87** (0.66 g, 2.85 mmol) in CH₂Cl₂:DMF (10 mL) and **85** (0.74 g, 2.85 mmol) in CH₂Cl₂:DMF (10 mL) and following flash chromatography (EtOAc:Hex - 20:80), the product was obtained as a yellow oil (1.10 g, 89%).

δ_{H} (400 MHz, CDCl₃): 7.37-7.21 (5H, m, Ar-CH), 6.60 (1H, d, J = 9.2 Hz, NH), 5.23 (1H, d, J = 8.8 Hz, NH), 4.66 (1H, dd, J = 2.2 Hz, J = 9.1 Hz, Thr α CH), 4.58 (1H, d, J = 11.9 Hz, CH₂OBn), 4.36 (1H, d, J = 11.9 Hz, CH₂OBn), 4.13 (1H, dddd, J = 2.2 Hz, J = 6.3 Hz, Thr β CH), 4.06 (1H, t, J = 7.7 Hz, Iso α CH), 3.64 (3H, s, OCH₃), 1.87 (1H, m, Iso β CH), 1.55 (1H, m, CH₂CH₃), 1.43 (9H, s, C(CH₃)₃), 1.21 (1H, m, CH₂CH₃), 1.20 (3H, d, J = 6.3 Hz, Thr CH₃), 0.96 (3H, d, J = 6.6 Hz, Iso CH₃CH), 0.91 (3H, t, J = 7.2 Hz, Iso CH₃CH₂)

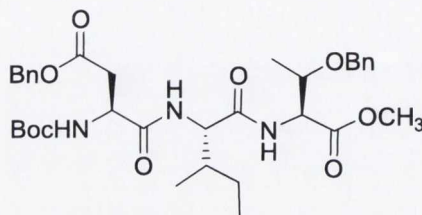
δ_{C} (100 MHz, CDCl_3):	170.9 (C=O), 155.6 (C=O), 172.2 (C=O), 138.0 (qC), 128.4, 127.8 (Ar-CH), 79.6 ($\text{C}(\text{CH}_3)_3$), 73.9 (Thr βCH), 70.8 (CH_2OBn), 59.5 (Iso αCH), 56.6 (Thr αCH), 52.3 (OCH_3), 37.8 (Iso βCH), 28.3 ($\text{C}(\text{CH}_3)_3$), 24.7 (CH_2CH_3), 16.1 (Thr CH_3), 15.4 (Iso CH_3CH), 11.4 (CH_3CH_2)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl_3):	-17
HRMS (m/z ESI $^+$):	Found: 437.2652 ($[\text{M} + \text{H}]^+$, $\text{C}_{23}\text{H}_{36}\text{N}_2\text{O}_6$) Requires: 437.2652
ν_{max} (film)/ cm^{-1} :	3320 (NH), 1751, 1690 (C=O), 1163 (CH)

H-Iso-Thr(OBzl)-OMe (89)

Prepared as per Procedure 3 using **88** (0.44 g, 1.01 mmol) in CH_2Cl_2 (6 mL), the product was obtained as a yellow oil (0.29 g, 76%).

δ_{H} (400 MHz, CD_3OD):	7.40-7.27 (5H, m, Ar-CH), 4.67 (1H, m, Thr αCH), 4.65 (1H, d, $J = 11.4$ Hz, CH_2OBn), 4.44 (1H, d, $J = 11.4$ Hz, CH_2OBn), 4.25 (1H, dddd, $J = 6.3$, $J = 2.7$ Hz, Thr βCH), 3.98 (1H, d, $J = 5.3$ Hz, Iso αCH), 3.70 (3H, s, OCH_3), 2.04 (1H, m, Iso βCH), 1.65 (1H, m, CH_2CH_3), 1.30 (1H, m, CH_2CH_3), 1.30 (3H, d, $J = 6.4$ Hz, Thr CH_3), 1.12 (3H, d, $J = 7.2$ Hz, CH_3CH), 1.01 (3H, t, $J = 7.5$ Hz, CH_3CH_2)
δ_{C} (100 MHz, CD_3OD):	170.8 (C=O), 169.3 (C=O), 138.2 (Ar-CH), 128.3 (Ar-CH), 128.0 (Ar-CH), 127.8 (Ar-CH), 74.2 (Thr βCH), 71.0 (CH_2OBn), 57.8 (Iso αCH), 57.5 (Thr αCH), 51.8 (OCH_3), 37.2 (Iso βCH), 23.8 (CH_2CH_3), 15.4 (Thr CH_3), 14.2 (Iso CH_3CH), 10.8 (CH_3CH_2)

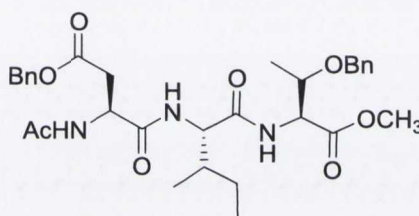
$[\alpha]_D^{20}$ (c 0.02, CH ₃ OH):	-38
HRMS (m/z ESI ⁺):	Found: 337.2120 ([M + H] ⁺ , C ₁₈ H ₂₈ N ₂ O ₄ Requires: 337.2127)
$\nu_{\max}$ (film)/cm ⁻¹ :	3213 (NH), 3032 (NH ₂), 1747, 1672 (C=O), 1211 (CH)

Boc-Asp(OBzl)-Iso-Thr(OBzl)-OMe (90)

Prepared as per Procedure 7 using **71** (0.20 g, 0.62 mmol) in CH₂Cl₂:DMF (5 mL) and **89** (0.23 g, 0.62 mmol) in CH₂Cl₂:DMF (5 mL) and following flash chromatography (EtOAc:Hex - 30:70), the product was obtained as a yellow oil (0.31 g, 79%).

δ_H (400 MHz, CDCl ₃):	7.37-7.22 (10H, m, Ar-CH), 7.16 (1H, d, J = 8.8 Hz, NH), 6.70 (1H, d, J = 8.9 Hz, NH), 5.79 (1H, d, J = 8.9 Hz, NH), 5.13 (2H, s, Asp CH ₂ OBn), 4.66 (1H, dd, J = 1.8, J = 9.0 Hz, Thr α CH), 4.45 (1H, m, Asp α CH), 4.45 (1H, t, J = 7.5 Hz, Iso α CH), 4.56 (1H, m, Thr CH ₂ OBn), 4.37 (1H, d, J = 11.9 Hz, Thr CH ₂ OBn), 4.14 (1H, m, Thr β CH), 3.65 (3H, s, OCH ₃), 3.01 (1H, dd, J = 4.7, J = 17.1 Hz, Asp CHCH ₂), 2.74 (1H, dd, J = 5.8, J = 17.1 Hz, Asp CHCH ₂), 1.95 (1H, m, Iso β CH), 1.46 (9H, s, C(CH ₃) ₃), 1.57 (1H, m, CH ₂ CH ₃), 1.23 (1H, m, CH ₂ CH ₃), 1.19 (3H, d, J = 6.3 Hz, Thr CH ₃), 0.98 (3H, d, J = 6.8 Hz, Iso CH ₃ CH), 0.93 (3H, t, J = 7.6 Hz, Iso CH ₃ CH ₂)
δ_C (100 MHz, CDCl ₃):	171.7 (C=O), 171.2 (C=O), 170.7 (C=O), 170.6 (C=O), 155.5 (C=O), 137.8 (qC), 135.5 (qC), 128.6 (Ar-CH), 128.4 (Ar-CH), 128.3 (Ar-CH),

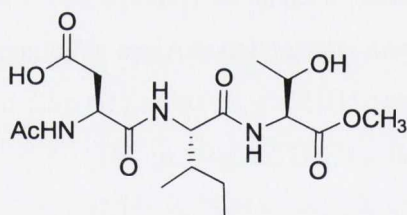
	128.2 (Ar-CH), 127.9 (Ar-CH), 127.8 (Ar-CH), 80.5 ($\underline{\text{C}}(\text{CH}_3)_3$), 73.9 (Thr βCH), 70.7 (Thr $\underline{\text{CH}_2\text{OBn}}$), 67.1 (Asp $\underline{\text{CH}_2\text{OBn}}$), 57.9 (Iso αCH), 56.7 (Thr αCH), 52.2 (OCH_3), 50.9 (Asp αCH), 37.8 (Iso βCH), 37.5 Asp $\text{CH}\underline{\text{CH}_2}$), 28.4 ($\text{C}(\underline{\text{CH}_3})_3$), 24.7 ($\underline{\text{CH}_2\text{CH}_3}$), 16.2 (Thr CH_3), 15.4 (Iso $\underline{\text{CH}_3\text{CH}}$), 11.4 ($\underline{\text{CH}_3\text{CH}_2}$)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CH_3OH):	-13
HRMS (m/z ESI ⁺):	Found: 664.3207 ($[\text{M} + \text{Na}]^+$, $\text{C}_{34}\text{H}_{47}\text{N}_3\text{O}_9$) Requires: 664.3210
ν_{max} (film)/ cm^{-1} :	3290 (NH), 1718, 1646 (C=O), 1161 (CH)

Ac-Asp(OBzl)-Iso-Thr(OBzl)-OMe (91)

Prepared as per Procedure 3 using **90** (0.25 g, 0.39 mmol) in CH_2Cl_2 (6 mL) the hydrochloride salt was obtained as a white solid and immediately subjected to Procedure 9 in sat. aq. NaHCO_3 solution (10 mL) to yield the product as a yellow oil (0.19 g, 83%).

δ_{H} (400 MHz, CDCl_3):	7.43-7.20 (10H, m, Ar-CH), 6.97 (1H, d, $J = 8.3$ Hz, NH), 6.74 (1H, d, $J = 8.3$ Hz, NH), 5.18 (2H, s, Asp $\underline{\text{CH}_2\text{OBn}}$), 4.94 (1H, m, Asp αCH), 4.71 (1H, dd, $J = 2.3$, $J = 9.1$ Hz, Thr αCH), 4.47 (1H, dd, $J = 6.4$, $J = 8.5$ Hz, Iso αCH), 4.62 (1H, d, $J = 11.4$ Hz, Thr $\underline{\text{CH}_2\text{OBn}}$), 4.41 (1H, d, $J = 11.4$ Hz, Thr $\underline{\text{CH}_2\text{OBn}}$), 4.18 (1H, m, Thr βCH), 3.70 (3H, s, OCH_3), 3.02 (1H, dd, $J = 6.8$, $J = 16.9$ Hz, Asp $\text{CH}\underline{\text{CH}_2}$), 2.76 (1H, dd, $J = 4.7$, $J = 16.9$ Hz, Asp $\text{CH}\underline{\text{CH}_2}$), 1.98 (1H, m, Iso βCH), 2.05 (3H, s, CH_3), 1.61 (1H, m, $\underline{\text{CH}_2\text{CH}_3}$), 1.24 (1H,
--	--

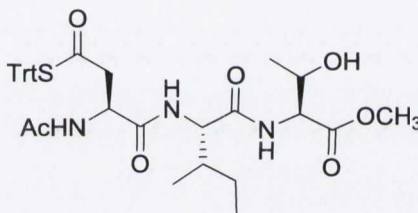
	m, $\underline{\text{CH}_2\text{CH}_3}$), 1.24 (3H, d, $J = 6.3$ Hz, Thr CH_3), 1.04 (3H, d, $J = 6.3$ Hz, Iso $\underline{\text{CH}_3\text{CH}}$), 0.99 (3H, t, $J = 7.3$ Hz, Iso $\underline{\text{CH}_3\text{CH}_2}$)
δ_{C} (100 MHz, CDCl_3):	171.9 (C=O), 171.2 (C=O), 170.8 (C=O), 170.4 (C=O), 170.3 (C=O), 137.8 (qC), 135.5 (qC), 129.1 (Ar-CH), 128.6 (Ar-CH), 128.4 (Ar-CH), 128.4 (Ar-CH), 128.3 (Ar-CH), 127.9 (Ar-CH), 73.9 (Thr βCH), 70.8 (Thr $\underline{\text{CH}_2\text{OBn}}$), 67.0 (Asp $\underline{\text{CH}_2\text{OBn}}$), 58.3 (Iso αCH), 56.8 (Thr αCH), 52.3 (OCH_3), 49.4 (Asp αCH), 37.3 (Iso βCH), 36.1 (Asp $\text{CH}\underline{\text{CH}_2}$), 24.9 ($\underline{\text{CH}_2\text{CH}_3}$), 23.2 (CH_3), 16.1 (Thr CH_3), 15.5 (Iso $\underline{\text{CH}_3\text{CH}}$), 11.5 ($\underline{\text{CH}_3\text{CH}_2}$)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl_3):	-19
HRMS (m/z ESI $^-$):	Found: 618.2581 ($[\text{M} + \text{Cl}]^-$, $\text{C}_{31}\text{H}_{41}\text{N}_3\text{O}_8$) Requires: 618.2582)
ν_{max} (film)/ cm^{-1} :	3281 (NH), 1740, 1640 (C=O), 1383 (CH)

Ac-Asp(OH)-Iso-Thr(OH)-OMe (92)

Prepared as per Procedure 2 using **91** (0.15 g, 0.26 mmol) in $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ (15 mL, 1:1) the product was obtained as a colourless oil (90 mg, 90%).

δ_{H} (400 MHz, CD_3OD):	4.77 (1H, t, $J = 7.2$ Hz, Asp αCH), 4.46 (1H, d, $J = 3.1$ Hz, Thr αCH), 4.35 (1H, d, $J = 7.3$ Hz, Iso αCH), 4.30 (1H, m, Thr βCH), 3.74 (3H, s, OCH_3), 2.86 (1H, dd, $J = 6.0$, $J = 16.8$ Hz, Asp $\text{CH}\underline{\text{CH}_2}$), 2.68 (1H, dd, $J = 7.6$, $J = 16.8$ Hz, Asp $\text{CH}\underline{\text{CH}_2}$), 1.91 (1H, m, Iso βCH), 2.00 (3H, s, CH_3), 1.58 (1H, m, $\underline{\text{CH}_2\text{CH}_3}$), 1.22 (1H, m, $\underline{\text{CH}_2\text{CH}_3}$), 1.19 (3H, d, $J = 6.3$ Hz, Thr CH_3),
---	--

	0.99 (3H, d, $J = 6.7$ Hz, Iso CH_3CH), 0.94 (3H, t, $J = 7.4$ Hz, Iso CH_3CH_2)
δ_{C} (100 MHz, CD_3OD):	172.6 (C=O), 172.4 (C=O), 172.0 (C=O), 171.7 (C=O), 170.8 (C=O), 67.0 (Thr βCH), 57.9 (Iso αCH), 57.9 (Thr αCH), 51.6 (OCH_3), 50.0 (Asp αCH), 36.7 (Iso βCH), 35.3 (Asp CHCH_2), 24.3 (CH_2CH_3), 21.0 (CH_3), 18.9 (Thr CH_3), 14.5 (Iso CH_3CH), 10.2 (CH_3CH_2)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CH_3OH):	-35
HRMS (m/z ESI $^{+}$):	Found: 426.1856 ($[\text{M} + \text{Na}]^{+}$, $\text{C}_{17}\text{H}_{29}\text{N}_3\text{O}_8$) Requires: 426.1852
ν_{max} (film)/ cm^{-1} :	3288 (NH), 2962 (OH), 1723, 1640 (C=O)

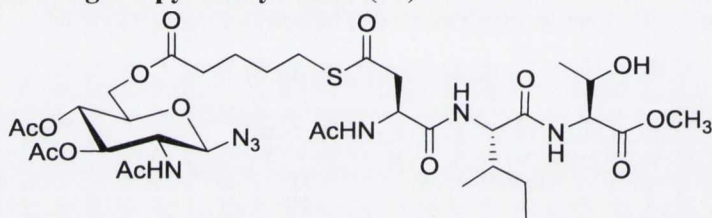
Ac-Asp(STrt)-Iso-Thr(OH)-OMe (93)

Prepared as per Procedure 4 using **92** (0.23 g, 0.57 mmol) in CH_2Cl_2 (15 mL) and following flash chromatography (EtOAc:Hex - 90:10), the product was obtained as a yellow oil (0.29 g, 76%).

δ_{H} (400 MHz, CDCl_3):	7.30-7.18 (15H, m, Ar-CH), 4.78 (1H, dd, $J = 4.5$, $J = 9.5$ Hz, Asp αCH), 4.45 (1H, d, $J = 3.1$ Hz, Thr αCH), 4.32 (1H, d, $J = 7.8$ Hz, Iso αCH), 4.29 (1H, dd, $J = 3.3$, $J = 6.6$ Hz, Thr βCH), 3.74 (3H, s, OCH_3), 3.04 (1H, dd, $J = 4.7$, $J = 15.9$ Hz, Asp CHCH_2), 2.92 (1H, dd, $J = 9.5$, $J = 15.9$ Hz, Asp CHCH_2), 1.96 (3H, s, CH_3), 1.88 (1H, m, Iso βCH), 1.88 (1H, m, CH_2CH_3), 1.56 (1H, m, CH_2CH_3), 1.17 (3H, d, $J = 6.3$ Hz, Thr CH_3), 0.97 (3H, d, $J = 6.9$ Hz, Iso CH_3CH), 0.91 (3H, t, $J = 7.2$ Hz, Iso CH_3CH_2)
--	--

δ_{C} (100 MHz, CDCl_3):	193.6 (C=O), 172.6 (C=O), 171.8 (C=O), 171.1 (C=O), 170.8 (C=O), 143.6 (qC), 129.6 (Ar-CH), 127.4 (Ar-CH), 126.8 (Ar-CH), 70.4 (SqC), 67.0 (Thr β CH), 58.0 (Iso α CH), 57.9 (Thr α CH), 51.5 (OCH_3), 50.1 (Asp α CH), 44.0 (Asp CHCH_2), 36.8 (Iso β CH), 24.5 (CH_2CH_3), 21.1 (CH_3), 19.0 (Thr CH_3), 14.5 (Iso CH_3CH), 10.1 (CH_3CH_2)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl_3):	-22
HRMS (m/z ESI $^{+}$):	Found: 684.2705 ($[\text{M} + \text{Na}]^{+}$, $\text{C}_{36}\text{H}_{43}\text{N}_3\text{O}_7\text{S}$ Requires: 684.2719)
ν_{max} (film)/ cm^{-1} :	3277 (NH), 1692, 1638 (C=O), 1264 (CH)

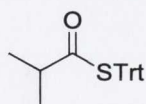
2-Deoxy-2-acetamido-3,4-di-*O*-acetyl-6-*O*-Ac-Asp-Iso-Thr(OH)-OMe-thio-pentanoate- α -D-glucopyranosyl azide (95)



Prepared as per Procedure 5 using **93** (0.12 g, 1.18 mmol) in CH_2Cl_2 (10 mL) to give the crude thioacid **94** which was then immediately subjected to Procedure 6 using **64** (50 mg, 0.12 mmol) in DMF (3 mL) and following flash chromatography (CH_2Cl_2 : CH_3OH - 97:3), the product was obtained as a yellow oil (82 mg, 82%).

δ_{H} (400 MHz, CDCl_3):	7.42 (1H, d, J = 8.4 Hz, NH), 7.37 (1H, d J = 9.1 Hz, NH), 7.04 (2H, m, NH), 5.42 (1H, app t, J = 9.9 Hz, H-3), 5.08 (1H, m, Asp α CH), 5.03 (1H, app t, J = 9.5 Hz, H-4), 4.85 (1H, d, J = 9.1 Hz, H-1), 4.61 (1H, dd, J = 2.8, J = 8.9 Hz, Thr α CH), 4.48 (1H, t, J = 8.2 Hz, Iso α CH), 4.40 (1H, dd, J = 2.5, J = 12.2 Hz, H-6), 4.35 (1H, dd, J = 3.1, J = 6.3 Hz, Thr β CH), 4.14 (1H, m, H-6'), 4.12 (1H, m, H-2), 3.89 (1H, m, H-5), 3.79 (3H, s, OCH_3), 3.18 (1H, dd, J = 6.8, J = 16.2
--	---

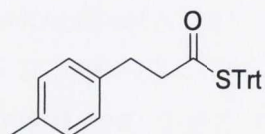
	Hz, Asp CHCH ₂), 2.98 (1H, dd, $J = 5.3$, $J = 16.2$ Hz, Asp CHCH ₂), 2.87 (2H, m, CH ₂), 2.50-2.34 (2H, m, CH ₂), 2.10 (3H, s, CH ₃), 2.08 (3H, s, CH ₃), 2.06 (3H, s, CH ₃), 1.98 (3H, s, CH ₃), 1.96 (1H, m, Iso β CH), 1.75 (1H, m, CH ₂), 1.64 (1H, m, CH ₂), 1.58 (1H, m, CH ₂ CH ₃), 1.22 (3H, d, $J = 6.4$ Hz, Thr CH ₃), 1.18 (1H, m, CH ₂ CH ₃), 0.97 (3H, d, $J = 6.7$ Hz, Iso CH ₃ CH), 0.93 (3H, t, $J = 7.4$ Hz, Iso CH ₃ CH ₂)
δ_C (100 MHz, CDCl ₃):	197.7 (C=O), 172.7 (C=O), 171.7 (C=O), 171.4 (C=O), 171.2 (C=O), 170.8 (C=O), 170.7 (C=O), 169.7 (C=O), 88.3 (H-1), 74.4 (C-5), 72.8 (C-3), 68.9 (C-4) 68.1 (Thr β CH), 62.6 (C-6), 58.3 (Iso α CH), 57.5 (Thr α CH), 53.6 (C-2), 52.6 (OCH ₃), 49.7 (Asp α CH), 45.1 (Asp CHCH ₂), 36.8 (Iso β CH), 33.5 (CH ₂), 28.9 (CH ₂), 28.7 (CH ₂), 24.8 (CH ₂ CH ₃), 24.3 (CH ₂), 23.1 (CH ₃), 23.0 (CH ₃), 20.7 (CH ₃), 20.7 (CH ₃), 20.0 (Thr CH ₃), 15.4 (Iso CH ₃ CH), 11.2 (CH ₃ CH ₂)
$[\alpha]_D^{20}$ (c 0.01, CHCl ₃):	-32
HRMS (m/z ESI):	Found: 866.3031 ($[M + Cl]^-$, C ₃₄ H ₅₃ N ₇ O ₇ S) Requires: 866.3009
$\nu_{\max}$ (film)/cm ⁻¹ :	3660 (OH), 2981 (CH), 2119 (N ₃) 1382 (C=O)

S-trityl-2-methylpropanethioate (100)

Prepared as per Procedure 4 using isobutyric acid (0.5 g, 5.67 mmol) in CH₂Cl₂ (10 mL) and following flash chromatography (EtOAc:Hex - 5:95), the product was obtained as a yellow oil (1.62 g, 84%).

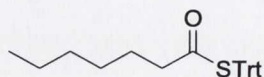
δ_H (400 MHz, CDCl ₃):	7.30-7.20 (15H, m, Ar-CH), 2.74 (1H, m, CH), 1.11 (3H, s, CH ₃), 1.10 (3H, s, CH ₃)
---	--

δ_{C} (100 MHz, CDCl_3):	201.2 (C=O), 144.0 (qC), 129.8 (Ar-CH), 127.7 (Ar-CH), 127.0 (Ar-CH), 69.9 (SqC), 43.0 (CH), 19.2 (CH_3)
HRMS (m/z ESI ⁺):	Found: 369.1299 ($[\text{M} + \text{Na}]^+$, $\text{C}_{23}\text{H}_{22}\text{OS}$) Requires: 369.1289)
ν_{max} (film)/ cm^{-1} :	1681 (C=O), 952 (CH)

S-trityl 3-(*p*-tolyl)propanethioate (101)

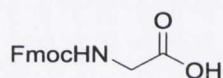
Prepared as per Procedure 4 using 3-(*p*-tolyl)propanoic acid (0.25 g, 1.52 mmol) in CH_2Cl_2 (10 mL) and following flash chromatography (EtOAc:Hex - 5:95), the product was obtained as a yellow oil (0.51 g, 80%).

δ_{H} (400 MHz, CDCl_3):	7.33-7.17 (15H, m, Ar-CH), 7.12 (2H, d, $J = 7.5$ Hz, Ar-CH), 7.04 (2H, d, $J = 7.5$ Hz, Ar-CH), 2.90-2.78 (4H, m, CH_2), 2.34 (3H, s, CH_3)
δ_{C} (100 MHz, CDCl_3):	196.1 (C=O), 143.8 (qC), 136.9 (qC), 135.8 (qC), 129.8 (Ar-CH), 129.2 (Ar-CH), 128.4 (Ar-CH), 127.8 (Ar-CH), 127.1 (Ar-CH), 70.4 (SqC), 45.3 (CH_2), 30.9 (CH_2), 21.1 (CH_3)
HRMS (m/z ESI ⁺):	Found: 445.1603 ($[\text{M} + \text{Na}]^+$, $\text{C}_{29}\text{H}_{26}\text{OS}$) Requires: 445.1602)
ν_{max} (film)/ cm^{-1} :	1693 (C=O), 1443 (CH)

S-tritylheptanethioate (102)

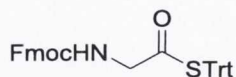
Prepared as per Procedure 4 using heptanoic acid (0.3 mL, 2.30 mmol) in CH_2Cl_2 (6 mL) and following flash chromatography (EtOAc:Hex - 5:95), the product was obtained as a yellow oil (1.59 g, 81%).

δ_{H} (400 MHz, CDCl_3):	7.34 (15H, m, Ar-CH), 2.52 (2H, t, $J = 7.3$ Hz, CH_2), 1.61 (2H, m, CH_2), 1.28 (2H, m, CH_2), 0.91 (3H, t, $J = 7.1$ Hz, CH_3)
δ_{C} (100 MHz, CDCl_3):	197.1 (C=O), 144.0 (qC), 129.8 (Ar-CH), 127.8 (Ar-CH), 127.1 (Ar-CH), 70.2 (SqC), 43.8 (CH_2), 31.4 (CH_2), 28.5 (CH_2), 25.7 (CH_2), 22.5 (CH_2), 14.0 (CH_3)
HRMS (m/z ESI ⁺):	Found: 411.1772 ($[\text{M} + \text{Na}]^+$, $\text{C}_{26}\text{H}_{28}\text{OS}$) Requires: 411.1759
ν_{max} (film)/ cm^{-1} :	1694 (C=O), 738 (CH)

Fmoc-Gly

To glycine (1.0 g, 13.32 mmol) in H_2O :dioxane (1:1, 20 mL) was added K_2CO_3 (2.95 g, 21.31 mmol) and FmocOSu (4.94 g, 14.65 mmol) and mixture was stirred at rt for 16 h. The dioxane was removed *in vacuo* and the mixture was washed with Et_2O (2 x 10 mL). The aqueous layer was acidified to pH2 with aq. HCl solution and extracted with Et_2O (2 x 20mL), dried over MgSO_4 , filtered, and solvent was removed *in vacuo* to yield the product as a white solid (3.42 g, 86%). M.p. 165-167 °C, Lit., 166-167 °C.³⁵⁶ The isolated compound was in good agreement with the literature.³⁵⁷

δ_{H} (400 MHz, CD_3OD):	7.77 (2H, d, $J = 7.2$ Hz, Ar-CH), 7.65 (2H, d, $J = 7.6$ Hz, Ar-CH), 7.36 (2H, t, $J = 7.2$ Hz, Ar-CH), 7.28 (2H, t, $J = 7.2$ Hz, Ar-CH), 4.32 (2H, d, $J = 7.1$ Hz, CH_2CH), 4.21 (1H, t, $J = 7.0$ Hz, CH_2CH), 3.82 (2H, s, $\text{CH}_2\text{C=O}$)
HRMS (m/z ESI ⁺):	Found: 320.0900 ($[\text{M} + \text{Na}]^+$, $\text{C}_{17}\text{H}_{15}\text{NO}_4$) Requires: 320.0899

Fmoc-Gly-STrt (103)

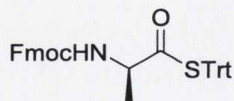
Prepared as per Procedure 4 using Fmoc-Gly (1.45 g, 4.88 mmol) in CH₂Cl₂ (20 mL) and following flash chromatography (EtOAc:Hex - 10:90), the product was obtained as a yellow solid (1.87 g, 69%).

δ_H (400 MHz, CDCl₃): 7.79 (2H, d, J = 7.5 Hz, Ar-CH), 7.60 (2H, d, J = 7.5 Hz, Ar-CH), 7.42 (2H, t, J = 7.5 Hz, Ar-CH), 7.35-7.23 (17H, m, Ar-CH), 5.32 (2H, d, J = 5.4 Hz, NH), 4.42 (2H, d, J = 7.1 Hz, CH₂CH), 4.23 (1H, t, J = 7.2 Hz, CH₂CH), 4.14 (2H, m, CH₂C=O)

δ_C (100 MHz, CDCl₃): 194.4 (C=O), 156.0 (C=O), 143.7 (qC), 143.4 (qC), 141.3 (qC), 129.8 (Ar-CH), 127.9 (Ar-CH), 127.8 (Ar-CH), 127.1 (Ar-CH), 125.1 (Ar-CH), 120.0 (Ar-CH), 70.9 (SqC), 67.0 (CH₂CH), 50.6 (CH₂C=O), 47.3 (CH₂CH)

HRMS (m/z ESI⁺): Found: 578.1779 ([M + Na]⁺, C₃₆H₂₉NO₃S)
Requires: 578.1766)

$\nu_{\max}$ (film)/cm⁻¹: 3410 (NH), 1697 (C=O), 1184 (CH)

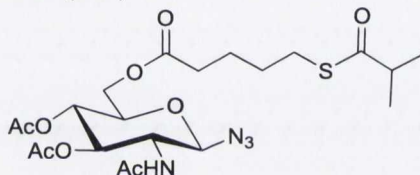
Fmoc-Ala-STrt (104)

Prepared as per Procedure 4 using Fmoc-Ala (1.0 g, 3.21 mmol) in CH₂Cl₂ (15 mL) and following flash chromatography (EtOAc:Hex - 20:80), the product was obtained as a yellow solid (1.51 g, 83%).

δ_H (400 MHz, CDCl₃): 7.79 (2H, d, J = 7.7 Hz, Ar-CH), 7.61 (2H, d, J = 7.5 Hz, Ar-CH), 7.42 (2H, t, J = 7.3 Hz, Ar-CH), 7.35-7.22 (17H, m, Ar-CH), 5.26 (2H, d, J = 7.9 Hz, NH), 4.49 (1H, CHCH₃), 4.49-4.35 (2H, m,

	CH_2CH), 4.25 (1H, m, CH_2CH), 1.36 (3H, d, $J = 7.2$ Hz, CH_3)
δ_{C} (100 MHz, CDCl_3):	198.0 (C=O), 143.4 (C=O), 141.3 (qC), 129.8 (qC), 127.9 (qC), 127.9 (Ar-CH), 127.7 (Ar-CH), 127.2 (Ar-CH), 127.2 (Ar-CH), 127.1 (Ar-CH), 125.1 (Ar-CH), 120.0 (Ar-CH), 70.6 (CHCH_3), 67.1 (SqC), 56.6 (CH_2CH), 47.1 (CH_2CH), 19.2 (CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.05, CHCl_3):	-10
HRMS (m/z ESI ⁺):	Found: 592.1921 ($[\text{M} + \text{Na}]^+$, $\text{C}_{37}\text{H}_{31}\text{NO}_3\text{S}$) Requires: 592.1922
ν_{max} (film)/ cm^{-1} :	1710 (C=O), 1211 (CH)

2-Deoxy-2-acetamido-3,4-di-*O*-acetyl-6-*O*-methylpropane-thio-pentanoate- β -D-glucopyranosyl azide (107)

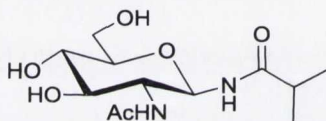


Prepared as per Procedure 5 using **100** (0.12 g, 0.36 mmol) in CH_2Cl_2 (8 mL) to give the crude thioacid **106** which was then immediately subjected to Procedure 6 using **64** (70 mg, 0.18 mmol) in DMF (4 mL) and following flash chromatography ($\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH} - 99:1$), the product was obtained as a yellow oil (80 mg, 88%).

δ_{H} (400 MHz, CDCl_3):	5.67 (1H, d, $J = 8.1$ Hz, NH), 5.26 (1H, app t, $J = 9.8$ Hz, H-3), 5.11 (1H, app t, $J = 9.8$ Hz, H-4), 4.79 (1H, d, $J = 9.3$ Hz, H-1), 4.28 (1H, dd, $J = 4.7$, $J = 12.3$ Hz, H-6), 4.21 (1H, dd, $J = 2.2$, $J = 12.3$ Hz, H-6'), 3.94 (1H, q, $J = 9.7$ Hz, H-2), 3.81 (1H, m, H-5), 2.89 (2H, t, $J = 6.9$ Hz, CH_2), 2.76 (1H, m, CH), 2.41 (2H, t, $J = 7.8$ Hz, CH_2), 2.06 (3H, s, CH_3), 2.06 (3H, s, CH_3), 2.00 (3H, s, CH_3), 1.74 (2H, m, CH_2), 1.64 (2H, m, CH_2), 1.22, 1.20 (3H, s, CHCH_3)
--	--

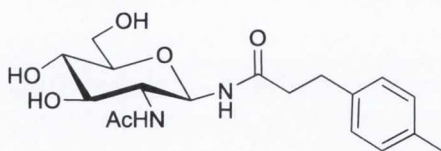
δ_{C} (100 MHz, CDCl_3):	204.2 (C=O), 172.9 (C=O), 171.0 (C=O), 170.4 (C=O), 169.2, (C=O), 88.6 (C-1), 74.2 (C-5), 72.2 (C-3), 68.2 (C-4), 62.0 (C-6), 54.4 (C-2), 43.1 (CH), 33.4 (CH_2), 29.1 (CH_2), 28.0 (CH_2), 23.9 (CH_2), 23.3 (CH_3), 20.6 (CH_3), 20.6 (CH_3), 19.4 (CHCH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl_3):	-55
HRMS (m/z ESI ⁺):	Found: 539.1782 ($[\text{M} + \text{Na}]^+$, $\text{C}_{21}\text{H}_{32}\text{N}_4\text{O}_9\text{S}$) Requires: 539.1788)
ν_{max} (film)/ cm^{-1} :	2981 (NH), 2116 (N_3), 1675 (C=O), 1234 (CH)

2-Deoxy-2-acetamido- β -D-glucopyranosyl-1-N-isobutyramide (**108**)



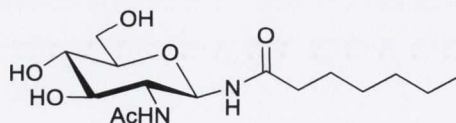
Prepared as per Procedure 8 using **65** (70 mg, 0.18 mmol) and **100** (90 mg, 0.27 mmol) in DMF (6 mL) and following flash chromatography (CH_2Cl_2 : CH_3OH - 91:9), the product was obtained as a colourless oil (31 mg, 61%).

δ_{H} (400 MHz, CD_3OD):	4.96 (1H, d, $J = 9.3$ Hz, H-1), 4.86 (1H, dd, $J = 1.2$, $J = 12.4$ Hz, H-6), 3.79 (1H, app t, $J = 9.9$ Hz, H-2), 3.69 (1H, m, H-6'), 3.48 (1H, m, H-3), 3.36 (1H, m, H-4), 3.35 (1H, m, H-5), 2.42 (1H, m, CH), 1.97 (3H, s, CH_3)
δ_{C} (100 MHz, CD_3OD):	179.4 (C=O), 173.3 (C=O), 79.5 (C-1), 78.8 (C-5), 75.2 (C-3), 70.9 (C-4), 61.6 (C-6), 55.4 (C-2), 35.5 (CH), 21.6 (CH_3), 19.0, 18.2 (CHCH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CH_3OH):	-38
HRMS (m/z ESI ⁻):	Found: 289.1396 ($[\text{M} - \text{H}]^-$, $\text{C}_{12}\text{H}_{22}\text{N}_2\text{O}_6$) Requires: 289.1400)
ν_{max} (film)/ cm^{-1} :	3301 (NH), 2982 (OH), 1666 (C=O)

2-Deoxy-2-acetamido-β-D-glucopyranosyl-1-N-(p-tolyl)propanamide (109)

Prepared as per Procedure 8 using **65** (50 mg, 0.13 mmol) and **101** (82 mg, 0.2 mmol) in DMF (5 mL) and following flash chromatography (CH₂Cl₂:CH₃OH - 90:10), the product was obtained as a colourless oil (28 mg, 59%).

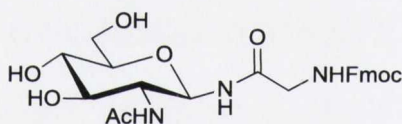
δ_{H} (600 MHz, CD ₃ OD):	7.09 (4H, s, Ar-CH), 4.98 (1H, d, $J = 9.8$ Hz, H-1), 3.86 (1H, dd, $J = 1.4$, $J = 11.9$ Hz, H-6), 3.75 (1H, app t, $J = 10.0$ Hz, H-2), 3.69 (1H, m, H-6'), 3.47 (1H, m, H-3), 3.35 (1H, m, H-5), 3.33 (1H, m, H-4), 2.85 (2H, m, CH ₂), 2.47 (2H, t, $J = 7.8$ Hz, CH ₂), 2.30 (3H, s, CH ₃), 1.91 (3H, s, Ar-CH ₃)
δ_{C} (150 MHz, CD ₃ OD):	174.5 (C=O), 173.0 (C=O), 137.5 (qC), 135.3 (qC), 128.7 (Ar-CH), 127.8 (Ar-CH), 80.0 (C-1), 78.4 (C-5), 75.1 (C-3), 70.5 (C-4), 61.3 (C-4), 54.7 (C-2), 38.0 (CH ₂), 20.5 (CH ₂), 21.4 (Ar-CH ₃), 19.4 (CH ₃)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CH ₃ OH):	-63
HRMS (m/z ESI ⁺):	Found: 389.1708 ([M + Na] ⁺ , C ₁₈ H ₂₆ N ₂ O ₆) Requires: 389.1689
ν_{max} (film)/cm ⁻¹ :	3301 (NH), 2981 (OH), 2981 (CH), 1655 (C=O)

2-Deoxy-2-acetamido-β-D-glucopyranosyl-1-N-heptaneamide (110)

Prepared as per Procedure 8 using **65** (50 mg, 0.13 mmol) and **102** (80 mg, 0.2 mmol) in DMF (5 mL) and following flash chromatography (CH₂Cl₂:CH₃OH - 91:9), the product was obtained as a colourless oil (25 mg, 58%).

δ_{H} (600 MHz, CD_3OD):	4.98 (1H, d, $J = 9.7$ Hz, H-1), 3.86 (1H, dd, $J = 1.3$, $J = 12.6$ Hz, H-6), 3.77 (1H, app t, $J = 9.6$ Hz, H-2), 3.69 (1H, m, H-6'), 3.48 (1H, m, H-3), 3.35 (1H, m, H-5), 3.34 (1H, m, H-4), 2.19 (2H, t, $J = 7.3$ Hz, CH_2), 1.60 (2H, m, CH_2), 1.32 (2H, m, CH_2), 0.93 (2H, t, $J = 6.9$ Hz, CH_2), 1.98 (3H, s, CH_3)
δ_{C} (150 MHz, CD_3OD):	175.4 (C=O), 172.8 (C=O), 79.2 (C-1), 78.3 (C-5), 75.0 (C-3), 70.4 (C-4), 61.2 (C-4), 54.7 (C-2), 21.5 (CH_3), 35.9 (CH_2), 31.3 (CH_2), 28.5 (CH_2), 25.4 (CH_2), 22.2 (CH_2), 13.3 (CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CH_3OH):	-49
HRMS (m/z ESI^+):	Found: 355.1841 ($[\text{M} + \text{Na}]^+$, $\text{C}_{15}\text{H}_{28}\text{N}_2\text{O}_6$) Requires: 355.1845
ν_{max} (film)/ cm^{-1} :	3309 (NH), 2949 (OH), 1654 (C=O)

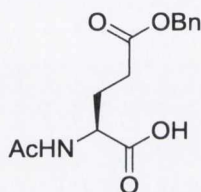
2-Deoxy-2-acetamido- β -D-glucopyranosyl-1-N-FmocGly amide (111)



Prepared as per Procedure 8 using **65** (50 mg, 0.13 mmol) and **103** (0.11 g, 0.2 mmol) in DMF (5 mL) and following flash chromatography (CH_2Cl_2 : CH_3OH - 92:8), the product was obtained as a colourless oil (41 mg, 64%).

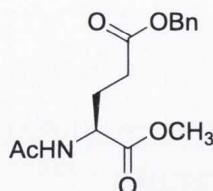
δ_{H} (400 MHz, CD_3OD):	7.82 (2H, d, $J = 7.3$ Hz, Ar-CH), 7.71 (2H, d, $J = 7.8$ Hz, Ar-CH), 7.41 (2H, t, $J = 7.6$ Hz, Ar-CH), 7.33 (2H, t, $J = 7.4$ Hz, Ar-CH), 4.96 (1H, d, $J = 9.9$ Hz, H-1), 4.40 (2H, m, CH_2CH), 4.28 (1H, t, $J = 6.9$ Hz, CH_2CH), 3.85 (1H, m, H-6), 3.80 (1H, app t, $J = 9.8$ Hz, H-2), 3.75 (2H, d, $J = 6.1$ Hz, $\text{CH}_2\text{C=O}$), 3.69 (1H, m, H-6'), 3.49 (1H, m, H-3), 3.36 (1H, m, H-4), 3.36 (1H, m, H-5)
δ_{C} (100 MHz, CD_3OD):	173.3 (C=O), 171.7 (C=O), 157.6 (C=O), 144.0 (qC), 143.8 (qC), 127.4 (Ar-CH), 126.7 (Ar-CH),

	124.9 (Ar-CH), 119.5 (Ar-CH), 79.4 (C-1), 78.4 (C-5), 74.7 (C-3), 70.7 (C-4), 66.8 ($\underline{\text{CH}_2\text{CH}}$), 61.3 (C-6), 54.5 (C-2), 47.0 ($\underline{\text{CH}_2\text{C=O}}$), 43.5 ($\underline{\text{CH}_2\text{C=O}}$), 21.1 (CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CH_3OH):	-70
HRMS (m/z ESI ⁺):	Found: 302.1009 ($[\text{M} + \text{Na}]^+$, $\text{C}_{14}\text{H}_{17}\text{NO}_5$) Requires: 302.1004
ν_{max} (film)/ cm^{-1} :	3600 (OH), 1867 (C=O), 1300 (CH)

Ac-Glu(OBzl)-OH (119)

Prepared as per Procedure 9 using Glu(OBzl)-OH **118** (1.5 g, 6.32 mmol) in sat. aq. NaHCO_3 solution (15 mL) to give the product as a colourless oil (1.75 g, 99%).

δ_{H} (600 MHz, CDCl_3):	7.38-7.28 (5H, m, Ar-CH), 6.59 (1H, d, $J = 6.1$ Hz, NH), 5.11 (2H, s, CH_2Ar), 4.53 (1H, m, αCH), 2.61 (1H, m, CH_2), 2.47 (1H, m, CH_2), 2.22 (1H, m, CH_2), 2.07 (1H, m, CH_2)
δ_{C} (150 MHz, CDCl_3):	173.7 (C=O), 171.6 (C=O), 170.2 (C=O), 135.7 (qC), 128.7 (Ar-CH), 128.5 (Ar-CH), 128.4 (Ar-CH), 66.9 ($\underline{\text{CH}_2\text{OBn}}$), 52.4 (αCH), 30.5 (CH_2), 26.4 (CH_2), 22.9 (CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.04, CHCl_3):	-25
HRMS (m/z ESI ⁺):	Found: 302.1010 ($[\text{M} + \text{Na}]^+$, $\text{C}_{14}\text{H}_{17}\text{NO}_5$) Requires: 302.1004
ν_{max} (film)/ cm^{-1} :	3665 (NH), 2772 (OH), 1849, 17528 (C=O)

Ac-Glu(OBzl)-OMe (120)

Prepared as per Procedure 1 using **119** (1.73 g, 6.19 mmol) in DMF (12 mL) to give the product as a colourless oil (1.61 g, 88%).

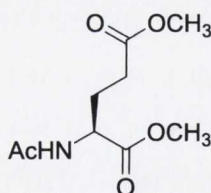
δ_{H} (400 MHz, CDCl_3): 7.36 (5H, m, Ar-CH), 6.57 (1H, br s, NH), 5.11 (2H, s, CH_2OBn), 4.63 (1H, m, αCH), 3.72 (3H, s, OCH_3), 2.46 (2H, m, CH_2), 2.21 (1H, m, CH_2), 2.02 (1H, m, CH_2), 1.99 (3H, s, CH_3)

δ_{C} (100 MHz, CDCl_3): 172.7 (C=O), 172.5 (C=O), 170.2 (C=O), 135.7 (qC), 128.6 (Ar-CH), 128.4 (Ar-CH), 128.3 (Ar-CH), 66.5 (CH_2OBn), 52.5 (OCH_3), 51.6 (αCH), 30.4 (CH_2), 27.3 (CH_2), 23.0 (CH_3)

$[\alpha]_{\text{D}}^{20}$ (c 0.05, CHCl_3): -39

HRMS (m/z ESI^+): Found: 316.1147 ($[\text{M} + \text{Na}]^+$, $\text{C}_{15}\text{H}_{19}\text{NO}_5$)
Requires: 316.1161

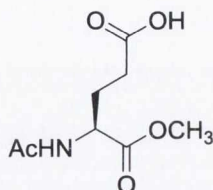
ν_{max} (film)/ cm^{-1} : 3661 (NH), 1722, 1578 (C=O)

Ac-Glu(OMe)-OMe (121)

Prepared as per Procedure 2 using **120** (0.25 g, 0.85 mmol) in $\text{CH}_3\text{OH}:\text{CH}_2\text{Cl}_2$ (1:1, 20 mL) to give the product as a colourless oil (0.18 g, >99%). The isolated compound was in good agreement with the literature.³⁵⁸

δ_{H} (400 MHz, CDCl_3): 4.46 (1H, s, αCH), 3.73 (OCH_3), 3.68 (3H, s, OCH_3), 2.44 (2H, m, CH_2), 2.16 (1H, m, CH_2), 1.95 (1H, m, CH_2), 2.00 (3H, s, CH_3)

HRMS (m/z ESI⁺): Found: 218.1023 ([M + H]⁺, C₉H₁₅NO₅ Requires: 218.1023)

Ac-Glu(OH)-OMe (122)

Prepared as per Procedure 2 using **120** (0.90 g, 3.07 mmol) in EtOAc (15 mL) to give the product as a colourless oil (0.6 g, 97%).

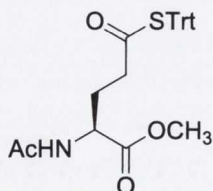
δ_H (400 MHz, CD₃OD): 4.46 (1H, s, α CH), 3.73 (3H, s, OCH₃), 2.44 (2H, m, CH₂), 2.17 (1H, m, CH₂), 1.94 (1H, m, CH₂), 2.00 (3H, s, CH₃)

δ_C (100 MHz, CD₃OD): 173.5 (C=O), 172.5 (C=O), 172.4 (C=O), 52.0 (α CH), 51.8 (OCH₃), 30.0 (CH₂), 26.5 (CH₂), 21.2 (CH₃)

$[\alpha]_D^{20}$ (c 0.02, CHCl₃): -19

HRMS (m/z ESI⁺): Found: 226.0699 ([M + Na]⁺, C₈H₁₃NO₅ Requires: 226.0691)

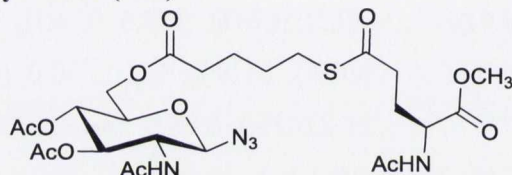
$\nu_{\max}$ (film)/cm⁻¹: 3288 (NH), 2954 (OH), 1831, 1738 (C=O), 1210 (CH)

Ac-Glu(STrt)-OMe (123)

Prepared as per Procedure 4 using **122** (0.5 g, 2.46 mmol) in CH₂Cl₂ (15 mL) and following flash chromatography (EtOAc:Hex - 20:80), the product was obtained as a yellow oil (0.98 g, 86%).

δ_{H} (400 MHz, CDCl_3):	7.37-7.22 (5H, m, Ar-CH), 6.45 (1H, m, NH), 4.57 (1H, m, αCH), 3.71 (3H, s, OCH_3), 2.65 (2H, m, CH_2), 2.12 (1H, m, CH_2), 1.98 (3H, s, CH_3), 1.97 (1H, m, CH_2)
δ_{C} (100 MHz, CDCl_3):	196.3 (C=O), 172.3 (C=O), 170.3 (C=O), 143.6 (qC), 129.8 (Ar-CH), 127.9 (Ar-CH), 127.2 (Ar- CH), 70.6 (SqC), 52.7 (OCH_3), 51.5 (αCH), 39.7 (CH_2), 27.4 (CH_2), 23.2 (CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl_3):	-42
HRMS (m/z ESI $^+$):	Found: 484.1553 ($[\text{M} + \text{Na}]^+$, $\text{C}_{27}\text{H}_{27}\text{NO}_4\text{S}$ Requires: 484.1559)
ν_{max} (film)/ cm^{-1} :	3287 (NH), 1742, 1691 (C=O), 1211 (CH)

2-Deoxy-2-acetamido-3,4-di-*O*-acetyl-6-*O*-Ac-Glu(OMe)-thio-pentanoate- β -D-glucopyranosyl azide (125)



Prepared as per Procedure 5 using **123** (0.17 g, 0.36 mmol) in CH_2Cl_2 (5 mL) to give the crude thioacid **124** which was then immediately subjected to Procedure 6 using **64** (0.1 g, 0.24 mmol) in DMF (5 mL) and following flash chromatography ($\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ - 99:1), the product was obtained as a yellow oil (0.14 g, 94%).

δ_{H} (600 MHz, $\text{DMSO}-d_6$):	8.31 (1H, d, $J = 7.4$ Hz, NH), 8.12 (1H, d, $J = 9.2$ Hz, NH), 5.10 (1H, app t, $J = 10.2$ Hz, H-3), 4.88 (2H, m H-1, H-4), 4.23 (1H, m, CHCH_2), 4.18 (1H, dd, $J = 4.6$, $J = 12.8$ Hz, H-6), 4.12 (1H, dd, $J = 1.2$, $J = 12.1$ Hz, H-6'), 3.99 (1H, m, H-5), 3.81 (1H, q, $J = 9.9$ Hz, H-2), 2.84 (2H, m, CH_2), 2.65 (2H, m, CH_2), 2.51 (3H, s, OCH_3), 2.33 (2H, m, CH_2), 2.00 (2H, m, CH_2), 1.97 (3H, s, CH_3), 1.92 (3H, s, CH_3), 1.85 (3H, s, CH_3), 1.81
--	---

(3H, s, CH₃), 1.85 (2H, m, CH₂), 1.55 (2H, m, CH₂)

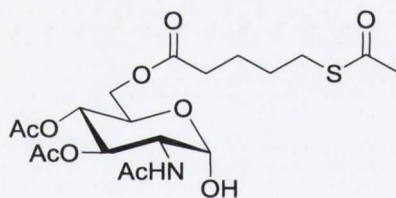
δ_C (150 MHz, DMSO-*d*₆): 198.3 (C=O), 172.7 (C=O), 172.4 (C=O), 170.2 (C=O), 170.2 (C=O), 170.0 (C=O), 169.6 (C=O), 87.7 (H-1), 73.2 (H-5), 72.6 (H-1), 68.3 (H-4), 61.6 (C-6), 52.9 (C-2), 51.2 (α CH), 39.5 (OCH₃), 39.5 (CH₂), 33.1 (CH₂), 28.6 (CH₂), 30.0 (CH₂), 26.5 (CH₂), 23.6 (CH₂), 22.8 (CH₃), 22.4 (CH₃), 20.6 (CH₃), 20.5 (CH₃)

$[\alpha]_D^{20}$ (c 0.01, CHCl₃): -50

HRMS (*m/z* ESI⁺): Found: 654.2060 ([M + Na]⁺, C₂₅H₃₇N₅O₁₂S)
Requires: 654.2057)

$\nu_{\max}$ (film)/cm⁻¹: 1737, 1684 (C=O), 1222 (CH)

2-Deoxy-2-acetamido-3,4-di-*O*-acetyl-6-*O*-acetylthiopentanoate- α -D-glucopyranose (131)

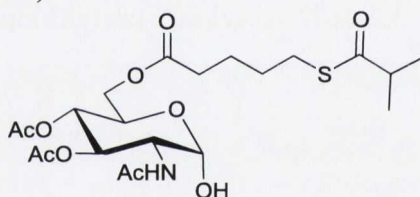


To **67** (50 mg, 0.10 mmol) in EtOAc (10 mL) was added Pd/C (10 mg, 0.10 mmol) was added and the suspension deoxygenated with a N₂ bubbler for 10 min. The solution was then placed under a H₂ atmosphere and stirred at rt for 2 h. The mixture was filtered through a plug of celite and the solvent removed *in vacuo* to give the product as a yellow oil (44 mg, 96%).

δ_H (600 MHz, CDCl₃): 5.92 (1H, d, *J* = 9.4 Hz, NH), 5.31 (1H, app t, *J* = 9.5 Hz, H-3), 5.27 (1H, br s, H-1), 5.09 (1H, app t, *J* = 9.5 Hz, H-4), 4.31 (1H, m, H-2), 4.25-4.18 (2H, m, H-6, H-6'), 4.24 (1H, m, H-5), 2.90 (1H, t, *J* = 7.5 Hz, CH₂), 2.40 (1H, t, *J* = 7.5 Hz, CH₂), 2.35 (3H, s, CH₃), 2.06 (3H, s, CH₃), 2.04 (3H, s,

	CH ₃), 1.98 (3H, s, CH ₃), 1.72 (2H, m, CH ₂), 1.64 (2H, m, CH ₂)
δ_{C} (150 MHz, CDCl ₃):	196.9 (C=O), 173.0 (C=O), 171.4 (C=O), 170.3 (C=O), 169.4 (C=O), 91.8 (C-1), 71.0 (C-3), 68.5 (C-4), 67.6 (C-5), 62.3 (C-6), 52.3 (C-2), 33.5 (CH ₂), 29.0 (CH ₂), 28.7 (CH ₂), 23.9 (CH ₂), 30.6 (CH ₃), 23.2 (CH ₃), 20.7 (CH ₃), 20.6 (CH ₃)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl ₃):	+72
HRMS (m/z ESI ⁺):	Found: 486.1415 ([M + Na] ⁺ , C ₁₉ H ₂₉ NO ₁₀ S) Requires: 486.1410
ν_{max} (film)/cm ⁻¹ :	3356 (NH), 2923 (OH), 1740, 1687 (C=O), 1219 (CH)

2-Deoxy-2-acetamido-3,4-di-*O*-acetyl-6-*O*-methylpropane-thio-pentanoate- α -D-glucopyranose (132)

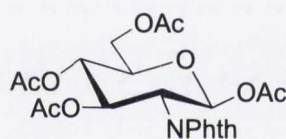


To **107** (70 mg, 0.14 mmol) in EtOAc (12 mL) was added Pd/C (10 mg, 0.10 mmol) was added and the suspension deoxygenated with a N₂ bubbler for 10 min. The solution was then placed under a H₂ atmosphere and stirred at rt for 2 h. The mixture was filtered through a plug of celite and the solvent was removed *in vacuo* to give the product as a yellow oil (63 mg, 95%).

δ_{H} (600 MHz, CDCl ₃):	5.85 (1H, d, J = 9.3 Hz, NH), 5.32 (1H, app t, J = 9.9 Hz, H-3), 5.28 (1H, d, J = 3.4 Hz, H-1), 5.09 (1H, app t, J = 9.9 Hz, H-4), 4.33 (1H, m, H-2), 4.26 (1H, m, H-5), 4.25-4.18 (2H, m, H-6, H-6'), 2.88 (1H, t, J = 7.2 Hz, CH ₂), 2.77 (1H, m, CH), 2.41 (1H, t, J = 7.5 Hz, CH ₂), 2.07 (3H, s, CH ₃), 2.05 (3H, s, CH ₃), 1.99 (3H, s, CH ₃), 1.72 (2H, m, CH ₂), 1.64 (2H, m, CH ₂), 1.73 (2H, m, CH ₂),
--	--

	1.65 (2H, m, CH ₂), 1.22 (3H, d, $J = 1.7$ Hz, CHCH ₃), 2.20 (3H, d, $J = 1.5$ Hz, CHCH ₃)
δ_{C} (150 MHz, CDCl ₃):	205.3 (C=O), 173.0 (C=O), 171.4 (C=O), 170.1 (C=O), 169.4, (C=O), 91.8 (C-1), 71.0 (C-3), 68.7 (C-4), 67.8 (C-5), 62.5 (C-6), 52.3 (C-2), 43.2 (CH), 33.5 (CH ₂), 29.1 (CH ₂), 28.1 (CH ₂), 24.0 (CH ₂), 23.2 (CH ₃), 20.8 (CH ₃), 20.7 (CH ₃), 19.4 (CHCH ₃)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl ₃):	+88
HRMS (m/z ESI ⁺):	Found: 514.1713 ([M + Na] ⁺ , C ₂₁ H ₃₃ NO ₁₀ S) Requires: 514.1723)
ν_{max} (film)/cm ⁻¹ :	3346 (NH), 2969 (OH), 1959, 1740 (C=O), 1219 (CH)

2-Deoxy-2-*N*-phthalimido-1,3,4,6-tetra-*O*-acetyl- β -D-glucopyranose (135)

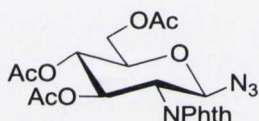


To a solution of D-glucosamine hydrochloride (5.0 g, 23.19 mmol) in CH₃OH (100 mL) was added a catalytic amount of freshly prepared NaOCH₃ and the reaction mixture was stirred at rt for 15 min. The resulting precipitate was filtered and phthalic anhydride (3.43 g, 23.19 mmol) was added to the filtrate. The solution was heated to 60 °C for 25 min resulting in the formation of a thick white precipitate. The reaction mixture was cooled to 0 °C and the precipitate was filtered and washed with hexane (2 x 100 mL) and dried *in vacuo* to afford a white solid. To this was added pyridine (36 mL, 0.46 mol) and the solution was cooled to 0 °C. Ac₂O (47 mL, 0.46 mol) was added slowly and the solution was stirred at rt for 16 h. The mixture was filtered and the filtrate was added to sat. aq. NaHCO₃ solution (300 mL). The product was extracted with CH₂Cl₂ (3 x 100 mL) and the combined organic layers were washed with H₂O (3 x 100 mL), dried over MgSO₄, filtered, and the solvent removed *in vacuo*. The product was recrystallised from EtOAc/hexane to yield the product a white solid (9.41 g, 85%). M.p. 92-95 °C, Lit., 90-94 °C.³⁵⁹ The isolated compound was in good agreement with the literature.³⁶⁰

δ_{H} (400 MHz, CDCl_3): 7.84 (2H, m, Ar-CH), 7.73 (2H, m, Ar-CH), 6.50 (1H, d, $J = 8.6$ Hz, H-1), 5.87 (1H, dd, $J = 10.5$, $J = 9.1$ Hz, H-3), 5.20 (1H, app t, $J = 9.3$ Hz, H-4), 4.45 (1H, dd, $J = 8.8$, $J = 10.5$ Hz, H-2), 4.35 (1H, dd, $J = 4.5$, $J = 12.5$ Hz, H-6), 4.12 (1H, m, H-6'), 4.00 (1H, m, H-5), 2.09 (3H, s, CH_3), 2.02 (3H, s, CH_3), 1.98 (3H, s, CH_3), 1.85 (3H, s, CH_3)

HRMS (m/z ESI $^+$): Found: 500.1170 ($[\text{M} + \text{Na}]^+$, $\text{C}_{22}\text{H}_{23}\text{NO}_{11}$)
Requires: 500.1169)

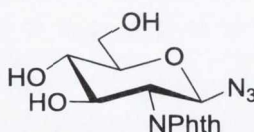
2-Deoxy-2-*N*-phthalimido-3,4,6-tri-*O*-acetyl- β -D-glucopyranosyl azide
(136)



To **135** (2.0 g, 4.19 mmol) and TMSN_3 (0.8 mL, 6.29 mmol) in anhydrous CH_3CN (20 mL) was added TMSOTf (0.1 mL, 0.419 mmol) and the solution was stirred at rt for 4 h. The mixture was diluted with EtOAc (20 mL), washed with H_2O (15 mL), sat. aq. NaHCO_3 solution (15 mL), dried over MgSO_4 , filtered and solvent removed *in vacuo* to give the product as a colourless oil (1.20 g, 62%). The isolated compound was in good agreement with the literature.³⁴⁶

δ_{H} (400 MHz, CDCl_3): 7.90 (2H, m, Ar-CH), 7.79 (2H, m, Ar-CH), 5.83 (1H, dd, $J = 9.2$, $J = 10.6$ Hz, H-3), 5.68 (1H, d, $J = 9.4$ Hz, H-1), 5.22 (1H, app t, $J = 9.6$ Hz, H-4), 4.38 (1H, dd, $J = 4.7$, $J = 12.5$ Hz, H-6), 4.27 (1H, app t, $J = 9.8$ Hz, H-2), 4.23 (1H, m, H-6'), 4.00 (1H, m, H-5), 2.16 (3H, s, CH_3), 2.06 (3H, s, CH_3), 1.89 (3H, s, CH_3)

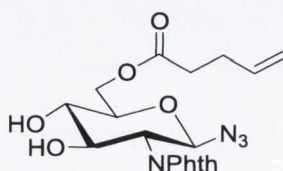
HRMS (m/z ESI $^-$): Found: 459.1147 ($[\text{M} - \text{H}]^-$, $\text{C}_{20}\text{H}_{20}\text{N}_4\text{O}_9$)
Requires: 459.1152)

2-Deoxy-2-*N*-phthalimido- β -D-glucopyranosyl azide (137)

A stock solution of guanidine/guanidinium nitrate (G/GHNO₃) was prepared by dissolving guanidium nitrate (0.62 g, 5.0 mmol) in CH₃OH:CH₂Cl₂ (50 mL, 9:1) and adding methanolic NaOCH₃ (1 mL, 1M solution). A solution of **136** (1.0 g, 2.35 mmol) in the previously prepared G/GHNO₃ stock solution (20 mL) was stirred at rt for 15 min. The mixture was neutralised by the addition of DOWEX-H⁺ resin and then filtered. The solvent was removed *in vacuo* and purified by flash chromatography (CH₂Cl₂ to CH₂Cl₂:CH₃OH - 95:5) to yield the product as a colourless oil (0.65 g, 83%). The isolated compound was in good agreement with the literature.³⁶¹

δ_{H} (400 MHz, CD₃OD): 7.92 (2H, m, Ar-CH), 7.86 (2H, m, Ar-CH), 5.44 (1H, d, $J = 9.6$ Hz, H-1), 4.31 (1H, dd, $J = 8.4$, $J = 10.4$ Hz, H-3), 3.99 (1H, m, H-6), 3.97 (1H, m, H-2), 3.81 (1H, dd, $J = 5.7$, $J = 12.0$ Hz, H-6'), 3.58 (1H, m, H-5), 3.47 (1H, app t, $J = 9.4$ Hz, H-4)

HRMS (m/z ESI): Found: 333.0835 ($[M - H]^+$, C₁₄H₁₄N₄O₆)
Requires: 333.0835)

2-Deoxy-2-*N*-phthalimido-6-*O*-pent-4-enoate- β -D-glucopyranosyl azide (138)

To a solution of **137** (0.36 g, 1.08 mmol) in anhydrous DMF (12 mL) was added NEt₃ (0.18 mL, 1.29 mmol) and the solution was cooled to 0 °C. To this was added 4-pentenoyl chloride (0.14 mL, 1.29 mmol) and the solution was allowed to warm to rt and stirred for 2 h under an argon atmosphere. The solvent was removed *in vacuo* and purified by flash chromatography (CH₂Cl₂ to CH₂Cl₂:CH₃OH - 98:2) to yield the product as a yellow oil (0.35 mg, 77%).

δ_H (400 MHz, CD₃OD): 7.91 (2H, m, Ar-CH), 7.87 (2H, m, Ar-CH), 5.90 (1H, m, CH=CH₂), 5.44 (1H, d, J = 9.7 Hz, H-1), 5.12 (1H, dd, J = 1.6, J = 12.1 Hz, CH=CH₂), 5.03 (1H, dd, J = 1.5, J = 12.1 Hz, CH=CH₂), 4.56 (1H, dd, J = 2.0, J = 11.9 Hz, H-6), 4.34 (1H, m, H-6'), 4.32 (1H, m, H-3), 3.97 (1H, app t, J = 10.4 Hz, H-2), 3.80 (1H, m, H-5), 3.49 (1H, app t, J = 9.5 Hz, H-4), 2.52 (2H, m, CH₂), 2.43 (2H, m, CH₂)

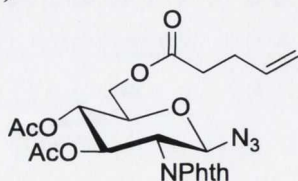
δ_C (100 MHz, CD₃OD): 173.5 (C=O), 168.2 (C=O), 134.7 (Ar-CH), 131.8 (qC), 123.5 (Ar-CH), 136.9 (CH=CH₂), 115.0 (CH=CH₂), 85.9 (C-1), 76.5 (C-5), 71.1 (C-4), 71.1 (C-3), 63.3 (C-6), 56.8 (C-2), 33.2 (CH₂), 29.0 (CH₂)

$[\alpha]_D^{20}$ (c 0.01, CH₃OH): -56

HRMS (m/z ESI): Found: 415.1246 ([M - H]⁻, C₁₉H₂₀N₄O₇)
Requires: 415.1254

$\nu_{\max}$ (film)/cm⁻¹: 3443 (OH), 2118 (N₃), 1710 (C=O), 1384 (CH)

2-Deoxy-2-*N*-phthalimido-3,4-di-*O*-acetyl-6-*O*-pent-4-enoate- β -D-glucopyranosyl azide (139**)**

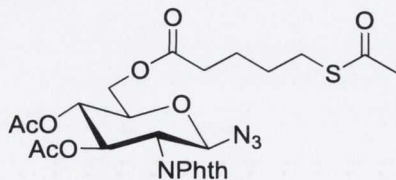


To a solution of **138** (0.22 g, 0.53 mmol) in CH₂Cl₂ (12 mL) was added pyridine (0.21 mL, 2.65 mmol) and Ac₂O (0.25 mL, 2.65 mmol) and the

resulting solution was stirred at rt for 16 h. The solvent was removed *in vacuo* to yield the product as a yellow oil (0.26 g, >99%).

δ_{H} (400 MHz, CDCl_3):	7.90 (2H, m, Ar-CH), 7.88 (2H, m, Ar-CH), 5.84 (1H, m, $\text{CH}=\text{CH}_2$), 5.84 (1H, m, H-3), 5.68 (1H, d, $J = 9.4$ Hz, H-1), 5.21 (1H, app t, $J = 9.5$ Hz, H-4), 5.10 (1H, dd, $J = 1.6$, $J = 1.1$ Hz, $\text{CH}=\text{CH}_2$), 5.05 (1H, dd, $J = 1.4$, $J = 10.2$ Hz, $\text{CH}=\text{CH}_2$), 4.36 (1H, dd, $J = 4.9$, $J = 12.4$ Hz, H-6), 4.26 (1H, m, H-6'), 4.25 (1H, m, H-5), 4.00 (1H, m, H-5), 2.53 (2H, m, CH_2), 2.44 (2H, m, CH_2), 2.06 (3H, s, CH_3), 1.89 (3H, s, CH_3)
δ_{C} (100 MHz, CDCl_3):	172.7 (C=O), 170.1 (C=O), 169.4 (C=O), 167.5 (C=O), 134.6 (qC), 129.0 (Ar-CH), 128.2 (Ar-CH), 123.9 (Ar-CH), 136.6 ($\text{CH}=\text{CH}_2$), 115.9 ($\text{CH}=\text{CH}_2$), 85.8 (C-1), 74.0 (C-5), 70.3 (C-3), 68.5 (C-4), 61.6 (C-6), 53.9 (C-2), 33.3 (CH_2), 28.7 (CH_2), 20.6 (CH_3), 20.5 (CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl_3):	-56
HRMS (m/z ESI $^{+}$):	Found: 523.1442 ($[\text{M} + \text{Na}]^{+}$, $\text{C}_{23}\text{H}_{24}\text{N}_4\text{O}_9$) Requires: 523.1441
ν_{max} (film)/ cm^{-1} :	2118 (N_3), 1745, 1715 (C=O), 1226 (CH)

2-Deoxy-2-*N*-phthalimido-3,4-di-*O*-acetyl-6-*O*-acetyl-thiopentanoate- β -D-glucopyranosyl azide (140)

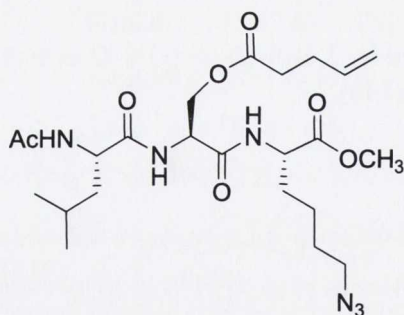


To a solution of **139** (50 mg, 0.10 mmol), DPAP (5 mg, 0.01 mmol) and MAP (4 mg, 0.01 mmol) in anhydrous DMF (5 mL) was added **66** (10 μL , 0.11 mmol) and the reaction mixture was irradiated in an UV oven at rt without agitation for 1 h. The solvent was removed *in vacuo* and purified by flash chromatography (EtOAc:Hex - 70:30) to yield the product as a colourless oil (55 mg, 92%).

δ_{H} (400 MHz, CDCl_3):	7.90 (2H, m, Ar-CH), 7.80 (2H, m, Ar-CH), 5.84 (1H, app t, $J = 9.7$ Hz, H-3), 5.69 (1H, d, $J = 9.4$ Hz, H-1), 5.21 (1H, app t, $J = 9.7$ Hz, H-4), 4.37 (1H, dd, $J = 4.9$, $J = 12.4$ Hz, H-6), 4.27 (1H, m, H-6'), 4.27 (1H, m, H-2), 4.01 (1H, m, H-5), 2.93 (2H, t, $J = 6.6$ Hz, CH_2), 2.45 (2H, t, $J = 7.2$ Hz, CH_2), 2.36, 2.07, 1.89 (3H, s, CH_3), 1.77 (2H, m, CH_2), 1.67 (2H, m, CH_2),
δ_{C} (100 MHz, CDCl_3):	195.9 (C=O), 172.9 (C=O), 170.1 (C=O), 169.4 (C=O), 167.7 (C=O), 133.7 (qC), 134.6 (Ar-CH), 123.8 (Ar-CH), 130.4 (Ar-CH), 85.5 (C-1), 74.2 (C-5), 70.5 (C-3), 68.5 (C-4), 61.7 (C-6), 54.0 (C-2), 33.3 (CH_2), 28.6 (CH_2), 30.8 (CH_3), 20.6 (CH_3), 20.4 (CH_3), 23.8 (CH_2), 28.9 (CH_2)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl_3):	-82
HRMS (m/z ESI $^{+}$):	Found: 599.1431 ($[\text{M} + \text{Na}]^{+}$, $\text{C}_{25}\text{H}_{28}\text{N}_4\text{O}_{10}\text{S}$ Requires: 599.1424)
ν_{max} (film)/ cm^{-1} :	2120 (N_3), 1716 (C=O), 1228 (CH)

6.6 Experimental details for Chapter 3

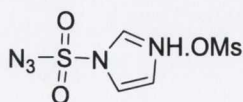
Ac-Leu-Ser-(4-pentenoate)-Lys-(azido)-OMe (144)



Prepared as per Procedure 10 using **159** (0.14 g, 0.33 mmol) in CH_2Cl_2 (8 mL) and following flash chromatography ($\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ - 88.5:1.5), the product was obtained as a colourless oil (0.12 g, 71%).

δ_{H} (400 MHz, CDCl_3):	7.26 (2H, d, $J = 7.9$ Hz, NH), 6.33 (1H, br s, NH), 5.82 (1H, m, $\text{CH}=\text{CH}_2$), 5.04 (2H, dd, $J = 10.3$, $J = 15.5$ Hz, $\text{CH}=\text{CH}_2$), 4.82 (1H, m, Ser αCH), 4.57 (2H, m, Leu αCH , Lys αCH), 4.43 (1H, dd, $J = 4.9$, $J = 10.9$ Hz, Ser CH_2), 4.31 (1H, dd, $J = 4.9$, $J = 11.1$ Hz, Ser CH_2), 3.77 (3H, s, OCH_3), 3.29 (2H, t, $J = 6.4$ Hz, CH_2N_3), 2.46 (2H, m, CH_2), 2.37 (2H, m, CH_2), 2.05 (3H, s, CH_3), 1.89 (1H, m, CH_2), 1.75 (1H, m, CH_2), 1.71-1.51 (5H, m, Leu CHCH_2 , CH_2), 1.42 (2H, m, CH_2), 0.96 (6H, m, Leu CH_3)
δ_{C} (100 MHz, CDCl_3):	173.0 (C=O), 172.5 (C=O), 172.2 (C=O), 170.5 (C=O), 168.4 (C=O), 136.5 ($\text{CH}=\text{CH}_2$), 115.7 ($\text{CH}=\text{CH}_2$), 63.6 (Ser CH_2), 52.5 (Ser αCH), 52.4 (Leu αCH), 52.3 (OCH_3), 52 (Lys αCH), 51 (CH_2N_3), 41.2 (CH_2), 33.2 (CH_2), 31.8 (CH_2), 28.6 (CH_2), 28.4 (CH_2), 24.9 (Leu CHCH_2), 23.1 (CH_3), 22.9 (Leu CH_3), 22.5 (CH_2), 22 (Leu CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.05, CHCl_3):	-42
HRMS (m/z APCI $^+$):	Found: 511.2875 ($[\text{M} + \text{H}]^+$, $\text{C}_{18}\text{H}_{32}\text{N}_6\text{O}_6$) Requires: 511.2864
ν_{max} (film)/ cm^{-1} :	3285 (NH), 2098 (N_3), 1742, 1642 (C=O), 1545 (CH)

3-Azidosulfonyl-3H-imidazol-1-ium methanesulfonate (145·MsOH)



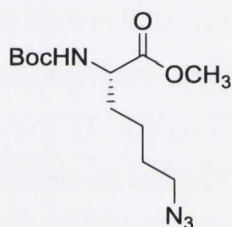
To a solution of NaN_3 (4.3 g, 66.67 mmol) in CH_3CN (60 mL) at 0 °C was added SO_2Cl_2 (5.4 mL, 66.67 mmol) and then the reaction mixture was allowed to warm to rt and stirred for 16 h. The resulting white slurry was cooled on ice and imidazole (8.63 g, 126.67 mmol) was added and the reaction mixture was stirred at rt for 4 h. The reaction was diluted with EtOAc (80 mL), washed with H_2O (2 x 20 mL), sat. aq. NaHCO_3 solution (20 mL), dried over MgSO_4 ,

filtered, and the solvent was removed *in vacuo*. The pale yellow oil was dissolved in Et₂O (20 mL), cooled to 0 °C and methanesulfonic acid (4.33 mL, 66.67 mmol) was added slowly while stirring to produce a solid which was filtered and washed with EtOH to give the product as a white solid (12.21 g, 68%). M.p. 76-78 °C, Lit., 75-78 °C.²⁶⁹ The isolated compound was in good agreement with the literature.²⁶⁹

δ_{H} (600 MHz, DMSO-*d*₆): 8.91 (1H, s, CH), 7.58 (2H, s, CH), 2.48 (3H, s, CH₃)

HRMS (*m/z* APCI⁺): Found: 174.0082 ([M + H]⁺, C₃H₄N₅O₂S)
Requires: 174.0080)

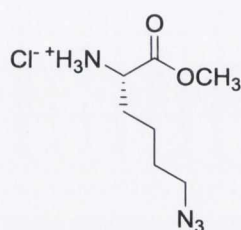
Boc-Lys(azido)-OMe (147)



To a solution of the Boc-Lys-OMe acetate salt **146** (1.77 g, 5.52 mmol), K₂CO₃ (1.53 g, 11.04 mmol) and CuSO₄·H₂O (0.28 g, 1.10 mmol) in CH₃OH (50 mL) was added **145·MsOH** (1.77 g, 5.52 mmol) and the reaction mixture was stirred at rt for 16 hr. H₂O was added (50 mL) and acidified with to pH2 with 1M aq. HCl solution (20 mL) and the product extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with H₂O (20 mL), dried over MgSO₄, filtered, and the solvent was removed *in vacuo*. The product was purified by flash chromatography (EtOAc:Hex - 40:60) to yield the product as a yellow oil (0.96 g, 61%).

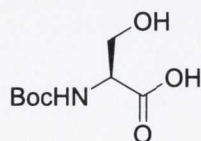
δ_{H} (400 MHz, CDCl₃): 5.28 (1H, d, *J* = 8.3 Hz, NH), 4.15 (1H, m, α CH), 3.59 (3H, s, CH₃), 3.14 (2H, t, *J* = 6.8 Hz, CH₂), 1.69 (1H, m, CH₂), 1.49 (3H, m, CH₂), 1.30 (2H, m, CH₂), 1.30 (9H, s, C(CH₃)₃)

δ_C (100 MHz, CDCl_3):	173.0 (C=O), 155.3 (C=O), 79.6 ($\text{C}(\text{CH}_3)_3$), 53.1 (αCH), 52.1 (CH_3), 51.0 (CH_2), 32.1 (CH_2), 28.3 (CH_2), 28.1 ($\text{C}(\text{CH}_3)_3$), 22.4 (CH_2)
$[\alpha]_D^{20}$ (c 0.05, CHCl_3):	-13
HRMS (m/z ESI^+):	Found: 309.1538 ($[\text{M} + \text{Na}]^+$, $\text{C}_{12}\text{H}_{22}\text{N}_4\text{O}_4$) Requires: 309.1539
ν_{max} (film)/ cm^{-1} :	2095 (N_3), 1709 (C=O), 1163 (C-O)

H-Lys(azido)-OMe (148)

Prepared as per Procedure 3 using **147** (0.68 g, 2.37 mmol) in CH_2Cl_2 (15 mL) to give the product as a colourless oil (0.53 g, >99%).

δ_H (400 MHz, CD_3OD):	4.08 (1H, br s, αCH), 3.86 (3H, s, CH_3), 3.37 (2H, br s, CH_2), 1.97 (2H, br s, CH_2), 1.66 (2H, br s, CH_2), 1.54 (2H, m, CH_2)
δ_C (100 MHz, CD_3OD):	169.6 (C=O), 53.1 (CH_3), 52.9 (αCH), 51.1 (CH_2), 30.0 (CH_2), 28.3 (CH_2), 22.2 (CH_2)
$[\alpha]_D^{20}$ (c 0.05, CH_3OH):	-58
HRMS (m/z ESI^+):	Found: 187.1192 ($[\text{M} + \text{H}]^+$, $\text{C}_7\text{H}_{15}\text{N}_4\text{O}_2$) Requires: 187.1195
ν_{max} (film)/ cm^{-1} :	3381 (NH_2), 2094 (N_3), 1743 (C=O), 1235 (C-O)

Boc-Ser-OH (149)

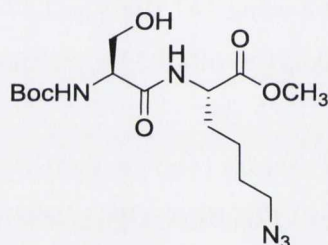
To a solution of L-Ser (5.0 g, 47.58 mmol) in H_2O :dioxane (100 mL, 1:1) was added NEt_3 (9.95 mL, 71.37 mmol) followed by $(\text{Boc})_2\text{O}$ (10.0 g, 95.16 mmol)

and the mixture was stirred at rt for 16 h. The dioxane was removed *in vacuo* and the aqueous layer was washed with Et₂O (2 x 15 mL). The aqueous layer was adjusted to pH2 with aq. HCl solution and then extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with H₂O (20 mL), dried over MgSO₄, filtered, and the solvent was removed *in vacuo* to give the product as colourless oil (7.03 g, 72%). The isolated compound was in good agreement with the literature.³⁶²

δ_{H} (400 MHz, CDCl₃): 5.64 (1H, d, J = 5.4 Hz, NH), 4.35 (1H, br s, α CH), 4.06 (1H, m, CH₂), 3.85 (1H, d, J = 10.4 Hz, CH₂), 1.45 (9H, s, C(CH₃)₃)

HRMS (m/z ESI): Found: 204.0873 ([M – H][–], C₈H₁₄NO₅ Requires: 204.0872)

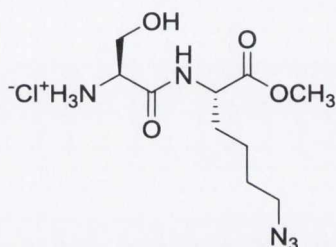
Boc-Ser(OH)-Lys-(azido)-OMe (150)



Prepared as per Procedure 7 using **149** (0.52 g, 2.56 mmol) in CH₂Cl₂:DMF (10 mL) and **148** (0.57 g, 2.56 mmol) in CH₂Cl₂:DMF (6 mL) and following flash chromatography (CH₂Cl₂:CH₃OH - 99:1), the product was obtained as colourless oil (0.71 g, 74%).

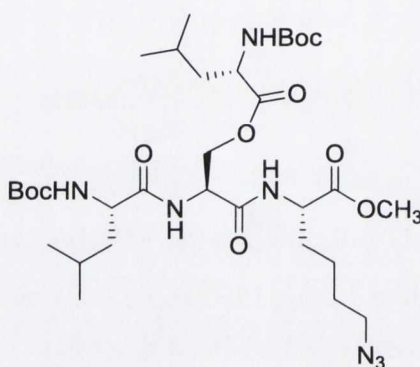
δ_{H} (400 MHz, CDCl₃): 7.33 (1H, d, J = 7.3 Hz, NH), 5.71 (1H, d, J = 6.6 Hz, NH), 4.57 (1H, d, J = 5.7 Hz, Lys α CH), 4.22 (1H, br s, Ser α CH), 3.99 (1H, d, J = 9.4 Hz, Ser CH₂), 3.74 (3H, s, OCH₃), 3.68 (1H, m, Ser CH₂), 3.26 (2H, t, J = 6.7 Hz, CH₂N₃), 1.87 (2H, m, CH₂), 1.71 (2H, m, CH₂), 1.59 (2H, m, CH₂), 1.44 (9H, s, C(CH₃)₃), 1.41 (2H, m, CH₂)

δ_{C} (100 MHz, CDCl_3):	172.6 (C=O), 171.3 (C=O), 156.0 (C=O), 80.4 ($\text{C}(\text{CH}_3)_3$), 62.8 (Ser CH_2), 55.1 (Ser αCH), 52.5 (OCH_3), 52.1 (Lys αCH), 51.0 (CH_2N_3), 31.4 (CH_2), 28.2 (CH_2), 28.2 ($\text{C}(\text{CH}_3)_3$), 22.5 (CH_2)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CDCl_3):	-8
HRMS (m/z ESI $^+$):	Found: 396.1858 ($[\text{M} + \text{Na}]^+$, $\text{C}_{15}\text{H}_{27}\text{N}_5\text{O}_6$) Requires: 396.1859
ν_{max} (film)/ cm^{-1} :	3312 (NH), 2095 (N_3), 1661 (C=O), 1164 (C-O)

H-Ser(OH)-Lys-(azido)-OMe (151)

Prepared as per Procedure 3 using **150** (0.65 g, 1.74 mmol) in CH_2Cl_2 (15 mL) to give the product as a colourless oil (0.54 g, >99%).

δ_{H} (400 MHz, CD_3OD):	4.50 (1H, dd, $J = 5.1$ Hz, $J = 8.9$ Hz, Lys αCH), 4.10 (1H, m, Ser αCH), 4.04 (1H, dd, $J = 4.1$ Hz, $J = 11.6$ Hz, Ser CH_2), 3.85 (1H, dd, $J = 6.7$ Hz, $J = 11.6$ Hz, Ser CH_2), 3.75 (3H, s, OCH_3), 3.34 (2H, t, $J = 6.6$ Hz, CH_2N_3), 1.91 (1H, m, CH_2), 1.78 (1H, m, CH_2), 1.63 (2H, m, CH_2), 1.50 (2H, m, CH_2)
δ_{C} (100 MHz, CD_3OD):	172.2 (C=O), 167.1 (C=O), 60.4 (Ser CH_2), 54.8 (Ser αCH), 52.5 (Lys αCH), 51.6 (OCH_3), 50.8 (CH_2), 30.6 (CH_2), 28.0 (CH_2), 22.7 (CH_2)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CH_3OH):	-21
HRMS (m/z ESI $^+$):	Found: 274.1503 ($[\text{M} + \text{H}]^+$, $\text{C}_{10}\text{H}_{20}\text{N}_5\text{O}_4$) Requires: 274.1515
ν_{max} (film)/ cm^{-1} :	3219 (NH_2), 2096 (N_3), 1736, 1679 (C=O), 1225 (C-O)

Boc-Leu-Ser-(Boc-Leu)-Lys-(azido)-OMe (153)

Prepared as per Procedure 7 using Boc-L-Leu **152** (0.33 g, 1.42 mmol) in CH_2Cl_2 :DMF (6 mL) and **151** (0.44 g, 1.42 mmol) in CH_2Cl_2 :DMF (6 mL) and following flash chromatography (CH_2Cl_2 : CH_3OH - 99.5:0.5), the product was obtained as a yellow oil (0.42 g, 42%).

δ_{H} (400 MHz, CDCl_3): 7.38 (1H, d, $J = 5.6$ Hz, NH), 6.99 (1H, d, $J = 5.6$ Hz, NH), 5.21 (1H, br s, NH), 4.95 (1H, br s, NH), 4.69 (1H, d, $J = 7.2$ Hz, Ser αCH), 4.62 (1H, m, Ser CH_2), 4.53 (1H, d, $J = 5.6$ Hz, Lys αCH), 4.19 (3H, m, Ser CH_2 , $\alpha\text{Leu CH}$, Leu αCH), 3.74 (3H, s, OCH_3), 3.28 (2H, t, $J = 5.9$ Hz, CH_2N_3), 1.87 (1H, m, CH_2), 1.78 (1H, m, CH_2), 1.76-1.39 (10 H, m, CH_2 , Leu CHCH_2), 1.45 (18H, s, $\text{C}(\text{CH}_3)_3$), 0.94 (12H, s, Leu CH_3)

δ_{C} (100 MHz, CDCl_3): 173.3 (C=O), 173.0 (C=O), 172.0 (C=O), 168.7 (C=O), 155.8 (C=O), 80.2 ($\text{C}(\text{CH}_3)_3$), 62.9 (Ser

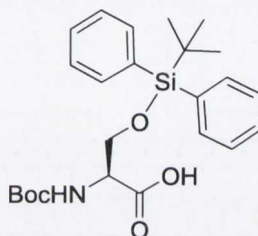
CH₂), 53.5 (Leu αCH), 52.4 (OCH₃), 52.3 (Lys αCH), 52.2 (Leu αCH), 51.9 (Ser αCH), 51.0 (CH₂N₃), 40.8, 40.2 (CH₂), 31.3 (CH₂), 28.4 (C(CH₃)₃), 28.3 (C(CH₃)₃), 24.8 (Leu CHCH₂), 23.1 (CH₂), 22.9 (Leu CH₃), 22.7 (CH₂), 21.6 (Leu CH₃), 21.5 (CH₂)

[α]_D²⁰ (c 0.02, CHCl₃): -37

HRMS (*m/z* ESI⁺): Found: 722.4072 ([M + Na]⁺, C₃₂H₅₇N₇O₁₀)
Requires: 722.4065

ν_{max} (film)/cm⁻¹: 3338 (NH), 2096 (N₃), 1672 (C=O), 1111 (C-O)

Boc-Ser-(*t*-butyl-dimethyl-silanyloxy)-OH (**154**)



To Boc-L-Ser **149** (2.0 g, 9.75 mmol) in DMF (15 mL) was added imidazole (2.12 g, 31.2 mmol) and the mixture was stirred for 15 min at rt. The mixture was cooled to 0 °C and TBDPSCl (3.8 mL, 14.62 mmol) was added in portions over 20 min, the reaction mixture was then allowed to warm to rt and stirred for 16 h. EtOAc (20 mL) was added and the mixture was poured into 1M aq. HCl solution (80 mL) and stirred for 15 min. The product was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with sat. aq. NaHCO₃ solution (30 mL), brine (30 mL), dried over MgSO₄, filtered, and the solvent was removed *in vacuo*. The product was purified by flash chromatography (EtOAc:Hex - 20:80) to yield the product as a colourless oil (3.22 g, 74%). The isolated compound was in good agreement with the literature.³⁶³

δ_H (400 MHz, CDCl₃): 7.63 (4H, m, Ar-CH), 7.39 (6H, m, Ar-CH), 5.42 (1H, d, *J* = 7.9 Hz, NH), 4.46 (1H, d, *J* = 7.6 Hz, CH), 4.12 (1H, d, *J* = 10 Hz, CH₂), 3.92 (1H, d,

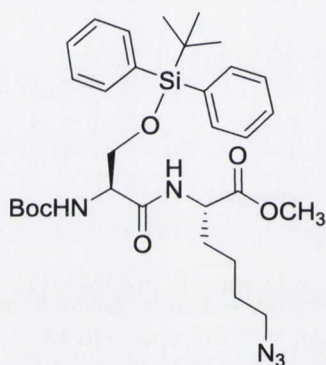
$J = 9.6$ Hz, CH₂), 1.47 (9H, s, C(CH₃)₃), 1.03 (9H, s, SiC(CH₃)₃)

HRMS (m/z ESI⁺):

Found: 466.2033 ([M + Na]⁺, C₂₄H₃₃NO₅Si

Requires: 466.2026)

Boc-Ser-(*t*-butyl-dimethyl-silanyloxy)-Lys-(azido)-OMe (155)



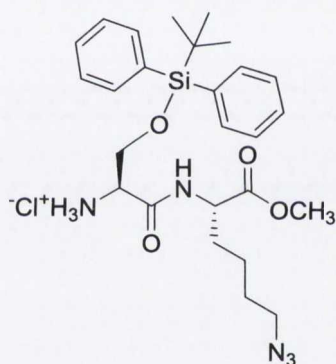
Prepared as per Procedure 7 using **154** (1.39 g, 3.13 mmol) in CH₂Cl₂:DMF (15 mL) and **148** (0.70 g, 3.13 mmol) in CH₂Cl₂:DMF (10 mL) and following flash chromatography (EtOAc:Hex - 20:80), the product was obtained as a white oil (1.70 g, 89%).

δ_H (400 MHz, CDCl₃):

7.67 (4H, m, Ar-CH), 7.43 (6H, m, Ar-CH), 7.09 (1H, d, $J = 7.1$ Hz, NH), 5.32 (1H, br s, NH), 4.67 (1H, m, Lys α CH), 4.30 (1H, br s, Ser α CH), 4.13 (1H, dd, $J = 3.7, J = 10$ Hz, Ser CH₂), 3.81 (1H, dd, $J = 5.9, J = 10.2$ Hz, Ser CH₂), 3.73 (3H, s, OCH₃), 3.20 (2H, m, CH₂N₃), 1.90 (1H, m, CH₂), 1.73 (1H, m, CH₂), 1.57 (2H, m, CH₂), 1.47 (9H, s, C(CH₃)₃), 1.40 (2H, m, CH₂), 1.08 (9H, s, SiC(CH₃)₃)

δ_{C} (100 MHz, CDCl_3):	172.3 (C=O), 170.3 (C=O), 155.5 (C=O), 135.5 (Ar-CH), 135.5 (Ar-CH), 130.0 (Ar-CH), 127.9 (Ar-CH), 132.7 (qC), 132.4 (qC), 80.3 ($\text{C}(\text{CH}_3)_3$), 64.0 (Ser CH_2), 55.8 (Ser αCH), 52.5 (OCH_3), 52.2 (Lys αCH), 51 (CH_2N_3), 32.2 (CH_2), 28.4 (CH_2), 28.3 ($\text{C}(\text{CH}_3)_3$), 26.8 ($\text{SiC}(\text{CH}_3)_3$), 22.5 (CH_2), 19.3 ($\text{SiC}(\text{CH}_3)_3$)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl_3):	-17
HRMS (m/z ESI $^{+}$):	Found: 634.3066 ($[\text{M} + \text{Na}]^{+}$, $\text{C}_{31}\text{H}_{45}\text{N}_5\text{O}_6\text{Si}$) Requires: 634.3037
ν_{max} (film)/ cm^{-1} :	3338 (NH), 2096 (N_3), 1672 (C=O), 1111 (C-O)

H-Ser-(*t*-butyl-dimethyl-silanyloxy)-Lys-(azido)-OMe (156)

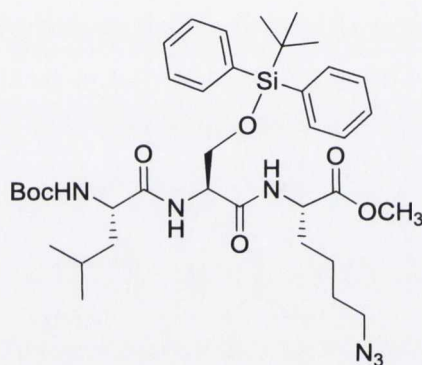


Prepared as per Procedure 3 using **155** (1.55 g, 2.53 mmol) in CH_2Cl_2 (20 mL) to give the product as a white oil (1.39 g, >99%).

δ_{H} (400 MHz, CD_3OD):	7.73 (4H, m, Ar-CH), 7.45 (6H, m, Ar-CH), 4.59 (1H, m, Lys αCH), 4.24 (1H, br s, Ser αCH), 4.12 (2H, s, Ser CH_2), 3.63 (3H, s, OCH_3), 3.31 (2H, t, $J = 6.3$ Hz, CH_2N_3), 1.90 (1H, m, CH_2), 1.79 (1H, m, CH_2), 1.61 (2H, m, CH_2), 1.48 (2H, m, CH_2), 1.09 (9H, s, $\text{SiC}(\text{CH}_3)_3$)
δ_{C} (100 MHz, CD_3OD):	171.8 (C=O), 166.6 (C=O), 135.5 (Ar-CH), 135.5 (Ar-CH), 129.9 (Ar-CH), 129.9 (Ar-CH), 127.8 (Ar-CH), 127.7 (Ar-CH), 132.1 (qC), 132.0 (qC), 62.9 (Ser CH_2), 54.3 (Ser αCH), 52.4

	(Lys α CH), 51.6 (OCH ₃), 50.8 (CH ₂ N ₃), 31.3 (CH ₂), 28.1 (CH ₂), 26.0 (SiC(CH ₃) ₃), 22.5 (CH ₂), 19.9 (SiC(CH ₃) ₃)
$[\alpha]_D^{20}$ (c 0.02, CH ₃ OH):	-59
HRMS (m/z ESI ⁺):	Found: 512.2688 ([M + H] ⁺ , C ₂₆ H ₃₈ N ₄ O ₅ Si) Requires: 512.2689)
$\nu_{\max}$ (film)/cm ⁻¹ :	3360 (NH ₂), 2096 (N ₃), 1743 (C=O), 1112 (C-O)

Boc-Leu-Ser-(*t*-butyl-dimethyl-silanyloxy)-Lys-(azido)-OMe (157)

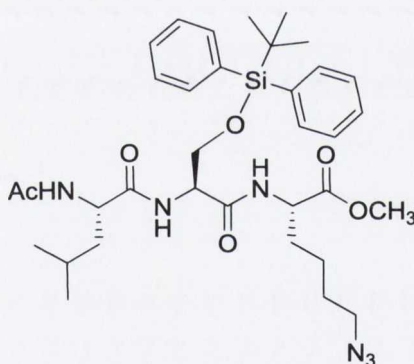


Prepared as per Procedure 7 using **152** (0.32 g, 1.39 mmol) in CH₂Cl₂:DMF (6 mL) and **156** (0.76 g, 1.39 mmol) in CH₂Cl₂:DMF (10 mL) and following flash chromatography (EtOAc:Hex - 30:70), the product was obtained as a white oil (0.92 g, 92%).

δ_H (400 MHz, CDCl ₃):	7.65 (4H, m, Ar-CH), 7.40 (6H, m, Ar-CH), 7.23 (1H, br s, NH), 6.97 (1H, br s, NH), 4.88 (1H, br s, NH), 4.58 (2H, m, Lys α CH, Ser α CH), 4.21 (1H, br s, Ser CH ₂), 4.10 (1H, m, Leu α CH), 3.75 (1H, dd, J = 6.1, J = 10.2 Hz, Ser CH ₂), 3.70 (3H, s, OCH ₃), 3.20 (2H, m, CH ₂ N ₃), 1.88 (1H, m, CH ₂), 1.94-1.50 (6H, m, CH ₂ , Leu CHCH ₂), 1.41 (12H, s, C(CH ₃) ₃ , CH ₂), 1.08 (9H, s, SiC(CH ₃) ₃), 0.94 (6H, m, Leu CH ₃)
---	--

δ_{C} (100 MHz, CDCl_3):	172.5 (C=O), 172.0 (C=O), 169.9 (C=O), 155.9 (C=O), 135.5 (Ar-CH), 130.0 (Ar-CH), 127.9 (Ar-CH), 132.7 (qC), 132.3 (qC), 80.4 ($\text{C}(\text{CH}_3)_3$), 63.6 (Ser CH_2), 54.1 (Ser αCH), 53.8 (Lys αCH), 52.4 (Leu αCH), 52.4 (OCH_3), 51.0 (CH_2N_3), 41.2 (CH_2), 31.8 (CH_2), 28.3 (CH_2), 28.3 ($\text{C}(\text{CH}_3)_3$), 26.8 ($\text{SiC}(\text{CH}_3)_3$), 24.8 (Leu CHCH_2), 23 (Leu CH_3), 22.6 (CH_2), 21.6 (Leu CH_3), 19.2 ($\text{SiC}(\text{CH}_3)_3$)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl_3):	-42
HRMS (m/z ESI $^+$):	Found: 747.3876 ($[\text{M} + \text{Na}]^+$, $\text{C}_{37}\text{H}_{56}\text{N}_6\text{O}_7\text{Si}$) Requires: 747.3876
ν_{max} (film)/ cm^{-1} :	2095 (N_3), 1648 (C=O), 1168, 1112 (C-O)

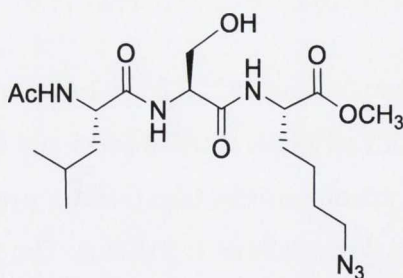
Ac-Leu-Ser-(*t*-butyl-dimethyl-silanyloxy)-Lys-(azido)-OMe (158)



Prepared as per Procedure 3 using **157** (0.85 g, 1.17 mmol) in CH_2Cl_2 (15 mL), the Boc group was removed in quantitative yield and the crude hydrochloride salt was dissolved in a mixture of CH_2Cl_2 (15 mL), pyridine (5 mL) and Ac_2O (5 mL) and the solution was stirred at rt for 16 h. The solvent was removed *in vacuo* to give the product as a yellow oil (0.78 g, >99%).

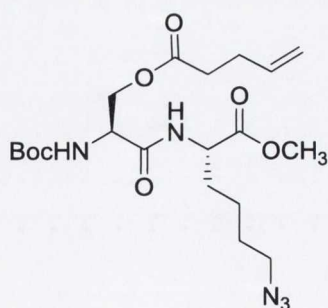
δ_{H} (400 MHz, CDCl_3):	7.68 (4H, m, Ar-CH), 7.45 (6H, m, Ar-CH), 7.19 (1H, br s, NH), 6.82 (1H, br s, NH), 5.94 (1H, br s, NH), 4.61 (1H, m, Lys αCH) 4.56 (1H, br s, Ser αCH), 4.42 (1H, br s, Leu αCH), 4.19 (1H, m, Ser CH_2), 3.80 (1H, dd, $J = 6.1$, $J = 9.3$ Hz,
--	---

	Ser CH ₂), 3.74 (3H, s, OCH ₃), 3.22 (2H, m, CH ₂ N ₃), 1.99 (3H, s, CH ₃), 1.89 (1H, m, CH ₂), 1.76 (1H, m, CH ₂), 1.68 (1H, m, CH ₂), 1.58 (2H, m, CH ₂), 1.53 (1H, m, Leu <u>CH</u> CH ₂), 1.42 (2H, m, CH ₂), 1.09 (9H, s, SiC(CH ₃) ₃), 0.94 (6H, m, Leu CH ₃)
δ_C (100 MHz, CDCl ₃):	172.1 (C=O), 171.9 (C=O), 170.5 (C=O), 169.8 (C=O), 135.5 (Ar-CH), 135.5 (Ar-CH), 130.2 (Ar-CH), 127.9 (Ar-CH), 132.7 (qC), 132.3 (qC), 63.6 (Ser CH ₂), 54.3 (Ser α CH), 52.4 (Lys α CH), 52.4 (Leu α CH), 52.4 (OCH ₃), 51.0 (CH ₂ N ₃), 41.3 (CH ₂), 31.8 (CH ₂), 28.4 (CH ₂), 26.8 (SiC(CH ₃) ₃), 24.9 (Leu <u>CH</u> CH ₂), 22.9 (Leu CH ₃), 22.9 (CH ₃), 22.7 (CH ₂), 22.0 (Leu CH ₃), 19.3 (SiC(CH ₃) ₃)
$[\alpha]_D^{20}$ (c 0.02, CHCl ₃):	-31
HRMS (m/z ESI ⁺):	Found: 689.3456 ([M + Na] ⁺ , C ₃₄ H ₅₀ N ₆ O ₆ Si Requires: 689.3459)
$\nu_{\max}$ (film)/cm ⁻¹ :	3264 (NH), 2096 (N ₃), 1631 (C=O), 1114 (C-O)

Ac-Leu-Ser-(OH)-Lys-(azido)-OMe (159)

To **158** (0.73 g, 1.10 mmol) in anhydrous THF (15 mL) was added 1M TBAF solution in THF (1.74 mL, 1.74 mmol) and the solution was stirred for 2 h at rt. H₂O (20 mL) was added and the product was extracted with EtOAc (2 x 15 mL). The combined organic extracts were washed with brine (2 x 10 mL), dried over MgSO₄, and the solvent was removed *in vacuo*. The product was purified by flash chromatography (gradient elution from CH₂Cl₂ to CH₂Cl₂:CH₃OH - 98:2) to give the product as a colourless oil (0.44g, 94%).

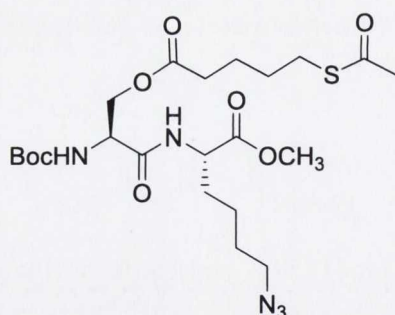
δ_{H} (400 MHz, CDCl_3):	7.83 (1H, d, $J = 7.9$ Hz, NH), 7.68 (1H, d, $J = 7.9$ Hz, NH), 7.33 (1H, d, $J = 7.9$ Hz, NH), 4.67 (1H, q, $J = 7.6$ Hz, Ser αCH), 4.57 (1H, q, $J = 7.6$ Hz, Leu αCH), 4.49 (1H, q, $J = 5.7$ Hz, Lys αCH), 4.34 (1H, br s, OH), 3.87 (1H, m, Ser CH_2), 3.75 (1H, m, Ser CH_2), 3.71 (3H, s, OCH_3), 3.26 (2H, t, $J = 6.9$ Hz, CH_2N_3), 2.02 (3H, s, CH_3), 1.85 (1H, m, CH_2), 1.76 (1H, m, CH_2), 1.68-1.54 (3H, m, CH_2 , Leu CHCH_2), 1.43 (4H, m, CH_2), 0.90 (6H, m, Leu CH_3)
δ_{C} (100 MHz, CDCl_3):	173.4 (C=O), 172.3 (C=O), 171.1 (C=O), 170.6 (C=O), 62.5 (Ser CH_2), 54.9 (Ser αCH), 52.4 (Leu αCH), 52.4 (OCH_3), 52.3 (Lys αCH), 51 (CH_2N_3), 41.5 (CH_2), 31.4 (CH_2), 28.3 (CH_2), 24.8 (Leu CHCH_2), 24.0 (CH_2), 23.1 (Leu CH_3), 21.9 (Leu CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl_3):	-25
HRMS (m/z APCI $^+$):	Found: 429.2451 ($[\text{M} + \text{H}]^+$, $\text{C}_{18}\text{H}_{32}\text{N}_6\text{O}_6$) Requires: 429.2456
ν_{max} (film)/ cm^{-1} :	2094 (N_3), 1663 (C=O), 1415, 1168 (C-O)

Boc-Ser-(4-pentenoate)-Lys-(azido)-OMe (160)

Prepared as per Procedure 10 using **150** (0.11g, 0.29 mmol) in CH_2Cl_2 (8 mL) and following flash chromatography (EtOAc:Hex - 20:80), the product was obtained as a colourless oil (0.10 g, 77%).

δ_{H} (400 MHz, CDCl_3):	6.94 (1H, d, $J = 7.7$ Hz, NH), 5.79 (1H, m, $\text{CH}=\text{CH}_2$), 5.41 (1H, d, $J = 7.4$ Hz, NH), 5.02 (2H, m, $\text{CH}=\text{CH}_2$), 4.58 (1H, m, Lys αCH), 4.42 (1H, m, Ser αCH), 4.38 (1H, m, Ser CH_2), 4.27 (1H, dd, $J = 4.8$, $J = 10.2$ Hz, Ser CH_2), 3.73 (3H, s, OCH_3), 3.25 (2H, t, $J = 6.7$ Hz, CH_2N_3), 2.42 (2H, m, CH_2), 2.35 (2H, m, CH_2), 1.86 (1H, m, CH_2), 1.70 (1H, m, CH_2), 1.58 (2H, m, CH_2), 1.44 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.38 (2H, m, CH_2)
δ_{C} (100 MHz, CDCl_3):	172.7 (C=O), 172.2 (C=O), 169.0 (C=O), 155.4 (C=O), 136.5 ($\text{CH}=\text{CH}_2$), 115.7 ($\text{CH}=\text{CH}_2$), 80.5 ($\text{C}(\text{CH}_3)_3$), 63.9 (Ser CH_2), 53.5 (Ser αCH), 52.5 (OCH_3), 52 (Lys αCH), 51.0 (CH_2N_3), 33.2 (CH_2), 31.9 (CH_2), 28.6 (CH_2), 28.3 (CH_2), 28.2 ($\text{C}(\text{CH}_3)_3$), 22.5 (CH_2)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl_3):	-19
HRMS (m/z APCI $^+$):	Found: 478.2288 ($[\text{M} + \text{Na}]^+$, $\text{C}_{20}\text{H}_{36}\text{N}_3\text{O}_7$) Requires: 478.2272)
ν_{max} (film)/ cm^{-1} :	3327 (NH), 2095 (N_3), 1740, 1664 (C=O), 1512 (CH), 1159 (C-O)

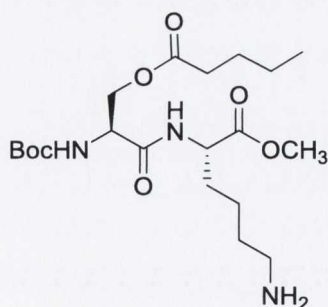
Boc-Ser-[(5-thioacetyl)pentenoate]-Lys-(azido)-OMe (161)



Prepared as per Procedure 6 using **160** (0.16 g, 0.35 mmol) and **66** (30 μL , 0.39 mmol) in DMF (5 mL) and following flash chromatography (EtOAc:Hex - 30:70), the product was obtained as a yellow oil (0.18 g, 96%).

δ_{H} (400 MHz, CDCl_3):	6.86 (1H, d, $J = 7.7$ Hz, NH), 5.38 (1H, m, NH), 4.62 (1H, m, Lys αCH), 4.43 (1H, br s, Ser αCH), 4.41 (1H, m, Ser CH_2), 4.28 (1H, m, Ser CH_2), 3.77 (3H, s, OCH_3), 3.29 (2H, t, $J = 6.6$ Hz, CH_2N_3), 2.89 (2H, t, $J = 6.3$ Hz, CH_2), 2.35 (2H, t, $J = 7.2$ Hz, CH_2), 2.34 (3H, s, SAc), 1.90 (1H, m, CH_2), 1.74 (3H, m, CH_2), 1.63 (4H, m, CH_2), 1.48 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.41 (2H, m, CH_2)
δ_{C} (100 MHz, CDCl_3):	196.0 ($\text{C}=\text{O}$), 172.9 ($\text{C}=\text{O}$), 172.2 ($\text{C}=\text{O}$), 168.9 ($\text{C}=\text{O}$), 155.4 ($\text{C}=\text{O}$), 80.6 ($\text{C}(\text{CH}_3)_3$), 63.8 (Ser CH_2), 53.5 (Ser αCH), 52.5 (OCH_3), 52.0 (Lys αCH), 51.0 (CH_2N_3), 33.2 (CH_2), 31.9 (CH_2), 30.6 (SAc), 28.9 (CH_2), 28.5 ($\text{C}(\text{CH}_3)_3$), (CH_2), 28.3 (CH_2), 28.2 ($\text{C}(\text{CH}_3)_3$), 23.6 (CH_2), 22.3 (CH_2)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl_3):	-5
HRMS (m/z APCI):	Found: 566.2042 ($[\text{M} + \text{Cl}]^-$, $\text{C}_{22}\text{H}_{37}\text{N}_5\text{O}_8\text{S}$ Requires: 566.2057)
ν_{max} (film)/ cm^{-1} :	3301 (NH), 2097 (N_3), 1747, 1648 ($\text{C}=\text{O}$), 1528 (CH), 1170 (C-O)

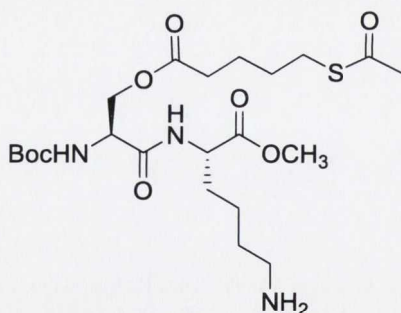
Boc-Ser-(pentanoate)-Lys-(amino)-OMe (162)



To a solution of **161** (50 mg, 0.09 mmol) in EtOAc (15 mL) was added Pd/C (10 mg, 0.09 mmol) and the mixture was stirred under an atmosphere of hydrogen using a balloon for 2 h. The Pd/C was removed by filtration and the solvent was removed *in vacuo* to give the product as a yellow oil (39 mg, >99%).

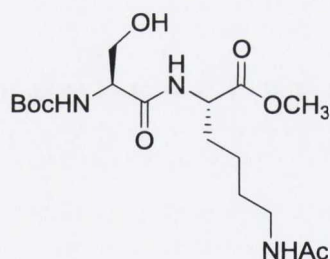
δ_{H} (400 MHz, CD_3OD):	4.49 (1H, dd, $J = 4.9$, $J = 9.0$ Hz, Lys αCH), 4.40 (1H, m, Ser αCH), 4.33 (1H, dd, $J = 4.7$, $J = 11.1$ Hz, Ser CH_2), 4.26 (1H, dd, $J = 7.1$, $J = 11.1$ Hz, Ser CH_2), 3.74 (3H, s, OCH_3), 2.94 (2H, t, $J = 7.4$ Hz, CH_2NH_2), 2.37 (2H, t, $J = 7.5$ Hz, CH_2), 1.93 (1H, m, CH_2), 1.78 (1H, m, CH_2), 1.70 (2H, m, CH_2), 1.62 (2H, t, $J = 7.4$ Hz, CH_2), 1.48 (9H, s, $\text{C}(\text{CH}_3)_3$), 1.38 (2H, m, CH_2), 1.35 (2H, m, CH_2), 0.95 (3H, t, $J = 7.3$ Hz, CH_2CH_3)
δ_{C} (100 MHz, CD_3OD):	173.6 (C=O), 172.1 (C=O), 170.6 (C=O), 167.6 (C=O), 79.9 ($\text{C}(\text{CH}_3)_3$), 63.2 (Ser CH_2), 53.6 (Ser αCH), 51.8 (Lys αCH), 51.4 (OCH_3), 39.1 (CH_2NH_2), 33.1 (CH_2), 30.6 (CH_2), 29.4 (CH_2), 27.4 ($\text{C}(\text{CH}_3)_3$), 26.6 (CH_2), 26.5 (CH_2), 22.2 (CH_2), 21.8 (CH_2), 12.7 (CH_2CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl_3):	-21
HRMS (m/z APCI $^+$):	Found: 432.2706 ($[\text{M} + \text{H}]^+$, $\text{C}_{20}\text{H}_{37}\text{N}_3\text{O}_7$ Requires: 432.2704)
ν_{max} (film)/ cm^{-1} :	3309 (NH_2), 1728, 1679 (C=O), 1162 (C-O)

Boc-Ser-[(5-thioacetyl)pentenoate]-Lys-OMe (163)



Prepared as per Procedure 11 using **161** (0.1 g, 0.19 mmol) in CH_3CN (7 mL). To the reaction mixture was added DMF (6 mL) and the reaction was stirred for 16 h at rt. The solvent was removed *in vacuo* and following flash chromatography ($\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH} - 96:4$), the product was obtained as a yellow oil (22 mg, 23%).

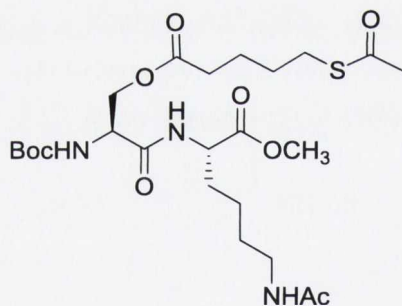
δ_{H} (400 MHz, CDCl_3):	4.69 (1H, m, Lys αCH), 4.58 (1H, m, Ser αCH), 4.46 (1H, m, Ser CH_2), 4.31 (1H, m, Ser CH_2), 3.74 (3H, s, OCH_3), 3.16 (2H, br s, CH_2NH_2), 2.87 (2H, t, $J = 7.0$ Hz, CH_2), 2.38 (2H, m, CH_2), 2.35 (3H, s, SAc), 1.89 (4H, m, CH_2), 1.74-1.58 (6H, m, CH_2), 1.45 (9H, s, $\text{C}(\text{CH}_3)_3$)
δ_{C} (100 MHz, CDCl_3):	196.0 ($\text{C}=\text{O}$), 173.2 ($\text{C}=\text{O}$), 172.1 ($\text{C}=\text{O}$), 169.3 ($\text{C}=\text{O}$), 155.8 ($\text{C}=\text{O}$), 80.5 ($\text{C}(\text{CH}_3)_3$), 64.7 (Ser CH_2), 52.8 (Ser αCH), 52.8 (OCH_3), 52.0 (Lys αCH), 39.2 (CH_2NH_2), 33.2 (CH_2), 30.7 (SAc), 30.5 (CH_2), 28.9 (CH_2), 28.4 (CH_2), 28.4 ($\text{C}(\text{CH}_3)_3$), 26.4 (CH_2), 23.7 (CH_2), 21.9 (CH_2)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl_3):	-63
HRMS (m/z ESI $^-$):	Found: 540.2149 ($[\text{M} + \text{Cl}]^-$, $\text{C}_{22}\text{H}_{39}\text{N}_3\text{O}_8\text{S}$) Requires: 540.2146
ν_{max} (film)/ cm^{-1} :	3356 (NH_2), 1681 ($\text{C}=\text{O}$), 1106 ($\text{C}-\text{O}$)

Boc-Ser-(OH)-Lys-(N-acetyl)-OMe (165)

Prepared as per Procedure 11 using **161** (70 mg, 0.13 mmol) in CH_3CN (5 mL). To the reaction mixture was added DMF (5 mL) and the reaction was stirred for 16 h at rt. The solvent was removed *in vacuo* and CH_3OH (5 mL) was added. The reaction mixture was cooled to 0 °C and to this was added KOH (1.0 mg, 0.15 mmol) and the reaction mixture was stirred at rt for 15 min. DOWEX- H^+ was added, filtered, the solvent was removed *in vacuo* and following flash chromatography ($\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH} - 96:4$), the product was obtained as a colourless oil (23 mg, 45%).

δ_{H} (400 MHz, CDCl_3):	4.29 (1H, m, Lys αCH), 4.25 (1H, m, Ser αCH), 4.23 (1H, m, Ser CH_2), 4.04 (1H, m, Ser CH_2), 3.62 (3H, s, OCH_3), 3.01 (2H, m, CH_2NHAc), 1.71 (2H, m, CH_2), 1.65 (2H, m, CH_2), 1.63 (2H, m, CH_2), 1.79 (3H, s, Ac), 1.39 (9H, s, $\text{C}(\text{CH}_3)_3$),
δ_{C} (100 MHz, CDCl_3):	172.3 ($\text{C}=\text{O}$), 172.3 ($\text{C}=\text{O}$), 168.9 ($\text{C}=\text{O}$), 155.3 ($\text{C}=\text{O}$), 79.1 ($\text{C}(\text{CH}_3)_3$), 63.4 (Ser CH_2), 52.2 (OCH_3), 51.8 (Ser αCH), 51.7 (Lys αCH), 38.3 (CH_2NHAc), 28.8 ($\text{C}(\text{CH}_3)_3$), 30.5 (CH_2), 27.9 (CH_2), 23.1 (CH_2), 22.9 (CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.03, CHCl_3):	-26
HRMS (m/z APCI):	Found: 424.1865 ($[\text{M} + \text{Cl}]^-$, $\text{C}_{17}\text{H}_{31}\text{N}_3\text{O}_7$ Requires: 424.1856)
ν_{max} (film)/ cm^{-1} :	2095 (N_3), 1742, 1652 ($\text{C}=\text{O}$), 1555 (CH)

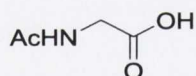
Boc-Ser-[(5-thioacetyl)pentenoate]-Lys-(N-acetyl)-OMe (166)



Prepared as per Procedure 11 using **161** (0.1 g, 0.19 mmol) in CH_3CN (7 mL). To the reaction mixture was added DMF (6 mL) and the reaction was stirred for 16 h at rt. The solvent was removed *in vacuo* and following flash chromatography ($\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ – 99:1), the product was obtained as a yellow oil (49 mg, 48%).

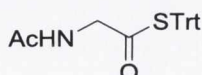
δ_{H} (400 MHz, CDCl_3):	6.99 (1H, d, $J = 7.8$ Hz, NH), 5.82 (1H, br s, NH), 5.57 (1H, br s, NH), 4.59 (1H, m, Lys αCH), 4.47 (1H, m, Ser αCH), 4.44 (1H, m, Ser CH_2), 4.31 (1H, m, Ser CH_2), 3.76 (3H, s,
--	---

	OCH ₃), 3.26 (2H, m, CH ₂ NHAc), 2.89 (2H, t, <i>J</i> = 6.8 Hz, CH ₂), 2.35 (2H, t, <i>J</i> = 7.6 Hz, CH ₂), 2.35 (3H, s, SAc), 2.02 (3H, s, NHAc), 1.89 (1H, m, CH ₂), 1.76 (H, m, CH ₂), 1.72 (4H, m, CH ₂), 1.59 (2H, m, CH ₂), 1.48 (9H, s, C(CH ₃) ₃), 1.37 (2H, m, CH ₂)
δ_C (100 MHz, CDCl ₃):	196.1 (C=O), 173.0 (C=O), 172.3 (C=O), 170.5 (C=O), 169.1 (C=O), 155.4 (C=O), 80.8 (C(CH ₃) ₃), 64.1 (Ser CH ₂), 53.6 (Ser α CH), 52.6 (OCH ₃), 52.1 (Lys α CH), 38.8 (CH ₂ NHAc), 33.3 (CH ₂), 31.8 (CH ₂), 30.6 (SAc), 29.8 (CH ₂), 29.0 (CH ₂), 28.6 (CH ₂), 28.3 (C(CH ₃) ₃), 23.7 (CH ₂), 23.3 (NHAc), 22.1 (CH ₂)
$[\alpha]_D^{20}$ (c 0.02, CHCl ₃):	-32
HRMS (<i>m/z</i> ESI ⁺):	Found: 791.3046 ([M + Na] ⁺ , C ₃₇ H ₄₈ N ₆ O ₁₀ S Requires: 791.3050)
$\nu_{\max}$ (film)/cm ⁻¹ :	3311 (NH), 1649 (C=O), 1011 (C-O)

***N*-Acetylglycine (169)**

To glycine (2.5 g, 33.3 mmol) in H₂O (50 mL) was added Ac₂O (6 mL, 66.6 mmol) and the mixture was vigorously stirred at rt for 4 hr. The solvent was removed *in vacuo* to produce the product as a white solid (3.89 g, >99%). The isolated compound was in good agreement with the literature.³⁶⁴

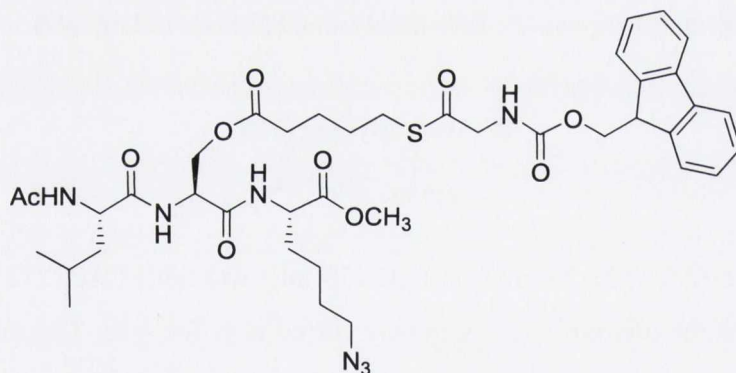
δ_H (400 MHz, CD ₃ OD):	3.88 (2H, s, CH ₂), 1.97 (3H, s, CH ₃)
HRMS (<i>m/z</i> APCI ⁻):	Found: 116.0357 ([M - H] ⁻ , C ₄ H ₇ NO ₃ Requires: 116.0353)

***N*-Ac-Gly-STrt (170)**

Prepared as per Procedure 4 using **169** (1.16 g, 9.91 mmol) in CH₂Cl₂:DMF (2:1, 20 mL) and following flash chromatography (EtOAc:Hex - 50:50), the product was obtained as a white solid (3.12 g, 84%).

δ_{H} (400 MHz, CDCl ₃):	7.24 (15H, m, Ar-CH), 6.11 (1H, br s, NH), 4.13 (2H, d, J = 5.3 Hz, CH ₂), 1.92 (3H, s, CH ₃)
δ_{C} (100 MHz, CDCl ₃):	194.2 (C=O), 170.1 (C=O), 143.4 (qC), 129.7 (Ar-CH), 127.9 (Ar-CH), 127.3 (Ar-CH), 70.9 (SqC), 49.1 (CH ₂), 22.9 (CH ₃)
HRMS (m/z ESI ⁺):	Found: 398.1194 ([M + Na] ⁺ , C ₂₃ H ₂₁ NO ₂ S Requires: 398.1191)
ν_{max} (film)/cm ⁻¹ :	3256 (NH), 1654 (C=O)

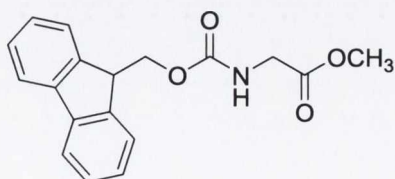
Ac-Leu-Ser-[(5-FmocGly)pentenoate]-Lys-(azido)-OMe (173**)**



Prepared as per Procedure 5 using **103** (0.50 g, 0.90 mmol) in CH₂Cl₂ (10 mL) the crude thioacid **172** was then immediately subjected to Procedure 6 using **144** (0.31 g, 0.60 mmol) in DMF (6 mL) and following flash chromatography (CH₂Cl₂:CH₃OH - 98.5:1.5), the product was obtained as a yellow oil (0.45 g, 92 %).

δ_{H} (400 MHz, CDCl ₃):	7.79 (2H, d, J = 7.3 Hz, Ar-CH), 7.64 (2H, d, J = 7.3 Hz, Ar-CH), 7.42 (2H, t, J = 7.3 Hz, Ar-CH), 7.42 (2H, t, J = 7.3 Hz, Ar-CH), 7.20 (1H, d, J = 7.4 Hz, NH), 7.14 (1H, d, J = 6.8 Hz, NH), 7.05
--	--

	(1H, d, $J = 7.4$ Hz, NH), 6.15 (1H, d, $J = 7.4$ Hz, NH), 4.72 (1H, m, Ser α CH), 4.57 (1H, m, Lys α CH), 4.45 (3H, m, Fmoc CH ₂ , Leu α CH), 4.27 (1H, t, $J = 6.5$ Hz, Fmoc CH), 4.16 (2H, m, Gly CH ₂), 3.76 (3H, s, OCH ₃), 3.29 (2H, t, $J = 6.6$ Hz, CH ₂ N ₃), 2.95 (2H, t, $J = 6.6$ Hz, CH ₂), 2.40 (2H, m, CH ₂), 2.04 (3H, s, NHAc). 1.88 (1H, m, CH ₂), 1.76 (1H, m, CH ₂), 1.93-1.24 (9H, m, CH ₂ , Leu CHCH ₂), 0.95 (6H, t, $J = 6.1$ Hz, Leu CH ₃)
δ_C (100 MHz, CDCl ₃):	198.2 (C=O), 173.2 (C=O), 172.4 (C=O), 172.2 (C=O), 170.7 (C=O), 168.6 (C=O), 156.4 (C=O), 143.7 (Ar-CH), 141.3 (Ar-CH), 127.8 (Ar-CH), 127.1 (Ar-CH), 125.1 (Ar-CH), 120.0 (Ar-CH), 67.3 (Fmoc CH ₂), 63.6 (Ser CH ₂), 52.6 (Ser α CH), 52.3 (Lys α CH), 52.3 (Leu α CH), 52.1 (OCH ₃), 51.1 (CH ₂ N ₃), 50.8 (Gly CH ₂), 47.1 (Fmoc CH), 40.7 (CH ₂), 33.2 (CH ₂), 31.7 (CH ₂), 28.8 (CH ₂), 28.4 (CH ₂), 28.0 (CH ₂), 24.8 (Leu CHCH ₂), 23.1 (Ac), 23.1 (CH ₂), 22.9 (Leu CH ₃), 22.5 (Leu CH ₃)
$[\alpha]_D^{20}$ (c 0.02, CHCl ₃):	-81
HRMS (m/z ESI ⁺):	Found: 846.3484 ([M + Na] ⁺ , C ₄₀ H ₅₃ N ₇ O ₁₀ S) Requires: 846.3472)
$\nu_{\max}$ (film)/cm ⁻¹ :	3300 (NH), 2097 (N ₃), 1735, 1646 (C=O), 1534 (CH)

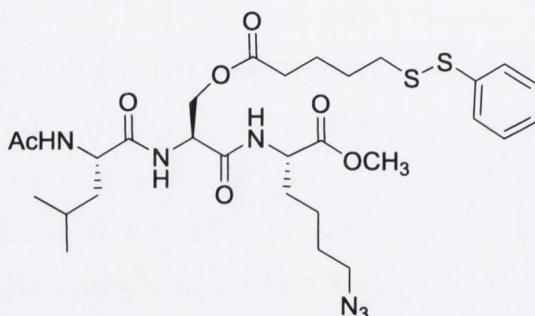
N-Fmoc-Gly-OMe (174)

Prepared as per Procedure 11 using **173** (0.15 g, 0.18 mmol) in CH₃CN (10 mL). Following flash chromatography (CH₂Cl₂:CH₃OH – 98:2), the product was obtained as a colourless oil (35 mg, 62%).

δ_{H} (400 MHz, CDCl_3): 7.78 (2H, d, $J = 7.7$ Hz, Ar-CH), 7.62 (2H, d, $J = 7.4$ Hz, Ar-CH), 7.42 (2H, t, $J = 7.4$ Hz, Ar-CH), 7.33 (2H, t, $J = 7.4$ Hz, Ar-CH), 5.43 (1H, br s, NH), 4.42 (2H, d, $J = 7.2$ Hz, CH_2NH), 4.26 (1H, t, $J = 6.8$ Hz, Fmoc CH), 4.02 (2H, d, $J = 5.6$ Hz, Fmoc CH_2), 3.78 (3H, s, OCH_3)

HRMS (m/z ESI^+): Found: 312.1233 ($[\text{M} + \text{H}]^+$, $\text{C}_{18}\text{H}_{17}\text{NO}_4$
Requires: 312.1230)

**Ac-Leu-Ser-[(5-FmocGly)pentenoate]-Lys-(azido)-OMe thiophenol
disulfide (175)**

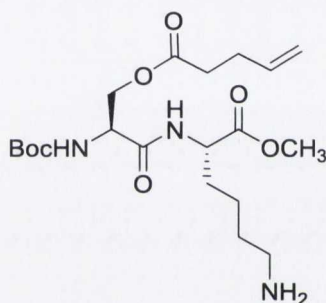


Prepared as per Procedure 11 using **173** (0.15 g, 0.18 mmol) in CH_3CN (10 mL). Following flash chromatography ($\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH} - 98:2$), the product was obtained as a yellow oil (0.95 g, 79%).

δ_{H} (400 MHz, CDCl_3): 7.54 (2H, d, $J = 7.8$ Hz, Ar-CH), 7.34 (2H, d, $J = 7.9$ Hz, Ar-CH), 4.77 (1H, m, Ser αCH), 4.56 (1H, m, Leu αCH), 4.50 (1H, m, Lys αCH), 4.46 (1H, m, Ser CH_2), 4.34 (1H, m, CH_2), 3.76 (3H, s, OCH_3), 3.29 (2H, t, $J = 6.4$ Hz, CH_2N_3), 2.75 (2H, m, CH_2), 2.48 (1H, m, CH_2), 2.38 (1H, m, CH_2), 2.05 (3H, s, NHAc), 1.98-1.29 (13H, m, CH_2 , Leu CHCH_2), 0.96 (6H, m, Leu CH_3)

δ_{C} (100 MHz, CDCl_3): 173.2 (C=O), 172.4 (C=O), 172.1 (C=O), 170.5 (C=O), 168.4 (C=O), 138.1 (qC), 129.1 (Ar-CH), 127.2 (Ar-CH), 63.7 (Ser CH_2), 52.5 (Ser αCH),

	52.4 (Leu α CH), 52.4 (OCH ₃), 52.3 (Lys α CH), 52.2 (CH ₂ N ₃), 41.1 (CH ₂), 38.2 (CH ₂), 33.3 (CH ₂), 33.2 (CH ₂), 31.7 (CH ₂), 29.7 (CH ₂), 28.6 (CH ₂), 28.4 (CH ₂), 24.8 (Leu CH), 23.4 (CH ₂), 23.1 (CH ₂), 23.1 (NHAc), 22.9 (Leu CH ₃), 22.5 (CH ₂), 21.8 (Leu CH ₃)
$[\alpha]_D^{20}$ (c 0.02, CHCl ₃):	-34
HRMS (m/z ESI ⁺):	Found: 675.2620 ([M + Na] ⁺ , C ₂₉ H ₄₄ N ₆ O ₇ S ₂ Requires: 675.2611)
$\nu_{\max}$ (film)/cm ⁻¹ :	2097 (N ₃), 1741, 1661 (C=O), 1529 (CH)

Boc-Ser-(4-pentenoate)-Lys-(amino)-OMe (176)

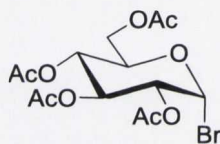
Prepared as per Procedure 11 using **160** (60 mg, 0.13 mmol) in CH₃CN (5 mL) and following flash chromatography (CH₂Cl₂:CH₃OH - 85:15), the product was obtained as a colourless oil (32 mg, 59%).

δ_H (400 MHz, CD ₃ OD):	5.86 (1H, m, $\underline{CH=CH_2}$), 5.04 (2H, m, $CH=\underline{CH_2}$), 4.48 (1H, m, Lys α CH), 4.40 (1H, m, Ser α CH), 4.34 (1H, dd, $J = 4.8$, $J = 11.2$ Hz, Ser CH ₂), 4.27 (1H, dd, $J = 6.7$, $J = 11.1$ Hz, Ser CH ₂), 3.74 (3H, s, OCH ₃), 2.86 (2H, br s, $\underline{CH_2}NH_2$), 2.46 (2H, m, CH ₂), 2.38 (2H, m, CH ₂), 1.90 (1H, m, CH ₂), 1.78 (1H, m, CH ₂), 1.64 (2H, m, CH ₂), 1.49 (2H, m, CH ₂), 1.47 (9H, s, C(CH ₃) ₃)
δ_C (100 MHz, CD ₃ OD):	173.2 (C=O), 172.8 (C=O), 172.6 (C=O), 170.9 (C=O), 137.0 ($\underline{CH=CH_2}$), 114.9 ($CH=\underline{CH_2}$), 79.9 ($\underline{C(CH_3)_3}$), 63.7 (Ser CH ₂), 53.9 (Ser α CH), 52.4

	(Lys α CH), 51.8 (OCH ₃), 40.0 (CH ₂ NH ₂), 33.2 (CH ₂), 31.1 (CH ₂), 28.8 (CH ₂), 27.7 (CH ₂), 27.7 (C(CH ₃) ₃), 22.8 (CH ₂)
$[\alpha]_D^{20}$ (c 0.02, CH ₃ OH):	-47
HRMS (m/z ESI ⁺):	Found: 430.2560 ([M + Na] ⁺ , C ₂₀ H ₃₆ N ₃ O ₇) Requires: 430.2553)
$\nu_{\max}$ (film)/cm ⁻¹ :	3310 (NH ₂), 1660 (C=O), 1526 (CH), 1167 (C-O)

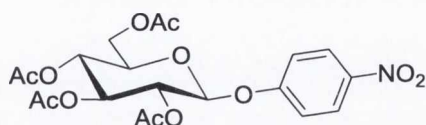
6.7 Experimental details for Chapter 4

2,3,4,6-Tetra-*O*-acetyl- α -D-glucopyranosylbromide (185)



To a solution of 1,2,3,4,6-penta-*O*-acetyl- β -D-glucopyranoside (3.0 g, 7.69 mmol) in CH₂Cl₂ (15 mL) at 0 °C was added 33% solution of HBr/AcOH (11.11 mL, 192.25 mmol) dropwise and the solution was stirred at rt for 3 h. The solution was poured onto crushed ice (100 mL) and stirred for 15 min. The solution was extracted with CH₂Cl₂ (2 x 50 mL), washed with sat. aq. NaHCO₃ solution (3 x 50 mL) and H₂O (2 x 40 mL). The organic layer was dried over MgSO₄, filtered and solvent removed *in vacuo*. The product was recrystallised from EtOAc/Hex to give colourless crystals (2.86 g, 91%). M.p. 78-79 °C, Lit., 77-79 °C. The isolated compound was in good agreement with the literature.³⁶⁵

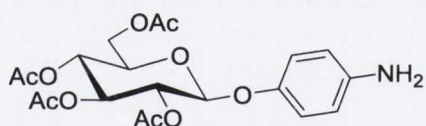
δ_H (600 MHz, CDCl ₃):	6.61 (1H, d, J = 3.8 Hz, H-1), 5.56 (1H, t, J = 9.6 Hz, H-2), 5.17 (1H, t, J = 10.1 Hz, H-4), 4.84 (1H, dd, J = 9.9, J = 3.75 Hz, H-3), 4.33 (1H, m, H-6), 4.31 (1H, m, H-5), 4.14 (1H, dd, J = 12.5, J = 1.5 Hz, H-6'), 2.11 (3H, s, CH ₃), 2.10 (3H, s, CH ₃), 2.06 (3H, s, CH ₃), 2.04 (3H, s, CH ₃)
HRMS (m/z ESI ⁺):	Found: 433.0115 ([M + Na] ⁺ , C ₁₄ H ₁₉ BrO ₉) Requires: 433.0105)

4-Nitrophenyl-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside (186)

Prepared as per Procedure 12 using 1,2,3,4,6-penta-*O*-acetyl- β -D-glucopyranoside (1.0 g, 2.43 mmol) in CH_2Cl_2 (20 mL) to give the product as colourless crystals (1.06 g, 74%). M.p. 176-178 °C, Lit., 176-177 °C.³⁶⁶ The isolated compound was in good agreement with the literature.³⁶⁷

δ_{H} (600 MHz, CDCl_3): 8.24 (2H, d, $J = 9.3$ Hz, Ar-CH), 7.10 (2H, d, $J = 9.3$ Hz, Ar-CH), 5.35 (1H, m, H-2), 5.33 (1H, m, H-3), 5.24 (1H, d, $J = 7.4$ Hz, H-1), 5.21 (1H, app t, $J = 9.7$ Hz, H-4), 4.31 (1H, dd, $J = 12.5$ Hz, $J = 5.7$ Hz, H-6), 4.21 (1H, dd, $J = 12.4$ Hz, $J = 2.4$ Hz, H-6'), 3.96 (1H, m, H-5), 2.10 (3H, s, CH_3), 2.09 (3H, s, CH_3), 2.09 (3H, s, CH_3), 2.07 (3H, s, CH_3)

HRMS (m/z ESI⁺): Found: 492.1118 ($[\text{M} + \text{Na}]^+$, $\text{C}_{20}\text{H}_{23}\text{NO}_{12}$ Requires: 492.1111)

4-Aminophenyl-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside (187)

Prepared as per Procedure 13 using **186** (0.9 g, 1.92 mmol) in $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ (20 mL) to give the product as a yellow oil (0.76 g, 90%). The isolated compound was in good agreement with the literature.³⁶⁸

δ_{H} (600 MHz, CDCl_3): 6.87 (2H, d, $J = 8.8$ Hz, Ar-CH), 6.67 (2H, d, $J = 8.8$ Hz, Ar-CH), 5.29 (1H, app t, $J = 8.9$ Hz, H-2), 5.24 (1H, app t, $J = 9.3$ Hz, H-3), 5.18 (1H, app t, $J = 9.5$ Hz, H-4), 4.94 (1H, d, $J = 7.6$ Hz, H-1), 4.31 (1H, dd, $J = 12.2$ Hz, $J = 5.1$ Hz, H-6), 4.19 (1H, dd, $J = 12.5$ Hz, $J = 2.2$ Hz, H-6'),

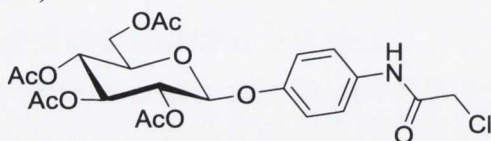
3.81 (1H, m, H-5), 4.02 (2H, br s, NH₂), 2.11 (3H, s, CH₃), 2.10 (3H, s, CH₃), 2.06 (3H, s, CH₃), 2.05 (3H, s, CH₃)

HRMS (*m/z* ESI⁺):

Found: 462.1366 ([M + Na]⁺, C₂₀H₂₅NO₁₀)

Requires: 462.1376)

4-(Chloromethylcarbonylamino)phenyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (188)



Prepared as per Procedure 14 using **187** (0.8 g, 1.82 mmol) in CH₂Cl₂ (10 mL) and following flash chromatography (EtOAc:Hex - 60:40), the product was obtained as a yellow oil (0.62 g, 66%).

δ_H (400 MHz, CDCl₃):

8.20 (1H, br s, NH), 7.49 (2H, d, *J* = 9.1 Hz, Ar-CH), 7.02 (2H, d, *J* = 9.1 Hz, Ar-CH), 5.31 (1H, dd, *J* = 14.2 Hz, *J* = 5.2 Hz, H-3), 5.27 (1H, m, H-2), 5.19 (1H, app t, *J* = 9.5 Hz, H-4), 5.07 (1H, d, *J* = 7.6 Hz, H-1), 4.31 (1H, dd, *J* = 12.3 Hz, *J* = 5.3 Hz, H-6), 4.21 (2H, s, CH₂Cl), 4.19 (1H, dd, *J* = 12.4 Hz, *J* = 2.4 Hz, H-6'), 3.87 (1H, m, H-5), 2.10 (3H, s, CH₃), 2.09 (3H, s, CH₃), 2.07 (3H, s, CH₃), 2.06 (3H, s, CH₃)

δ_C (100 MHz, CDCl₃):

170.5 (C=O), 170.2 (C=O), 169.4 (C=O), 169.2 (C=O), 166.4 (C=O), 156.4 (qC), 137.9 (qC), 128.5 (Ar-CH), 118.4 (Ar-CH), 98.7 (C-1), 72.7 (C-3), 72.1 (C-5), 71.2 (C-2), 68.3 (C-4), 61.9 (C-6), 42.8 (CH₂Cl), 20.7 (CH₃), 20.6 (CH₃), 20.6 (CH₃), 20.5 (CH₃)

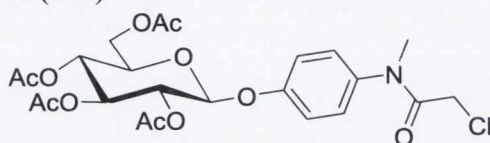
HRMS (*m/z* ESI⁻):

Found: 514.1124 ([M - H]⁻, C₂₂H₂₆ClNO₁₁)

Requires: 514.1116)

$\nu_{\max}$ (film)/ cm^{-1} : 1749, 1510 (C=O), 1209 (CH)

4-(Chloromethylcarbonylamino)-*N*-methyl-phenyl-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside (189)



Prepared as per Procedure 15 using **188** (0.88 g, 1.71 mmol) in THF (15 mL) to give the product as an orange oil (0.62 g, 69%).

δ_{H} (600 MHz, CDCl_3): 7.22 (2H, d, $J = 8.9$ Hz, Ar-CH), 7.07 (2H, d, $J = 8.9$ Hz, Ar-CH), 5.35 (1H, m, H-2), 5.33 (1H, m, H-3), 5.21 (1H, app t, $J = 9.5$ Hz, H-4), 5.15 (1H, d, $J = 7.3$ Hz, H-1), 4.32 (1H, dd, $J = 12.4$ Hz, $J = 5.5$ Hz, H-6), 4.22 (1H, dd, $J = 12.3$ Hz, $J = 2.2$ Hz, H-6'), 3.92 (1H, m, H-5), 3.85 (2H, s, CH_2Cl), 3.30 (3H, s, N- CH_3), 2.11 (3H, s, CH_3), 2.10 (3H, s, CH_3), 2.09 (3H, s, CH_3), 2.07 (3H, s, CH_3)

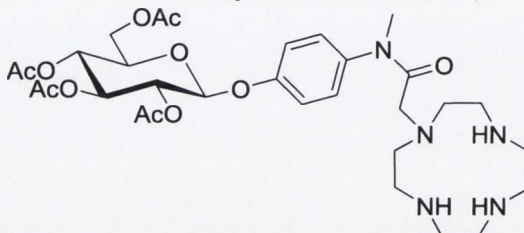
δ_{C} (150 MHz, CDCl_3): 170.3 (C=O), 170.1 (C=O), 169.2 (C=O), 169.1 (C=O), 166.3 (C=O), 156.3 (qC), 137.7 (qC), 128.4 (Ar-CH), 118.0 (Ar-CH), 98.8 (C-1), 72.1 (C-5), 72.1 (C-2), 70.1 (C-3), 67.9 (C-4), 61.7 (C-6), 41.2 (CH_2Cl), 37.9 (N- CH_3), 20.5 (CH_3), 20.5 (CH_3), 20.4 (CH_3), 20.4 (CH_3)

$[\alpha]_{\text{D}}^{20}$ (c 0.04, CHCl_3): -16

HRMS (m/z ESI $^+$): Found: 530.1428 ($[\text{M} + \text{H}]^+$, $\text{C}_{22}\text{H}_{26}\text{ClNO}_{11}$)
Requires: 530.1429

$\nu_{\max}$ (film)/ cm^{-1} : 1747, 1673 (C=O), 1221 (CH)

1-{*N*-methylcarbonylamino)-phenyl-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside}-4,7,10-tetraaza-cyclododecane (190**)**



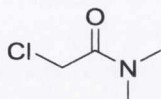
To a solution of cyclen (0.26 g, 1.51 mmol) in freshly distilled CH_3CN (15 mL) was added **189** (0.2 g, 0.38 mmol) and NEt_3 (0.05 mL, 0.46 mmol) and the reaction was refluxed for 16 h. The reaction was diluted with CH_2Cl_2 (20 mL), washed with H_2O (2 x 20 mL), dried over MgSO_4 , filtered and the solvent was removed *in vacuo* to give the product as a yellow oil (0.73 g, 74%).

δ_{H} (400 MHz, CDCl_3): 7.10 (2H, d, $J = 8.3$ Hz, Ar-CH), 7.03 (2H, d, $J = 8.3$ Hz, Ar-CH), 5.32 (2H, m, H-2, H-3), 5.21 (1H, app t, $J = 9.3$ Hz, H-4), 5.13 (1H, d, $J = 7.2$ Hz, H-1), 4.33 (1H, dd, $J = 12.3$ Hz, $J = 5$ Hz, H-6), 4.22 (1H, dd, $J = 12.3$ Hz, $J = 1.3$ Hz, H-6'), 3.91 (1H, m, H-5), 3.22 (5H, s, CH_2 , N- CH_3), 2.93-2.54 (16H, m, cyclen CH_2), 2.11 (3H, s, CH_3), 2.11 (3H, s, CH_3), 2.08 (3H, s, CH_3), 2.07 (3H, s, CH_3)

δ_{C} (100 MHz, CDCl_3): 171.0 (C=O), 170.5 (C=O), 170.2 (C=O), 169.4 (C=O), 169.3 (C=O), 156.1 (qC), 138.4 (qC), 128.5 (Ar-CH), 118.2 (Ar-CH), 99.0 (C-1), 72.6 (C-5), 72.2 (C-2), 71.1 (C-3), 68.9 (C-4), 61.9 (C-6), 55.6 (CH_2), 51.7 (CH_2), 47.0 (CH_2), 46.3 (CH_2), 45.8 (CH_2), 37.3 (N- CH_3)

$[\alpha]_D^{20}$ (c 0.05, CHCl_3):	-39
HRMS (m/z ESI ⁺):	Found: 666.3356 ($[\text{M} + \text{H}]^+$, $\text{C}_{31}\text{H}_{48}\text{N}_5\text{O}_{11}$) Requires: 666.3350
ν_{max} (film)/ cm^{-1} :	1742, 1656 (C=O), 1255 (CH)

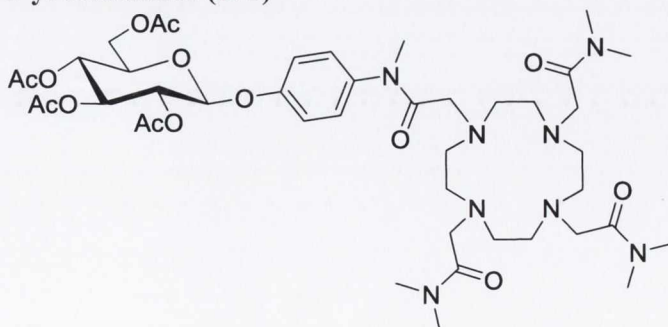
2-Chloro-*N,N*-dimethylacetamide (191)



A solution of *N,N*-dimethylamine 40% solution (3.13 mL, 22.54 mmol) in CH_2Cl_2 (10 mL) and NaOH (1.88 g, 47.08 mmol) was cooled to 0 °C. To this was added a solution of chloroacetyl chloride (5 mL, 22.54 mmol) in CH_2Cl_2 (10 mL) dropwise over 1 h and the reaction was stirred for 16 h at rt. H_2O (40 mL) was added and the product was extracted with CH_2Cl_2 (2 x 20 mL). The organic layers were combined and washed with 1M aq. HCl solution (20 mL), dried over MgSO_4 , filtered and the solvent was removed *in vacuo* to give the product as a colourless oil (0.73 g, 58%). The isolated compound was in good agreement with the literature.³⁶⁹

δ_{H} (400 MHz, CDCl_3):	4.05 (2H, s, CH_2), 3.07, 2.96 (3H, s, CH_3)
HRMS (m/z ESI ⁺):	Found: 122.0370 ($[\text{M} + \text{H}]^+$, $\text{C}_4\text{H}_8\text{ClNO}$) Requires: 122.0373

1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside]-1,4,7,10-tetraazacyclododec-1-yl-acetamide (192)

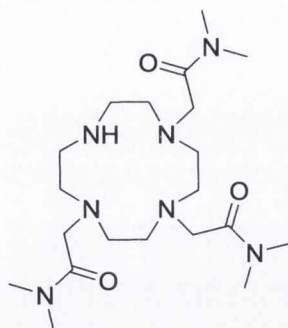


Prepared as per Procedure 16 using **189** (0.46 g, 0.89 mmol) in CH_3CN (12 mL) and following alumina column chromatography (gradient elution from

CH₂Cl₂ to CH₂Cl₂:CH₃OH - 95:5), the product was obtained as a yellow oil (0.56 g, 62%).

δ_{H} (600 MHz, CDCl ₃):	7.14 (2H, d, J = 8.5 Hz, Ar-CH), 7.03 (2H, d, J = 8.5 Hz, Ar-CH), 5.33 (1H, d, J = 9.0 Hz, H-2), 5.27 (1H, app t, J = 9.1 Hz, H-3), 5.22 (1H, d, J = 7.4 Hz, H-1), 5.19 (1H, d, J = 9.5 Hz, H-4), 4.33 (1H, dd, J = 12.5 Hz, J = 4.7 Hz, H-6), 4.21 (1H, dd, J = 12.1 Hz, J = 1.7 Hz, H-6'), 3.99 (1H, m, H-5), 3.43-2.32 (45H, br m, cyclen CH ₂ + CH ₂ CON(CH ₃) ₂ + CH ₂ CON-CH ₃ + N-CH ₃), 2.05 (3H, s, CH ₃), 2.05 (3H, s, CH ₃), 2.04 (3H, s, CH ₃), 2.02 (CH ₃)
δ_{C} (150 MHz, CDCl ₃):	171.2, (C=O) 171.0 (C=O), 170.1 (C=O), 170.3 (C=O), 170.0 (C=O), 169.3 (C=O), 169.2 (C=O), 155.9 (qC), 137.8 (qC), 128.6 (Ar-CH), 118.0 (Ar-CH), 98.6 (C-1), 72.7 (C-2), 72.0 (C-5), 71.2 (C-3), 68.2 (C-4), 61.9 (C-6), 56.0 (CH ₂), 55.0 (CH ₂), 54.9 (CH ₂), 53.3 (CH ₂), 37.5 (CH ₂), 36.3 (CH ₃), 36.2 (CH ₃), 36.1 (N-CH ₃), 35.4 (CH ₃), 35.4 (CH ₃), 20.8 (CH ₃), 20.7 (CH ₃), 20.6 (CH ₃), 20.6 (CH ₃)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl ₃):	-20
HRMS (m/z ESI ⁺):	Found: 943.4750 ([M + Na] ⁺ , C ₄₃ H ₆₈ N ₈ O ₁₄) Requires: 943.4753)
ν_{max} (film)/cm ⁻¹ :	2818 (CH), 1752, 1648 (C=O), 1223, 1104 (CH)

**1,4,7-Tris(dimethylcarbamoylmethyl)-1,4,7,10-tetraazacyclododecane
(193)**

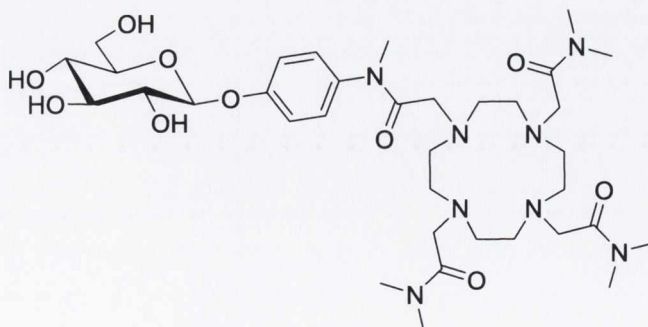


A solution of cyclen (0.3 g, 1.74 mmol) and NaHCO_3 (0.44 g, 5.22 mmol) in CH_3CN (20 mL) was cooled to 0°C and 2-chlorodimethylacetamide (0.64 g, 5.22 mmol) was added and the reaction was refluxed for 72 h. The mixture was cooled to rt and filtered through a plug of celite and the solvent was removed *in vacuo*. The product was purified by alumina flash chromatography (gradient elution from CH_2Cl_2 to $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ - 90:10) to give the product as a white solid (0.46 g, 64%). The isolated compound was in good agreement with the literature.³⁷⁰

δ_{H} (600 MHz, CDCl_3): 10.09 (1H, br s, NH), 3.60 (2H, s, $\text{CH}_2\text{C}=\text{O}$), 3.57 (4H, s, $\text{CH}_2\text{C}=\text{O}$), 3.10 (8H, s, cyclen CH_2), 3.03 (3H, s, CH_3), 2.95 (6H, s, CH_3), 2.89 (9H, s, CH_3), 2.83 (8H, s, cyclen CH_2)

HRMS (m/z ESI⁺): Found: 428.3346 ($[\text{M} + \text{H}]^+$, $\text{C}_{20}\text{H}_{41}\text{N}_7\text{O}_3$)
Requires: 428.3349)

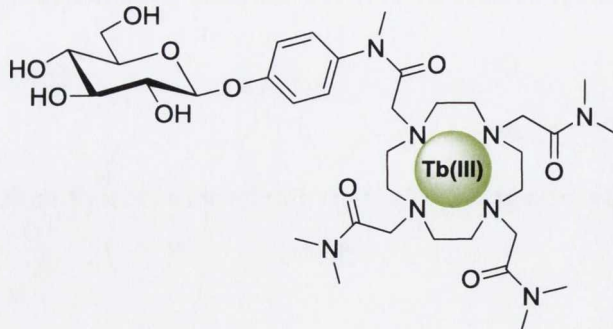
1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-N-methylcarbonylamino)-phenyl- β -D-glucopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (195)



Prepared as per Procedure 17 using **192** (0.5 g, 0.54 mmol) in CH_3OH (6 mL) to give the product as a colourless oil (0.4 g, 98%).

δ_{H} (600 MHz, D_2O):	7.23 (2H, d, $J = 8.4$ Hz, Ar-CH), 7.13 (2H, d, $J = 8.4$ Hz, Ar-CH), 5.09 (1H, d, $J = 7.6$ Hz, H-1), 3.89 (1H, dd, $J = 12.5$ Hz, $J = 2.1$ Hz, H-6), 3.70 (1H, dd, $J = 12.5$ Hz, $J = 5.7$ Hz, H-6'), 3.60 (1H, m, H-5), 3.57 (1H, m, H-2), 3.54 (1H, m, H-3), 3.46 (1H, dd, $J = 18.8$ Hz, $J = 9.22$ Hz, H-4), 3.32-2.64 (45H, br m, cyclen CH_2 + $\text{CH}_2\text{CON}(\text{CH}_3)_2$ + $\text{CH}_2\text{CON}-\text{CH}_3$ + $\text{N}-\text{CH}_3$), 3.29 (3H, s, $\text{N}-\text{CH}_3$)
δ_{H} (150 MHz, D_2O):	173.0 (C=O), 172.9 (C=O), 172.8 (C=O), 156.0 (qC), 137.3 (qC), 128.5 (Ar-CH), 117.4 (Ar-CH), 99.9 (C-1), 76.0 (C-5), 75.4 (C-2), 72.8 (C-3), 69.3 (C-4), 60.5 (C-6), 55.2 (CH_2), 54.8 (CH_2), 54.8 (CH_2), 54.7 (CH_2), 37.1 (CH_2), 48.7 (CH_3), 36.0 (CH_3), 35.9 ($\text{N}-\text{CH}_3$), 35.4 (CH_3), 35.3 (CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.04, CH_3OH):	-40
HRMS (m/z ESI $^+$):	Found: 775.4336 ($[\text{M} + \text{Na}]^+$, $\text{C}_{35}\text{H}_{60}\text{N}_8\text{O}_{10}$) Requires: 775.4330
ν_{max} (film)/ cm^{-1} :	3364 (OH), 2825 (CH), 1751, 1639 (C=O), 1238 (CH)

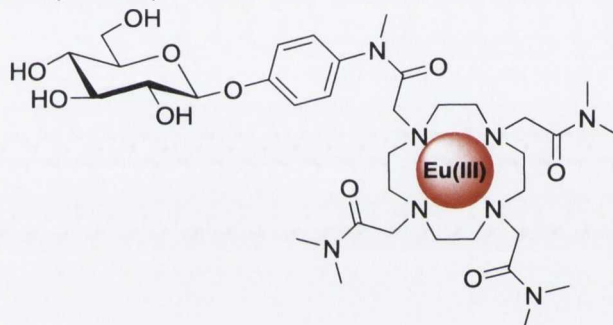
1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl- β -D-glucopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide Tb(III) complex (195.Tb**)**



Prepared as per Procedure 18 using **195** (15 mg, 0.02 mmol) in CH_3OH (4 mL) to give the product as a fine pale yellow solid (22 mg, 84%). M.p. decomposed above 175 $^{\circ}\text{C}$.

δ_{H} (600 MHz, D_2O):	86.42, 65.71, 59.77, 51.19, 45.96, 43.59, 31.17, 27.36, 25.26, 20.48, 18.70, 16.21, 15.76, 12.82, 11.00, 10.72, 8.03, 7.56, 6.22, 3.50, 2.15, -47.78, -56.22, -61.87, -73.15, -81.52
$[\alpha]_{\text{D}}^{20}$ (c 0.05, CH_3OH):	-132
HRMS (m/z MALDI+):	Found: 1209.2775 ($[\text{M} + 2(\text{CF}_3\text{SO}_3)]^+$, $\text{C}_{37}\text{H}_{60}\text{F}_6\text{N}_8\text{O}_{16}\text{S}_2\text{Tb}$ Requires: 1209.2726)
ν_{max} (film)/ cm^{-1} :	3358 (OH), 1734, 1622 (C=O), 1206 (CH)

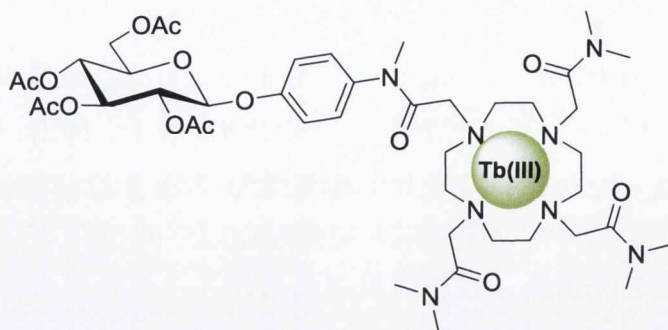
1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl- β -D-glucopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide Eu (III) complex (195.Eu)



Prepared as per Procedure 18 using **195** (46 mg, 0.06 mmol) in CH_3OH (6 mL) to give the product as a fine pale yellow solid (70 mg, 82%). M.p. decomposed above 170 °C.

δ_{H} (400 MHz, CD_3OD):	32.70, 11.11, 7.61, 7.28, 6.52, 2.38, 2.19, 1.97, 1.65, 1.33, 0.93, 0.13, -7.54, -9.01, -10.75
$[\alpha]_{\text{D}}^{20}$ (c 0.07, CH_3OH):	-152
HRMS (m/z MALDI+):	Found: 1203.2698 ($[\text{M} + 2(\text{CF}_3\text{SO}_3)]^+$, $\text{C}_{37}\text{H}_{60}\text{F}_6\text{N}_8\text{O}_{16}\text{S}_2\text{Eu}$ Requires: 1203.2685)
ν_{max} (film)/ cm^{-1} :	3425 (OH), 1618 (C=O), 1249 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide Tb(III) complex (192.Tb)



Prepared as per Procedure 18 using **192** (50 mg, 0.05 mmol) in CH₃OH (4 mL) to give the product as a fine orange solid (66 mg, 87%). M.p. decomposed above 190 °C.

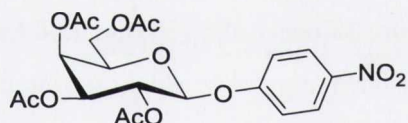
δ_{H} (400 MHz, CDCl₃): 63.75, 45.17, 44.43, 43.97, 33.77, 31.34, 22.87, 21.25, 13.34, 8.79, 4.57, 3.30, 2.97, 2.78, 0.1

$[\alpha]_{\text{D}}^{20}$ (c 0.05, CH₃OH): -112

HRMS (m/z ESI): Found: 1077.3922 ($[\text{M} - 2\text{H}]^+$, C₄₃H₆₆N₈O₁₄Tb)
Requires: 1077.3952)

ν_{max} (film)/cm⁻¹: 1741, 1625 (C=O), 1223 (CH)

4-Nitrophenyl-2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranoside (**199**)

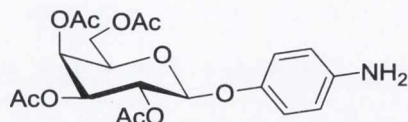


Prepared as per Procedure 12 using 1,2,3,4,6-penta-*O*-acetyl-β-*D*-galactopyranoside (5.0 g, 12.81 mmol) in CH₂Cl₂ (30 mL) to give the product as colourless crystals (3.85 g, 64%). M.p. 141-143 °C, Lit., 143-144 °C.³⁶⁶ The isolated compound was in good agreement with the literature.³⁷¹

δ_{H} (600 MHz, CDCl₃): 8.19 (2H, d, $J = 9.2$ Hz, Ar-CH), 7.06 (2H, d, $J = 9.2$ Hz, Ar-CH), 5.50 (1H, m, H-2), 5.46 (1H, d, $J = 3.3$ Hz, H-4), 5.15 (1H, d, $J = 7.9$ Hz, H-1), 5.12 (1H, dd, $J = 10.3$ Hz, $J = 3.3$ Hz, H-3), 4.23-4.09 (3H, m, H-5, H-6, H-6'), 2.17 (3H, s, CH₃), 2.05 (3H, s, CH₃), 2.05 (3H, s, CH₃), 2.00 (3H, s, CH₃)

HRMS (m/z ESI⁻): Found: 468.1159 ($[M - H]^-$, C₂₀H₂₂NO₁₂)
Requires: 468.1142)

4-Aminophenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (200)

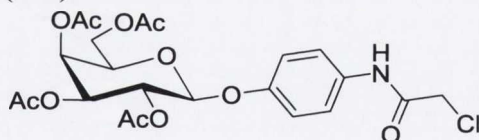


Prepared as per Procedure 13 using **199** (3.5 g, 7.46 mmol) in CH₂Cl₂:CH₃OH (20 mL) to give the product as a yellow oil (2.78 g, 85%). The isolated compound was in good agreement with the literature.³⁷¹

δ_H (600 MHz, CDCl₃): 6.82 (2H, d, J = 8.9 Hz, Ar-CH), 6.59 (2H, d, J = 8.9 Hz, Ar-CH), 5.29 (2H, m, H-2, H-4), 5.24 (1H, dd, J = 10.6 Hz, J = 3.5 Hz, H-3), 4.85 (1H, d, J = 7.9 Hz, H-1), 4.21 (1H, dd, J = 11.2 Hz, J = 6.9 Hz, H-6), 4.13 (1H, dd, J = 11.2 Hz, J = 6.9 Hz, H-6'), 3.97 (1H, app t, J = 8.5 Hz, H-5), 2.16 (3H, s, CH₃), 2.06 (3H, s, CH₃), 2.03 (3H, s, CH₃), 1.99 (3H, s, CH₃)

M/z HRMS (ESI⁺): Found: 462.1366 ($[M + Na]^+$, C₂₀H₂₅NO₁₀)
Requires: 462.1375)

4-(Chloromethylcarbonylamino)phenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (201)

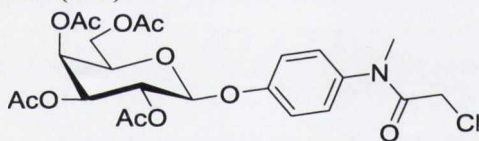


Prepared as per Procedure 14 using **200** (2.44 g, 5.55 mmol) in CH₂Cl₂ (20 mL) and following flash chromatography (EtOAc:Hex - 50:50), the product was obtained as a yellow oil (1.92 g, 67%).

δ_H (400 MHz, CDCl₃): 8.41 (1H, br s, NH), 7.44 (2H, d, J = 8.4 Hz, Ar-CH), 6.96 (2H, d, J = 8.4 Hz, Ar-CH), 5.47-5.39 (2H, m, H-2, H-4), 5.09 (1H, dd, J = 10.5 Hz, J =

	3.4 Hz, H-3), 5.00 (1H, d, $J = 8$ Hz, H-1), 4.19-4.12 (2H, m, H-6, H-6'), 4.14 (2H, s, CH ₂ Cl), 4.06 (1H, m, H-5), 2.14 (3H, s, CH ₃), 2.04 (3H, s, CH ₃), 2.01 (3H, s, CH ₃), 1.98 (3H, s, CH ₃)
δ_{C} (100 MHz, CDCl ₃):	170.4 (C=O), 170.3 (C=O), 170.1 (C=O), 169.5 (C=O), 164.2 (C=O), 154.1 (qC), 132.3 (qC), 121.9 (Ar-CH), 117.5 (Ar-CH), 99.8 (C-1), 71.0 (C-5), 70.7 (C-3), 68.6 (C-2), 67.0 (C-4), 61.4 (C-6), 42.9 (CH ₂ Cl), 20.7 (CH ₃), 20.7 (CH ₃), 20.6 (CH ₃), 20.6 (CH ₃)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl ₃):	-64
HRMS (m/z ESI ⁺):	Found: 538.1095 ([M + Na] ⁺ , C ₂₂ H ₂₆ ClNO ₁₁) Requires: 538.1092)
ν_{max} (film)/cm ⁻¹ :	1750, 1610 (C=O), 1220 (CH)

4-(Chloromethylcarbonylamino)-*N*-methyl-phenyl-2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranoside (202)



Prepared as per Procedure 15 using **201** (0.74 g, 1.43 mmol) in THF (10 mL) to give the product as an orange oil (0.55 g, 72%).

δ_{H} (400 MHz, CDCl ₃):	7.22 (2H, d, $J = 8.5$ Hz, Ar-CH), 7.09 (2H, d, $J = 8.5$ Hz, Ar-CH), 5.52 (2H, m, H-2, H-4), 5.16 (1H, dd, $J = 10.5$ Hz, $J = 3.1$ Hz, H-3), 5.11 (1H, d, $J = 7.8$ Hz, H-1), 4.26 (1H, m, H-6), 4.19 (1H, m, H-6'), 4.12 (1H, m, H-5), 4.21 (2H, s, CH ₂ Cl), 3.30 (3H, s, N-CH ₃), 2.21 (3H, s, CH ₃), 2.11 (3H, s, CH ₃), 2.09 (3H, s, CH ₃), 2.05 (3H, s, CH ₃)
δ_{C} (100 MHz, CDCl ₃):	170.2 (C=O), 170.0 (C=O), 169.9 (C=O), 169.2 (C=O), 166.3 (C=O), 156.3 (qC), 137.6 (qC), 128.3 (Ar-CH), 117.0 (Ar-CH), 99.2 (C-1), 71.0

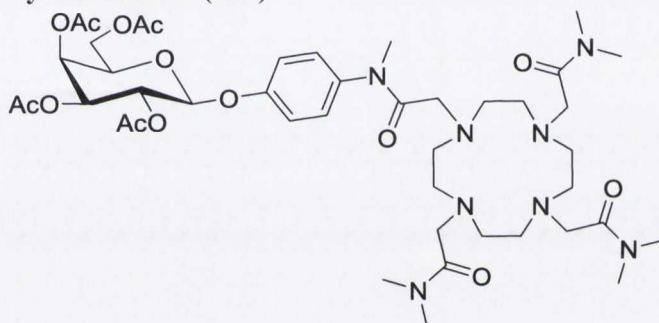
(C-5), 70.6 (C-3), 68.3 (C-2), 66.6 (C-4), 61.2 (C-6), 41.2 (CH₂Cl), 38.0 (N-CH₃), 20.6 (CH₃), 20.5 (CH₃), 20.5 (CH₃), 20.4 (CH₃)

$[\alpha]_D^{20}$ (c 0.05, CHCl₃): -16

HRMS (m/z ESI⁺): Found: 552.1251 ([M + Na]⁺, C₂₃H₂₈ClNO₁₁)
Requires: 552.1249

$\nu_{\max}$ (film)/cm⁻¹: 1756, 1650 (C=O), 1225 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl-2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (203)



Prepared as per Procedure 16 using **202** (0.39 g, 0.59 mmol) in CH₃CN (10 mL) and following alumina column chromatography (gradient elution from CH₂Cl₂ to CH₂Cl₂:CH₃OH - 95:5), the product was obtained as a yellow oil (0.38 g, 72%).

δ_H (600 MHz, CDCl₃): 7.19 (2H, d, J = 8.8 Hz, Ar-CH), 7.07 (2H, d, J = 8.8 Hz, Ar-CH), 5.51 (2H, m, H-2, H-4), 5.18 (2H, m, H-1, H-3), 4.28-4.08 (3H, m, H-5, H-6, H-6'), 3.82-2.33 (45H, br m, cyclen CH₂ + CH₂CON(CH₃)₂ + CH₂CON-CH₃ + N-CH₃), 2.20 (3H, s, CH₃), 2.11 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.04 (3H, s, CH₃)

δ_C (150 MHz, CDCl₃): 171.3 (C=O), 171.1 (C=O), 170.3 (C=O), 170.1 (C=O), 169.9 (C=O), 169.4 (C=O), 156.0 (qC), 137.9 (qC), 128.6 (Ar-CH), 118.0 (Ar-CH), 99.1

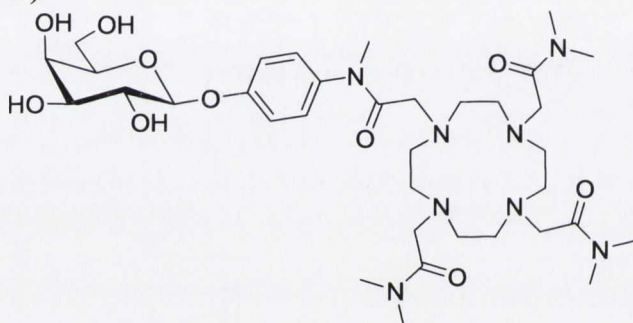
(C-1), 71.0 (C-5), 70.7 (C-3), 68.6 (C-2), 66.8 (C-4), 61.6 (C-6), 56.1 (CH₂), 55.1 (CH₂), 55.0 (CH₂), 53.3 (CH₂), 37.6 (CH₂), 36.4 (CH₃), 36.3 (CH₃), 36.0 (N-CH₃), 35.5 (CH₃), 29.6 (CH₃), 25.6 (CH₃), 20.8 (CH₃), 20.7 (CH₃) 20.6 (CH₃), 20.5 (CH₃)

$[\alpha]_D^{20}$ (c 0.05, CHCl₃): -29

HRMS (m/z ESI⁺): Found: 943.4776 ([M + Na]⁺, C₄₃H₆₈N₈O₁₄)
Requires: 943.4774)

$\nu_{\max}$ (film)/cm⁻¹: 2890 (CH), 1764, 1664 (C=O), 1225, 1231 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl- β -D-galactopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (204)



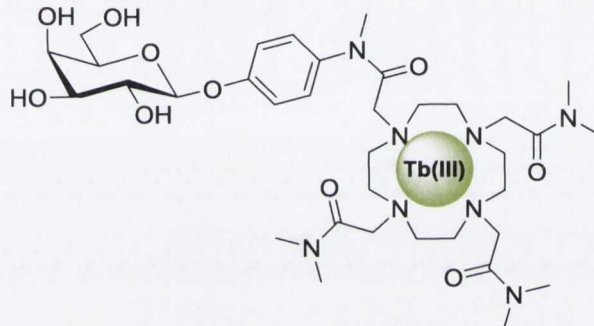
Prepared as per Procedure 17 using **203** (0.3 g, 0.33 mmol) in CH₃OH (6 mL) to give the product as a colourless oil (0.24 g, 98%).

δ_H (600 MHz, D₂O): 7.13 (2H, d, J = 8.9 Hz, Ar-CH), 7.05 (2H, d, J = 8.9 Hz, Ar-CH), 4.93 (1H, d, J = 7.2 Hz, H-1), 3.86 (1H, m, H-2), 3.73 (1H, m, H-4), 3.68-3.57 (4H, m, H-3, H-5, H-6, H-6'), 3.36-1.87 (45H, br m, cyclen CH₂ + CH₂CON(CH₃)₂ + CH₂CON-CH₃ + N-CH₃)

δ_H (150 MHz, D₂O): 173.1 (C=O), 164.7 (C=O), 172.9 (C=O), 163.8 (C=O), 156.2 (qC), 137.3 (qC), 128.5 (Ar-CH), 117.5 (Ar-CH), 100.5 (C-1), 75.4 (C-2), 72.5 (C-5), 70.4 (C-3), 68.4 (C-4), 60.7 (C-6), 55.3 (CH₂), 54.9 (CH₂), 54.8 (CH₂), 37.2 (N-CH₃), 36.1 (CH₃), 36.1 (CH₃), 35.5 (CH₃), 35.4 (CH₃)

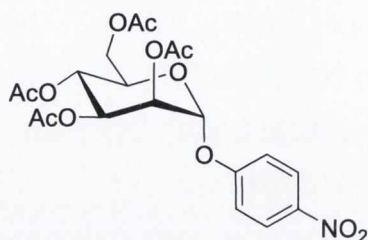
$[\alpha]_D^{20}$ (c 0.05, CH ₃ OH):	-47
HRMS (m/z ESI ⁺):	Found: 775.4337 ([M + Na] ⁺ , C ₃₅ H ₆₀ N ₈ O ₁₀) Requires: 775.4330)
$\nu_{\max}$ (film)/cm ⁻¹ :	3361 (OH), 2830 (CH), 1754, 1678 (C=O), 1229 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl- β -D-galactopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide Tb (III) complex (204.Tb**)**



Prepared as per Procedure 18 using **204** (30 mg, 0.04 mmol) in CH₃OH (4 mL) to give the product as a fine pale yellow solid (48 mg, 89%). M.p decomposed above 175 °C.

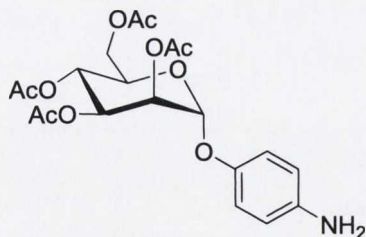
δ_H (400 MHz, D ₂ O):	71.93, 65.47, 62.73, 62.07, 56.77, 52.96, 50.71, 47.82, 47.24, 45.41, 38.54, 34.23, 31.24, 29.92, 26.10, 18.48, 16.57, 15.99, 15.58, 15.08, 10.86, 9.28, 3.89, 3.76, 3.21, 2.87, 2.83, 2.37, 1.63, 1.31, 0.95
$[\alpha]_D^{20}$ (c 0.05, CH ₃ OH):	-132
HRMS (m/z MALDI ⁺):	Found: 909.3560 ([M - 2H] ⁺ , C ₃₅ H ₅₈ N ₈ O ₁₀ Tb) Requires: 909.3569)
$\nu_{\max}$ (film)/cm ⁻¹ :	3349 (OH), 1731, 1642 (C=O), 1225 (CH)

4-Nitrophenyl-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside(205)

Prepared as per Procedure 12 using 1,2,3,4,6-penta-*O*-acetyl- α -D-mannopyranoside (4 g, 10.25 mmol) in CH_2Cl_2 (25 mL) and following recrystallisation, the product was obtained as off-white crystals (4.19 g, 87%). M.p. 143-145 °C, Lit., 145.1-145.1 °C.³⁷¹ The isolated compound was in good agreement with the literature.³⁷¹

δ_{H} (600 MHz, CDCl_3): 8.25 (2H, d, $J = 9.2$ Hz, Ar-CH), 7.23 (2H, d, $J = 9.2$ Hz, Ar-CH), 5.65 (1H, br s, H-1), 5.56 (1H, dd, $J = 10$ Hz, $J = 3.6$ Hz, H-3), 5.48 (1H, dd, $J = 3.2$ Hz, $J = 1.6$ Hz, H-2), 5.40 (1H, app t, $J = 9.9$ Hz, H-4), 4.29 (1H, dd, $J = 12.5$ Hz, $J = 5.5$ Hz, H-6), 4.10 (1H, dd, $J = 12.3$ Hz, $J = 2.1$ Hz, H-6'), 4.03 (1H, m, H-5), 2.24 (3H, s, CH_3), 2.08 (3H, s, CH_3), 2.07 (3H, s, CH_3), 2.04 (3H, s, CH_3)

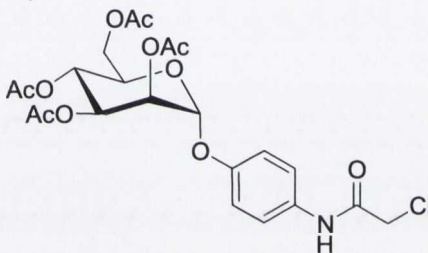
HRMS (m/z ESI): Found: 504.0907 ($[\text{M} + \text{Cl}]^+$, $\text{C}_{20}\text{H}_{23}\text{NO}_{12}$ Requires: 504.0909)

4-Aminophenyl-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (206)

Prepared as per Procedure 13 using **205** (4.4 g, 9.37 mmol) in $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ (30 mL) to give the product as a yellow oil (3.62 g, 88%). The isolated compound was in good agreement with the literature.³⁷²

δ_{H} (600 MHz, CDCl_3):	7.02 (2H, d, $J = 8.8$ Hz, Ar-CH), 7.02 (2H, d, $J = 8.8$ Hz, Ar-CH), 5.56 (1H, dd, $J = 3.5$ Hz, $J = 10.1$ Hz, H-3), 5.45 (1H, m, H-2), 5.44 (1H, br s, H-1), 5.38 (1H, app t, $J = 9.9$ Hz, H-4), 4.30 (1H, dd, $J = 12.1$ Hz, $J = 5.1$ Hz, H-6), 4.13 (1H, m, H-5), 4.10 (1H, dd, $J = 12.1$ Hz, $J = 2.2$ Hz, H-6'), 2.21 (3H, s, CH_3), 2.19 (3H, s, CH_3), 2.08 (3H, s, CH_3), 2.05 (3H, s, CH_3)
HRMS (m/z ESI $^+$):	Found: 462.1371 ($[\text{M} + \text{Na}]^+$, $\text{C}_{20}\text{H}_{25}\text{NO}_{10}$) Requires: 462.1373)

4-(Chloromethylcarbonylamino)phenyl-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (207)

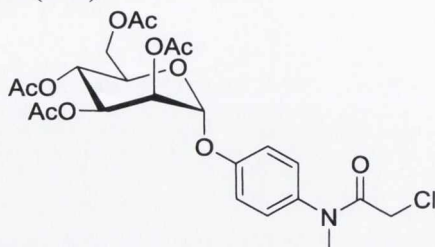


Prepared as per Procedure 14 using **206** (3.9 g, 8.88 mmol) in CH_2Cl_2 (15 mL) and following flash chromatography (EtOAc:Hex - 50:50), the product was obtained as a yellow oil (3.25 g, 71%). The isolated compound was in good agreement with the literature.³¹⁵

δ_{H} (600 MHz, CDCl_3):	8.20 (1H, s, NH), 7.51 (2H, d, $J = 8.8$ Hz, Ar-CH), 7.12 (2H, d, $J = 8.8$ Hz, Ar-CH), 5.58 (1H, dd, $J = 10.1$ Hz, $J = 3.4$ Hz, H-3), 5.52 (1H, br s, H-1), 5.47 (1H, m, H-2), 5.39 (1H, app t, $J = 10.1$ Hz, H-4), 4.31 (1H, dd, $J = 12.5$ Hz, $J = 5.5$ Hz, H-6), 4.22 (1H, s, CH_2Cl), 4.13 (1H, m, H-5), 4.11 (1H, m, H-6'), 2.23 (3H, s, CH_3), 2.08 (3H, s, CH_3), 2.07 (3H, s, CH_3), 2.07 (3H, s, CH_3)
HRMS (m/z APCI $^-$):	Found: 514.1100 ($[\text{M} - \text{H}]^-$, $\text{C}_{22}\text{H}_{26}\text{ClNO}_{11}$) Requires: 514.1122)

$\nu_{\max}$ (film)/ cm^{-1} : 1741, 1683 (C=O), 1210 (CH)

4-(Chloromethylcarbonylamino)-*N*-methyl-phenyl-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (208)



Prepared as per Procedure 15 using **207** (1.38 g, 2.67 mmol) in THF (20 mL) to give the product as an orange oil (1.02 g, 72%).

δ_{H} (400 MHz, CDCl_3): 7.16 (4H, m, Ar-CH), 5.59 (1H, m, H-3), 5.57 (1H, m, H-1), 5.47 (1H, m, H-2), 5.43 (1H, app t, $J = 10.1$ Hz, H-4), 4.32 (1H, dd, $J = 12.6$ Hz, $J = 5.4$ Hz, H-6), 4.12 (1H, m, H-5), 4.09 (1H, m, H-5), 3.86 (2H, s, CH_2Cl), 3.31 (3H, s, N- CH_3), 2.24 (3H, s, CH_3), 2.07 (3H, s, CH_3), 2.07 (3H, s, CH_3)

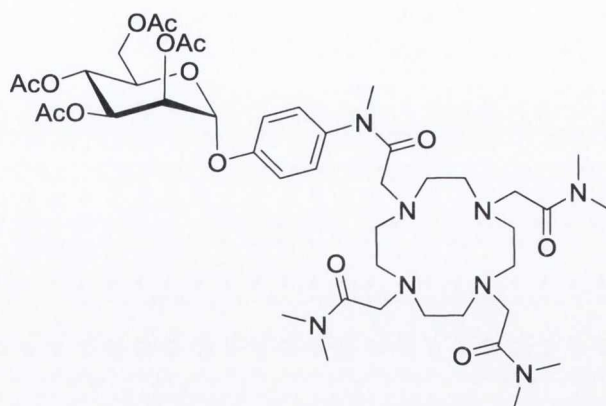
δ_{C} (100 MHz, CDCl_3): 171.2 (C=O), 170.5 (C=O), 170.1 (C=O), 169.7 (C=O), 166.5 (C=O), 155.6 (qC), 137.7 (qC), 128.5 (Ar-CH), 117.8 (Ar-CH), 95.9 (C-1), 69.4 (C-5), 69.2 (C-2), 68.7 (C-3), 65.9 (C-4), 62.0 (C-6), 41.4 (CH_2Cl), 38.2 (N- CH_3), 21.1 (CH_3), 20.9 (CH_3), 20.7 (CH_3), 20.7 (CH_3)

$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl_3): +96

HRMS (m/z ESI $^{+}$): Found: 530.1427 ($[\text{M} + \text{H}]^{+}$, $\text{C}_{23}\text{H}_{29}\text{ClNO}_{11}$)
Requires: 530.1429)

$\nu_{\max}$ (film)/ cm^{-1} : 1749, 1665 (C=O), 1222 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (209)



Prepared as per Procedure 16 using **208** (0.96 g, 1.81 mmol) in CH₃CN (10 mL) and following alumina column chromatography (gradient elution from CH₂Cl₂ to CH₂Cl₂:CH₃OH - 95:5), the product was obtained as a yellow oil (1.03 g, 62%).

δ_{H} (600 MHz, CDCl₃): 7.19 (2H, d, J = 8.6 Hz, Ar-CH), 7.14 (2H, d, J = 8.6 Hz, Ar-CH), 5.57 (1H, dd, J = 9.8, J = 3.4 Hz, H-3), 5.54 (1H, br s, H-1), 5.44 (1H, m, H-2), 5.43 (1H, m, H-4), 4.30 (1H, dd, J = 12.5 Hz, J = 4.9 Hz, H-6), 4.16 (1H, m, H-6'), 4.14 (1H, m, H-5), 3.56-2.54 (45H, br m, cyclen CH₂ + CH₂CON(CH₃)₂ + CH₂CON-CH₃ + N-CH₃), 2.23 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.07 (3H, s, CH₃), 2.07 (3H, s, CH₃)

δ_{C} (150 MHz, CDCl₃): 171.3 (C=O), 171.3 (C=O), 171.2 (C=O), 170.4 (C=O), 170.1 (C=O), 169.9 (C=O), 169.6 (C=O), 155.2 (qC), 137.9 (qC), 128.7 (Ar-CH), 117.8 (Ar-CH), 96.2 (C-1), 69.4 (C-2), 69.3 (C-5), 68.6 (C-3), 65.7 (C-4), 61.9 (C-6), 56.2 (CH₂), 55.1 (CH₂), 55.0 (CH₂), 37.6 (CH₂), 36.4 (CH₂), 36.3 (CH₃), 36.3 (CH₃), 35.6 (N-CH₃), 35.6 (CH₃), 30.9 (CH₃), 20.9 (CH₃), 20.7 (CH₃), 20.7 (CH₃), 20.6 (CH₃)

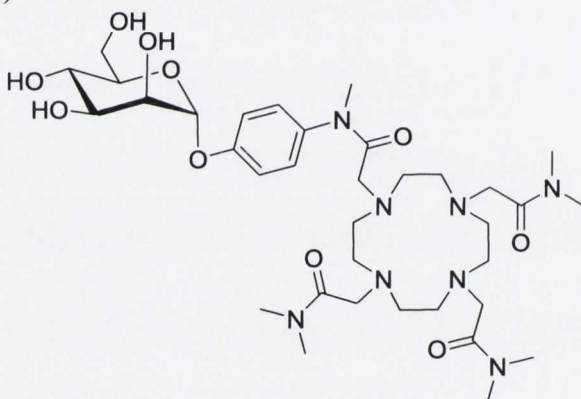
$[\alpha]_{\text{D}}^{20}$ (c 0.06, CHCl₃): +114

HRMS (m/z ESI⁺): Found: 943.4764 ([M + Na]⁺, C₄₃H₆₈N₈O₁₄)
Requires: 943.4747)

$\nu_{\max}$ (film)/ cm^{-1} :

1745, 1647 (C=O), 1216 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl- α -D-mannopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (210)



Prepared as per Procedure 17 using **209** (0.82 g, 0.89 mmol) in CH_3OH (10 mL) to give the product as a colourless oil (0.65 g, 97%).

 δ_{H} (600 MHz, D_2O):

7.22 (2H, d, $J = 8.8$ Hz, Ar-CH), 7.16 (2H, d, $J = 8.8$ Hz, Ar-CH), 5.56 (1H, br s, H-1), 4.10 (1H, m, H-2), 3.96 (1H, dd, $J = 9.4$ Hz, $J = 3.2$ Hz, H-3), 3.73 (1H, m, H-6), 3.70 (1H, m, H-4), 3.68 (1H, m, H-6'), 3.64 (1H, m, H-5), 3.40-2.76 (45H, br m, cyclen CH_2 + $\text{CH}_2\text{CON}(\text{CH}_3)_2$ + $\text{CH}_2\text{CON}-\text{CH}_3$ + N- CH_3)

 δ_{C} (150 MHz, D_2O):

173.1 (C=O), 173.0 (C=O), 172.9 (C=O), 154.9 (qC), 137.2 (qC), 128.5 (Ar-CH), 118.1 (Ar-CH), 98.7 (C-1), 73.6 (C-5), 70.6 (C-3), 69.9 (C-2),

66.6 (C-4), 60.7 (C-6), 58.0 (CH₂), 55.3 (CH₂),
54.9 (CH₂), 54.7 (CH₂), 43.9 (CH₂), 37.2 (CH₃),
36.2 (CH₃), 36.1 (N-CH₃), 35.5 (CH₃), 35.4
(CH₃)

$[\alpha]_D^{20}$ (c 0.05, CH₃OH):

+74

HRMS (*m/z* MALDI+):

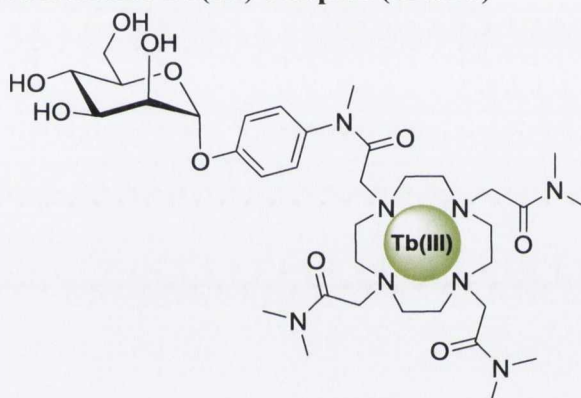
Found: 775.4335 ([M + Na]⁺, C₃₅H₆₀N₈O₁₀

Requires: 775.4330)

$\nu_{\max}$ (film)/cm⁻¹:

3376 (OH), 1624 (C=O), 1229 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide Tb(III) complex (210.Tb)



Prepared as per Procedure 8 using **210** (0.26 g, 0.35 mmol) in CH₃OH (10 mL) to give the product as a fine pale yellow solid (0.39 g, 84%). M.p. decomposed above 178 °C.

δ_H (400 MHz, CD₃OD):

51.57, 46.40, 44.91, 43.56, 34.63, 32.16, 24.95,
20.11, 18.48, 12.38, 10.80, 7.94, 7.26, 3.77, 3.53,
3.04, 2.20, 1.91, 1.35, 1.21, -59.74, -82.24, -
82.00, -88.41, -88.18, -95.39, -103.74

$[\alpha]_D^{20}$ (c 0.06, CH₃OH):

+153

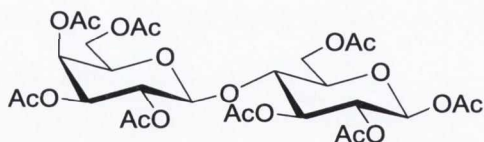
HRMS (*m/z* MALDI+):

Found: 1059.3160 ([M + CF₃SO₃]²⁺, C₃₆H₅₉F₉N₈
O₁₃SF₉Tb Requires: 1059.3128)

$\nu_{\max}$ (film)/cm⁻¹:

3436 (OH), 1616 (C=O), 1226 (CH)

2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-1,2,3,6-tetra-*O*-acetyl- β -D-glucopyranoside

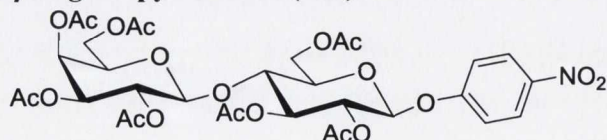


To a suspension of NaOAc (0.79 g, 9.64 mmol) in Ac₂O (10 mL) stirring at reflux was slowly added D-lactose (3 g, 8.76 mmol) and the mixture was refluxed for an additional 30 min. The now clear solution was added to ice H₂O (300 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were washed with H₂O (50 mL), sat. aq. NaHCO₃ solution (50 mL) and brine (50 mL), dried over MgSO₄, filtered, and the solvent was removed *in vacuo*. The crude product was recrystallised from EtOAc/Hex to give the product as colourless crystals (4.8 g, 81%). M.p. 140-142 °C, Lit., 138-142 °C.³⁷³ The isolated compound was in good agreement with the literature.³⁷⁴

δ_{H} (400 MHz, CDCl₃): 5.70 (1H, d, $J = 8.3$ Hz, H-1_{Glc}), 5.37 (1H, app t, $J = 5.3$ Hz, H-4_{Gal}), 5.27 (1H, app t, $J = 5.3$ Hz, H-3_{Glc}), 5.17-5.03 (2H, m, H-2_{Gal}, H-2_{Glc}), 4.97 (1H, dd, $J_{3,4} = 3.5$ Hz, $J_{3,2} = 10.4$ Hz, H-3_{Gal}), 4.53-4.45 (2H, m, H-1_{Gal}, H-6_{Glc}), 4.21-4.07 (3H, m, H-6'_{Glc}, H-6_{Gal}, H-6'_{Gal}), 3.93-3.83 (2H, m, H-4_{Glc}, H-5_{Gal}), 3.79 (1H, m, H-5_{Glc}), 2.25 (3H, s, CH₃), 2.15 (3H, s, CH₃), 2.12 (3H, s, CH₃), 2.09 (3H, s, CH₃), 2.08 (3H, s, CH₃), 2.07 (3H, s, CH₃), 2.06 (3H, s, CH₃), 1.99 (3H, s, CH₃)

HRMS (m/z ESI⁺): Found: 701.1898 ([M + Na]⁺, C₂₈H₃₈O₁₉)
Requires: 701.1899)

4-Nitrophenyl-2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tetra-*O*-acetyl- β -D-glucopyranoside (211)



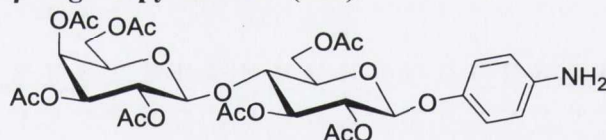
Prepared as per Procedure 12 using 2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-1,2,3,6-tetra-*O*-acetyl- β -D-glucopyranoside (1.51 g, 10.87 mmol) in CH₂Cl₂ (20 mL) to give the product as colourless crystals (2.73 g,

68%). M.p. 138-140 °C, Lit., 133.3-135.4 °C.³⁷⁵ The isolated compound was in good agreement with the literature.³⁶⁶

δ_{H} (600 MHz, CDCl_3): 8.23 (2H, d, $J = 9.2$ Hz, Ar-CH), 7.08 (2H, d, $J = 9.2$ Hz, Ar-CH), 5.39 (1H, d, $J = 3.1$ Hz, H-4_{Gal}), 5.33 (1H, app t, $J = 8.2$ Hz, H-3_{Glc}), 5.22 (1H, m, H-1_{Gal}), 5.22 (1H, m, H-2_{Glc}), 5.16 (1H, dd, $J = 7.9$ Hz, $J = 10.5$ Hz, H-3_{Gal}), 5.00 (1H, dd, $J = 3.1$, $J = 10.5$ Hz, H-2_{Gal}), 4.54 (1H, m, H-1_{Glc}), 4.53 (1H, m, H-6_{Glc}), 4.16 (3H, m, H-6'_{Glc}, H-6_{Gal}, H-6'_{Gal}), 3.94 (1H, m, H-5_{Gal}), 3.92 (1H, m, H-5_{Glc}), 3.88 (1H, m, H-4_{Glc}), 2.20 (3H, s, CH_3), 2.19 (3H, s, CH_3), 2.11 (3H, s, CH_3), 2.10 (3H, s, CH_3), 2.09 (3H, s, CH_3), 2.09 (3H, s, CH_3), 2.00 (3H, s, CH_3)

HRMS (m/z ESI⁻): Found: 792.1768 ($[\text{M} + \text{Cl}]^-$, $\text{C}_{32}\text{H}_{39}\text{NO}_{20}\text{Cl}$)
Requires: 792.1754)

4-Aminophenyl-2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tetra-*O*-acetyl- β -D-glucopyranoside (212**)**



Prepared as per Procedure 13 using **211** (1.43 g, 1.93 mmol) in CH_2Cl_2 : CH_3OH (20 mL) to give the product as a yellow oil (1.25 g, 89%). The isolated compound was in good agreement with the literature.³⁷⁶

δ_{H} (400 MHz, CDCl_3): 6.80 (2H, d, $J = 8.3$ Hz, Ar-CH), 6.67 (2H, d, $J = 8.3$ Hz, Ar-CH), 5.33 (1H, d, $J = 3.1$ Hz, H-4_{Gal}), 5.23 (1H, app t, $J = 9.2$ Hz, H-3_{Glc}), 5.21 (2H, m, H-2_{Gal}, H-2_{Glc}), 4.94 (1H, dd, $J = 7.9$ Hz, $J = 10.5$ Hz, H-3_{Gal}), 4.87 (1H, d, $J = 7.7$ Hz, H-1_{Glc}), 4.48 (2H, m, H-1_{Gal}, H-6_{Glc}), 4.08 (3H, m, H-6'_{Glc}, H-6_{Gal}, H-6'_{Gal}), 3.86 (2H, m, H-5_{Gal}, H-4_{Glc}), 3.69

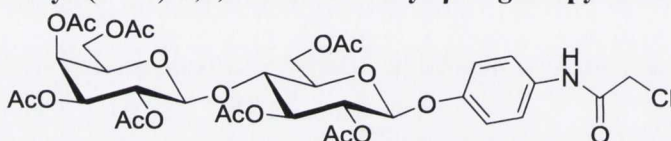
(1H, m, H-5_{Glc}), 2.13 (3H, s, CH₃), 2.07 (3H, s, CH₃), 2.04 (3H, s, CH₃), 2.04 (3H, s, CH₃), 2.04 (3H, s, CH₃), 2.02 (3H, s, CH₃), 1.94 (3H, s, CH₃)

HRMS (*m/z* APCI⁺):

Found: 728.2386 ([M + H]⁺, C₃₂H₄₁NO₁₈)

Requires: 728.2396)

4-(Chloromethylcarbonylamino)phenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tetra-*O*-acetyl-β-D-glucopyranoside (213)



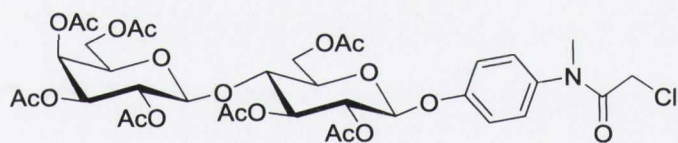
Prepared as per Procedure 14 using **212** (1.21 g, 1.66 mmol) in CH₂Cl₂ (20 mL) and following flash chromatography (EtOAc:Hex - 50:50), the product was obtained as a yellow oil (1.14 g, 86%).

δ_H (600 MHz, CDCl₃):

8.19 (1H, s, NH), 7.48 (2H, d, *J* = 9.1 Hz, Ar-CH), 7.00 (2H, d, *J* = 9.1 Hz, Ar-CH), 5.38 (1H, d, *J* = 3.6 Hz, H-4_{Gal}), 5.30 (1H, app t, *J* = 8.9 Hz, H-3_{Glc}), 5.19 (1H, dd, *J* = 9.1 Hz, *J* = 7.7 Hz, H-2_{Glc}), 5.15 (1H, dd, *J* = 7.6 Hz, *J* = 10.2 Hz, H-3_{Gal}), 5.04 (1H, d, *J* = 7.5 Hz, H-1_{Glc}), 4.99 (1H, dd, *J* = 10.5 Hz, *J* = 3.2 Hz, H-2_{Gal}), 4.54 (1H, d, *J* = 7.9 Hz, H-1_{Gal}), 4.52 (1H, d, *J* = 12.8 Hz, H-6_{Glc}), 4.21 (2H, s, CH₂Cl), 4.17 (1H, dd, *J* = 12.3 Hz, *J* = 5.9 Hz, H-6'_{Glc}), 4.17 (1H, m, H6_{Gal}), 4.12 (1H, dd, *J* = 11.1 Hz, *J* = 7.3 Hz, H-6'_{Gal}), 3.91 (1H, app t, *J* = 9.3 Hz, H-4_{Glc}), 3.90 (1H, m, H-5_{Gal}), 3.80 (1H, m, H-5_{Glc}), 2.18, (3H, s, CH₃), 2.12 (3H, s, CH₃), 2.10 (3H, s, CH₃), 2.09 (3H, s, CH₃), 2.09 (3H, s, CH₃), 2.08 (3H, s, CH₃), 1.99 (3H, s, CH₃)

δ_{C} (150 MHz, CDCl_3):	170.3 (C=O), 170.2 (C=O), 170.1 (C=O), 170.0 (C=O), 169.7 (C=O), 169.6 (C=O), 169.1 (C=O), 163.7 (C=O), 154.1 (qC), 132.1 (qC), 121.8 (Ar-CH), 117.7 (Ar-CH), 101.4 (C-1 _{Gal}), 99.2 (C-1 _{Glc}), 76.2 (C-4 _{Glc}), 72.9 (C-5 _{Glc}), 72.8 (C-3 _{Glc}), 71.5 (C-2 _{Glc}), 70.9 (C-4 _{Gal}), 70.8 (C-5 _{Gal}), 69.2 (C-3 _{Gal}), 66.6 (C-2 _{Gal}), 62.0 (C-6 _{Glc}), 60.8 (C-6 _{Gal}), 42.8 (CH_2Cl), 20.8 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 20.6 (CH_3), 20.6 (CH_3), 20.6 (CH_3), 20.5 (CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.02, CHCl_3):	+65
HRMS (m/z MALDI ⁺):	Found: 826.1937 ($[\text{M} + \text{Na}]^+$, $\text{C}_{34}\text{H}_{42}\text{NO}_{19}\text{Cl}$ Requires 826.1944)
ν_{max} (film)/ cm^{-1} :	1750 (C=O), 1222 (CH)

4-(Chloromethylcarbonylamino)-*N*-methyl-phenyl-2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tetra-*O*-acetyl- β -D-glucopyranoside (214)

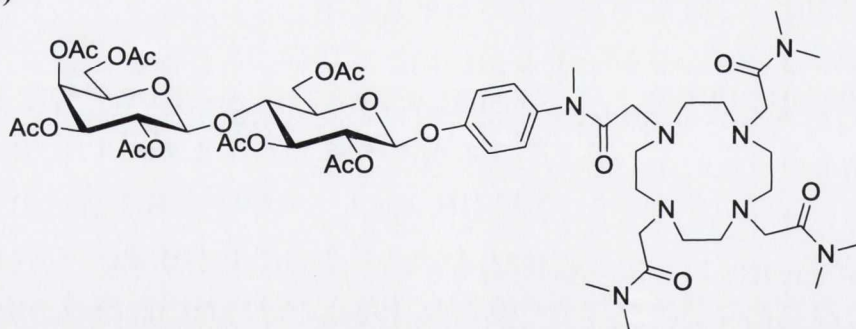


Prepared as per Procedure 15 using **213** (0.7 g, 0.87 mmol) in THF (12 mL) to give the product as an orange oil (0.56 g, 79%).

δ_{H} (600 MHz, CDCl_3):	7.20 (2H, d, $J = 8.7$ Hz, Ar-CH), 7.05 (2H, d, $J = 8.7$ Hz, Ar-CH), 5.39 (1H, d, $J = 3.1$ Hz, H-4 _{Gal}), 5.32 (1H, app t, $J = 8.9$ Hz, H-3 _{Glc}), 5.21 (1H, app t, $J = 8.9$ Hz, H-2 _{Glc}), 5.16 (1H, dd, $J = 7.7$ Hz, $J = 10.7$ Hz, H-3 _{Gal}), 5.11 (1H, d, $J = 7.5$ Hz, H-1 _{Glc}), 5.00 (1H, dd, $J = 10.5$ Hz, $J = 3.3$ Hz, H-2 _{Gal}), 4.55 (1H, d, $J = 7.8$ Hz, H-1 _{Gal}), 4.55 (1H, m, H-6 _{Glc}), 4.18 (1H, m, H-6' _{Glc}), 4.18 (1H, m, H-6 _{Gal}), 4.13 (1H, m, H-6' _{Gal}), 3.94 (1H, m, H-4 _{Glc}), 3.92 (1H, m, H-5 _{Gal}), 3.85 (2H, s, CH_2Cl),
--	---

	3.84 (1H, m, H-5 _{Glc}), 3.30 (3H, s, N-CH ₃), 2.19 (3H, s, CH ₃), 2.19 (3H, s, CH ₃), 2.12 (3H, s, CH ₃), 2.11 (3H, s, CH ₃), 2.10 (3H, s, CH ₃), 2.08 (3H, s, CH ₃), 2.00 (3H, s, CH ₃)
δ_{C} (150 MHz, CDCl ₃):	170.3 (C=O), 170.2 (C=O), 170.1 (C=O), 170.0 (C=O), 169.7 (C=O), 169.5 (C=O), 169.0 (C=O), 166.4 (C=O), 156.4 (qC), 137.7 (qC), 128.5 (Ar-CH), 118.2 (Ar-CH), 101.2 (C-1 _{Gal}), 98.6 (C-1 _{Glc}), 80.0 (C-5 _{Glc}), 76.1 (C-4 _{Glc}), 72.7 (C-3 _{Glc}), 71.4 (C-2 _{Glc}), 70.9 (C-5 _{Gal}), 70.8 (C-2 _{Gal}), 69.1 (C-3 _{Gal}), 66.6 (C-4 _{Gal}), 61.9 (C-6 _{Glc}), 60.8 (C-6 _{Gal}), 41.4 (CH ₂ Cl), 38.1 (N-CH ₃), 20.8 (CH ₃), 20.8 (CH ₃), 20.7 (CH ₃), 20.6 (CH ₃), 20.6 (CH ₃), 20.6 (CH ₃), 20.5 (CH ₃)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl ₃):	+78
HRMS (m/z ESI ⁺):	Found: 840.2075 ([M + Na] ⁺ , C ₃₅ H ₄₄ NO ₁₉ Cl) Requires: 840.2094)
ν_{max} (film)/cm ⁻¹ :	1749 (C=O), 1224 (CH)

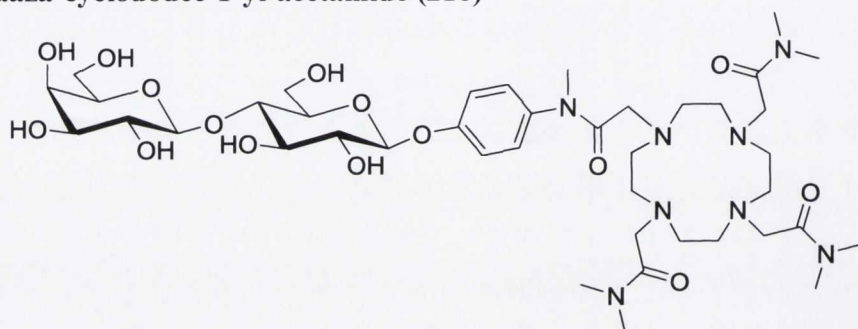
1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl-2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2,3,6-tetra-*O*-acetyl- β -D-glucopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (215)



Prepared as per Procedure 16 using **214** (0.21 g, 0.26 mmol) in CH₃CN (12 mL) and following alumina column chromatography (gradient elution from CH₂Cl₂ to CH₂Cl₂:CH₃OH - 95:5), the product was obtained as a yellow oil (0.22 g, 71%).

δ_{H} (600 MHz, CDCl_3):	7.17 (2H, d, $J = 8.4$ Hz, Ar-CH), 7.03 (2H, d, $J = 8.4$ Hz, Ar-CH), 5.39 (1H, d, $J = 3.1$ Hz, H-4 _{Gal}), 5.32 (1H, app t, $J = 8.8$ Hz, H-3 _{Glc}), 5.20 (1H, m, H-2 _{Glc}), 5.16 (1H, m, H-1 _{Glc}), 5.16 (1H, m, H-3 _{Gal}), 5.00 (1H, dd, $J = 3.3$ Hz, $J = 10.5$ Hz, H-2 _{Gal}), 4.57 (1H, m, H-6 _{Glc}), 4.56 (1H, m, H-1 _{Gal}), 4.18 (1H, m, H-6' _{Glc}), 4.17 (1H, m, H-6 _{Gal}), 4.12 (1H, m, H-6' _{Gal}), 3.96 (1H, m, H-5 _{Glc}), 3.93 (1H, m, H-5 _{Gal}), 3.88 (1H, m, H-4 _{Glc}), 3.48-2.24 (45H, br m, cyclen $\text{CH}_2 + \text{CH}_2\text{CON}(\text{CH}_3)_2 + \text{CH}_2\text{CON-CH}_3 + \text{N-CH}_3$), 2.14 (3H, s, CH_3), 2.06 (3H, s, CH_3), 2.06 (3H, s, CH_3), 2.05 (3H, s, CH_3), 2.05 (3H, s, CH_3), 2.04 (3H, s, CH_3), 1.95 (3H, s, CH_3)
δ_{C} (150 MHz, CDCl_3):	171.2 (C=O), 171.1 (C=O), 171.1 (C=O), 170.2 (C=O), 170.1 (C=O), 170.0 (C=O), 169.9 (C=O), 169.5 (C=O), 169.5 (C=O), 168.9 (C=O), 156.0 (qC), 137.9 (qC), 128.5 (Ar-CH), 118.0 (Ar-CH), 101.0 (C-1 _{Gal}), 98.2 (C-1 _{Glc}), 75.8 (C-5 _{Glc}), 72.7 (C-4 _{Glc}), 72.6 (C-3 _{Glc}), 71.4 (C-2 _{Glc}), 70.8 (C-2 _{Gal}), 70.6 (C-5 _{Gal}), 69.3 (CH_2), 68.9 (C-3 _{Gal}), 66.6 (C-4 _{Gal}), 61.9 (C-6 _{Glc}), 60.7 (C-6 _{Gal}), 56.0 (CH_2), 55.0 (CH_2), 54.9 (CH_2), 37.5 (CH_2), 36.2 (CH_3), 36.2 (N- CH_3), 36.1 (CH_3), 35.4 (CH_3), 35.4 (CH_3), 35.4 (CH_3), 20.7 (CH_3), 20.6 (CH_3), 20.6 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 20.5 (CH_3), 20.3 (CH_3)
$[\alpha]_{\text{D}}^{20}$ (c 0.05, CHCl_3):	+62
HRMS (m/z MALDI ⁺):	Found: 1209.5759 ($[\text{M} + \text{H}]^+$, $\text{C}_{55}\text{H}_{84}\text{N}_8\text{O}_{22}$ Requires: 1209.5778)
ν_{max} (film)/ cm^{-1} :	2970 (CH), 1747, 1648 (C=O), 1218, 1047 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (216)



Prepared as per Procedure 17 using **215** (0.15 g, 0.12 mmol) in CH₃OH (10 mL) to give the product as a colourless oil (0.11 g, 96%).

δ_{H} (600 MHz, D₂O):

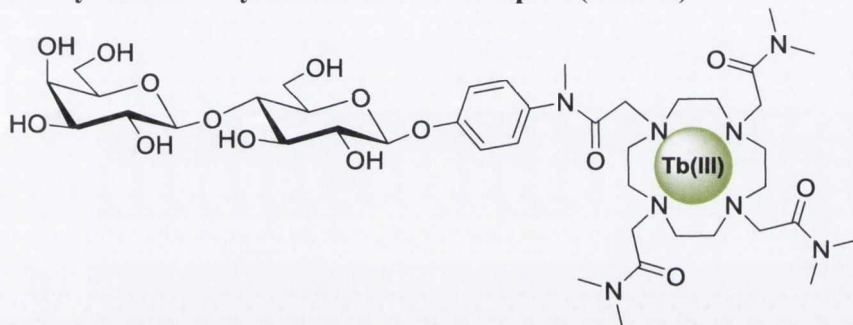
7.25 (2H, d, J = 8.6 Hz, Ar-CH), 7.16 (2H, d, J = 8.6 Hz, Ar-CH), 5.13 (1H, br s, H-1_{Glc}), 4.45 (1H, d, J = 7.7 Hz, H-1_{Gal}), 3.95 (1H, m, H-6_{Glc}), 3.89 (1H, d, J = 2.9 Hz, H-4_{Gal}), 3.82 (1H, m, H-6'_{Glc}), 3.76 (2H, m, H-6_{Gal}, H-6'_{Gal}), 3.74 (3H, m, H-3_{Glc}, H-4_{Glc}, H-5_{Glc}), 3.70 (1H, m, H-5_{Gal}), 3.63 (1H, m, H-3_{Gal}), 3.59 (1H, m, H-2_{Glc}), 3.52 (1H, m, H-2_{Gal}), 3.48-2.27 (45H, br m, cyclen CH₂ + CH₂CON(CH₃)₂ + N-CH₃)

δ_{C} (150 MHz, D₂O):

172.8 (C=O), 168.3 (C=O), 156.3 (qC), 137.1 (qC), 128.4 (Ar-CH), 117.4 (Ar-CH), 103.8 (C-1_{Gal}), 100.9 (C-1_{Glc}), 78.7 (C-5_{Glc}), 76.0 (C-5_{Gal}), 75.8 (C-4_{Glc}), 73.7 (C-3_{Glc}), 71.1 (C-2_{Glc}), 71.1 (C-2_{Gal}), 69.2 (C-3_{Gal}), 63.9 (C-4_{Gal}), 61.3 (C-6_{Gal}), 60.4 (C-6_{Glc}), 55.3 (CH₂), 54.7 (CH₂), 54.7

	(CH ₂), 37.2 (N-CH ₃), 36.1 (CH ₃), 35.4 (CH ₃), 35.3 (CH ₃), 23.7 (CH ₃)
$[\alpha]_D^{20}$ (c 0.04, CH ₃ OH):	+76
HRMS (<i>m/z</i> MALDI ⁺):	Found: 915.5042 ([M + H] ⁺ , C ₄₁ H ₇₁ N ₈ O ₁₅ Requires: 915.5039)
$\nu_{\max}$ (film)/cm ⁻¹ :	3661 (OH), 2981 (CH), 1698 (C=O), 1252 (CH)

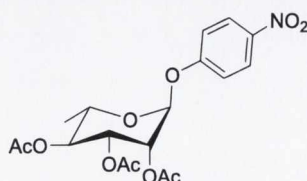
1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide Tb complex (216.Tb)



Prepared as per Procedure 18 using **216** (60 mg, 0.07 mmol) in CH₃OH (6 mL) to give the product as a fine pale yellow solid (78 mg, 78%). M.p. decomposed above 180 °C.

δ_H (400 MHz, CDCl ₃):	65.88, 50.97, 45.75, 43.95, 31.01, 27.01, 25.06, 24.29, 20.40, 16.14, 15.30, 13.70, 10.79, 9.96, 9.67, 8.29, 7.16, 7.09, 2.24, 1.16
$[\alpha]_D^{20}$ (c 0.05, CH ₃ OH):	+96
HRMS (<i>m/z</i> MALDI ⁺):	Found: 1221.3662 ([M + CF ₃ SO ₃] ²⁺ , C ₄₂ H ₆₉ N ₈ O ₁₈ SF ₃ Tb Requires 1221.3656)
$\nu_{\max}$ (film)/cm ⁻¹ :	3431 (OH), 2981 (CH), 1624 (C=O), 1252 (CH)

4-Nitrophenyl-2,3,4,6-tetra-*O*-acetyl- α -L-rhamnopyranoside (217)

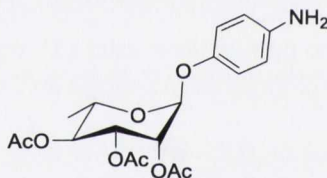


Prepared as per Procedure 12 using 1,2,3,4,6-Penta-*O*-acetyl-L-rhamnopyranoside (0.9 g, 2.71 mmol) in CH₂Cl₂ (10 mL) and following recrystallisation, the product was obtained as colourless crystals (1.03 g, 92%). M.p. 142-145 °C, Lit., 146-147 °C.³⁷⁷ The isolated compound was in good agreement with the literature.³⁷⁸

δ_{H} (400 MHz, CDCl₃): 8.21 (2H, d, $J = 9.1$ Hz, Ar-CH), 7.17 (2H, d, $J = 9.1$ Hz, Ar-CH), 5.59 (1H, br s, H-1), 5.54 (1H, m, H-3), 5.47 (1H, m, H-2), 5.16 (1H, app t, $J = 10.2$ Hz, H-4), 3.98 (1H, m, H-5), 2.19 (3H, s, CH₃), 2.04 (3H, s, CH₃), 2.02, (3H, s, CH₃), 1.19 (3H, d, $J = 5.7$ Hz, H-6)

HRMS (m/z ESI⁺): Found: 434.1058 ([M + Na]⁺, C₁₈H₂₁NO₁₀)
Requires: 434.1059)

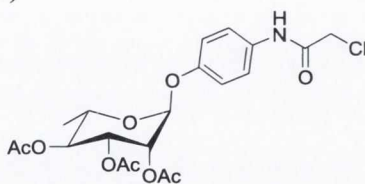
4-Aminophenyl-2,3,4-Tri-*O*-acetyl- α -L-rhamnopyranoside (218)



Prepared as per Procedure 13 using **217** (0.85 g, 2.06 mmol) in CH₂Cl₂:CH₃OH (15 mL) to give the product as a yellow oil (0.73 g, 92%). The isolated compound was in good agreement with the literature.³⁷⁹

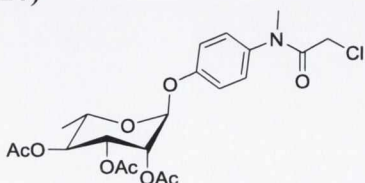
δ_{H} (600 MHz, CDCl₃): 7.25 (2H, d, $J = 6.4$ Hz, Ar-CH), 7.06 (2H, d, $J = 8.6$ Hz, Ar-CH), 5.50 (1H, dd, $J = 2.8$, $J = 10.2$ Hz, H-3), 5.45 (1H, m, H-2), 5.44 (1H, m, H-1), 5.18 (1H, app t, $J = 10.1$ Hz, H-4), 3.91 (1H, m, H-5), 2.17 (3H, s, CH₃), 2.04 (3H, s, CH₃), 2.01, (3H, s, CH₃), 1.17 (3H, d, $J = 6.2$ Hz, H-6)

HRMS (m/z ESI⁺): Found: 382.1510 ([M + H]⁺, C₁₈H₂₃NO₈)
Requires: 382.1502)

4-(Chloromethylcarbonylamino)phenyl-2,3,4,6-tetra-*O*-acetyl- α -L-rhamnopyranoside (219)

Prepared as per Procedure 14 using **218** (4.83 g, 12.66 mmol) in CH₂Cl₂ (25 mL) and following flash chromatography (EtOAc:Hex - 50:50), the product was obtained as a yellow oil (4.12 g, 71%).

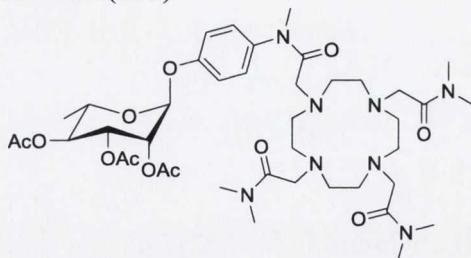
δ_{H} (600 MHz, CDCl ₃):	8.20 (1H, br s, NH), 7.49 (2H, d, $J = 8.8$ Hz, Ar-CH), 7.09 (2H, d, $J = 8.8$ Hz, Ar-CH), 5.52 (1H, dd, $J = 2.6$ Hz, $J = 10.2$ Hz, H-3), 5.44 (1H, m, H-2), 5.44 (1H, br s, H-1), 5.16 (1H, br s, H-1), 5.16 (1H, app t, $J = 9.9$ Hz, H-4), 4.20 (2H, s, CH ₂ Cl), 3.99 (1H, m, H-5), 2.21 (3H, s, CH ₃), 2.08 (3H, s, CH ₃), 2.05 (3H, s, CH ₃), 1.22 (3H, d, $J = 6.3$ Hz, H-6)
δ_{C} (150 MHz, CDCl ₃):	169.9 (C=O), 169.9 (C=O), 169.8 (C=O), 163.6 (C=O), 153.0 (qC), 131.5 (qC), 121.8 (Ar-CH), 116.8 (Ar-CH), 95.7 (C-1), 70.8 (C-4), 69.5 (C-2), 68.7 (C-3), 67.2 (C-5), 42.5 (CH ₂ Cl), 20.7 (CH ₃), 20.6 (CH ₃), 20.6 (CH ₃), 17.3 (CH ₃)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl ₃):	-22
HRMS (m/z ESI ⁺):	Found: 480.1034 ([M + Na] ⁺ , C ₂₀ H ₂₄ ClNO ₉) Requires: 480.1037)
ν_{max} (film)/cm ⁻¹ :	2981 (CH), 1748 (C=O), 1218 (CH)

4-(Chloromethylcarbonylamino)-*N*-methyl-phenyl-2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranoside (220)

Prepared as per Procedure 15 using **219** (1.24 g, 2.71 mmol) in THF (12 mL) to give the product as an orange oil (0.93 g, 73%).

δ_{H} (600 MHz, CDCl_3):	7.21 (2H, d, $J = 8.6$ Hz, Ar-CH), 7.16 (2H, d, $J = 8.6$ Hz, Ar-CH), 5.53 (1H, dd, $J = 3.3$ Hz, $J = 10.1$ Hz, H-3), 5.49 (1H, s, H-1), 5.45 (1H, s, H-2), 5.20 (1H, app t, $J = 10.1$ Hz, H-4), 4.01 (1H, m, H-5), 3.86 (2H, s, CH_2Cl), 3.31 (3H, s, N- CH_3), 2.23 (3H, s, CH_3), 2.09 (3H, s, CH_3), 2.07 (3H, s, CH_3), 1.26 (3H, d, $J = 6.1$ Hz, H-6)
δ_{C} (150 MHz, CDCl_3):	170.1 (C=O), 170.0 (C=O), 169.9 (C=O), 169.5 (C=O), 155.8 (qC), 137.3 (qC), 128.5 (Ar-CH), 117.7 (Ar-CH), 95.9 (C-1), 70.8 (C-4), 69.6 (C-2), 68.8 (C-3), 67.5 (C-5), 41.5 (CH_2Cl), 38.1 (N- CH_3), 20.9 (CH_3), 20.8 (CH_3), 20.2 (CH_3), 17.5 (C-6)
$[\alpha]_{\text{D}}^{20}$ (c 0.01, CHCl_3):	-20
HRMS (m/z ESI $^+$):	Found: 494.1196 ($[\text{M} + \text{Na}]^+$, $\text{C}_{21}\text{H}_{26}\text{ClNO}_9$ Requires: 494.1194)
ν_{max} (film)/ cm^{-1} :	2981 (CH), 1745 (C=O), 1214 (CH)

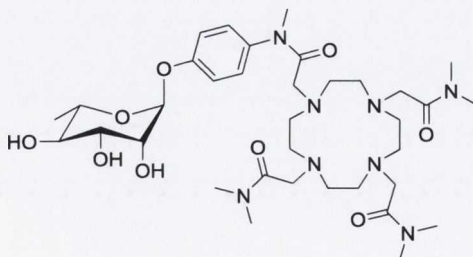
1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl-2,3,4,6-tetra-*O*-acetyl- α -D-rhamnopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (221)



Prepared as per Procedure 16 using **220** (0.56 g, 0.92 mmol) in CH_3CN (15 mL) and following alumina column chromatography (gradient elution from CH_2Cl_2 to $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH} - 95:5$), the product was obtained as a yellow oil (0.55 g, 70%).

δ_{H} (600 MHz, CDCl_3):	7.17 (2H, d, $J = 8.3$ Hz, Ar-CH), 7.12 (2H, d, $J = 8.3$ Hz, Ar-CH), 5.52 (1H, dd, $J = 3.3$ Hz, $J = 10.2$ Hz, H-3), 5.48 (1H, br s, H-1), 5.42 (1H, m, H-2), 5.19 (1H, app t, $J = 9.7$ Hz, H-4), 4.03 (1H, m, H-5), 3.26-2.81 (45H, br m, cyclen CH_2 + $\text{CH}_2\text{CON}(\text{CH}_3)_2$ + $\text{CH}_2\text{CON}-\text{CH}_3$ + N- CH_3), 2.22 (3H, s, CH_3), 2.09 (3H, s, CH_3), 2.06 (3H, s, CH_3), 1.26 (3H, d, $J = 6.2$ Hz, H-6)
δ_{C} (150 MHz, CDCl_3):	171.4 (C=O), 171.2 (C=O), 171.2 (C=O), 171.2 (C=O), 170.2 (C=O), 170.1 (C=O), 170.1 (C=O), 169.9, (C=O), 155.6 (qC), 137.6, (qC), 128.5 (Ar-CH), 117.6 (Ar-CH), 95.9 (C-1), 70.8 (C-4), 69.7 (C-2), 68.8 (C-3), 67.4 (C-5), 56.2 (CH_2), 55.1 (CH_2), 55.1 (CH_2), 37.6 (CH_2), 36.3 (CH_3), 36.3 (CH_3), 35.6 (N- CH_3), 35.6 (CH_3), 30.9 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 20.5 (CH_3), 20.6 (C-6)
$[\alpha]_{\text{D}}^{20}$ (c 0.04, CHCl_3):	-89
HRMS (m/z ESI $^+$):	Found: 885.4686 ($[\text{M} + \text{Na}]^+$, $\text{C}_{41}\text{H}_{66}\text{N}_8\text{O}_{12}$) Requires: 885.4698
ν_{max} (film)/ cm^{-1} :	2940 (CH), 1745, 1651 (C=O), 1218 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl- α -D-rhamnopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (222)

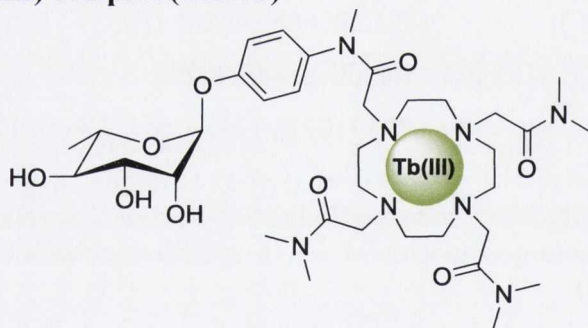


Prepared as per Procedure 17 using **221** (0.15 g, 0.17 mmol) in CH_3OH (10 mL) to give the product as a colourless oil (0.12 g, 92%).

δ_{H} (600 MHz, D_2O):	7.23 (2H, d, $J = 8.5$ Hz, Ar-CH), 7.14 (2H, d, $J = 8.5$ Hz, Ar-CH), 5.51 (1H, br s, H-1), 4.13 (1H,
---	---

	m, H-2), 3.95 (1H, dd, $J = 3.5$ Hz, $J = 9.7$ Hz, H-3), 3.74 (1H, m, H-5), 3.49 (1H, app t, $J = 9.7$ Hz, H-4), 3.34-2.59 (45H, br m, cyclen CH_2 + $\text{CH}_2\text{CON}(\text{CH}_3)_2$ + $\text{CH}_2\text{CON-CH}_3$ + N- CH_3), 1.18 (3H, d, $J = 6.2$ Hz, H-6)
δ_{C} (150 MHz, D_2O):	173.0 (C=O), 172.9 (C=O), 172.8 (C=O), 154.9 (qC), 137.3, (qC), 128.5 (Ar-CH), 118.1 (Ar-CH), 98.2 (C-1), 71.8 (C-4), 69.9 (C-3), 69.8 (C-2), 69.4 (C-5), 54.5 (CH_2), 51.0 (CH_2), 48.7 (CH_2), 37.1 (N- CH_3), 36.0 (CH_3), 35.4 (CH_3), 35.3 (CH_3), 17.1 (C-6)
$[\alpha]_{\text{D}}^{20}$ (c 0.04, CH_3OH):	-74
HRMS (m/z ESI $^{+}$):	Found: 737.4559 ($[\text{M} + \text{H}]^{+}$, $\text{C}_{35}\text{H}_{60}\text{N}_8\text{O}_9$ Requires: 737.4562)
ν_{max} (film)/ cm^{-1} :	3372 (OH), 2936 (CH), 1627 (C=O), 1103 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-[(4-*N*-methylcarbonylamino)-phenyl- α -D-rhamnopyranoside]-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide Tb(III) complex (222.Tb**)**

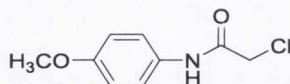


Prepared as per Procedure 18 using **222** (50 mg, 0.07 mmol) in CH_3OH (6 mL) to give the product as a fine white solid (75 mg, 80%). M.p. decomposed above 164 °C.

δ_{H} (400 MHz, D_2O):	51.18, 45.96, 43.59, 31.17, 27.35, 25.25, 20.48, 16.70, 16.21, 15.76, 12.82, 11.00, 10.71, 8.26, 8.03, 7.56, 6.22, 3.50, 3.25, 1.11
$[\alpha]_{\text{D}}^{20}$ (c 0.04, CH_3OH):	-45
HRMS (m/z ESI $^{+}$):	Found: 1044.3254 ($[\text{M} + \text{CF}_3\text{SO}_3]^{2+}$, $\text{C}_{35}\text{H}_{60}\text{N}_8\text{O}_{12}\text{F}_3\text{STb}$ Requires 1044.3257)

$\nu_{\max}$ (film)/ cm^{-1} : 3358 (OH), 2972 (CH), 1591 (C=O), 1108 (CH)

2-chloro-*N*-(4-methoxy-phenyl)acetamide (223)

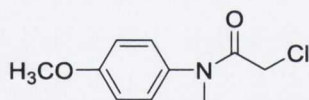


To a solution of *p*-anisidine (1.0 g, 8.12 mmol) in H_2O (40 mL) was added NaOH (0.1 g, 24.36 mmol) and the solution was cooled to 0 °C. Chloroacetyl chloride (1.94 mL, 24.36 mmol) was added dropwise over 1 h and the reaction was stirred for at rt 16 h. The solution was filtered and the resulting precipitate was washed with H_2O (100 mL) and Hex (100 mL) to give the product as a grey solid (1.01 g, 62%). The isolated compound was in good agreement with the literature.³⁸⁰

δ_{H} (400 MHz, CDCl_3): 8.11 (1H, br s, NH), 7.42 (2H, d, $J = 8.9$ Hz, Ar-CH), 6.87 (2H, d, $J = 8.9$ Hz, Ar-CH), 4.16 (2H, s, CH_2Cl), 3.78 (3H, s, CH_3)

HRMS (m/z ESI^+): Found: 198.0321 ($[\text{M} + \text{H}]^+$, $\text{C}_9\text{H}_{10}\text{ClNO}_2$)
Requires: 198.0322)

2-Chloro-*N*-(4-methoxy-phenyl)-*N*-methylacetamide (224)

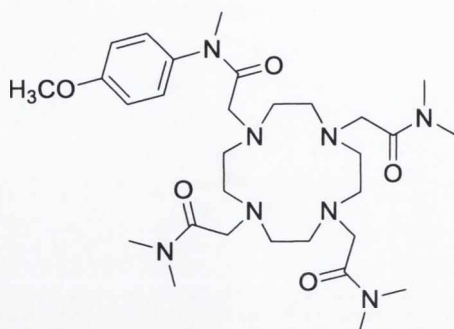


Prepared as per Procedure 15 using **223** (0.68 g, 3.41 mmol) to give the product as a yellow oil (0.61 g, 84%). The isolated compound was in good agreement with the literature.³⁸¹

δ_{H} (600 MHz, CDCl_3): 7.19 (2H, d, $J = 8.9$ Hz, Ar-CH), 6.97 (2H, d, $J = 8.9$ Hz, Ar-CH), 3.87 (2H, s, CH_2Cl), 3.86 (3H, s, N- CH_3), 3.31 (3H, s, OCH_3)

HRMS (m/z ESI^+): Found: 214.0632 ($[\text{M} + \text{H}]^+$, $\text{C}_{10}\text{H}_{13}\text{NO}_2$)
Requires: 214.0635)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-*N*-methyl-(4-methoxyphenyl)-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (225)



Prepared as per Procedure 16 using **224** (0.26 g, 0.94 mmol) in CH₃CN (6 mL) following alumina column chromatography (gradient elution from CH₂Cl₂ to CH₂Cl₂:CH₃OH - 96:4), the product was obtained as a yellow oil (0.49 g, 86%).

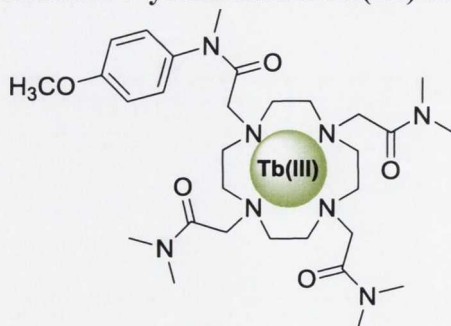
δ_{H} (600 MHz, CDCl₃): 7.10 (2H, d, $J = 8.1$ Hz, Ar-CH), 6.91 (2H, d, $J = 8.1$ Hz, Ar-CH), 3.83 (3H, s, OCH₃), 3.02-2.85 (45H, br m, cyclen CH₂ + CH₂CON(CH₃)₂ + CH₂CON-CH₃ + N-CH₃)

δ_{C} (100 MHz, CDCl₃): 171.3 (C=O), 170.9 (C=O), 170.9 (C=O), 158.9 (qC), 135.4 (qC), 128.3 (Ar-CH), 135.4 (Ar-CH), 55.9 (CH₂), 55.0 (CH₂), 55.0 (CH₂), 54.9 (CH₂), 37.4 (CH₂), 55.5 (OCH₃), 36.2 (CH₃), 36.2 (N-CH₃), 36.1 (CH₃), 35.4 (CH₃), 35.4 (CH₃)

HRMS (m/z ESI⁺): Found: 605.4132 ([M + H]⁺, C₉H₁₀ClNO₂)
Requires: 605.4139)

ν_{max} (film)/cm⁻¹: 2816 (CH), 1645 (C=O), 1250 (CH)

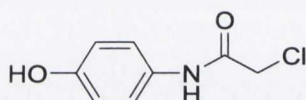
1,4,7-Tris(dimethylcarbamoylmethyl)-10-N-methyl-(4-methoxyphenyl)-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide Tb(III) complex (225.Tb)



Prepared as per Procedure 18 using **225** (20 mg, 0.05 mmol) in CH₃CN (4 mL) to give the product was a fine yellow solid (51 mg, 85%). M.p. decomposed above 190 °C.

δ_{H} (400 MHz, CDCl ₃):	78.42, 68.71, 63.34, 56.19, 50.60, 48.26, 47.09, 45.97, 38.35, 37.88, 29.25, 27.87, 22.37, 20.94, 16.32, 15.86, -54.16, -59.58, -62.33, -82.23, -87.87, -92, -95.09, -97.31, -102.66
HRMS (m/z ESI ⁺):	Found: 1061.2375 ([M + 2(CF ₃ SO ₃)] ⁺ , C ₃₂ H ₅₂ F ₆ N ₈ O ₁₁ S ₂ Tb Requires: 1061.2355)
ν_{max} (film)/cm ⁻¹ :	3498 (CH), 1620 (C=O), 1251 (CH)

2-chloro-*N*-(4-hydroxyphenyl)acetamide (**226**)



To sat. aq. NaOAc solution (50 mL) was added *p*-aminophenol (3.0 g, 27.49 mmol) followed by AcOH (50 mL). The suspension was cooled to 0 °C and chloroacetyl chloride (2.19 mL, 27.49 mmol) was added slowly and the reaction was stirred at rt for 2 h. The white precipitate was filtered, washed with H₂O (2 x 50 mL), and dried *in vacuo* to yield the product as a white solid (3.15 g, 62%). The isolated compound was in good agreement with the literature.³²⁴

δ_{H} (400 MHz, DMSO- <i>d</i> ₆):	10.05 (1H, br s, OH), 9.29 (1H, br s, NH), 7.37 (2H, d, J = 8.7 Hz, Ar-CH), 6.71 (2H, d, J = 8.7 Hz, Ar-CH), 4.19 (2H, s, CH ₂ Cl)
HRMS (m/z ESI ⁻):	Found: 184.0173 ([M - H] ⁻ , C ₈ H ₈ ClNO ₂ Requires: 184.0165)

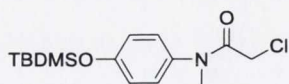
2-Chloro-*N*-[4-(*tert*-Butyl-dimethyl-silanyloxy)-phenyl]acetamide (**227**)



To **226** (1.5 g, 8.08 mmol) in anhydrous DMF (15 mL) at 0 °C was added imidazole (1.44 g, 21.15 mmol) and TBDMSCl (1.58 g, 10.48 mmol) and the mixture was stirred at rt for 16 h. The reaction was quenched with sat. aq. NH₄Cl solution (15 mL) and the product extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with water (20 mL), sat. aq. NaHCO₃ solution (20 mL), brine (20 mL), dried over MgSO₄, filtered, and the solvent was removed *in vacuo*. The product was purified by flash chromatography (EtOAc:Hex - 20:80) to give the product as a white solid (1.97 g, 81%).

δ_{H} (400 MHz, CDCl ₃):	8.15 (1H, br s, NH), 7.42 (2H, d, J = 8.8 Hz, Ar-CH), 6.86 (2H, d, J = 8.8 Hz, Ar-CH), 4.21 (2H, s, CH ₂ Cl), 1.00 (9H, s, SiC(CH ₃) ₃), 0.22 (6H, s, Si(CH ₃) ₂)
δ_{C} (100 MHz, CDCl ₃):	163.6 (C=O), 153.1 (qC), 130.3 (qC), 121.9 (Ar-CH), 120.5 (Ar-CH), 43.1 (CH ₂ Cl), 25.6 (SiC(CH ₃) ₃), 17.4 (SiC(CH ₃) ₃), -4.5 (Si(CH ₃) ₂)
HRMS (m/z ESI ⁺):	Found: 298.1042 ([M - H] ⁺ , C ₁₄ H ₂₂ ClNO ₂ Si Requires 298.1030)
ν_{max} (film)/cm ⁻¹ :	3360 (OH), 2954 (CH), 1896 (C=O), 1230 (CH)

2-Chloro-*N*-[4-(*tert*-Butyl-dimethyl-silanyloxy)-phenyl]-*N*-methylacetamide (228**)**

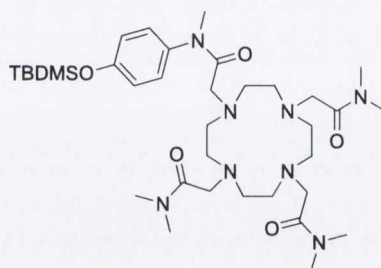


Prepared as per Procedure 15 using **227** (1.42 g, 4.67 mmol) in THF (15 mL) to give the product as an orange oil (0.92 g, 63%).

δ_{H} (400 MHz, CDCl ₃):	7.07 (2H, d, J = 8.7 Hz, Ar-CH), 6.84 (2H, d, J = 8.7 Hz, Ar-CH), 3.82 (2H, s, CH ₂ Cl), 3.25 (3H, s, N-CH ₃), 0.97 (9H, s, SiC(CH ₃) ₃), 0.20 (6H, s, Si(CH ₃) ₂)
δ_{C} (100 MHz, CDCl ₃):	165.8 (C=O), 155.2 (qC), 135.3 (qC), 127.6 (Ar-CH), 120.6 (Ar-CH), 40.9 (CH ₂ Cl), 37.6 (N-

	CH ₃), 25.0 (SiC(CH ₃) ₃), 17.6 (Si(CH ₃) ₃), -4.8 (Si(CH ₃) ₂)
HRMS (<i>m/z</i> ESI ⁺):	Found: 314.1341 ([M + H] ⁺ , C ₁₅ H ₂₅ ClNO ₂ Si) Requires: 314.1343)
$\nu_{\max}$ (film)/cm ⁻¹ :	2956 (CH), 1672 (C=O), 1255 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-*N*-methyl-[4-[1,1-dimethylethyl)dimethyl-silyl]oxy]phenyl-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (229)

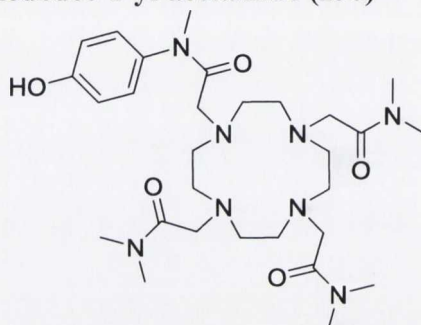


Prepared as per Procedure 16 using **228** (0.47 g, 1.5 mmol) in CH₃CN (8 mL) and following alumina column chromatography (gradient elution from CH₂Cl₂ to CH₂Cl₂:CH₃OH - 95:5), the product was obtained as a colourless oil (0.73 g, 69%).

δ_{H} (600 MHz, CDCl ₃):	7.07 (2H, d, <i>J</i> = 8.4 Hz, Ar-CH), 6.86 (2H, d, <i>J</i> = 8.4 Hz, Ar-CH), 3.49-2.40 (45H, br m, cyclen CH ₂ + CH ₂ CON(CH ₃) ₂ + CH ₂ CON-CH ₃ + N-CH ₃), 1.00 (9H, s, SiC(CH ₃) ₃), 0.24 (6H, s, Si(CH ₃) ₂)
δ_{C} (150 MHz, CDCl ₃):	171.3 (C=O), 171.1 (C=O), 170.9 (C=O), 155.2 (qC), 136.0 (qC), 128.2 (Ar-CH), 120.9 (Ar-CH), 55.9 (CH ₂), 55.0 (CH ₂), 55.0 (CH ₂), 54.9 (CH ₂), 37.4 (CH ₂), 36.3 (CH ₃), 36.3 (CH ₃), 36.2 (N-CH ₃), 35.4 (CH ₃), 35.4 (CH ₃) 25.6 (SiC(CH ₃) ₃), 18.0 (Si(CH ₃) ₂), -4.4 (Si(CH ₃) ₂)

HRMS (m/z ESI ⁺):	Found: 705.4829 ([M + H] ⁺ , C ₃₅ H ₆₄ N ₈ O ₅ Si) Requires: 705.4847)
$\nu_{\max}$ (film)/cm ⁻¹ :	2844 (CH), 1649 (C=O), 1264 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-*N*-methyl-4-hydroxyphenyl-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide (230)

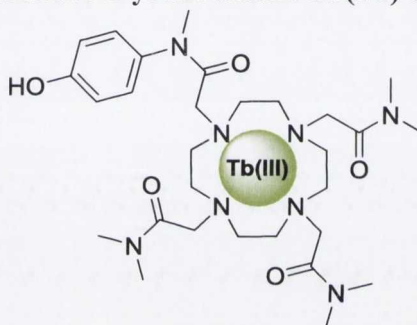


To **229** (0.35 g, 0.43 mmol) in anhydrous THF (15 mL) was added 1M TBAF in THF (0.18 mL, 0.64 mmol) and the reaction was stirred for 12 h at rt. CaCO₃, DOWEX-H⁺ and CH₃OH (4 mL) was added and the suspension was stirred at rt for 1 h. The suspension was filtered and insoluble materials washed with CH₃OH (5 mL) and the solvent was removed *in vacuo*. The product was purified by alumina column chromatography (gradient elution from CH₂Cl₂ to CH₂Cl₂:CH₃OH - 85:15), the product was obtained as a colourless oil (0.13 g, 72%).

δ_{H} (400 MHz, D ₂ O):	7.06 (2H, d, J = 8.4 Hz, Ar-CH), 6.81 (2H, d, J = 8.4 Hz, Ar-CH), 3.49-2.29 (45H, br m, cyclen CH ₂ + $\underline{\text{CH}}_2\text{CON}(\underline{\text{CH}}_3)_2$ + $\underline{\text{CH}}_2\text{CON-CH}_3$ + N-CH ₃)
δ_{C} (100 MHz, D ₂ O):	173.1 (C=O), 172.9 (C=O), 172.7 (C=O), 157.2 (qC), 134.1 (qC), 128.3 (Ar-CH), 116.6 (Ar-CH), 55.0 (CH ₂), 55.0 (CH ₂), 54.8 (CH ₂), 54.7 (CH ₂),

	37.3 (CH ₂), 48.7 (CH ₃), 36.1 (CH ₃), 36.1 (N-CH ₃), 35.4 (CH ₃), 35.3 (CH ₃)
HRMS (<i>m/z</i> ESI ⁺):	Found: 613.3802 ([M + Na] ⁺ , C ₂₉ H ₅₀ N ₈ O ₅ Requires: 613.3796)
$\nu_{\max}$ (film)/cm ⁻¹ :	3462 (OH), 1616 (C=O), 1247 (CH)

1,4,7-Tris(dimethylcarbamoylmethyl)-10-*N*-methyl-4-hydroxyphenyl-1,4,7,10-tetraaza-cyclododec-1-yl-acetamide Tb(III) complex (230.Tb)



Prepared as per Procedure 18 using **37** (30 mg, 0.05 mmol) in CH₃OH (4 mL) to give the product as a fine white solid (48 mg, 80%). M.p. decomposed above 190 °C.

δ_{H} (400 MHz, CD ₃ OD):	158.25, 109.07, 85.64, 65.80, 62.07, 59.23, 57.99, 48.54, 45.07, 43.91, 35.79, 28.44, 26.21, 19.19, 18.40, 17.67, 10.11, 8.87, 7.18, 6.92, -56.40, -58.16, -61.71, -66.73, -70.16, -71.96, -86.04, -90.78, -97.91, -99.59, -103.88, -188.97
HRMS (<i>m/z</i> MALDI ⁺):	Found: 1047.2245 ([M + 2CF ₃ SO ₃] ⁺ , C ₃₁ H ₅₀ F ₆ N ₈ O ₁₁ S ₂ Tb Requires 1047.2198)
$\nu_{\max}$ (film)/cm ⁻¹ :	3355, 1617, 1516, 1458, 1411, 1250, 1159, 1082, 958, 845, 758

7.0 References

- 1 C. R. Drake, D. C. Miller and E. F. Jones, *Curr. Org. Synth.*, 2011, **8**, 498.
- 2 J. Kalia and R. T. Raines, *Curr. Org. Chem.*, 2010, **14**, 138.
- 3 S. van Kasteren, *Biochem. Soc. Trans.*, 2012, **40**, 929.
- 4 S. V. Moradi, W. M. Hussein, P. Varamini, P. Simerska and I. Toth, *Chem. Sci.*, 2016, **7**, 2492.
- 5 O. T. Avery and W. F. Goebel, *J. Exp. Med.*, 1929, **50**, 533.
- 6 P. M. Rudd, H. C. Joao, E. Coghill, P. Fiten, M. R. Saunders, G. Opdenakker and R. A. Dwek, *Biochemistry*, 1994, **33**, 17.
- 7 T. J. Boltje, T. Buskas and G.-J. Boons, *Nat. Chem.*, 2009, **1**, 611.
- 8 D. J. Craik, D. P. Fairlie, S. Liras and D. Price, *Chem. Biol. Drug Des.*, 2013, **81**, 136.
- 9 M. Sato, R. Sadamoto, K. Niikura, K. Monde, H. Kondo and S.-I. Nishimura, *Angew. Chem., Int. Ed.*, 2004, **43**, 1516.
- 10 J. C. Egrie, E. Dwyer, J. K. Browne, A. Hitz and M. A. Lykos, *Exp. Hematol.*, 2003, **31**, 290.
- 11 H. Susaki, K. Suzuki, M. Ikeda, H. Yamada and H. K. Watanabe, *Chem. Pharm. Bull.*, 1998, **46**, 1530.
- 12 M. A. Robinson, S. T. Charlton, P. Garnier, X.-t. Wang, S. S. Davis, A. C. Perkins, M. Frier, R. Duncan, T. J. Savage, D. A. Wyatt, S. A. Watson and B. G. Davis, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 14527.
- 13 D. P. Gamblin, E. M. Scanlan and B. G. Davis, *Chem. Rev.*, 2009, **109**, 131.
- 14 A. Muppidi, K. Doi, S. Edwardraja, E. J. Drake, A. M. Gulick, H.-G. Wang and Q. Lin, *J. Am. Chem. Soc.*, 2012, **134**, 14734.
- 15 A. Muppidi, K. Doi, S. Edwardraja, S. V. S. R. K. Pulavarti, T. Szyperski, H.-G. Wang and Q. Lin, *Bioconjugate Chem.*, 2014, **25**, 424.
- 16 H. M. Burke, T. Gunnlaugsson and E. M. Scanlan, *Chem. Commun.*, 2015, **51**, 10576.
- 17 D. S. Hameed, A. Sapmaz and H. Ovaa, *Bioconjugate Chem.*, 2016, 10.1021/acs.bioconjchem.6b00140.
- 18 I. Human Genome Sequencing Consortium, *Nature*, 2004, **431**, 931.
- 19 C. T. Walsh, S. Garneau-Tsodikova and G. J. Gatto, Jr., *Angew. Chem., Int. Ed.*, 2005, **44**, 7342.
- 20 D. Ribet and P. Cossart, *FEBS Lett.*, 2010, **584**, 2748.
- 21 T. M. Karve and A. K. Cheema, *J. Amino Acids*, 2011, 207691.
- 22 Y.-C. Wang, S. E. Peterson and J. F. Loring, *Cell Res.*, 2014, **24**, 143.
- 23 A. Ciechanover, *Angew. Chem., Int. Ed.*, 2005, **44**, 5944.
- 24 O. Kerscher, R. Felberbaum and M. Hochstrasser, *Annu. Rev. Cell Dev. Biol.*, 2006, **22**, 159.

- 25 J. Peng, D. Schwartz, J. E. Elias, C. C. Thoreen, D. Cheng, G. Marsischky, J. Roelofs, D. Finley and S. P. Gygi, *Nat. Biotechnol.*, 2003, **21**, 921.
- 26 A. Hershko and A. Ciechanover, *Annu. Rev. Biochem.*, 1998, **67**, 425.
- 27 L. Spasser and A. Brik, *Angew. Chem., Int. Ed.*, 2012, **51**, 6840.
- 28 A. M. Weissman, N. Shabek and A. Ciechanover, *Nat. Rev. Mol. Cell Biol.*, 2011, **12**, 605.
- 29 V. N. Uversky, *J. Neurochem.*, 2007, **103**, 17.
- 30 B. I. Giasson and V. M. Y. Lee, *Cell*, **114**, 1.
- 31 E. Leroy, R. Boyer, G. Auburger, B. Leube, G. Ulm, E. Mezey, G. Harta, M. J. Brownstein, S. Jonnalagada, T. Chernova, A. Dehejia, C. Lavedan, T. Gasser, P. J. Steinbach, K. D. Wilkinson and M. H. Polymeropoulos, *Nature* 1998, **395**, 451.
- 32 F. E. Reyes-Turcu, K. H. Ventii and K. D. Wilkinson, *Annu. Rev. Biochem.*, 2009, **78**, 363.
- 33 S. Bergink and S. Jentsch, *Nature*, 2009, **458**, 461.
- 34 R. Apweiler, H. Hermjakob and N. Sharon, *Biochim. Biophys. Acta, Gen. Subj.*, 1999, **1473**, 4.
- 35 R. G. Spiro, *Glycobiology*, 2002, **12**, 43R.
- 36 R. A. Dwek and T. D. Butters, *Chem. Rev.*, 2002, **102**, 283.
- 37 R. A. Dwek, *Chem. Rev.*, 1996, **96**, 683.
- 38 P. M. Rudd, T. Elliott, P. Cresswell, I. A. Wilson and R. A. Dwek, *Science*, 2001, **291**, 2370.
- 39 G. Opdenakker, P. M. Rudd, C. P. Ponting and R. A. Dwek, *FASEB J.*, 1993, **7**, 1330.
- 40 D. F. Wyss, J. S. Choi, J. Li, M. H. Knoppers, K. J. Willis, A. R. N. Arulanandam, A. Smolyar, E. L. Reinherz and G. Wagner, *Science* 1995, **269**, 1273.
- 41 T. Conroy, K. A. Jolliffe and R. J. Payne, *Org. Biomol. Chem.*, 2010, **8**, 3723.
- 42 M. R. Pratt and C. R. Bertozzi, *Chem. Soc. Rev.*, 2005, **34**, 58.
- 43 R. S. Rush, P. L. Derby, D. M. Smith, C. Merry, G. Rogers, M. F. Rohde and V. Katta, *Anal. Chem.*, 1995, **67**, 1442.
- 44 M. N. Fukuda, H. Sasaki, L. Lopez and M. Fukuda, *Blood*, 1989, **73**, 84.
- 45 S. Erbayraktar, G. Grasso, A. Sfacteria, Q.-w. Xie, T. Coleman, M. Kreilgaard, L. Torup, T. Sager, Z. Erbayraktar, N. Gokmen, O. Yilmaz, P. Ghezzi, P. Villa, M. Fratelli, S. Casagrande, M. Leist, L. Helboe, J. Gerwein, S. Christensen, M. A. Geist, L. O. Pedersen, C. Cerami-Hand, J.-P. Wuerth, A. Cerami and M. Brines, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 6741.
- 46 N. Subiros, D. Garcia del Barco and R. M. Coro-Antich, *Ther. Adv. Neurol. Disord.*, 2012, **5**, 161.

- 47 Z. Shriver, S. Raguram and R. Sasisekharan, *Nat. Rev. Drug Discovery*, 2004, **3**, 863.
- 48 R. A. Laine, *Glycobiology*, 1994, **4**, 759.
- 49 B. G. Davis, *J. Chem. Soc., Perkin Trans. 1*, 1999, 3215.
- 50 K. Ohtsubo and J. D. Marth, *Cell*, 2006, **126**, 855.
- 51 K. J. Doores, D. P. Gamblin and B. G. Davis, *Chem. Eur. J.*, 2006, **12**, 656.
- 52 C. R. Bertozzi and L. L. Kiessling, *Science*, 2001, **291**, 2357.
- 53 A. Varki, H. H. Freeze and A. E. Manzi, *Curr. Protoc. Protein Sci.*, 2009, **Chapter 12**, Unit 12.1 12.1.1.
- 54 A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart, M. E. Etzler and Editors, *Essentials of Glycobiology; Second Edition*, Cold Spring Harbor Laboratory Press, 2009.
- 55 G. Arsequell and G. Valencia, *Tetrahedron: Asymmetry*, 1999, **10**, 3045.
- 56 G. Arsequell and G. Valencia, *Tetrahedron: Asymmetry*, 1997, **8**, 2839.
- 57 P. Van den Steen, P. M. Rudd, R. A. Dwek and G. Opdenakker, *Crit. Rev. Biochem. Mol. Biol.*, 1998, **33**, 151.
- 58 T. Pesnot, R. Jorgensen, M. M. Palcic and G. K. Wagner, *Nat. Chem. Biol.*, 2010, **6**, 321.
- 59 H. Lodish and a. et, *Molecular Cell Biology, 5th Edition*, W. H. Freeman, 2003.
- 60 D. E. Levy and P. Fügedi, *The organic chemistry of sugars*, CRC Press, 2005.
- 61 J. Guo and X.-S. Ye, *Molecules*, 2010, **15**, 7235.
- 62 A. V. Demchenko, *Curr. Org. Chem.*, 2003, **7**, 35.
- 63 G.-J. Boons, *Contemp. Org. Synth.*, 1996, **3**, 173.
- 64 B. Mukhopadhyay, S. V. Maurer, N. Rudolph, R. M. Van Well, D. A. Russell and R. A. Field, *J. Org. Chem.*, 2005, **70**, 9059.
- 65 K. Shimawaki, Y. Fujisawa, F. Sato, N. Fujitani, M. Kurogochi, H. Hoshi, H. Hinou and S.-I. Nishimura, *Angew. Chem., Int. Ed.*, 2007, **46**, 3074.
- 66 K. A. Winans, D. S. King, V. R. Rao and C. R. Bertozzi, *Biochemistry*, 1999, **38**, 11700.
- 67 S. A. Svarovsky and J. J. Barchi, *Carbohydr. Res.*, 2003, **338**, 1925.
- 68 A. Helenius and M. Aebe, *Annu. Rev. Biochem.*, 2004, **73**, 1019.
- 69 M. Taylor and K. Drickamer, *Introduction to Glycobiology*, Oxford University Press, 2003.
- 70 A. Helenius and M. Aebe, *Science*, 2001, **291**, 2364.
- 71 S. T. Anisfeld and P. T. Lansbury, Jr., *J. Org. Chem.*, 1990, **55**, 5560.
- 72 S. T. Cohen-Anisfeld and P. T. Lansbury, Jr., *J. Am. Chem. Soc.*, 1993, **115**, 10531.

- 73 L. M. Likhoshesterov, O. S. Novikova, V. A. Derevitskaya and N. K. Kochetkov, *Carbohydr. Res.*, 1986, **146**, C1.
- 74 P. Wang, B. Aussedat, Y. Vohra and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2012, **51**, 11571.
- 75 R. Subiros-Funosas, A. El-Faham and F. Albericio, *Tetrahedron*, 2011, **67**, 8595.
- 76 K. J. Doores, Y. Mimura, R. A. Dwek, P. M. Rudd, T. Elliott and B. G. Davis, *Chem. Commun.*, 2006, 1401.
- 77 A. J. Ratcliffe, P. Konradsson and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1990, **112**, 5665.
- 78 A. L. Handlon and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1993, **115**, 3796.
- 79 C. M. Kaneshiro and K. Michael, *Angew. Chem., Int. Ed.*, 2006, **45**, 1077.
- 80 R. Joseph, F. B. Dyer and P. Garner, *Org. Lett.*, 2013, **15**, 732.
- 81 P. Wang, X. Li, J. Zhu, J. Chen, Y. Yuan, X. Wu and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2011, **133**, 1597.
- 82 V. R. Pattabiraman and J. W. Bode, *Nature*, 2011, **480**, 471.
- 83 X. Zhu and R. R. Schmidt, *Chem. Eur. J.*, 2004, **10**, 875.
- 84 J. E. Moses and A. D. Moorhouse, *Chem. Soc. Rev.*, 2007, **36**, 1249.
- 85 F. Friscourt and G.-J. Boons, in *Click Chemistry in Glycoscience*, John Wiley & Sons, Inc., 2013, pp. 211-233.
- 86 S. Caddick, D. Hamza, S. N. Wadman and J. D. Wilden, *Org. Lett.*, 2002, **4**, 1775.
- 87 T. Posner, *Ber. Dtsch. Chem. Ges.*, 1905, **38**, 646.
- 88 A. Dondoni, *Angew. Chem., Int. Ed.*, 2008, **47**, 8995.
- 89 A. B. Lowe, *Polym. Chem.*, 2014, **5**, 4820.
- 90 A. Dondoni, *Angew Chem Int Ed Engl*, 2008, **47**, 8995.
- 91 M. Fiore, M. Lo Conte, S. Pacifico, A. Marra and A. Dondoni, *Tetrahedron Lett.*, 2011, **52**, 444.
- 92 N. Floyd, B. Vijayakrishnan, J. R. Koeppe and B. G. Davis, *Angew. Chem., Int. Ed.*, 2009, **48**, 7798.
- 93 A. Dondoni, A. Massi, P. Nanni and A. Roda, *Chem. Eur. J.*, 2009, **15**, 11444.
- 94 A. El-Faham and F. Albericio, *Chem. Rev.*, 2011, **111**, 6557.
- 95 A. Isidro-Llobet, M. Álvarez and F. Albericio, *Chem. Rev.*, 2009, **109**, 2455.
- 96 C. A. G. N. Montalbetti and V. Falque, *Tetrahedron*, 2005, **61**, 10827.
- 97 F. R. M. de, J.-S. Suppo and J.-M. Campagne, *Chem. Rev.*, 2016, **116**, 12029.
- 98 R. B. Merrifield, *J. Am. Chem. Soc.*, 1963, **85**, 2149.
- 99 M. Amblard, J.-A. Fehrentz, J. Martinez and G. Subra, *Mol. Biotechnol.*, 2006, **33**, 239.
- 100 P. Thapa, R.-Y. Zhang, V. Menon and J.-P. Bingham, *Molecules*, 2014, **19**, 14461.

- 101 S. Chandrudu, P. Simerska and I. Toth, *Molecules*, 2013, **18**, 4373.
- 102 J. A. Borgia and G. B. Fields, *Trends Biotechnol.*, 2000, **18**, 243.
- 103 B. L. Bray, *Nat. Rev. Drug Discovery*, 2003, **2**, 587.
- 104 L. Liu, C. S. Bennett and C.-H. Wong, *Chem. Commun.*, 2006, 21.
- 105 S. B. H. Kent, *Chem. Soc. Rev.*, 2009, **38**, 338.
- 106 R. J. Payne and C.-H. Wong, *Chem. Commun.*, 2010, **46**, 21.
- 107 P. E. Dawson, T. W. Muir, I. Clark-Lewis and S. B. Kent, *Science*, 1994, **266**, 776.
- 108 T. Wieland, E. Bokelmann, L. Bauer, H. U. Lang, H. Lau and W. Schafer, *Justus Liebigs Ann. Chem.*, 1953, **583**, 129.
- 109 P. E. Dawson, M. Churchill, M. R. Ghadiri and S. B. H. Kent, *J. Am. Chem. Soc.*, 1997, **119**, 4325.
- 110 E. C. B. Johnson and S. B. H. Kent, *J. Am. Chem. Soc.*, 2006, **128**, 6640.
- 111 T. M. Hackeng, J. H. Griffin and P. E. Dawson, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 10068.
- 112 V. Y. Torbeev and S. B. H. Kent, *Angew. Chem., Int. Ed.*, 2007, **46**, 1667.
- 113 S. Bondalapati, M. Jbara and A. Brik, *Nat. Chem.*, 2016, **8**, 407.
- 114 D. Macmillan, *Angew. Chem., Int. Ed.*, 2006, **45**, 7668.
- 115 J. Offer, C. N. C. Boddy and P. E. Dawson, *J. Am. Chem. Soc.*, 2002, **124**, 4642.
- 116 L. E. Canne, S. J. Bark and S. B. H. Kent, *J. Am. Chem. Soc.*, 1996, **118**, 5891.
- 117 A. Brik and C.-H. Wong, *Chem. Eur. J.*, 2007, **13**, 5670.
- 118 L. Z. Yan and P. E. Dawson, *J. Am. Chem. Soc.*, 2001, **123**, 526.
- 119 F. W. Hoffmann, R. J. Ess, T. C. Simmons and R. S. Hanzel, *J. Am. Chem. Soc.*, 1956, **78**, 6414.
- 120 C. T. Walling and R. Rabinowitz, *J. Am. Chem. Soc.*, 1957, **79**, 5326.
- 121 Q. Wan and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2007, **46**, 9248.
- 122 D. Crich and A. Banerjee, *J. Am. Chem. Soc.*, 2007, **129**, 10064.
- 123 J. Chen, Q. Wan, Y. Yuan, J. Zhu and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2008, **47**, 8521.
- 124 K. S. Ajish Kumar, M. Haj-Yahya, D. Olschewski, H. A. Lashuel and A. Brik, *Angew. Chem., Int. Ed.*, 2009, **48**, 8090.
- 125 D. Bang, B. L. Pentelute and S. B. H. Kent, *Angew. Chem., Int. Ed.*, 2006, **45**, 3985.
- 126 D. Bang, B. L. Pentelute, Z. P. Gates and S. B. Kent, *Org. Lett.*, 2006, **8**, 1049.
- 127 D. Bang and S. B. H. Kent, *Angew. Chem., Int. Ed.*, 2004, **43**, 2534.
- 128 M. Patek and M. Lebl, *Biopolymers*, 1999, **47**, 353.
- 129 G. W. Kenner, J. R. McDermott and R. C. Sheppard, *J. Chem. Soc. D.*, 1971, 636.
- 130 B. J. Backes and J. A. Ellman, *J. Org. Chem.*, 1999, **64**, 2322.

- 131 J. B. Blanco-Canosa and P. E. Dawson, *Angew. Chem., Int. Ed.*, 2008, **47**, 6851.
- 132 Y. Shin, K. A. Winans, B. J. Backes, S. B. H. Kent, J. A. Ellman and C. R. Bertozzi, *J. Am. Chem. Soc.*, 1999, **121**, 11684.
- 133 S. Mezzato, M. Schaffrath and C. Unverzagt, *Angew. Chem., Int. Ed.*, 2005, **44**, 1650.
- 134 P. Wang, S. Dong, J.-H. Shieh, E. Peguero, R. Hendrickson, M. A. S. Moore and S. J. Danishefsky, *Science* 2013, **342**, 1357.
- 135 A. Brik, Y.-Y. Yang, S. Ficht and C.-H. Wong, *J. Am. Chem. Soc.*, 2006, **128**, 5626.
- 136 A. Brik, S. Ficht, Y.-Y. Yang, C. S. Bennett and C.-H. Wong, *J. Am. Chem. Soc.*, 2006, **128**, 15026.
- 137 S. Ficht, R. J. Payne, A. Brik and C.-H. Wong, *Angew. Chem., Int. Ed.*, 2007, **46**, 5975.
- 138 R. J. Payne, S. Ficht, S. Tang, A. Brik, Y.-Y. Yang, D. A. Case and C.-H. Wong, *J. Am. Chem. Soc.*, 2007, **129**, 13527.
- 139 M.-Y. Lutsky, N. Nepomniaschiy and A. Brik, *Chem. Commun.*, 2008, 1229.
- 140 L. Spasser, K. S. Ajish Kumar and A. Brik, *J. Pept. Sci.*, 2011, **17**, 252.
- 141 T. W. Muir, D. Sondhi and P. A. Cole, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 6705.
- 142 T. W. Muir, *Annu. Rev. Biochem.*, 2003, **72**, 249.
- 143 L. Berrade and J. A. Camarero, *Cell. Mol. Life Sci.*, 2009, **66**, 3909.
- 144 R. R. Flavell and T. W. Muir, *Acc. Chem. Res.*, 2009, **42**, 107.
- 145 J. A. Camarero and Y. Kwon, *Int. J. Pept. Res. Ther.*, 2008, **14**, 351.
- 146 C. P. R. Hackenberger and D. Schwarzer, *Angew. Chem., Int. Ed.*, 2008, **47**, 10030.
- 147 R. M. Hofmann and T. W. Muir, *Curr. Opin. Biotechnol.*, 2002, **13**, 297.
- 148 B. L. Nilsson, M. B. Soellner and R. T. Raines, *Annu. Rev. Biophys. Biomol. Struct.*, 2005, **34**, 91.
- 149 R. M. Hofmann, G. J. Cotton, E. J. Chang, E. Vidal, D. Veach, W. Bornmann and T. W. Muir, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 3091.
- 150 H. P. Hemantha and A. Brik, *Bioorg. Med. Chem.*, 2013, **21**, 3411.
- 151 T. Fekner, X. Li and M. K. Chan, *ChemBioChem*, 2011, **12**, 21.
- 152 D. L. Zechel and S. G. Withers, *Acc. Chem. Res.*, 2000, **33**, 11.
- 153 P. M. Coutinho, E. Deleury and B. Henrissat, *J. Appl. Glycosci.*, 2003, **50**, 241.
- 154 K. A. Stubbs, *Carbohydr. Res.*, 2014, **390**, 9.
- 155 B. Henrissat and G. Davies, *Curr. Opin. Struct. Biol.*, 1997, **7**, 637.
- 156 V. Lombard, H. Golaconda Ramulu, E. Drula, P. M. Coutinho and B. Henrissat, *Nucleic Acids Res.*, 2014, **42**, D490.
- 157 T. M. Gloster and D. J. Vocadlo, *Nat. Chem. Biol.*, 2012, **8**, 683.
- 158 D. E. Koshland, Jr., *Biol. Rev. Cambridge Philos. Soc.*, 1953, **28**, 416.

- 159 C. S. Rye and S. G. Withers, *Curr. Opin. Chem. Biol.*, 2000, **4**, 573.
- 160 K. M. Koeller and C. H. Wong, *Nature*, 2001, **409**, 232.
- 161 D. H. G. Crout and G. Vic, *Curr. Opin. Chem. Biol.*, 1998, **2**, 98.
- 162 B. Li, H. Song, S. Hauser and L.-X. Wang, *Org. Lett.*, 2006, **8**, 3081.
- 163 C.-H. Wong, *J. Org. Chem.*, 2005, **70**, 4219.
- 164 L. Weignerova, P. Bojarova and V. Kren, *Carbohydr. Chem.*, 2009, **35**, 310.
- 165 L.-X. Wang, *Carbohydr. Res.*, 2008, **343**, 1509.
- 166 S. M. Hancock, M. D. Vaughan and S. G. Withers, *Curr. Opin. Chem. Biol.*, 2006, **10**, 509.
- 167 L. F. Mackenzie, Q. Wang, R. A. J. Warren and S. G. Withers, *J. Am. Chem. Soc.*, 1998, **120**, 5583.
- 168 M. Jahn, D. Stoll, R. A. J. Warren, L. Szabo, P. Singh, H. J. Gilbert, V. M. A. Ducros, G. J. Davies and S. G. Withers, *Chem. Commun.*, 2003, 1327.
- 169 M. D. Vaughan, K. Johnson, S. DeFrees, X. Tang, R. A. J. Warren and S. G. Withers, *J. Am. Chem. Soc.*, 2006, **128**, 6300.
- 170 P. Bojarova and V. Kren, *Trends Biotechnol.*, 2009, **27**, 199.
- 171 R. J. Desnick and E. H. Schuchman, *Nat. Rev. Genet.*, 2002, **3**, 954.
- 172 B. Ernst and J. L. Magnani, *Nat. Rev. Drug Discovery*, 2009, **8**, 661.
- 173 L. I. Willems, J. Jiang, K.-Y. Li, M. D. Witte, W. W. Kallemijn, T. J. N. Beenakker, S. P. Schroeder, J. M. F. G. Aerts, G. A. van der Marel, J. D. C. Codee and H. S. Overkleeft, *Chem. Eur. J.*, 2014, **20**, 10864.
- 174 E. Farkas and J. Thiem, *Eur. J. Org. Chem.*, 1999, 3073.
- 175 J. Kiernan, *Biotech. Histochem.*, 2007, **82**, 73.
- 176 J. A. Mead, J. N. Smith and R. T. Williams, *Biochem. J.*, 1954, **58**, xxxv.
- 177 J. A. R. Mead, J. N. Smith and R. T. Williams, *Biochem. J.*, 1955, **61**, 569.
- 178 M. Engstler, J. W. Talhouk, R. E. Smith and R. Schauer, *Anal. Biochem.*, 1997, **250**, 176.
- 179 O. Motabar, Z.-D. Shi, E. Goldin, K. Liu, N. Southall, E. Sidransky, C. P. Austin, G. L. Griffiths and W. Zheng, *Anal. Biochem.*, 2009, **390**, 79.
- 180 Z.-D. Shi, O. Motabar, E. Goldin, K. Liu, N. Southall, E. Sidransky, C. P. Austin, G. L. Griffiths and W. Zheng, *Anal. Bioanal. Chem.*, 2009, **394**, 1903.
- 181 W. H. Porter, C. D. Jennings, Jr. and H. D. Wilson, *Clin. Chem.*, 1986, **32**, 652.
- 182 X. Chen, T. Pradhan, F. Wang, J. S. Kim and J. Yoon, *Chem. Rev.*, 2012, **112**, 1910.
- 183 B. Rotman, J. A. Zderic and M. Edelstein, *Proc. Natl. Acad. Sci. U. S. A.*, 1963, **50**, 1.
- 184 G. P. Nolan, S. Fiering, J. F. Nicolas and L. A. Herzenberg, *Proc. Natl. Acad. Sci. U. S. A.*, 1988, **85**, 2603.

- 185 V. A. Rakhmanova and R. C. Macdonald, *Anal. Biochem.*, 1998, **257**, 234.
- 186 I. Hemmilä and V. Laitala, *J. Fluoresc.*, 2005, **15**, 529.
- 187 L. Armelao, S. Quici, F. Barigelli, G. Accorsi, G. Bottaro, M. Cavazzini and E. Tondello, *Coord. Chem. Rev.*, 2010, **254**, 487.
- 188 V. V. Zherdeva and A. P. Savitsky, *Biochemistry*, 2012, **77**, 1553.
- 189 D. Parker, *Coord. Chem. Rev.*, 2000, **205**, 109.
- 190 J.-C. G. Bunzli, *Chem. Lett.*, 2009, **38**, 104.
- 191 P. Mukherjee, C. M. Shade, A. M. Yingling, D. N. Lamont, D. H. Waldeck and S. Petoud, *J. Phys. Chem. A*, 2011, **115**, 4031.
- 192 J.-C. G. Bunzli and C. Piguet, *Chem. Soc. Rev.*, 2005, **34**, 1048.
- 193 E. Pershagen, J. Nordholm and K. E. Borbas, *J. Am. Chem. Soc.*, 2012, **134**, 9832.
- 194 K. E. Borbas and J. I. Bruce, *Org. Biomol. Chem.*, 2007, **5**, 2274.
- 195 P. Caravan, J. J. Ellison, T. J. McMurphy and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293.
- 196 M. C. Heffern, L. M. Matosziuk and T. J. Meade, *Chem. Rev.*, 2014, **114**, 4496.
- 197 E. Toth, L. Helm and A. E. Merbach, John Wiley & Sons Ltd., 2001, pp. 45-119.
- 198 S. Faulkner and O. A. Blackburn, in *The Chemistry of Molecular Imaging*, John Wiley & Sons, Inc, 2014, pp. 179-197.
- 199 R. A. Moats, S. E. Fraser and T. J. Meade, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 726.
- 200 J. A. Duimstra, F. J. Femia and T. J. Meade, *J. Am. Chem. Soc.*, 2005, **127**, 12847.
- 201 R. M. Schmaltz, S. R. Hanson and C.-H. Wong, *Chem. Rev.*, 2011, **111**, 4259.
- 202 C. S. Bennett and C.-H. Wong, *Chem. Soc. Rev.*, 2007, **36**, 1227.
- 203 C. Unverzagt and Y. Kajihara, *Chem. Soc. Rev.*, 2013, **42**, 4408.
- 204 B. Colak, J. C. S. Da Silva, T. A. Soares and J. E. Gautrot, *Bioconjugate Chem.*, 2016, **27**, 2111.
- 205 T. Buskas, E. Soederberg, P. Konradsson and B. Fraser-Reid, *J. Org. Chem.*, 2000, **65**, 958.
- 206 D. Ramos, P. Rollin and W. Klaffke, *J. Org. Chem.*, 2001, **66**, 2948.
- 207 Y.-Y. Yang, S. Ficht, A. Brik and C.-H. Wong, *J. Am. Chem. Soc.*, 2007, **129**, 7690.
- 208 J. T. Edward, *Chem. Ind.*, 1955, 1102.
- 209 Lemieux, R. U. & Chu, P. in 133rd National Meeting of the American Chemical Society 31N (American Chemical Society, 1958).
- 210 G. F. Bauerfeldt, T. M. Cardozo, M. S. Pereira and C. O. da Silva, *Org. Biomol. Chem.*, 2013, **11**, 299.
- 211 Lemieux, R. U. & Chu, P. in 133rd National Meeting of the American Chemical Society 31N (American Chemical Society, 1958).

- 212 H. Staudinger and J. Meyer, *Helv. Chim. Acta*, 1919, **2**, 635.
- 213 Y. G. Gololobov and L. F. Kasukhin, *Tetrahedron*, 1992, **48**, 1353.
- 214 J. Kovacs, I. Pinter, A. Messmer and G. Toth, *Carbohydr. Res.*, 1985, **141**, 57.
- 215 J. G. Ulan, E. Kuo, W. F. Maier, R. S. Rai and G. Thomas, *J. Org. Chem.*, 1987, **52**, 3126.
- 216 A. Aravind, M. G. Sankar, B. Varghese and S. Baskaran, *J. Org. Chem.*, 2009, **74**, 2858.
- 217 P. G. Reddy, T. V. Pratap, G. D. K. Kumar, S. K. Mohanty and S. Baskaran, *Eur. J. Org. Chem.*, 2002, 3740.
- 218 P. R. Sridhar, K. R. Prabhu and S. Chandrasekaran, *J. Org. Chem.*, 2003, **68**, 5261.
- 219 L. Markey, S. Giordani and E. M. Scanlan, *J. Org. Chem.*, 2013, **78**, 4270.
- 220 M. Uygun, M. A. Tasdelen and Y. Yagci, *Macromol. Chem. Phys.*, 2010, **211**, 103.
- 221 D. Crich, K. Sana and S. Guo, *Org. Lett.*, 2007, **9**, 4423.
- 222 J. T. Edward, G. Welch and S. C. Wong, *Can. J. Chem.*, 1978, **56**, 935.
- 223 N. Assem and A. K. Yudin, *Nat. Protoc.*, 2012, **7**, 1327.
- 224 D. Crich and I. Sharma, *Angew. Chem., Int. Ed.*, 2009, **48**, 7591.
- 225 P. Lloyd-Williams, F. Albericio and E. Giralt, *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC, 1997.
- 226 F. K. Hansen, K. Ha, E. Todadze, A. Lillicotch, A. Frey and A. R. Katritzky, *Org. Biomol. Chem.*, 2011, **9**, 7162.
- 227 S. S. Panda, C. El-Nachef, K. Bajaj, A. O. Al-Youbi, A. Oliferenko and A. R. Katritzky, *Chem. Biol. Drug Des.*, 2012, **80**, 821.
- 228 B. Capon, *Chem. Rev.*, 1969, **69**, 407.
- 229 B. Capon and B. E. Connett, *J. Chem. Soc.*, 1965, 4497.
- 230 W. Pigman, E. A. Cleveland, D. H. Couch and J. H. Cleveland, *J. Am. Chem. Soc.*, 1951, **73**, 1976.
- 231 H. S. Isbell and H. L. Frush, *J. Am. Chem. Soc.*, 1950, **72**, 1043.
- 232 *J. Org. Chem.*, 1958, **23**, 1309.
- 233 P. E. Dawson, T. W. Muir, I. Clark-Lewis and S. B. H. Kent, *Science* 1994, **266**, 776.
- 234 W. H. Pan, M. A. Harmer, T. R. Halbert and E. I. Stiefel, *J. Am. Chem. Soc.*, 1984, **106**, 459.
- 235 M. D. Argyle and C. H. Bartholomew, *Catalysts*, 2015, **5**, 145.
- 236 J. K. Dunleavy, *Platinum Met. Rev.*, 2006, **50**, 110.
- 237 S. L. Rawe, D. Doyle, V. Zaric, I. Rozas, K. McMahon, M. Tosin, H. M. Bunz, E. P. Murphy, K. M. O'Boyle and P. V. Murphy, *Carbohydr. Res.*, 2006, **341**, 1370.
- 238 A. Dan, M. Lergenmuller, M. Amano, Y. Nakahara, T. Ogawa and Y. Ito, *Chem. Eur. J.*, 1998, **4**, 2182.
- 239 U. Ellervik and G. Magnusson, *Tetrahedron Lett.*, 1997, **38**, 1627.

- 240 A. Bianchi and A. Bernardi, *J. Org. Chem.*, 2006, **71**, 4565.
- 241 D. Komander, *Biochem. Soc. Trans.*, 2009, **37**, 937.
- 242 K.-H. Lim and K.-H. Baek, *Curr. Pharm. Des.*, 2013, **19**, 4039.
- 243 J.-P. Briand, A. Van Dorselaer, B. Raboy and S. Muller, *Pept. Res.*, 1989, **2**, 381.
- 244 R. Ramage, J. Green and O. M. Ogunjobi, *Tetrahedron Lett.*, 1989, **30**, 2149.
- 245 D. Bang, G. I. Makhatadze, V. Tereshko, A. A. Kossiakoff and S. B. Kent, *Angew. Chem., Int. Ed.*, 2005, **44**, 3852.
- 246 C. G. Benitez-Cardoza, K. Stott, M. Hirshberg, H. M. Went, D. N. Woolfson and S. E. Jackson, *Biochemistry*, 2004, **43**, 5195.
- 247 M. Hejjaoui, M. Haj-Yahya, K. S. A. Kumar, A. Brik and H. A. Lashuel, *Angew. Chem., Int. Ed.*, 2011, **50**, 405.
- 248 M. Hasegawa, H. Fujiwara, T. Nonaka, K. Wakabayashi, H. Takahashi, V. M. Y. Lee, J. Q. Trojanowski, D. Mann and T. Iwatsubo, *J. Biol. Chem.*, 2002, **277**, 49071.
- 249 Y. Kravtsova-Ivantsiv, T. Sommer and A. Ciechanover, *Angew. Chem., Int. Ed.*, 2013, **52**, 192.
- 250 D. Komander and M. Rape, *Annu. Rev. Biochem.*, 2012, **81**, 203.
- 251 J. Piotrowski, R. Beal, L. Hoffman, K. D. Wilkinson, R. E. Cohen and C. M. Pickart, *J. Biol. Chem.*, 1997, **272**, 23712.
- 252 R. M. Hofmann and C. M. Pickart, *J. Biol. Chem.*, 2001, **276**, 27936.
- 253 A. Bremm, S. M. V. Freund and D. Komander, *Nat. Struct. Mol. Biol.*, 2010, **17**, 939.
- 254 K. S. A. Kumar, L. Spasser, L. A. Erlich, S. N. Bavikar and A. Brik, *Angew. Chem., Int. Ed.*, 2010, **49**, 9126.
- 255 F. El Oualid, R. Merckx, R. Ekkebus, D. S. Hameed, J. J. Smit, A. de Jong, H. Hilkmann, T. K. Sixma and H. Ovaas, *Angew. Chem., Int. Ed.*, 2010, **49**, 10149.
- 256 S. Virdee, Y. Ye, D. P. Nguyen, D. Komander and J. W. Chin, *Nat. Chem. Biol.*, 2010, **6**, 750.
- 257 C. Castaneda, J. Liu, A. Chaturvedi, U. Nowicka, T. A. Cropp and D. Fushman, *J. Am. Chem. Soc.*, 2011, **133**, 17855.
- 258 S. B. Gunnoo and A. Madder, *ChemBioChem*, 2016, **17**, 529.
- 259 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004.
- 260 E. M. Valkevich, R. G. Guenette, N. A. Sanchez, Y.-c. Chen, Y. Ge and E. R. Strieter, *J. Am. Chem. Soc.*, 2012, **134**, 6916.
- 261 G. H. Pham, A. S. J. B. Rana, E. N. Korkmaz, V. H. Trang, Q. Cui and E. R. Strieter, *Protein Sci.*, 2016, **25**, 456.
- 262 V. H. Trang, E. M. Valkevich, S. Minami, Y.-C. Chen, Y. Ge and E. R. Strieter, *Angew. Chem., Int. Ed.*, 2012, **51**, 13085.
- 263 S. S. Panda, C. D. Hall, A. A. Oliferenko and A. R. Katritzky, *Acc. Chem. Res.*, 2014, **47**, 1076.

- 264 A. Catic, C. Collins, G. M. Church and H. L. Ploegh, *Bioinformatics*, 2004, **20**, 3302.
- 265 S. Brase, C. Gil, K. Knepper and V. Zimmermann, *Angew. Chem., Int. Ed.*, 2005, **44**, 5188.
- 266 E. F. V. Scriven and K. Turnbull, *Chem. Rev.*, 1988, **88**, 297.
- 267 A. K. Pandiakumar, S. P. Sarma and A. G. Samuelson, *Tetrahedron Lett.*, 2014, **55**, 2917.
- 268 E. D. Goddard-Borger and R. V. Stick, *Org. Lett.*, 2011, **13**, 2514.
- 269 N. Fischer, E. D. Goddard-Borger, R. Greiner, T. M. Klapoetke, B. W. Skelton and J. Stierstorfer, *J. Org. Chem.*, 2012, **77**, 1760.
- 270 R. D. Crouch, *Synthetic Communications*, 2013, **43**, 2265.
- 271 A. Arsianti, H. Tanimoto, T. Morimoto, A. Bahtiar, T. Takeya and K. Kakiuchi, *Tetrahedron*, 2012, **68**, 2884.
- 272 S. Braun, a. et and Editors, *100 And More Basic NMR Experiments: A Practical Course*, VCH, 1996.
- 273 M. Bartra, F. Urpi and J. Vilarrasa, *Tetrahedron Lett.*, 1987, **28**, 5941.
- 274 M. Bartra, P. Romea, F. Urpi and J. Vilarrasa, *Tetrahedron*, 1990, **46**, 587.
- 275 J. W. Lee and P. L. Fuchs, *Org. Lett.*, 1999, **1**, 179.
- 276 D. S. Hays and G. C. Fu, *J. Org. Chem.*, 1998, **63**, 2796.
- 277 S. M. Mali and H. N. Gopi, *J. Org. Chem.*, 2014, **79**, 2377.
- 278 V. Popov, S. S. Panda and A. R. Katritzky, *J. Org. Chem.*, 2013, **78**, 7455.
- 279 C. Haase and O. Seitz, *Eur. J. Org. Chem.*, 2009, **2009**, 2096.
- 280 S. Park, M.-R. Lee and I. Shin, *Chem. Soc. Rev.*, 2008, **37**, 1579.
- 281 L. L. Lairson, B. Henrissat, G. J. Davies and S. G. Withers, *Annu. Rev. Biochem.*, 2008, **77**, 521.
- 282 M. Jmoudiak and A. H. Futerman, *Br. J. Haematol.*, 2005, **129**, 178.
- 283 H. Ishizuka, T. Nakayama, S. Matsuoka, I. Gotoh, M. Ogawa, K. Suzuki, N. Tanaka, K. Tsubaki, H. Ohkubo, Y. Arakawa and T. Okano, *Intern Med*, 1999, **38**, 927.
- 284 D. L. Meany and D. W. Chan, *Clin. Proteomics*, 2011, **8**, 7.
- 285 K. Matsumoto, C. Shimizu, T. Arao, M. Andoh, N. Katsumata, T. Kohno, K. Yonemori, F. Koizumi, H. Yokote, K. Aogi, K. Tamura, K. Nishio and Y. Fujiwara, *J. Proteome Res.*, 2009, **8**, 457.
- 286 S. Faulkner, S. J. A. Pope and B. P. Burton-Pye, *Appl. Spectrosc. Rev.*, 2005, **40**, 1.
- 287 W. J. Evans, *Inorg. Chem.*, 2007, **46**, 3435.
- 288 Generalic, Eni. "Download printable materials." EniG. Periodic Table of the Elements. KTF-Split, 2016.
<<http://www.periodni.com/download.html>>
- 289 P. Hänninen and H. Härmä, *Lanthanide luminescence: photophysical, analytical and biological aspects*, Springer Science & Business Media, 2011.

- 290 S. I. Weissman, *J. Chem. Phys.*, 1942, **10**, 214.
- 291 J.-C. G. Bunzli, *Acc. Chem. Res.*, 2006, **39**, 53.
- 292 A. J. Amoroso and S. J. A. Pope, *Chem. Soc. Rev.*, 2015, **44**, 4723.
- 293 K. Binnemans, *Chem. Rev.*, 2009, **109**, 4283.
- 294 D. L. Dexter, *J. Chem. Phys.*, 1953, **21**, 836.
- 295 T. Forster, *Discuss. Faraday Soc.*, 1959, **No. 27**, 7.
- 296 J.-C. G. Bunzli and S. V. Eliseeva, *Springer Ser. Fluoresc.*, 2011, **7**, 1.
- 297 J.-C. G. Bunzli and C. Piguet, *Chem. Soc. Rev.*, 2005, **34**, 1048.
- 298 W. D. Horrocks, Jr. and D. R. Sudnick, *Acc. Chem. Res.*, 1981, **14**, 384.
- 299 A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams and M. Woods, *J. Chem. Soc., Perkin Trans. 2*, 1999, 493.
- 300 S. Shinoda and H. Tsukube, *Analyst* 2011, **136**, 431.
- 301 B. Jastrzebska, R. Lebel, H. Therriault, J. O. McIntyre, E. Escher, B. Guerin, B. Paquette, W. A. Neugebauer and M. Lepage, *J. Med. Chem.*, 2009, **52**, 1576.
- 302 M. Querol, J. W. Chen, R. Weissleder and A. Bogdanov, Jr., *Org. Lett.*, 2005, **7**, 1719.
- 303 J. W. Chen, W. Pham, R. Weissleder and A. Bogdanov, Jr., *Magn. Reson. Med.*, 2004, **52**, 1021.
- 304 M. Giardiello, M. P. Lowe and M. Botta, *Chem. Commun.*, 2007, 4044.
- 305 S. Mizukami, K. Tonai, M. Kaneko and K. Kikuchi, *J. Am. Chem. Soc.*, 2008, **130**, 14376.
- 306 I. Rahman, A. Kode and S. K. Biswas, *Nat. Protoc.*, 2006, **1**, 3159.
- 307 B. K. McMahon and T. Gunnlaugsson, *J. Am. Chem. Soc.*, 2012, **134**, 10725.
- 308 J.-C. G. Bunzli, *Chem Rev*, 2010, **110**, 2729.
- 309 R. M. Izatt, K. Pawlak, J. S. Bradshaw and R. L. Bruening, *Chem. Rev.*, 1995, **95**, 2529.
- 310 G. Muller, *Dalton Trans.*, 2009, 9692.
- 311 J. Bruno, W. D. Horrocks, Jr. and R. J. Zauhar, *Biochemistry*, 1992, **31**, 7016.
- 312 W. Koenigs and E. Knorr, *Ber. Dtsch. Chem. Ges.*, 1901, **34**, 957.
- 313 H. M. Christensen, S. Oscarson and H. H. Jensen, *Carbohydr. Res.*, 2015, **408**, 51.
- 314 D. E. Green, C. L. Ferreira, R. V. Stick, B. O. Patrick, M. J. Adam and C. Orvig, *Bioconjugate Chem.*, 2005, **16**, 1597.
- 315 J. J. Garcia-Lopez, F. Hernandez-Mateo, J. Isac-Garcia, J. M. Kim, R. Roy, F. Santoyo-Gonzalez and A. Vargas-Berenguel, *J. Org. Chem.*, 1999, **64**, 522.
- 316 H. K. R. Santhapuram, A. Datta, O. E. Hutt and G. I. Georg, *J. Org. Chem.*, 2008, **73**, 4705.
- 317 J. Massue, S. E. Plush, C. S. Bonnet, D. A. Moore and T. Gunnlaugsson, *Tetrahedron Lett.*, 2007, **48**, 8052.

- 318 T. Gunnlaugsson and A. J. Harte, *Org. Biomol. Chem.*, 2006, **4**, 1572.
- 319 J. W. Akitt and B. E. Mann, *NMR and Chemistry: An introduction to modern NMR spectroscopy*, CRC Press, 2000.
- 320 B. Yan, H. J. Zhang, S. B. Wang and J. Z. Ni, *Monatsh. Chem.*, 1998, **129**, 567.
- 321 S. A. Weissman and D. Zewge, *Tetrahedron*, 2005, **61**, 7833.
- 322 E. H. Vickery, L. F. Pahler and E. J. Eisenbraun, *J. Org. Chem.*, 1979, **44**, 4444.
- 323 J. Selvakumar, A. Makriyannis and C. R. Ramanathan, *Org. Biomol. Chem.*, 2010, **8**, 4056.
- 324 L. Miao, L. Xu, K. W. Narducy and M. L. Trudell, *Org. Process Res. Dev.*, 2009, **13**, 820
- 325 Y. Kaburagi and Y. Kishi, *Org. Lett.*, 2007, **9**, 723.
- 326 T. Watanabe, T. Sato, S. Yoshioka, T. Koshijima and M. Kuwahara, *Eur. J. Biochem.*, 1992, **209**, 651.
- 327 H. Michlmayr, C. Schuemann, N. M. B. Braz da Silva, K. D. Kulbe and A. M. del Hierro, *J. Appl. Microbiol.*, 2010, **108**, 550.
- 328 R. Brooks, Z. Du, G. Borlace, D. Brooks and S. Plush, *J. of Inorg. Chem.*, 2013, **2013**, 8.
- 329 A. Varki, *Glycobiology*, 1993, **3**, 97.
- 330 K. T. Pilobello, D. E. Slawek and L. K. Mahal, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 11534.
- 331 F. P. Schwarz, K. D. Puri, R. G. Bhat and A. Surolia, *J. Biol. Chem.*, 1993, **268**, 7668.
- 332 K. Kikuchi, H. Takakusa and T. Nagano, *TrAC, Trends Anal. Chem.*, 2004, **23**, 407.
- 333 P. R. Selvin, *IEEE J. Sel. Top. Quantum Electron.*, 1996, **2**, 1077.
- 334 T. Heyduk, *Curr. Opin. Biotechnol.*, 2002, **13**, 292.
- 335 E. Martin Rodriguez, N. Bogdan, J. A. Capobianco, S. Orlandi, M. Cavazzini, C. Scalera and S. Quici, *Dalton Trans.*, 2013, **42**, 9453.
- 336 O. Rusin, V. Kral, J. O. Escobedo and R. M. Strongin, *Org. Lett.*, 2004, **6**, 1373.
- 337 K. Sato and J.-i. Anzai, *Anal. Bioanal. Chem.*, 2006, **384**, 1297.
- 338 M. Delbianco, P. Bharate, S. Varela-Aramburu and P. H. Seeberger, *Chem. Rev.*, 2016, **116**, 1693.
- 339 K.-R. Wang, H.-W. An, R.-X. Rong, Z.-R. Cao and X.-L. Li, *Macromol. Rapid Commun.*, 2014, **35**, 727.
- 340 B. Guo, X. Cai, S. Xu, S. M. A. Fateminia, J. Liu, J. Liang, G. Feng, W. Wu and B. Liu, *J. Mater. Chem. B*, 2016, **4**, 4690.
- 341 Y. Hong, J. W. Y. Lam and B. Z. Tang, *Chem. Soc. Rev.*, 2011, **40**, 5361.
- 342 J. Mei, N. L. C. Leung, R. T. K. Kwok, J. W. Y. Lam and B. Z. Tang, *Chem. Rev.*, 2015, **115**, 11718.

- 343 T. Sanji, K. Shiraishi, M. Nakamura and M. Tanaka, *Chem. - Asian J.*, 2010, **5**, 817.
- 344 H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512.
- 345 H. P. R. Mangunuru, J. R. Yerabolu and G. Wang, *Tetrahedron Lett.*, 2015, **56**, 3361
- 346 S. B. Salunke, N. S. Babu and C.-T. Chen, *Chem. Commun.*, 2011, **47**, 10440
- 347 Hammer, Loranger and Schein, *J. Org. Chem.*, 1981, **46**, 1521
- 348 F. Fazio and C.-H. Wong, *Tetrahedron Lett.*, 2003, **44**, 9083
- 349 E. A. Englund, H. N. Gopi and D. H. Appella, *Org. Lett.*, 2004, **6**, 859.
- 350 J. M. Travins and F. A. Etzkorn, *J. Org. Chem.*, 1997, **62**, 8387.
- 351 S. Kim, J. I. Lee and Y. C. Kim, *J. Org. Chem.*, 1985, **50**, 560
- 352 M. Maiti, M. Maiti, J. Rozenski, J. S. De and P. Herdewijn, *Org. Biomol. Chem.*, 2015, **13**, 5158.
- 353 P. Lloyd-Williams, A. Sanchez, N. Carulla, T. Ochoa and E. Giralt, *Tetrahedron*, 1997, **53**, 3369
- 354 G. Giorgi, L. Guideri and F. Ponticelli, *Tetrahedron*, 2011, **67**, 1463
- 355 D. A. Evans, Y. Aye and J. Wu, *Org. Lett.*, 2006, **8**, 2071
- 356 S. N. Khattab, R. Subiros-Funosas, A. El-Faham and F. Albericio, *Eur. J. Org. Chem.*, 2010, 3275.
- 357 N. B. Palakurthy, D. Dev, S. Paikaray, S. Chaudhury and B. Mandal, *RSC Adv.*, 2014, **4**, 7952.
- 358 A. G. Avent, H. M. E. Duggan and D. W. Young, *Org. Biomol. Chem.*, 2005, **3**, 2327
- 359 D. Bednarczyk, A. Walczewska, D. Grzywacz, A. Sikorski, B. Liberek and H. Myszka, *Carbohydr. Res.*, 2013, **367**, 10
- 360 D. Chatterjee, A. Paul, Rajkamal and S. Yadav, *RSC Advances*, 2015, **5**, 29669
- 361 M. Niemietz, L. Perkams, J. Hoffman, S. Eller and C. Unverzagt, *Chem. Commun.*, 2011, **47**, 10485
- 362 S. S. Bag, S. Jana, A. Yashmeen, Senthilkumar and R. Bag, *Chem. Commun.*, 2014, **50**, 433
- 363 J. C. Anderson, J. G. Ford and M. Whiting, *Org. Biomol. Chem.*, 2005, **3**, 3734.
- 364 T. J. Williams, A. J. Reay, A. C. Whitwood and I. J. S. Fairlamb, *Chem. Commun.*, 2014, **50**, 3052
- 365 Y. A. Lin, J. M. Chalker and B. G. Davis, *J. Am. Chem. Soc.*, 2010, **132**, 16805
- 366 P. R. Patil and K. Ravindranathan, *J. Carbohydr. Chem.*, 2008, **27**, 411
- 367 N. Baggett and B. J. Marsden, *Carbohydr. Res.*, 1983, **119**, 285
- 368 J. H. Jung, J. A. Rim, E. J. Cho, S. J. Lee, I. Y. Jeong, N. Kameda, M. Masuda and T. Shimizu, *Tetrahedron*, 2007, **63**, 7449

- 369 T. Miyazawa, K. Tanaka, E. Ensatsu, R. Yanagihara and T. Yamada, *J. Chem. Soc. Perkin Trans. 1*, 2001, 87
- 370 S. E. Plush, N. A. Clear, J. P. Leonard, A.-M. Fanning and T. Gunnlaugsson, *Dalton Trans.*, 2010, **39**, 3644
- 371 S.-I. Kawano, S.-I. Tamaru, N. Fujita and S. Shinkai, *Chem. Eur. J.*, 2004, **10**, 343
- 372 L. Yu, M. Huang, P. G. Wang and X. Zeng, *Anal. Chem.*, 2007, **79**, 8979
- 373 Y. Yang, M. Yu, T.-T. Yan, Z.-H. Zhao, Y.-L. Sha and Z.-J. Li, *Bioorg. Med. Chem.*, 2010, **18**, 5234
- 374 S. Kopitzki, K. J. Jensen and J. Thiem, *Chem. Eur. J.*, 2010, **16**, 7017
- 375 R. Roy, F. D. Tropper, T. Morrison and J. Boratynski, *J. Chem. Soc., Chem. Commun.*, 1991, 536
- 376 N. Laurent, D. Lafont, F. Dumoulin, P. Boullanger, G. Mackenzie, P. H. J. Kouwer and J. W. Goodby, *J. Am. Chem. Soc.*, 2003, **125**, 15499
- 377 Garegg and et al., *Carbohydr. Res.*, 1978, **62**, 173.
- 378 S.-S. Chang, C.-C. Lin, Y.-K. Li and K.-K. T. Mong, *Carbohydr. Res.*, 2009, **344**, 432.
- 379 D. R. Jayasundara, T. Duff, M. D. Angione, J. Bourke, D. M. Murphy, E. M. Scanlan and P. E. Colavita, *Chem. Mater.*, 2013, **25**, 4122
- 380 L. Ma, C. Xie, Y. Ma, J. Liu, M. Xiang, X. Ye, H. Zheng, Z. Chen, Q. Xu, T. Chen, J. Chen, J. Yang, N. Qiu, G. Wang, X. Liang, A. Peng, S. Yang, Y. Wei and L. Chen, *J. Med. Chem.*, 2011, **54**, 2060
- 381 K. S. Lee, K. K. Adhikary, H. W. Lee, B.-S. Lee and I. Lee, *Org. Biomol. Chem.*, 2003, **1**, 1989