Synthesis of *S*-glycolipids and peptidomimetics

By

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Department of Chemistry,

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Under the supervision and direction of

Prof. Paul V. Murphy

National University of Ireland,

Galway.

For my mother and father

Declaration

This thesis has not been submitted before, in whole or in part, to this or any other university for any degree, and is, except where otherwise stated, the original work of the author.

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Ciarán O'Reilly

Abstract

Chapters one and two of this thesis describe the synthesis of two novel glycosphingolipid derivatives. Glycosphingolipids are amphiphilic molecules consisting of a carbohydrate head group glycosidically linked to a sphingoid lipid chain. Our interest is in the synthesis of α-glycosphingolipids of bacterial origin, particularly those isolated from the cell walls of *Sphingomonas* bacteria. These glycolipid derivatives bear a striking resemblance to the known immunostimulant KRN7000 and have shown activity in both human and mouse models. The Murphy group has previously reported the synthesis of a bacterial glycosphingolipid termed PBS-59 and its glucuronic acid derivative; therefore, this thesis focuses on our efforts to synthesise thio-linked analogues of these lipid derivatives in the hope of improving their *in vivo* stability and immunostimulatory activity. The synthetic route includes a stereoselective anomerisation reaction to form α-glycosyl thiol precursors as well as a new route to sphinganine chains starting from the Myers pseudoephedrine chiral auxiliary.

Chapters three and four investigate the use of carbohydrates as scaffolds for the development of novel biologically active peptidomimetics. Of particular interest is the synthesis of a novel macrocyclic structure with embedded carbohydrates capable of mimicking the α-helical domain. The hydroxyl groups of the carbohydrate act as a functional handle onto which pharmacophoric groups can be grafted. Two key protein-protein interactions (Bcl-2 family of proteins and the p53-MDM2 interaction) were identified as targets for peptidomimetic development. The synthesis of these macrocyclic structures was developed, a route which includes a rare example of the use of a reductive amination/ring closing macrocyclisation sequence, and a number of novel α-helical peptidomimetics were obtained. Some of these mimetics have shown activity in triggering apoptosis in ML-1 cells.

Presented in chapter five is the synthesis of a novel bicyclic iminocyclitol derivative. This compound was synthesised via a novel one pot nucleophilic substitution, Huisgen cycloaddition, triazoline decomposition, aziridine formation and aziridine ring opening by an azide anion. The conversion of this compound into a tricyclic derivative is also described showing its potential for use in peptidomimetic development or as a building block in the synthesis of more complex molecules.

Acknowledgements

Firstly, I would like to say a heartfelt thank you to my supervisor and mentor, Professor Paul V. Murphy, to whom I owe a huge debt of gratitude. It has been a real privilege and an honour to have been part of his group for the last four years. His dedication and knowledge of chemistry is truly inspirational and his guidance, advice and support to me personally throughout my time in his group was second to none and is something I will never forget.

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To Amy Lynch, thanks so much for being so supportive and putting up with me over the last year! You are amazing.

Finally I would like to say a special thank you to the most amazing parents and brother in the world. To my parents, Carmel and Martin O'Reilly and my brother Aaron, I'd like to dedicate this thesis to you all. Your unwavering support, belief, kindness and love has made me the person I am today. I could never have achieved any of this without you. I love you all and I hope I have made you proud.

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Chapter 3: Novel peptidomimetics: synthesis of Bcl-2 family inhibitors

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Symbols and Abbreviations

α	Alpha
ADDP	1,1'-(azidodicarbonyl)dipiperidine
Ar	Aromatic
BAIB	Bisacetoxyiodobenzene
Bcl	B-Cell lymphoma
BH	Bcl-2 homology
BuLi	Butyllithium
Cer	Ceramide
COSY	Correlation Spectroscopy
δ	Chemical shift in ppm downfield from TMS
d	Doublet (spectral)
dd	Doublet of doublets (spectral)
ddd	Doublet of doublets of doublets (spectral)
DEPT	Distortionless Enhancement by Polarisation Transfer
DIPEA	Diisopropylethylamine
DMJ	Deoxymannojirimycin
DNA	Deoxyribonucleic acid
DNJ	Deoxynojirimycin
dt	Doublet of triplets (spectral)
dr	Diastereomeric ratio
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

eq.	Equivalents
er	Enantiomeric ratio
Et	Ethyl
ES-HRMS	High-Resolution Mass Spectrometry - Electrospray Ionization
FTIR	Fourier transform infrared (spectroscopy)
Gal	Galactose
Glc	Glucose
GSL	Glycosphingolipid
HMBC	Heteronuclear multiple bond correlation
HMDS	Hexamethyldisilylazide
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single quantum correlation
Hz	Hertz
IFN-γ	Interferon-γ
IL	Interleukin
IR	Infrared (spectroscopy)
J	Coupling constant (nmr), in Hz
kDa	Kilo Dalton
lit.	Literature reference
LHMDS	Lithium hexamethyldisilazide
N	Normal
m	Multiplet
M	Molar

M^+	Mass of the molecular ion (mass spectrometry)
MDM2	Murine double-minute 2
Mcl	Myeloid cell leukaemia
Me	Methyl
Ms	Mesyl
MHz	Megahertz
mL, μL	Milliliter, microliter
mol, mmol	Mole, millimole
mM, μM	Millimolar, micromolar
Mp	Melting point
NK	Natural Killer
NMM	*N*-methylmorpholine
NMO	*N*-Methylmorpholine-*N*-oxide
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
PTSA	para-toluenesulfonic acid
ppm	Parts per million (NMR)
Prop	Propyl
q	Quartet (spectral)
R_f	Retention factor
rt	Room temperature
$[\alpha]_D$	Specific rotation

s	Singlet (spectral)
S_N2	Bimolecular nucleophilic substitution
t	Triplet (spectral)
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TMS	Tri-methylsilyl
TIPS	Tri-isopropysilyl
TBS	tert-butyl-di-methylsilyl
Ts	Tosyl
td	Triplet of doublets (spectral)
t-Bu	tert-butyl
TCR	T cell receptor
T_h	T helper
TLC	Thin Layer Chromatography

Chapter 1: Introduction to glycosphingolipids

1.1 Biology of Natural Killer T-Cells

CD1d restricted Natural Killer T cells (NKT cells) constitute an exclusive subset of natural killer (NK) lymphocytes. Possessing a number of unique characteristics, these lymphocytes play a key role in the regulation of immune responses[1-2]. NKT cells present in the liver and spleen are capable of co-expressing an invariant T cell receptor (TCR). It has been shown that in both mouse and human models TCR's can recognize glycosphingolipid antigens presented by the monomorphic antigen presenting glycoprotein known as CD1d[3]. This glycoprotein is expressed on the cell surface of an antigen presenting cell or APC. Upon activation, NKT cells stimulate the production of numerous signalling peptides (cytokines) such as interferon-γ (IFN-γ) and members of the interleukin family including IL-4 and IL-12. These cytokines are capable of exerting rapid and substantial immune responses. The response triggered can be of two distinct types; T helper 1 (T_h1) responses and T helper 2 (T_h2) responses. The effects on the immune system that these responses promote are summarised below[4]:

1. T_h1 response: this constitutes a pro-inflammatory response which assists in the control of various bacterial, parasitic and viral infections and can have an effect against certain tumours. These responses are triggered by cytokines such as IFN-γ. The major caveat of this response however, is that a number of autoimmune diseases such as diabetes, multiple sclerosis, lupus and rheumatoid arthritis are also T_h1 mediated.

2. T_h2 response: this constitutes an immunomodulatory response brought about by cytokines such as IL-4. This response is antagonistic and hinders the T_h1 response meaning T_h2 responses could help alleviate the effects of autoimmune diseases.

Stimulation of NKT cells by glycosphingolipid antigens can lead to production of both T_h1 and T_h2 cytokines simultaneously. Should this happen, the antagonistic nature of these cytokines means that any immunogenic response will be effectively abrogated[5]. This means that any potential medicinal use of NKT cell stimulation by glycosphingolipid antigens must be under strict control in order to elicit a biased response towards the production of either T_h1 or T_h2 cytokines.

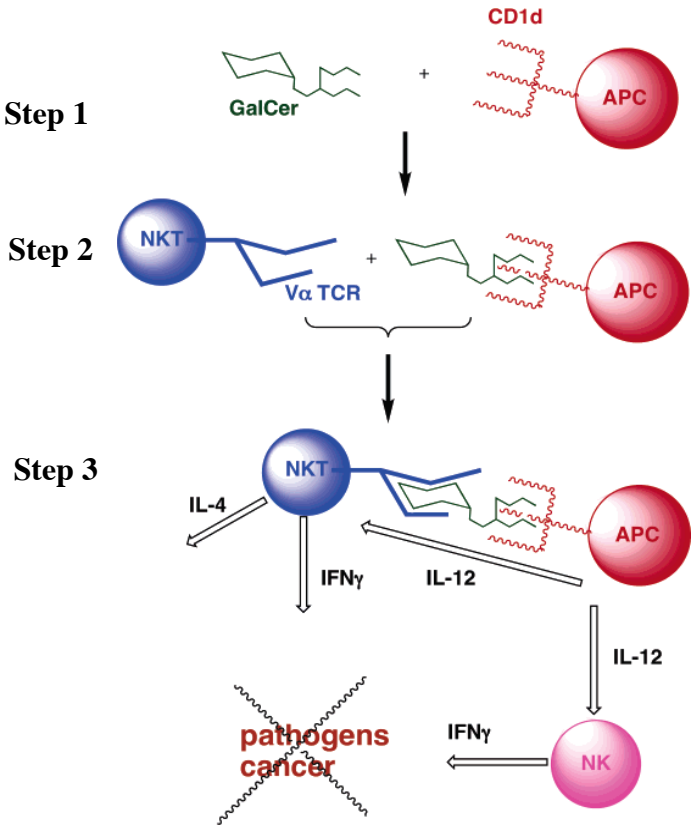


Figure 1 Simplified overview of NKT cell stimulation (Reprinted with permission by Accounts of Chemical Research, copyright 2006, American Chemical Society)

Presented in Figure 1[6] is a simplified overview of the interaction between glycosphingolipids and NKT cells. In step 1 an antigen presenting cell (APC) of the innate immune system expressing the CD1d protein on its cell surface binds the hydrophobic chain of the glycolipid and displays the sugar head group on the cell surface. The sugar groups are recognised by TCR's on the surface of NKT cells (step 2). Recognition leads to the rapid expression of cytokines (IFN-γ and IL-4 by NKT cells and IL-12 from the CD1d bearing APC) which in turn elicit an immune response (step 3).

1.2 Structure of glycosphingolipids

Glycosphingolipids are a diverse subset of glycolipids, many of which have varied and complex biological roles. These compounds have been shown to be involved in many cellular interactions including cell signalling, host-pathogen interactions, migration and apoptosis. Typically made up of a sphingoid base glycosidically liked to a single sugar residue or oligosaccharide moiety examples of glycosphingolipids include[7]:

1. Cerebrosides Containing one sugar residue
2. Sulfatides Containing one sulfated sugar residue
3. Neutral glycosphingolipids Containing more than one sugar residue
4. Gangliosides Containing neuraminic acid residues

Much of the diversity found in glycosphingolipid structure comes as a result of the sphingoid portion of the molecule. There are three main classes of sphingoid base present in nature. The most abundant of which are sphinogsines, which contain an amino-diol and are unsaturated at C4-C5. Phytosphingosines are 2-amino-1,2,4-triols and sphinganines (dihydrosphingosines) are amino-diols without the double bond at C4-C5. Being the most abundant, the synthesis of sphingosines[8] and phytosphingosines[9] has been extensively reviewed. This thesis will focus on synthetic efforts towards sphinganines and their use in the synthesis of bacterial glycosphingolipid mimics.

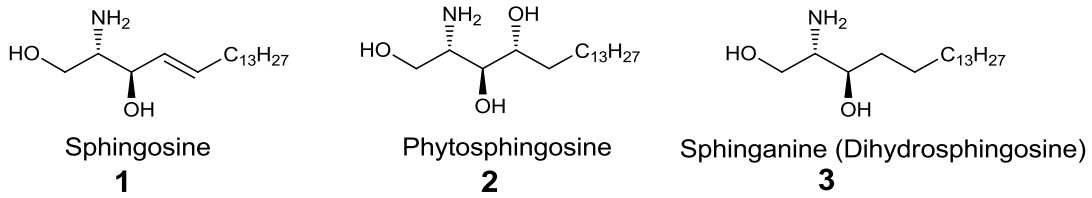


Figure 2 The three most common sphingoid bases present in nature

1.3 Discovery of glycolipid ligands for NKT cell stimulation

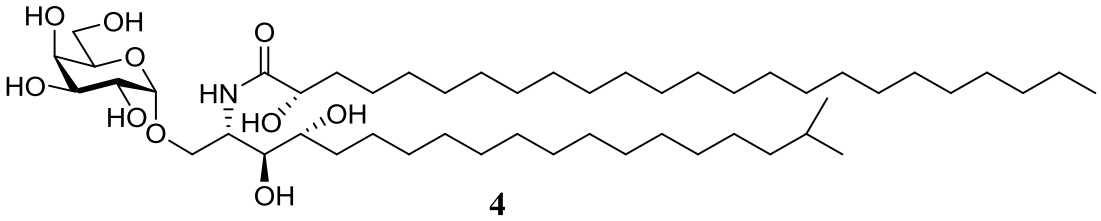


Figure 3 Structure of Agelasphin 9b

In 1993, workers at the Kirin brewery in Japan isolated a number of unusual glycolipids from the Okinawan marine sponge *Agelas mauritianus* [10-11]. These compounds were called agelasphins (Figure 3) and a biological evaluation showed that they possessed potent anti-tumour properties due to their ability to stimulate NKT cells. These agelasphins were seen as unusual because they contained an α-glycosidic bond between the sugar and ceramide moieties (glycolipids in higher organisms are typically β-linked with respect to their glycosidic bonds).

Extensive structure activity relationships were carried out on both the sugar and ceramide portions of the molecules in order to elucidate the reasons for the high biological potency of these compounds. Modifications of the sugar portion showed that α-galactosyl ceramide **5** was more active than α-glucosylceramide **6** (see figure 4)[12]. It also showed that both α-mannosylceramide **7** and β-galactosylceramide **8** were inactive. This suggests that both the nature of the glycone and the presence of an α-glycosidic linkage are important factors in the ability of glycolipids to stimulate NKT cells.

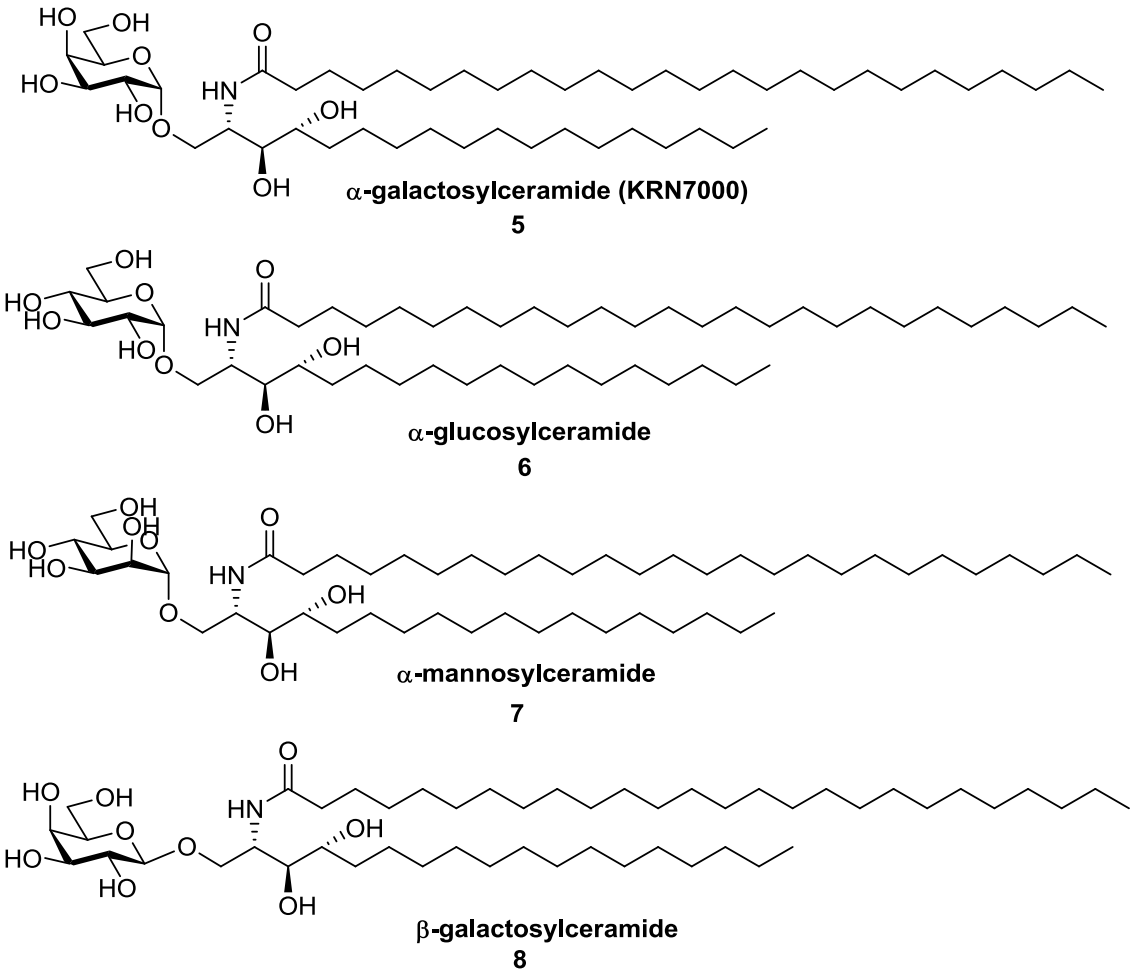


Figure 4 Structures of monoglycosylceramides

Investigations into the ceramide portion showed that the C2' hydroxyl group does not significantly alter the activity of the bound glycolipid. The C4 hydroxyl group only plays a minor role, however, the C3 hydroxyl group is vital to maximise the biological activity of the lipid derivatives[13] (Figure 5).

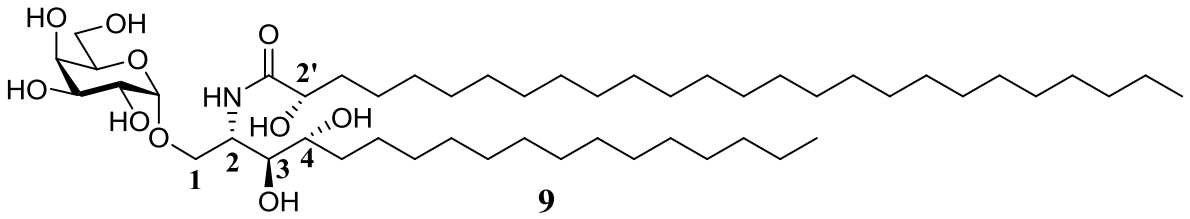


Figure 5 Numbering of ceramide chain

From these studies α-GalCer **9** or KRN7000 was identified as a lead drug candidate and has been extensively studied *in vivo* for its immunostimulatory properties. Phase 1 clinical trials showed KRN7000 to be safe and active as an immunostimulant, however, the results of further trials were less than optimistic. As previously stated, stimulation of NKT cells by glycolipids can trigger both T_h1 and T_h2 responses simultaneously. The antagonistic nature of these immune responses could be a reason for the impaired efficacy of KRN7000.

1.4 The CD1d/KRN7000 interaction

In recent years structure activity relationships have been aided by crystal structures of lipid bound mouse[14] and human[15] CD1d and even more recently by the ternary structure of the TCR/CD1d/KRN7000 complex[16]. The crystal structure of the CD1d/KRN7000 interaction is shown in Figure 6. The lipid-binding pocket of CD1d is particularly well adapted to bind both self and microbial glycosphingolipids. As shown in Figure 6 C the acyl chain occupies A' hydrophobic pocket while the sphingosine chain occupies the F' hydrophobic pocket. For KRN7000 and the closely related α-glycuronylceramides, the α1 helix Arg79 and Asp80 establish hydrogen bonds with the hydroxyl groups of the sphingosine. The α2 helix Asp153 stabilises the galactose through hydrogen bonds with the 2'' and 3'' hydroxyl groups, this anchors the sugar in a position parallel to the plain of the α helices (Figure 6 B) and therefore allows it to sit atop the binding groove. This is in sharp contrast to β-GalCer (Figure 6 B), in which the galactose moiety is orientated away from the binding groove indicating why an α-linkage is so important for NKT cell stimulation. The α2 helix also shows a hydrogen bond between the anomeric oxygen and Thr156, this is interesting as this hydrogen bond is absent from the ternary structure published recently (Figure 6 E)[16].

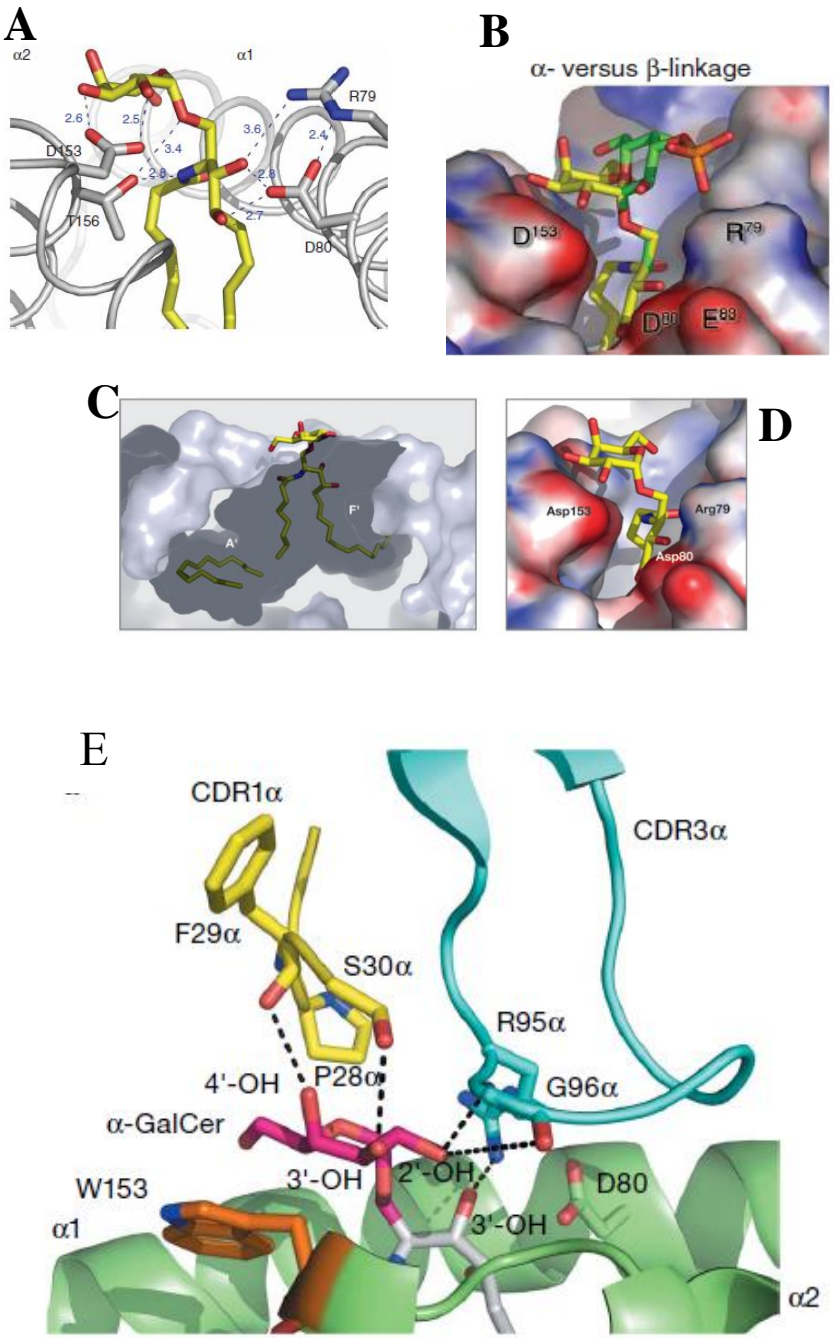


Figure 6 A.) Crystal structure of the KRN7000/CD1d complex showing hydrogen bonding to sphingosine chain. B.) KRN7000 (yellow) and β-GalCer (green) bound to CD1d. C.) sphingosine and acyl chains occupying F and A' hydrophobic pockets respectively. E.) Ternary structure TCR/CD1d/KRN7000. (Reprinted by permission from Macmillan Publishers Ltd: Nature, Ref. 16, copyright 2007)

1.5 Polarising the immune response

Recently, research has focused on the design and synthesis of glycolipid derivatives which are capable of eliciting biased immune responses. These compounds offer an enormous challenge to synthetic chemists, not only are there inherent challenges in the synthesis of the sphingoid portion of the molecule, controlling the stereochemistry of the anomeric linkage poses a great challenge in itself. Therefore the development of novel strategies towards α-glycolipids has been of great interest in recent years. Several synthetic analogues have been developed and a number of these including OCH **10**[17] and α-*C*-GalCer **11**[18-19] (Figure 7) have been successful in polarizing the type of response triggered.

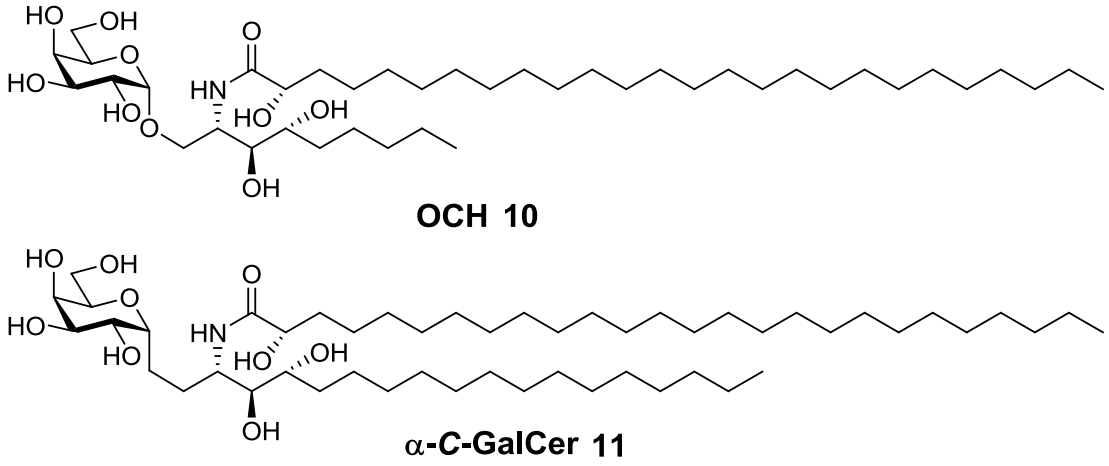


Figure 7 Structures of important glycolipid analogues

OCH **10** is a glycolipid derivative which is truncated in the sphingosine chain of KRN7000. This compound showed a bias in the production of IL-4 cytokines from NKT cells. It is believed the reason for this biased response is that OCH is less stable in the binding site of the CD1d protein and therefore exerts a short lived NKT cell stimulation[20]. On the other hand, α-*C*-GalCer **11** induces an IFN-γ biased immune response presumably due to a more prolonged NKT-cell stimulation time (IFN-γ production requires longer TCR stimulation than that of IL-4). α-*C*-GalCer is an analogue of KRN7000 in which the anomeric oxygen is replaced with a methylene group. Interest in compound **11** has been immense in recent years due, in part, to its superiority to KRN7000 in cure ratios for malaria (1000/1 α-*C*-GalCer/KRN7000) and melanoma (100/1 α-*C*-GalCer/KRN7000). The reason for this dramatic increase in NKT cell stimulation is poorly understood at present. Crystal structures show that the KRN7000/CD1d complex has a key hydrogen bond to the anomeric oxygen (however, as pointed out previously, this hydrogen bond is absent in the ternary structure). As

α-*C*-GalCer lacks this oxygen it would be anticipated that the binding interaction would not be as strong and that a decrease in cytokine production would be observed, however, this is not the case. This implies that, although crystal structures and considerable SAR information is available for some of these compounds, it is still difficult to predict how modified glycolipids will bind. The success of α-*C*-GalCer as an NKT cell agonist suggests that anomeric modifications/replacements could be important in both biasing responses and also as mechanistic tools to further elucidate the mechanism by which NKT cell stimulation occurs.

1.6 Bacterial glycolipids as natural NKT cell antigens

Detection and response to microbial infection is one of the main aspects of innate immunity, therefore it is reasonable to suggest that NKT cells play a key role in the hunt for pathogenic bacteria or parasites *in vivo*. In 2004 Schaible and co-workers[21] showed that glycolipids isolated from mycobacterium such as PIM_4 **12** (Figure 8) could stimulate NKT cells causing the modest release of cytokines compared to KRN7000. This was the first report of a glycolipid of bacterial origin stimulating NKT cells.

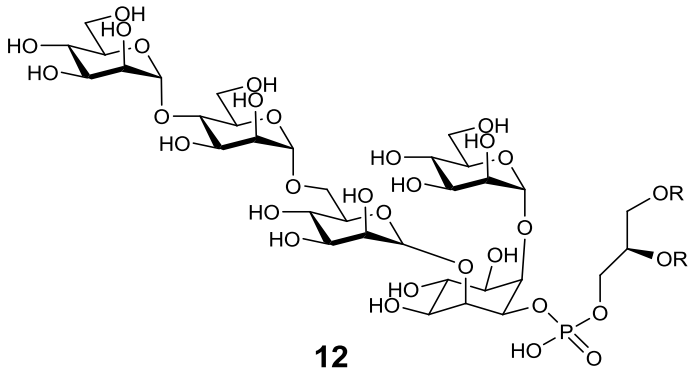


Figure 8 Structure of PIM_4, a glycolipid from mycobacterium that stimulates NKT cells

In recent years it has been shown that glycolipids isolated from certain gram-negative bacteria can also trigger an immune response. Gram-negative bacteria are ubiquitous in nature. In general, the majority of these bacteria have cell walls comprised of lipopolysaccharide (LPS). The innate immune system is capable of recognizing these pathogens and NKT cells play an indirect role in this process. However numerous gram-negative bacteria have cell walls which do not contain LPS (Figure 9)[22], so the question arises: how does the innate immune system address this problem?

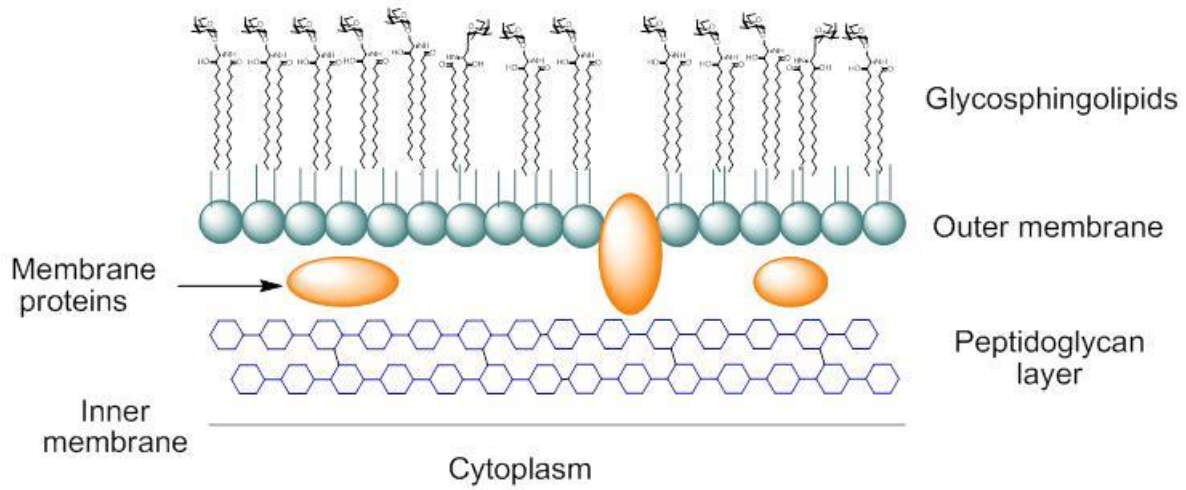


Figure 9 Graphical representation of the *Sphingomonas* cell wall

Investigations into the structure of the membranes of non-LPS-producing gram-negative bacteria of the *Sphingomonadacaea*[23-24] family turned up some very interesting results. Zahringer[25], along with contributions from Kawasaki[26] and co-workers managed to elucidate the structures of a number of glycosphingolipids from the cell walls of these bacteria which they termed GSL-1 **13**, GSL-3 **15** and GSL-4. A GSL-1 (GSL-1' **14**) derivative containing a galacturonic acid residue in place of the glucuronic acid residue (Figure 10)[27] has also been isolated. Similar glycolipids have also been isolated from members of the *Ehrlichia* family which belong to the same class of α-proteobacteria[28].

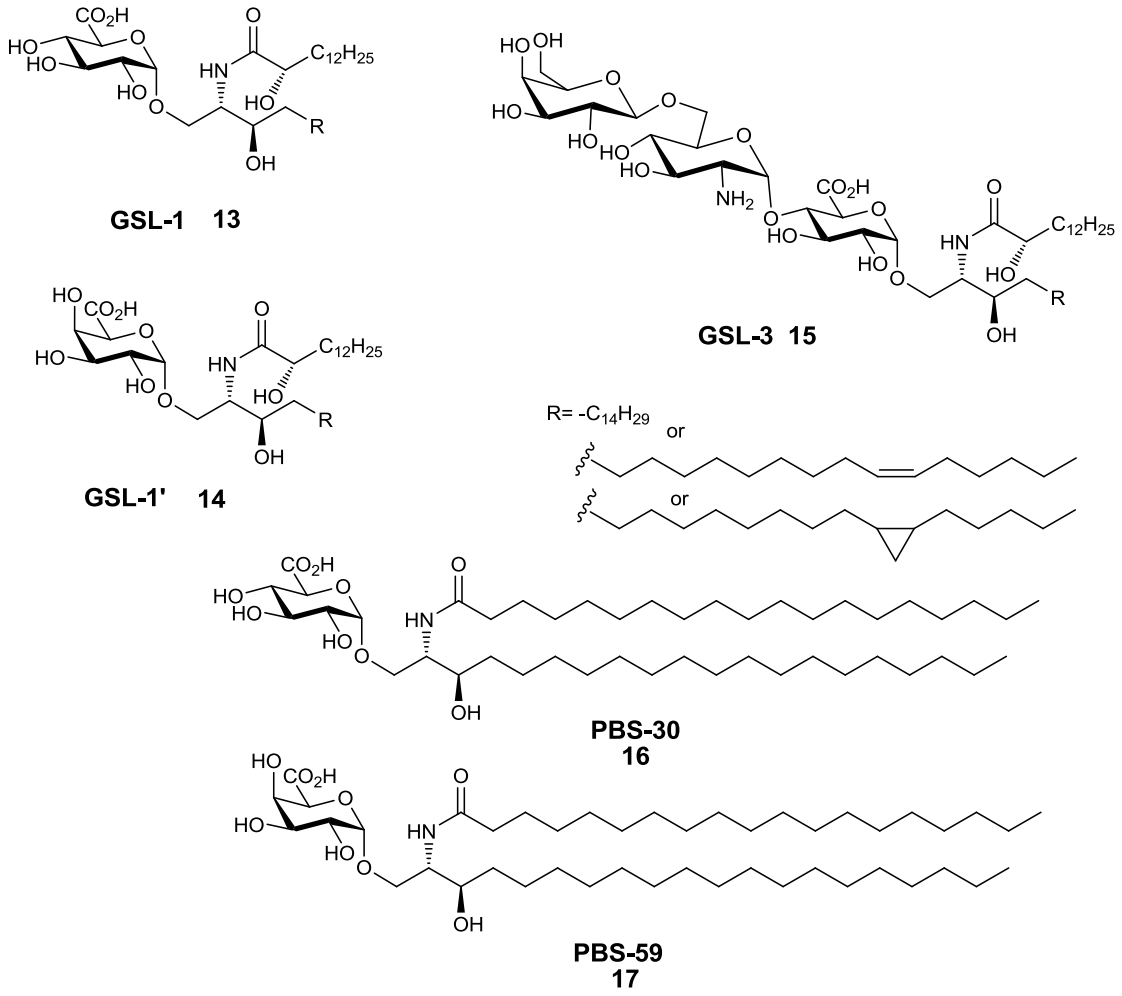


Figure 10 Examples of natural and synthetic bacterial glycolipids

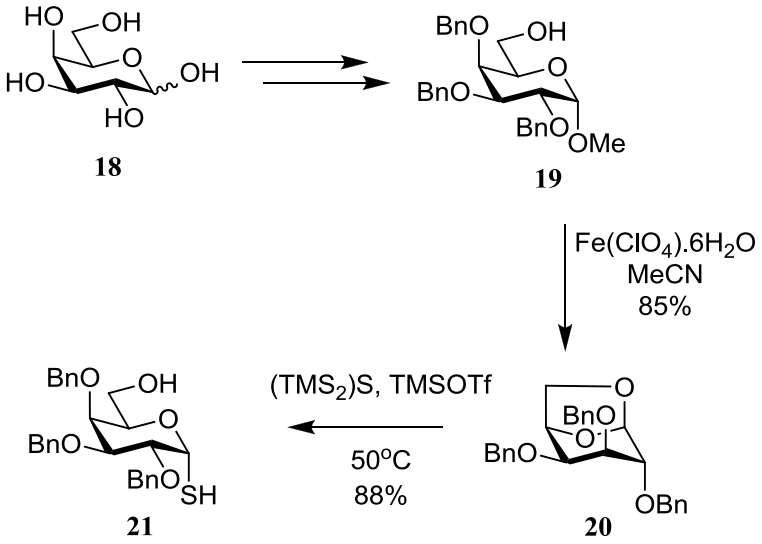
It can be clearly seen that some of these compounds bear a striking resemblance to KRN7000 differing only in the presence of uronic acid residues in the sugar moiety. These similarities led a number of groups to test bacterial glycolipids for their ability to activate NKT cells. As expected it has been shown that these compounds and some synthetic analogues such as PBS-30 **16** and PBS-59 **17** (Figure 10) strongly activated NKT cells in a CD1d dependant fashion[28-29]. For example, injection of *Sphingomonas* into mice triggered septic shock followed by bacterial clearance, however, in NKT cell-deficient mice; a marked decrease in bacterial clearance was noted. Although a number of syntheses of GSL-1 derivatives have been reported, there is still a lack of SAR evaluation on these glycolipids.

1.7 α-*S*-glycolipids

With the impressive results obtained from the biological evaluations of α-*C*-GalCer[6] it comes as no surprise that many groups have become interested in modifying the anomeric linkage. *S*-glycosides have long been used as *O*-glycoside isosteres owing to their similar properties and enhanced stability against the action of glyco-processing enzymes *in vivo*. Although a C-S bond is longer than a C-O bond, the C-S-C bond angle is a lot smaller than the corresponding C-O-C bond angle. This means that there is a relatively small difference in their positions within a glycosidic bond. These properties have led a number of groups, including our own, to pursue *S*-linked glycolipids as novel lipid derivatives. It is believed that these compounds could provide an invaluable mechanistic insight into NKT cell activation by glycolipids as well as an extra degree of stability *in vivo* over their *O*-linked counterparts

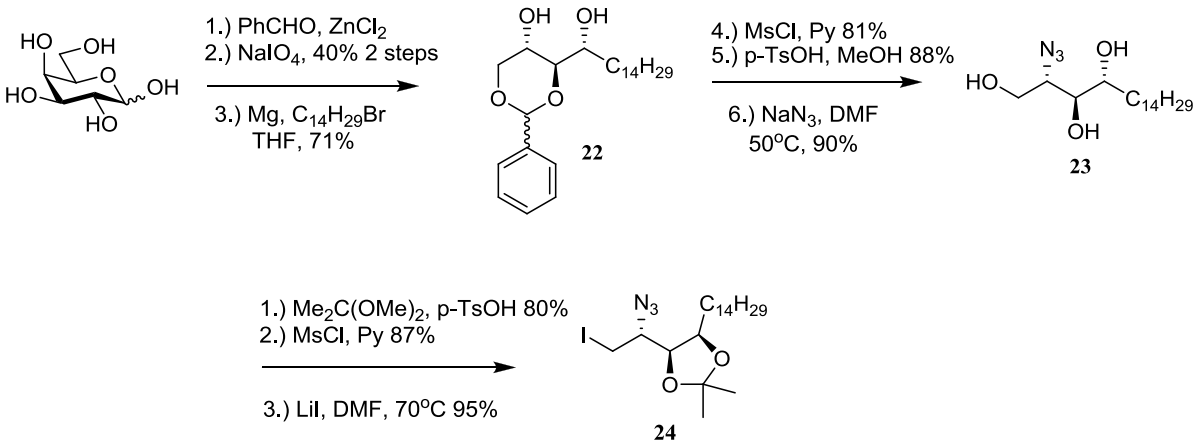
1.7.1 Previous synthesis of *S*-linked analogues of KRN7000

At present, only two reports have appeared containing the synthesis of thioglycoside analogues of KRN7000, the first of which was reported by Zhu and Dere[30]. Their synthetic approach includes a novel synthesis of α-glycosyl thiols via the treatment of 1,6-anhydrogalactose **20** with hexamethyldisilathiane and TMSOTf at reflux to provide the corresponding α-glycosyl thiol **21** exclusively in 88% yield (Scheme 1). This reaction is believed to proceed via an S_N2 displacement on the 1,6 anhydrosugar[31].



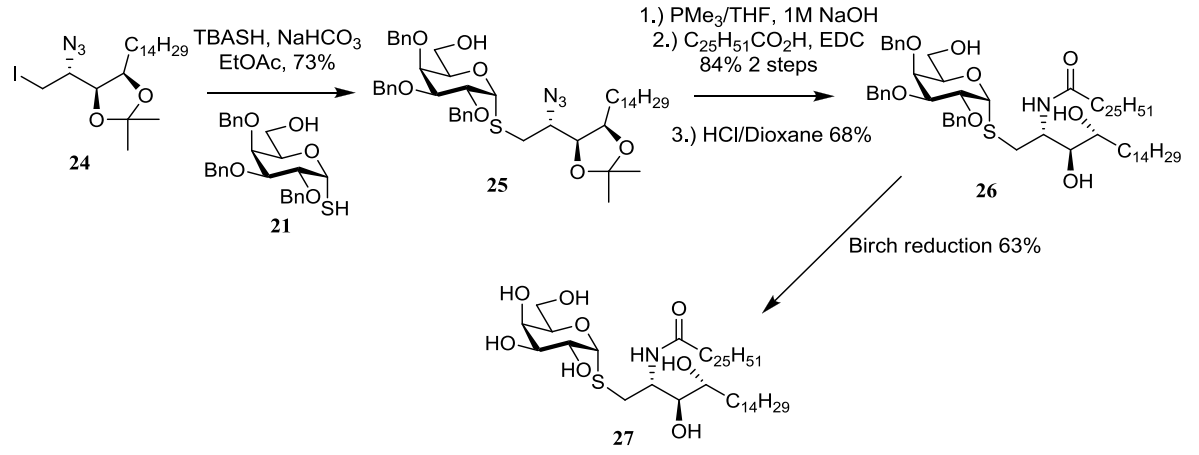
Scheme 1 Novel synthesis of α-glycosyl thiols

With the α-glycosyl thiols in hand, attention was focused on the synthesis of the phytosphingosine chain (Scheme 2). Following the procedure outlined by Schmidt, compound **23** was synthesised from D-galactose via intermediate **22** in 6 steps. Protection of **23** as an isopropylidene followed by mesylation and Finkelstein type reaction gave the iodide derivative **24** in 95% yield.



Scheme 2 Zhu's approach to the synthesis of phytosphingosines

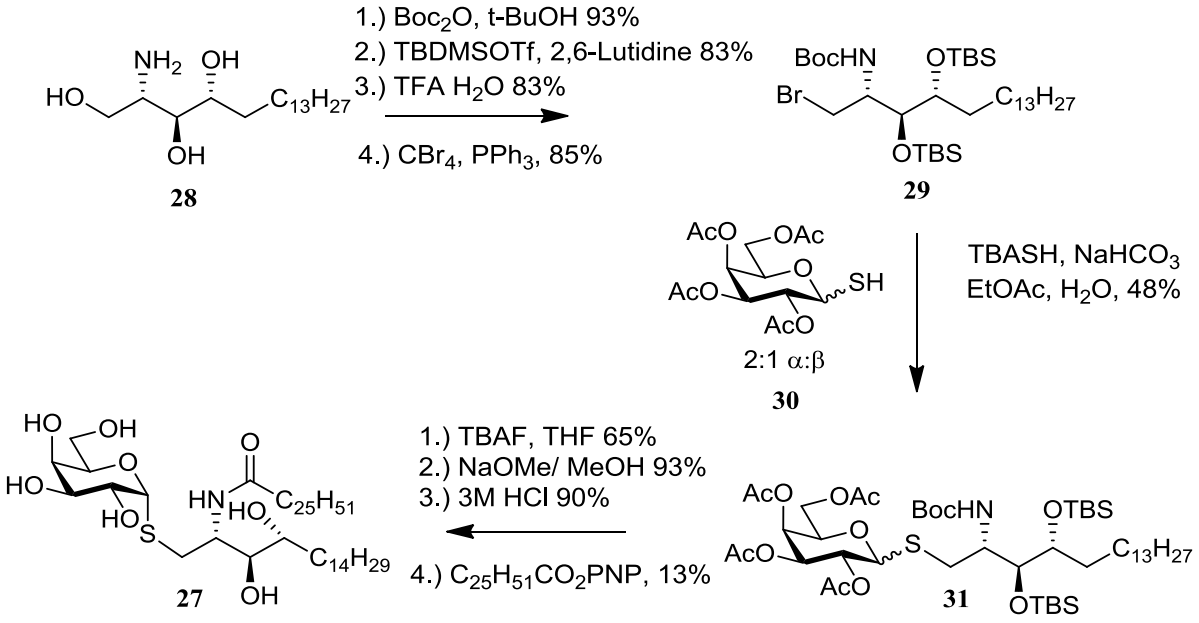
Coupling of glycosyl thiol **21** and the phytosphingosine derivative **24** under phase transfer conditions gave the glycolipid derivative **25** in a 73% yield. Staudinger reduction of the azide followed by amide coupling and subsequent deprotection under Birch conditions gave α-*S*-GalCer **27** in a 63% yield.



Scheme 3 Zhu's synthesis of α-*S*-GalCer

Around the same time as the paper from Zhu's group appeared in the literature, a second total synthesis of the thioglycoside analogue of KRN7000 appeared[32]. Howells group synthesised the bromide derivative **29** in 4 steps from ribo-phytosphingosine in excellent yield (Scheme

4). The glycosyl thiol **30** was synthesised in 3 steps using the procedure described by Yamamoto[33] as a 2:1 α:β mixture of anomers. Coupling of the fragments **29** and **30** under phase transfer conditions gave glycolipid derivative **31** in modest yield. Subsequent deprotection followed by amide formation gave α-*S*-GalCer **27**.



Scheme 4 Howells synthesis of α-*S*-GalCer

Despite the obvious similarities between KRN7000 and its thioglycoside analogue, the Howell group detected no immune response or cytokine production for α-*S*-GalCer in both *in vivo* and *in vitro* tests. In order to decipher a possible reason for this lack of NKT stimulation, they carried out a docking study between α-*S*-GalCer and the ternary crystal structure of the CD1d/TCR complex (Figure 11). Although several key hydrogen bonds were retained between the two complexes, three key hydrogen bonds were either absent or greatly weakened in the α-*S*-GalCer model. KRN7000 forms two hydrogen bonds to Asp 151, one to the carbonyl of Gly 96 and another to the hydroxyl group of Thr 154. In α-*S*-GalCer, only one bond exists with Asp 151 although the other could be possible if there was a slight shift of the sugar residue. The hydrogen bond to the Gly 96 carbonyl is completely absent and the bond to the Thr 154 hydroxyl group is very weak. However a new hydrogen bond does exist between α-*S*-GalCer and Asp 80. This evidence demonstrates that binding is indeed possible between α-*S*-GalCer and CD1d, however, the reason for the lack of NKT cell stimulation is still unclear and further investigation is needed.

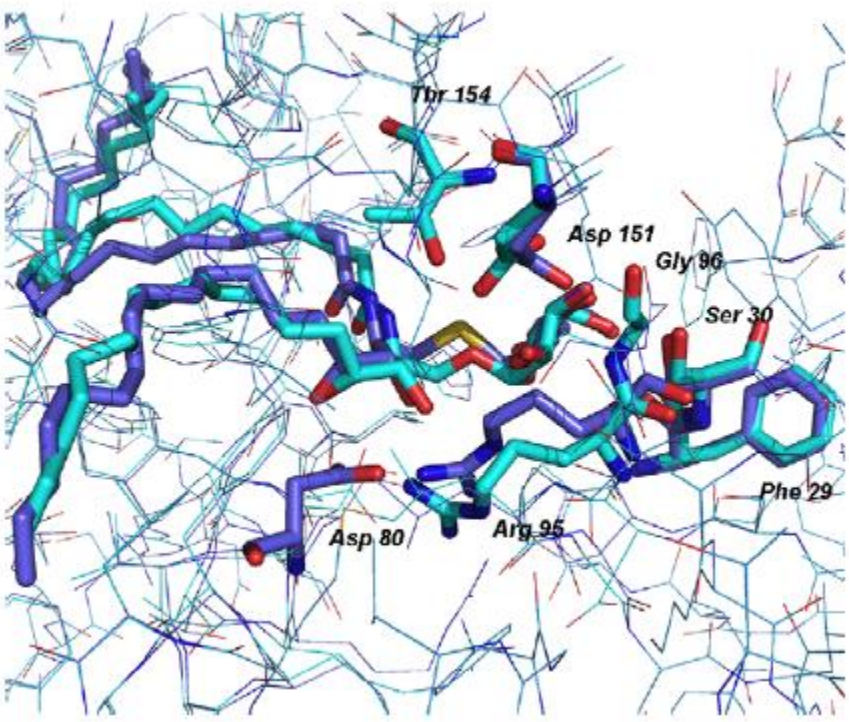


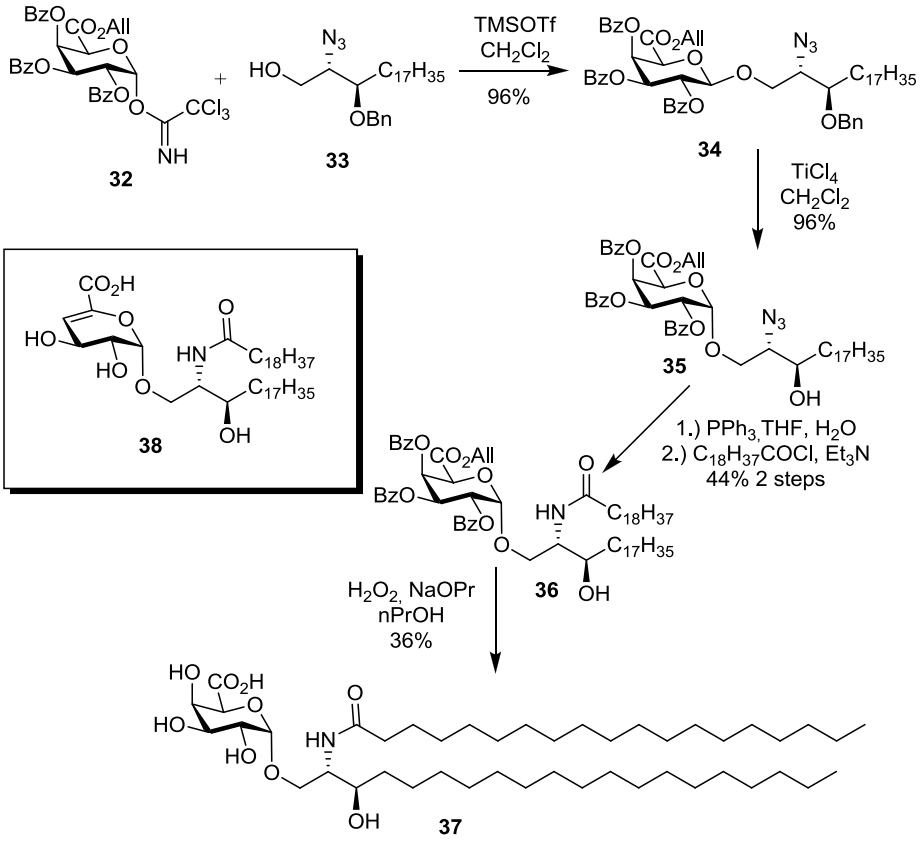
Figure 11 Howells model of α-*S*-GalCer (purple) and KRN7000 (cyan) overlapping and docked into the ternary structure of CD1d-NKT-TCR (reprinted from Ref. 32 with permission from Elsevier, copyright 2008)

1.7.2 Bacterial *S*-linked glycosphingolipid mimics

Since 2005 a number of total syntheses of GSL-1' and PBS-59 have appeared in the literature[34]. Although these compounds are not as active[29,35] as the highly potent α-GalCer, there exists the opportunity for modification of their structures in an effort to improve their immunostimulatory properties. Amide bond formation for instance, (to maintain a hydrogen bonding group in the structure) has yet to be explored and to the best of our knowledge, there is only one report which attempts to modify the anomeric linkage[36].

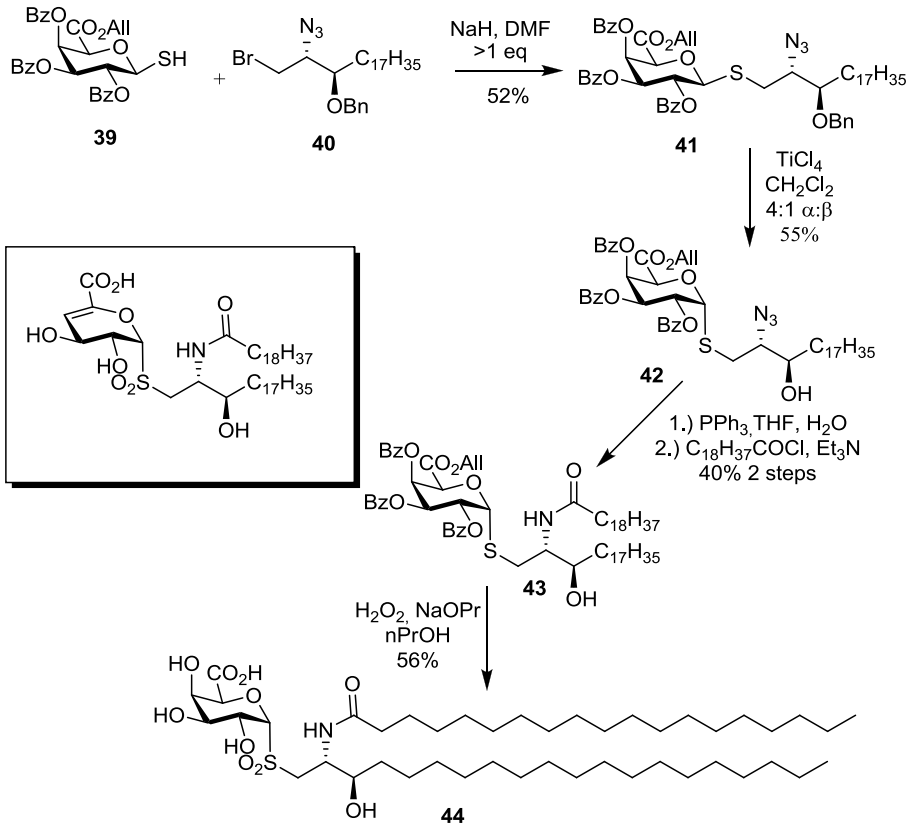
Within the Murphy group, there has been a long standing interest in the stereoselective synthesis of glycuronic acid derivatives. With this in mind, PBS-59 and its analogues presented ideal synthetic targets. Vital to this work was the development of a novel strategy to gain stereoselective control in the synthesis of α-linked glycuronic acid derivatives. It was known within the group that treatment of glycuronic acid derivatives with Lewis acids such as $TiCl_4$ and $SnCl_4$ highly favoured the formation of α-anomers by way of a chelation induced anomerisation reaction. This reaction will be discussed further in the next chapter. Recently, Pilgrim and Murphy have successfully applied this reaction to the synthesis of

PBS-59 and a sulfone analogue[36] (Scheme 5). Glycosidation of the benzoylated galacturonic acid trichloroacetimidate donor **32** and the sphinganine acceptor **33**, synthesised in 14 steps from D-galactal, gave the β-glycolipid derivative **34** in a 96% yield. Chelation induced anomerisation was facilitated through treatment with a catalytic amount of $TiCl_4$ to generate the α-sphingolipid derivative **35** in high α:β ratio and 96% yield. Staudinger reduction of the azide followed by amide formation gave the ceramide derivative **36** in modest yield over 2 steps. Unfortunately, deprotection of these compounds proved challenging. It is well known that under basic conditions, glycuronic acid derivatives (particularly galacturonic acid derivatives) undergo elimination reactions across C4 and C5 to give unsaturated derivatives such as **38** via an E1cB type elimination process. This elimination reaction poses a challenge to any synthetic route involving the use of uronic acid derivatives. All attempts to remove the benzoate protecting groups from **36** under basic conditions led to this elimination process. After the evaluation of a number of deprotection conditions, successful deprotection of **36** was facilitated via treatment with hydrogen peroxide and sodium propoxide. The PBS-59 derivative **37** was isolated in 36% yield.



Scheme 5 Pilgrim and Murphy' synthesis of PBS-59 via chelation induced anomerisation reaction

Also in this report, Murphy and Pilgrim also made an initial attempt to modify the anomeric linkage to give access to an *S*-linked analogue of a bacterial glycosphingolipid (Scheme 6). Chelation induced anomerisation reactions of *S*-glycosides is rare in the literature and this paper provides a rare glimpse at the potential such reactions could have in the stereoselective synthesis of *S*-linked glycosides. The β-thioglycolipid **41** was synthesised via the coupling of bromide **40** with the β-glycosyl thiol derivative **39** in a 55% yield. With compound **41** in hand, the chelation induced anomerisation reaction was attempted using 2 equivalents of $TiCl_4$. Gratifyingly the anomerisation reaction proceeded to give the α-glycolipid derivative **42** in 55% yield with a 4:1 α:β selectivity. Staudinger reduction and amide coupling gave ceramide derivative **43** in 40% yield over 2 steps. However, deprotection of **43** under the conditions set out for compound **36** led to oxidation of the sulfur atom to give the sulfone derivative **44** in 56% yield and all efforts to preclude this oxidation reaction proved unsuccessful.



Scheme 6 First attempt to synthesise *S*-linked bacterial glycosphingolipids via chelation induced anomerisation reaction

1.8 Target glycolipids and objectives

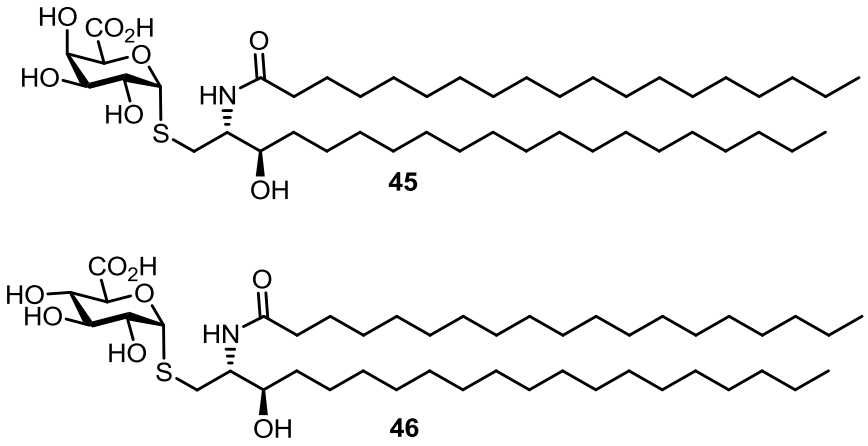


Figure 12 Target glycosphingolipids

Although the sulfone **44** is in itself an interesting and novel glycosphingolipid derivative, the thio-analogue of PBS-59 was still seen as a desirable and very achievable goal. The next chapter of this thesis will focus on attempts made to synthesise the novel *S*-linked PBS-59 derivative **45** and the glucuronic derivative **46**. A second generation route will be provided and deprotection conditions to preclude sulfur oxidation will be explored. It is hoped these glycolipid derivatives may help to further the current understanding of NKT cell stimulation by lipid antigens.

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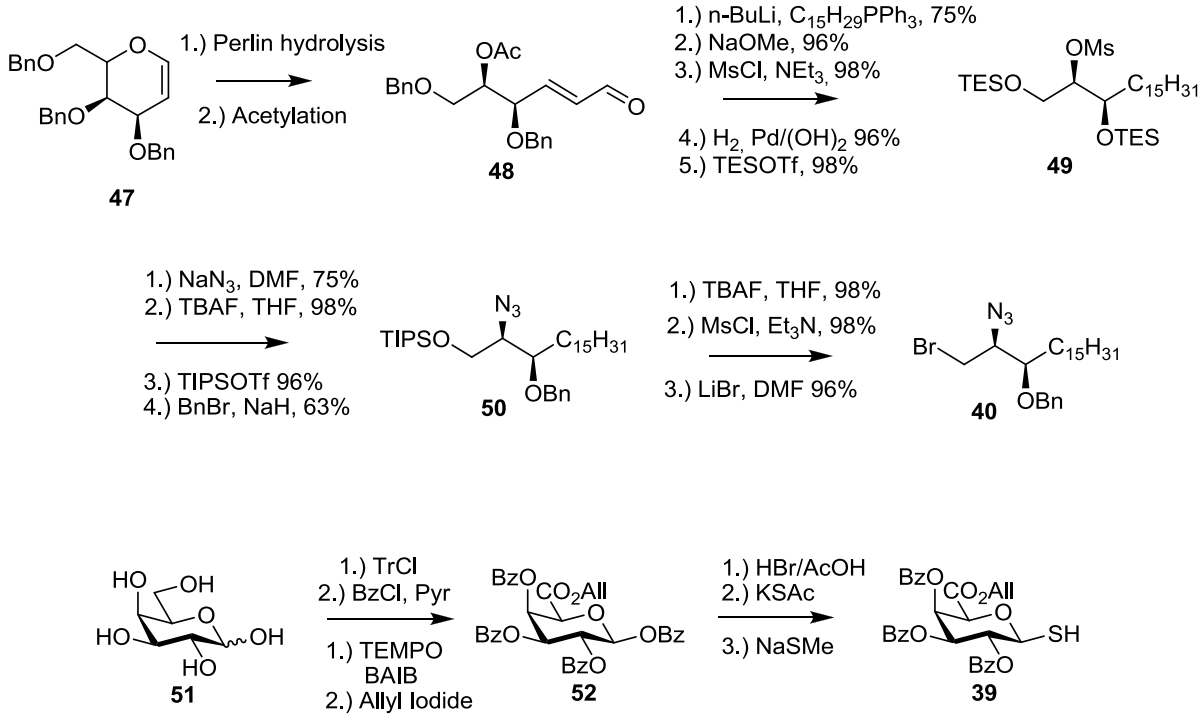
Chapter 2: Synthesis of *S*-linked bacterial glycosphingolipid derivatives

2.1 Retrosynthetic analysis

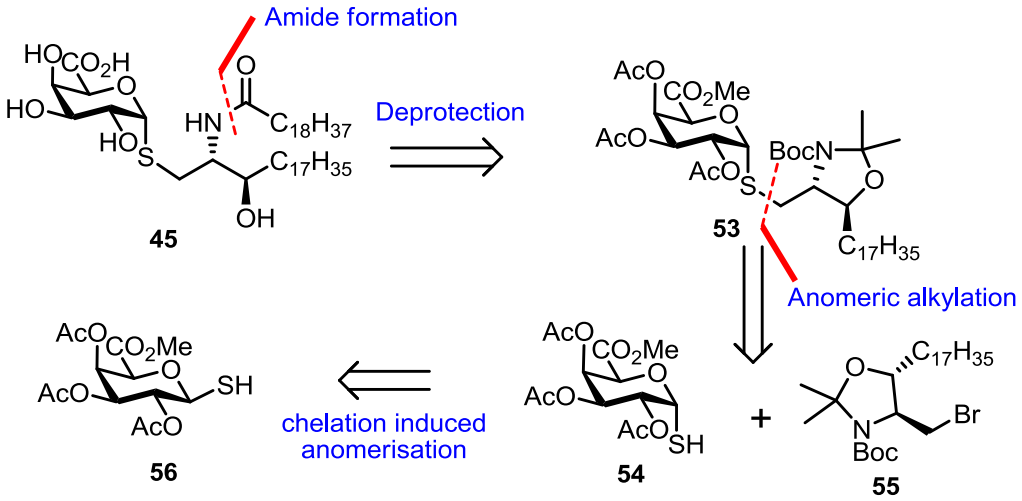
Although the route devised by Pilgrim and Murphy was highly successful in delivering a number of glycosphingolipid derivatives, it did, however, suffer from a number of drawbacks. The route to the sphinganine chain **40** from D-galactal (Scheme 7) was a lengthy process, consisting of 14 steps. Synthesis of the benzoylated glycosyl thiol **39** also proved difficult. A 7 step synthetic sequence involved an unreliable allylation reaction which often led to low yields and poor reproducibility. It was our opinion that a shorter route to these key intermediates was possible which would allow for faster access to the desired thioglycolipid derivatives.



Scheme 7 Previous syntheses of a sphinganine chain and β-glycosyl thiols from the Murphy group

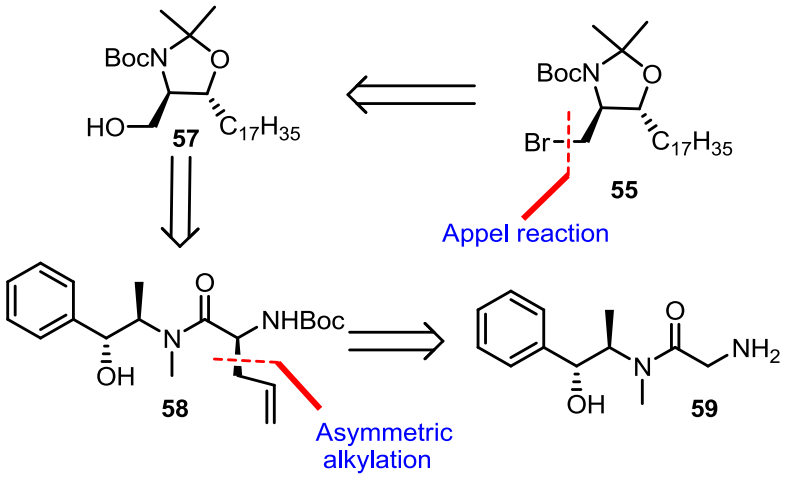
A re-evaluation of the retrosynthetic scheme led us to a number of conclusions (Scheme 8). As the rate of saponification for acetate protecting groups is much greater than that of benzoate protecting groups, it was believed that a change in the protecting group strategy could considerably aid the efforts in finding suitable deprotection conditions for the final compounds. It was also envisaged that the glycolipid derivative **53** could be obtained through an anomeric alkylation of α-glycosyl thiol **54** and the bromo-sphinganine derivative **55**. Amide formation and subsequent deprotection would then give the α-*S*-linked bacterial

glycosphingolipid derivative **45**. It was proposed that α-glycosyl thiols such as **54** could be prepared via a chelation induced anomerisation reaction of the easily obtainable β-glycosyl thiol derivative **56**. This would offer a key intermediate of considerable value, not only in the synthesis of glycolipids but also in the synthesis of other α-*S*-linked glycoconjugates.



Scheme 8 Proposed "2nd generation" retrosynthetic analysis

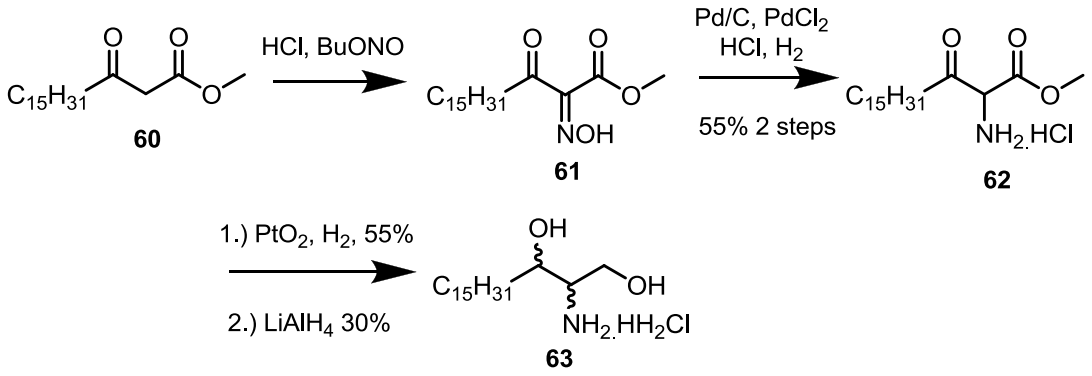
It was proposed that a route to the alkyl bromide **55** starting from the so called 'Myers auxiliary' **59** would also be examined[1]. A number of simple organic transformations would give the sphinganine derivative **57** which could then be treated under Appel conditions to obtain the desired bromide derivative **55**.



Scheme 9 Retrosynthesis of sphinganine derivative

2.2 Previous syntheses of sphinganine chains

The 1950's saw a flurry of interest in the synthesis of sphinganines. Over a period of 5 years, several groups working independently reported the synthesis of sphinganines via similar methods. Gregory and Malkin[2] were the first to show that oxaminating the β-ketoester **60** gave oxime derivative **61**, subsequent hydrogenation of **61** gave amine derivative **62**. A double reduction then gave access to sphinganine **63** (Scheme 10). Both Shapiro[3] and Fischer[4] used a similar reaction sequence to obtain compound **63**. Each of these syntheses gave rise to mixtures of both the threo and erythro isomers and it was not until the work of Grob and Jenny that pure samples of each isomer were obtained[5].



Scheme 10 Gregory and Malkin's synthesis of sphinganines

Since those early pioneering efforts, a number of new approaches have been developed for the stereoselective synthesis of sphinganines. These approaches can be broken down into a number of categories[6]:

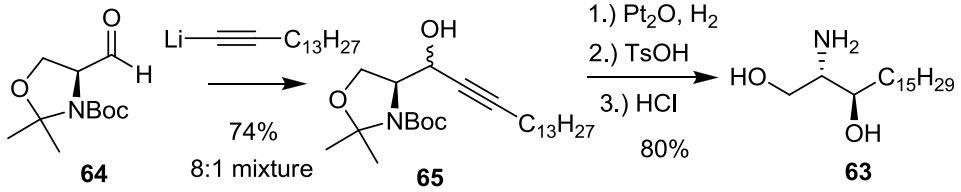
1. Serine derived syntheses
2. Carbohydrate based syntheses
3. Asymmetric synthesis
4. Chiral auxiliary based approaches

Some important examples will be discussed in this section.

2.2.1 Syntheses from L-serine

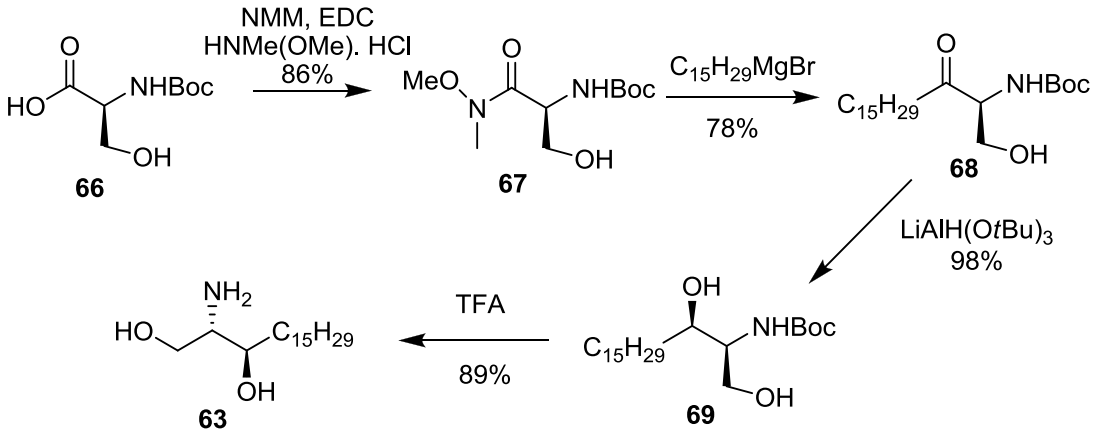
In general, serine is the most widely used starting material for the synthesis of sphinganines. Of the many routes reported with serines, the two most successful are those using the Garner aldehydes and those using serine derived Weinreb amides.

Garner's aldehyde **64** was first reported in the 1980s[7] and is still the most common method to synthesise 1,2 amino alcohols and 2-amino-1,3-diols. In one example D-erythro-sphinganine was synthesised through the addition of Lithium pentadecyne to Garners aldehyde to give **65** as a mixture of diastereomers (8:1). Catalytic hydrogenation followed by deprotection under acidic conditions gave sphinganine **63** in 80% yield[8].



Scheme 11 Synthesis of sphinganines from Garner aldehyde

In 2004 Howell *et al*. reported a highly efficient synthesis of sphinganines from serine derived Weinreb amide **67** in five steps. Displacement of tertiary amide using an alkyl magnesium bromide gave ketone derivative **68** which underwent the stereoselective reduction conditions set out by Hoffman to give alcohol **69**. Finally treatment with TFA gave sphinganine **63** in good yield[9].

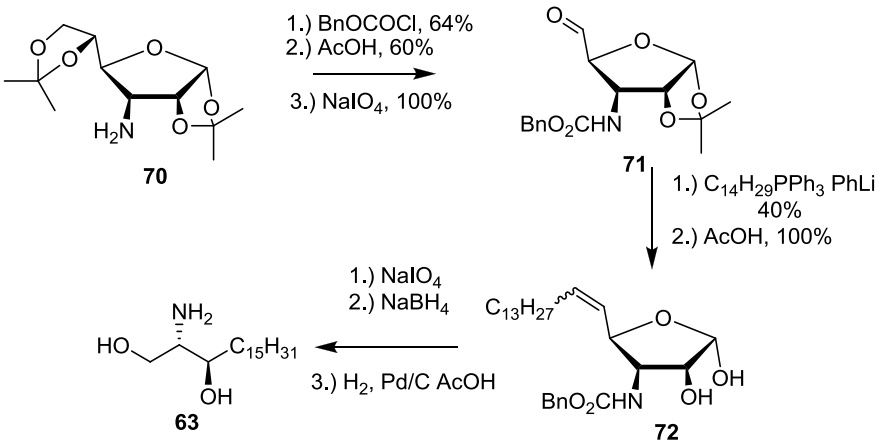


Scheme 12 Synthesis of sphinganines from serine derived Weinreb amides

2.2.2 Synthesis from carbohydrates

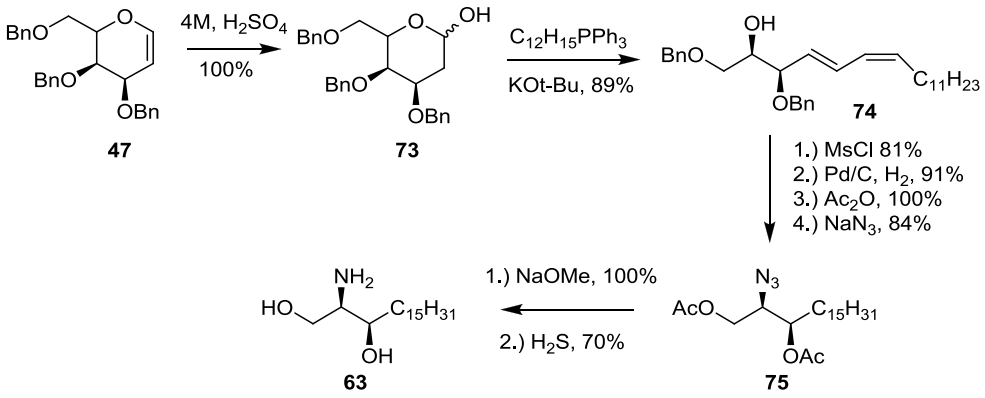
Carbohydrates offer convenient chiral templates for the synthesis of sphingoid bases. The main drawback of this approach is the requirement for the introduction of an amino group onto the molecule. This is usually achieved through the introduction of an azide functionality onto the molecule which can then be reduced at a later stage in the synthesis.

Reist and Christie[10] have reported the use of a protected α-allofuranose for the synthesis of sphinganines. Protection of the amino group of **70** followed by removal of the more labile isopropylidene group gave a diol intermediate which was oxidatively cleaved using $NaIO_4$ to give aldehyde derivative **71**. Wittig reaction followed by deprotection of the remaining isopropylidene gave **72** as a mixture of isomers. Oxidative cleavage, reduction of the subsequent carbonyl, catalytic hydrogenation and deprotection gave sphinganine **63**.



Scheme 13 Reist and Christie's synthesis of sphinganines from α-allofuranose

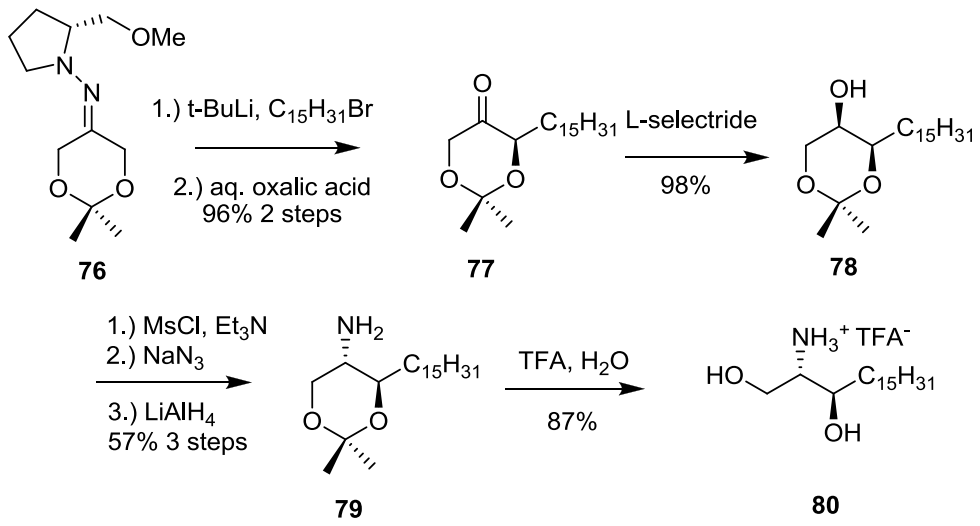
In the early nineties Schmidt reported the synthesis of sphinganines from commercially available perbenzylated D-galactal **47** [11]. Treatment of **47** with sulfuric acid gave hemiacetal derivative **73**. Wittig reaction of **73** gave compound **74** with concomitant loss of the benzyl group at C4 of the galactose residue. Mesylation followed by catalytic hydrogenation, acetate protection and displacement of the azide using NaN_3 gave sphinganine derivative **75** in high yield. Finally, saponification of the acetate groups followed by reduction of the azide gave sphinganine **63**. D-galactal was also used in the synthesis of sphinganines in the Murphy group as shown Scheme 7[12].



Scheme 14 Schmidt's synthesis of sphinganines from D-galactal

2.2.3 Chiral auxiliary based approaches

Chiral auxiliaries have also been employed in the synthesis of sphinganines. One such example shows the utility of the known RAMP/SAMP hydrazones to prepare sphinganines in excellent yields with a high degree of stereochemical control[13]. Alkylation of RAMP hydrazone **76** followed by cleavage of the hydrazone using oxalic acid gave ketone **77** in 96% *ee*. Selective reduction gave syn alcohol **78**. Introduction of the amino group by way of an azide reduction gave anti amine **79**. Finally deprotection gave sphinganine **80** in high yield and high overall enantiomeric excess (*ee*).

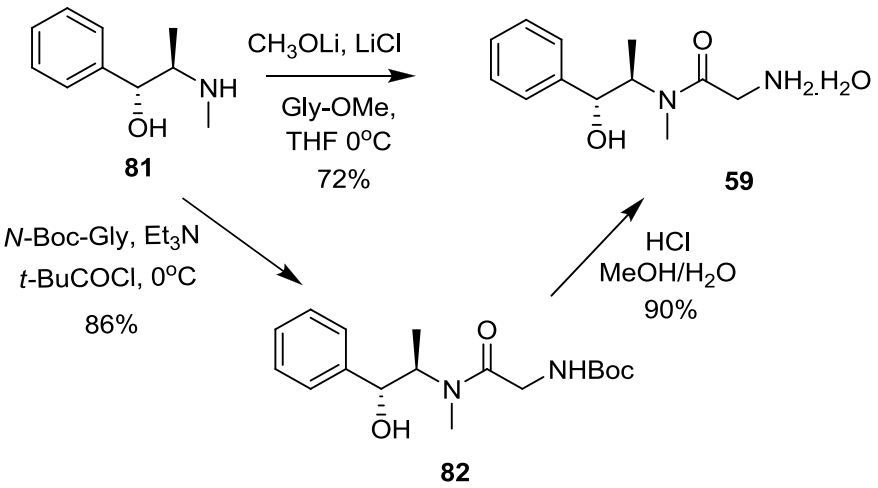


Scheme 15 RAMP/SAMP chiral auxiliary approach to sphinganines

2.3 Novel approach to sphinganines from pseudoephedrine glycinamide

Myers and co-workers have demonstrated the utility of the inexpensive amino alcohol pseudoephedrine (which is readily available in both enantiomeric forms) as a chiral auxiliary in stereoselective α-alkylation reactions of amino acid derivatives[1]. Of particular interest was the highly selective alkylation reactions carried out on pseudoephedrine glycinamide, usually used for the formation of unnatural amino acids. However, these pseudoephedrine auxiliaries can also be readily displaced with alkyl lithium or Grignard reagents, giving access to highly enantioenriched amino ketones[14]. With this in mind, it was decided to exploit these useful properties in the synthesis of sphinganine chains. It was believed this new approach could be

useful in obtaining new sphinganine derivatives in an efficient manner. An important aspect of this proposed route is the potential to modify the stereochemistry of the substrate at almost any step in the process to give access to numerous isomers of erythro-sphinganine for SAR studies.



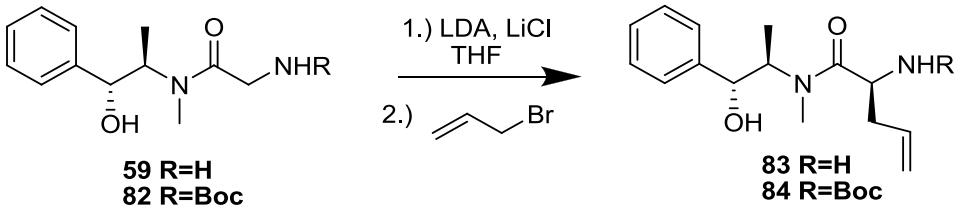
Scheme 16 Synthesis of pseudoephedrine glycinamide monohydrate

To investigate this hypothesis, pseudoephedrine glycinamide **59** was prepared in a one step procedure from glycine methyl ester. Under basic conditions a direct condensation between the amino group of the pseudoephedrine moiety and the carbonyl group of glycine occurs without substantial competition from the unprotected primary amine on glycine to give access to pseudoephedrine glycinamide in 72% yield. This process is believed to occur through an initial transesterification between glycine methyl ester and the secondary hydroxyl group of pseudoephedrine, followed by a rapid *N-O* acyl transfer[1]. The product is highly crystalline and pure **59** can be obtained via recrystallization from THF. Alternatively **59** can also be synthesised in a 2 step process involving the mixed anhydride of *N*-Boc-glycine to give compound **82**. Hydrolysis and recrystallisation then give **59** in high yield (Scheme 16).

The drawback of this particular procedure is the rather substantial drying process of **59** which is vital to the attainment of high yields in subsequent alkylation reactions. The anhydrous compound is also a white solid; however, we have found it to be highly hygroscopic and can be extremely difficult to keep dry leading to incomplete alkylation reactions. Considerable care must also be taken when treating **59** with strong bases such as n-butyllithium during the course of the alkylation reactions as to prevent decomposition of the starting material. A recent paper from the Myers group has reported a way of circumventing

these problems through the use of lithium hexamethyldisilazide (LHMDS) as a base in alkylation reactions with the monohydrate of **59**[15]. Unfortunately, in our hands the alkylation reactions using this reagent were extremely slow and poor isolated yields were obtained.

It has been shown that compound **82** can also be used directly in the alkylation reactions. This compound proved less susceptible to decomposition under the reaction conditions and maintained the same high yields and good diastereomeric ratios (*dr*) (this is in good agreement with the results of Myers work); however, unlike compound **59**, **82** does not have the advantage of being a crystalline solid.

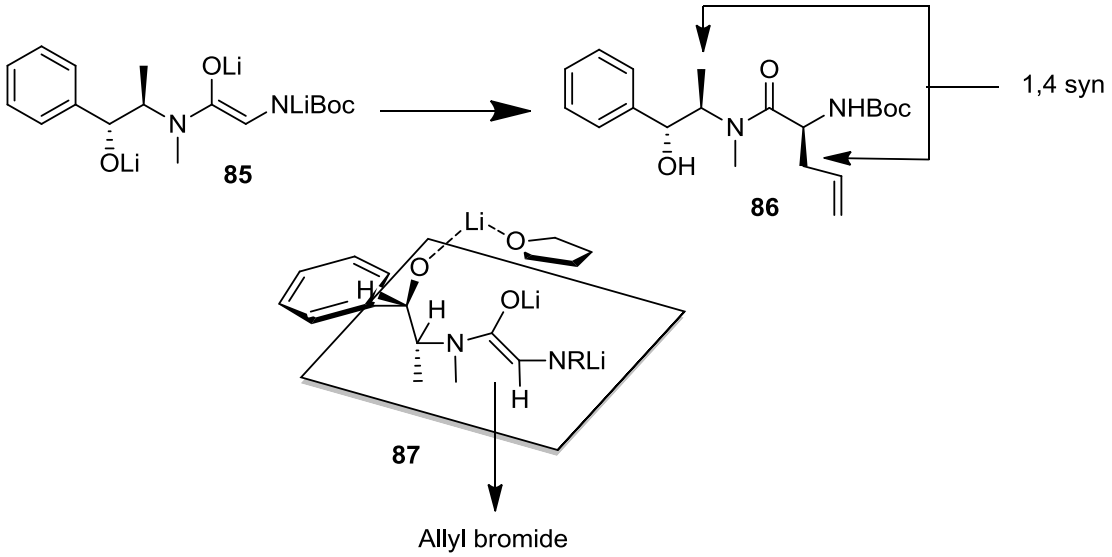


Scheme 17 Synthesis of *N*-Boc pseudoephedrine allyl glycinamide

The asymmetric alkylation of compound **59** was next examined. Initial focus concentrated on choosing a suitable electrophilic partner for use in the alkylation reaction. Using paraformaldehyde as an electrophile gave a serine derivative in 26% yield which could then be protected, for example, as a silyl ether. The low yield of this reaction coupled with the extra protection step involved in the synthesis forced us to consider alternative electrophiles. Alkylation of **59** and **82** was also attempted with (chloromethoxy)triisopropylsilane. It was hoped that this would lead to the introduction of a silylated methylene group which could then undergo a late stage oxidation under Tamao-Fleming conditions to reveal the desired sphinganine chain. Unfortunately, this alkylation reaction failed to yield any of the desired products. The failure of this reaction is probably due to the lack of electrophilic character of such substrates in alkylation reactions coupled with the steric hindrance provided by the bulky isopropyl groups. The use of large excesses of potentially carcinogenic oxymethylchlorides when conducting the reaction on large scales was also considered a major drawback of this proposed route.

It was finally decided that the use of allyl bromide as an electrophile would provide a pseudoephedrine allyl glycinamide derivative such as **83** (Scheme 17), a compound which is well known in the literature. This compound was identified as an ideal starting material for the synthesis of sphinganines. The presence of the allylic alkene would allow for further

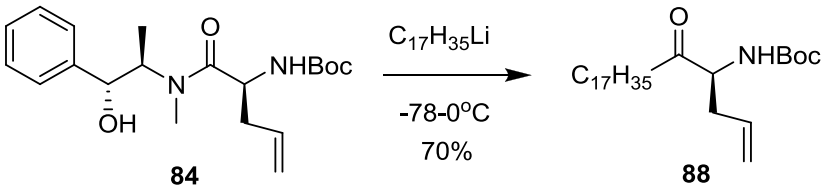
functionalization of the lipid chain in order to obtain natural sphinganines as well as novel sphinganine derivatives. A late stage isomerisation of the allylic bond followed by oxidative cleavage would give access to the desired sphinganine derivative. This route could also provide easier access to *C*-glycolipid derivatives and glycolipid derivatives which contain an extra methylene group in the sphinganine chain. Derivatives of this type could provide valuable SAR information.

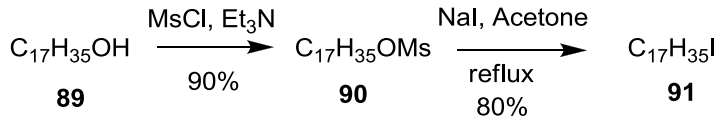


Scheme 18 Proposed reactive conformation of pseudoephedrine amides

Therefore treatment of **82** with LDA following the procedures outlined in the literature[16] gave diastereomerically pure (99:1 *dr*) L-allyglycinamide in 58% yield after recrystallisation. *N*-Boc protection was then achieved through treatment with Boc_2O to give compound **84** in 95% yield. Alternatively, treatment of **83** with 3.2 equivalents of LDA at -78^{o}C followed by addition of 1.2 equivalents of allyl bromide gave *N*-Boc-pseudoephedrine allyl-glycinamide **84** in 70% yield with a 96:4 *dr*. Treatment of either compound with LDA presumably gives rise to a trianion such as **85**. In simple terms, the asymmetric induction is as a result of the electrophile entering on the same side as the methyl group of the auxiliary giving a 1,4 syn addition. The reason for the high diastereoselectivities observed in these reactions is not obvious and still remains open for debate. As pseudoephedrine is a linear auxiliary, it lacks a lot of the characteristics of a 'traditional' chiral auxiliary[17]. One proposed reason for this highly diastereoselective reaction is shown in Scheme 18[1]. Myers has suggested a reactive conformation such as **87**. The secondary lithium alkoxide on the pseudoephedrine, potentially with the aid of some chelating THF molecules, effectively blocks the π-face of the enolate **87**[18]. This model also takes into account the allylic strain present in the molecule as the C-H

bond α to the nitrogen atom lies in plane with the enolate oxygen therefore forcing the pseudoephedrine to adopt a staggered conformation[19].





Scheme 19 Synthesis of enantiomerically enriched amino ketone and the iodoheptadecane intermediate

With compound **84** in hand, attention was focused on displacement of the auxiliary to give access to the ketone derivative **88**. It is well known that treatment of tertiary carboxamides with organometallic nucleophiles gives rise to a stable tetrahedral intermediate[20]. This intermediate breaks down upon aqueous work-up to give ketone products. This tetrahedral intermediate is key to the success of this reaction, premature breakdown of this intermediate can allow the ketone to react further giving rise to tertiary alcohol by-products. The conversion of *N*-Boc pseudoephedrine derivatives into ketones is well documented in the literature, however, the use of longer chain organometallic reagents is as yet untested[14]. As a 17 carbon chain organolithium reagent was required to test this reaction, heptadecanol **89** was treated with mesyl chloride in CH_2Cl_2 to give the mesylate derivative **90**. Subsequent Finkelstein type reaction using sodium iodide in refluxing acetone gave 1-iodoheptadecane **91** in 80% yield over 2 steps. The organolithium reagent was then generated according to the procedure of Bailey[21], treatment of iodoheptadecane with 2.2 equivalents of *t*-BuLi in Et_2O-pentane at -78°C gave a 0.5 M solution of lithiated heptadecane. Pseudoephedrine derivative **84** was treated with 3.1 equivalents of 0.5M lithiated heptadecane at -78°C. The reaction was warmed to room temperature for 2 hours and quenched with aqueous NH_4Cl. The product ketone was obtained in 70% yield after flash chromatography. In general we have found that organolithium reagents are far superior to Grignard reagents for these reactions. Treatment of **84** with the Grignard reagent derived from **91** gave the ketone in 45% yield and required a much longer reaction time.

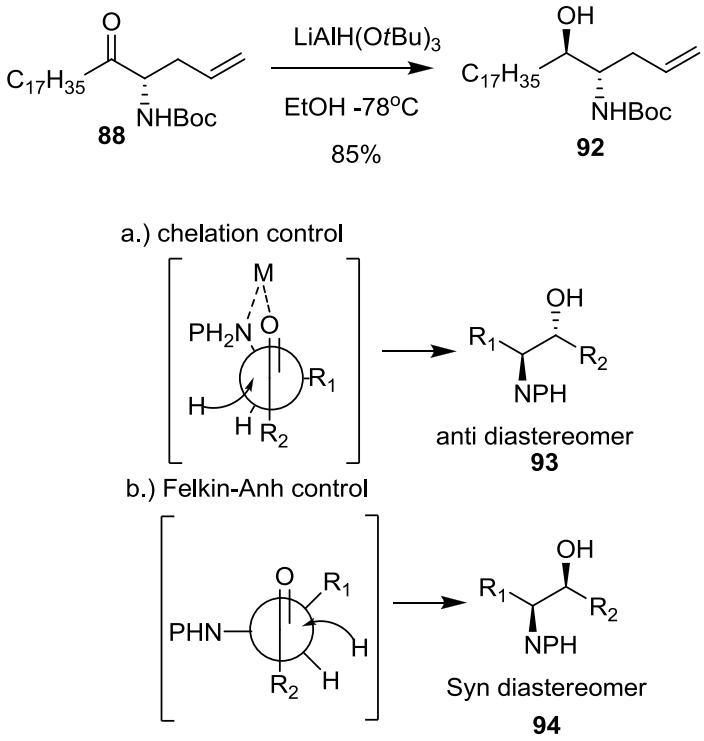


Figure 13 Stereoselective reduction to give access to anti-amino alcohols

Hoffman has shown that 1,2 anti amino alcohols can be synthesised from 1,2 amino ketones via a highly stereoselective reduction with $LiAlH(OtBu)_3$ in EtOH at -78°C[22]. This method has been used successfully in a number of previous sphinganine syntheses [6,23]. In the stereoselective reduction of any 1,2 protected amino ketone; the stereochemical outcome of the reaction is under control of two different modes (Figure 13). In the case of carbamate protected amino ketones, chelation control enforces a syn-periplanar relationship between the carbonyl and the amino group due to the presence of a Lewis acid or counterion. This chelation effect gives rise to anti-selectivity in the reduction e.g. **93**. Felkin-Anh control can be gained by placing steric bulk onto the nitrogen through the use of trityl or *N*,*N* benzyl protecting groups. This orientates the amino group perpendicular to the carbonyl in order to minimize repulsions in the transition state. This effect gives rise to syn-amino alcohols upon reduction e.g. **94**.

Hoffman has observed that carbamate protected amino alcohols undergo highly selective reductions under chelation control when treated with $LiAlH(O\text{-}t\text{-}Bu)_3$ in a protic solvent such as ethanol at low temperatures. With regards to the mechanism of such a selective reduction, it is believed that the lithium counter-ion does not play a role in the stereochemical outcome

of the reaction and that chelation occurs between the amino ketone and the aluminium of the reducing agent through various exchange-disproportionation reactions in ethanol (Figure 14). As aluminium metals form weak complexes with neutral species and much stronger complexes with anionic species it has been postulated that small amounts of ethoxide generated during the course of the reaction deprotonate the carbamate nitrogen and facilitate chelation through ligand exchange to aluminium.

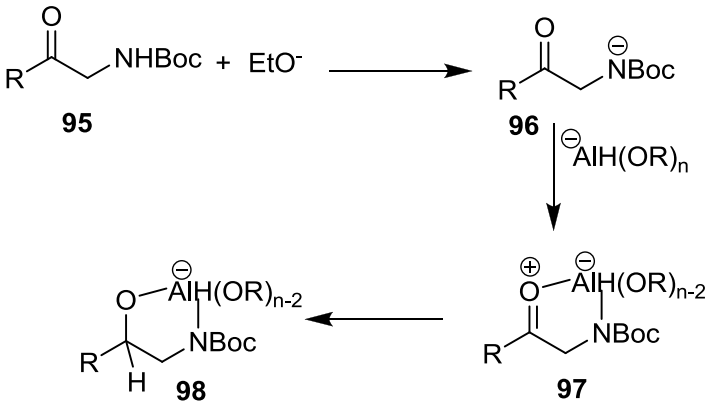
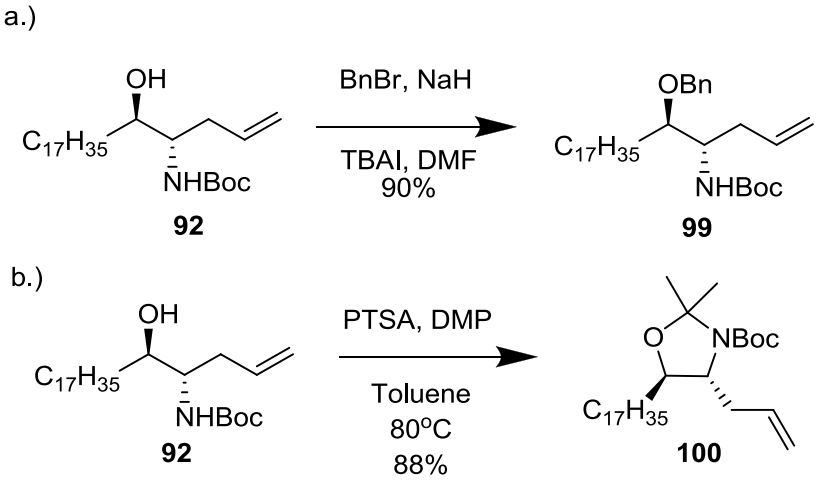


Figure 14 Proposed mechanism for reduction reaction

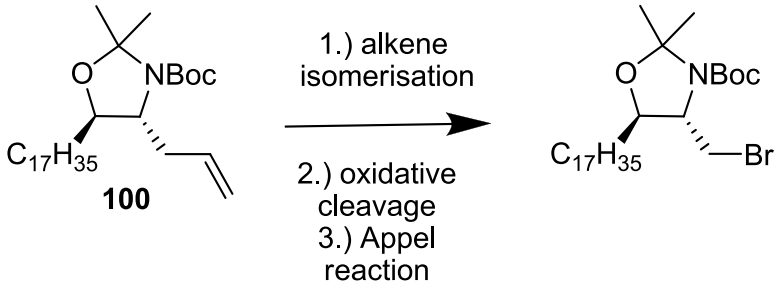
As expected, this reaction proved extremely reliable giving the anti amino alcohol product **92** in 85% yield. There were some slight problems with the solubility of the ketone in EtOH at such low temperatures; however, the reaction proceeded to completion when left to stir for 24 hours.



Scheme 20 Protection of 1,2-amino alcohol

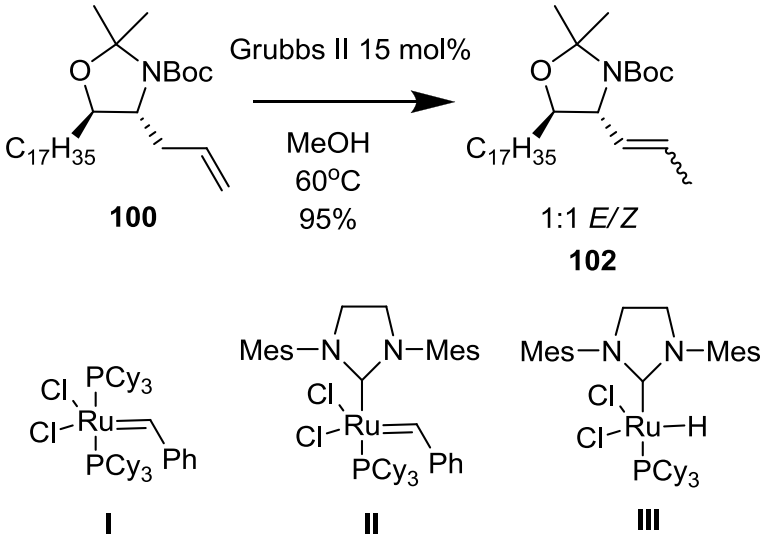
The amino alcohol derivative **92** was then protected, initially, as a benzyl ether via treatment with benzyl bromide and NaH to give the benzylated derivative **99** in 90% yield. It was

anticipated that this protecting group strategy may lead to problems at a later stage in the synthesis as the sulfur atom present in the final glycolipid derivatives would prevent the use of catalytic hydrogenation conditions for debenzylation due to poisoning of the palladium catalyst. As an alternative approach, the protection of the secondary hydroxyl group as an *N*,*O*-acetal by treatment of **92** with dimethoxypropane and catalytic pyridinium *p*-toluenesulfonate in refluxing toluene gave the acetal derivative **100** in 88% yield[24].



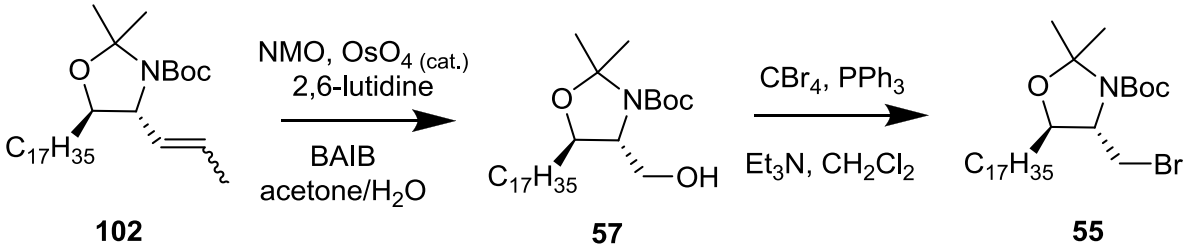
Scheme 21 Synthesis of bromosphinganines for use in alkylation reactions

In order to synthesise the desired sphinganine derivative **55**, it was first necessary to isomerise the allylic double bond of **100** to give the propenyl derivative **102**. This propenyl derivative could then be subjected to oxidative cleavage followed by an Appel reaction to give the desired bromo-sphinganine derivative **55**. Compound **100** could also be useful in the synthesis of glycolipid derivatives possessing an extra methylene group in the sphinganine moiety for SAR studies.



Scheme 22 Thermally modified Grubbs II catalysed isomerisation reaction

Hanessian has recently reported an efficient method for the isomerisation of allylic double bonds to propenyl derivatives via treatment with a thermally modified Grubbs II catalyst[25]. Grubbs catalysts (Scheme 22 **I** and **II**) have revolutionised strategic planning in organic synthesis due to their success in ring closing metathesis reactions (RCM). It has been reported however, that some substrates undergo unwanted isomerisation side reactions during the RCM process. Hanessian's group saw the potential to develop this unwanted side reaction into a useful and mild method for the isomerisation of terminal double bonds. In our experience, the reaction proceeds very smoothly using 15 mol% Grubbs II catalyst (the paper reports the reaction using 10 mol% however we have found that in many cases the reaction does not proceed to completion) in MeOH at 60°C. The reaction is believed to proceed via a ruthenium-hydrido species (Scheme 22 **III**) (also reported by Grubbs et al.) however, due to the complex nature of these ruthenium species an exact mechanism or catalytic cycle has yet to be elucidated. Compound **100** was therefore treated with commercially available Grubbs II at 60°C overnight. Upon purification the propenyl derivative **102** was obtained in 71% yield as a 1:1 *E*/*Z* mixture of isomers with no detection of dimerization products arising from potential cross coupling reactions. NMR spectra of these compounds were further complicated due to the presence of rotational isomer due to the *N*-Boc group. Interestingly when the isomerisation reaction was performed using freshly prepared Grubbs II catalyst (prepared according to the procedure of Scholl et. al[26]), the yield of the reaction increased to 95%. Propenyl derivative **102** could be viewed a valuable intermediate in the synthesis of *C*-linked-glycolipids. To the best of our knowledge this is the shortest route to obtain sphinganine derivatives of this type. This compound could therefore prove a valuable intermediate which could be used in cross metathesis reactions to give access *C*-glycolipids.



102 **57** **55**

Scheme 23 Oxidative cleavage and Appel reaction

With propenyl derivative **102** in hand, oxidative cleavage of the double bond was attempted in order to obtain the protected sphinganine derivative **57**. It was envisaged that ozonolysis followed by a reductive work-up would give access to compound **57**; however, the ozonolysis

reaction of **102** was extremely unreliable and failed on a number of occasions. In the rare occasions when the reaction did proceed to completion, a complex mixture of products was obtained along with substantial decomposition of the starting material. Another common method of carrying out oxidative cleavage reactions is the use of $NaIO_4$ and catalytic OsO_4, the so called Lemieux-Johnson oxidation[27]. It has been reported that in many cases these reactions are often low yielding and give rise to substantial by-products such as α-hydroxy ketones. Recently, Yu has reported a modified Lemieux-Johnson in which a stoichiometric amount of 2,6-lutidine was added to the reaction mixture[28]. This led to a dramatic improvement in the yield of the reactions due to the suppression of by-product formation. To this end, **102** was treated under Yu's modified conditions. The reaction went to completion after four days and the crude aldehyde was treated with $NaBH_4$. Purification gave compound **55** in a disappointing 30% yield. Attempts were made to modify this procedure with the hope of improving the reaction yield. The reaction time was changed and the equivalents of the reagents used were adjusted with no discernable effect on the outcome of the reaction. An attempted Upjohn dihydroxylation (NMO, OsO_4), followed by treatment with $NaIO_4$ also gave the product alcohol in low yield. Nicolaou has recently reported the use of hypervalent iodine compounds in oxidative cleavage reactions[29]. BAIB is an excellent reagent for the cleavage of 1,2-diols and when combined with OsO_4 becomes a very useful tool in the oxidative cleavage of double bonds. Unlike Lemieux-Johnson type reactions, this reaction is completely homogenous and it was believed that this homogeneity could be key to increasing the yield of the oxidative cleavage of **102**. Therefore compound **102** was treated with NMO, catalytic OsO_4 and 2,6-lutidine in acetone-water and stirred for 3 days. BAIB was added and the reaction mixture was stirred for a further 3 hours. Reduction of the crude product with $NaBH_4$ and purification gave the desired primary alcohol in 54% yield over 2 steps.

Conversion of the primary alcohol **57** into the bromide derivative **55** was then carried out under standard Appel conditions to give the bromide derivative in high yields[30].

2.4 Synthesis of α-glycosyl thiols

Novel methods for the stereoselective synthesis of α-glycosyl thiols have garnered considerable interest in recent years. The inherent stability of the thioglycoside bond against the action of carbohydrate processing enzymes has led to widespread interest in the synthesis of *S*-linked glycomimetics. Glycosyl thiols, unlike sugar hemiacetals, do not undergo the same mutarotation processes under basic conditions, meaning that their stereochemistry can

be maintained during the course of anomeric alkylation reactions. Although many methods exist to synthesise β-glycosyl thiols, there are currently very few reports on the synthesis of α-glycosyl thiols. In general, β-glycosyl thiols are synthesised through S_N2 displacement reactions of glycosyl halides with either thiourea or sodium thioacetate, hydrolysis of the former or selective deacetylation of the latter gives β-glycosyl thiols in high yields.

The method mentioned in chapter 1 by Zhu shows a rare example of direct synthesis of α-thiols. β-glycosyl chlorides have also been used in multistep procedures however it has been reported that the reproducibility of this method is low[31]. Recently Davis reported the use of Lawesson reagent to produce anomeric mixtures of glycosyl thiols from sugar hemiacetals[32].

It was our belief that chelation induced anomerisation reactions could be used to control the stereoselectivity of glycosyl thiols as the thermodynamically favoured α-anomer would be formed in preference to the β-anomer. The work reported by Murphy and Pilgrim[33] recently shows a rare example of anomerisation reactions of *S*-glycosides. Aside from this report, very few instances of *S*-glycoside anomerisation have appeared in the literature and there are no accounts of the use of anomerisation reactions in the stereoselective synthesis of glycosyl thiols. From previous studies on the rates of Lewis acid catalysed anomerisation reactions, it was well known that:

1.) Anomerisation of *S*-glycosides is generally faster than that of *O*-glycosides
2.) The rates generally follow the trend galacturonide > glucuronide > galactoside > glucoside
3.) Anomerisation reactions are faster with $TiCl_4$

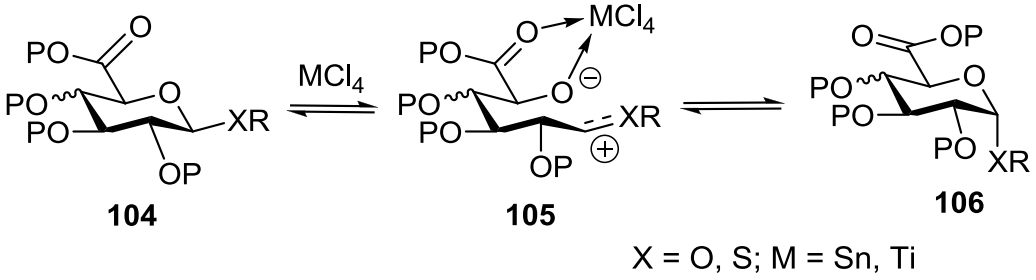
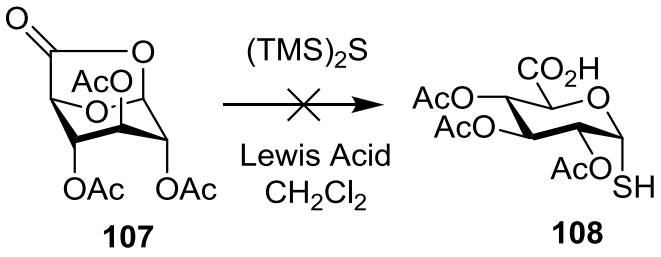


Figure 15 Proposed mechanism for anomerisation reaction which involves an endocyclic cleavage followed by equilibration to thermodynamically favoured α-anomer.

It has been suggested that these anomerisation reactions proceed via chelation induced endocyclic cleavage between C1 and the pyranose oxygen (**105**) which then allows for

equilibration to the thermodynamically favoured α-anomer **106**. This chelation comes as a result of an interaction between the pyranose oxygen and the substituent on C6. In general chelation is much faster for galacturonides and glucuronides due to the presence of a carbonyl group at C6. The high α:β ratios for compounds of this type is due to an increased anomeric effect.



107 **108**

Scheme 24 Glycosidation of lactone **107** with hexamethyldisilathiane

It was anticipated that the glycosidation of 1,6-lactone **107** with hexamethyldisilathiane in the presence of a Lewis acid would lead to the formation of β-glycosyl thiols which would be followed by anomerisation to the desired α-glycosyl thiols. The reaction of this particular lactone with silylated nucleophiles is well known from our own group and typically gives a highly stereoselective reaction favouring the α-anomer[34]. To this end, the 1,6-lactone **107** was synthesised as previously described[35] and treated with hexamethyldisilathiane and $SnCl_4$. The reaction was left stirring overnight, however both TLC and mass spectrum analysis showed no product formation. With this disappointing result, a range of Lewis acids were screened with the hope of obtaining the desired glycosyl thiol **108**. The results of these experiments are summarised in Table 1. Unfortunately, all efforts ultimately resulted in failure of the *S*-glycosidation, even the addition of small amounts of TBAF to try to generate a thiolate anion and heating the reaction at reflux proved ineffective.

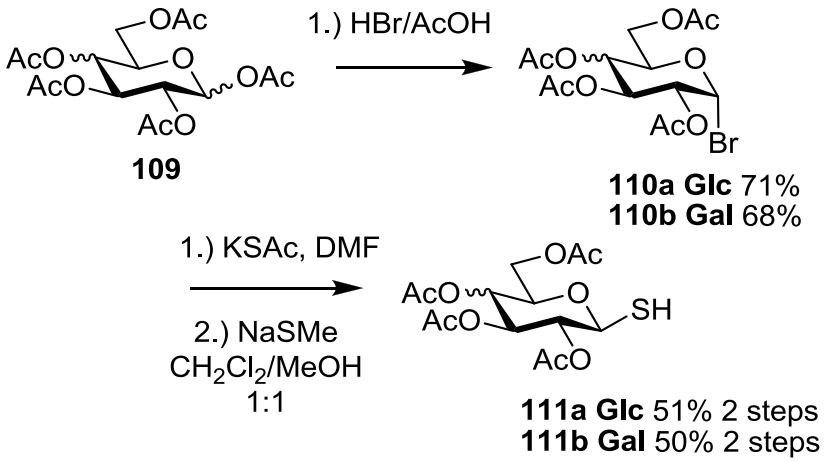
	Lewis acid equivalents	$(TMS)_2S$ equivalents	Additive	Yield	Temp (°C)
$SnCl_4$	0.5	5	-	S.M	RT
$SnCl_4$	3.5	5	TBAF (0.5eq)	S.M	RT
$TiCl_4$	0.5	5	-	S.M	RT
$TiCl_4$	0.5	5	TBAF (0.5eq)	S.M	RT

$BF_3.Et_2O$	3.5	5	-	S.M	RT
$Sc(OTf)_2$	3.5	5	-	S.M	Reflux
$Yb(OTf)_2$	3.5	5	-	S.M	RT
TMSOTf	3.5	5	-	S.M	RT
TMSOTf	3.5	5	-	S.M	Reflux

Table 1 Summary of the Lewis acid catalysed glycosidation conditions which were used in the attempts to facilitate the formation of **108**

Although the direct synthesis of thiols from 1,6 glucuronolactone would be a useful synthetic protocol to develop, difficulties were encountered when attempting to synthesise the corresponding 1,6-galacturonolactone. Only one synthesis of this compound has appeared in the literature through an indirect 5 step route[36] and the conditions reported within our own group for the synthesis of the 1,6-glucuronolactone failed to yield the desired galacturonolactone derivative.

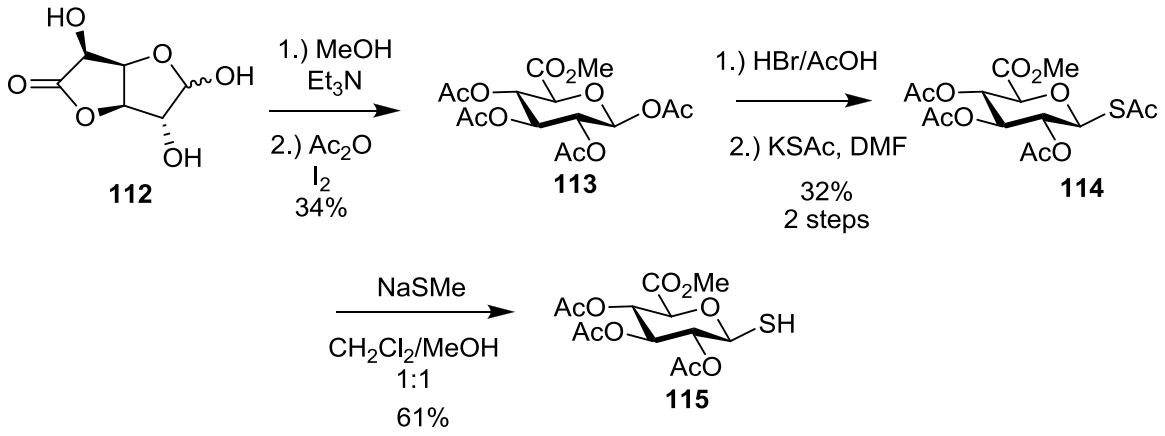
Due to the problems encountered with both the formation of the galacturonolactone and the failure of the *S*-glycosidation reaction, an alternative approach to glycosyl thiols was devised. It was decided to focus on the synthesis of pure β-glycosyl thiols which we could then attempt to anomerise, under Lewis acid catalysed conditions, to the desired α-glycosyl thiol derivatives.



Scheme 25 Synthesis of β-glycosyl thiols

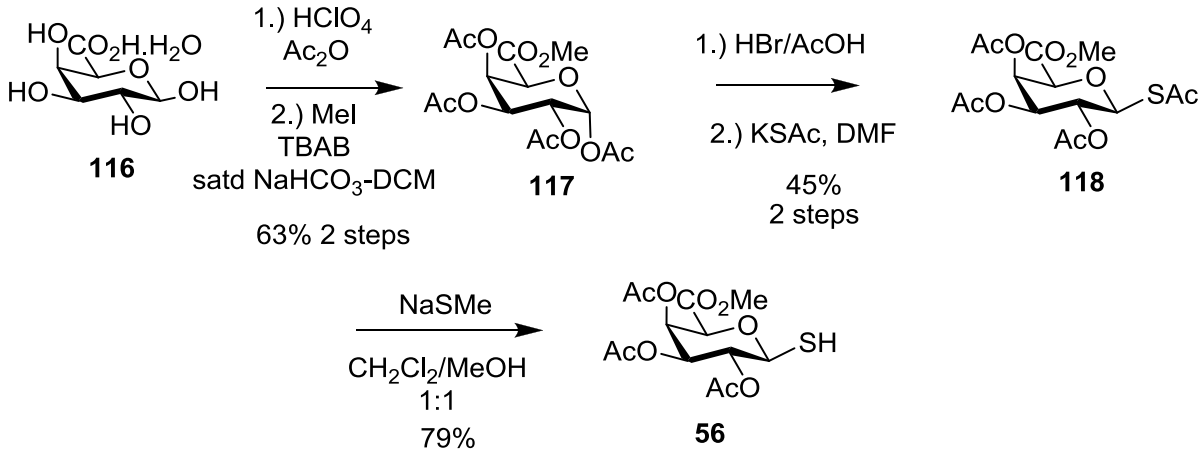
The peracetylated glucosyl and galactosyl thiols were prepared in good yields from commercially available peracetylated glucose and galactose. Treatment of the peracetylated

glycosides with hydrogen bromide in acetic acid gave glycosyl bromides **110a** and **110b**. S_N2 displacement of the bromide with potassium thioacetate gave the fully protected β-thioglycoside derivatives. Selective *S*-deacetylation via thiolysis with sodium thiomethoxide gave the β-glycosyl thiols **111a** and **111b** in good yield[37].



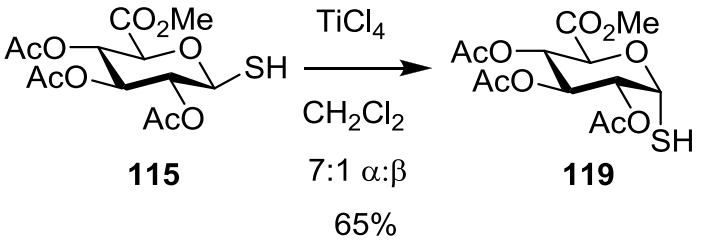
Scheme 26 Synthesis of 2,3,4-Tri-*O*-acetyl-β-thio-D-glucopyranosiduronic acid, methyl ester

The synthesis of the β-glucuronic acid thiol derivatives started from commercially available D-glucurono-6,3-lactone **112**. Opening of lactone **112** using Et_3N in methanol, followed by iodine catalysed acetylation gave **113** in 34% yield. The α-bromide of **113** was generated through treatment with hydrobromic acid in acetic acid followed by recrystallization from ethanol. Displacement of the bromine with potassium thioacetate and selective *S*-deacetylation as previously described gave the pure β-glucuronic thiol derivative **115** as a single anomer[37].



Scheme 27 Synthesis of 2,3,4-Tri-*O*-acetyl-β-thio-D-galactopyranosiduronic acid, methyl ester

The galacturonosyl thiol **56** was synthesised in a similar sequence to the above. D-galacturonic acid monohydrate **116** was acetylated with Ac_2O and catalytic perchloric acid (other acid catalysts were tried but gave the desired galacturonic acid derivative in poor yields) to give 1,2,3,4-tetra-*O*-acetyl-galacturonic acid[38]. Esterification with methyl iodide under phase transfer conditions gave the fully protected galacturonic acid methyl ester **117** in high yields[39]. Compound **117** was subjected to an identical reaction sequence as outlined above to give the β-galacturonosyl thiol **56** in good yield after purification by flash chromatography.



Scheme 28 Lewis acid catalysed anomerisation of **115**

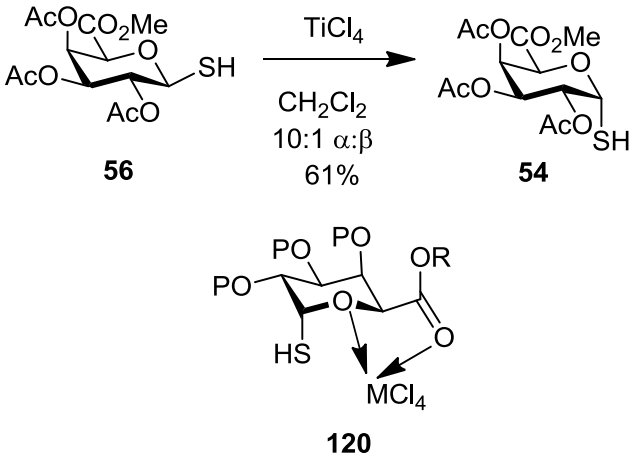
With the four β-glycosyl thiols in hand, attention was turned to the Lewis acid catalysed anomerisation reactions. As an initial experiment, compound **115** was treated with 0.5 equivalents of $TiCl_4$ in anhydrous CH_2Cl_2 at room temperature for 20 hours. Gratifyingly a 1:1 mixture of anomers was obtained. Evidence had suggested that increasing the number of equivalents of $TiCl_4$ and lowering the reaction temperature could give a higher α:β ratio.

Therefore a number of experiments were conducted at 0°C while varying the amount of $TiCl_4$. The results of these experiments are given in table 2.

eq.$TiCl_4$	Time (h)	Temp. (°C)	Ratio (α:β)
0.5	24	0	60:40
1.5	24	0	80:20
2.5	24	0	88:11
3.5	24	0	83:17
4.5	24	0	78:22

Table 2 Effects of Lewis acid concentration on anomerisation

The results in the table indicate that the anomerisation reaction reached its peak ratio after about 2.5 equivalents of $TiCl_4$ after which the α:β ratio begins to decline. Cooling the reaction further to -20°C did not have a further influence on the ratio obtained. The results obtained here are in very good agreement with previous observations in the group[33].

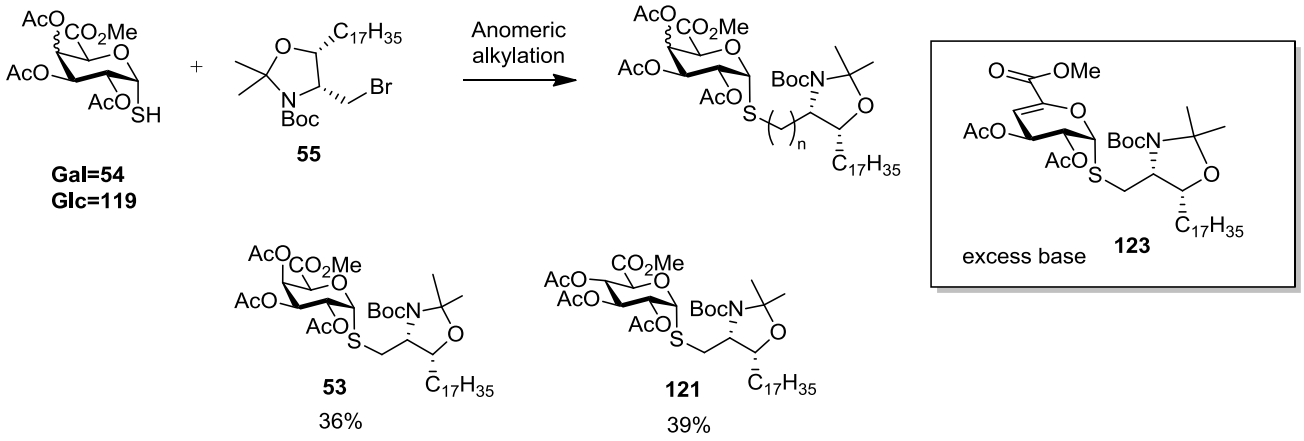


Scheme 29 Application of anomerisation conditions in the synthesis of **54**

The optimised conditions were then applied to the galacturonic thiol. As expected under these conditions the galacturonic acid thiol **56** gave a higher α:β ratio than the glucuronic thiols. on 50-100 mg scale the ratio was determined to be > 95% α-anomer, however when the reaction was scaled up to 0.5-1 gram scale, the ratio was slightly lower giving 9:1 ratio α:β.

Interestingly the anomerisation reaction with both peracetylated galactose thiol **111b** and peracetylated glucose thiol **111a** failed to give any of the desired α-thiols indicating that the presence of the carbonyl at C6 was pivotal to the success of these anomerisation reactions, perhaps giving rise to an intermediate such as **120**.

2.5 Coupling reactions and endgame



Scheme 30 Anomeric alkylation reactions

With the bromide **55** in hand, the direct anomeric alkylation was attempted with thiols **54** and **119** in the hope of obtaining the 2 glycolipid derivatives **53** and **121**. Deprotonation of the thiols with less than 1 equivalent of NaH gave the glycolipid derivatives in 35-40% yield (The use of more than 1 equivalent in model reactions led to unsaturation across the C4-C5 bond via elimination of acetic acid to give compounds such as **123**)[37]. Addition of additives such as tetra butyl ammonium iodide (TBAI) did not increase the yield of the reaction. In an effort to improve the yield a number of other coupling conditions were attempted. The use of K_2CO_3 and $NaHCO_3$ as a base led to lower yields of the desired glycolipid derivatives. Interestingly in one model reaction, treatment of the β-glucosyl thiol and alkyl halide in the presence of $CsCO_3$ and TBAI[40] brought about an alkylation reaction followed by an anomerisation reaction which gave the coupled product in 4:1 α:β mixture.

Toth and co-workers have recently reported a modified Mitsunobu type reaction to couple glycosyl thiols and alcohols[41]. The proposed mechanism for this transformation is shown in Figure 16. Initially, an ADDP-PMe_3 salt (**124)** is formed (indicated by a colour change from yellow to clear). Reaction of alcohol **125** with the ADDP-PMe_3 salt **124** gives rise to an

oxyphosphonium ion such as **126.** Nucleophilic displacement with glycosyl thiol **54** then gives the final coupled product with release of trimethylphosphine as a by-product.

Therefore, the alcohol **57** and the thiols **54** and **119** were added to stirring solutions of PMe_3 and 1,1'-(azidodicarbonyl)dipiperidine (ADDP) in THF. The product glycolipids **53** and **121** were obtained in modest yields after chromatography, although this reaction gave the product lipid derivatives in a similar low yield to the direct anomeric alkylation reactions, it reduced the synthetic Scheme by 1 step.

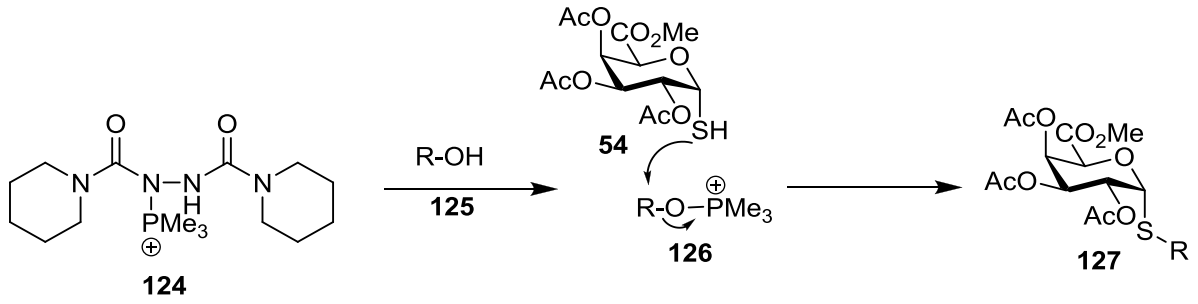
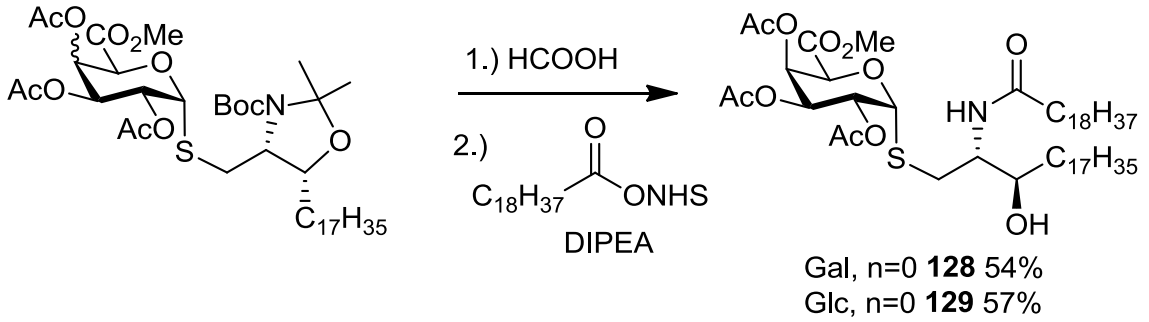


Figure 16 Toth's proposed mechanism for the modified Mitsunobu reaction

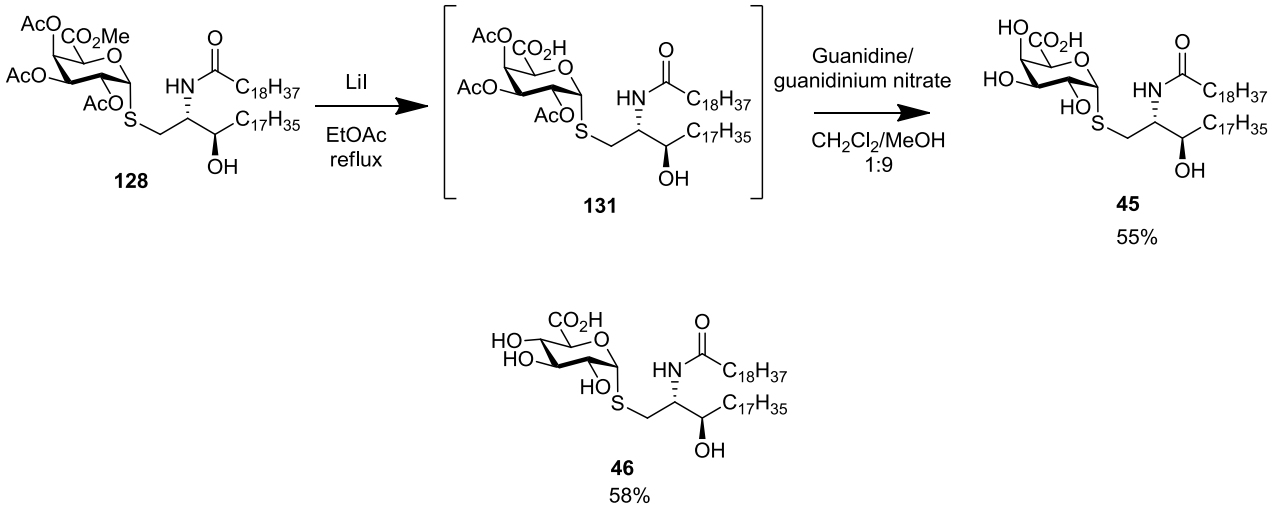
Treatment of the glycolipid derivatives with Formic acid removed the Boc and isopropylidene groups concomitantly. Acylation of the crude amine with the *N*-hydroxysuccinimide ester of nonadecanoic acid in the presence of DIPEA[42] gave the fully protected glycolipid derivatives **128** and **129** in good yields.



Scheme 31 Removal of Boc and isopropylidene groups and ceramide formation

In an attempt to find suitable deprotection conditions a number of small scale reactions were carried out on compound **128** and monitored by mass spectrum analysis. As was the case with Dr. Pilgrims's synthesis, all attempts to directly deprotect the glycolipid derivative under basic conditions led to unsaturation across the C4-C5 bond via elimination of acetic acid to

give unsaturated compounds such as **123**. It was then proposed that a 2 step deprotection sequence be evaluated to deprotect compound **128**. The basis for this proposal was due to a recent report by Mayato *et al*.,[43] in which they reported the selective deprotection of methyl esters using lithium iodide in refluxing EtOAc. It was our belief that, under the basic conditions required for the removal of acetate protecting groups, the presence of a carboxylate anion may suppress the unwanted E1Cb side reaction. Therefore, treatment of glycolipid derivative **128** with 5 equivalents of lithium iodide in refluxing EtOAc gave the free carboxylic acid derivative **131**. Removal of the acetates was then achieved under mildly basic conditions (~pH 8) via treatment with a solution of guanidine-guanidinium nitrate as described by Ellervik and co-workers[44]. Gratifyingly the fully deprotected bacterial glycolipid **45** was obtained in 55% yield over the two steps. The same deprotection sequence was then applied to compound **129** to give the fully deprotected novel bacterial glycolipid derivative **46.**



Scheme 32 Two step deprotection of glycolipid derivatives

2.6 Conclusion

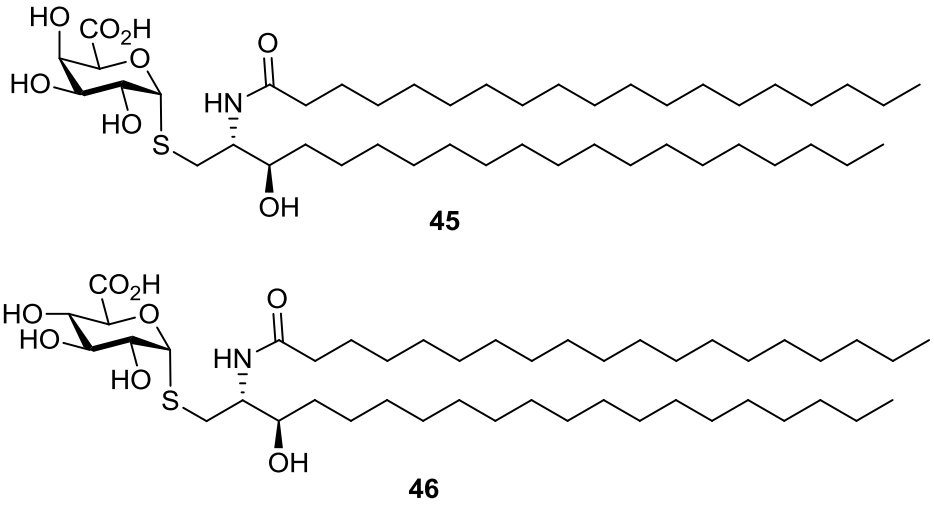


Figure 17 Synthesised glycolipid derivatives

In summary the synthesis of two novel bacterial glycosphingolipids has been achieved. It is our belief that this represents the first synthesis of *S*-linked bacterial glycosphingolipid mimetics based on uronic acids. The synthetic route illustrates the use of chelation induced anomerisation reactions to generate α-glycosyl thiols of uronic acids. It is believed this reaction could find widespread use in the synthesis of novel *S*-linked glycoconjugates. In addition, these results also provide scope for further investigation into the anomerisation reactions of glycosyl thiols in the Murphy group in the hope of finding suitable conditions for the anomerisation of glycosyl thiols **111a** and **111b.** This is currently under investigation by Michelle McKinney and Shane O'Sullivan.

We have also reported a new and versatile route to sphinganine chains from the Myers auxiliary. We believe this route has the potential to give easy access to further glycolipid derivatives via manipulation of chain length and stereochemistry which could provide useful insights into the mechanism of NKT cell stimulation via SAR studies. The glycolipids **45** and **46** are currently awaiting biological testing in order to establish their immunostimulatory activity.

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Chapter 3: Novel peptidomimetics: synthesis of Bcl-2 family inhibitors

3.1 The role of the Bcl-2 family of proteins in cancer

Apoptosis or programmed cell death is the process by which unwanted, damaged or compromised cells are removed in mammalian systems. This process is vital during embryogenesis and in maintaining cellular homeostasis[1]. Cells die in response to numerous stimuli in a controlled and regulated manner. These properties distinguish apoptosis from other mechanisms of cell death such as necrosis, in which the mechanism of cell death is uncontrolled and can lead to serious health problems. Apoptosis is therefore sometimes referred to as cell suicide as the cell plays an active role its own death. Cell death through apoptosis can proceed via two distinct mechanisms (Figure 18[2]), triggering of cell surface death receptors (extrinsic pathway) or perturbation of the mitochondria (intrinsic or Bcl-2 regulated pathway)[3].

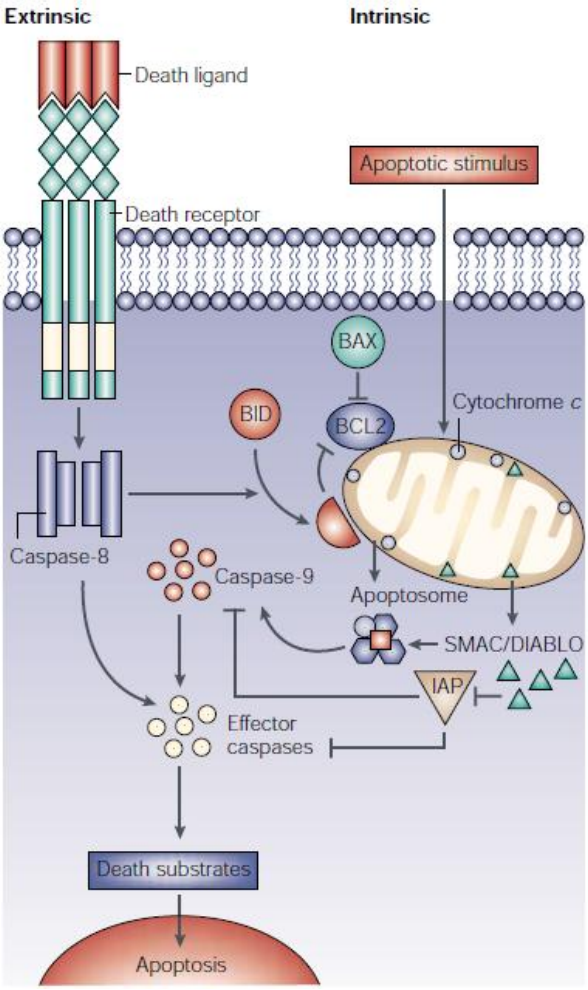

Figure 18[2] Schematic representation of the intrinsic and extrinsic apoptosis pathways

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These apoptotic pathways play an important role in fighting pathogenic infections and in executing the triggers from cytotoxic agents used in chemotherapy. Resistance by tumour cells to chemotherapeutic agents is a major problem in modern cancer therapy. This resistance is usually attributed to dysfunctional or impaired apoptosis[4]. This theory was hypothesised by the discovery that B-cell lymphoma 2 (Bcl-2) proteins are over-expressed in many tumour cells and lead to cell survival but not cell proliferation. It has been suggested that targeting the apoptotic machinery of this intrinsic pathway of apoptosis could provide novel cancer therapies which would circumvent the resistance problems[5]. Therefore, recent years has seen a flurry of research in developing drug like molecules capable of promoting cell death by antagonizing members of the Bcl-2 family of proteins.

The intrinsic pathway of apoptosis is governed by members of the Bcl-2 family of proteins. This family of proteins can be divided into 2 subsets; pro-apoptotic family members and anti-apoptotic (pro-survival) members. Members of the pro-apoptotic family include the proteins BAK and BAX. BAK and BAX cause the mitochondrial membrane to become permeable in response to death signals. In turn, the mitochondrion releases cytochrome c into the cytosol which causes a proteolytic cascade (caspase cascade) that induces apoptosis. Pro-survival members of the Bcl-2 family bind to these mitochondrial pro-apoptotic proteins which leads to inhibition of apoptosis[6]. Members of the pro-survival family include Bcl-2, Bcl-X_L, Mcl-1, Bcl-w and A1. Interestingly each of the proteins mentioned share a remarkably similar sequence of homology known as the Bcl-2 homology. The Bcl-2 homology is made up of four domains, BH1, BH2, BH3 and BH4[7].

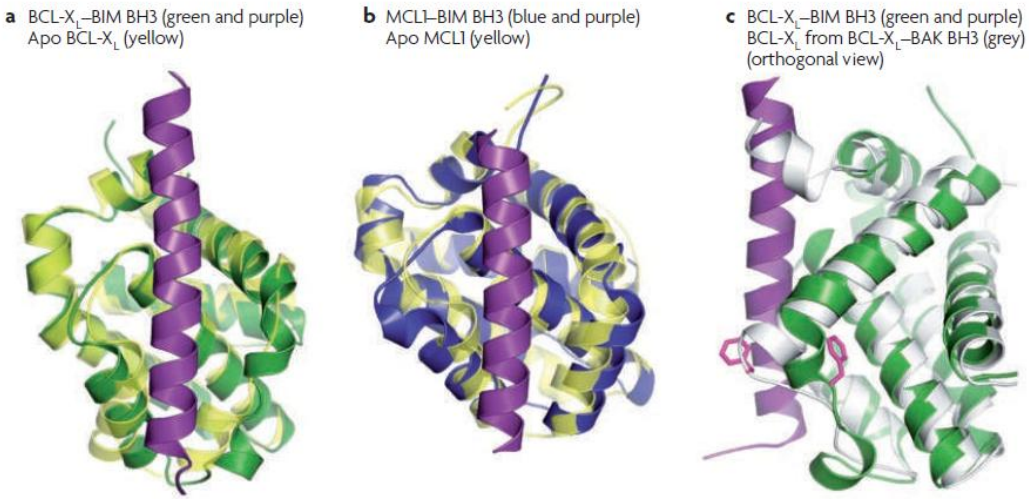


Figure 19 Crystal structures of pro-apoptotic proteins bound to pro-survival proteins (Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Drug Discovery, Ref. 1, copyright 2008)

Aside from these proteins, a number of similar pro-apoptotic proteins exist which only share the BH3 domain. These 'BH3 only' proteins are up-regulated in response to cellular stress signals and eight of these are known to exist in mammals, BIM, BID, PUMA, NOXA, BAD, BMF, HRK and BIK[8]. These proteins are capable of binding selectively into a hydrophobic groove made up of the BH1, BH2 and BH4 domains on pro-survival proteins leading to BAK/BAX activation. Crystal structures of a number of these binding interactions have been elucidated (Figure 19) [9-12]. It has also been demonstrated that a number of these 'BH3 only' proteins bind to specific pro-survival proteins[13]. For instance, BIM and PUMA can bind all pro-survival proteins tightly, BAD interacts with Bcl-2, Bcl-X_w and Bcl-w and NOXA will only bind to Mcl-1 and A1. The reasons for this selectivity are currently still unclear and are the subject of much debate. Therefore, this makes the synthesis of BH3 domain mimetics an important goal in medicinal chemistry as they can be employed as mechanistic probes to further elaborate current understanding of the interactions of Bcl-2 family proteins. In many tumour cells pro-survival members of the Bcl-2 family are over expressed leading to cell survival[14]. It is therefore reasonable to suggest the idea that molecular mimicry of the BH3 domain should provide drug-like molecules which can bind to pro-survival proteins and in turn, trigger apoptosis.

3.2 Synthetic and naturally occurring Bcl-2 family inhibitors

Development of novel BH3 mimetics can be broken down into 3 categories:

1. Small molecule antagonists
2. Modified peptides
3. Peptidomimetics

3.2.1 Small molecule antagonists

Recently a number of small molecule compounds have been discovered which show binding to members of the Bcl-2 family (Figure 20). (-)-Gossypol, also known as AT-101 is currently being developed as a potential Bcl-2 inhibitor by Ascenta®. Studies have shown **134** to have high affinity for Bcl-2 (Ki 230nM), Bcl-Xl (Ki 480nM) and Mcl-1 (Ki 180 nM)[15,16]. This compound also shows good pharmacokinetics and has entered clinical trials[17]. Extensive investigation of Gossypol derivatives has also proved successful and have allowed for the

identification of a number of analogues with submicromolar affinities for Bcl-2 and Mcl-1 including apogossypolone **135**[18].

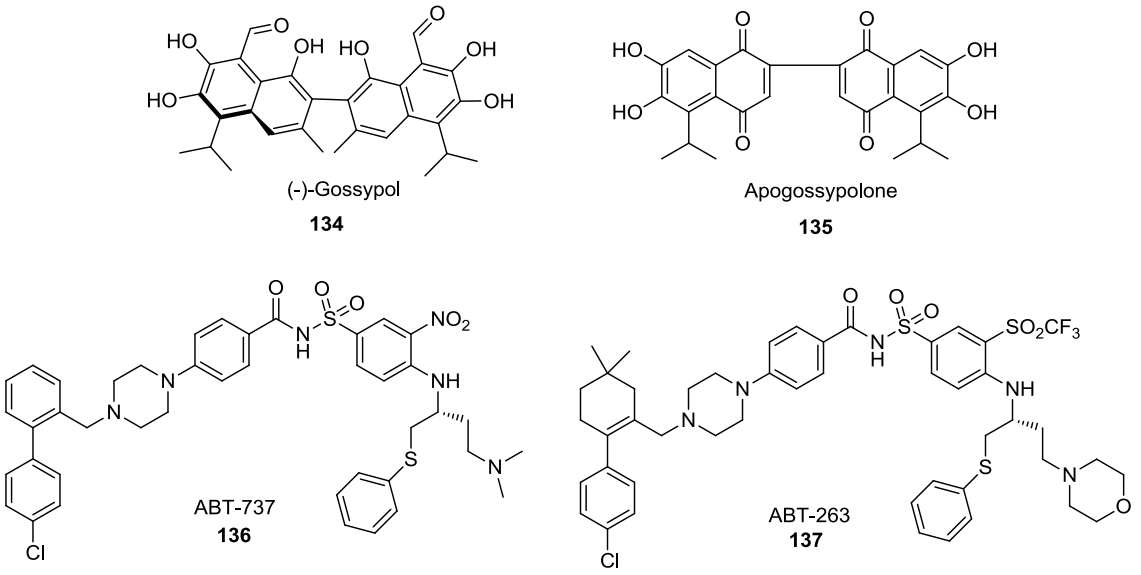


Figure 20 Structures of some important Bcl-2 family inhibitors

Abbott pharmaceuticals have recently reported two Bcl-2 inhibitors called ABT-737 and ABT-263. These compounds are the benchmark synthetic inhibitors of Bcl-2 proteins. Developed via a combination of NMR based structure activity relationships and fragment based screening, multiple iterations of medicinal chemistry led to the development of ABT-737 **136**[19,20]. This compound showed extremely high affinity for Bcl-2 (IC_{50} 1nM) and Bcl-X$_L$ (IC_{50} 0.5nM). Critically however, it does not have any affinity for Mcl-1[21]. Further studies led to the discovery of ABT-263 **137** which retains the activity of ABT-737 but is advantageous as it can be administered orally[22]. ABT-263 has currently entered phase I clinical trials. The lack of interaction with Mcl-1, however, is the main drawback of the Abbott compounds as they only show single agent efficacy against malignancies with low Mcl-1 levels and would require the use of combination therapies against many cancers.

3.2.2 Modified peptides

α-helices are the most common form of protein secondary structure[23] and account for a high proportion of the secondary structures in proteins. Surprisingly, there has been little interest in α-helical mimics for therapeutic use even though these secondary structures play an important role in many protein-protein interactions. This has recently changed however and interest in the synthesis of α-helices is growing. Initially, small modified peptides were

designed and synthesised to represent the BH3 domain, however, these compounds failed to show any binding affinity, this is possibly due to their lack of helicity[24]. In order to combat this, a number of efforts were made to reinforce their helical structures. Walensky *et al*. have reported the use of ring closing metathesis reaction (RCM) to effectively staple the peptide and force it to adopt a helical type structure. These "stapled" helical mimics show good cell membrane permeability and are resistant to the action of proteases. These mimics have currently entered preclinical trials[25]. Cell based assays have shown a reduction in leukaemia progression and an extended median time of death in animal models. Recently, the same group have shown an Mcl-1 helix mimic which is an exclusive Mcl-1 inhibitor and apoptosis inducer[26] indicating that targeting specific pro-survival proteins is a very achievable goal.

3.2.3 Peptidomimetics

Owing to the importance that the α-helix plays in protein-protein interactions it is reasonable to assume that mimicking the α-helical structure on a suitable backbone or scaffold would allow for the identification of novel biologically active structures (Figure 21). Hamilton and co workers have been successful in attaching a range of alkyl or aryl groups to a terphenyl backbone (Figure 21) in an effort to mimic the display of key amino acid epitopes on an α-helix[27]. By examining crystal structures between Bcl-X_L and Bak BH3 they were able to identify a number of 'hot residues' which are key to the binding interaction between the two proteins[28]. In this case the so-called 'hot residues' are present at the i, i+3 and i+7 positions on an α-helix. These compounds have shown binding affinities in the nanomolar range (K_i 114 nM) against Bcl-X_L. However the compounds are poorly soluble due to their high lipophilicity and attempts to overcome these issues have resulted in a loss of binding affinity[29,30].

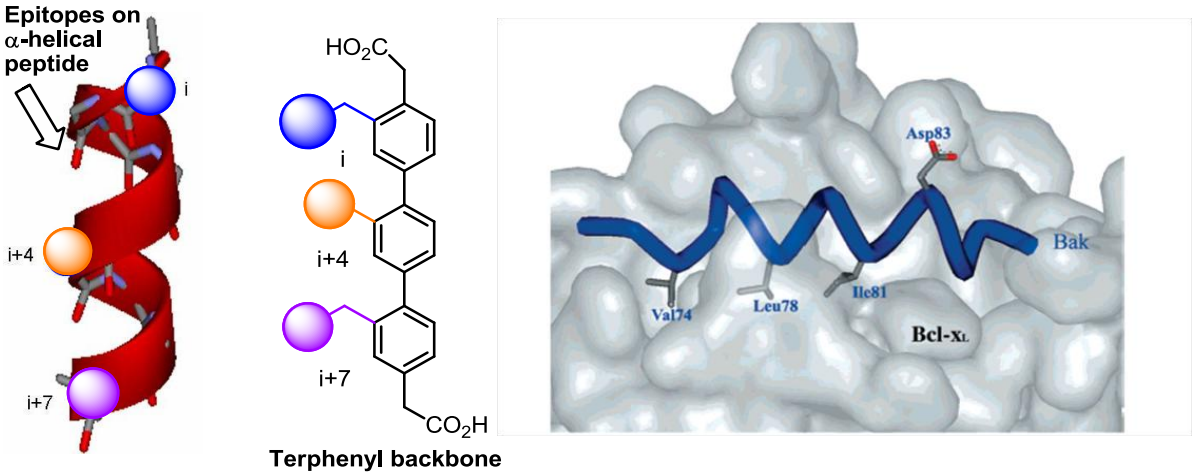
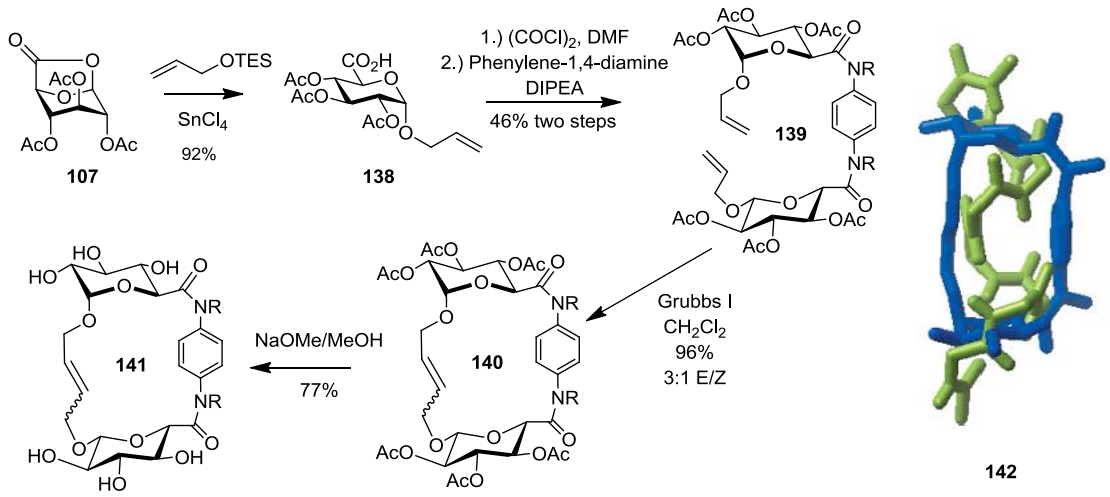


Figure 21 Hamilton's terphenyl peptidomimetics and the crystal structure of the BAK/Bcl-X_L interaction

3.3 Macrocycles with embedded carbohydrates as peptidomimetic scaffolds

Recently, carbohydrate structures have been introduced and validated as biologically relevant scaffolds on which pharmacophoric groups can be grafted[31]. The Murphy group has recently reported the synthesis of the rigid bivalent saccharide structure **141** (Scheme 33). Glycosidation of lactone **107** with the silylated nucleophile in the presence of $SnCl_4$ gave the α-carboxylic acid derivative **138** via a glycosidation-anomerisation reaction as described in previous chapter in high yield. Acid chloride formation followed by amide coupling gave dimeric compound **139** which underwent ring closing metathesis with the Grubbs 1st generation catalyst in CH_2Cl_2 to give the macrocyclic structure **140** in high yield as a mixture of cis and trans isomers. Deprotection then gave macrocycle **141** in 77% yield. The use of monosaccharides as scaffolds for the development of peptidomimetics such as somatostatin and β-turn mimetics has been well documented[32]. With this in mind macrocycle **141** was investigated for its potential in peptidomimetic design. Molecular modelling indicated that these structures do have a relationship to the α-helix peptide backbone and could be used to display the i, i+1, i+2, i+5, i+6 and i+7 residues.



Scheme 33 Murphy group synthesis of bivalent saccharide macrocycles and their comparison to the α-helix (structure **142**)

Inspired by this work, we set about designing and synthesising a number of novel type III peptidomimetic derivatives which we felt could mimic the BH3 α-helical domain with the aim of triggering apoptosis. Type-III peptidomimetics are considered the ideal in peptidomimetic development[33]. Even though they appear unrelated to the original peptide, they contain the necessary groups grafted onto a novel non-peptide scaffold which serve as geometrical mimetics (Figure 22). In this case the scaffold will be represented by a macrocycle with embedded monosaccharides. Each hydroxyl group can then act as a functional handle onto which the binding epitopes (amino acid side chains /or related motifs) can be grafted. The presence of a macrocycle will allow for the confinement of the amino acid epitopes into a strict spatial orientation which will mimic the display of an α-helix. This means that the three hot residues, i, i+4 and i+7, aligned along one side of the BH3 helix will be transferred onto one side of the novel scaffold**.**

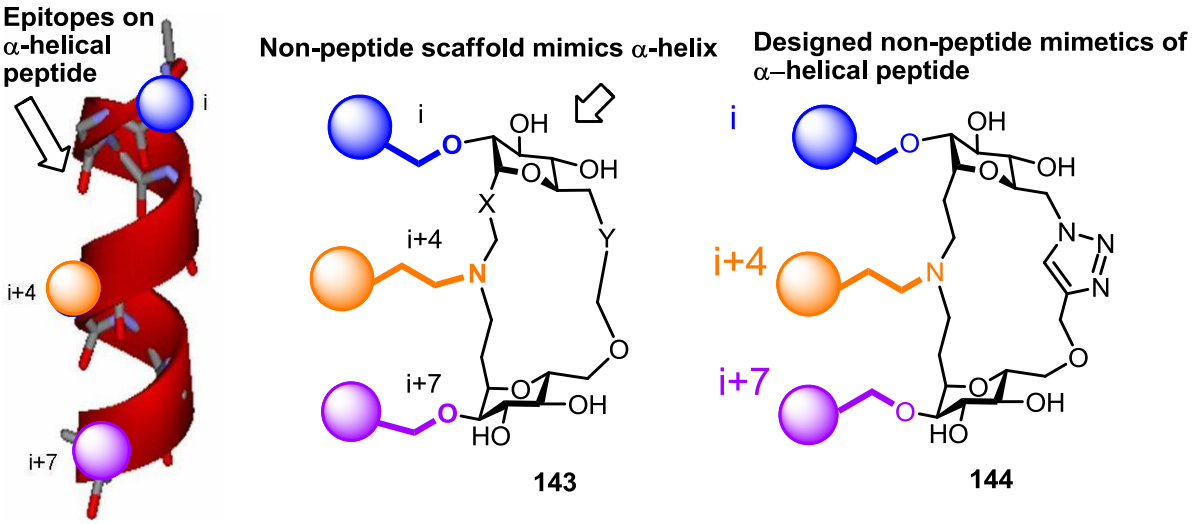
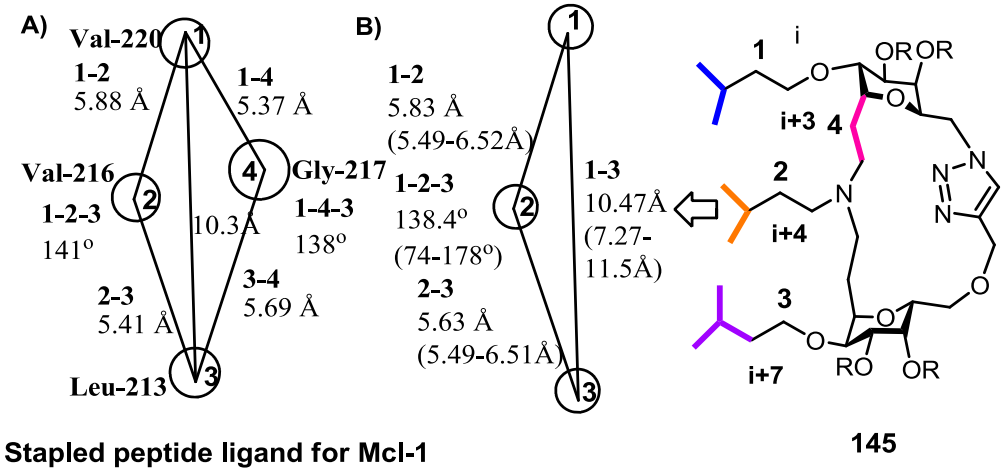


Figure 22 Structures of the proposed macrocyclic peptidomimetics

The preliminary molecular modelling studies on compound **144** were carried out by Professor Murphy using Macromodel® software and the results are shown in Figure 23. The macrocyclic structure was compared to the stapled peptide Mcl-1 sensitizer synthesised recently by Walensky *et al*.[26].The important hydrophobic residues that facilitate binding of this peptide to Mcl-1 are Leu213, Val216, Gly217 and Val220 (i, i+3, i+4 and i+7). Placing these residues onto the macrocyclic scaffold generated compound **145**. It is evident that the, i (**1**), i+4 (**2**) and i+7 (**3**) residues are presented along one side of the scaffold and perhaps the methylene group (**4**) could be considered a glycine mimic. The average distances (1-2, 2-3, 1-3) and bond angles (1-2-3) for **145** and the stapled helical mimic were calculated. Also shown in parenthesis are the range of interatomic distances and angles that were observed for the retained structures. Although not identical to the α-helix, the results are within a reasonable range to suggest that **145** could mimic the α-helix. Similar results were obtained when **145** was modelled with other anti-apoptotic members of the Bcl-2 family.



C.)

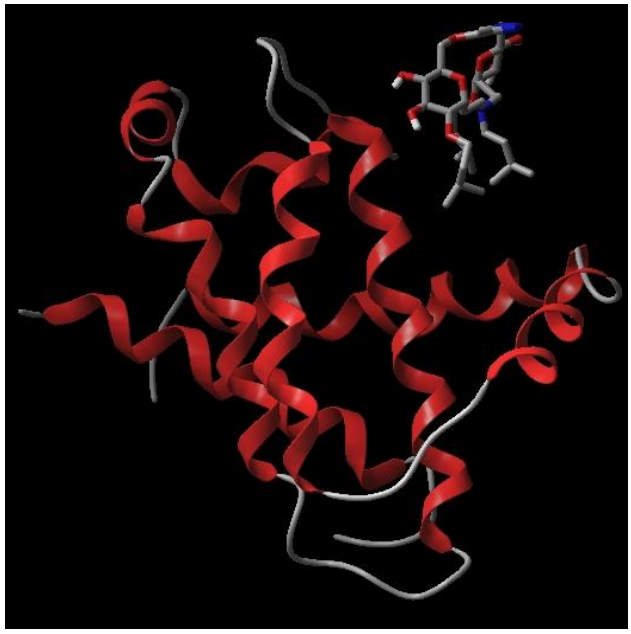
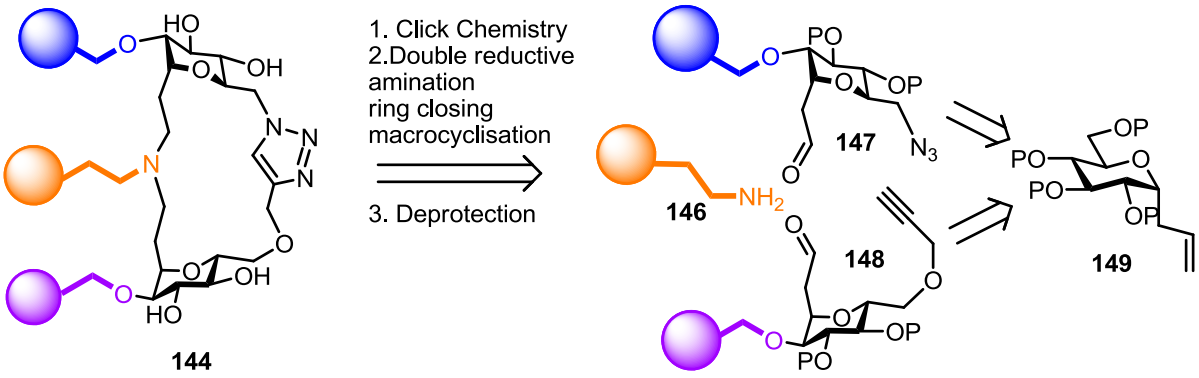


Figure 23 Results of molecular modelling **A.)** Bond angles and distances for stapled peptidomimetics by Walensky. **B.)** Bond angles and distances of **145 C.) 145** docked into the binding site of Mcl-1

3.4 Retrosynthetic analysis

With the molecular modeling data in hand and the previous success in the synthesis of macrocyclic carbohydrate structures, a novel approach to carbohydrate α-helical peptidomimetics was developed. In order to achieve this objective, a number of structural modifications needed to be made to the initial macrocyclic structures. The proposed retrosynthetic analysis is shown in Scheme 34. It was envisaged that replacement of the *O*-glycosidic linkage with a *C*-glycosidic linkage would provide enhanced stability of the

compounds towards the action of carbohydrate processing enzymes in the body and provide an extra degree of rigidity to the structures. Easier access to these macrocycles could be gained through the use of a copper catalyzed azide-alkyne cycloaddition reaction[34], this would insert a triazole into the backbone of the macrocycle, replacing the phenylenediamine backbone present in previous structures. A suitable orthogonal protecting strategy was needed in order to introduce the desired amino acid epitopes on the ith and i+7 residues. It was suggested that the i+4 residue could be introduced through the use of an under-utilized double reductive amination-ring closing macrocyclisation sequence.



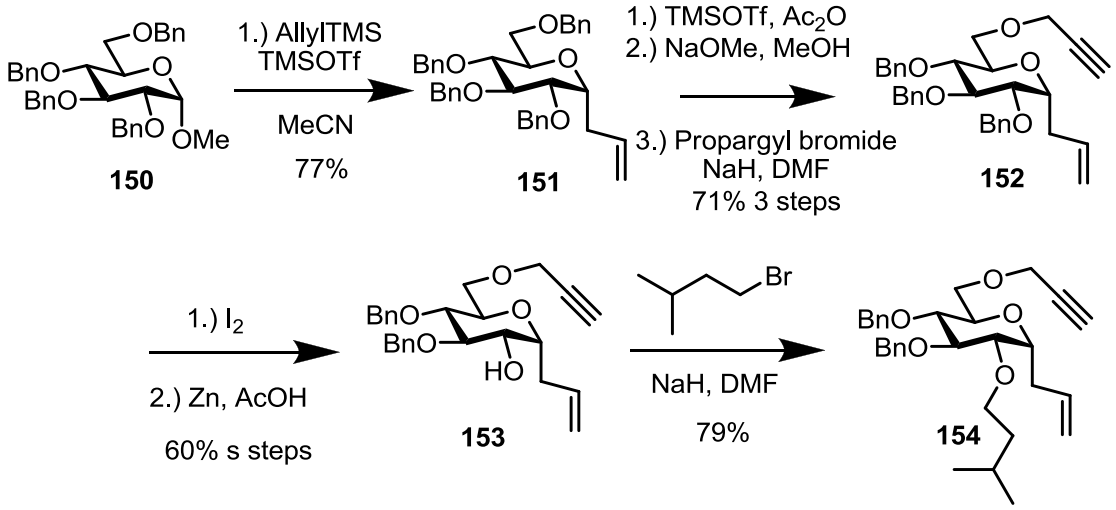
Scheme 34 Proposed route to peptidomimetics starting from D-glucose

3.5 Synthesis of novel macrocyclic peptidomimetics with embedded carbohydrates

3.5.1 Synthesis of Alkyne monomers

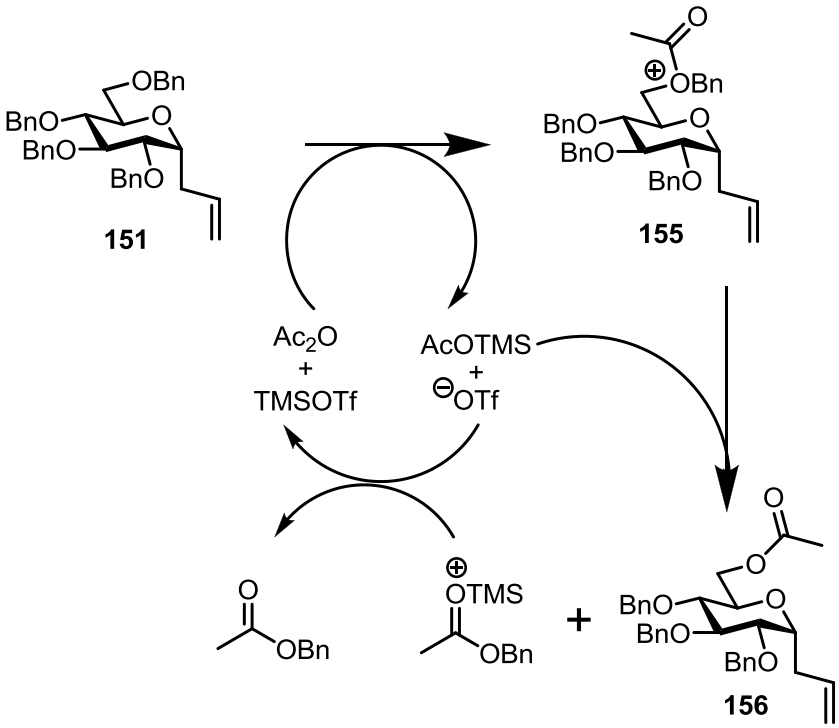
Working concurrently with Dr. Dilip Jarikote, the synthesis of these macrocycles starting from D-glucose was explored. Dr. Jarikote had already devised a route to the desired azide and alkyne monomers starting from commercially available methyl α-D-glucopyranoside. The route to the alkyne monomers is shown in Scheme 35. Methyl α-D-glucopyranoside was benzylated via treatment with benzyl bromide and sodium hydride to give the fully protected glucose derivative **150** in 83% yields. Allylation of **150** was achieved by treatment with allyltrimethylsilane and catalytic TMSOTf in acetonitrile gave the *C*-glycoside derivative **151** in 77% yield as a mixture of anomers (11:1 α:β)[35]. The use of acetonitrile as a solvent in these reactions is key to the high α ratios obtained, Schmidt[36] and later Fraser-Reid[37] have suggested that acetonitrile interacts with the oxocarbenium ion to form equatorial isonitrilium species which then undergo S_N2 type displacement with inversion to give the observed axial

attack. One explanation provided for this solvent effect is that the β-isonitrilium salt is much more stable than the α-isonitrillium salt and does not revert to the axial isomer.



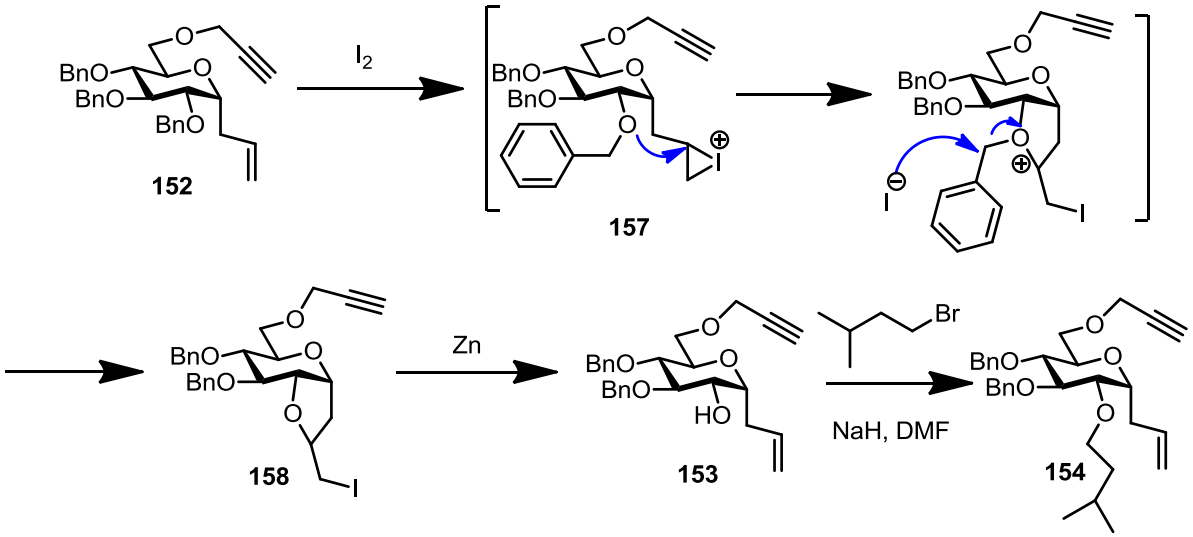
Scheme 35 Synthesis of the alkyne monomer

With the *C*-allylated derivatives in hand, attention then focused on selective manipulation of the C2 and C6 benzyloxy groups. Wong has shown that the 6-*O*-benzyl group of a glycoside can be regioselectively converted into an acetate group via treatment with TMSOTf and Ac_2O at low temperatures[38]. A proposed mechanism for this reaction is shown in Scheme 36[39]. It is believed that **151** reacts with Ac_2O in the presence of TMSOTf giving the cationic intermediate **155**, AcOTMS and the triflate anion. **155** then reacts with AcOTMS to give the acetylated product **156** along with a second cationic intermediate. Reaction of this cationic intermediate with the triflate anion then regenerates the catalyst. A Zemplén deacetylation gave the primary alcohol derivative in high yield.



Scheme 36 Proposed mechanism for regioselective acetylation of **151**

Alkylation of the primary alcohol with sodium hydride and propargyl alcohol gave propargyl derivative **152** in 90% yield. As alkylation at the C2 position was desired in order to mimic the amino acid side-chain, selective de-*O*-benzylation via reductive ring opening using the conditions described by Nicotra[40] was carried out on compound **152**. Treatment of **152** with I_2 affords the iodonium ion **157** (Scheme 37). It is well known that the oxygen of a benzyl ether can react with an iodonium ion. In this case the only benzyloxy group close enough to interact with the iodonium ion is that of the C2 position. Attack of benzylic oxygen followed by loss of the subsequent benzyl cation affords cyclic iodoether **158**. Zinc mediated reductive elimination restores the allylic double bond and reveals compound **153**.

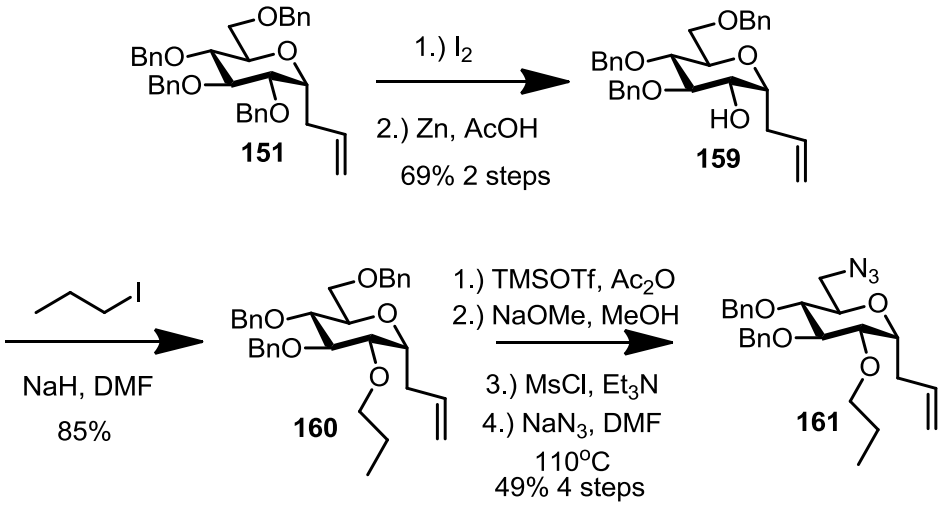


Scheme 37 Mechanism for the formation of **153**

Alkylation of **153** with 1-bromo-3-methyl-butane afforded the benzylated monomer **154** in 79% yield.

3.5.2 Synthesis of azide monomers

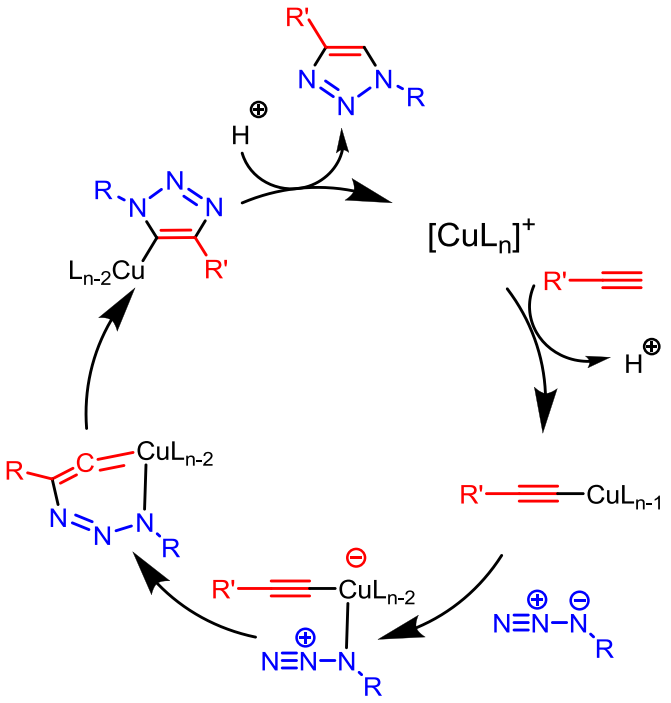
Access to azide monomers was achieved through a similar reaction sequence (Scheme 38); regioselective deprotection of the C2 benzyloxy group was carried out on compound **151** as described for compound **153**. Alkylation with iodopropane gave the benzylated derivative **160**. Regioselective deprotection of the C6 benzyloxy group via treatment with Ac_2O and TMSOTf followed by Zemplén deacetylation gave a primary alcohol intermediate. Mesylation of the primary alcohol followed by S_N2 displacement with NaN_3 gave the azide derivative **161**.



Scheme 38 Synthesis of the azide monomers

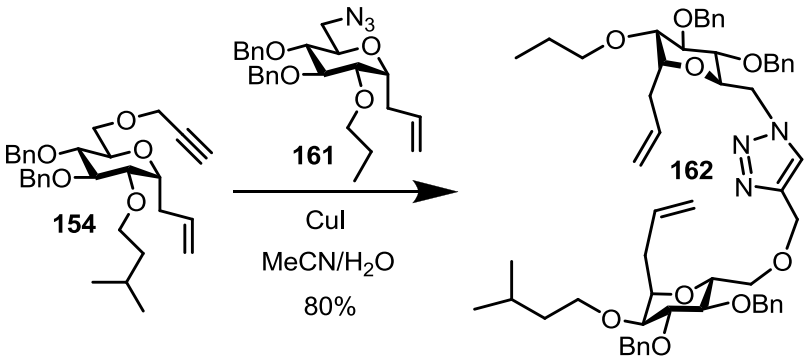
3.5.3 Coupling of monomers, macrocyclisation and deprotection

In 2001, K. Barry Sharpless coined the term "click chemistry" in reference to reactions which are wide in scope, high yielding, give minimal by-products, are stereospecific, easy to perform and can be carried out in volatile removable solvents[41]. One such reaction which has become synonymous with click chemistry is the 1,3 dipolarcycloaddition reaction which occurs between an azide and a terminal alkyne. Initially introduced by Rolf Huisgen[42], the thermal 1,3-dipolarcycloadditon of azides and alkynes fulfilled many of the criteria for a click reaction, however, the classical reaction fell short due, in part, to the elevated temperatures required for the reaction to occur and the mixture of regioisomers obtained after purification. Development of this reaction into a fully-fledged click reaction was realised in 2002 when the groups of Mendel[43] and Sharpless[44], working independently, introduced the copper catalysed variant. This reaction is facilitated via a different mechanism to the classical reaction and can even be conducted in aqueous solutions at room temperature. The copper catalysed variant also has the advantage of giving rise to one regioisomer, the 1,4 disubstituted triazole. More recently, a ruthenium based cycloaddition has also been introduced which gives the 1,5 disubstituted regioisomers[45]. Sharpless has described this reaction as the "cream of the crop" in click chemistry and so it comes as no surprise then that there has been considerable interest in this area in recent years. The mechanism for the copper catalysed azide alkyne cycloaddition is presented in Scheme 39[46].



Scheme 39 Mechanism of copper catalysed azide-alkyne cycloaddition

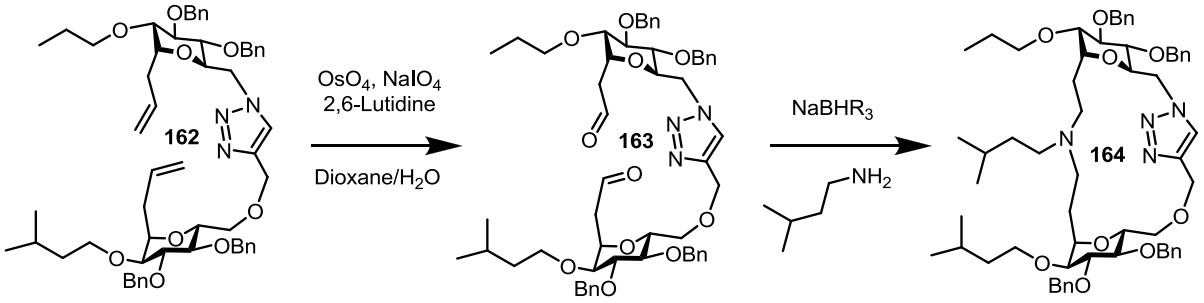
To exploit the useful properties of this reaction, alkyne **154** and azide **161** were treated with CuI in MeCN-H_2O at reflux for 6 hours. After work-up and purification the triazole derivative **162** was obtained in 80% yield.



Scheme 40 Synthesis of triazole **162**

With compound **162** in hand, attention turned into macrocyclisation and the introduction of the final amino acid epitope. It was suggested that the final amino acid epitope could be introduced by a double reductive amination reaction. Oxidative cleavage of dialkene **162** would give a bisaldehyde intermediate which could then undergo a double reductive amination/macrocyclisation when treated with an amine to deliver the desired macrocyclic peptidomimetics. At the conception of this route, a double reductive amination, ring closing

macrocyclisation reaction between a bisaldehyde and an amine was unprecedented in the literature, however, recently Madsen *et al*. have reported the use of such reactions in the synthesis of some novel macrocyclic compounds[47].

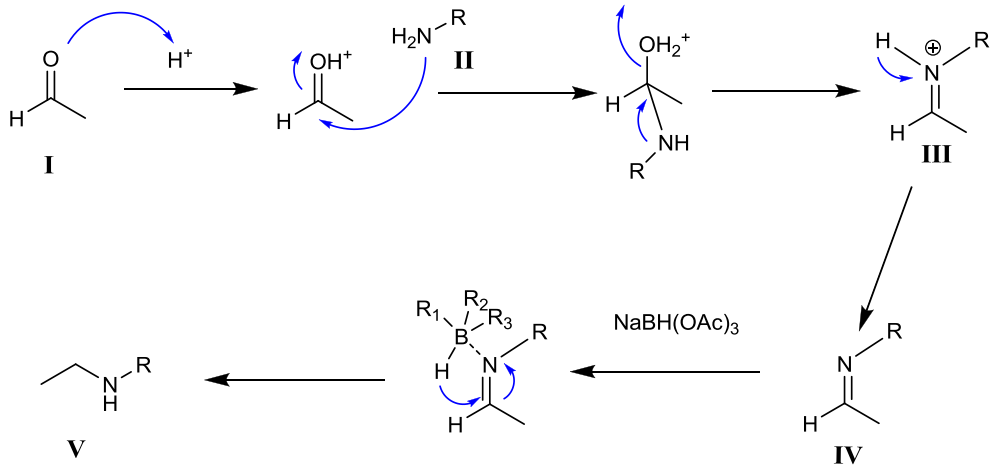


Scheme 41 Oxidative cleavage and reductive amination/macrocyclisation sequence

Compound **162** was treated with a catalytic amount of OsO_4, $NaIO_4$ and 2,6-lutidine in dioxane-H_2O[48] giving the bisaldehyde intermediate **163**. Reaction of crude **163** with $NaCNBH_3$ and isopentylamine in THF for 24 hours gave compound **164** according to mass spectrum analysis. The solvents were removed and purification via flash chromatography was attempted. Unfortunately the yield of the macrocyclic structures using this method was poor (~10-15%) and it proved very difficult to purify the compounds to a high degree. In addition to the poor yield, the NMR analysis of the compound was also complicated due to poorly resolved spectra. As a result, it became very difficult to characterise the macrocyclic structures. At this point, an extensive investigation into this reaction conditions was undertaken. NMR spectra of the crude bisaldehyde **163** showed well resolved clean spectra which were relatively free from impurities. Evidence pointed to a problem during the reductive amination reaction. In an effort to improve the reaction, alternative reducing agents were first examined. $NaCNBH_3$ has a number of undesirable properties including its toxicity and the potential for toxic by products such as NaCN and HCN to be generated during work-up, meaning it is not ideal for large scale processes. It can also, in some cases, contaminate products with cyanide. A cleaner and milder method for reductive amination has been reported by Abdel-Magid and co-workers[49], in which sodium triacetoxyborohydride was used as the reducing agent. Following their procedure, the bisaldehyde **163** was treated with isopentylamine and $NaBH(OAc)_3$ in dichloroethane (dichloromethane can also be used in this reaction, making removal of solvent easier). Interestingly, upon work-up with aqueous sodium bicarbonate, compound **164** was isolated in 75% yield. The NMR spectra obtained after purification were much cleaner and had a higher degree of resolution than those

obtained when using $NaCNBH_3$. When this reaction was carried out without work up with sodium bicarbonate, a poor yield was observed and the spectra were also poorly resolved. This suggests that, perhaps, there is a chelation between the nitrogen of the macrocycle and the boron of the reducing agent which leads to complicated NMR spectra. Upon work-up, however, this interaction is broken down and **164** was revealed.

The mechanism for reductive amination is shown in Scheme 42. Reaction of an aldehyde (**I**) under acid catalysed conditions with an amine (**II**) generates an iminium ion (**III**). Loss of a proton then generates the imine (**IV**) which undergoes a reduction to give the secondary amine (**V**). In the case above this secondary amine then undergoes a second intramolecular reductive amination to close the ring.



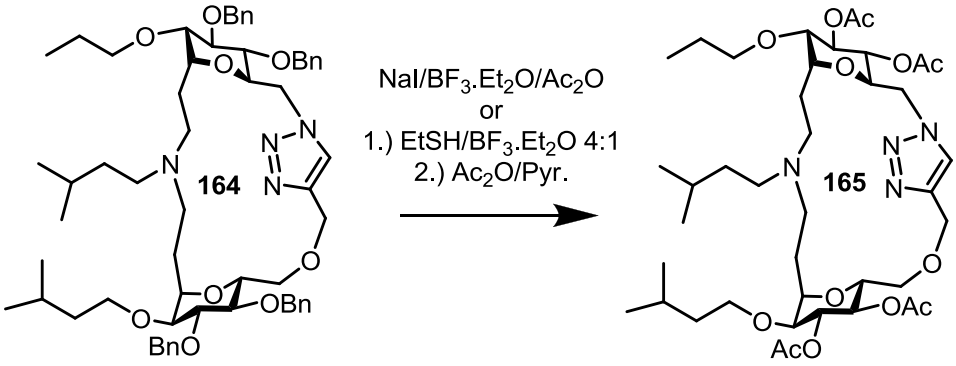
Scheme 42 Mechanism for reductive amination

With the macrocyclic structure in hand, attention was then focused on deprotection to yield the final polyhydroxylated macrocyclic structures. The most common method to remove benzyl ethers is through catalytic hydrogenation. Unfortunately, with this substrate, all attempts to remove the benzyl ethers under catalytic hydrogenation conditions failed to deliver the desired deprotected macrocycles. The hydrogenation was attempted with numerous palladium catalysts and solvents, these efforts are summarised in table 3. In each case, only unreacted starting material was recovered. Initially, it was thought that the nitrogen atoms present on the scaffolds could potentially poison the palladium catalyst. In an effort to overcome this potential problem, a number of reactions were performed in the presence of organic acids such as TFA and formic acid, however, these attempts also failed to deliver the desired product.

Catalyst	Additive	Solvent	Yield
Pd/C 20%		THF	S.M
Pd/C 20%	1% TFA	THF	S.M
Pd/C 20%		EtOH	S.M
Pd/C 20%	1% TFA	EtOH	S.M
$Pd(OH)_2$		$CHCl_3$-MeOH	S.M
Degussa type		THF	S.M
$Pd/C/Pd(OH)_2$		THF	S.M
Pd/C 30%		THF	S.M

Table 3 Summary of catalytic hydrogenation conditions attempted for the deprotection of **164**

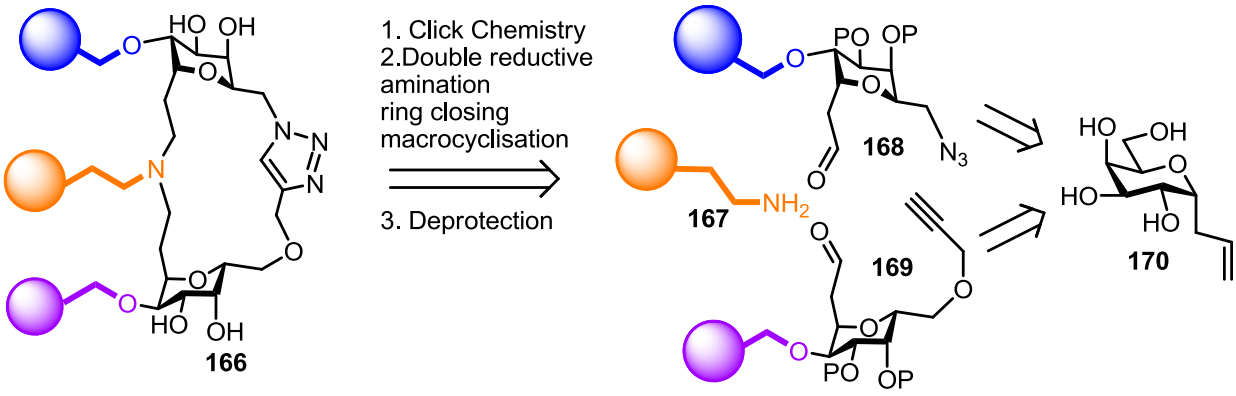
While working on similar macrocyclic structures, Dr. Jarikote was able to successfully achieve a global debenzylation using the Birch reduction conditions[50]. In this case the macrocycles were obtained in low yields. The low yield of these reactions coupled with the harsh conditions under which the Birch reduction is carried out led us to explore alternative ways to deprotect the macrocyclic derivatives.



Scheme 43 Lewis acid mediated deprotection of **164**

Lewis acids have been reported in the literature for the deprotection of benzyl ethers. Recently Brar *et al*. reported the use of a reagent system consisting of $BF_3.Et_2O$, NaI and Ac_2O to facilitate a deprotective acetylation reaction[51]. When **164** was subjected to these conditions, the presence of the fully acetylated derivative **165** was detected by both mass spectrum and TLC analysis after 24 hours. Gratifyingly, purification gave the macrocyclic

derivative **165** in 57% yield. Although this reaction worked well on small scale, larger scale reactions were less successful, complex mixtures were obtained. Prolonged reaction times and the use of excesses of reagents failed to push the reaction to completion. The minor success of this reaction suggested that Lewis acids are particularly useful in the deprotection of these macrocyclic substrates. $BF_3.Et_2O$ used in conjunction with ethane thiol has shown to be an excellent reagent system for the debenzylation of sugar derivatives[52]. Therefore, treatment of **164** with this reagent system gave fully deprotected derivative in 85% crude yield. Acetylation of the crude product gave the macrocycle derivative **165** in 90% yield. It was believed that the acetylated macrocyclic derivatives would be more suitable for biological evaluation owing to the greater lipophilicity of the compounds being advantageous when crossing cell membranes.

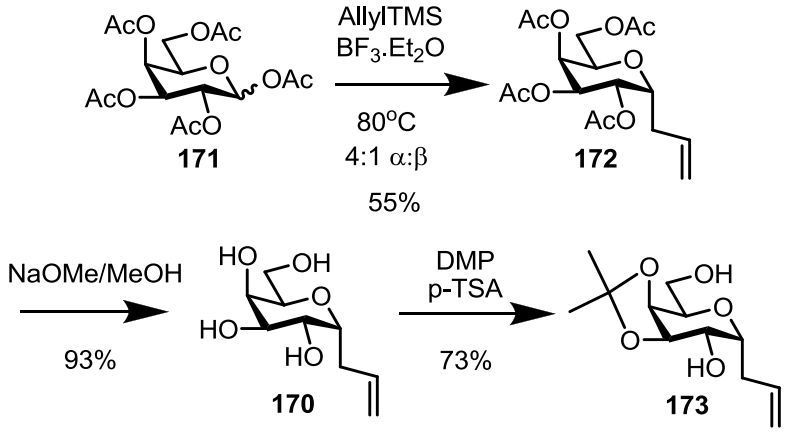


Scheme 44 Approach to macrocyclic peptidomimetics from D-galactose

3.6 A second generation approach to macrocyclic peptidomimetics

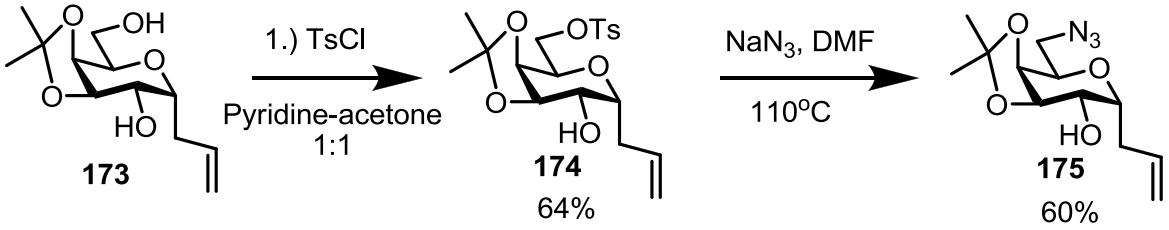
3.6.1 Synthesis of 1-*C*-Allyl-1-deoxy-3,4-*O*-isopropylidene-α-*D*-galactopyranoside

Although the route from D-glucose did yield some positive results, it was decided that a more concise and flexible route should be developed. It was proposed that selective protecting group manipulations would be easier on macrocycles derived from D-galactose. A revised retrosynthesis is shown in Scheme 44. Selective introduction of an isopropylidene group onto compound **170** would give the diol **173** which could be further manipulated into the desired azide and alkyne derivatives.



Scheme 45 Synthesis of key intermediate **173**

The synthesis of **173** started from commercially available peracetylated D-galactose. Allylation of **171** was achieved through treatment with allyltrimethylsilane and 5 equvalents of $BF_3.Et_2O$ at 80°C for 6 hours to give **172** as a 4:1 mixture of anomers[53]. Zemplén deacetylation of **172** gave the compound **170** in 93% yield on a multi-gram scale. Selective introduction of the isopropylidene was then achieved under thermodynamic conditions using conditions set out by Ben and co-workers. Treatment of **170** with dimethoxypropane and p-toluenesulfonic acid in acetone gave **173** in 73% yield[53]. With this key intermediate in hand, attention was then focused on conversion of this material into the desired alkyne and azide monomers.

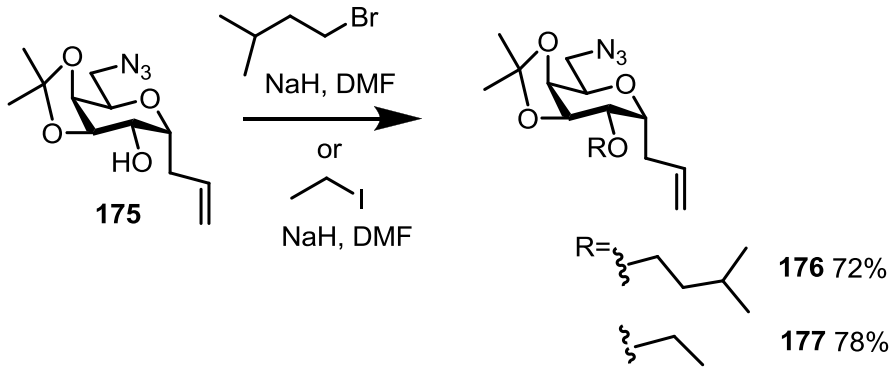


Scheme 46 Synthetic route to azide **175**

3.6.2 New approach to azide monomers

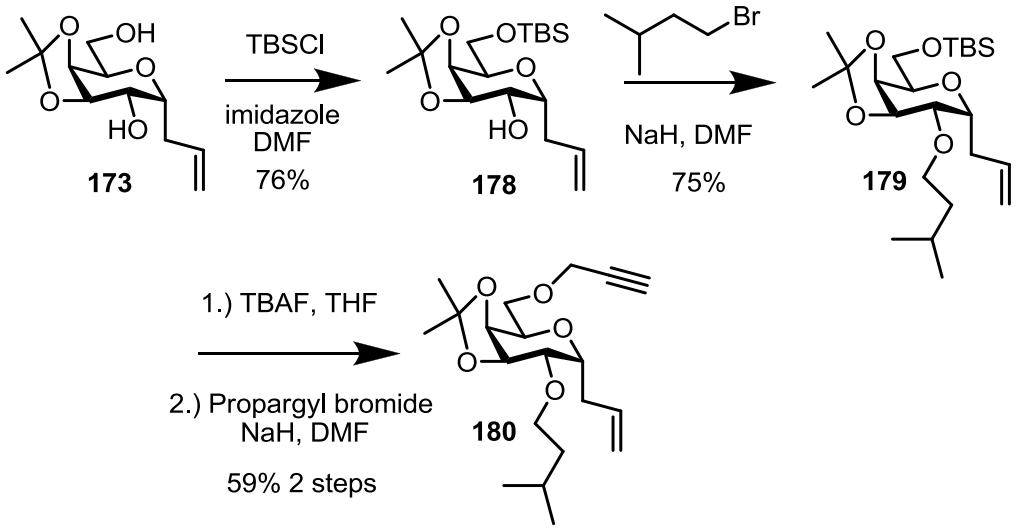
Initially, attempts were made to introduce a mesylate selectively onto the primary alcohol of **173** in order to facilitate an S_N2 displacement reaction with NaN_3. **173** was treated with MsCl and Et_3N in CH_2Cl_2, this however, led to a mixture of the di-mesylate, mono-mesylate and unreacted starting material. As an alternative, the less reactive tosylate group was selectively introduced onto the primary alcohol to give **174** in excellent yield. Displacement of the tosylate was achieved through treatment with NaN_3 in DMF to give the azide derivative **175**

in good yield. This new route to azide monomers represents a significant improvement over the previous route in both the number of steps required and also overall yield of the sequence. The presence of the free secondary hydroxyl group at C2 also offers the possibility to develop a library of azide monomers for use in macrocycle synthesis.



Scheme 47 Synthesis of azide monomers **176** and **177**

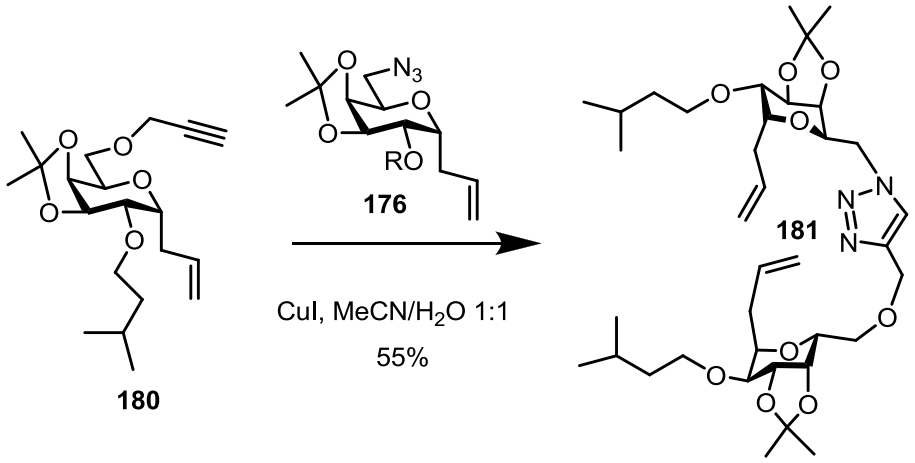
In order to obtain a small library of potential Bcl-2 inhibitors for biological screening, alkylation of azide **175** was carried out with iodoethane and 1-bromo-3-methyl-butane to give the alkylated derivatives **176** and **177**. *O*-alkylation was also attempted with 1-bromo-2-methyl-propane however it is believed that a competing elimination reaction prevents the alkylation from occurring on this particular substrate.



Scheme 48 Synthesis of alkyne monomer **180**

3.6.3 Revised approach to alkyne monomers

With the azide monomers now in hand, attention was turned to the synthesis of the alkyne monomer (Scheme 48). It was initially believed that a propargyl group could be introduced selectively on the free primary alcohol of **173**. Treatment of compound **173** with propargyl bromide gave a mixture of both the di-alkylated and mono-alkylated products as well as a considerable amount of unreacted starting material. As an alternative approach, the primary alcohol was selectively protected as a silyl ether via treatment with TBSCl and imidazole to give **178** in 76% yield. Alkylation of the free secondary hydroxyl group at C2 was then achieved through treatment with 1-bromo-3-methyl-butane in DMF to give **179** in good yield. Removal of the silyl ether with TBAF followed by alkylation with propargyl bromide gave the alkyne derivative **180** in 59% yield over 2 steps. Again, this represents a marked improvement over the previous approach in terms of both the yield and the number of steps required to obtain the desired alkyne monomer.

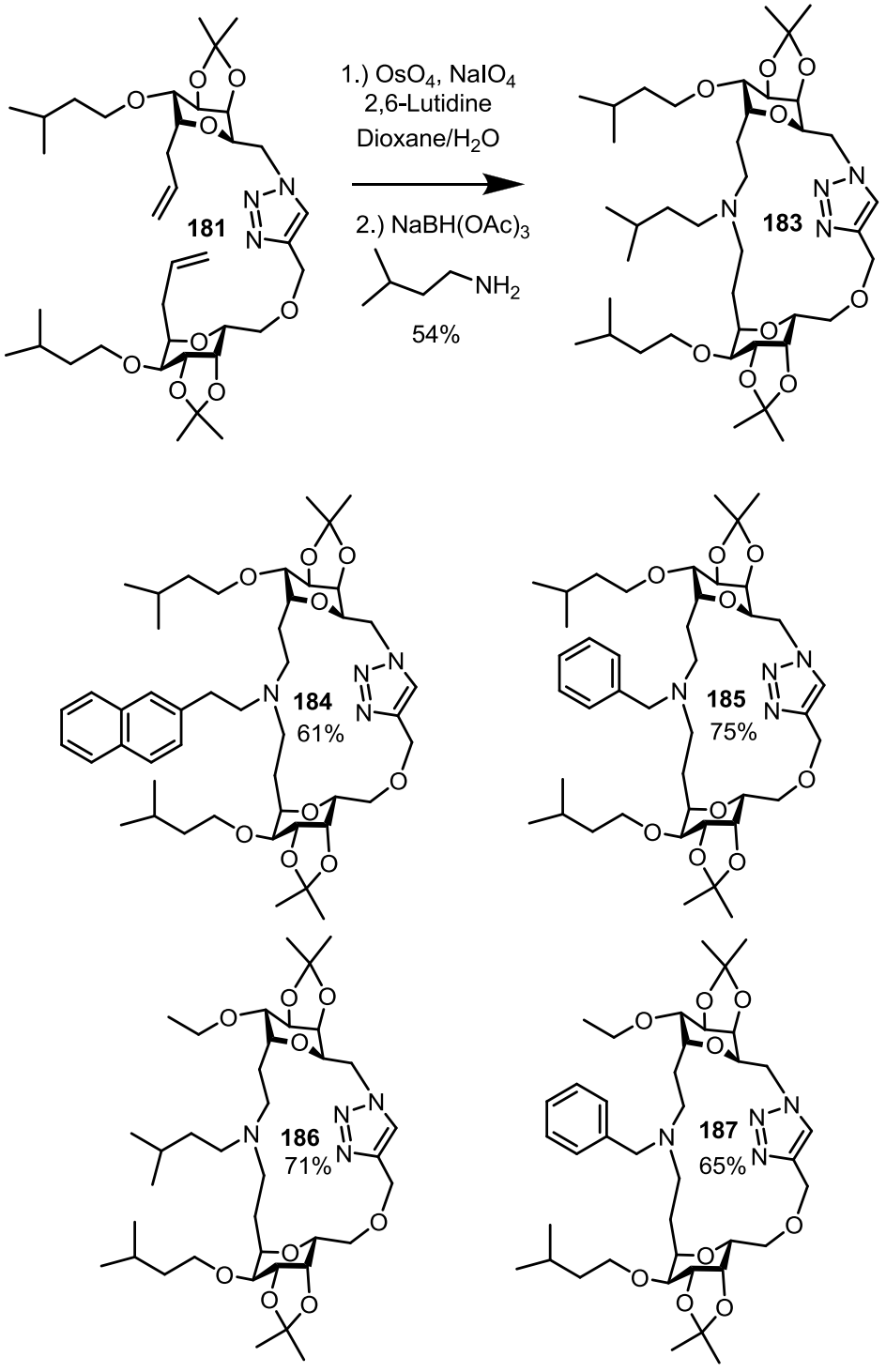


Scheme 49 Click reaction of **176** and **180**

3.6.4 Coupling reaction, macrocyclisation and final manipulations

Coupling of the azide **176** with alkyne **180** under copper catalysed conditions gave compounds **181** in good yield. An identical reaction sequence was conducted with azide **177** to generate the dialkene derivative **182.** Gratifyingly, treatment of dialkene derivative **181** with the conditions outlined previously for the double reductive amination macrocyclisation reaction gave macrocycle **183** in 55% yield. Replacement of isopentylamine with 2-(2-Naphthyl)ethylamine hydrochloride and benzyl amine in the reductive amination reaction generated the macrocyclic derivatives **184** and **185** respectively. Treatment of compound **182**

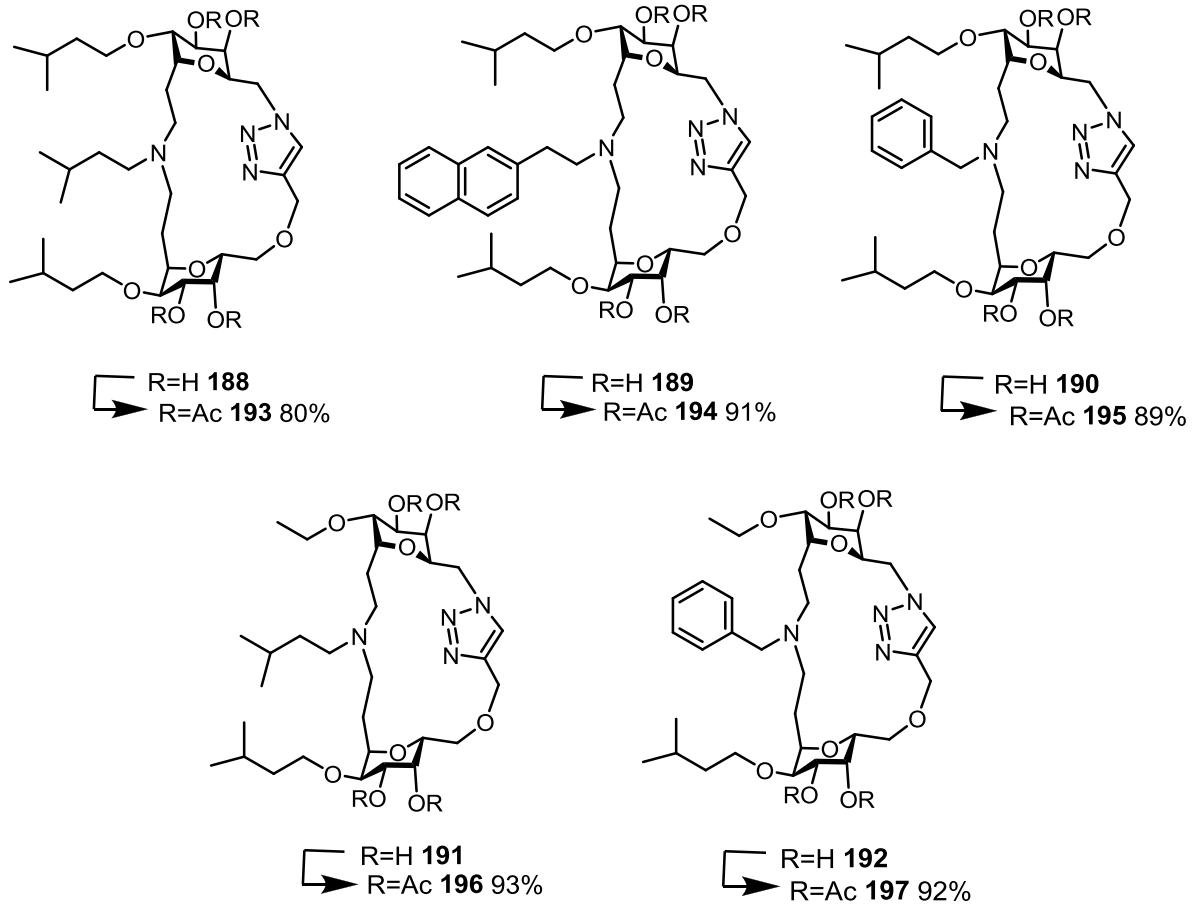
with isopentylamine and benzylamine generated two further macrocyclic derivatives **186** and **187.**



Scheme 50 Macrocyclisation reaction and protected peptidomimetic compounds

Deprotection of macrocyclic derivatives **183-187** was facilitated via treatment with TFA-H_2O 4:1 to give the fully deprotected macrocycles **188-192** in excellent yields. In order to improve

the bioavailability of the compounds, acetylation of **188-192** was achieved through treatment with pyridine-acetic anhydride to give acetylated macrocycles **193-197** in high yields.



Scheme 51 Summary of macrocyclic peptidomimetics synthesised

3.7 Preliminary biological results

Some preliminary cell viability testing has been carried out on a number of the proposed BH3 mimics by the cell stress and apoptosis research group of Professor Afshin Samali at the National University of Ireland, Galway. A series comprised of the isopropylidene protected compound **183**, its polyhydroxylated analogue **188** and the acetylated derivative **193** were tested (alongside the two acetylated derivatives **196** and **197**) and the results were compared to the known Bcl-2 inhibitor ABT 737. The candidate compounds were incubated with ML-1 acute myeloid leukaemia cells for 24 hours and the loss of cell viability was measured by an MTT assay. The results are shown in Figure 24.

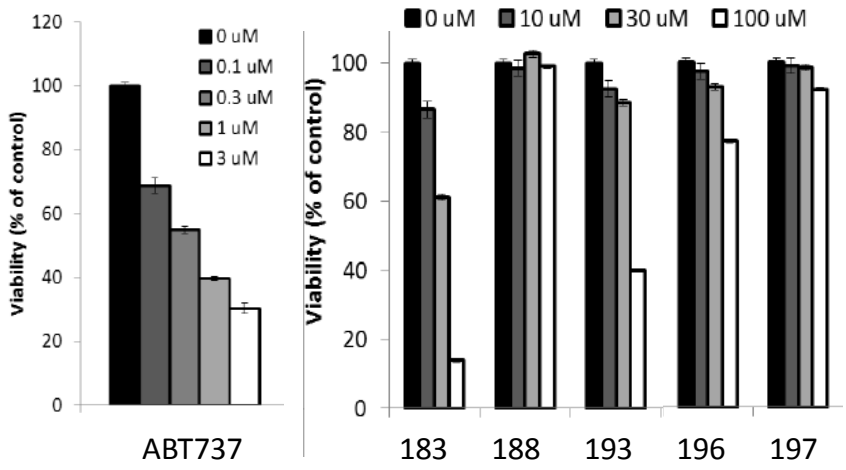


Figure 24 Candidate compounds are active in ML-1 acute myeloid leukaemia cells. ML-1 cells were treated with the indicated concentrations of the candidate BH3-mimetic compounds or ABT737 for 24 h after which loss of cell viability was assessed with MTT assay

The results show that, although not as potent as the highly optimised compound ABT737, there is activity in acute myeloid leukaemia cells. As expected the protected derivatives **183** and **193** were much more active than the related polyhydroxylated compound **188.** It is believed that the increased hydrophobicity of these compounds allows them to cross the cell membrane easier. Interestingly the isopropylidene protected compound **183** showed a much higher degree of activity than the acetylated derivative indicating the choice of protecting group may be pivotal to obtaining good biological activity (although each compound only showed good activity at the 100 μM range). Compounds **196** and **197** showed little activity, perhaps indicating the ethyl side chain is not long enough to elicit suitable binding in the active site of the protein. Although these preliminary results show throw up some promising results, it must be noted that some solubility issues were identified with these compounds during these experiments and this must be addressed in the future.

3.8 Conclusion

The synthesis of six novel macrocyclic α-helical peptidomimetics has been successfully achieved. These compounds provide the first example of the use of bivalent carbohydrate macrocycles as rigid scaffolds for the synthesis of α-helical peptidomimetics. These scaffolds could find use in drug design and as mechanistic probes to further understand the basis for protein-protein interactions. The route provided is quick and flexible and allows for the generation of numerous macrocyclic derivatives in high yields for biological evaluation. With the promising biological data obtained for compounds such as **183**, the future will allow for

the use of rational design and molecular modelling to identify potential lead compounds and targets for drug development. Improvements to the solubility of the compounds must be addressed and could lead to improvements in the biological results, this work is on going in the Murphy group and is being carried out by Dr. Jian Zhou and Mark Farrell.

3.9 References

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Chapter 4: Novel peptidomimetics: disrupting the p53/MDM2 interaction

4.1 The p53/MDM2 interaction as a target for cancer therapy

p53 ("p" for protein and 53 for its molecular weight of 53 kDa) is a well known transcription factor and tumour suppressor protein[1]. In times of cellular stress (e.g. DNA damage, hypoxia), an increase in the expression of wild type p53 induces cell cycle arrest[2] or apoptosis[3] (Figure 25).

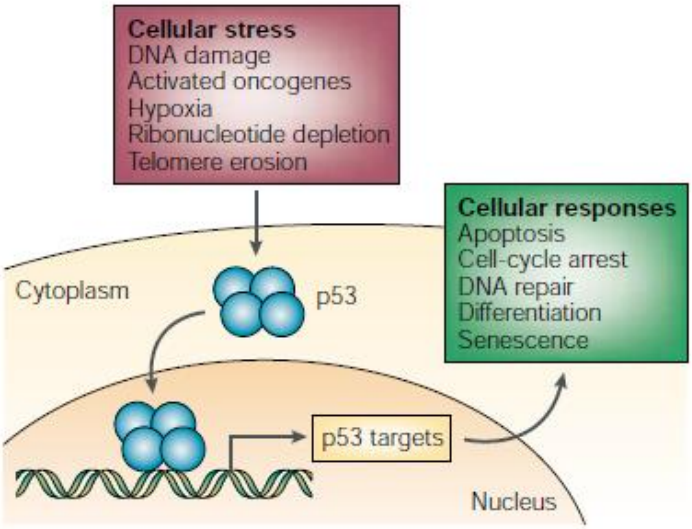

Figure 25 The p53 mediated response

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In normal cells p53 is present in low levels and is kept under strict control by the murine double minute 2 protein (MDM2) (the human form of this protein is called HDM2, however for the purposes of this thesis the MDM2 terminology will be used). The regulation of p53 by MDM2 is represented in Figure 26. Binding of p53 to MDM2 blocks the DNA binding affinity of p53 and helps export it away from the nucleus where it can be targeted by proteases and broken down via the ubiqutin-dependent proteasome pathway[4,5]. Under times of cellular stress, p53 is phosphorylated at or near to its MDM2 binding site thereby decreasing its affinity for MDM2 and activating it as a transcription factor[1]. In many tumours and especially soft-tissue cancers, osteosarcomas and oesophageal carcinomas[6,7], MDM2 expression is found to be highly amplified. This overexpression inhibits the ability of p53 to cause cell cycle arrest and/or apoptosis and leads to uncontrolled cell-proliferation[8]. Overexpression of MDM2 causes many modern cancer treatments to show a decrease in potency as the tumour cells are much less susceptible to the signals which trigger apoptosis through the p53 pathway. This in turn, leads to poor patient prognosis. Therefore, molecules

which disrupt the interaction of p53 and MDM2 have attracted interest from many research groups and have become a major goal in anticancer drug development[9].

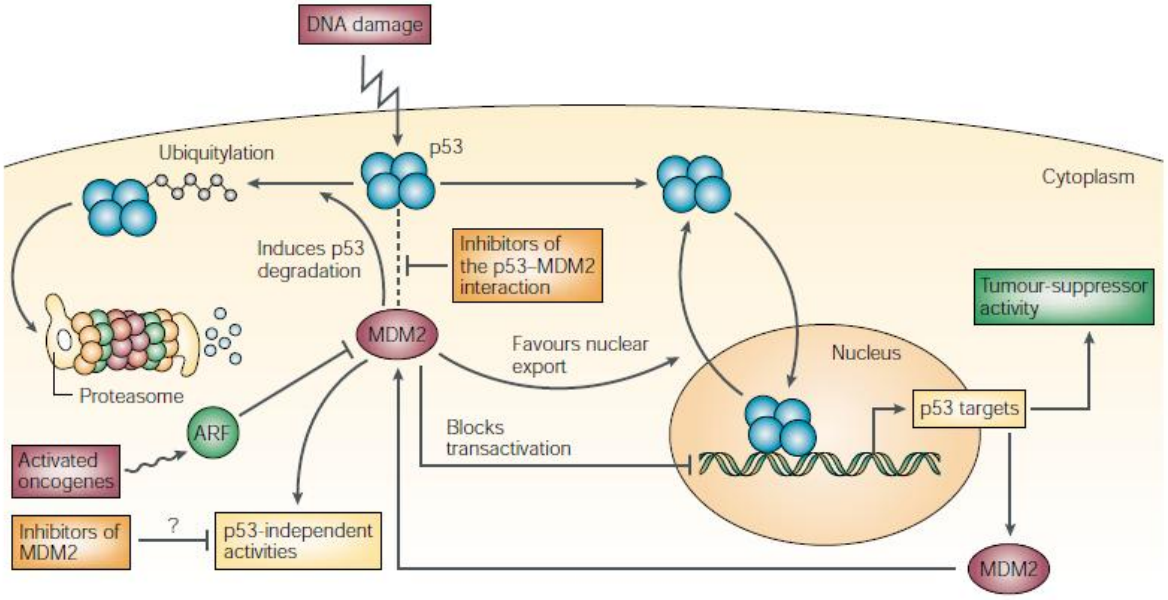

Figure 26 Regulation of p53 by MDM2

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4.2 Characteristics of the p53/MDM2 interaction

Interest in disruption of the p53/MDM2 interaction was sparked in 1996 when the co-crystal structure was published showing the exact protein-protein interface[10]. This structure showed that binding occurs in a hydrophobic cleft of MDM2 consisting of amino acid residues 18-102 and an α-helical portion of p53 consisting of residues 16-28. Three key 'hot residues' corresponding to Phe 19, Trp 23, and Leu 26 projected on one side of the p53 α-helical domain are ultimately responsible for this strong binding interaction[1] (Figure 27).

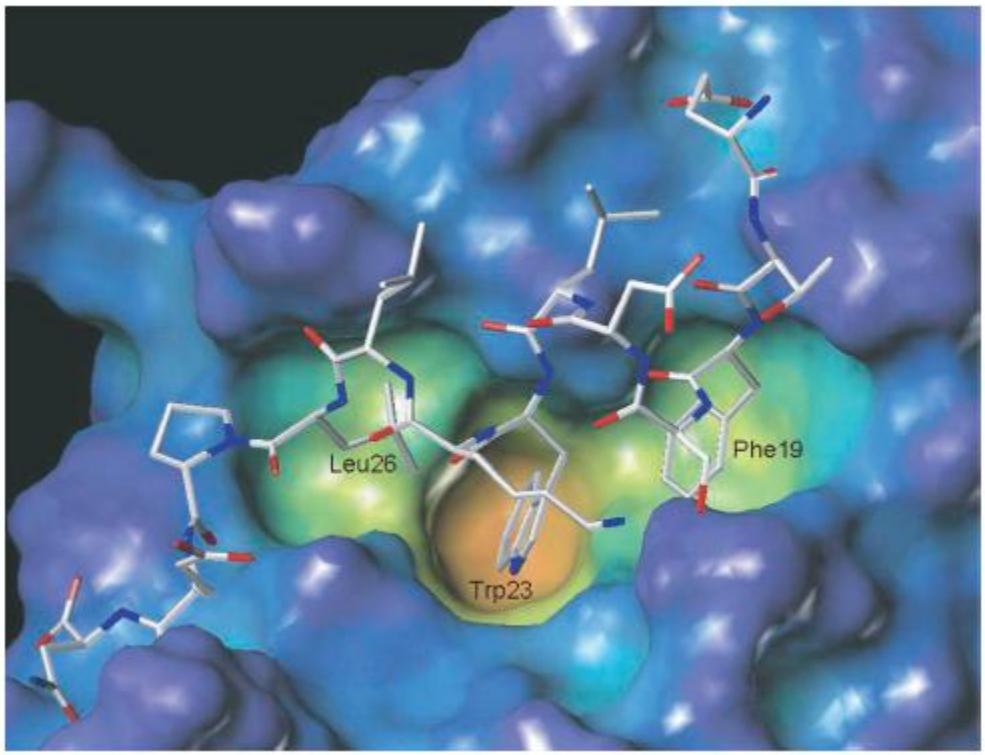


Figure 27[1] Crystal structure of p53 bound to MDM2. PDB, code 1YCR

4.3 Synthesis of macrocyclic peptidomimetics as potential MDM2 inhibitors

Since the elucidation of the p53/MDM2 crystal structure, numerous synthetic and natural inhibitors which show a disruption of this interaction have appeared in the literature.

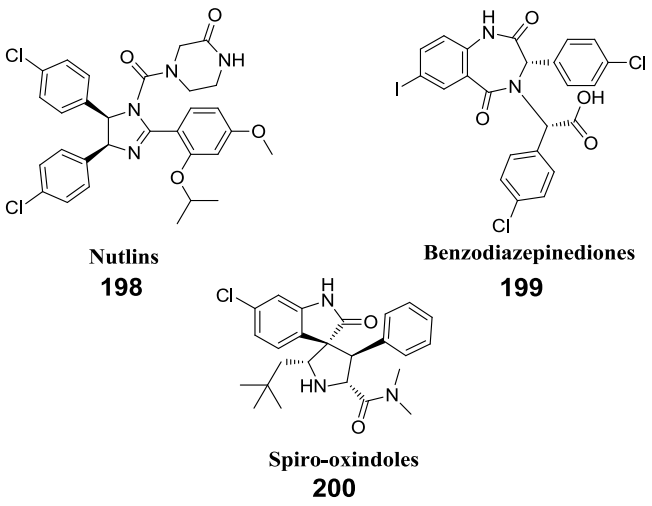
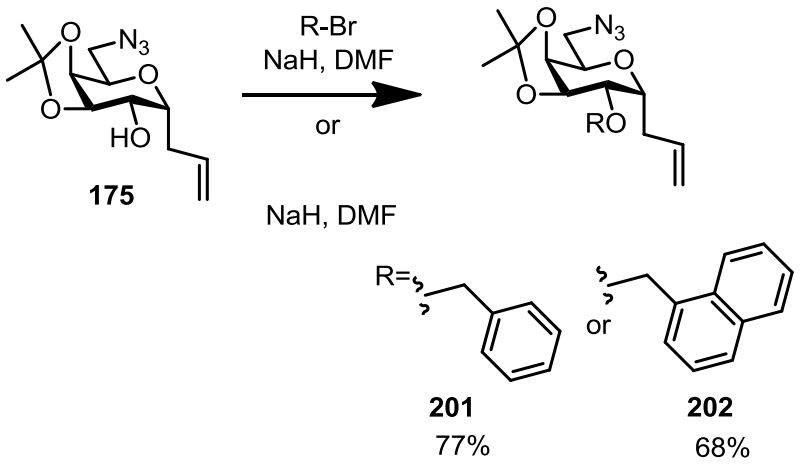


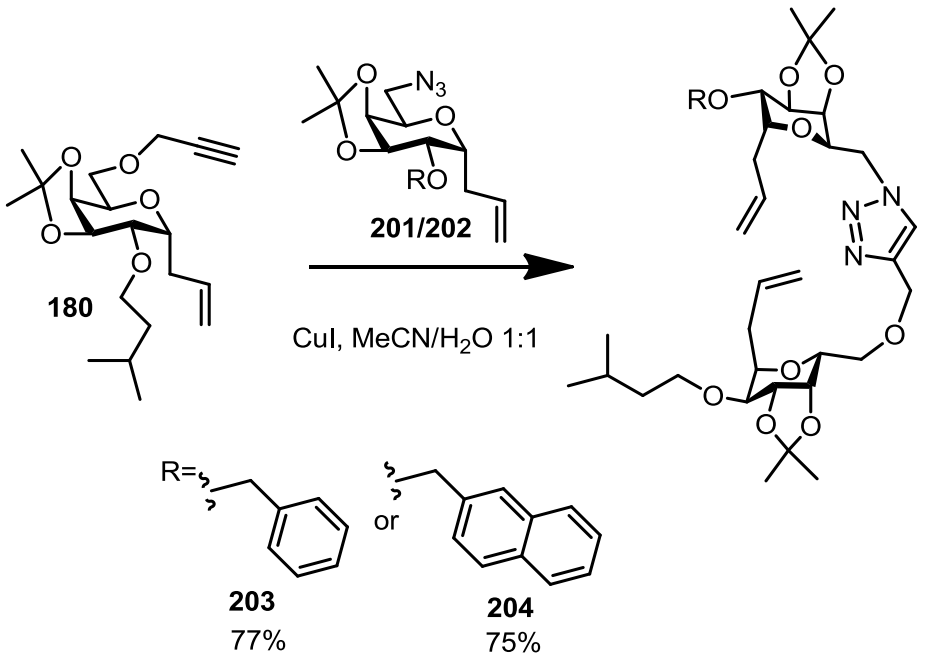
Figure 28 representative examples of known small molecule p53/MDM2 disrupters

Successful disruption of the p53/MDM2 interaction has been achieved with small molecule inhibitors such as nutlins (**198**)[11], benzodiazepinediones (**199**)[12] and spiro-oxindoles (**200**)[13] (Figure 28) as well as oligomeric α-helical peptidomimetics[14-16]. It is the latter type of inhibitor which is of interest to us. With the success of using carbohydrate based macrocycles in the synthesis of Bcl-2 family peptidomimetics as described in the previous chapter, it was decided to show the versatility of the novel macrocyclic scaffold in the synthesis of other α-helical peptidomimetics. The three 'hot residues', i, i+4 and i+7, that facilitate the p53/MDM2 interaction presented an ideal target for peptidomimetic development. The alkyne **180** was chosen to mimic the Leu 26 residue on the α-helix and was readily available through our previous work on Bcl-2 family inhibitors. With this monomer already in hand, attention was focused on synthesis of suitable azide monomers possessing aromatic groups on the C2 hydroxyl group. Following a similar reaction sequence to those described in section 3.6.2 the azide monomers **201** and **202** were synthesised via treatment of azide **175** with benzyl bromide and 2-(bromomethyl)naphthalene to give the aromatic derivatives in 77% and 68% yield respectively. The synthesis of a third aromatic derivative was also attempted via treatment of azide **175** with 2-(bromoethyl)benzene. This reaction failed to give any of the desired product, this is possibly due to a β-elimination process being more favoured under the basic reaction conditions.



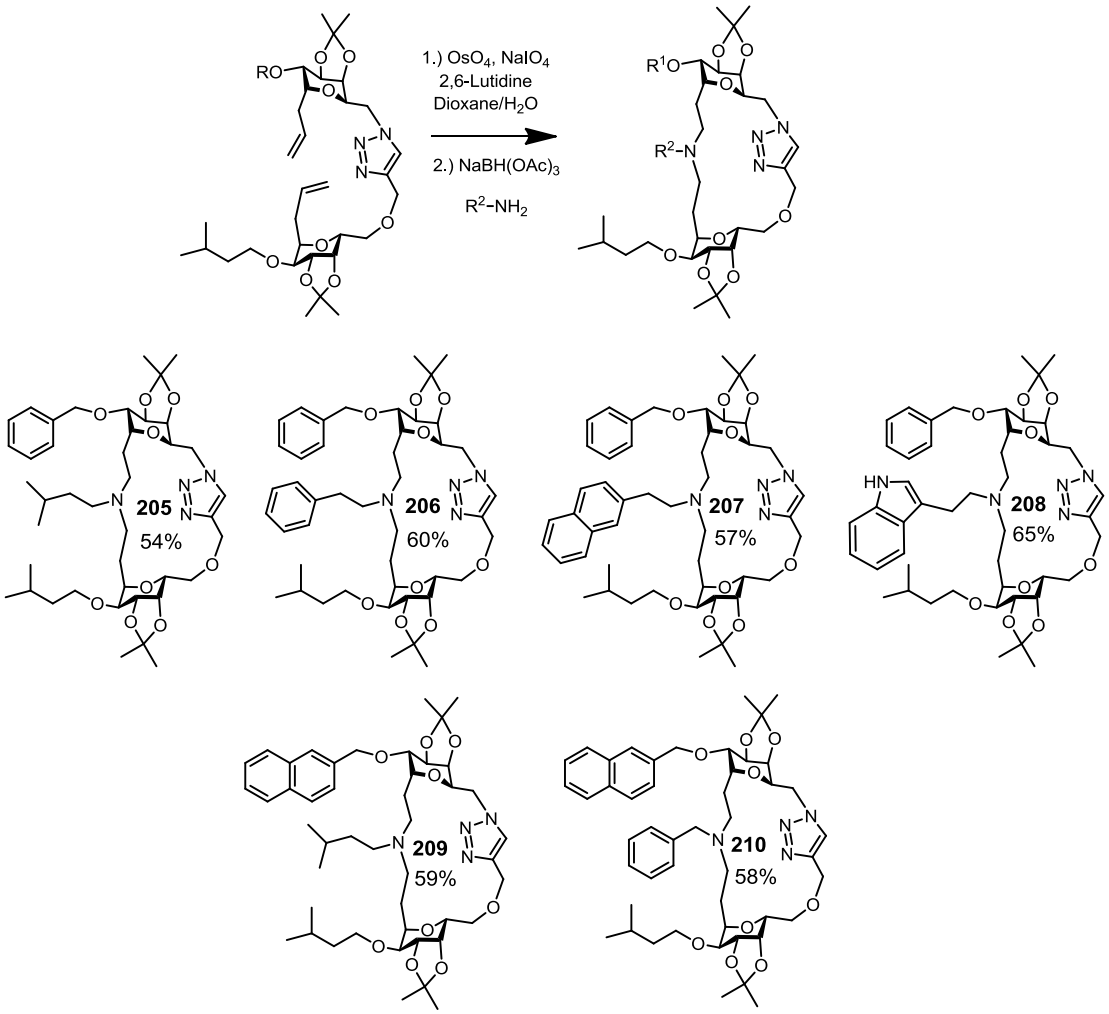
Scheme 52 Synthesis of aromatic monomers

Azides **201** and **202** were coupled with alkyne **180** under copper catalysed azide-alkyne cycloaddition conditions to give the dialkene derivatives **203** and **204** in high yields.



Scheme 53 Click reactions to give triazole derivatives **200** and **201**

Oxidative cleavage of dialkenes **203** and **204** using conditions described previously followed by the reductive amination macrocyclisation sequence gave access to the potential MDM2 inhibitors **205**-**210** in good yields (Scheme 54).



Scheme 54 Reductive amination/macrocyclisation sequence in the synthesis of potential p53/MDM2 inhibitors and a summary of the macrocycles synthesised

Compounds **205-210** were deprotected using TFA-H_2O 4:1 to give the polyhydroxylated macrocycles **211-216** in high yields (Figure 29).

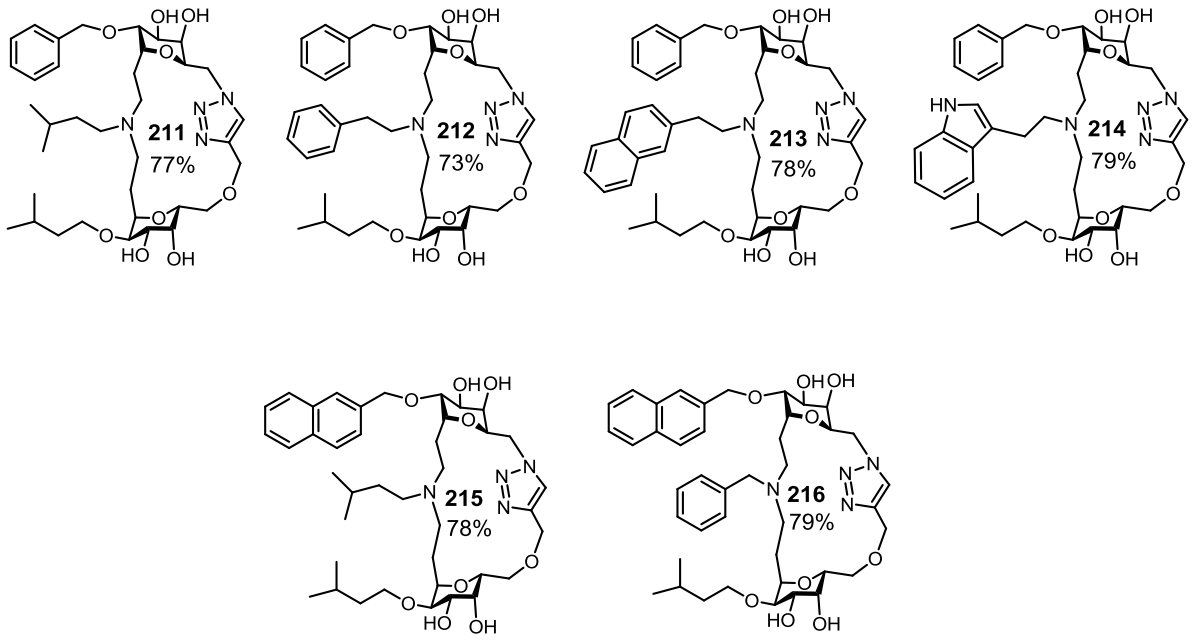


Figure 29 Polyhydroxylated p53/MDM2 inhibitors

In an effort to increase their cell permeability the compounds were acetylated with acetic anhydride in pyridine to give protected derivatives **217-221** in excellent yield (Figure 30).

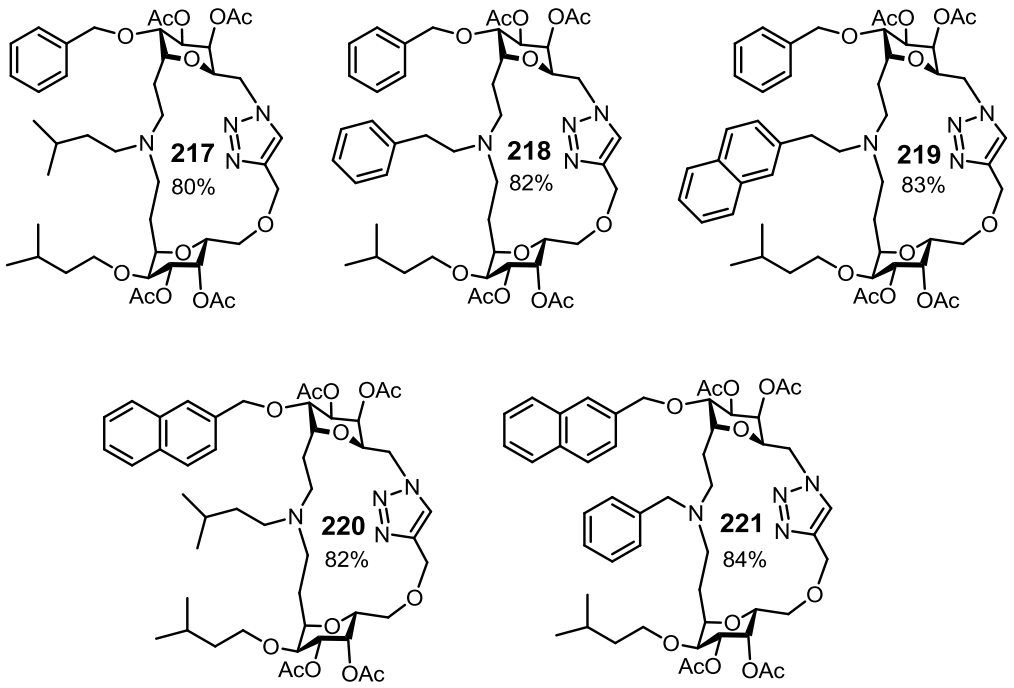
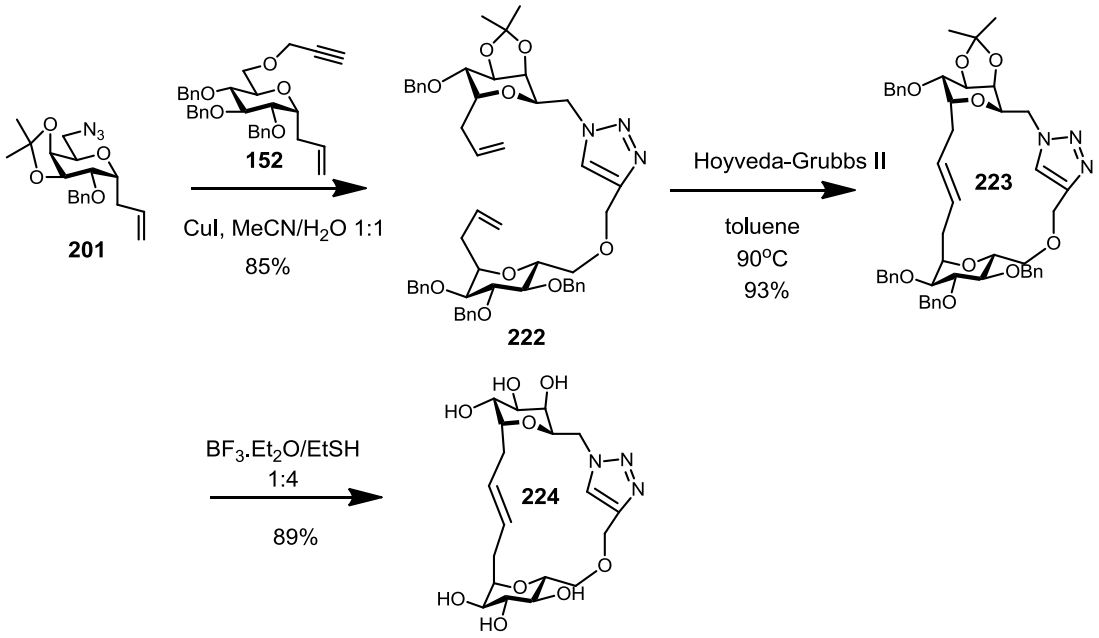


Figure 30 Acetylated p53/MDM2 inhibitors

4.4 Synthesis of a polyhydroxylated macrocycle

The hydrophobicity of such macrocycles means that there is a potential for them to participate in non-specific interactions in biological systems. In order to discount this possibility, a macrocycle lacking the amino acid epitopes at i, i+4 and i+7 was synthesised. The route to this compound is presented in Scheme 55.



Scheme 55 Synthesis of polyhydroxylated macrocycle **224**

Azide **201** and alkyne **152** were coupled under copper catalysed conditions to give the dialkene derivative **222** in 85% yield. As there was no desire to mimic the i+4 residue in this compound, macrocyclisation was achieved via the venerable ring closing metathesis reaction using the Hoveyda-Grubbs II catalyst in toluene at 90°C to give the protected macrocyclic derivative **223**. The compound was isolated as the trans isomer exclusively, indicated by *J* values of 15 Hz between the two alkenyl protons. Chauvin's mechanism for the ring closing metathesis reaction is shown in Figure 31[17]. [2+2] cycloaddition between an alkene and a transition metal alkylidene forms a metallocyclobutane intermediate. This metallocyclobutane then undergoes cycloreversion to give the initial alkylidene or a new alkylidene species. A second metallocyclobutane intermediate is then formed between this new alkylidene complex and a second alkene derivative. Cycloreversion of this metallocyclobutane releases the desired coupled product and gives a new alkylidene complex to restart the cycle.

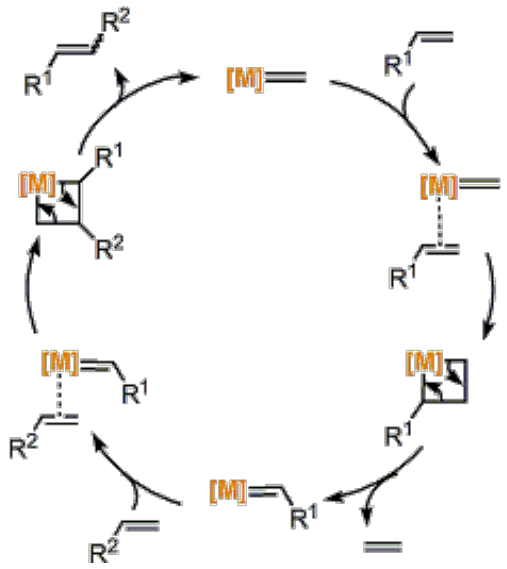


Figure 31 Chauvin's mechanism for ring closing metathesis

Finally, debenzylation with concomitant removal of the isopropylidene was facilitated via treatment with $BF_3.Et_2O$ and ethane thiol 1:4[18] to give the fully deprotected macrocycle derivative **224** in 89% yield.

4.5 Conclusion

In an effort to show the utility of the novel macrocyclic scaffold designed and synthesised in the previous chapter, six novel p53 mimics have been developed in the hope of disrupting the p53/MDM2 interaction. These compounds are currently awaiting biological evaluation. From the results obtained, it can be seen how easily the macrocyclic scaffold we have developed can be adapted to target other important protein-protein interactions. It is hoped that these initial compounds can provide a valuable insight into the p53/MDM2 interaction which in turn will lead to optimisation of the epitopes on the scaffold to provide α-helical mimetics with high potency. The synthesis of a macrocycle free of any amino acid epitopes, **224**, has also been achieved. This compound will be used as a negative control in the biological studies which will be performed in order to discount the possibility of non-specific interactions taking place.

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Chapter 5: Synthesis of a novel bicyclic iminocyclitol

5.1 Introduction to iminosugars

Iminosugars or iminocyclitols, make up a diverse family of alkaloids. These alkaloids are sugar analogues in which the endocyclic oxygen is replaced by a nitrogen atom. Recent years has seen a huge interest in both synthetic and naturally occurring iminosugars as biological tools and as potential therapeutic agents. This interest is due in part to the ability of iminosugars to interfere with carbohydrate processing enzymes. It is thought that these sugar analogues are capable of mimicking the transition state in the active site of pyranosidic or furanosidic glycosidase inhibitors thereby endowing them with potent biological activity. The inhibition of such glycosidase enzymes can cause wide and varied responses *in vivo* and can have considerable effects against numerous disease states including diabetes, bacterial infections, cancer and some sphingolipid storage diseases. A number of both natural and non-natural iminosugars have subsequently found their way into clinical use[1,2]. The three main families of naturally occurring iminosugars are: polyhydroxylated piperidines e.g. **225** & **226**, indolizidines (fused piperidine and pyrrolidine) e.g. **227** & **228**, and nortropanes e.g. **229** & **230** (Figure 32).

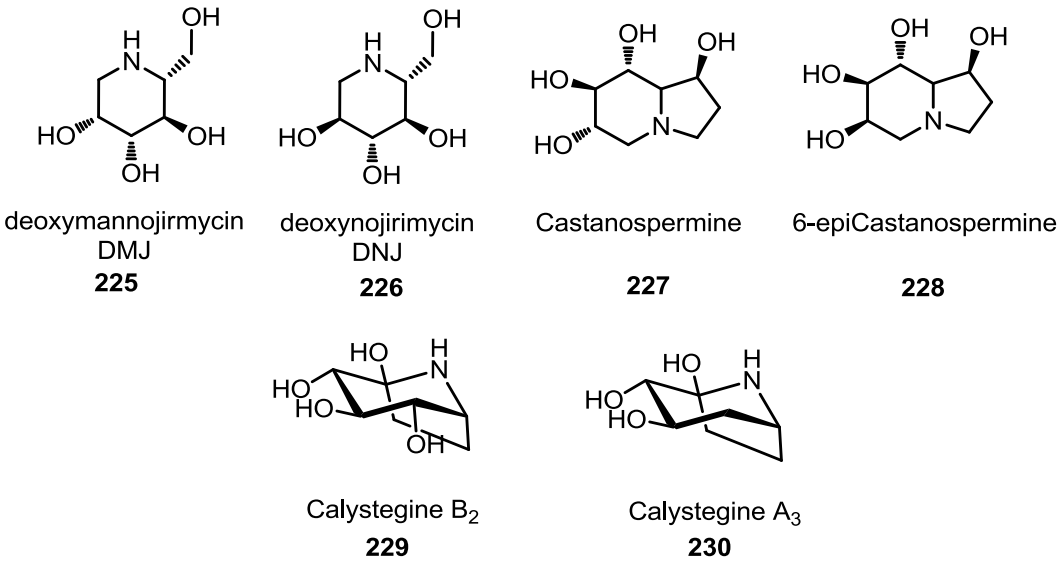


Figure 32 Naturally occurring iminosugars: Piperidines (DMJ & DNJ), indolizidines (Castanospermine & 6-epi-Castanospermine) and nortropanes (Calystegines B2 &A3)

5.2 Use of iminosugars as scaffolds for peptidomimetic development

As stated in chapters 3 and 4, recent years has seen widespread interest in the use of sugars as scaffolds for peptidomimetic development. The pioneering work of Nicolaou and Hirschmann showed that β-D-glucopyranoside can be used as a suitable scaffold onto which pharmacophoric amino acid side chains can be grafted. This strategy was used to great effect in the synthesis of somatostatin mimics such as **232**[3]. Monosaccharides have a number of advantages which make them ideal for peptidomimetic development, they are chiral, have rigid conformations and contain multiple sites on which to graft pharmacophoric groups[4]. Iminosugars however, are under-utilised as scaffolds, perhaps due to the difficulties often encountered during their synthesis. These sugar analogues would offer a number of advantages over other monosaccharide scaffolds as the presence of the nitrogen atom in the ring would allow for the introduction of pharmacophoric groups at a very useful site on the molecule and also for the possibility of incorporating a charged nitrogen atom which could led to increased hydrogen bonding interactions between ligand and receptor. Indeed, some success has already been achieved within the Murphy group through the synthesis of novel somatostatin mimics using both DNJ (**233**) and DMJ (**234**) as scaffolds (Figure 33)[5-7].

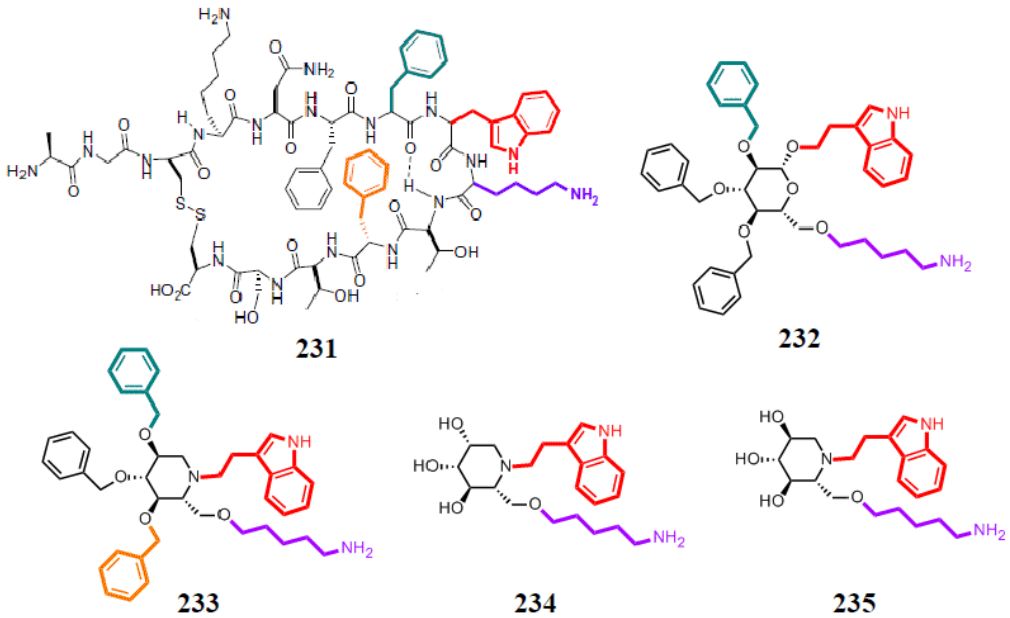


Figure 33 Somatostatin 230, β-D-glucopyranoside mimic **232** and representative Murphy group iminosugar derivatives **233**, **234** and **235**

5.3 Novel access to iminosugar derivatives

There is, however, still a great desire to develop new and more efficient approaches to the synthesis of both natural and novel iminosugar derivatives. This would allow for their widespread use in medicinal and bioorganic chemistry. Recently, intense efforts within in the Murphy group have focused on the development of novel methodology to access iminosugar derivatives.

The intramolecular Huisgen 1,3-dipolarcycloaddition reaction[8] between an azide and an alkene has recently been utilised by Dr. Ying Zhou as part of a sequence of reactions in the synthesis of DNJ derivatives[9].

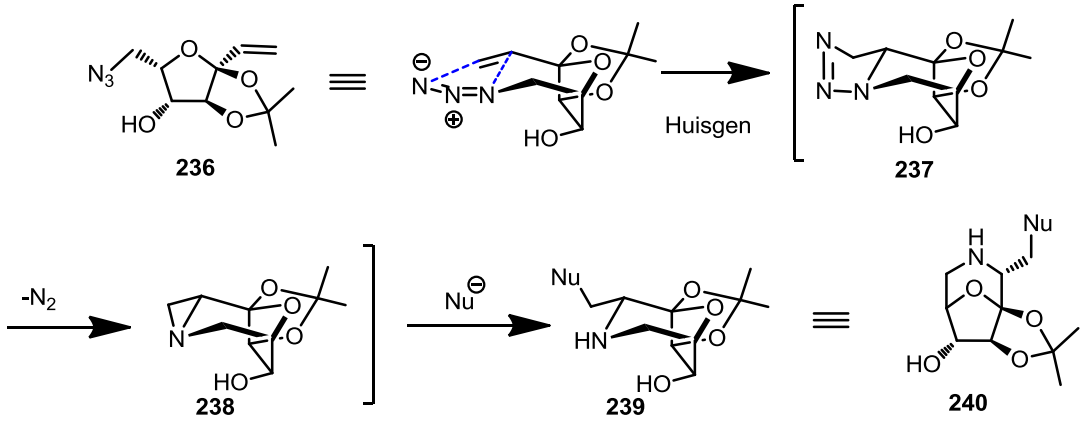
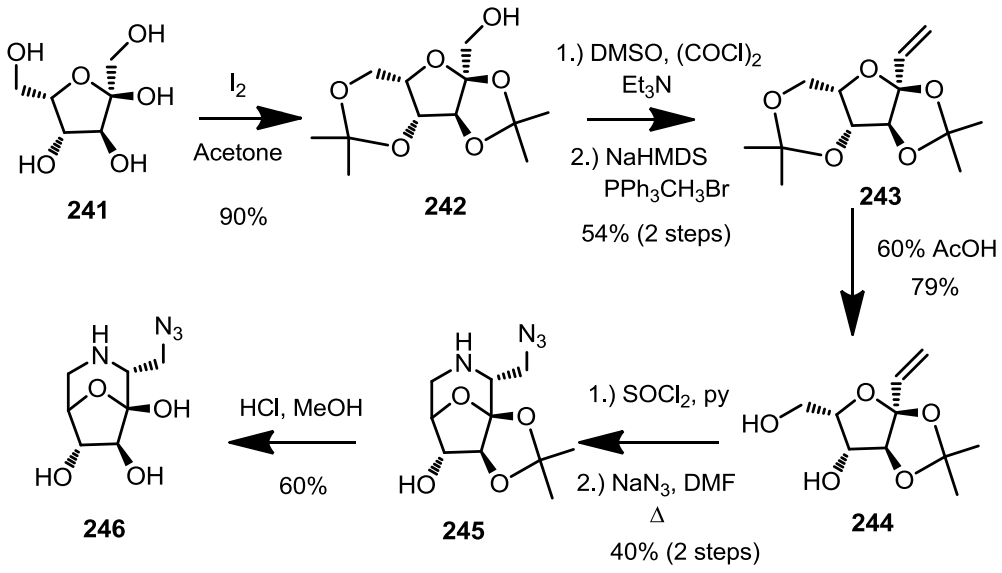


Figure 34 Proposed synthesis of iminosugars via use of azide alkene cycloaddition reaction

As an extension of this methodology, it was envisaged that a novel polyhydroxylated iminosugar **240** (Figure 34) could be derived from the intramolecular Huisgen cycloaddition of the azide derivative **236**. It was our belief that a thermally promoted 1,3-dipolarcycloadditon would give a triazoline derivative such as **237**. As with the DNJ synthesis it was anticipated that minimization of allylic strain would lead to the formation of a single triazoline stereoisomer **237**. Extrusion of molecular nitrogen was then expected to give aziridine derivative **238**[10]. It was then anticipated that **238** would then undergo nucleophilic ring opening to give novel polyhydroxylated iminosugar derivatives. It was proposed that the synthesis of **236** could be achieved from commercially available L-sorbose.



Scheme 56 Synthesis of novel iminosugar derivative from L-sorbose

5.4 Synthesis of a novel bicyclic iminosugar derivative

Treatment of L-sorbose **241** (Scheme 56) with I_2 in acetone gave di-isopropylidene-L-sorbose **242** in high yield and multi-gram quantities[11]. Oxidation of the remaining free primary alcohol under Swern conditions followed by Wittig olefination gave alkene derivative **243**[12]. Regioselective ring opening of the more labile isopropylidene group was achieved through treatment with 60% acetic acid at 60°C to give the diol **244** in good yield. In order to facilitate the introduction of the azide, the diol **244** was converted into a cyclic sulfite via treatment with thionyl chloride and pyridine. The crude cyclic sulfite was treated with 5 equivalents of sodium azide at 120°C in the hopes of obtaining **236**. However upon isolation, compound **245** was obtained as a white crystalline solid and no other by-products were detected. The formation of **245** can be explained by a cascade sequence which can occur under the reaction conditions. The proposed mechanism for this transformation is shown in Scheme 57. The structure of **245** was confirmed through x-ray analysis (Figure 35)[13].

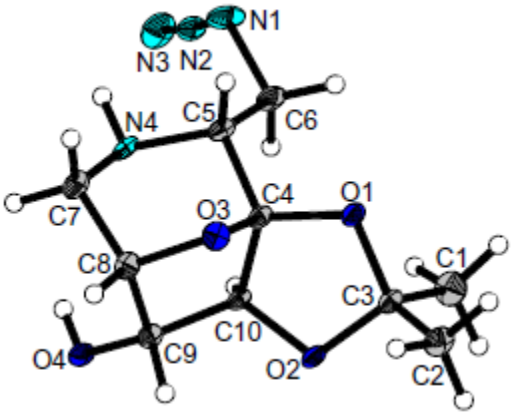
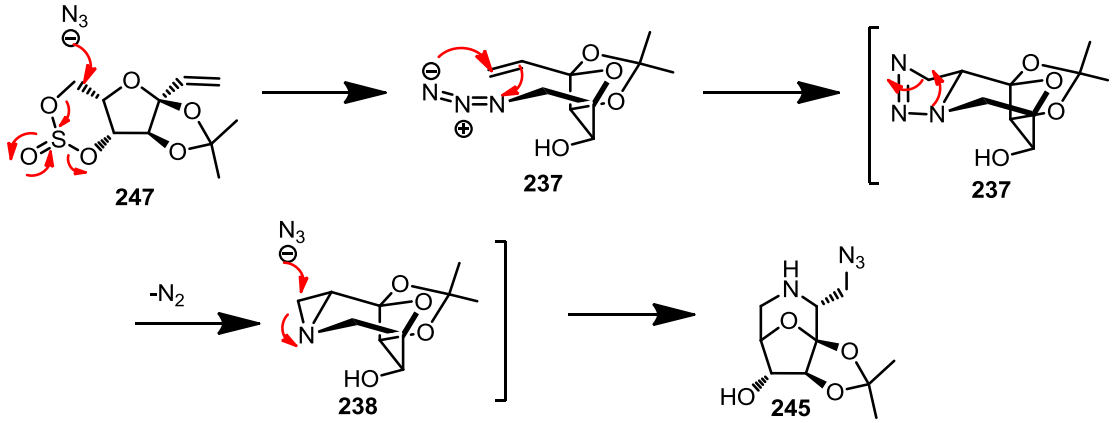


Figure 35 ORTEP representation of the crystal structure of **245**. The atomic displacement parameters are at the 50% level. CCDC 731460

The reaction was repeated using differing numbers of equivalents of sodium azide in the hope of trapping the aziridine intermediate however these attempts all failed to deliver the desired compound **238**. Removal of the remaining isopropylidene from **245** was facilitated with HCl in methanol to give the bicyclic iminocyclitol derivative **246** in 60% yield.

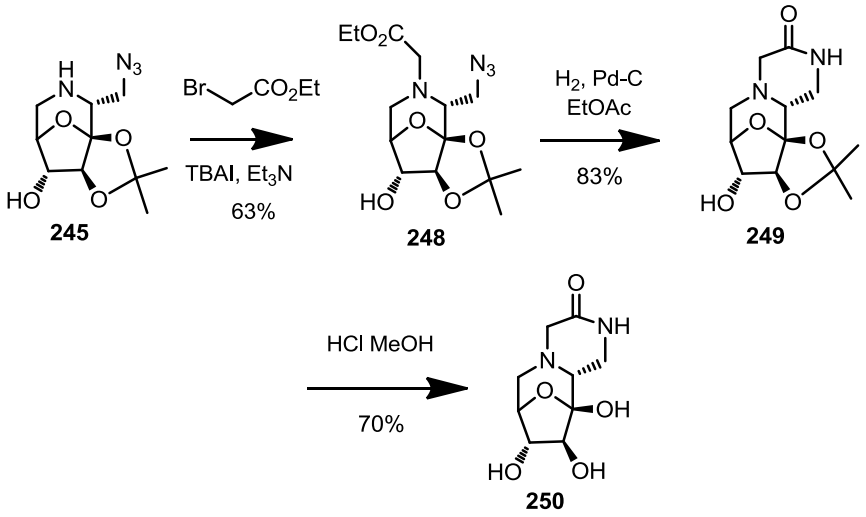


Scheme 57 Proposed mechanism for the formation of **245**

5.5 Synthesis of a novel tricyclic framework

In an effort to show the potential utility of compound **245** as a starting material for the synthesis of more complex molecules, alkylation of the secondary amino group with ethyl bromoacetate was carried out in the presence of catalytic tetrabutylammonium iodide at reflux to give the protected sugar amino acid derivative in 61% yield (Scheme 58). Compound **248** could be considered a protected sugar amino acid[14]. Catalytic hydrogenation of **248** led to spontaneous lactam formation to give the novel tricyclic derivative **249** in high

yield. Deprotection with methanolic HCl as before gave polyhydroxylated tricycle **250** in 70% yield.



Scheme 58 conversion of **245** in a novel tricyclic framework

5.6 Conclusion

A novel iminocyclitol containing an ether bridge has been synthesised in a stereoselective and efficient manner starting from L-sorbose. The route involves a one pot nucleophilic substitution, Huisgen cycloaddition, triazoline decomposition, aziridine formation and aziridine ring opening by an azide anion. This provides a molecule which could be of great interest to medicinal chemists. 8-oxa-3-azabicyclo[3.2.1]-octanes such as **246** are of medicinal interest[15,16]. Cope has reported the achiral 8-oxa-3-azabicyclo[3.2.1]-octane as an analgesic and anti-inflammatory agent which shows efficacy *in vivo*[17]. The protected derivative **245** can be considered a conformationally constrained morpholine type structure[18] and possess a similar structural motif to nortropane type iminosugars which have shown to be potent glycosidase inhibitors[19]. The constrained structure of this amine means that this molecule could also be of use as an organocatalyst although this has not been examined. The protected derivative also gives the opportunity for further development as a scaffold for peptidomimetic research, both the secondary alcohol and the secondary amine can be selectively alkylated, the presence of the azido group means this compound could be used in 'click reactions' or alternatively reduction to the primary amino group and alkylation would give access to further derivatives. Also shown is the utility of this compound as a scaffold for the synthesis of the tricyclic lactam **250**.

5.7 References

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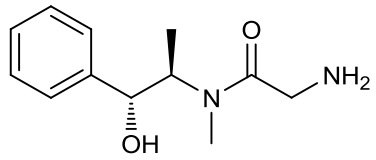
Chapter 6: Experimental Data

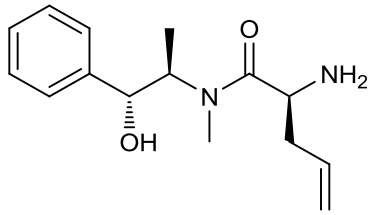
6.1 General Experimental Conditions

Optical rotations were determined at the sodium D line at 20°C. NMR spectra were recorded with 600, 500 and 400 MHz Varian spectrometers. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ (δ 0.0), HOD for D_2O (δ 4.84) or CD_2HOD (δ 3.31) for ^1H and Me_4Si in $CDCl_3$ (δ 0.0) or $CDCl_3$ (δ 77.0) or CD_3OD (δ 49.05) for ^{13}C. ^1H NMR signals were assigned with the aid of COSY. ^{13}C NMR signals were assigned with the aid of DEPT, gHSQCAD and/or gHMBCAD. Coupling constants are reported in hertz. The IR spectra were recorded using thin film on a NaCl plate or with ATR attachment. Low and high resolution mass spectra were in positive and/or negative mode as indicated in each case. Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel and spots visualized by UV and charring with H_2SO_4-EtOH (1:20), or cerium molybdate. Flash chromatography was carried out with silica gel 60 (0.040-0.630 mm) and using a stepwise solvent polarity gradient correlated with TLC mobility. CH_2Cl_2, MeOH, and THF reaction solvents were used as obtained from a Pure Solv™ Solvent Purification System. Anhydrous DMF, pyridine, and toluene were used as purchased from Sigma-Aldrich. Chromatography solvents, petroleum ether, cyclohexane and ethyl acetate were used as obtained from suppliers (Sigma-Aldrich).

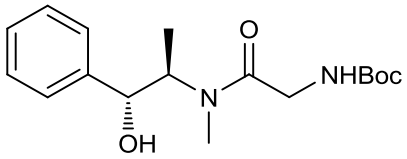
6.2 Chapter 2-Experimental



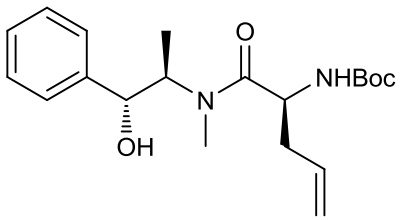
2-Amino-*N*-((1*R*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)-*N*-methylacetamide (Pseudoephedrine glycinamide)[1] (59). A flame dried round bottom flask stored under argon was charged with anhydrous LiCl (5.13 g, 121 mmol), (*R*,*R*)-(-)-pseudoephedrine (10 g, 61 mmol) and THF (90 mL). The resulting slurry was stirred at 0°C for 15 min. Solid lithium methoxide (1.1 g, 30 mmol) was then added to the slurry and the reaction mixture stirred for a further 10 mins. A solution of glycine methyl ester (6.7 g, 76 mmol) in THF (20 mL) was then added dropwise over 1 h and the reaction mixture was stirred at 0°C for 8 h. The reaction was terminated via the addition of H_2O (100 mL) and the bulk of the THF was removed under reduced pressure. The aqueous solution was then diluted with H_2O and extracted into CH_2Cl_2. The combined organic extracts were then dried with K_2CO_3 and the solvents were removed under reduced pressure. The crude product was then recrystallized from THF-H_2O to give (*R*,*R*)-(-)-pseudoephedrine glycinamide monohydrate as a white crystalline solid (10.3 g, 72%). The monohydrate was then dissolved in CH_2Cl_2 and stirred for 1 h. To this was added anhydrous K_2CO_3 and the reaction stirred until translucent. The mixture was then filtered and concentrated under reduced pressure. The oily residue was recrystallised from hot toluene to give anhydrous **59** as a white solid. Analytical data were in good agreement with those reported in the literature; $[\alpha]_D$ -103.1° (c 1.1, MeOH); IR (film) cm^{-1}: 3360, 2989, 1630, 1486, 1454, 1312, 1126, 1040, 926; ^1H NMR (500 MHz, 1:1 mixture of rotamers, $CDCl_3$) δ 7.37-7.26 (5H, m, Ar-H), 4.64-4.55 (1.5H, m), 3.78 (0.5H, m), 3.67 (0.5H, d, *J* 15.4), 3.30 (1.5H, m), 2.91 (1.5H, s), 2.76 (1.5H, s), 0.99 (1.5H, d, J 6.6), 0.93 (1.5H, d, 6.6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.0, 173.5, 142.1, 141.8, 128.5, 128.4, 128.1, 126.7, 126.6, 75.6, 74.9, 57.4, 57.2, 43.5, 43.2, 26.9, 15.3, 14.1; ESI-HRMS calcd for $C_{12}H_{19}N_2O_2$ 223.1447, found *m*/*z* 223.1440 $[M+H]^+$



(*S*)-2-Amino-*N*-((1*R*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)-*N*-methylpent-4-enamide[1] (83). A flame dried round bottom flask stored under argon was charged with THF (30 mL) and diisopropylamine (5 mL, 36 mmol). The solution was cooled to 0°C and deoxygenated under high vacuum. To this was added n-BuLi (12 mL of 2.5 M in hexanes) dropwise over 20 min. After a further 20 min the resulting LDA solution was transferred via cannula to a separate flask containing a solution of anhydrous lithium chloride (4.6 g, 107 mmol) and **59** (4 g, 18 mmol) in THF (40 mL) at 0°C. The rate of addition was carefully monitored so as the internal temperature of the mixture did not rise above 5°C. The yellow enolate solution was stirred for a further 30 min and allyl bromide (1.7 mL, 20 mmol) was added dropwise while keeping the temperature below 5°C. After stirring for 1 h the reaction was terminated via the addition of H_2O. The biphasic mixture was then extracted into EtOAc. Phases were separated and the organic phase was extracted into 3M HCl solution. The aqueous layers were combined and cooled to 0°C. The solution was basified via the addition of cold 50% NaOH solution. The basic solution was then extracted into CH_2Cl_2. The combined organic layers were then dried over K_2CO_3 and the solvents were concentrated under reduced pressure. The crude residue was recrystallized from hot toluene to give (*R*,*R*)-(-)-Pseudoephedrine-L-allylglycinamide (2.7 g, 58%) as a white solid. NMR data (^1H and ^{13}C) was in agreement with reported literature data; $[\alpha]_D$ -79.0° (c 1.0 MeOH); IR (film) cm^{-1}: 3356, 3071, 2978, 1631, 1491, 1453, 1109, 1049, 703; ^1H NMR (500 MHz, 3:1 rotamer ratio, $CDCl_3$) major rotamer δ 7.38-7.23 (5H, m), 5.85-5.64 (1H, m, CH_2CH=CH_2), 5.14-5.07 (2H, m, CH_2CH=CH_2), 4.59-4.45 (2H, m), 3.65 (1H, dd, *J* 7.5, 5.3), 2.87 (3H, s, NCH_3), 2.23 (1H, m, CHHCH=CH_2), 2.13 (1H, m, CHHCH=CH_2), 1.03 (3H, d, *J* 6.4, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1 (C=O), 142.1 (CH_2CH=CH_2), 133.7, 128.2, 127.6, 126.5, 118.1 (CH_2CH=CH_2), 75.5 (CHOH), 57.6 (CHN), 51.2 (CH), 39.6 (CH_2CH=CH_2), 31.4 (CH_3N), 14.4 (CH_3); ESI-HRMS calcd for $C_{15}H_{23}N_2O_2$ 263.1759, found *m*/*z* 263.1749 $[M+H]^+$

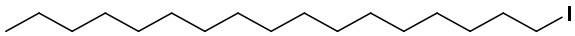


***N*-Boc-2-amino-*N*-((1*R*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)-*N*-methylacetamide[1] (82).** Trimethyl acetyl chloride (10.5 mL, 85.7 mmol) was added dropwise to a stirring solution of *N*-Boc-glycine (15 g, 85.7 mmol) and triethylamine (13 mL, 94 mmol) in CH_2Cl_2 (300 mL) at 0°C. After 40 min a second portion of triethylamine (13 mL, 94 mmol) was added, followed by the rapid addition of solid (*R*,*R*)-(-)-pseudoephedrine (14 g, 85.7 mmol). The reaction mixture was stirred for 1 h, diluted with 1M HCl solution, brine and EtOAc. Phases were separated and the aqueous layer was extracted into EtOAc. The combined organic phases were washed with satd K_2CO_3, dried over $MgSO_4$ and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:1) to give **82** (23.8 g, 86%) as a viscous oil; ^{1}H NMR (500 MHz, 1:1 rotamer ratio, $CDCl_3$) δ 7.39 – 7.29 (5H, m, Ar-H), 5.62 (1H, m, RC*H*OH), 4.66 – 4.57 (1H, m), 4.16 (1H, d, *J* 16.4), 4.00 (1H, dd, *J* 16.5, 4.6), 3.88 (1H, t, *J* 11.3, C*H*HNH$_2$), 3.86 – 3.78 (1H, m, CH*H*NH$_2$), 2.92 (1H, d, *J* 11.1), 2.81 (2H, s), 1.45 (9H, s, Boc), 1.02 (2H, d, *J* 6.6), 0.95 (1H, d, *J* 6.6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 169.6*, 156.0, 155.9*, 141.7, 141.5*, 128.7, 128.4*, 128.4, 127.9, 126.8, 126.5*, 79.6, 79.5*, 75.8, 75.1, 57.5, 57.2*, 42.8, 42.5*, 28.3, 28.3*, 27.12, 15.1, 14.2*; ESI-HRMS calcd for $C_{17}H_{27}N_2O_4$ 323.1971, found *m/z* 323.1966 $[M+H]^+$

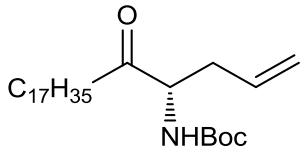


***N*-Boc-(*S*)-2-amino-*N*-((1*R*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)-*N*-methylpent-4-enamide (84).** n-BuLi (28.8 mL of 2.5 M in hexanes) was added dropwise to a stirred suspension of anhydrous lithium chloride (5.9 g, 139.6 mmol) and diisopropylamine (10.5 mL, 74.4 mmol) in THF (140 mL) at -78 °C. After stirring for 10 min a solution of **82** (7.5 g, 23.3 mmol) in THF (130 mL) was added via cannula. The resulting yellow solution was stirred for 20 min at -78 °C and then warmed to 0 °C. After 20 min at 0 °C, allyl bromide was added dropwise. After 2 h the reaction was terminated by the addition of 1 M aqueous HCl.

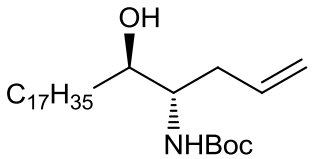
The phases were separated and the aqueous phase was extracted with EtOAc. The combined organic phases were then washed with satd $NaHCO_3$, dried over $MgSO_4$ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc, 1:1) gave **84** (5.9 g**,** 70%) as a yellow oil; $[\alpha]_D$ -26.1° (c 0.79, $CHCl_3$); ^1H NMR (500 MHz, 2:1 ratio of rotamers, the asterisk denotes signals of the minor rotamer, $CDCl_3$); δ 7.35-7.24 (5H, m, Ar-H), 5.76* (1H, ddt, *J* CH=CH_2), 5.68 (1H, ddt, *J* , CH=CH_2), 5.55 (1H, d, *J* 8.1,), 5.45 (1H, d, *J* 9.1), 5.08 (2H, m), 4.68* (1H, m), 4.57 (3H, m), 4.17* (1H, m), 2.89 (3H, s), 2.54* (1H, m), 2.37* (1H, m), 2.25 (1H, m), 1.40 (9H, s, *t*-Bu), 0.98 (3H, d, *J* 6.5), 0.90* (3H, d, *J* 6.6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 172.4*, 155.3, 155.1*, 141.7, 141.4*, 133.7*, 132.6, 128.5, 128.1*, 127.5, 126.8*, 126.5, 118.3, 117.6*, 79.5, 79.2*, 75.3, 75.1*, 57.8, 50.4, 49.7*, 37.1,* 36.9, 28.1, 26.7, 15.3*, 14.1; IR (film) cm^{-1}: 3411(br), 2978, 2933, 1697, 1625, 1493, 1453, 1366, 1249, 1165; ESI-HRMS calcd for $C_{20}H_{31}N_2O_4$ 363.2283, found *m/z* 363.2285 $[M+H]^+$



1-Iodoheptadecane (91). Iodoheptadecanol (25 g, 97 mmol) and triethylamine (40.7 mL, 292 mmol) were dissolved in CH_2Cl_2 (200 mL) and cooled to 0°C. To this was added methanesulfonyl chloride (9 mL, 116 mmol) dropwise. The solution was allowed warm to room temperature and stirred for 4 h. The reaction was diluted with H_2O and washed with 1M HCl solution. The organic phase was washed with brine, dried over $MgSO_4$ and the solvents were concentrated under reduced pressure. The crude mesylate was dissolved in acetone (250 mL) and NaI (72 g, 485 mmol) was added and the reaction was heated at reflux overnight. H_2O and EtOAc were added and the phases were separated. The organic phase was washed with water, brine dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified via flash chromatography (petroleum ether-EtOAc 19:1) to give iodide **91** (28.4 g, 80%) as a white solid; ^1H NMR (500 MHz, $CDCl_3$) δ 3.17 (2H, t, *J* 7.5, CH_2I), 1.82-1.77 (2H, m, CH_2), 1.39 (2H, dd, *J* 14.9, 7.0, CH_2), 1.24 (26H, s, each alkyl CH_2), 0.88 (3 H, t, *J* 6.9, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 31.9, 30.9, 29.6, 29.6, 29.6, 29.5, 29.3, 29.0, 28.7, 25.5, 24.5, 22.6 (each CH_2), 14.1 (CH_3)

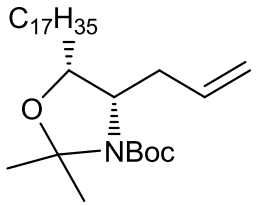


(*S*)-*t*-Butyl 5-oxodocos-1-en-4-ylcarbamate (88). To a stirred solution of **91** (5.4 g, 14.86 mmol) in pentane-Et_2O (50 mL, 3:2) at -78 °C was added t-BuLi (18.2 mL, 1.6M in hexanes) dropwise. The solution was stirred for 10 min at -78°C, warmed to room temperature and stirred for 1 h. The resultant suspension was then added via cannula to a stirred solution of **84** (4.4 g, 11.4 mmol) in THF (25 mL) at -78 °C. After a further 10 min the reaction flask was transferred to an ice-bath and stirred for 2 h. The reaction mixture was then poured slowly onto a mixture of crushed ice and satd NH_4Cl and extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 15:1) gave **88** (3.7 g, 75%) as a white solid. R_f 0.75 (petroleum ether-EtOAc, 9:1); $[\alpha]_D$ +31.5° (c 1.4, $CHCl_3$); IR (film) cm^{-1}: 2925, 2854, 1706, 1493, 1367, 1264, 1167 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.66 (1H, ddt, *J* 17.1, 9.9, 7.1, H-2), 5.22 (1H, d, *J* 6.7, NH), 5.11 (2H, d, *J* 13.5, H-1), 4.36 (1H, dd, *J* 12.1, 5.9, H-4), 2.64 – 2.54 (1H, m, H-3a), 2.48 (2H, dd, *J* 9.2, 5.6, H-6), 2.43 – 2.33 (1H, m, H-3b), 1.63 – 1.53 (2H, m, H-7), 1.44 (9H, s, *t*-Bu), 1.26 (28H, s, each CH_2), 0.88 (3H, t, *J* 6.8, CH_3). NMR (125 MHz, $CDCl_3$) δ 208.8 (C=O), 155.3 (C=O), 132.4 (C-2), 118.9 (C-1), 79.6 (tBu), 58.6 (C-4), 40.0 (C-6), 36.0 (C-3), 31.9, 29.7 (4s) 29.6, 29.5 (each CH_2), 29.4 (*t*-Bu), 29.2, 28.3, 23.4, 22.7 (each CH_2), 14.1 (CH_3); ESI-HRMS calcd for $C_{27}H_{51}NO_3Na$ 460.3767, found *m/z* 460.3759 $[M+Na]^+$

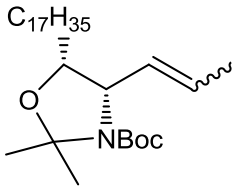


***t*-Butyl (4*S*,5*R*)-5-hydroxydocos-1-en-4-ylcarbamate (92).** The ketone **88** (1.94 g, 4.4 mmol) was taken up in dry EtOH (35 mL) and cooled to -78 °C. LiAl(O-*t*-Bu)$_3$H (2.88 g, 26.6 mmol) was added portion-wise over 1 h. After stirring at -78 °C overnight the reaction was then diluted with CH_2Cl_2 and treated with a 10% citric acid (60 mL). The mixture was stirred at room temperature for 2 h and extracted into CH_2Cl_2. The combined organic layers were washed with H_2O, brine, dried over $MgSO_4$ and the solvent removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 6:1) gave the alcohol

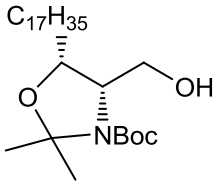
92 (1.65 g, 85%) as a white solid; R_f 0.25 (petroleum ether-EtOAc 9:1) $[\alpha]_D$ +7.6° (c 1.2, $CHCl_3$); IR (film) cm^{-1}: 3345, 2917, 2849, 1684, 1527, 1262, 1172, 1014; ^1H NMR (500MHz, $CDCl_3$) δ 5.82 (1H, ddt, *J* 17.1, 9.9, 7.1, H-2), 5.14 – 5.06 (2H, m, H-1), 4.68 (1H, br s, OH), 3.65 (2 H, overlapping signals, H-4 & H-5), 2.32 (1H, m, H-3a), 2.23 – 2.13 (1H, m, H-3b), 1.51 (1H, s), 1.44 (12H, overlapping signals, CH_2 & *t*-Bu), 1.26 (30H, s, each alkyl CH_2), 0.88 (3H, t, *J* 6.8, CH_3);NMR (125MHz, $CDCl_3$): δ 156.2 (C=O), 134.9 (*C*H=CH_2), 117.5 (CH=*C*H_2), 79.4, 74.1 (-CH-), 54.6 (-CH-), 34.1, (*C*H_2-CH=CH_2), 33.3, 32.0, 29.7 (3s), 29.6 (2s), 29.4 (each CH_2), 28.4 (*t*-Bu), 26.1, 22.7 (each CH_2), 14.1 (CH_3); ESI-HRMS calcd for $C_{27}H_{53}NO_3Na$ 462.3923, found *m/z* 462.3924 $[M+Na]^+$



(4*S*,5*R*)-*t*-Butyl 4-allyl-5-heptadecyl-2,2-dimethyloxazolidine-3-carboxylate (100). The alcohol **92** (1.16 g, 2.64 mmol) was taken up in toluene (4 mL) and 2,2-dimethoxypropane (0.81 mL, 6.6 mmol) was added along with pyridinium p-toluenesulfonate (7 mg). The resulting suspension was stirred at 85 °C for 6 h. Upon cooling the reaction was neutralised with solid $NaHCO_3$ (10 mg) and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1) gave **100** (1.18 g, 88%) as a colourless oil; $[\alpha]_D$ +1.8° (c 0.7, $CHCl_3$); IR (film) cm^{-1}: 2923, 2853, 1698, 1384, 1364, 1256, 1178, 1075; ^1H NMR (500MHz, $CDCl_3$) δ 5.78 (1H, ddt, *J* 16.9, 10.1, 6.8, H-2), 4.99 (2H, m, H-1), 3.97 – 3.91 (1H, m), 3.77 (1H, dd, *J* 11.1, 5.7), 2.40 – 2.28 (1H, m, H-3a), 2.17 (1H, ddd, *J* 20.2, 13.9, 6.5, H-3b), 1.53 (2H, s), 1.48 (2H, s), 1.47 (2H, s), 1.43 (2H, d, *J* 3.9), 1.42 (9H, s, *t*-Bu), 1.38 (2H, s), 1.23 (30H, s, each CH_2), 0.83 (3H, t, *J* 6.9). ; ^{13}C NMR (125MHz, $CDCl_3$) δ 152.2*, 151.7 (C=O), 135.9 (*C*H=CH_2), 116.7 (CH=*C*H_2), 116.4*, 92.6, 92.1*, 79.7*, 79.3 (*t*-Bu), 77.1 (C*H*OCR_3), 76.8*, 59.0 (CHN), 58.8*, 34.9, 34.4* (*C*H_2CH=CH_2), 32.0, 29.8, 29.7 (2s), 29.6, 29.5, 29.4, 29.1 (2s) (each CH_2), 28.5, 28.4 (2 s), 27.8, 27.0, 26.5, 26.4, 24.9, 23.6 (each CH_3), 22.8 (CH_2), 14.2 (CH_3); ESI-HRMS calcd for $C_{30}H_{57}NO_3Na$ 502.4236, found *m/z* 502.4231 $[M+Na]^+$

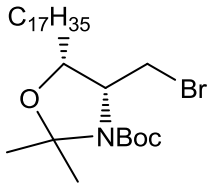


(4*S*,5*R*)-*t*-Butyl-5-heptadecyl-2,2-dimethyl-4-(prop-1-enyl)oxazolidine-3-carboxylate (102). Oxazolidine **100** (0.49 g, 1.0 mmol) was taken up in methanol (14 mL) and the Grubbs-II catalyst, (9 mg, 0.1 mmol)) was added. The reaction mixture was placed in a pre-heated oil bath at 60 °C for 12 h. After cooling the solvent was removed under reduced pressure and the residue purified by flash chromatography (petroleum ether-EtOAc 9:1) to give compound **102** (467 mg, 95 %) as a colourless oil; IR (film) cm^{-1}: 2922, 2853, 1696, 1384, 1375, 1364, 1251, 1176, 1075; ^{1}H NMR (500MHz, $CDCl_3$ 1:1 *E,Z* mixture) δ 5.56 (1H, ddd, *J* 17.5, 12.0, 6.9), 5.46 (1H, m), 5.24 (2H, dd, *J* 15.1, 8.8), 4.15 (1H, d, *J* 5.9), 3.98 (1H, dd, *J* 8.4, 5.3), 3.88 (2H, dd, *J* 11.5, 5.8), 1.63 (3H, d, *J* 6.5), 1.54 (2H, s), 1.49 (2H, s), 1.43 (5H, s), 1.40 (7H, s), 1.33 (9H, s), 1.18 (51H, s), 0.80 (6H, t, *J* 6.8); ^{13}C NMR (125MHz, $CDCl_3$): δ 151.8, 129.0*, 128.7, 126.8, 126.34*, 92.7, 92.2*, 79.7*, 78.9, 76.8, 76.7*, 62.6, 62.3*, 56.6, 32.0, 29.7 (3s), 29.6, 29.5, 29.4, 28.45, 27.3, 25.7, 23.8, 22.7, 17.8*, 17.6, 14.1.; ESI-HRMS calcd for $C_{30}H_{57}NO_3Na$ 502.4236, found *m/z* 502.4234$[M+Na]^+$

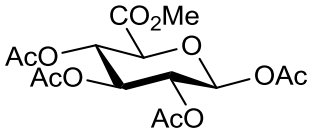


(4*S*,5*R*)-*t*-Butyl 5-heptadecyl-4-(hydroxymethyl)-2,2-dimethyloxazolidine-3-carboxylate (57). To a stirred solution of alkene **102** (0.3 g, 0.63 mmol) in 10:1 acetone-water (6 mL) were added 2,6-lutidine (0.15 mL, 1.25 mmol), 4-methylmorpholine-*N*-oxide (0.11 g, 0.9 mmol) and a catalytic amount of osmium tetroxide (0.16 mL, 2 mol %,). The solution was stirred vigorously for 2 days at room temperature. Then $PhI(OAc)_2$ was added and the reaction mixture was stirred for a further 3 h. The reaction was quenched with aqueous sodium thiosulfate and extracted with EtOAc. The combined organic extracts were washed with water, brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was taken up in THF (8 mL) and cooled to 0 °C. Sodium borohydride was added and the reaction mixture allowed to attain room temperature over 1 h. Brine was then added and the mixture was extracted with EtOAc. The combined organic phases were dried over

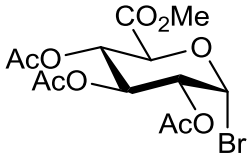
$MgSO_4$ and the solvent was removed under reduced pressure. Flash chromatography of the residue(petroleum ether-EtOAc 9:1, R_f 0.20) gave the title compound (189 mg, 64%) as a colourless oil; $[\alpha]_D$ +3.6° (c 0.2, $CHCl_3$); IR (film) cm^{-1}: 3514 (br), 2922, 2853, 1698, 1466, 1392, 1366, 1256, 1176, 1049; ^1H NMR (500MHz, $CDCl_3$) δ 4.03 (2H, overlapping signals, H-2 & H-3), 3.80 (1H, m, H-1a), 3.63 (1H, m, H-1b), 1.56 (4H, s, CH_3 & C*H*H), 1.50 (4H, m, CH_3 CH*H*), 1.48 (9H, s, *t*-Bu), 1.25 (30H, s, each CH_2), 0.87 (3H, t, *J* 6.9); ^{13}C NMR (125MHz, $CDCl_3$) δ 154.8 (C=O), 92.8, 81.3, 75.8 (CH), 63.5 (CH_2), 61.35 (CH), 32.1, 29.8 (3 s), 29.7, 29.6, 29.5, 29.1, 28.5 (each CH_2), 28.0, 26.6, 24.7, 22.8, 14.3 (each CH_3); ESI-HRMS calcd for $C_{28}H_{56}NO_4$ 470.4209, found *m/z* 470.4220 $[M+H]^+$



(4*R*,5*R*)-tert-butyl-4-(bromomethyl)-5-heptadecyl-2,2-dimethyloxazolidine-3-carboxylate (55). (4S,5R)-t-Butyl 5-heptadecyl-4-(hydroxymethyl)-2,2-dimethyloxazolidine-3-carboxylate (529 mg, 1.1 mmol) was dissolved in CH_2Cl_2 (40 mL). To this was added carbon tetrabromide (710 mg, 2.15 mmol), triphenylphosphine (4.30 mmol), and triethylamine (300µl, 2.15 mL). The solution was stirred for 5 h. Silica gel was added and solvents were removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1) gave bromide **55** (511mg, 85%) as a colourless oil. $[\alpha]_D$ +16.8 (c 1.1, $CHCl_3$); ^1H NMR (500MHz, $CDCl_3$; the asterisk denotes the minor rotamer.) δ 4.15* (1H, s), 4.05 (2H, d, *J* 8.0, H-2 & H-3 overlapping), 3.49* (1H, m), 3.42 (1H, dd, *J* 16.0, 8.8, H-1a), 3.31 (1H, d, *J* 10.2, H-1b), 1.76 (1H, m), 1.64 (1H, m) (CH_2), 1.56 (3H, s, CH_3), 1.49 (12H, overlapping signals, *t*-Bu & CH_3), 1.25 (30H, s), 0.87 (3H, t, *J* 6.7, CH_3); ^{13}C NMR (125MHz, $CDCl_3$): δ 152.21*, 151.40 (C=O), 92.7, 92.35*, 80.8*, 80.4, 76. 6, 76.3* (CH), 60.5, 60.2* (CH), 32.1, 29.8 (2s), 29.7 (2s), 29.65, 29.5, 28.6 (each CH_2), 28.5, 27.9, 27.3, 27.2, 26.9, 24.6 23.35, 22.8, 14.3 (CH_3)

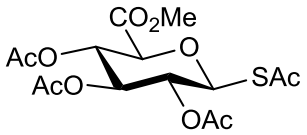


1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranosiduronic acid, methyl ester[2] (113). Glucuronolactone (10 g, 56.8 mmol) was suspended in dry MeOH (160 mL). Dimethylethylamine (0.1 mL) was added and the reaction was stirred for 16 h until all the glucuronolactone had dissolved. The solvent was evaporated and the foam was used without purification. Acetic anhydride (50 mL) and sodium acetate (5 g, 61.0 mmol) were added and the suspension was stirred for 8 days. The reaction was poured onto ice water (300 mL) and stirred overnight. The β-acetate was separated by filtration, washed with water and recrystallised from Et_2O-petroleum ether to give **113** as a white solid (8.5 g, 39%). R_f 0.57 (1:1 EtOAc-cyclohexane); NMR data (^1H and ^{13}C) was in agreement with reported literature data; $[\alpha]_D$ +9.3° (c 1.16, $CHCl_3$); IR (film) cm^{-1}: 2958, 1757, 1439, 1370, 1215, 1039; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.77 (1H, d, *J* 7.7 Hz, H-1), 5.31 (1H, t, *J* 9.3 Hz, H-3), 5.25 (1H, t, *J* 9.3 Hz, H-4), 5.14 (1H, dd, *J* 9.3 Hz, *J* 7.7 Hz, H-2), 4.18 (1H, d, 9.3 Hz, H-5), 3.76 (3H, s, OMe), 2.13 (3H, s), 2.05 (6H, s), 2.04 (3H, s) (each OAc); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.9, 169.4, 169.2, 168.8, 166.8 (each C=O), 91.4 (C-1), 73.0 (C-5), 71.8 (C-3), 70.1 (C-2), 68.9 (C-4), 53.0 (OMe), 20.8, 20.6, 20.5, 20.5 (each OAc); ESI-HRMS calcd for $C_{15}H_{20}O_{11}Na$ 399.0903, found *m/z* 399.0885 $[M+Na]^+$

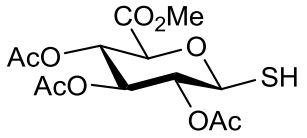


1-Bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranosiduronic acid, methyl ester[3]. Methyl ester **113** (4.0 g, 10.5 mmol) was dissolved in CH_2Cl_2 (8 mL) and cooled to 0 °C. To this HBr (33% in AcOH, 16 mL) was added and the reaction allowed to warm to room temperature over 4 h. The solvent was removed under reduced pressure and the residue was dissolved in chloroform. The organic layer was washed with satd $NaHCO_3$, water, brine, dried over $MgSO_4$, filtered and the solvents were removed under reduced pressure. Recrystallisation of the residue from absolute EtOH gave the bromide precursor (2.66 g, 63%) as a white solid; R_f 0.69 (1:1 EtOAc-cyclohexane); IR (film) cm^{-1}: 2975, 1752, 1370, 1213, 1115, 1044; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.64 (1H, d, *J* 4.1 Hz, H-1), 5.61 (1H, t, *J* 10.0 Hz, H-3), 5.24 (1H, t, *J* 10.0 Hz, H-4), 4.86 (1H, dd, 10.0, 4.1 Hz, H-2), 4.58 (1H, d,

10.0 Hz, H-5), 3.76 (3H, s, OMe), 2.10 (3H, s), 2.06 (3H, s), 2.05 (3H, s) (each OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.6, 169.5, 169.3, 166.6 (each C=O), 85.3 (C-1), 72.0, 70.3, 69.3, 68.5, 53.0 (OMe), 20.6 (2s), 20.4 (each OAc)

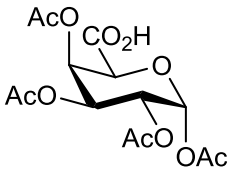


1-*S*-Acetyl-2,3,4-tri-*O*-acetyl-1-β-thio-D-glucopyranuronic acid, methyl ester[4] (114). Bromide precursor (2.66 g, 6.62 mmol) was dissolved in DMF (20 mL) and KSAc (0.92 g, 8.07 mmol) was added. The reaction stirred at room temperature for 3 h, diluted with EtOAc and the solvent was removed under reduced pressure. The residue filtered through silica (EtOAc-cyclohexane 1:1), solvents were removed and the title compound was recovered by recrystallising from absolute EtOH to give **114** (1.34 g, 51%) as an off white solid; R_f 0.59 (EtOAc-cyclohexane 3:1); $[\alpha]_D$ +17.2° (c 2.16, $CHCl_3$); Mp 163.6-164.0 °C; IR (film) cm^{-1}: 2956, 1654, 1711, 1375, 1217, 1077, 1036; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.33 (1H, t, *J* 9.7 H, H-3), 5.30 (1H, d, *J* 10.4 Hz, H-1), 5.20 (1H, t, *J* 9.7 Hz, H-4), 5.14 (1H, dd, *J* 10.4 Hz, *J* 9.7 Hz, H-2), 4.16 (1H, d, *J* 9.7 Hz, H-5), 3.73 (3H, s, OMe), 2.38 (3H, s, SAc), 2.03 (3H, s, OAc), 2.02 (6H, s, OAc); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 191.7, 169.8, 169.3, 169.2, 166.7 (each C=O), 80.2 (C-1), 76.5 (C-5), 73.1 (C-3), 69.3 (C-4), 68.7 (C-2), 52.9 (OMe), 30.8 (SAc), 20.5 (2s), 20.4 (each OAc); ESI-HRMS calcd for $C_{15}H_{20}O_{10}SNa$ 415.0675, found *m/z* 415.0656 $[M+Na]^+$

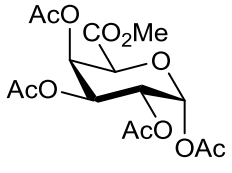


2,3,4-Tri-*O*-acetyl-1-β-thio-D-glucopyranosiduronic acid, methyl ester[4] (115). Thioacetate **114** (400 mg, 1.01 mmol) was dissolved in $CHCl_3$-MeOH 1:1 (8 mL) and cooled to 0 °C. Nitrogen was bubbled through the solution for 5 min, followed by the addition of NaSMe (70 mg, 1.01 mmol). The reaction was stirred for 5 min at 0 °C and then poured onto 1% aqueous HCl and extracted with CH_2Cl_2.The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Recrystallisation from absolute EtOH gave **115** as a yellow solid (217 mg, 61%); R_f 0.52 (EtOAc-cyclohexane 3:1); $[\alpha]_D$ 2.7° (c 0.94, $CHCl_3$); Mp 122.6-122.9 °C; IR (film) cm^{-1}: 2955, 2559, 1752, 1375, 1218, 1072, 1036 ; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.24 (2H, m, H-3, H-4), 5.00 (1H, m, H-2),

4.58 (1H, t, *J* 9.9 Hz, H-1), 4.05 (1H, d, *J* 9.6 Hz, H-5), 3.76 (3H, s, OMe), 2.38 (1H, d, *J* 9.9 Hz, SH), 2.08 (3H, s), 2.03 (3H, s), 2.02 (3H, s) (each OAc); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9, 169.5, 169.3, 166.7 (each C=O), 79.0 (C-1), 76.6 (C-5), 73.2 (C-2), 72.8, 69.3, 53.0 (OMe), 20.7, 20.6, 20.5 (each OAc)

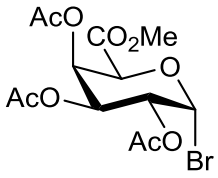


1,2,3,4-Tetra-*O*-acetyl-α-D-galactopyranosiduronic acid[5]. To a stirred solution of $HClO_4$ (500 μL) in Ac_2O (130 mL) at 0°C was added D-galacturonic acid monohydrate (25 g, 118 mmol). The reaction was warmed to room temperature and stirred for 3 h. The reaction was then re-cooled to 0°C and MeOH (15 mL) was added cautiously. After stirring for 30 mins the reaction was partitioned between EtOAc and H_2O. The aqueous layer was extracted into EtOAc and the combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The product was recrystallised from EtOAc-pentane to give the acid derivative (38g, 89%) as a white solid; R_f 0.27 (EtOAc-cyclohexane 1:1); IR (film) cm^{-1}: 3507, 2945, 1756, 1370, 1217, 1039; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.31 (1H, bs, OH), 6.37 (1H, d, *J* 3.4 Hz, H-1), 5.75 (1H, m, H-4), 5.30 (1H, dd, *J* 10.8 Hz, *J* 3.1 Hz, H-3), 5.23 (1H, dd, *J* 10.8 Hz, *J* 3.4 Hz, H-2), 4.71 (1H, d, *J* 0.7 Hz, H-5), 2.07 (3H, s), 2.02 (3H, s), 1.92 (3H, s), 1.90 (3H, s) (each OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.4, 170.2, 170.1, 169.1, 168.6 (each C=O), 89.5 (C-1), 70.5 (C-5), 68.7 (C-4), 67.3 (C-3), 66.2 (C-2), 20.8, 20.7, 20.6 (2s) (each OAc); ESI-HRMS calcd for $C_{14}H_{18}O_{51}Na$ 385.0747, found *m/z* 385.0755 $[M+Na]^+$

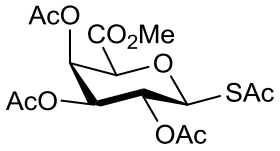


1,2,3,4-Tetra-*O*-acetyl-α-D-galactopyranosiduronic acid, methyl ester[6] (117). To a solution of 1,2,3,4-tetra-*O*-α-D-galactopyranuronic acid (18 g, 50 mmol) in H_2O (35 mL) was added solid $NaHCO_3$ (4.6 g, 55 mmol). After the cessation of gas evolution, CH_2Cl_2 (90 mL) was added followed by tetra-*n*-butyl ammonium bromide (17.5 g, 55 mmol) and methyl iodide (4.1 ml, 65 mmol). The resulting suspension was stirred overnight at room temperature. The phases were separated and the aqueous phase was extracted into CH_2Cl_2.

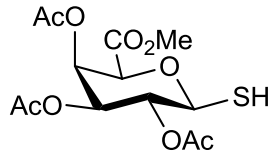
The combined organic phases were washed with water, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The residue was dissolved in warm EtOAc and cooled to -5°C where the ammonium salts precipitated and were removed by filtration. The filtrate and washings were then concentrated under reduced pressure. Recrystallization from Et_2O gave **117** (13.4 g, 71%). NMR data (^1H and ^{13}C) was in agreement with reported literature data; R_f 0.27 (EtOAc-cyclohexane 1:1); IR (film) cm^{-1}: 2958, 1755, 1438, 1372, 1224, 1078, 940, 730; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.37 (1H, d, *J* 2.8 Hz, H-1), 5.68 (1H, m, H-4), 5.25 (2H, overlapping signals, H-2, H-3), 4.67 (1H, d, *J* 1.2 Hz, H-5), 3.63 (3H, s, OMe), 2.03 (3H, s), 1.99 (3H, s), 1.90 (3H, s), 1.89 (3H, s) (each OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.6, 169.4, 169.3, 168.2, 166.3 (each C=O), 89.2 (C-1), 70.4 (C-5), 68.3 (C-4), 66.7, 65.7, 52.4 (OMe), 20.4, 20.2, 20.1 (2s) (each OAc); ESI-HRMS calcd for $C_{15}H_{20}O_{10}Na$ 399.0903, found *m/z* 399.9012 $[M+Na]^+$



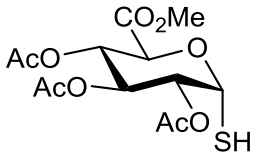
1-Bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-galactopyranosiduronic acid, methyl ester[6] (**117**) (10 g, 27 mmol) was dissolved in CH_2Cl_2 (30 mL). To this was added Ac_2O (3.3 mL), AcOH (13 mL) and AcBr (18 mL). The reaction mixture was brought to 0°C and a solution of H_2O (4.2 mL) in AcOH (14 mL) was added dropwise. After 10 min the reaction was warmed to room temperature and stirred for 3 h. The mixture was poured onto ice, the layers were separated and the aqueous layer was washed into CH_2Cl_2. The combined organic layers were washed with ice water, cold satd $NaHCO_3$, water, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude material (80%) was used without further purification; NMR data (^1H and ^{13}C) was in good agreement with the reported literature data; R_f 0.58 (EtOAc-cyclohexane 1:1); IR (film) cm^{-1}: 2992, 2957, 1755, 1372, 1220, 1093, 1013; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.72 (1H, d, *J* 3.9 Hz, H-1), 5.77 (1H, dd, *J* 3.2 Hz, *J* 1.2 Hz, H-4), 5.40 (1H, dd, *J* 10.6 Hz, *J* 3.2 Hz, H-3), 5.05 (1H, dd, *J* 10.6 Hz, *J* 3.9 Hz, H-2), 4.84 (1H, d, *J* 1.2 Hz, H-5), 3.73 (3H, s, OMe), 2.06 (3H, s), 1.97 (6H, s) (each OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.7, 169.5, 169.3, 165.7 (each C=O), 87.2 (C-1), 72.3 (C-5), 67.8 (C-4), 67.5 (C-3), 67.1 (C-2), 52.8 (OMe), 20.5, 20.4, 20.3 (each OAc)



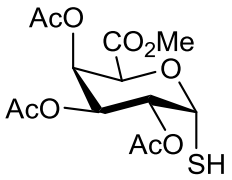
1-*S*-Acetyl-2,3,4-tri-*O*-acetyl-β-thio-D-galactopyranosiduronic acid, methyl ester (118). The bromide precursor **117** (5 g, 12.6 mmol) was dissolved in DMF (40 mL) and KSAc (1.72 g, 15.1 mmol) was added. The reaction was stirred at room temperature for 3 h, diluted with EtOAc and washed with water, brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. Flash chromatography (petroleum ether-EtOAc, 1:1) gave the thioacetate **118** (2.87 g, 56%) as an off white solid; R_f 0.57 (EtOAc-cyclohexane 3:1); $[\alpha]_D$ +53.5° (c 2.2, $CHCl_3$); IR (film) cm^{-1}: 3021, 1753, 1721, 1372, 1214, 1086, 1061; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.77 (1H, d, *J* 3.0, H-4), 5.41 – 5.32 (2H, overlapping signals, H-2 and H-1), 5.27 (1H, dd, *J* 8.7, 3.2, H-3), 4.61 (1H, s, H-5), 3.75, 2.41, 2.11, 2.04, 1.99 (each s, each 3H, each CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.1, 169.7 (2s), 169.4, 166.0 (each C=O), 80.4 (C-1), 76.1 (C-5), 71.5 (C-4), 68.5 (C-3), 65.9 (C-2), 52.8 (OMe), 30.7, 20.6, 20.5 (2s, each CH_3); ESI-HRMS calcd for $C_{15}H_{20}O_{10}SNa$ 415.0675, found *m/z* 415.0656 $[M+Na]^+$



2,3,4-Tri-*O*-acetyl-β-thio-D-galactopyranosiduronic acid, methyl ester (56). Thioacetate **118** (1.5 g, 3. 7 mmol) was dissolved in $CHCl_3$-MeOH 1:1 (30 mL) and cooled to 0 °C. Nitrogen was bubbled through the solution for 5 min, followed by the addition of NaSMe (257 mg, 3.67 mmol). The reaction was stirred for 5 min at 0 °C and then poured onto 1M HCl (20 mL), and extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. Flash chromatography (EtOAc-petroleum ether 3:1) gave **56** as an off-white solid (1.01 g, 79%); R_f 0.52 (EtOAc-cyclohexane 3:1); $[\alpha]_D$ +39.7° (c 1.85, $CHCl_3$); IR (film) cm^{-1}: 1750, 1372, 1220, 1059; ^1H NMR (500 MHz, $CDCl_3$) δ 5.76 (1H, dd, *J* 3.4, 1.3, H-4), 5.22 (1H, t, *J* 9.9, H-2), 5.09 (1H, dd, *J* 10.1, 3.4, H-3), 4.56 (1H, t, *J* 9.8, H-1), 4.35 (1H, d, *J* 1.2, H-5), 3.77 (3H, s, OCH_3), 2.50 (1H, d, *J* 9.9, SH), 2.13 (3H, s), 2.09 (3H, s), 2.00 (3H, s) (each OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.8, 169.7, 166.1 (each C=O), 79.3, 76.2, 71.2, 70.4, 68.4, 52. 9 (OMe) 20.6, 20.5 (each 3H, each s, each CH_3). ESI-HRMS calcd for $C_{13}H_{17}O_9S$ 349.0593, found *m/z* 349.0588 $[M-H]^-$

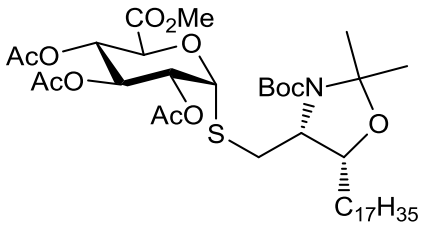


2,3,4-Tri-*O*-acetyl-α-thio-D-glucopyranosiduronic acid, methyl ester (119). To a stirred solution of **115** (100 mg, 0.28 mmol) in CH_2Cl_2 (3 mL) was added $TiCl_4$ (76 µL, 0.7mmol) dropwise. The reaction mixture was stirred for 10 min at room temperature and then cooled to 0°C overnight. The reaction was diluted with CH_2Cl_2 and quenched with satd NH_4Cl. Phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated under reduced pressure to give **119** (65mg, 65%) as a white foam; IR (film) cm^{-1}: 2955, 2572, 1747, 1438, 1370, 1214, 1077, 1042; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.98 (1H, t, *J* 5.7, H-1), 5.39 (1H, t, *J* 9.0, H-3), 5.17 (1H, t, *J* 8.9, H-4), 5.02 (1H, dd, *J* 9.3, 5.2, H-2), 4.76 (1H, d, *J* 9.2, H-5), 3.76 (3H, s, OCH_3), 2.09 (3H,s), 2.06 (3H, s,), 2.05 (3H, s) (each OAc), 2.02 (1H, s, SH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.5, 169.4, 167.7 (each C=O), 76.4 (C-1), 69.8 (C-2), 69.4 (C-5), 68.9 (C-4) 68.6 (C-3), 52.8 (OMe), 20.6, 20.5, 20.5 (each OAc); ESI-HRMS calcd for $C_{13}H_{17}O_9S$ 349.0593, found *m/z* 349.0584 $[M-H]^-$

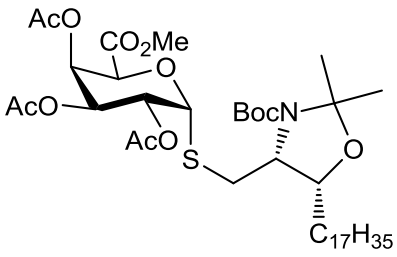


2,3,4-Tri-*O*-acetyl-α-thio-D-galactopyranosiduronic acid, methyl ester (54). To a stirred solution of **56** (100 mg, 0.28 mmol) in CH_2Cl_2 (3 mL) was added $TiCl_4$ (76 µL, 0.7 mmol) dropwise. The reaction mixture was stirred for 10 min at room temperature and was then cooled and left at 0 °C overnight. The reaction was then diluted with CH_2Cl_2 and quenched with satd NH_4Cl. The phases were separated and the aqueous phase was extracted into CH_2Cl_2. The combined organic layers were washed with water, brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure to give **54** (61 mg, 61%) as a white foam; $[\alpha]_D$ +186.4° (c 3.3, $CHCl_3$); IR (film) cm^{-1}: 3059, 1748, 1439, 1370, 1266, 1217, 1125, 1069; ^1H NMR (500 MHz, $CDCl_3$) δ 6.08 (1H, t, *J* 5.1, H-1), 5.72 (1H, s, H-4), 5.25 (1H, dd, *J* 10.8, 5.2, H-2), 5.21 (1H, dd, *J* 10.8, 3.0, H-3), 5.02 (1H, s, H-5), 3.71 (3H, s, OCH_3), 2.05 (3H, s), 2.03 (3H, s), 1.95 (3H, s) (each OAc), 1.89 (1H, d, *J* 5.1, SH). ^{13}C NMR (125 MHz,

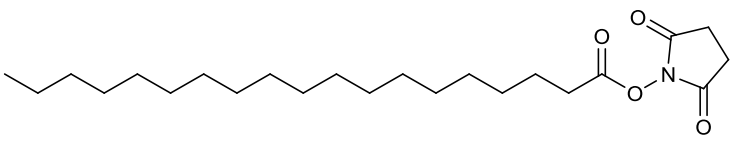
$CDCl_3$) δ 169.7, 169.6, 167.0 (each C=O), 77.7 (C-1), 69.0 (C-5), 68.6 (C-4), 67.1 (C-3), 66.9 (C-2), 52.7 (OMe), 20.6, 20.5, 20.4 (each CH_3); ESI-HRMS calcd for $C_{13}H_{17}O_9S$ 349.0593, found *m/z* 349.0588 $[M-H]^-$



1-*S*-(((4*R*,5*R*)-3-(tert-Butoxycarbonyl)-5-heptadecyl-2,2-dimethyloxazolidin-4-yl)methyl)-2,3,4-Tri-*O*-acetyl-α-thio-D-glucopyranosiduronic acid, methyl ester (121). Compound **119** (155 mg, 0.44 mmol) was dissolved in dry DMF (3 mL) and cooled to 0°C. NaH (60% in mineral oil, 16 mg, 0.4 mmol) was added slowly and the reaction stirred for 30 min. A solution of bromide **55** (179 mg, 0.15 mmol) in DMF was then added dropwise and the reaction mixture allowed warm to room temperature overnight. EtOAc and water were added and the aqueous layer was washed with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$ and concentrated under reduced pressure. Purification via flash chromatography (petroleum ether-EtOAc 4:1) gave **121** (42mg, 39%) as a colourless oil. $[\alpha]_D$ +59.5° (c, $CHCl_3$); IR (film) cm^{-1}: 2923, 2853, 1752, 1697, 1457, 1375, 1177, 1040; ^1H NMR (500MHz, $CDCl_3$) δ 5.71 (1H, m, H-1), 5.41 – 5.31 (1H, m, H-3), 5.24 – 5.14 (1H, m, H-4), 5.03 (1H, dd, *J* 9.8, 5.5, H-2), 4.72 (1H, m, H-5), 3.96 (2H, overlapping signals, H-2' & H-3'), 3.74 (3H, s, OMe), 2.08 – 2.00 (9H, overlapping signals, OAc), 1.54 (6H, m), 1.48 (9H, s, *t*-Bu), 1.45 (3H, s), 1.25 (30H, s, each CH_2), 0.88 (3H, t, *J* 6.7, CH_3); ^{13}C NMR (125MHz, $CDCl_3$) δ 169.8, 169.5, 169.4, 167.9 (C=O), 92.5, 83.3 (C-1), 80.1, 76.6 (C-3'), 70.0 (C-2), 69.5 (C-3), 69.1 (C-4), 68.9 (C-5), 58.6 (C-2'), 52.9 (OMe), 32.1, 29.9, 29.7, 29.5, 28.6 (each CH_2), 22.8, 20.8, 20.7 20.7, 14.3 (each CH_3); ESI-HRMS calcd for $C_{41}H_{71}NO_{12}SNa$ 824.4595, found *m/z* 824.4586 $[M+Na]^+$

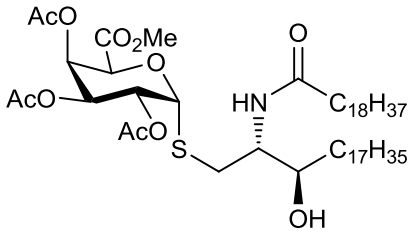


1-*S*-(((4*R*,5*R*)-3-(tert-Butoxycarbonyl)-5-heptadecyl-2,2-dimethyloxazolidin-4-yl)methyl)-2,3,4-Tri-*O*-acetyl-α-thio-D-galactopyranosiduronic acid, methyl ester (53). Trimethylphosphine (330 μL, 1.0M in THF) and ADDP (84 mg, 0.33 mmol) were stirred for 30 min at 0 °C in THF. Compound **54** (58 mg, 0.165 mmol) and alcohol **57** (60 mg, 0.127 mmol) were added and the resulting mixture was stirred at room temperature for 2 days. The mixture was filtered and diluted with EtOAc. Remaining hydrazide was precipitated from hexane and removed via filtration. The filtrate was washed with H_2O, brine, satd $NaHCO_3$, dried over $MgSO_4$ and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc, 4:1) gave **53** (36 mg, 36%) as a colourless oil; $[\alpha]_D$ +58.4° (c, $CHCl_3$); IR (film) cm^{-1}: 2923, 2853, 1752, 1697, 1457, 1375, 1177, 1040; ^1H NMR (500MHz, $CDCl_3$): δ 5.87 (1H, d, *J* 5.4 H-1), 5.76 (1H brs, H-4), 5.22 (1H, m, H-2), 5.05 (1H, m, H-3), 4.96 (1H, m, H-5), 4.01 (1H, m, H-3'), 3.81 (1H, m, H-2'), 3.74 (3H, s, OMe), 2.84 (1H, dd, J 12.6, 7.4), 2.78 (1H, dd, *J* 13.1, 8.9), 2.70 (1H, dd, *J* 12.7, 3.7), 2.60 (1H, dd, *J* 12.7, 1.1), 2.08 – 2.00 (9H, m, OAc), 1.56 (9H, brs, *t*-Bu), 1.50 (6H, s,), 1.47 (4H, s), 1.25 (27H, s, each CH_2), 0.88 (3H, t, *J* 6.7, CH_3); ^{13}C NMR (125MHz, $CDCl_3$): δ 169.7, 167.35, 167.1, 151.3 (each C=O), 92.7, 92.3*, 83.6*, 82.6 (C-1), 80.4 , 76.1 (C-3'), 68.9 (C-4), 68.8 (C-3), 68.6 (C-5), 67.7 (C-2), 59.2 (C-2'), 52.6 (OMe), 31.9, 29.7 (2s), 29.6 (2s), 29.4, 28.4, 28.3, 27.1, 26.7, 24.6, 23.4, 22.7, 20.7, 20.6, 20.55, 14.1; ESI-HRMS calcd for $C_{41}H_{71}N\,O_{12}S$ Na 824.4595, found *m/z* 824.4586 $[M+Na]^+$



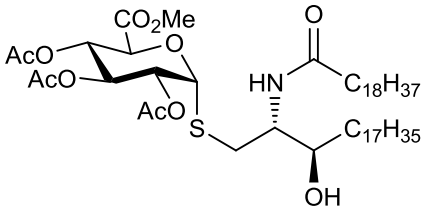
1-*N*-succinimidyl-nonadecanoate. *N*-hydroxysuccinimide (0.76 g, 6.56 mmol) and EDC (1.25 g, 6.56 mmol) were added to a solution of nonadecanoic acid (1.87 g, 6.56 mmol) in CH_2Cl_2 (50 mL) and the mixture was stirred overnight at room temperature. The solvent was concentrated under reduced pressure and the resulting residue was dissolved in CH_2Cl_2. The

solution was washed with water, dried over $MgSO_4$ and concentrated under reduced pressure to yield the title compound as a white solid (2.45 g, 98%). The compound was used without further purification; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.83 (4H, s, O=CC*H*$_2$C*H*$_2$C=O), 2.60 (2H, t, *J* 7.5, CH_2C=O), 1.79 – 1.69 (2H, m, CH_2), 1.39 (2H, dd, *J* 14.9, 7.0, CH_2), 1.27 (29H, s, each alkyl CH_2), 0.88 (3H, t, *J* 6.9, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 168.6 (each C=O), 31.9, 30.9, 29.6, 29.6, 29.6, 29.5, 29.3, 29.0, 28.8, 25.6, 24.6, 22.7 (each CH_2), 14.1 (CH_3)

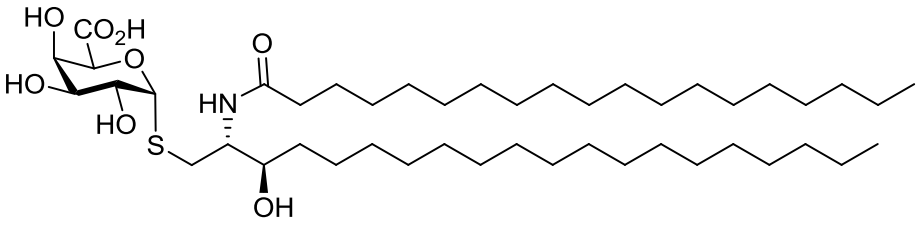


1-*S*-((2*R*,3*R*)-3-Hydroxy-2-nonadecanamidoicosyl)-2,3,4-Tri-*O*-acetyl-α-thio-D-galactopyranosiduronic acid, methyl ester (128). Compound **53** (30 mg, 0.037 mmol) was taken up in formic acid (2 mL) and the reaction mixture was stirred vigorously for 30 min. Toluene (5 mL) was added and the volatile components were then removed under reduced pressure. The resulting residue was dissolved in water and basified with solid $NaHCO_3$. The mixture was then extracted into CH_2Cl_2, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was then taken up in CH_2Cl_2 and DIPEA (31 μL, 0.18 mmol) was added. To this was added a solution of 1-*N*-succinimidyl nonadecanoate (50 mg, 0.13 mmol) in CH_2Cl_2 and the mixture was stirred at room temperature for 16 h. The reaction mixture was partitioned between EtOAc and satd $NaHCO_3$. Phases were separated and the aqueous phase extracted into EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 3:1) gave **128** (19 mg, 54% over 2 steps) as a white solid; $[\alpha]_D$ +51.5 (c, $CHCl_3$); IR (film) cm^{-1}: 3293 br, 2917, 2850, 1751, 1648, 1467, 1371, 1223, 1080; ^{1}H NMR (500MHz, $CDCl_3$) δ 6.04, (1H, d, *J* 8.9, -NH-), 5.84 (1H, d, *J* 5.6, H-1), 5.79 (1H, br s, H-4), 5.31 – 5.25 (1H, m, H-2), 5.18 (1H, dd, *J* 10.9, 3.3, H-3), 5.05 (1H, brs, H-5), 4.07 (1H, brs, H-2'), 3.76 (3H, s, OMe), 3.62 (1H, m, H-3'), 2.94 (1H, dd, *J* 14.2, 8.4, -C*H*HS-), 2.82 (1H, m,-CH*H*S-), 2.24 – 2.18 (2H, m, CH_2), 2.10, 2.08, 2.00 (each 3H, each s, acetate CH_3) , 1.63 (4H, m, each CH_2), 1.46 (2H, m, CH_2), 1.28 (58 H, s each CH_2), 0.88 (6H, t, *J* 6.9); ^{13}C NMR (125MHz, $CDCl_3$): δ 173.5, 170.0, 169.7, 169.6, 167.25 (each C=O), 84.7 (C-1), 73.7 (C-3'), 68.8 (C-5), 68.5 (C-4), 67.7 (C-3), 67.5 (C-2),

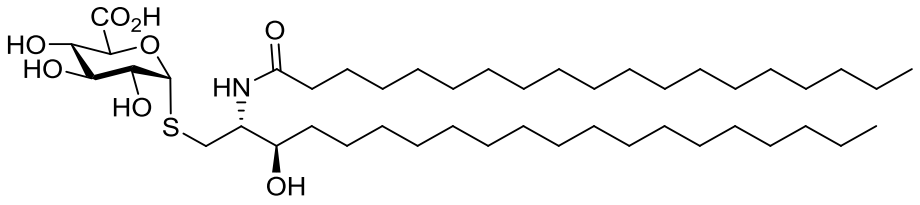
53.6 (C-2'), 52.8 (OMe), 36.75, 34.0, 32.4, 31.9, 29.7, 29.7, 29.63, 29.6, 29.4, 25.9, 25.8 (each CH_2), 22.7, 20.8, 20.6, 20.5, 14.1 (each CH_3) ; ESI-HRMS calcd. for $C_{52}H_{94}N\ O_{11}S$ 940.6548, found *m/z* 940.6558 $[M-H]^-$



1-*S*-((2*R*,3*R*)-3-Hydroxy-2-nonadecanamidoicosyl)-2,3,4-Tri-*O*-acetyl-α-thio-D-glucopyranosiduronic acid, methyl ester (129). Compound **121** (30 mg, 0.037 mmol) was taken up in formic acid (3 mL) and stirred vigorously for 30 min. Toluene (5 mL) was added and the solvents were removed under reduced pressure. The resulting residue was taken up in water and basified with solid $NaHCO_3$. The mixture was then extracted into CH_2Cl_2, dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was taken up in CH_2Cl_2 (2 mL) and DIPEA (22 µL, 0.131 mmol) was added. To this was added a solution of 1-*N*-succinimidyl nonadecanoate (37 mg, 0.094 mmol) in CH_2Cl_2 (1.5 mL) and the mixture was stirred at room temperature for 16 h. The reaction mixture was partitioned between EtOAc and satd $NaHCO_3$. Phases were separated and the aqueous phase extracted into EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. Purification via flash chromatography (petroleum ether-EtOAc 3:1) gave **129** (19 mg, 57% over two steps) as a white solid; $[\alpha]_D$ +47.8° (c 1.0 in $CHCl_3$); IR (film) cm^{-1}: 3294 br, 2917, 2850, 1751, 1650, 1467, 1219, 1043; ^1H NMR (500MHz, $CDCl_3$) δ 5.69 (1H, d, *J* 5.3, H-1), 5.33 – 5.27 (1H, m, H-3), 5.21 (1H, t, *J* 8.8, H-4), 4.99 (1H, dd, *J* 9.3, 5.2, H-2), 4.74 (1H, d, *J* 9.1, H-5), 4.01 (1H, s, H-3'), 3.79 – 3.73 (3H, m, OMe), 3.68 (1H, brs, H-2'), 3.03 (1H, dd, *J* 14.0, 8.0, C*H*$_2$CHOH), 2.84 (1H, dd, *J* 14.0, 3.5, C*H*$_2$CHOH), 2.19 (2H, d, *J* 4.3, CH_2), 2.08 (3H, s), 2.04 (3H, s), 2.03 (3H, s) (each CH_3), 1.62 (4H, s, 2 x CH_2), 1.45 (2H, m, CH_2), 1.32 – 1.22 (60H, m, each CH_2), 0.87 (6H, t, *J* 6.9, each CH_3); ^{13}C NMR (125MHz, $CDCl_3$) δ 173.68, 169.76, 169.49, 169.42, 167.94 (each C=O), 83.52 (C-1), 73.55 (C-3'), 70.02 (C-2), 69.23 (C-3), 69.18 (C-5), 68.77 (C-4), 54.04 (C-2'), 52.95 (OMe), 36.74, 34.02, 31.93 (each CH_2), 29.72, 29.68, 29.67, 29.56, 29.41, 29.37, 25.96, 25.72, 22.70 (each CH_2), 20.71, 20.62, 20.60, 14.13 (each CH_3); ESI-HRMS calcd. for $C_{52}H_{94}NO_{11}S$ 940.6548, found *m/z* 940.6578 $[M-H]^-$



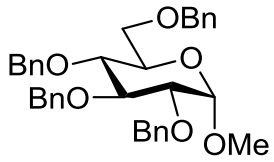
1-*S*-((2*R*,3*R*)-3-Hydroxy-2-nonadecanamidoicosyl)-α-thio-D-galactopyranosiduronic acid (45). Protected lipid derivative **128** (9 mg, 0.92 μmol) was dissolved in anhydrous EtOAc (20μL) and LiI (13 mg, 0.09 mmol) was added. The reaction mixture was heated at reflux for 6 h. Upon cooling the reaction mixture was washed with H_2O, brine, dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was then taken up in a guanidine-guanidinium nitrate solution (2 mL, 1M in CH_2Cl_2-MeOH 1:9) the reaction was stirred at room temperature for 1h. The reaction was neutralised by the addition of Amberlite® IR-20, filtered and concentrated under reduced pressure. The crude product was purified via lipophilic Sephadex® LH-20 to give the title compound (4.2 mg, 55%) as a white powder; ^{1}H NMR (500 MHz, $CDCl_3$-MeOD 2:1); δ ^{1}H NMR (500 MHz, $CDCl_3$-MeOD 2:1) δ 5.43 (1H, d, *J* 5.5, H-1), 4.73 (1H, m, H-5), 4.26 (1H, m, H-4) 4.08 (1H, m, H-2), 3.95 (1H, dd, *J* 11.2, 6.3, C*H*NHR), 3.61 (1H, dd, *J* 10.2, 3.3, H-3), 3.51 (1H, m, CHOH), 2.85 (1H, m, C*H*HS), 2.60 (1H, m, CH*H*S) 2.32 – 2.23 (1H, m), 2.18 (2H, t, *J* 7.5), 1.58 (3H, m, CH_2), 1.47 (3H, m, CH_2), 1.25 (56H, s, each CH_2), 0.85 (6H, t, *J* 6.9, each CH_3); ^{13}C NMR (125 MHz, $CDCl_3$-MeOD 2:1) δ 175.2, 171.8 (each C=O), 87.9 (C1), 73.1 (CHOH), 71.4 (C5), 70.7 (C3), 70.1 (C4), 68.5 (C2), 54.8 (CHN), 36.6, 32.3 (*C*H_2S), 34.1, 29.6, 26.1, 26.0, 22.9, 22.8, 19.4 (each CH_2), 14.1 (CH_3); ESI-HRMS calcd. for $C_{45}H_{86}NO_8S$ 800.6074, found *m*/*z* 800.6072 $[M-H]^-$



1-*S*-((2*R*,3*R*)-3-Hydroxy-2-nonadecanamidoicosyl)-α-thio-D-glucopyranosiduronic acid (46). Protected lipid derivative **129** (3 mg, 0.3 μmol) was dissolved in anhydrous EtOAc (200 μL) and LiI (15 mg, 0.11 mmol) was added. The reaction mixture was heated at reflux for 6 h. Upon cooling the reaction mixture was washed with H_2O, brine, dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was then taken up in a guanidine-guanidinium nitrate solution (2 mL, 1M in CH_2Cl_2-MeOH 1:9) the reaction was stirred at

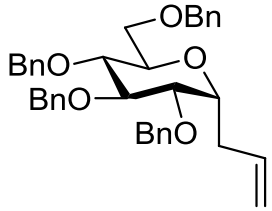
room temperature for 1 h. The reaction was neutralised by the addition of Amberlite® resin IR-20, filtered and the solvents was removed under reduced pressure. The crude product was purified via lipophilic Sephadex® LH-20 to give the title compound (1.4 mg, 58% over 2 steps) as a white powder; ^{1}H NMR (500 MHz, $CDCl_3$-MeOD 2:1) δ 5.17 (1H, d, *J* 4.7), 4.31 (1H, s), 3.78 (1H, s), 3.58 (2H, s), 3.45 (1H, s), 3.38 (1H, s), 2.80 – 2.72 (1H, m), 2.66 (1H, s), 2.10 (1H, s), 1.97 (2H, s), 1.83 (1H, s), 1.46 (1H, s), 1.39 (2H, s), 1.28 (2H, s), 1.04 (40H, s), 0.65 (7H, d, *J* 6.6); ^{13}C NMR (125 MHz, $CDCl_3$-MeOD 2:1) δ 175.5 (C=O), 87.6 (C1), 73.5 (CHOH), 71.4 (C2), 70.4 (C-3), 69.0 (C-5), 67.2 (C-4), 53.6 (CHN), 35.3, 31.3 (CH_2S), 34.0, 29.4, 26.1, 26.0, 22.9, 22.7, 19.3 (each CH_2), 14.1 (CH_3); ESI-HRMS calcd for $C_{45}H_{86}NO_8S$ 800.6074, found *m/z* 800.6077 $[M-H]^-$

6.3 Chapter 3-Experimental

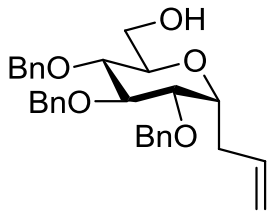


Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (150). Methyl α-D-glucopyranoside (10 g, 51.5 mmol) was dissolved in DMF (250 mL) and cooled to 0 °C. To this was added sodium hydride (60% in mineral oil dispersion, 10.3 g, 257 mmol) portion-wise over 1 h. Benzyl bromide (31 mL, 257 mmol) was then added dropwise and the reaction was allowed to attain room temperature over 24 h. The reaction was quenched via the slow addition of MeOH and diluted with EtOAc. The organic layer was washed with H_2O, brine, dried over $MgSO_4$ and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (EtOAc-cyclohexane 1:8) gave **150** (23.7 g, 83%) as a yellow oil; IR (film) cm^{-1}: 3032, 1605, 1495, 1161, 1048, 736; ^{1}H NMR (500MHz, $CDCl_3$) δ 7.12–7.38 (20H, m, Ar-H), 4.99 (1H, d, *J* 10.9, PhCH_2O), 4.84 (1H, d, *J* 10.7, PhCH_2O), 4.83 (1H, d, *J* 10.9, PhCH_2O), 4.81 (1H, d, *J* 12.1, PhCH_2O), 4.68 (1H, d, *J* 12.1, PhCH_2O), 4.64 (1H, d, *J* 3.6, H-1), 4.62 (1H, d, *J* 12.4 Hz, PhCH_2O), 4.49 (1H, d, *J* 12.4, PhCH_2O), 4.48 (1H, d, *J* 10.7, PhCH_2O), 3.99 (1H, t, *J* 9.2 Hz, H-3), 3.71–3.77 (2H, overlapping signals, H-5 & H-6a), 3.62–3.68 (2H, overlapping signals, H-4 & H-6b), 3.57 (1H, dd, *J* 5.6, 9.6, H-2), 3.39 (3H, s, OCH_3); ^{13}C NMR (125MHz, $CDCl_3$) δ 138.7, 138.2, 138.1, 138.0 (each Ar-C), 128.7 (2s), 128.6, 128.5, 128.2, 128.2, 128.1 (2s), 128.0, 127.96, 127.8 (2s) (each Ar-*C*H), 98.2 (C-1),

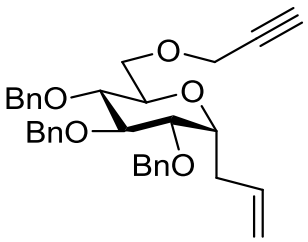
82.1 (C-3), 79.9 (C-2), 77.7 (C-4), 75.7, 75.0, 73.4, 73.3 (each O*C*H_2Ph), 70.1 (C-5), 68.6 (C-6), 55.1 (O*C*H_3); ESI-HRMS calcd for $C_{35}H_{39}O_6$ 555.2746, found *m*/*z* 555.2740 $[M+H]^+$



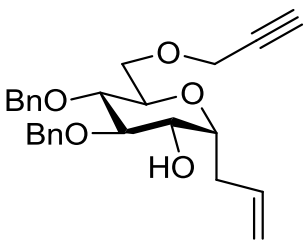
1-*C*-Allyl-1-deoxy-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside[7] (151). Compound **150** (15 g, 27 mmol) was placed under high vacuum and heated to 60 °C for 2 h. The resulting syrup was kept under an atmosphere of argon. Acetonitrile (300 mL) was added and the solution was cooled to 0 °C. Allyltrimethylsilane (12.8 mL, 81 mmol) was added and the mixture stirred for five min. Trimethylsilyl triflate (2.44 mL, 13.5 mmol) was added dropwise and the reaction was left to stir overnight at room temperature. Satd. $NaHCO_3$ was added and the aqueous layer was extracted into EtOAc. The organic layers were combined and washed with H_2O, brine and dried over $MgSO_4$ and filtered. The solvent was concentrated under reduced pressure and the crude residue was purified via flash chromatography (EtOAc-Cyclohexane 1:7) to give **151** (11.7 g, 77%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.00 (20H, m, Ar-H), 5.86-5.76 (1 H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.15-5.05 (2H, m, CH_2CH=CH_2), 4.92 (1H, d, *J* 10.9 Hz, OCH_2Ph), 4.80 (2H, dd, *J* 2.9, 7.7, OCH_2Ph), 4.69 (1H, d, *J* 11.6 Hz, OCH_2Ph), 4.63-4.60 (2H, dd, OCH_2Ph), 4.48-4.45 (2H, dd, OCH_2Ph), 4.15-4.10 (1H, m, H-1), 3.82-3.73 (2H, overlapping signals, H-3 & H-2), 3.71-3.68 (1H, ddd, *J* 9.8, 4.3, 2.5, H-5), 3.65-3.60 (3H, overlapping signals, H-4 & H-6), 2.55-2.42 (2H, m, CH_2CH=CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9, 138.4, 138.4, 138.3 (each Ar-C), 134.9 (CH_2CH=CH_2), 128.7, 128.6 (2s), 128.5, 128.2, 128.1 (2s), 128.0 (2s),127.9, 127.8 (2s) (Ar-C), 117.1 (CH_2CH=CH_2) 82.6, 80.3, 78.4, 75.8, 75.3 (OCH_2Ph), 73.9 (CH), 73.7, 73.3 (OCH_2Ph), 71.4 (CH), 69.2 (C-6), 30.1 (CH_2CH=CH_2); ESI-HRMS calcd for $C_{37}H_{41}O_5$ 565.2954, found *m*/*z* 565.2958 $[M+H]^+$



1-*C*-Allyl-1-deoxy-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside[7]. Compound **151** (5 g, 8.8 mmol) was dissolved in Ac_2O-CH_2Cl_2 (80 mL, 1:1) and cooled to -78 °C. To this was added Trimethylsilyl triflate (477 µL, 2.6 mmol) dropwise. After 3 h, the reaction was brought to 0°C and quenched with satd. $NaHCO_3$. Phases were separated and the aqueous phase was extracted into CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give the 6-*O*-acetylated intermediate **156** as a brown oil. The crude product was dried for 3 h under high vacuum and dissolved in MeOH (40 mL). To this was added freshly prepared solution of NaOMe-MeOH (10 mL of a 1M solution). The reaction mixture was stirred at room temperature overnight. Solvents were removed and the crude residue was taken up in CH_2Cl_2 and washed with water, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 9:1) to give the title compound as a white solid (3.3 g, 79 % over two steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.27 (15 H, m, Ar-H), 5.80-5.72 (1 H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.12-5.07 (2H, m, CH_2CH=CH_2), 4.93 (1H, d, *J* 10.9, OCH_2Ph), 4.86 (1 H, d, *J* 10.9, OCH_2Ph), 4.81 (1H, d, *J* 10.9, OCH_2Ph), 4.71-4.69 (1H, d, *J* 10.9, OCH_2Ph), 4.63-4.61 (2H, each d, OCH_2Ph), 4.06-4.03 (1H, m, H-1), 3.80 (1H, apt t, *J* 8.8, H-3), 3.75 (1H, ddd, *J* 11.6, 4.4, 2.3, H-6a), 3.69 (1H, dd, *J* 9.3, 5.8, H-2), 3.65-3.59 (1H, m, H-6b), 3.54 (1H, ddd, J 9.6, 4.2, 2.5, H-5), 3.49 (1H, t, *J* 8.5, H-4), 2.52-2.42 (2H, m, CH_2CH=CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.7, 138.1 (2s) (each Ar-C), 134.5 (CH_2CH=CH_2), 128.5, 128.4 (2s), 128.0, 127.9, 127.8 (2s), 127.79, 127.61 (each Ar-CH), 117.16 (CH_2CH=CH_2) 82.2 (C-3), 80.1 (C-2), 78.1 (C-4), 75.4, 75.1 (each OCH_2Ph), 73.6 (C-1), 73.1 (CH), 71.6 (C-6), 29.9 (CH_2CH=CH_2); ESI-HRMS calcd for $C_{30}H_{35}O_5$ 475.2484, found *m/z* 475.2480 $[M+H]^+$

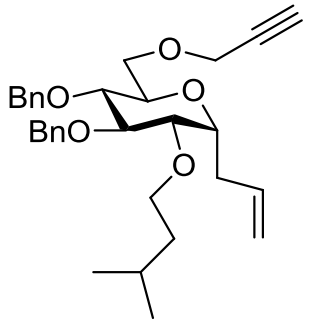


1-*C*-Allyl-1-deoxy-2,3,4-tri-*O*-benzyl-6-*O*-propargyl-α-D-glucopyranoside (152). Primary alcohol (1 g, 2.1 mmol) was dissolved in DMF (20 mL) and cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 127 mg, 3.2 mmol) was added to the reaction mixture portion-wise and stirring was continued for 10 min. Propargyl bromide (341 µL, 3.2 mmol, 80% solution in toluene) was added dropwise and the reaction mixture was allowed warm to room temperature overnight. The reaction was quenched via the slow addition of MeOH at 0 °C. EtOAc was added and the reaction mixture was washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 6:1) to give **152** (973 mg, 90%) as a yellow oil; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.36-7.26 (15H, m, Ar-H), 5.84-5.78 (1 H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.13-5.07 (2H, m, CH_2CH=CH_2), 4.90 (1H, d, *J* 11.0 OCH_2Ph), 4.86 (1H, d, *J* 10.8, OCH_2Ph), 4.82 (1H, d, *J* 11.0 Hz, OCH_2Ph), 4.70 (2H, dd, *J* 4.2, 10.8, OCH_2Ph), 4.62 (1H, d, *J* 11.6, OCH_2Ph), 4.24 (1H, dd, *J* 4.2, 15.9 alkyne CH_2), 4.15 (1H, d, *J* 4.2 alkyne CH_2), 4.11 (1H, ddd, *J* 8.0, 1.8, 8.4, H-1), 3.84 (1H, dd, *J* 7.7, 2.5, H-3) 3.81-3.78 (1H, m, H-6a), 3.74 (1H, dd, *J* 9.4, 5.8, H-2), 3.64 (3H, overlapping signals, H-6b, H-5 & H-4), 2.49-2.48 (2H, m, CH_2CH=CH2), 2.36 (1H, t, *J* 2.36, alkyne C-H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.8, 138.4, 138.2 (each Ar-C), 134.6 (CH_2CH=CH_2), 128.4 (2s), 128.3, 128.0, 127.8 (2s), 127.7, 127.5 (each Ar-CH), 116.9 (CH_2CH=CH_2) 82.3 (C-3), 80.0 (C-2), 79.6 (C-4), 77.9 (alkyne C), 77.2, 77.0, 76.0 (OCH_2Ph), 75.4, 75.1 (CH), 74.7 (C-1), 73.8 (CH_2), 70.9 (C-5), 68.5 (alkyne CH_2), 58.5 (C-6), 29.8 (alkyne CH), 29.7 (CH_2CH=CH_2); ESI-HRMS calcd for $C_{33}H_{36}O_5Na$ 535.2460, found *m/z* 535.2465 $[M+Na]^+$



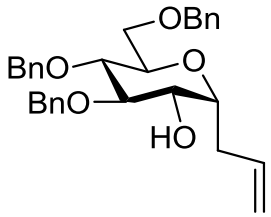
1-*C*-Allyl-1-deoxy-3,4-di-*O*-benzyl-6-*O*-propargyl-α-D-glucopyranoside (153). Compound **152** (800 mg, 1.56 mmol) was taken up in THF (10 mL) and cooled to 0 °C. I_2

(1.98 g, 7.8 mmol) was added and the reaction mixture was allowed to stir for 2 h. The reaction mixture was diluted with EtOAc and 1M $Na_2S_2O_3$ solution was added and stirring was continued for 10 min. Phases were separated and aqueous layer was extracted into EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified via flash chromatography (petroleum ether-EtOAc 9:1) to give a colourless syrup. The product was dissolved in MeOH-Et_2O 1:1 (12 mL). To this was added Zn dust (1.0 g, 15.3 mmol) and glacial acetic acid (100 µL). The reaction mixture was stirred at room temperature overnight, filtered through Celite® and the solvents were concentrated under reduced pressure. The crude residue was taken up in CH_2Cl_2, washed with 1M HCl, satd $NaHCO_3$, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1) gave **153** (395 mg, 60%) as a white solid; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.37-7.3 (10H, m, Ph-H), 5.88-5.80 (1H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.16-5.06 (2H, m, CH_2CH=CH_2), 4.70 (1H, d, *J* 11.7 OC*H*HPh), 4.68 (2H, d, *J* 11.4 OCH_2Ph), 4.62 (1H, d, *J* 11.7 OCH_2Ph), 4.23 (1H, dd, *J* 15.9, 2.3, alkyne CH_2), 4.20 (1H, dd, *J* 15.9, 2.3, alkyne CH_2), 4.01-3.98 (2H, overlapping signals, H-1 & H-3), 3.90 (1H, dd, *J* 10.2, 5.6, H-2), 3.76 (1H, t, *J* 5.8, H-6a), 3.72 (1H, dd, *J* 10.8, 4.6, H-6b), 3.70-3.68 (1H, m, H-5), 3.63 (1H, t, *J* 5.2, H-4), 2.47-2.40 (2H, m, CH_2CH=CH_2), 2.38 (1H, t, *J* 2.4 alkyne C-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 138.0, 137.5 (each Ar-C), 134.6 (CH_2CH=CH_2), 128.5, 128.4, 127.9 (2s), 127. 6, 127.4 (each Ar-C), 116.9 (CH_2CH=CH_2) 79.6, 77.9, 75.2 (CH), 75.2, 73.1, 73.0 (each OCH_2Ph), 71.4 (C-1), 73.1 (CH), 69.3 (C-5), 67.6 (alkyne C-H), 58.4 (CH), 32.8 (CH_2CH=CH_2); ESI-HRMS calcd for $C_{26}H_{31}O_5$ 423.2171, found *m/z* 423.2168 $[M+H]^+$



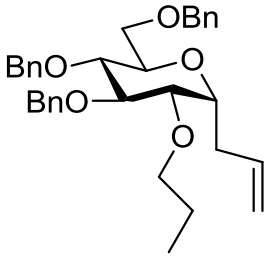
1-*C*-Allyl-1-deoxy-2-*O*-isopentyl-3,4-di-*O*-benzyl-6-*O*-propargyl-α-D-glucopyranoside (154). Sodium hydride (60% dispersion in mineral oil, 37 mg, 0.92 mmol) was added to a stirring solution of **153** (300 mg, 0.71 mmol) in DMF (7 mL) at 0 °C. After 10 min, 1-bromo-

3-methyl-butane (212 μL, 1.8 mmol) was added dropwise. The reaction mixture was allowed warm to room temperature overnight and quenched via the slow addition of MeOH at 0 °C. The reaction mixture was diluted with EtOAc, washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 6:1) to give **154** (276 mg, 79 %) as a white solid; $[\alpha]_D$ +32.4° (c 0.01 in $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): 7.38-7.28 (10 H, m Ar-H), 5.88-5.80 (1 H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.14-5.07 (2H, m, CH_2CH=CH_2), 4.93 (1H, d, *J* 11.1, OCH_2Ph), 4.86 (1H, d, *J* 10.8, OCH_2Ph), 4.80 (1H, d, *J* 11.06, OCH_2Ph), 4.68 (1H, d, *J* 11.8, OCH_2Ph), 4.24 (1H, dd, *J* 15.9, 2.3, alkyne CH_2), 4.19-3.16 (1H, m, H-1), 4.14 (1H, dd, *J* 16.0, 2.3, alkyne CH_2), 3.84 (1H, dd, *J* 10.4, 2.9, H-3), 3.72 (1H, t, *J* 8.8, H-2), 3.66-3.57 (4H, overlapping signals, H-5, H-6 & H-4 overlapping), 3.57 (2H, t, *J* 6.5, $OCH_2CH_2CH(CH_3)_2$), 2.50-2.36 (2H, m, CH_2CH=CH_2), 2.36 (1H, t, *J* 2.3, alkyne C-H), 1.75-1.67 (1H, m, $OCH_2CH_2CH(CH_3)_2$), 1.48 (1H, dd, *J* 6.7, 1.6, $OCH_2CH_2CH(CH_3)_2$), 1.48 (1H, dd, *J* 6.8, 1.8, $OCH_2CH_2CH(CH_3)_2$), 0.88 (6H, dd, *J* 6.6, 1.5, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.9, 138.4 (each Ar-*C*), 134.8 (CH_2CH=CH_2), 128.3 (2s), 128.0, 127.8, 127.6, 127.4 (each Ar-*C*H), 116.7 (CH_2CH=CH_2), 82.2, 80.6, 79.6 (CH), 77.8 ($OCH_2CH_2CH(CH_3)_2$), 75.2, 75.0, (each OCH_2Ph), 73.8 (C-1), 70.9 (alkyne CH_2), 69.3 (CH), 68.5, 58.5 (alkyne C-H), 39.0 ($OCH_2CH_2CH(CH_3)_2$), 29.7 (CH_2CH=CH_2), 24.8 ($OCH_2CH_2CH(CH_3)_2$), 22.6, 22.5 ($OCH_2CH_2CH(CH_3)_2$); ESI-HRMS calcd for $C_{31}H_{40}O_5Na$ 515.2773, found *m/z* 515.2770 $[M+Na]^+$



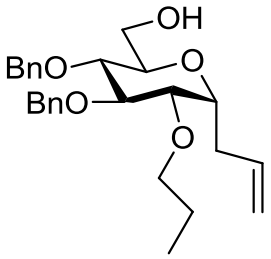
1-*C*-Allyl-1-deoxy-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside[8] (159). Compound **151** (1.0 g, 1.9 mmol) was taken up in THF (12 mL) under and cooled to 0 °C. I_2 (1.98 g, 7.8 mmol) was added and the reaction mixture was allowed to stir for 2 h. The reaction mixture was diluted with EtOAc and 1M $Na_2S_2O_3$ solution was added. Phases were separated and aqueous layer was extracted into EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude product was dissolved in MeOH-Et_2O (14 mL, 1:1). To this was added Zn dust (1.0 g, 15.3 mmol) and glacial acetic acid (0.110 mL). The reaction mixture stirred at room temperature overnight. The reaction mixture was then filtered through Celite® and the solvents were

concentrated under reduced pressure. The crude residue was taken up in CH_2Cl_2, washed with 1M HCl, satd $NaHCO_3$, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1) gave **159** (622 mg, 69%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 – 7.27 (10H, m, Ar-H), 7.25 – 7.22 (5H, m, Ar-H), 5.83 (1H, ddt, *J* 17.1, 10.2, 6.9, CH_2CH=CH_2), 5.16 – 5.03 (2H, m, CH_2CH=CH_2), 4.64 (1H, d, *J* 11.7, OC*H*HPh), 4.61 (1H, d, *J* 11.5, OC*H*HPh), 4.56 (3H, dd, *J* 11.9, 2.1, each OCH*H*Ph), 4.52 – 4.48 (1H, m, OCH*H*Ph), 4.05 (1H, dd, *J* 10.1, 5.2, CH), 3.93 (1H, ddd, *J* 8.7, 5.5, 3.0, H-1), 3.81 (1H, dd, *J* 10.2, 5.8, H-6a), 3.75 (1H, t, *J* 5.3, CH), 3.69 (1H, dd, *J* 10.2, 5.2, H-6b), 3.67 – 3.62 (2H, m, CH), 2.47 – 2.34 (2H, m, CH_2CH=CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.1, 137.9, 137.4 (each Ar-C), 134.7 (CH_2CH=CH_2), 128.5, 128.4, 128.3, 127.9 (3s), 127.7, 127.6, 127.5 (each Ar-CH), 116.9 (CH_2CH=CH_2), 77.4 (CH), 74.7 (CH), 73.5 (CH), 73.2 (OCH_2Ph), 72.7 (OCH_2Ph), 71.0 (C-1), 69.0 (CH), 68.0 (C-6), 33.2 (CH_2CH=CH_2); ESI-HRMS calcd for $C_{30}H_{35}O_5$ 475.2484, found *m/z* 475.2480 $[M+H]^+$



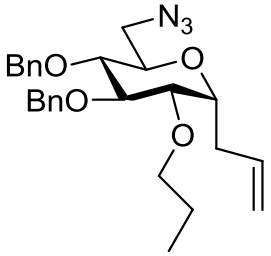
1-*C*-Allyl-1-deoxy-2-*O*-propyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (160). Sodium hydride (60% in mineral oil dispersion, 66 mg, 1.64 mmol) was added portionwise to a stirring solution of **160** (600 mg, 1.26 mmol) in DMF (13 mL) at 0 °C. After 10 min, 1-iodopropane (393 μL, 4.0 mmol) was added dropwise. The reaction mixture was allowed warm to room temperature overnight and quenched via the slow addition of MeOH at 0 °C. The reaction mixture was diluted with EtOAc, washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 6:1) gave **160** (553 mg, 71%) as a white solid; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38-7.26 (14H, m, Ar-H), 7.14 (1H, d, *J* 5.8, Ar-H), 5.89-5.81 (1H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.14-5.07 (2H, m, CH_2CH=CH_2), 4.93 (1H, d, J 10.8, OCH_2Ph), 4.79 (2H, dd, *J* 17.8, 10.8, OCH_2Ph), 4.62 (1H, d, *J* 12.0, OCH_2Ph), 4.48 (2H, d, *J* 12.3, OCH_2Ph), 4.20 (1H, m, H-1), 3.7 (2H, overlapping signals, each CH), 3.64-3.59 (6H, overlapping signals, CH, CH_2 & $OCH_2CH_2CH_3$), 2.45 (2H, m, CH_2CH=CH_2), 1.65 – 1.53 (2H, m, $OCH_2CH_2CH_3$), 0.94 (3H, t, *J* 7.4, $OCH_2CH_2CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ

138.9, 138.2, 138.1 (Ar-C), 134.8 (CH_2CH=CH_2), 128.3 (2s), 127.9, 128.8, 127.8, 127.6, 127.5 (2s) (each Ar-CH), 116.7 (CH_2CH=CH_2), 82.3, 80.6, 78.0 (CH), 75.0, 75.4 (each OCH_2Ph), 73.7 (C-1), 75.4 ($OCH_2CH_2CH(CH_3)_2$, 72.7 ($OCH_2CH_2CH_3$), 71.4 (CH), 62.4 (C-6), 29.9 (CH_2CH=CH_2), 23.4 ($OCH_2CH_2CH_3$), 10.7 ($OCH_2CH_2CH_3$); ESI-HRMS calcd for $C_{33}H_{41}O_5$ 517.2954, found *m/z* 517.2951 $[M+H]^+$

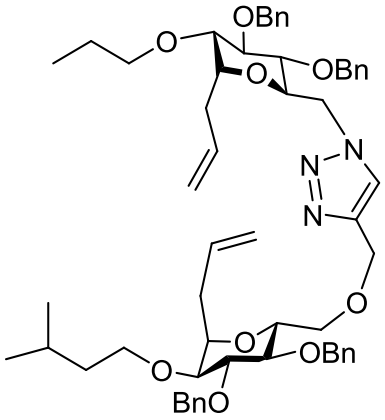


1-*C*-Allyl-1-deoxy-2-*O*-propyl-3,4-di-*O*-benzyl-α-D-glucopyranoside. Compound **160** (550 mg, 1.1 mmol) was dissolved in Ac_2O-CH_2Cl_2 (10 mL, 1:1) and cooled to -78 °C. To this was added trimethylsilyltriflate (60 µL, 0.33 mmol) dropwise. After 3 h, the reaction was brought to 0 °C and quenched with satd $NaHCO_3$. Phases were separated and the aqueous phase was extracted into CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give the 6-*O*-acetylated intermediate as a brown oil. The crude product was dried for 3 h under high vacuum and dissolved in MeOH (10 mL). To this was added freshly prepared solution of NaOMe-MeOH (2mL of a 1M solution) and the reaction mixture was stirred at room temperature overnight. Solvents were concentrated under reduced pressure and the crude residue was taken up in CH_2Cl_2 and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 9:1) to give the title compound (351 mg, 75%) as a white solid; $[\alpha]_D$ +18.1 (*c* 1.0 in $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.32 (10H, ddd, *J* 20.2, 14.0, 7.0, Ar-H), 5.85 – 5.74 (1H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.16 – 5.06 (2H, m, CH_2CH=CH_2), 4.95 (1H, d, *J* 11.0, OC*H*HPh), 4.86 (1H, d, *J* 10.9, OC*H*HPh), 4.79 (1H, d, *J* 11.0, OCH*H*Ph), 4.62 (1H, d, *J* 10.9, OCH*H*Ph), 4.16 – 4.10 (1H, m, H-1), 3.80 – 3.72 (2H, overlapping signals, H-6a & CH), 3.67 – 3.60 (1H, m, H-6b), 3.58 – 3.50 (4H, overlapping signals, $OCH_2CH_2CH_3$, CH & CH), 3.51 – 3.43 (1H, m, CH), 2.45 (2H, m, CH_2CH=CH_2), 1.65 – 1.53 (2H, m, $OCH_2CH_2CH_3$), 0.94 (3H, t, *J* 7.4, $OCH_2CH_2CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.7 (CH_2CH=CH_2), 138.0, 134.6 (each Ar-C), 128.4, 128.3, 128.0, 127.8, 127.5 (each Ar-CH), 117.0 (CH_2CH=CH_2), 82.0 (CH), 80.6 (CH), 77.9 (CH), 75.3 (OCH_2Ph), 75.1 (OCH_2Ph), 73.5 (CH), 72.7 ($OCH_2CH_2CH_3$), 71.4

(CH), 62.4 (C-6), 29.9 (*C*H_2CH=CH_2), 23.4 (OCH_2*C*H_2CH_3), 10.7 (OCH_2CH_2*C*H_3); ESI-HRMS calcd for $C_{26}H_{35}O_5$ 427.2484, found *m*/*z* 427.2479 $[M+H]^+$

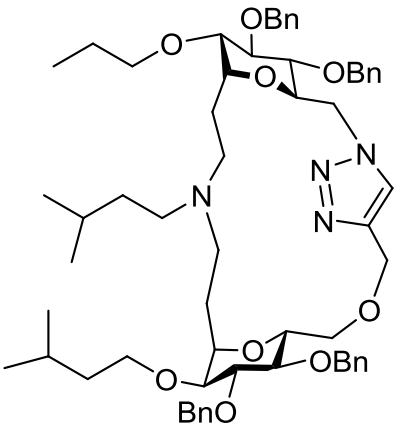


1-*C*-Allyl-1,6-dideoxy-2-*O*-propyl-3,4-di-*O*-benzyl-6-azido-α-D-glucopyranoside (161). Primary alcohol (350 mg, 0.82 mmol) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C. To this was added triethylamine (400 µL, 2.87 mmol) followed by the dropwise addition of MsCl (95 µL, 1.2 mmol). The reaction was stirred for 2 h, diluted with H_2O and extracted into EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was dissolved in DMF (10 mL) and NaN_3 (267, 4.1 mmol) was added. The reaction was heated to 110 °C for 26 h. Upon cooling the reaction was diluted with Et_2O and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 6:1) to give **161** (241 mg, 65%) as a white solid; $[\alpha]_D$ +39.1 (*c* 1.0 in $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.32 (10H, ddd, *J* 20.2, 14.0, 7.0, Ar-H), 5.85 – 5.74 (1H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.16 – 5.06 (2H, m, CH_2CH=CH_2), 4.95 (1H, d, *J* 11.0, OC*H*HPh), 4.86 (1H, d, *J* 10.9, OC*H*HPh), 4.79 (1H, d, *J* 11.0, OCH*H*Ph), 4.62 (1H, d, *J* 10.9, OCH*H*Ph), 4.16 – 4.10 (1H, m, H-1), 3.80 – 3.72 (2H, overlapping signals, H-6a & CH), 3.67 – 3.60 (1H, m, H-6b), 3.58 – 3.50 (4H, overlapping signals, OC$H_2$$CH_2CH_3$, CH & CH), 3.51 – 3.43 (1H, m, CH), 2.45 (2H, m, CH_2CH=CH_2), 1.65 – 1.53 (2H, m, OCH_2C$H_2$$CH_3$), 0.94 (3H, t, *J* 7.4, OCH_2CH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.7, 137.9 (Ar-C), 134.4 (CH_2CH=CH_2), 128.4, 128.3, 128.0, 127.9, 127.8, 127.6 (each Ar-CH), 116.9 (CH_2C=CH_2), 81.9, 80.5, 78.6 (CH & O*C*$H_2CH_2CH_3$ overlapping), 75.2, 75.1, (each OCH_2Ph), 73.6 (C-1), 70.9, 69.4 (each CH), 51.7 (C-6), 29.9 (*C*H_2CH=CH_2), 23.4 (OCH_2*C*H_2CH_3), 10.7 (OCH_2CH_2*C*H_3); ESI-HRMS calcd for $C_{26}H_{34}N_3O_4$ 452.2549, found *m*/*z* 452.2444 $[M+H]^+$



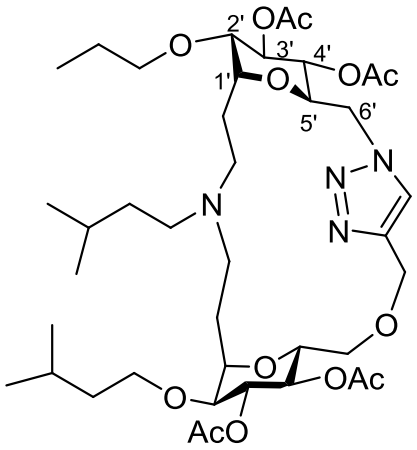
1-*C*-allyl-1,6-dideoxy-6-(4-(((1-*C*-allyl-1,6-dideoxy-2-*O*-isopentyl-3,4-di-*O*-benzyl-α-D-glucopyranos-6-yl)oxymethyl)-1H-1,2,3-triazol-1-yl)-2-*O*-propyl-3,4-di-*O*-benzyl-α-D-glucopyranoside (162). Alkyne **154** (200 mg, 0.41 mmol) and azide **161** (183 mg, 0.41mmol) were dissolved in a mixture of Acetonitrile-H_2O 1:1 (4 mL). To this was added CuI (41 mg, 0.22 mmol) and the reaction was heated at reflux for 24 h. Upon cooling the reaction was diluted with EtOAc, washed with H_2O, satd NH_4Cl, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:1) to give compound **162** (329 mg 85%) as a colourless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (1H, s, triazole H), 7.39 – 7.18 (20H, m, Ar-H), 5.82 (1H, ddt, *J* 16.9, 10.1, 6.8, $CH_2CH{=}CH_2$), 5.59 (1H, ddt, *J* 16.9, 10.1, 6.8, $CH_2CH{=}CH_2$), 5.14 – 5.03 (2H, m, $CH_2CH{=}CH_2$), 4.99 – 4.89 (4H, overlapping signals, $CH_2CH{=}CH_2$ & OC*H*HPh), 4.85 (1H, dd, *J* 10.7, 1.9, OC*H*HPh), 4.81 (1H, d, *J* 11.0, OC*H*HPh), 4.79 – 4.74 (1H, d, *J* 11.1 OCH*H*Ph), 4.72 (3H, overlapping signals, OC*H*HPh & OC*H*HC=C), 4.63 (1H, d, *J* 12.5, OCH*H*C=C), 4.50 (1H, d, *J* 10.8, OCH*H*Ph), 4.46 (1H, dd, *J* 14.2, 5.6, H-6'a), 4.37 (1H, dd, *J* 14.2, 2.6, H-6'b), 4.20 – 4.13 (1H, m, CH), 4.12 – 4.02 (1H, m, CH), 3.79 – 3.65 (5H, overlapping signals), 3.64 – 3.54 (5H, overlapping signals), 3.49 (2H, td, *J* 6.5, 3.0, CH_2), 3.43 (1H, dd, *J* 9.2, 5.8, CH), 3.08 (1H, dd, *J* 9.6, 8.8, CH), 2.51 – 2.33 (4H, overlapping signals, each CH_2CH=CH_2), 1.70 (1H, dt, *J* 13.4, 6.7, $OCH_2CH_2CH(CH_3)_3$), 1.57 (2H, dd, *J* 14.1, 6.9, $OCH_2CH_2CH(CH_3)_3$), 1.45 (2H, dt, *J* 19.6, 9.8, $OCH_2CH_2CH_3$), 0.91 (3H, t, *J* 7.4, $OCH_2CH_2CH_3$), 0.88 (6H, d, *J* 6.7, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.7, 138.8, 138.4, 138.2, 137.9 (each Ar-C), 134.7 ($CH_2CH{=}CH_2$), 134.1 ($CH_2CH{=}CH_2$), 128.5, 128.4, 128.3 (3s), 128.0, 127.9, 127.8 (3s), 127.7, 127.6, 127.5 (2s), (each Ar-CH), 124.3 (triazole *C*H=C), 117.1 ($CH_2CH{=}CH_2$), 82.2, 81.8, 80.5, 80.2, 78.0, 77.9 (each CH), 75.3, 75.2, 75.0 (2s), 74.7 (each OCH_2Ph), 73.7 (CH), 73.7 (CH), 72.6 (CH_2), 71.0 (CH), 70.8 (CH), 70.0 (CH_2), 69.3 (CH_2),

68.4, 65.0 (OCH_2C=C), 50.8 (C-6), 39.0 ($OCH_2CH_2CH_3$), 29.8 (CH_2CH=CH_2), 29.7 (CH_2CH=CH_2), 24.8 ($OCH_2CH_2CH(CH_3)_2$), 23.4 ($OCH_2CH_2CH(CH_3)_2$), 22.6, 22.5 (each $OCH_2CH_2CH(CH_3)_2$), 10.6 ($OCH_2CH_2CH_3$); ESI-HRMS calcd for $C_{57}H_{74}N_3O_9$ 944.5425, found *m/z* 944.5423 $[M+H]^+$



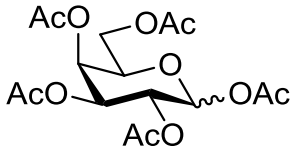
Macrocycle 164. To a stirred solution of **162** (200 mg, 0.212 mmol) in dioxane-H_2O (3:1, 2 mL) was added 2,6-lutidine (86 μL, 0.742 mmol), $NaIO_4$ (204 mg, 0.95 mmol) and a catalytic amount of OsO_4 (2 drops, 2.5% solution in *t*-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H_2O and CH_2Cl_2 were added, layers were separated and the aqueous layer was extracted into CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and isopentylamine (25 μL, 0.212 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (157 mg, 0.742 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **164** (160 mg, 75%) as an off white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (1H, s, triazole H), 7.40 – 7.26 (20H, m, Ar-H), 4.89 (4H, overlapping signals, each OCH_2Ph), 4.76 (4H, overlapping signals, OCH_2Ph, H-6'a and OC*H*HC=C), 4.65 (1H, d, *J* 11.2, OCH*H*Ph), 4.60 – 4.52 (2H, overlapping signals, OC*H*HPh & OCH*H*C=C), 4.06 (3H, overlapping signals, 2 x CH & H-6'b), 3.77 (3H, overlapping signals, 2 x CH & H-6a), 3.67 (2H, dd, *J* 18.1, 9.1, each CH), 3.55 (7H, overlapping signals), 3.32 – 3.22 (2H, overlapping signals, each CH), 2.46 (3H, m), 2.38 (2H,

m), 2.18 (1H, m) (each CH_2N), 1.73 (5H, overlapping signals), 1.60 (2H, m, $OCH_2CH_2CH(CH_3)_2$), 1.52 (1H, $NCH_2CH_2CH(CH_3)_2$) 1.45 (2H, m $OCH_2CH_2CH_3$), 1.25 (2H, m, $NCH_2CH_2CH(CH_3)_2$) 0.93 (3H, t, *J* 7.4, $OCH_2CH_2CH_3$), 0.88 (12H, d, *J* 6.2, $CH_2CH_2CH(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.3 (triazole CH=*C*), 138.0, 137.6 (each Ar-C), 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5 (each Ar-CH), 122.9 (triazole *C*H=C), 82.6, 81.8, 80.7, 80.4, 79.2, 78.7 (each CH), 75.3 (CH_2Ph), 75.1 (CH_2Ph), 74.8 (CH_2Ph), 72.7 (CH_2), 72.3, 72.0, 71.9, 70.7, 70.2, 69.3 (CH_2), 65.2 (OCH_2C=C), 51.8, 51.7, 51.3, 49.0 (each CH_2N), 39.1 (CH_2), 26.6 (CH), 24.9 (CH), 23.4 (CH_2), 22.6, 22.5 (each $CH_2CH_2CH(CH_3)_2$), 21.7 (CH_2), 10.7 ($OCH_2CH_2CH_3$); ESI-HRMS calcd for $C_{60}H_{83}N_4O_9$ 1003.6160, found *m/z* 1003.6158 $[M+H]^+$



Macrocycle 165. Compound **164** (50 mg, 0.049 mmol) was dissolved in EtSH-BF_3.Et_2O (2 mL, 4:1). The reaction was stirred at room temperature for 24 h. The solvents were removed under reduced pressure and the crude residue was dissolved in pyridine-Ac_2O (1:1, 1.5 mL). The reaction mixture was stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (MeOH-CH_2Cl_2, 1:9) gave **165** (30 mg, 76%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (1H, s, triazole H), 5.20 (2H, overlapping signals, H-3 & H-3'), 4.88 – 4.82 (1H, m, H-4'), 4.74 (3H, overlapping signals, H-4 & OCH_2C=C), 4.62 (2H, m, H-6'), 4.27 – 4.22 (1H, m, H-1'), 4.17 (1H, dd, *J* 14.1, 9.9, H-5'), 4.11 – 4.04 (1H, m, H-1), 3.93 – 3.86 (1H, m, H-5), 3.84 – 3.79 (1H, m, H-2), 3.61 – 3.50 (7H, overlapping signals, each CH_2, H-2 & H-2'), 2.52 – 2.41 (3H, m), 2.41 – 2.33 (3H, m) (each CH_2N), 2.11 (3H, s), 2.07 (3 H, s), 2.05 (6H, each s) (each acetate CH_3), 1.72 – 1.57 (4H, m), 1.54 (4H, m), 1.45 – 1.33 (2H, m, $OCH_2CH_2CH(CH_3)_2$), 1.23 (2H, m, $NCH_2CH_2CH(CH_3)_2$), 0.88 (15H,

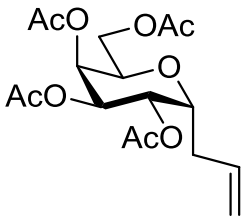
dd, *J* 17.4, 7.0, $CH_2CH_2CH(CH_3)_2$ & $CH_2CH_2CH_3$ overlapping); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 170.1, 169.9, 169.7 (each C=O), 146.3 (*C*=CH), 123.2 (C=*C*H), 77.4, 77.3 (each CH), 73.1 (CH_2), 72.4, 72.3, 72.2, 72.2, 71.8, 71.4, 71.1, 70.9, 69.9, 69.9 (each CH), 69.5 (CH_2), 69.4 (3s) 68.2, 65.4, 58.6, 51.6, 51.5, 51.1, 38.7 (each CH_2), 26.2, 25.9, 24.6 (CH), 23.1 (CH_2), 22.5, 22.4, 22.3, 21.4, 20.8 (2s), 20.7 (2s), 10.36 (each CH_3); ESI-HRMS calcd for $C_{40}H_{67}N_4O_{13}$ 811.4705, found *m/z* 811.4703 $[M+H]^+$



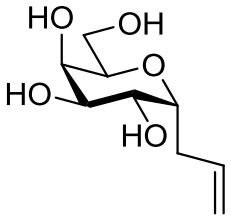
1,2,3,4,6-Penta-*O*-acetyl-α/β-D-galactopyranose (171). To a suspension of D-galactose (100 g, 554 mmol) in Ac_2O (500 mL) was added I_2 (7 g, 55 mmol) . The reaction was cooled to 0 °C in an ice bath and strirred for 3 h. The reaction was quenched with sodium thiosulphate and extracted into CH_2Cl_2. The combined organic layers were washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting yellow oil was recrystallised from ethanol to give **171** (205 g, 95%) (4:1 α:β ratio) as a white solid. NMR data (^1H and ^{13}C) was in agreement with reported literature data.

α-anomer: ^1H NMR (500 MHz, $CDCl_3$) δ 6.36 (1H, d, *J* 1.7, H-1), 5.48 (1H, s, H-4), 5.32 (2H, overlapping signals, H-2 & H-3), 4.32 (1H, t, *J* 6.6, H-5), 4.12 – 4.06 (2H, m, H-6), 2.14 (6H, s, CH_3), 2.02 (3H, s, CH_3), 2.00 (3H, s, CH_3), 1.98 (3H, s, CH_3); ^{13}C NMR (126 MHz, $cdcl_3$) δ 170.4, 170.2 (2s), 169.9, 169.0 (each C=O), 89.8 (C-1), 68.8 (C-5), 67.5 (C-4), 67.4, 66.5, 61.3 (C-6), 20.9, 20.7 (3s), 20.6 (each CH_3)

β-anomer: ^1H NMR (500 MHz, $CDCl_3$) δ 5.72 (1H, d, *J* 8.3, H-1), 5.4 (1H, d, *J* 3.3, H-4), 5.3 (1H, dd, *J* 8.8, 9.8, H-2), 5.1 (1H, dd, *J* 10.3, 3.5, H-3), 4.35 (1H, m, H-5), 4.13 (2H, m, H-6), 2.20 (3H, s, CH_3), 2.15 (3H, s, CH_3), 2.09 (3H, s, CH_3), 1.99 (3H, s, CH_3), 1.95 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.2, 169.6, 169.4, 169.2, 167.7 (each C=O), 92.3 (C-1), 70.2, 68.4, 68.0, 65.1, 61.7, 20.5, 20.4 (2s), 20.3, 20.2 (each CH_3)

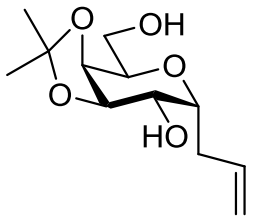


1-*C*-Allyl-1-deoxy-2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (172). To a stirred suspension of **171** (30 g, 76 mmol) and allyltrimethylsilane (36 mL, 230 mmol) in Acetonitrile (150 mL) was added $BF_3.Et_2O$ (47 mL, 380 mmol). The reaction was heated at reflux overnight, diluted with EtOAc and quenched with satd $NaHCO_3$. Phases were separated and the aqueous layer was washed with EtOAc. The combined organic phases were washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. Purification of the crude residue via flash chromatography gave **172** (21 g, 75%) as a viscous oil. NMR data (^1H and ^{13}C) was in agreement with reported literature data. R_f 0.6 (petroleum ether-EtOAc 2:1); $[\alpha]_D$ +84° (c 0.01 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.80–5.69 (1H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.42–5.40 (1H, m, H-4), 5.28–5.25 (1H, dd, *J* 4.8, 9.3 Hz, H-2), 5.22–5.21 (1H, dd, *J* 3.2, 9.3 Hz, H 3), 5.15–5.08 (2H, m, CH_2CH=CH_2), 4.31– 4.28 (1H, q, *J* 4.8, 10.0 Hz, H-1), 4.22–4.17 (1H, dd, *J* 8.8, 12.8 Hz, H-6), 4.12–4.06 (2H, overlapping signals, H-5 & H-6), 2.52–2.41 (1H, m, $CHHCH$=CH_2), 2.32–2.22 (1H, m, $CHHCH$=CH_2), 2.12 (3H, s, CH_3), 2.07 (3H, s, CH_3), 2.04 (3H, s, CH_3), 2.03 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.1, 167.6, 167.5, 167.3 (each C=O) 130.8 (CH_2CH=CH_2), 115.3 (CH_2CH=CH_2), 69.1 (CH), 65.9 (CH), 65.6 (CH), 65.3 (CH), 59.2 (C-6), 28.7 (CH_2CH=CH_2), 18.7, 18.6, 18.5 (each CH_3); ESI-HRMS calcd for $C_{17}H_{25}O_9$ 373.1498, found *m/z* 373.1492 $[M+H]^+$

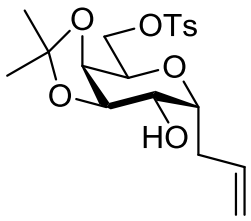


1-*C*-Allyl-1-deoxy-α-D-galactopyranoside[9] (170). Compound **172** (25 g, 67 mmol) was dissolved in MeOH (250 mL) and cooled to 0 °C. To this was added a solution of NaOMe-MeOH (13.4 mL of a 1M solution) and the resulting mixture was stirred at room temperature for 4 h. The reaction was acidified with Amberlite® resin, filtered and the solvents were concentrated under reduced pressure to give **170** (12.7, 93%) as a white solid. NMR data (^1H and ^{13}C) was in agreement with reported literature data; ^1H NMR (500 MHz, CD_3OD) δ 5.90

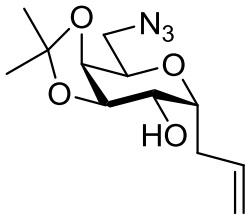
(1H, ddt, *J* 17.1, 10.1, 6.9, CH_2CH=CH_2), 5.10 (2H, m, CH_2CH=CH_2), 4.04 – 3.99 (1H, m), 3.97 (1H, s), 3.91 (1H, dd, *J* 8.6, 5.2), 3.77 (2H, dt, *J* 11.8, 8.1), 3.71 (1H, dd, *J* 7.8, 4.6), 3.35 – 3.31 (1H, m), 2.47 (1H, m, CH_2CH=CHH), 2.40 (1H, m, CH_2CH=CHH); ^{13}C NMR (125 MHz, CD_3OD) δ 135.3 (CH_2CH=CH_2), 115.4 (CH_2CH=CH_2), 74.2, 72.7, 70.5, 68.7, 68.5, 60.5, 29.7 (CH_2CH=CH_2); ESI-HRMS calcd for $C_9H_{17}O_5$ 205.1076, found *m/z* 205.1071 $[M+H]^+$



1-*C*-Allyl-1-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranoside[9] (173). To a solution of **170** (10 g, 49 mmol) and dimethoxypropane (24 mL, 195 mmol) in acetonitrile (80 mL), was added p-TsOH monohydrate (186 mg, 1 mmol) and the reaction mixture was stirred for 2 h. H_2O (15 mL) was then added and after 30 min the reaction was neutralized with triethylamine and the solvents were removed under reduced pressure. Flash chromatography of the crude residue (CH_2Cl_2-EtOAc 1:9) afforded **173** (8.4 g, 70%) as white crystalline solid. NMR data (^{1}H and ^{13}C) was in agreement with reported literature data. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.86 (1H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.15-5.09 (2H, m, CH_2CH=CH_2) 4.33-4.25 (2H, overlapping signals), 4.08-4.02 (2H, overlapping signals), 3.87-3.77 (2H, overlapping signals), 2.45-2.30 (2H, overlapping signals), 2.20 (1H, m, C*H*HCH=CH_2), 2.00 (1H, m, CH*H*CH=CH_2), 1.47 (3H, s, isopropylidene CH_3), 1.32 (3H, s, isopropylidene CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.3 (CH_2CH=CH_2), 117.7 (CH_2CH=CH_2), 109.7 (isopropylidene C), 74.8 (C-2), 73.1 (C-4), 70.6 (C-3), 69.6 (C-1), 69.1 (C-5), 63.6 (C-6), 34.9 (CH_2CH=CH_2), 26.8 (CH_3), 24.6 (CH_3); ESI-HRMS: calcd for $C_{12}H_{21}O_5Na$ 267.1162, found 267.1208 $[M+Na]^+$

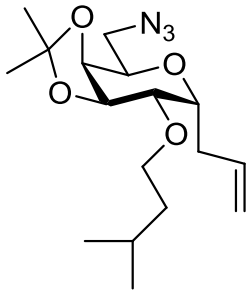


1-*C*-Allyl-1-deoxy-3,4-*O*-isopropylidene-6-*O*-*p*-toluenesulfonyl-α-D-galactopyranoside (174). A solution of **173** (1.00 g, 4.27 mmol) in pyridine-acetone (1:1, 25 mL) was treated at 0 °C with p-toluenesulfonyl chloride (896 mg, 4.70 mmol). The solution was allowed to warm to room temperature and stirred for 6 h. The reaction mixture was repeatedly azeotroped with toluene and the crude residue was taken up in CH_2Cl_2 and H_2O. The aqueous phase was extracted with CH_2Cl_2 and the organic extracts were combined, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. Flash chromatography of the crude residue (petroleum ether-EtOAc 2:1) gave the tosylate **174** (1.07 g, 64%) as a white solid. Rf 0.51 (EtOAc). $[\alpha]_D$ +27.1° (c 0.01 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (2H, d, *J* 8.3, Ar-H), 7.32 (2H, d, *J* 8.0, Ar-H), 5.77 (1H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.11 (1H, dd, *J* 17.2, 1.5, CH_2CH=CHH), 5.05 (1H, d, *J* 10.2, CH_2CH=CHH), 4.23 (2H, overlapping signals, H-4 & H-3), 4.21 (1H, apt t, *J* 6.5, H-5), 4.16 – 4.05 (2H, m, H-6), 3.92 (1H, td, *J* 7.1, 2.2, H-1), 3.74 (1H, brs, H-2), 2.42 (3H, s, Ar-CH_3), 2.35 – 2.22 (2H, m, CH_2CH=CH_2), 1.38 (3H, s, isopropylidene CH_3), 1.25 (3H, s, isopropylidene CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.8 (Ar-C), 134.2 (CH_2CH=CH_2), 132.8, 129.8, 128.0 (each Ar-*C*H), 117.5 (CH_2CH=CH_2), 109.6 (isopropylidene C), 74.1 (C-4), 71.6 (C-3), 70.5 (C-1), 69.4 (C-6), 68.4 (C-2), 67.5 (C-5), 35.2 (CH_2CH=CH_2), 26.5 (isopropylidene CH_3), 24.4 (isopropylidene CH_3), 21.6 (Ar-CH_3); ESI-HRMS calcd for $C_{19}H_{27}O_7S$ 399.1478, found *m/z* 399.1474 $[M+H]^+$



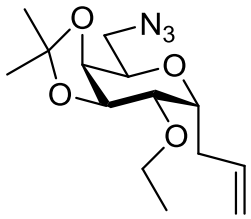
1-*C*-Allyl-1,6-dideoxy-3,4-*O*-isopropylidene-6-azido-α-D-galactopyranoside (175). The tosylate **174** (5.0 g, 12.5 mmol) was dissolved in DMF-H_2O (10:1). To this was added NaN_3 (4.07 g, 62.7 mmol) and the reaction heated to 120 °C for 24 h. Upon cooling to room temperature, the reaction mixture was partitioned between EtOAc and H_2O. The organic

phase was washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was purified via flash chromatography (petroleum ether-EtOAc 1:1) to give the title compound **175** (2 g, 60%) as a colourless oil. $[\alpha]_D$ +32.3° (c 0.03 in $CHCl_3$); IR (film) cm^{-1}: 3446, 2917, 2101 1642, 1376, 1211, 1062; ^1H NMR (500 MHz, $CDCl_3$) δ 5.86 (1H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.16 (1H, ddd, *J* 17.0, 3.2, 1.4, CH_2CH=CHH), 5.10 (1H, ddt, *J* 10.3, 2.0, 1.0, CH_2CH=CHH), 4.28 (1H, dd, *J* 7.4, 3.3, H-3), 4.23 (1H, dd, *J* 7.3, 2.0, H-4), 4.13 (1H, ddd, *J* 7.4, 5.2, 2.0, H-5), 4.03 (1H, td, *J* 7.2, 2.5, H-1), 3.80 (1H, brs, H-2), 3.51 (1H, dd, *J* 12.6, 7.8, H-6a), 3.26 (1H, dd, *J* 12.6, 5.2, H-6b), 2.45 – 2.32 (2H, m, CH_2CH=CH_2), 1.48 (3H, s, isopropylidene CH_3), 1.32 (3H, s, isopropylidene CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 133.2 (CH_2CH=CH_2), 116.6 (CH_2CH=CH_2), 108.7 (isopropylidene C), 73.5 (C-2), 71.5 (C-4), 69.8 (C-3), 68.0 (C-1), 67.7 (C-5), 51.0 (C-6), 34.0 (CH_2CH=CH_2), 25.7 (isopropylidene CH_3), 23.5 (isopropylidene CH_3); ESI-HRMS calcd for $C_{12}H_{20}N_3O_4$ 270.1376, found *m/z* 270.1374 $[M+H]^+$



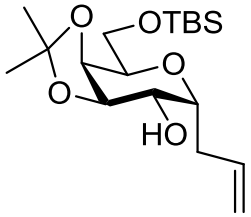
1-*C*-Allyl-1,6-dideoxy-2-*O*-isopentyl-3,4-*O*-isopropylidene-6-azido-α-D-galactopyranoside (176). To a stirred suspension of **175** (1.76 g, 6.5 mmol) in DMF (25 mL) at 0 °C was added sodium hydride (60% in mineral oil dispersion, 3.9 g, 9.7 mmol) slowly with vigorous stirring. After 15 min, 1-bromo-3-methylbutane (2.5 mL, 20.8 mmol) was added and the reaction was warmed to room temperature and stirred for 14 h. The reaction was quenched by the slow addition of MeOH (5 mL) and partitioned between EtOAc and H_2O. Phases were separated and the organic phase was washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 4:1) to give compound **176** (1.58g, 72%) as a colourless oil. $[\alpha]_D$ +9.3° (c 0.05 in $CHCl_3$); IR (film) cm^{-1}: 2925, 2098 1642, 1372, 1210, 1061; ^1H NMR (500 MHz, $CDCl_3$) δ 5.81 (1H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.12 (1H, ddd, *J* 17.5, 3.1, 1.6, CH_2CH=CHH), 5.07 (1H, ddt, *J*

10.4, 2.1, 1.1, $CH_2CH{=}CHH$), 4.37 (1H, dd, *J* 7.5, 3.3, H-3), 4.20 (1H, dd, *J* 7.5, 1.7, H-4), 4.07 – 4.00 (2H, overlapping signals, H-1 & H-5), 3.66 (1H, dt, *J* 9.2, 6.7, $OCHHCH_2CH(CH_3)_2$), 3.52 – 3.43 (2H, overlapping signals, $OCHHCH_2CH(CH_3)_2$ & H-6a), 3.37 (1H, t, *J* 3.1, H-2), 3.26 (1H, dd, *J* 12.5, 5.5, H-6b), 2.45 – 2.38 (1H, m, $CHHCH{=}CH_2$), 2.37 – 2.30 (1H, m, $CHHCH{=}CH_2$), 1.71 (1H, dq, *J* 20.1, 6.7, $OCH_2CH_2CH(CH_3)_2$), 1.49 (3H, s, isopropylidene CH_3), 1.46 (2H, m, $OCH_2CH_2CH(CH_3)_2$), 1.34 (3H, s, isopropylidene CH_3), 0.90 (6H, dd, *J* 6.7, 0.9, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.7 ($CH_2CH{=}CH_2$), 117.2 ($CH_2CH{=}CH_2$), 109.9 (isopropylidene C), 75.8 (C-2), 73.1 (C-4), 71.7 (C-3), 71.4 (C-5), 69.6 (C-1), 69.0 ($OCH_2CH_2CH(CH_3)_2$), 52.1 (C-6), 39.0, 35.2, 26.8, 25.0, 24.7, 22.7; ESI-HRMS calcd for $C_{17}H_{29}N_3O_4$ 339.2258, found *m/z* 339.2258 $[M+Na]^+$

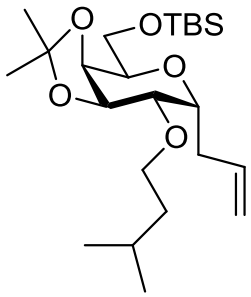


1-*C*-Allyl-1,6-dideoxy-2-*O*-ethyl-3,4-*O*-isopropylidene-6-azido-α-D-galactopyranoside (177). To a stirred suspension of **175** (870 mg, 3.23 mmol) in DMF (7 mL) at 0°C was added sodium hydride (60% dispersion in mineral oil 193 mg, 4.85 mmol) slowly with vigorous stirring. After 15 min, iodoethane (904 μL, 11.3 mmol) was added and the reaction was warmed to room temperature and stirred for 14 h. EtOAc and H_2O were added and the phases were separated. The organic phase was washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 4:1) to give **177** (749 mg, 78%) as a colourless oil; $[\alpha]_D$ +14.8° (c 0.06 in $CHCl_3$); IR (film) cm^{-1}: 2986, 2101 1641, 1375, 1059 905; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.79 (1H, ddt, *J* 17.1, 10.1, 7.0, $CH_2CH{=}CH_2$) , 5.07 (2H, ddd, *J* 13.7, 11.0, 1.2, $CH_2CH{=}CH_2$), 4.34 (1H, dd, *J* 7.4, 3.3, H-3), 4.19 (1 H, dd, *J* 7.4, 1.8, H-4), 4.06 – 3.98 (2H, overlapping signals, H-1 & H-5), 3.68 (1H, dq, *J* 9.2, 7.0, $OCHHCH_3$), 3.51 – 3.43 (2H, overlapping signals, H-6a & $OCHHCH_3$), 3.36 (1H, t, *J* 3.1, H-2), 3.24 (1H, dd, *J* 12.5, 5.5, H-6b), 2.39 (1H, ddd, *J* 14.1, 7.7, 6.3, $CH_2CH{=}CHH$), 2.32 (1H, dt, *J* 14.3, 7.3, $CH_2CH{=}CHH$), 1.47 (3H, s, isopropylidene CH_3), 1.32 (3H, s, isopropylidene CH_3), 1.18 (H, t, *J* 7.0, OCH_2CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.4 ($CH_2CH{=}CH_2$), 117.0 ($CH_2CH{=}CH_2$), 109.6 (isopropylidene C), 75.4 (C2), 72.8 (C4), 71.7 (C3), 71.1 (C-5), 68.8 (C1), 66.3 (OCH_2CH_3), 51.9 (C-6), 34.9 ($CH_2CH{=}CH_2$), 26.6 (isopropylidene CH_3),

24.5 (isopropylidene CH_3), 15.5 (OCH_2CH_3); ESI-HRMS calcd for $C_{14}H_{23}N_3O_4Na$ 320.1689 found *m/z* 320.1683 $[M+Na]^+$

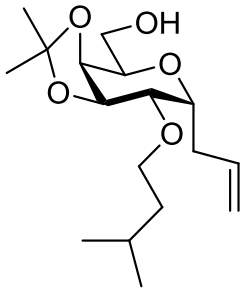


1-*C*-Allyl-1-deoxy-3,4-*O*-isopropylidene-6-*O*-dimethyltertbutylsilyl-α-D-galactopyranoside[9] (178). Compound **173** (8.8 g, 36 mmol) was dissolved in DMF (70 mL) and cooled to 0 °C. Imidazole (8.56 g, 126 mmol) was added followed by the addition of TBDMSCl (7.05 g, 46.8 mmol). The reaction mixture was stirred for 2 h at room temperature, diluted with EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography to give **178** (9.8 g, 76%) as a colourless oil. NMR data (^1H and ^{13}C) was in agreement with reported literature data; ^1H NMR (500 MHz, $CDCl_3$) δ 5.82 (1H, ddt, *J* 17.1, 10.2, 7.0, CH_2CH=CH_2), 5.09 (1H, ddd, *J* 17.2, 3.3, 1.5, CHHCH=CH_2), 5.02 (1H, ddt, *J* 10.1, 2.0, 1.1, CHHCH=CH_2) 4.37 (1H, dd, *J* 7.1, 1.9, H-3), 4.20 (1H, dd, *J* 7.1, 3.4, H-4), 4.02 – 3.96 (2H, overlapping signals, H-5 & H-1), 3.79 – 3.67 (3H, overlapping signals, H-2 & H-6), 2.34 (2H, ddd, *J* 7.2, 2.4, 1.2, CH_2CH=CH_2), 1.46 (3H, s, isopropylidene CH_3), 1.31 (3H, s, isopropylidene CH_3), 0.91 – 0.85 (9H, m), 0.04 (6H, d, *J* 1.5); ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.5 (C2), 117.5 (C-1), 109.1 (C), 74.9 (C-7), 71.9 (C-6), 70.8, 69.9, 69.6 (C-5), 62.8 (C-9), 34.8 (C-3), 27.2, 25.9, 25.7, 24.7, 18.4, -5.2, -5.3 (each SiCH_3)



1-*C*-Allyl-1-deoxy-2-*O*-isopentyl-3,4-*O*-isopropylidene-6-*O*-dimethyltertbutylsilyl-α-D-galactopyranoside (179). To a stirred suspension of **178** (4.2 g, 11.7 mmol) in DMF (15 mL) at 0°C was added sodium hydride (60% dispersion in mineral oil, 609 mg, 15.2 mmol) slowly with vigorous stirring. After 15 min, 1-bromo-3-methylbutane (4 mL, 35.1 mmol) was added and the reaction was warmed to room temperature and stirred for 14 h. MeOH (5 mL) was

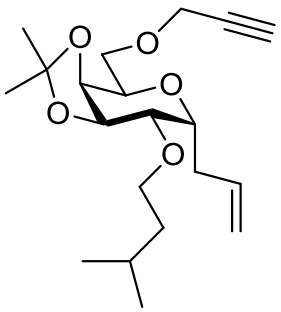
added to quench the reaction and EtOAc and H_2O were added. The phases were separated and the organic phase was washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 6:1) to give compound **179** (3.76 g, 75%) as a colourless oil; $[\alpha]_D$ +19.4° (c 0.03 in $CHCl_3$); IR (film) cm^{-1}: 2954, 2905, 2869, 1477, 1361, 1198, 882; ^1H NMR (500 MHz, $CDCl_3$) δ 5.79 (1H, ddt, *J* 17.1, 10.1, 7.1, CH_2CH=CH_2), 5.10 (1H, ddd, *J* 16.8, 3.1, 1.4, CH_2CH=C*H*H), 5.05 (1H, ddt, *J* 10.4, 2.1, 1.0, CH_2CH=CH*H*), 4.37 (1H, dd, *J* 7.3, 1.6, H-4), 4.29 (1H, dd, *J* 7.3, 3.2, H-3), 3.99 (1H, td, *J* 7.3, 2.8, H-1), 3.96 – 3.90 (1H, m, H-5), 3.73 (2H, dt, *J* 9.6, 6.7, H-6), 3.66 (1H, dt, *J* 9.2, 7.0, OC*H*H$CH_2CH(CH_3)_2$), 3.44 (1H, dt, *J* 9.2, 6.7, OCH*H*$CH_2CH(CH_3)_2$), 3.31 (1H, t, *J* 3.0, H-5), 2.42 – 2.30 (2H, m, C*H*$_2$CH=CH_2), 1.72 (1H, tt, *J* 13.4, 6.7, $OCH_2CH_2CH(CH_3)_2$), 1.49 (3H, s, isopropylidene CH_3), 1.45 (2H, dd, *J* 13.8, 7.1, $OCH_2CH_2CH(CH_3)_2$), 1.33 (3H, s, isopropylidene CH_3), 0.89 (15H, overlapping signals, $OCH_2CH_2CH(CH_3)_2$ & *t*-Bu-H), 0.07 (6H, dd, *J* 5.1, 2.8, tBuSi(CH_3)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.9 (CH_2CH=CH_2), 117.1 (CH_2CH=CH_2), 109.1 (isopropylidene C), 76.6 (C-2), 72.1 (C-4), 71.7 (C-3), 71.0 (C-1), 69.8 (C-5), 69.2 ($OCH_2CH_2CH(CH_3)_2$), 62.6 (C-6), 39.0 ($OCH_2CH_2CH(CH_3)_2$), 35.0 (CH_2CH=CH_2),), 27.1, 26.0, 25.0 (each CH_3), 24.7 ($OCH_2CH_2CH(CH_3)_2$), 22.7 (2s), 18.4, 14.2, -5.1, -5.2 (each CH_3). ESI-HRMS calcd for $C_{23}H_{45}O_5Si$ 429.3035 found *m/z* 429.3036 $[M+H]^+$



1-*C*-Allyl-1-deoxy-2-*O*-isopentyl-3,4-*O*-isopropylidene-α-D-galactopyranoside.

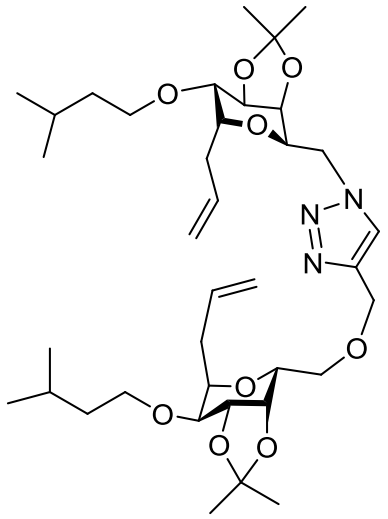
Compound **179** (1.4 g, 3.3 mmol) was dissolved in THF (25 mL) and TBAF (10 mL of a 1.0 M solution in THF) was added dropwise. The resulting solution was stirred for 3 h at room temperature. EtOAc was added and the organic layer was washed with 1M HCl, H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was used without further purification (955 mg, 92% crude yield). IR (film) cm^{-1}: 3500 br, 2926, 1381, 1210, 1060; ^1H NMR (500 MHz, $CDCl_3$) δ 5.78 (1H, ddt, *J* 17.1, 10.1, 7.1, CH_2CH=CH_2), 5.08 (2H, dd, *J* 20.7, 13.8, CH_2CH=C*H*$_2$), 4.34 (1H, dd, *J* 7.4, 3.4,

H-3), 4.26 (1H, dd, *J* 7.4, 1.5, H-4), 4.03 (1H, td, *J* 7.3, 3.0, H-1), 4.00 – 3.95 (1H, m, H-5), 3.80 (1H, dd, *J* 11.3, 6.8, H-6a), 3.69 (1H, dd, *J* 11.6, 4.7, H-6b), 3.66 – 3.61 (1H, m, OC*H*HCH$_2$CH(CH$_3$)$_2$), 3.44 (1H, dt, *J* 9.0, 6.6, OCH*H*CH$_2$CH(CH$_3$)$_2$), 3.37 (1H, t, *J* 3.2, H-2), 2.37 (2H, m, C*H*$_2$CH=CH$_2$), 1.69 (1H, tt, *J* 13.4, 6.7, OCH$_2$CH$_2$C*H*(CH$_3$)$_2$), 1.47 (3H, s, isopropylidene CH$_3$), 1.44 (2H, dd, *J* 13.5, 6.8, OCH$_2$C*H*$_2$CH(CH$_3$)$_2$), 1.33 (3H, s, isopropylidene CH$_3$), 0.89 (6H, s, CH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 134.7 (CH$_2$*C*H=CH$_2$), 117.2 (CH$_2$CH=*C*H$_2$), 109.7 (isopropylidene C), 76.0 (C-2), 73.5 (C-4), 71.9 (C-3), 71.1 (C-1), 69.6 (C-5), 69.4 (O*C*H$_2$CH$_2$CH(CH$_3$)$_2$), 63.5 (C-6), 38.9 (OCH$_2$*C*H$_2$CH(CH$_3$)$_2$), 34.9 (*C*H$_2$CH=CH$_2$), 26.8 (CH$_3$), 25.7 (OCH$_2$CH$_2$*C*H(CH$_3$)$_2$), 24.9, 24.7, 22.6 (each CH$_3$); ESI-HRMS calcd for C$_{17}$H$_{34}$NO$_5$ 332.2437, found *m/z* 332.2433 [M+NH$_4$]$^+$



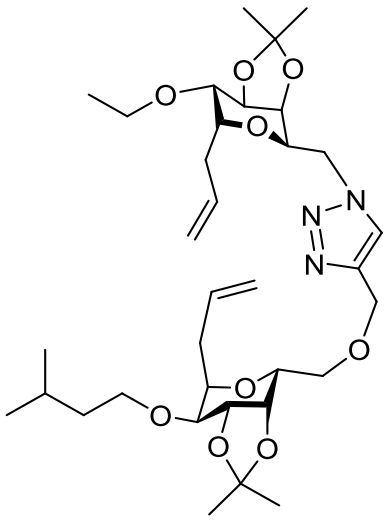
1-*C*-Allyl-1-deoxy-2-*O*-isopentyl-3,4-*O*-isopropylidene-6-*O*-propargyl-α-D-galactopyranoside (180). To a stirred suspension of alcohol (955 mg, 3 mmol) in DMF (6 mL) at 0°C was added sodium hydride (60% dispersion in mineral oil, 145 mg, 3.6 mmol) slowly and with vigorous stirring. After 15 min, propargyl bromide (80% solution in toluene, 620μL, 9 mmol) was added and the reaction was warmed to room temperature and stirred for 14 h. EtOAc and H$_2$O were added, phases were separated and the organic phase was washed with H$_2$O, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography to give compound **180** (676 mg 64%) as a colourless oil; [α]$_D$ +21.7° (c 0.03 in CHCl$_3$); IR (film) cm^{-1}: 3529 sharp, 2954, 2905, 2869, 1477, 1361, 1198, 882; ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.84 – 5.75 (1H, ddt, *J* 17.1, 10.1, 7.0, CH$_2$C*H*=CH$_2$), 5.08 (2H, ddd, *J* 13.7, 11.0, 1.3, CH$_2$CH=C*H*$_2$), 4.33 (1H, dd, *J* 7.4, 3.2, H-3), 4.29 (1H, dd, *J* 7.4, 1.5, H-4), 4.21 (2H, qd, *J* 15.7, 2.4, alkyne CH$_2$), 4.11 (1H, td, *J* 6.4, 1.6), 4.01 (1H, td, *J* 7.3, 2.9, H-1), 3.71 (1H, dd, *J* 9.8, 6.0, H-6a), 3.69 – 3.62 (2H, overlapping signals, OC*H*HCH$_2$CH(CH$_3$)$_2$ & H-6b), 3.44 (1H, dt, *J* 9.1, 6.6, OCH*H*CH$_2$CH(CH$_3$)$_2$), 3.35 (1H, t, *J* 3.0, H-2), 2.43 (1H, t, *J* 2.3, alkyne C-H), 2.37 (2H, td, *J* 14.0, 7.0, C*H*$_2$CH=CH$_2$), 1.76 – 1.67 (1H, m, OCH$_2$CH$_2$C*H*(CH$_3$)$_2$), 1.49 (3H, s,

isopropylidene CH_3), 1.45 (2H, q, *J* 6.7, $OCH_2CH_2CH(CH_3)_2$), 1.34 (3H, s, isopropylidene CH_3), 0.90 (6H, d, *J* 6.7, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.8 ($CH_2CH=CH_2$), 117.2 ($CH_2CH=CH_2$), 109.5 (isopropylidene C), 79.9, 76.0 (C-2), 74.5, 73.0 (C-4), 71.6 (C-3), 71.1 (C-1), 70.1 ($OCH_2CH_2CH(CH_3)_2$), 69.3 (C-6), 68.5 (C-2), 58.7 (C-10), 39.0 ($CH_2CH=CH_2$), 35.1 ($OCH_2CH_2CH(CH_3)_2$), 26.9 (CH_3), 24.9 ($OCH_2CH_2CH(CH_3)_2$), 24.8 (CH_3), 22.7 (CH_3); ESI-HRMS calcd for $C_{20}H_{32}O_5Na$ 375.2147, found *m/z* 375.2143 $[M+Na]^+$



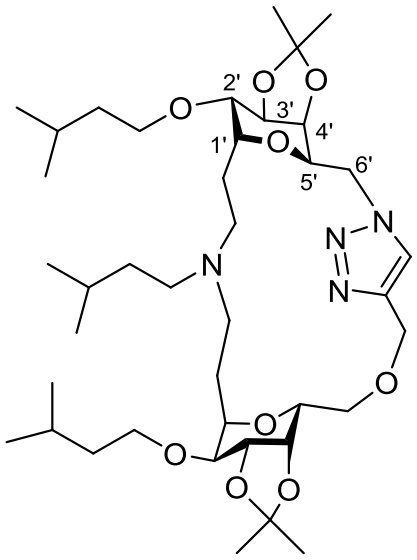
1-*C*-Allyl-1,6-dideoxy-6-(4-(((1-*C*-allyl-1,6-dideoxy-2-*O*-isopentyl-3,4-*O*-isopropylidene-α-D-galactopyranos-6-yl)oxymethyl)-1H-1,2,3-triazol-1-yl)-2-*O*-isopentyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (181). Alkyne **180** (110 mg, 0.31 mmol) and azide **176** (106 mg, 0.31mmol) were dissolved in acetonitrile-H_2O (6 mL, 1:1). To this was added CuI (41 mg, 0.22 mmol) and the reaction was heated at reflux for 24 h. Upon cooling the reaction was diluted with EtOAc, washed with H_2O, satd NH_4Cl, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:1) to give compound **181** (118 mg 55%) as a colourless oil. $[\alpha]_D$ +24.2° (c 0.002 in $CHCl_3$); IR (film) cm^{-1}: 3572, 2955, 2871, 1466, 1361, 1200, 882; ^1H NMR (500 MHz, $CDCl_3$) δ 7.71 (1H, s, triazole H), 5.84 – 5.74 (1H, ddt, *J* 17.1, 10.1, 7.0, $CH_2CH=CH_2$), 5.71 (1H, ddd, *J* 17.3, 8.7, 5.3, $CH_2CH=CH_2$), 5.13 – 4.99 (4H, m, each $CH_2CH=CH_2$), 4.70 (2H, q, *J* 12.4,-C=C-CH_2-O), 4.60 (1H, dd, *J* 13.9, 3.4, H-6'a), 4.39 (2H, overlapping signals, H6'b & H-4'), 4.34 – 4.28 (2H, overlapping signals, H-3' & H-4), 4.21 (1H, dd, *J* 9.7, 2.3, H-3), 4.18 (1H, brd, H-5), 4.11 (1H, t, *J* 6.2, H-5'), 4.09 – 4.04 (1H, m, H-1'), 4.01 (1H, td, *J* 7.3, 2.7, H-1), 3.73 – 3.59 (4H, overlapping

signals, OC*H*$_2$CH$_2$CH(CH$_3$)$_2$ & H-6), 3.42 (3H, overlapping signals, OC*H*$_2$CH$_2$CH(CH$_3$)$_2$ & H-2), 3.33 (1H, apt t, *J* 2.7, H-2'), 2.43 – 2.34 (2H, m, C*H*$_2$CH=CH$_2$), 2.29 (1H, dd, *J* 14.6, 7.4, C*H*HCH=CH$_2$), 2.22 (1H, dd, *J* 13.9, 6.9, CH*H*CH=CH$_2$), 1.74 – 1.61 (2H, m, each OCH$_2$CH$_2$C*H*(CH$_3$)$_2$), 1.52 (3H, s, isopropylidene CH$_3$), 1.48 (3H, s, isopropylidene CH$_3$), 1.43 (4H, dq, *J* 13.6, 6.8, each OCH$_2$C*H*$_2$CH(CH$_3$)$_2$), 1.36 (3H, s, isopropylidene CH$_3$), 1.34 (3H, s, isopropylidene CH$_3$), 0.88 (12H, dd, *J* 7.8, 6.8, each OCH$_2$CH$_2$CH(C*H*$_3$)$_2$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 134.4 (CH$_2$*C*H=CH$_2$), 124.1 (triazole C-H), 117.2 (CH$_2$CH=*C*H$_2$), 110.0 (C), 109.3 (C), 76.1 (CH), 75.5 (CH), 73.1 (CH), 72.9 (CH), 71.6 (CH), 71.6 (CH), 71.5 (CH), 71.0 (CH), 70.5 (CH), 69.8 (CH), 69.3 (CH$_2$), 68.9 (CH), 68.5 (CH), 65.0 (CH$_2$), 51.9 (CH$_2$), 39.0 (OCH$_2$*C*H$_2$CH(CH$_3$)$_2$), 35.2 (*C*H$_2$CH=CH$_2$), 27.0 (isopropylidene CH$_3$), 26.7 (isopropylidene CH$_3$), 24.9 (OCH$_2$CH$_2$*C*H(CH$_3$)$_2$), 24.9 (isopropylidene CH$_3$), 24.8 (isopropylidene CH$_3$), 22.7 (2s), 22.6 (2s), (each OCH$_2$CH$_2$CH(*C*H$_3$)$_2$); ESI-HRMS calcd for C$_{37}$H$_{62}$N$_3$O$_9$ 692.4486, found *m*/*z* 692.4472 [M+H]$^+$



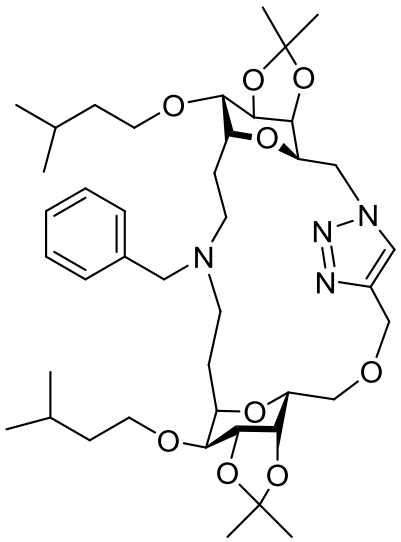
1-*C*-Allyl-1,6-dideoxy-6-(4-(((1-C-allyl-1,6-dideoxy-2-*O*-ethyl-3,4-*O*-isopropylidene-α-D-galactopyranos-6-yl)oxymethyl)-1H-1,2,3-triazol-1-yl)-2-*O*-isopentyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (182). Alkyne **180** (189 mg, 0.54 mmol) and azide **177** (159 mg, 0.54 mmol) were dissolved in a mixture of acetonitrile-H$_2$O (6 mL, 1:1). To this was added CuI (51 mg, 0.27 mmol) and the reaction was heated at reflux for 6 h. Upon cooling the reaction was diluted with EtOAc, washed with 1M HCl, H$_2$O, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether, 1:1) to give the title compound (288 mg, 82%) as a colourless oil; [α]$_D$ +35.5° (c 0.004 in CHCl$_3$); IR (film) cm^{-1}:

2923, 1559, 1372, 1098, 935; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (1H, s, triazole H), 5.85 – 5.65 (2H, m, each CH_2CH=CH_2), 5.14 – 4.99 (4H, m, each CH_2CH=CH_2), 4.71 (2H, q, *J* 12.4, O-CH_2C=C), 4.62 (1H, dd, *J* 14.0, 3.8, H-6'a), 4.42 – 4.36 (2H, overlapping signals, H-6'b & H-3'), 4.34 – 4.27 (2H, overlapping signals, H-3 & H-4'), 4.23 (1H, dd, *J* 8.2, 3.3, H-5), 4.20 (1H, d, *J* 7.6, H-4), 4.11 (1H, apt t, *J* 6.2, H-5'), 4.08 – 4.03 (1H, m, H-1'), 4.01 (1H, apt t, *J* 7.2, H-1), 3.73 – 3.61 (4H, overlapping signals, H-6, OC*H*H$CH_2CH(CH_3)_2$ & OC*H*HCH_3), 3.44 (2H, overlapping signals, OCH*H*$CH_2CH(CH_3)_2$ & OCH*H*CH_3), 3.40 (1H, t, *J* 2.7, H-2'), 3.33 (1H, apt s, H-2), 2.36 (2H, m, CH_2CH=CH_2), 2.30 (1H, dd, *J* 14.3, 7.0, C*H*HCH=CH_2), 2.23 (1H, dd, *J* 13.9, 6.9, CH*H*CH=CH_2), 1.70 (1H, dt, *J* 13.4, 6.7, $OCH_2CH_2CH(CH_3)_2$), 1.52 (3H, s, isopropylidene CH_3), 1.48 (3H, s, isopropylidene CH_3), 1.44 (2H, dd, *J* 13.4, 6.7, $OCH_2CH_2CH(CH_3)_2$), 1.35 (3H, s, isopropylidene CH_3), 1.33 (3H, s, isopropylidene CH_3), 1.16 (3H, t, *J* 6.9, OCH_2CH_3), 0.89 (6H, d, *J* 6.6, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.89 (triazole CH=*C*), 134.38 (CH_2CH=CH_2), 124.23 (triazole *C*H=C), 117.28 (CH_2CH=CH_2), 110.03 (isopropylidene C), 109.33 (isopropylidene C), 76.01 (C-2), 75.21 (C-2), 72.91 (C-4), 72.81 (C-4'), 71.57 (C-3'), 71.48 (C-3), 71.35 (C-1'), 70.84 (C-1), 70.44 (C-6), 69.20 (OCH_2), 68.76 (C-5), 68.37 (C-5'), 66.57 (OCH_2), 64.88 (OCH_2C=C), 51.88 (C-6'), 38.86 ($OCH_2CH_2CH(CH_3)_2$), 35.07 (CH_2CH=CH_2), 26.85 (isopropylidene CH_3), 26.62 (isopropylidene CH_3), 24.82 (isopropylidene CH_3), 24.65 ($OCH_2CH_2CH(CH_3)_2$), 24.62 (isopropylidene CH_3), 22.58 ($OCH_2CH_2CH(CH_3)_2$), 15.50 (OCH_2CH_3); ESI-HRMS calcd for $C_{34}H_{55}N_3O_9Na$ 672.3836, found *m/z* 672.3861 $[M+Na]^+$



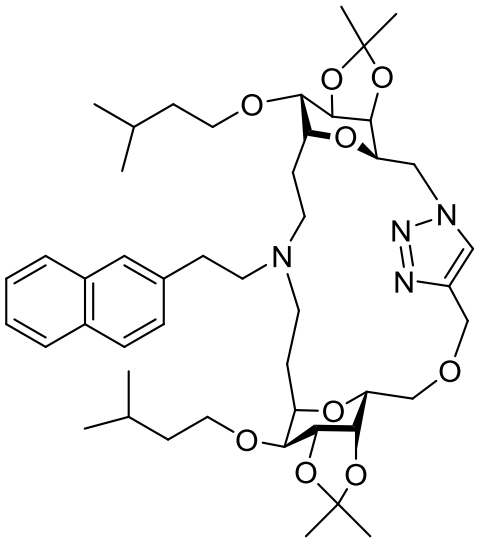
Macrocycle 183. To a stirred solution of **181** (118 mg, 0.171 mmol) in dioxane-H_2O (3:1, 2 mL) was added 2,6-lutidine (69 μL, 0.599 mmol), $NaIO_4$ (165 mg, 0.77 mmol) and a catalytic

amount of OsO_4 (2 drops, 2.5% solution in *t*-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H_2O and CH_2Cl_2 were added, layers were separated and the aqueous layer was extracted into CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and isopentylamine (20 µL, 0.171 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **183** (69 mg, 54%) as an off white solid; $[\alpha]_D$ +50.7° (c 0.002 in $CHCl_3$); IR (film) cm^{-1}: 2957, 1466, 1382, 1212, 1099, 905; ^1H NMR (500 MHz, $CDCl_3$) δ 7.81 (1H, s, triazole H), 4.77 (1H, d, *J* 11.7, OC*H*HC=C), 4.70 (1H, d, *J* 11.7, OCH*H*C=C), 4.65 (1H, d, *J* 13.2, H-6'a), 4.40 (1H, dd, *J* 7.6, 3.2, H-3), 4.36 (1H, dd, *J* 14.3, 10.0, H-6'b), 4.27 (1H, dd, *J* 7.7, 1.4, H-3'), 4.24 (1H, apt d, *J* 6.1, H-4'), 4.14 (1H, dd, *J* 6.4, 1.9, H-4), 4.09 (2H, overlapping signals, H-5 & H-5'), 4.06 – 4.00 (1H, m, H-1'), 3.98 (1H, dt, *J* 9.6, 3.2, H-1), 3.80 – 3.70 (2H, m, H-6'), 3.64 (2H, ddd, *J* 21.7, 9.3, 6.8, OC*H*$_2$CH$_2$CH(CH$_3$)$_2$), 3.51 (2H, overlapping signals, OC*H*HCH$_2$CH(CH$_3$)$_2$ & H-2'), 3.46 – 3.40 (1H, m, OCH*H*CH$_2$CH(CH$_3$)$_2$), 3.36 – 3.32 (1H, m, H-2), 2.72 (3H, d, *J* 11.3), 2.52 (2H, dd, *J* 19.5, 11.0), 2.44 (1H, dd, *J* 17.4, 10.4), 1.87 – 1.74 (2H, m) (each CH$_2$C*H*$_2$N), 1.67 (5H, overlapping signals, 3 x CH and CH$_2$), 1.53 (3H, s, isopropylidene CH$_3$), 1.52 (3H, s, isopropylidene CH$_3$), 1.50 – 1.40 (4H, overlapping signals, OCH$_2$C*H*$_2$CH(CH$_3$)$_2$), 1.38 (3H, s, isopropylidene CH$_3$), 1.33 (5H, overlapping signals, CH$_2$ & isopropylidene CH$_3$), 0.94 – 0.82 (18H, m, OCH$_2$CH$_2$CH(C*H*$_3$)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.0 (triazole C=C), 123.2 (triazole CH=C), 110.2 (isopropylidene C), 109.7 (isopropylidene C), 76.5, 74.6 (each C-2), 73.9, 73.6 (each C-4), 71.6 (C-3), 71.3 (C-1), 71.1 (C-6), 70.4 (C-1), 69.9 (O*C*H$_2$CH$_2$CH(CH$_3$)$_2$), 69.5 (O*C*H$_2$CH$_2$CH(CH$_3$)$_2$), 69.1 (C-5), 68.7 (C-5), 65.9 (O*C*H$_2$C=C) 52.7 (C-6), 51.6 (CH$_2$*C*H$_2$N), 51.1 (CH$_2$*C*H$_2$N), 49.6 (CH$_2$*C*H$_2$N), 38.9 (CH$_2$), 38.7 (CH$_2$), 27.6, 26.7, 26.6, 25.8, 25.0, 24.8, 24.6, 22.9, 22.7 (3s), 22.6, 22.5; ESI-HRMS calcd for $C_{40}H_{71}N_4O_9$ 751.5221, found *m/z* 751.5220 $[M+H]^+$



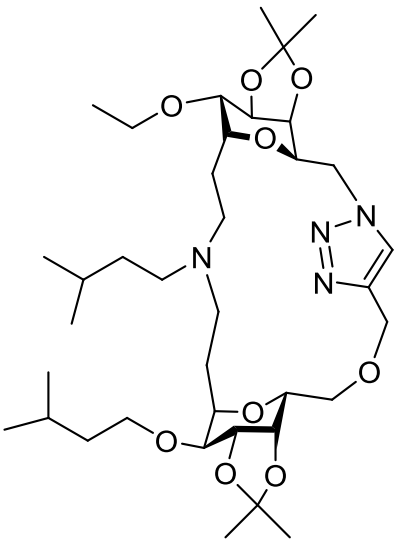
Macrocycle 185. To a stirred solution of **181** (110 mg, 0.159 mmol) in dioxane-H_2O (3:1, 2 mL) was added 2,6-lutidine (65 μL, 0.565 mmol), $NaIO_4$ (152 mg, 0.715 mmol) and a catalytic amount of OsO_4 (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H_2O and CH_2Cl_2 were added, the layers were separated and the aqueous layer was extracted into CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and benzyl amine (17 μL, 0.159 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **185** (92 mg, 75%) as an off white solid; $[\alpha]_D$ +50.7° (c 0.002 in $CHCl_3$); IR (film) cm^{-1}: 2957, 1466, 1382, 1212, 1099, 905; ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 (1H, s, triazole H), 7.37 – 7.19 (5H, m, Ar-H), 4.75 (2H, q, *J* 12.0, OCH_2C=C), 4.64 (1H, dd, *J* 14.3, 1.3, H-6'a), 4.37 (1H, dd, *J* 7.6, 3.3, H-3,), 4.32 (1H, dd, *J* 14.4, 9.9, H-6'b), 4.27 – 4.22 (2H, overlapping signals, H3 & H-4'), 4.17 (1H, dd, *J* 6.6, 1.9, H-4), 4.12 – 4.08 (1H, m, H-1), 4.0 (2H, overlapping signals, H-5 and H-5'), 3.95 – 3.90 (1H, m, H-1'), 3.77 – 3.72 (1H, m, H-6a), 3.70 (1H, dd, *J* 10.3, 3.0), 3.62 (1H, dt, *J* 9.4, 6.7, OC*H*$HCH_2CH(CH_3)_2$), 3.59 – 3.53 (2H, m, PhCH_2N), 3.54 – 3.47 (1H, m, OCH*H*$CH_2CH(CH_3)_2$), 3.45 (1H, dd, *J* 6.7, 2.7, OC*H*$HCH_2CH(CH_3)_2$), 3.42 (1H, dd, *J* 8.8, 3.8, OCH*H*$CH_2CH(CH_3)_2$), 3.27 (1H, m, H-2'), 3.23 (1H, apt t, *J* 3.2, H-2), 2.75 – 2.67 (1H, m), 2.67 – 2.54 (2H, m), 2.53 – 2.45 (1H, m) (each CH_2N), 1.87 – 1.75 (2H, m), 1.69 (2H, dt,

J 18.8, 6.1, $OCH_2CH_2CH(CH_3)_2$), 1.64 – 1.58 (1H, m, $CH_2CH_2CH(CH_3)_2$), 1.51 (3H, s, isopropylidene CH_3), 1.49 (3H, s, isopropylidene CH_3), 1.43 (4H, dt, *J* 9.6, 5.9, each $OCH_2CH_2CH(CH_3)_2$), 1.35 (3H, s, isopropylidene CH_3), 1.34 (3H, s, isopropylidene CH_3), 0.87 (12H, ddd, *J* 14.1, 6.6, 2.1, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.7 (triazole CH=*C*), 128.6, 128.3, 128.1, 128.1, 126.9, 126.7 (each Ar-C), 123.3 (triazole *C*H=C), 110.1 (isopropylidene C), 109.4 (isopropylidene C), 77.4 (C-2), 76.0 (C-2), 74.0 (C-4), 73.7 (C-4), 73.5 (C-3), 71.5 (C-3'), 71.1, 70.3 (C-1), 70.3 (C-1'), 69.7 (C-5), 69.3 (C-5'), 69.0 ($OCH_2CH_2CH(CH_3)_2$), 68.7 ($OCH_2CH_2CH(CH_3)_2$), 65.9 (OCH_2C=C), 56.8 ($PhCH_2N$), 53.1 (C-6), 51.5 (CH_2N), 50.8 (CH_2N), 38.8 (CH_2), 38.6 (CH_2), 29.6, 27.3 (isopropylidene CH_3), 26.5 (isopropylidene CH_3), 25.5 (isopropylidene CH_3), 24.8 (isopropylidene CH_3), 24.7, 24.5, 22.6, 22.5, 22.3; ESI-HRMS calcd for $C_{42}H_{66}N_4O_9$ 771.4908, found *m/z* 771.4901 $[M+H]^+$



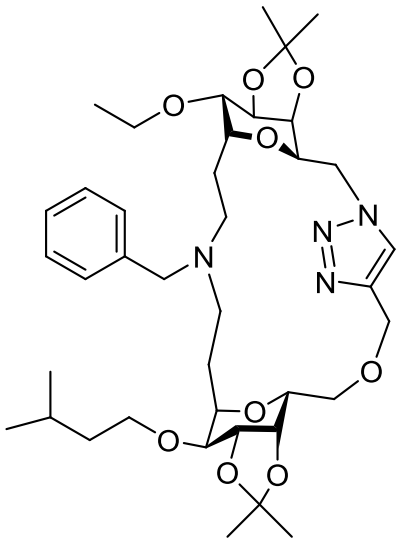
Macrocycle 184. To a stirred solution of **181** (120 mg, 0.173 mmol) in dioxane-H_2O (3:1, 2 mL) was added 2,6-lutidine (71 μL, 0.607 mmol), $NaIO_4$ (165 mg, 0.77 mmol) and a catalytic amount of OsO_4 (2 drops, 2.5% solution in *t*-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H_2O and CH_2Cl_2 were added, the layers were separated and the aqueous layer was extracted into CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and 2-(2-Naphthyl)ethylamine hydrochloride (36 mg, 0.173 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by

the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **184** (88 mg, 61%) as an off white solid; $[\alpha]_D$ +40.7° (c 0.001 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.80 (1H, s, Ar-H), 7.80 – 7.73 (3H, m, Ar-H), 7.61 (1H, s, Ar-H), 7.42 (2H, tt, *J* 13.4, 6.7, Ar-H), 7.31 (1H, dd, *J* 8.4, 1.2, Ar-H), 4.77 (1H, d *J* 11.7, OC*H*HC=C), 4.70 (1H, d *J* 11.7, OCH*H*C=C), 4.64 (1H, d, *J* 13.6, H-6'a), 4.35 (2H, overlapping signals, H-3 & H-6'b), 4.22 – 4.18 (1H, m, H-4), 4.15 (1H, apt t, *J* 6.1, H-4'), 4.03 (5H, overlapping signals, H-5, H-5', H-1 & H-1'), 3.77 – 3.71 (1H, m, H-6a), 3.68 (1H, dd, *J* 10.3, 2.2, H-6b), 3.65 – 3.56 (2H, m, OC*H*$_2$CH$_2$CH(CH$_3$)$_2$), 3.48 (2H, overlapping signals, H-2' & OC*H*HCH$_2$CH(CH$_3$)$_2$), 3.42 – 3.36 (1H, m, OCH*H*CH$_2$CH(CH$_3$)$_2$), 3.30 (1H, t, *J* 3.0, H-2), 2.92 – 2.85 (2H, m), 2.84 – 2.71 (5H, m), 2.61 (1H, m) (each CH_2N), 1.79 (1H, dd, *J* 16.9, 6.9), 1.69 (4H, dt, *J* 13.3, 6.7), 1.61 (1H, dt, *J* 20.0, 6.7), 1.52 (3H, s, isopropylidene CH_3), 1.50 (3H, s, isopropylidene CH_3), 1.48 – 1.37 (4H, m, each CH_2), 1.35 (3H, s, isopropylidene CH_3), 1.32 (3H, s, isopropylidene CH_3), 0.85 (12H, ddd, *J* 9.0, 6.6, 2.8, OCH$_2$CH$_2$CH(C*H*$_3$)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.0 (triazole CH=*C*), 138.1, 133.6, 132.0, 127.9, 127.6, 127.5, 127.4, 126.8, 126.0, 125.3 (each Ar-C), 123.1 (triazole *C*H=C), 110.1 (isopropylidene CH_3), 109.6 (isopropylidene CH_3), 76.6 (C-2), 74.6 (C-2), 73.8 (C-4), 73.5 (C-4), 71.7 (C-3), 71.3 (C-3), 71.1 (C-6), 70.4 (C-1), 69.8 (C-1), 69.4 (C-5), 69.0 (C-5'), 68.6 (OCH$_2$CH$_2$CH(C*H*$_3$)$_2$), 66.1 (OCH$_2$C=C), 53.3 (CH_2N), 52.7 (C-6), 52.0 (CH_2N), 50.9 (CH_2N), 38.8 (CH_2), 38.7 (CH_2), 27.6 (isopropylidene CH_3), 26.7 (isopropylidene CH_3), 25.8 (isopropylidene CH_3), 24.9 (isopropylidene CH_3), 24.8, 24.6, 22.7, 22.6 (2s), 22.4; ESI-HRMS calcd for $C_{47}H_{71}N_4O_9$ 835.5221, found *m/z* 835.5218 $[M+H]^+$



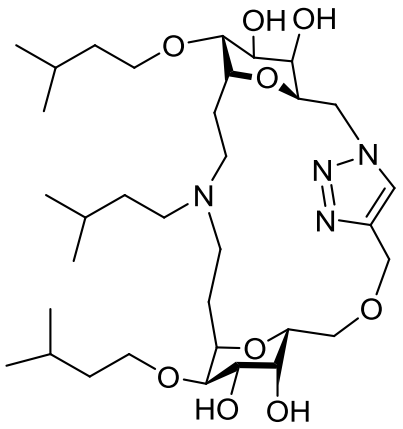
Macrocycle 186. To a stirred solution of **182** (112 mg, 0.172 mmol) in dioxane-H_2O (3:1, 2 mL) was added 2,6-Lutidine (69 µL, 0.599 mmol), $NaIO_4$ (165 mg, 0.77 mmol) and a catalytic amount of OsO_4 (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H_2O and CH_2Cl_2 were added, the layers were separated and the aqueous layer was extracted into CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and isopentylamine (20 µl, 0.171 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **186** (86 mg, 71%) as an off white solid; $[\alpha]_D$ +43.3° (c 0.003 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (1H, s, triazole H), 4.75 (1H, d, *J* 11.8, OC*H*HC=C), 4.68 (1H, d, *J* 11.8, OCH*H*C=C), 4.64 (1H, apt d, *J* 13.9, H-6'a), 4.37 (1H, dd, *J* 7.6, 3.2, H-3), 4.32 (1H, dd, *J* 14.4, 10.0, H-6'b), 4.26 (1H, dd, *J* 7.6, 1.2, H-3), 4.21 (1H, t, *J* 6.1, H-4), 4.11 (1H, dd, *J* 6.4, 1.8, H-4), 4.10 – 4.03 (2H, overlapping signals, H-5 & H-5'), 4.03 – 3.98 (1H, m, H-1), 3.97 – 3.92 (1H, m, H-1), 3.76 – 3.67 (2H, m, H-6), 3.63 (2H, overlapping signals OC*H*HCH$_3$ & OC*H*HCH$_2$CH(CH$_3$)$_2$), 3.47 (3H, overlapping signals, H-2', OCH*H*CH$_3$ & OCH*H*CH$_2$CH(CH$_3$)$_2$), 3.31 (1H, t, *J* 2.5, H-2), 2.67 (2H, dd, *J* 24.4, 12.4, C*H*$_2$N), 2.49 (2H, m, C*H*$_2$N), 2.45 – 2.33 (2H, m, C*H*$_2$N), 1.82 – 1.60 (5H, overlapping signals, CH$_2$N & OCH$_2$CH$_2$C*H*(CH$_3$)$_2$), 1.54 (1H, m, CH) 1.51 (3H, s, isopropylidene CH$_3$), 1.49 (3H, s, isopropylidene CH$_3$), 1.47 – 1.38 (4H, m,

$OCH_2CH_2CH(CH_3)_2$), 1.35 (3H, s, isopropylidene CH_3), 1.31 (3H, s, isopropylidene CH_3), 1.13 (3H, t, *J* 7.0, OCH_2CH_3), 0.91 – 0.82 (12H, m, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.9 (triazole *C*=CH), 122.0 (triazole C=*C*H), 109.0 (isopropylidene C), 108.5 (isopropylidene C), 76.4 (C-2), 75.3 (C-2), 73.6 (C-4), 72.8 (C-4), 72.5 (C-3'), 70.6 (C-3), 70.2 (C-6), 70.0 (C-1'), 69.2 (C-1), 68.3 (C-5'), 68.0 ($OCH_2CH_2CH(CH_3)_2$), 67.5 (C-5), 65.7 (OCH_2CH_3), 65.0 (OCH_2C=C), 51.7 (C-6'), 50.7, 50.2 , 48.6 (each CH_2N), 37.8 ($OCH_2CH_2CH(CH_3)_2$), 26.5, 25.6, 25.5, 24.7, 23.9, 23.4, 21.8, 21.6 (2s), 21.5 ($OCH_2CH_2CH(CH_3)_2$), 14.5 (OCH_2CH_3); ESI-HRMS calcd for $C_{37}H_{65}N_4O_9$ 709.4751, found *m/z* 709.4745 $[M+H]^+$

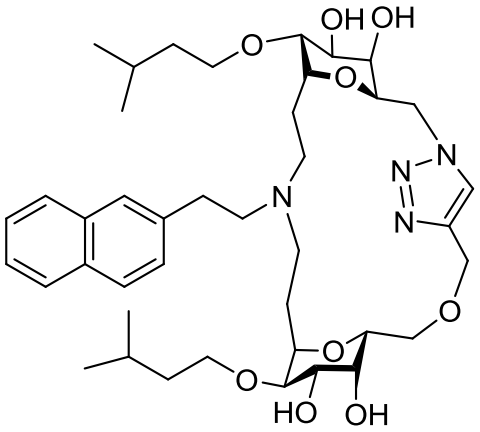


Macrocycle 187. To a stirred solution of **182** (100 mg, 0.154 mmol) in dioxane-H_2O (3:1, 2 mL) was added 2,6-lutidine (63 μL, 0.539 mmol), $NaIO_4$ (148 mg, 0.69 mmol) and a catalytic amount of OsO_4 (2 drops, 2.5% solution in *t*-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H_2O and CH_2Cl_2 were added, layers were separated and the aqueous layer was extracted into CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and benzyl amine (17 μL, 0.154 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash

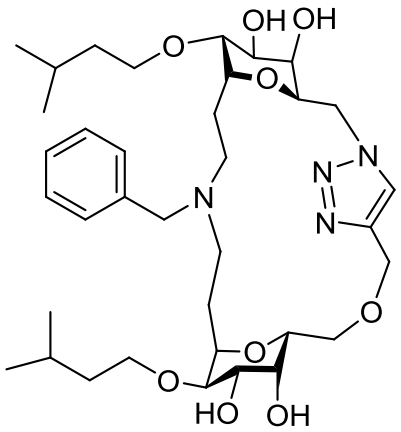
chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **187** (73 mg, 65%) as an off white solid; $[\alpha]_D$ +33.4° (c 0.004 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (1H, s, triazole H), 7.30 – 7.12 (5H, m, Ar-H), 4.68 (2H, q, *J* 12.1, O-C*H*$_2$C=C), 4.56 (1H, d, *J* 14.0, H-6'a), 4.31 – 4.27 (1H, m, H-3), 4.27 – 4.21 (1H, m, H-6'b), 4.20 – 4.15 (2H, overlapping signals, H-3' & H-4), 4.10 (1H, dd, *J* 6.6, 1.2, H-4), 4.02 (3H, overlapping signals, H-1', H-5 & H-5'), 3.87 – 3.80 (1H, m, H-1), 3.64 (2H, dt, *J* 10.2, 8.8, H-6), 3.57–3.50 (2H, m, OC*H*HCH$_2$CH(CH$_3$)$_2$), 3.50 – 3.42 (2H, overlapping signals, NC*H*HPh & OC*H*HCH$_3$), 3.42 – 3.34 (1H, m, NCH*H*Ph), 3.33 (1H, t, *J* 4.8, H-2'), 3.24 – 3.14 (2H, overlapping signals, H-2 & OCH*H*CH$_3$), 2.60 (2H, d, *J* 6.7, C*H*HN), 2.48 (1H, dd, *J* 15.6, 8.3, C*H*H-N), 2.45 – 2.36 (1H, m, CH*H*N), 1.74 (2H, m, C*H*$_2$CH$_2$N) 1.67 – 1.54 (2H, overlapping signals, C*H*HCH$_2$N & OCH$_2$CH$_2$C*H*(CH$_3$)$_2$), 1.47 (1H, m, CH*H*CH$_2$N), 1.43 (3H, s, isopropylidene CH$_3$), 1.42 (3H, s, isopropylidene CH$_3$), 1.36 (2H, dd, *J* 13.4, 6.6, OCH$_2$C*H*$_2$CH(CH$_3$)$_2$), 1.27 (3H, s, isopropylidene CH$_3$), 1.26 (3H, s, isopropylidene CH$_3$), 1.00 (3H, t, *J* 7.0, OCH$_2$C*H*$_3$), 0.81 (6H, dt, *J* 17.1, 8.6, OCH$_2$CH$_2$CH(C*H*$_3$)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.8 (Ar-C), 140.3 (*C*=CH), 128.7, 128.4, 128.2, 128.2, 127.0 (each Ar-C), 123.4 (C=*C*H), 110.2 (isopropylidene C), 109.5 (isopropylidene C), 77.5 (C-2), 75.8 (C-2'), 74.0 (C-4), 73.8 (C-4), 73.6 (C-3'), 71.7 (C-3'), 71.2 (C-6), 70.3, (C-1') 70.2 (C-1), 69.4 (C-5), 69.1 (C-5'), 68.7 (O*C*H$_2$CH$_2$CH(CH$_3$), 66.7 (O*C*H$_2$CH$_3$), 65.9 (O*C*H$_2$C=C), 57.0 (PhCH$_2$N), 53.2 (C-6'), 51.4 (CH$_2$*C*H$_2$N), 50.9 (CH$_2$*C*H$_2$N), 38.9 (OCH$_2$*C*H$_2$CH(CH$_3$), 27.4 (isopropylidene CH$_3$), 26.6 (isopropylidene CH$_3$), 25.5 (isopropylidene CH$_3$), 24.9 (isopropylidene CH$_3$), 24.6 (OCH$_2$CH$_2$*C*H(CH$_3$), 22.7 (OCH$_2$CH$_2$CH(*C*H$_3$), 15.5 (OCH$_2$C*H*$_3$); ESI-HRMS calcd for $C_{39}H_{61}N_4O_9$ 729.4438, found *m/z* 729.4431 $[M+H]^+$



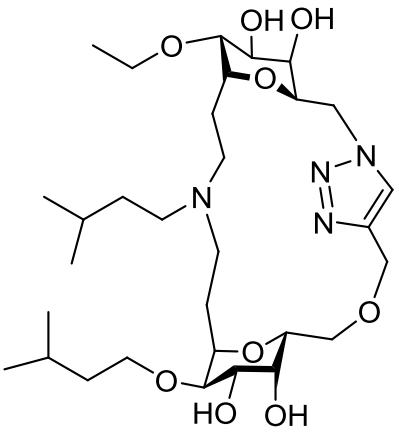
Macrocycle 188. Compound **183** (50 mg, 0.066 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give **183** (34 mg, 75%) as a white powder. ^{1}H NMR (500 MHz, CD_3OD) δ 7.84 (1H, s, triazole-H), 4.62 (1H, dd, *J* 14.3, 10.3, C*H*HN), 4.54 (2H, q, *J* 11.9, OC*H*$_2$C=CH), 4.51 – 4.43 (1H, m, CH*H*N), 4.04 – 3.98 (1H, m, CH), 3.91 (3H, overlapping signals, each CH), 3.73 (1H, br s, CH), 3.68 (3H, overlapping signals, CH), 3.63 – 3.51 (6H, overlapping signals, each CH_2), 3.47 (2H, dd, *J* 15.4, 7.0, CH_2), 2.49 – 2.24 (6H, m, each C*H*$_2$N), 1.72 – 1.53 (7H, overlapping signals, 2 x CH_2 and 3 x $OCH_2CH_2CH(CH_3)_2$), 1.45 – 1.32 (6H, m, $OCH_2CH_2CH(CH_3)_2$), 0.81 (18H, brs, each $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 145.7 (*C*=CH), 124.0 (C=*C*H), 77.0 (CH), 76.9 (CH), 72.0 (*C*H_2C=CH), 71.5, 71.3, 70.6, 70.0, 69.9, 69.3, 69.2 (each *C*H), 69.1 (CH_2), 69.0 (CH), 64.1, 51.5, 51.4, 50.9, 48.4, 38.7 (each CH_2), 33.9, 26.6, 24.7 (CH), 22.1, 21.8, 21.8 (each CH_2), 21.7, 21.6, 21.5, 21.4 (each CH_3); ESI-HRMS calcd for $C_{34}H_{63}N_4O_9$ 671.4595, found *m/z* 671.4592 $[M+H]^+$



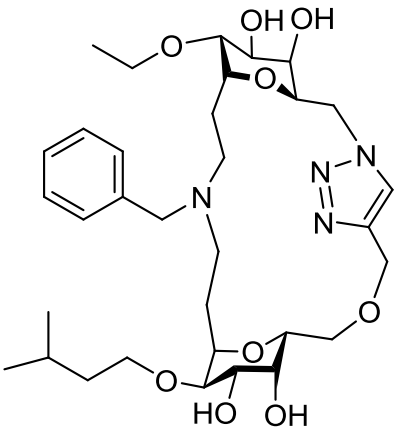
Macrocycle 189. Compound **184** (70 mg, 0.084 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and the solvents were concentrated under reduced pressure to give **189** (48 mg, 76%) as a white powder. ^{1}H NMR (500 MHz, CD_3OD) δ 7.84 (1H, s, triazole H), 7.68 (3H, dd, *J* 18.1, 8.0, ArH), 7.51 (1H, s, ArH), 7.32 (2H, td, *J* 13.2, 6.3, ArH), 7.20 (1H, d, *J* 8.4, ArH), 4.61 (1H, dd, *J* 14.3, 10.2, H-6'a), 4.56 (2H, m, CH_2), 4.51 – 4.41 (1H, m, H-6'b), 4.03 (2H, overlapping signals, each CH), 3.90 – 3.84 (2H, m), 3.69 (4H, dd, *J* 18.3, 9.0), 3.63 – 3.50 (5H, overlapping signals), 3.50 – 3.41 (3H, overlapping signals), 2.73 (4H, overlapping signals, CH_2N), 2.53 (2H, d, *J* 6.6, CH_2N), 2.33 (1H, m, C*H*H), 2.16 (1H, m, CH*H*), 1.66 (6H, overlapping signals, 2 x CH & 2 x CH_2), 1.32 (4H, dt, *J* 21.6, 6.9), 0.80 – 0.72 (6H, m), 0.68 (6H, dd, *J* 13.9, 8.6); ^{13}C NMR (125 MHz, CD_3OD) δ 145.6 (*C*=CH), 136.0, 133.3, 133.1 (each Ar-C), 127.7, 127.5, 127.2, 126.4, 125.7, 125.6 (each Ar-CH), 124.1 (C=*C*H), 76.9, 76.7, 72.9, 71.3, 71.2, 70.0, 69.9, 69.5, 69.2 (each CH), 69.0, 64.0, 51.4, 51.2, 50.9, 48.3, 38.8 (Each CH_2), 33.8, 26.4 (CH), 24.7, 21.8, 21.7 (each CH_2), 21.5, 21.4 (each CH_3); ESI-HRMS calcd for $C_{41}H_{63}N_4O_9$ 755.4595, found *m/z* 755.4592 $[M+H]^+$



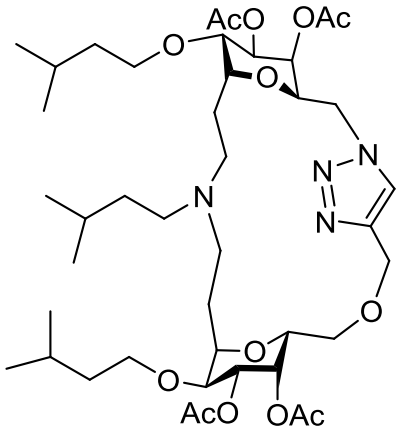
Macrocycle 190. Compound **185** (50 mg, 0.065 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 9 using Dowex® M-43 ion exchange resin, filtered and the solvents were concentrated under reduced pressure to give **190** (32 mg, 71%) as a white powder. ^{1}H NMR (500 MHz, CD_3OD) δ 7.94 (1H, s, triazole-H), 7.34 (3H, d, *J* 3.9, ArH), 7.29 (2H, d, *J* 6.3, ArH), 4.74 (1H, d, *J* 10.6, H-6'a), 4.68 (1H, d, *J* 12.2, H-6'b), 4.63 – 4.55 (2H, brs, OCH_2C=C), 4.53 (1H, m, CH), 4.09 (1H, brs, CH), 4.01 (2H, overlapping signals, each CH), 3.81 (4H, overlapping signals, each CH), 3.70 (2H, overlapping signals, each CH), 3.67 – 3.47 (6H, overlapping signals, each CH_2), 3.47 – 3.41 (2H, m, CH_2), 2.51 (2H, m), 2.47 – 2.39 (1H, m), 2.20 – 2.11 (1H, m) (each CH_2N), 1.74 (2H, m, CH_2), 1.65 (4H, overlapping signals, each CH_2), 1.47 – 1.34 (4H, overlapping signals, each CH_2), 0.86 (12H, dd, *J* 10.0, 6.6, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 145.6 (*C*=CH), 138.5 (Ar-C), 127.9, 127.6, 127.3 (each Ar-*C*H), 124.1 (C=*C*H), 76.9, 76.5, 72.8, 71.5, 71.3, 70.5, 69.8, 69.4, 69.2 (each CH), 69.0 (CH_2), 64.0 (CH), 59.2, 51.4, 51.3, 50.9, 38.8 (each CH_2), 33.6, 26.4, 24.7 (each CH_2), 21.8, 21.7, 21.5, 21.4 (each CH_3); ESI-HRMS calcd for $C_{36}H_{59}N_4O_9$ 791.4282, found *m/z* 791.4275 $[M+H]^+$



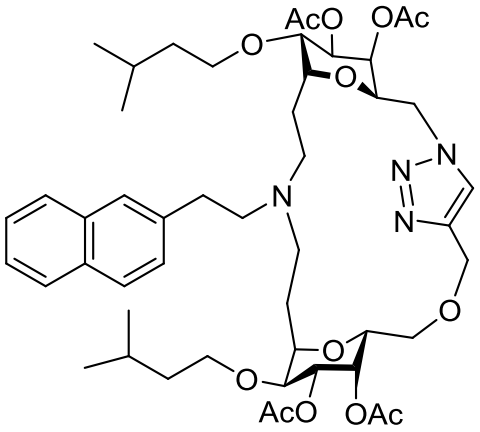
Macrocycle 191. Compound **186** (50 mg, 0.070 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and the solvents were concentrated under reduced pressure to give **191** (30 mg, 69%) as a white powder. ^{1}H NMR (500 MHz, CD_3OD) δ 7.88 (1H, s, triazole-H), 4.70 – 4.62 (1H, m, H-6'a), 4.58 – 4.48 (3H, overlapping signals, OCH_2C=C & H-6'b), 4.02 (2H, overlapping signals, each CH), 3.93 – 3.86 (2H, overlapping signals, each CH), 3.77 (2H, overlapping signals, each CH), 3.69 (2H, overlapping signals, each CH), 3.64 – 3.45 (7H, overlapping signals, 3 x CH & 2 x CH_2), 2.56 (4H, m), 2.06 (1H, m), 1.84 (1H, m) (each CH_2N), 1.63 (3H, td, *J* 13.5, 6.8), 1.47 (1H, dd, *J* 13.2, 6.6), 1.42 – 1.32 (2H, m, each $OCH_2CH_2CH(CH_3)_2$), 1.32 – 1.22 (4H, overlapping signals, each $OCH_2CH_2CH(CH_3)_2$), 1.09 (3H, t, *J* 7.0, OCH_2CH_3), 0.83 (12H, ddd, *J* 8.9, 6.5, 3.5, each $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 145.7 (*C*=CH), 124.0 (C=*C*H), 78.4, 78.1, 73.3, 73.1, 73.0, 72.4, 71.8, 71.1, 70.6, 70.5 (each *C*H), 67.5, 65.4, 53.4, 52.6, 52.2, 40.2 (each CH_2), 27.7, 26.1 (CH), 23.2 (CH), 23.1, 22.9, 22.8 (each CH_3), 16.0 (CH_3); ESI-HRMS calcd for $C_{31}H_{56}N_4O_9$ 628.4047, found *m*/*z* 628.4040 $[M+H]^+$



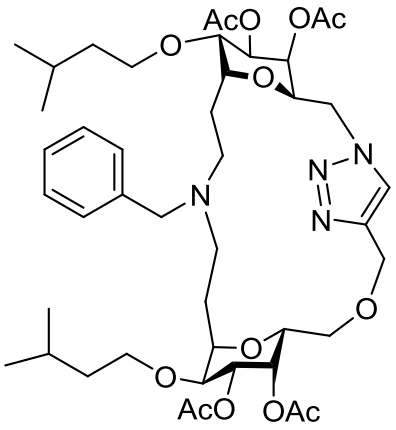
Macrocycle 192. Compound **187** (62 mg, 0.085 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and the solvents were concentrated under reduced pressure to give **192** (42 mg, 76%) as a white powder. ^{1}H NMR (500 MHz, CD_3OD) δ 7.85 (1H, s, triazole H), 7.34 – 7.03 (5H, m, Ar-H), 4.65 (1H, dd, *J* 14.4, 10.5, H-6'a), 4.59 (1H, d, *J* 12.1, OC*H*H*C*=C), 4.52 (1H, d, *J* 12.4, OCH*H*C=C), 4.45 (1H, d, *J* 15.2, H-6'b), 4.01 – 3.96 (1H, m, CH), 3.96 – 3.87 (3H, overlapping signals, each CH), 3.74 (1H, s, CH), 3.72 – 3.59 (4H, overlapping signals), 3.56 (1H, dd, *J* 9.3, 3.2), 3.53 – 3.43 (3H, m), 3.42 (2H, d, *J* 6.1), 3.36 (3H, ddd, *J* 16.3, 12.2, 6.2), 2.40 (2H, m), 2.30 (1H, m), 2.05 (1H, m) (each CH_2N), 1.75 – 1.60 (2H, m, CH_2), 1.60 – 1.49 (3H, overlapping signals, CH_2 & $OCH_2CH_2CH(CH_3)_2$), 1.31 (2H, tt, *J* 13.6, 6.7, $OCH_2CH_2CH(CH_3)_2$), 1.01 (3H, t, *J* 7.0, OCH_2CH_3), 0.84 – 0.75 (6H, m, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 145.7 (*C*=CH), 128.6, 128.2, 128.0, 127.9, 126.6, 124.1 (C=*C*H), 76.9, 76.7, 71.6, 71.4, 71.1, 70.3, 69.8, 69.7, 69.1 (each *C*H), 68.9, 65.8, 63.9, 59.2, 56.2, 52.1, 51.9, 51.7, 50.7, 38.7 (each CH_2), 24.6 (*C*H), 21.7 (CH_3), 21.4 (CH_3), 16.0 (CH_3); ESI-HRMS calcd for $C_{33}H_{53}N_4O_9$ 649.3812, found *m/z* 641.3811 $[M+H]^+$



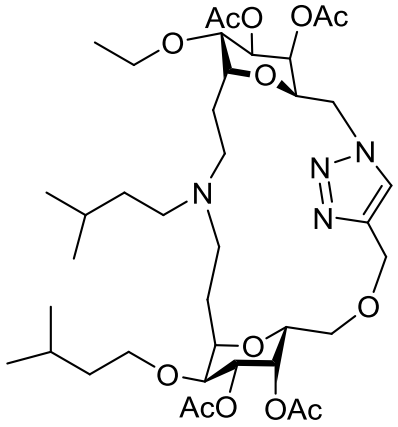
Macrocycle 193. Compound **188** (20 mg, 0.029 mmol) was dissolved in pyridine-Ac_2O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give **193** (20mg, 80%) as a white solid; IR (film) cm^{-1}: 2956, 2253, 1745, 1370, 1239, 1107, 903; ^1H NMR (500 MHz, $CDCl_3$) δ 7.70 (1H, s, triazole H), 5.51 – 5.47 (1H, m, H-4'), 5.37 (1H, dd, *J* 2.7, 1.2, H-4), 5.15 (1H, dd, *J* 9.5, 3.4, H-3'), 5.05 (1H, dd, *J* 9.7, 3.3, H-3), 4.68 (2H, q, *J* 12.5, CH_2), 4.55 (1H, dd, *J* 14.1, 0.9, H-6'a), 4.37 (1H, dd, *J* 14.1, 10.2, H-6'b), 4.27 – 4.20 (1H, m, H-1'), 4.19 – 4.08 (2H, overlapping signals, H-5' & H-1), 3.87 (1H, d, *J* 6.9, H-5), 3.79 – 3.72 (2H, overlapping signals, H-2 & H-2'), 3.66 – 3.58 (2H, m, $OCH_2CH_2CH(CH_3)_2$), 3.59 – 3.50 (4H, overlapping signals, $OCH_2CH_2CH(CH_3)_2$ & C-6), 2.47-2.25 (6H, m, each CH_2N), 2.20, 2.14, 2.06, 2.03 (each 3H, each s, each acetate CH_3), 1.84 – 1.59 (6H, overlapping signals, 2 x CH_2 & CH), 1.41 (4H, qd, *J* 13.7, 6.9, $OCH_2CH_2CH(CH_3)_2$), 1.23 (2H, m, $-NCH_2CH_2CH(CH_3)_2$), 0.88 (18H, t, *J* 6.6, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.4, 170.1, 170.0 (each C=O), 146.3 (triazole CH=*C*), 123.5 (triazole *C*H=C), 74.4 (C-2), 74.3 (C-2'), 72.4 (C-1), 72.0 (C-1'), 70.3 (C-3), 70.2 (C-5), 70.0 (CH_2), 69.7 (CH_2), 69.5 (C-4), 69.4 (C-3'), 69.3 (C-5'), 69.0 (C-4'), 65.2 (CH_2), 51.7 (CH_2N), 51.6 (CH_2N), 50.4 (C-6'), 49.5 (CH_2N), 38.9 (CH_2), 30.4 (CH), 29.7, 29.5, 26.7 (CH), 24.9 (CH), 23.2, 22.9, 22.8 (each CH_2), 22.7, 22.6, 21.0, 20.9 (2s), 20.8 (each CH_3); ESI-HRMS calcd for $C_{42}H_{71}N_4O_{13}$ 839.5017, found *m/z* 839.5015 $[M+H]^+$



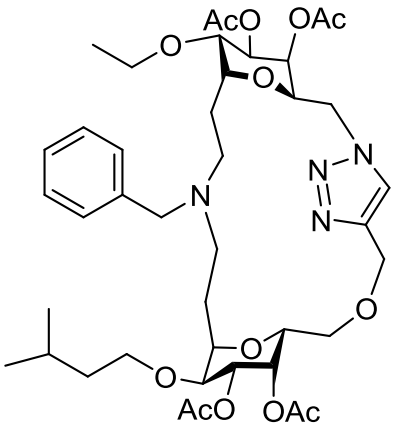
Macrocycle 194. Compound **189** (30 mg, 0.039 mmol) was dissolved in pyridine-Ac_2O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give **194** (33 mg, 91%) as a white solid; $[\alpha]_D$ +60° (c 0.006 in $CHCl_3$); IR (film) cm^{-1}: 2956, 2253, 1745, 1370, 1239, 1107, 903; ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 – 7.73 (4H, m, ArH), 7.59 (1H, s, triazole H), 7.43 (2H, td, *J* 13.1, 6.2, ArH), 7.30 – 7.27 (1H, m, ArH), 5.45 – 5.41 (1H, m, H-4'), 5.35 (1H, dd, *J* 3.0, 1.2, H-4), 5.14 (1H, dd, *J* 9.2, 3.4, H-3'), 5.06 (1H, dd, *J* 9.7, 3.4, H-3), 4.72 – 4.65 (2H, q, *J* 12.5, $OCH_2C{=}C$), 4.49 (1H, dd, *J* 14.1, 1.4, H-6'a), 4.39 (1H, dd, *J* 13.8, 10.6, H-6'b), 4.29 – 4.22 (1H, m, H-1'), 4.18 – 4.09 (2H, m, H-1 & H-5'), 3.87 (1H, d, *J* 7.0, H-5), 3.80 – 3.71 (2H, overlapping signals, H-2 & H-2'), 3.69 – 3.60 (1H, m), 3.59 – 3.51 (5H, overlapping signals) (each CH_2), 2.90 – 2.78 (4H, m), 2.69 – 2.61 (2H, m), 2.54 – 2.46 (1H, m), 2.40 – 2.32 (1H, m), 2.19, 2.15, 2.06, 2.04 (each 3H, each s, each acetate CH_3), 1.93 – 1.81 (3H, m), 1.66 (2H, ddt, *J* 27.0, 13.4, 6.7, $OCH_2CH_2C\mathit{H}(CH_3)_2$), 1.41 (4H, dtd, *J* 20.8, 13.7, 6.9, $OCH_2C\mathit{H}_2CH(CH_3)_2$), 0.89 – 0.81 (12H, m, $OCH_2CH_2CH(C\mathit{H}_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.3, 170.1, 170.0 (each C=O), 146.1 (triazole CH=*C*), 133.6, 132.1 (each Ar-C), 128.2, 127.7, 127.5, 127.2, 126.9, 126.1, 125.4 (each Ar-CH), 123.7 (triazole *C*H=C), 74.4 (C-2), 74.3 (C-2), 72.3 (C-1), 71.8 (C-1), 70.3, 70.2 (C-5), 70.0 (C-3), 69.8 (CH_2), 69.6 (CH), 69.3 (CH), 69.3 (CH), 68.8 (CH), 65.2 ($OCH_2C{=}C$), 52.3, 51.4, 51.1, 50.3 (each CH_2N), 38.9, 38.9 (each CH_2), 24.9 (CH), 24.9 (CH), 22.7, 22.6, 22.5 (2s) (each CH_3), 21.0, 20.9 (2s), 20.8 (each acetate $C\mathit{H}_3$); ESI-HRMS calcd for $C_{49}H_{71}N_4O_{13}$ 923.5017, found *m/z* 923.5016 $[M+H]^+$



Macrocycle 195. Compound **190** (25 mg, 0.036 mmol) was dissolved in pyridine-Ac_2O (1:1, 2.5 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give **195** (27 mg, 89%) as a white solid; $[\alpha]_D$ +51.2° (c 0.009 in $CHCl_3$) IR (film) cm^{-1}: 2956, 2253, 1745, 1370, 1239, 1107, 903; ^1H NMR (500 MHz, $CDCl_3$) δ 7.72 (1 H, s, triazole H), 7.41 – 7.30 (2H, m, ArH), 7.19 (3H, m, ArH), 5.49 – 5.46 (1H, m, H-4'), 5.38 – 5.35 (1H, m, H-4), 5.13 (1H, dd, *J* 8.9, 3.3, H-3'), 5.04 (1H, dd, *J* 9.4, 3.4, H-3), 4.71 (1H, d, *J* 12.6, OC*H*HC=C), 4.64 (1H, d, *J* 12.6, OCH*H*C=C), 4.54 (1H, d, *J* 13.0, H-6'a), 4.41 (1H, m, H-6'b), 4.24 – 4.16 (2H, overlapping signals, H-1 & H-5), 4.07 – 4.01 (1H, m, H-1), 3.88 (1H, d, *J* 7.6, H-5), 3.70 – 3.63 (3H, overlapping signals, H-2, H-2' & H-6a), 3.58 – 3.53 (3H, overlapping signals, H-6b & NCH_2Ph), 3.47 (2H, ddd, *J* 16.1, 11.4, 4.6, OC$H_2$$CH_2CH(CH_3)_2$), 3.41 – 3.36 (2H, m, OC$H_2$$CH_2CH(CH_3)_2$), 2.55 – 2.39 (2H, m, CH_2N), 2.32 (2H, dd, *J* 9.6, 7.3, CH_2N), 2.19, 2.13, 2.07, 2.04 (each 3H, each s, each acetate CH_3), 1.79 (2H, m, CH_2), 1.70 (2H, m, CH_2) 1.60 (2H, td, *J* 13.5, 6.8, $OCH_2CH_2CH(CH_3)_2$), 1.34 (4H, dtd, *J* 20.6, 13.7, 6.8, $OCH_2CH_2CH(CH_3)_2$), 0.87 – 0.82 (12H, m, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 170.2, 169.9, 169.8 (each C=O), 146.1 (triazole CH=*C*), 128.9 (Ar-C), 128.5, 128.2, 128.2, 126.3 (each Ar-CH), 123.4 (triazole *C*H=C), 74.2, 74.2 (each C-2), 71.8, 71.6 (each C-1), 70.0 (C-5), 69.9 (C-3), 69.7 (CH_2), 69.5 (CH_2), 69.4 (C-5), 69.4 (C-3), 69.1 (C-6), 68.9 (C-4), 68.6 (C-4'), 65.1 (OCH_2C=C), 56.5 (NCH_2Ph) 51.8, 51.6, 50.7 (each -CH_2N-), 38.7, 38.6 (each CH_2), 24.7 (CH), 22.5, 22.5 (CH_3), 22.4 (CH_2), 22.4, 21.6, 20.8, 20.8, 20.7, 20.7 (each acetate CH_3). ESI-HRMS calcd for $C_{44}H_{67}N_4O_{13}$ 859.4704, found *m/z* 859.4710 $[M+H]^+$

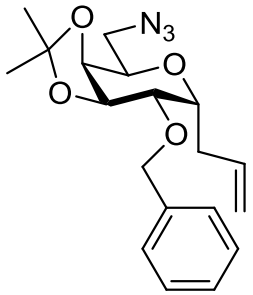


Macrocycle 196. Compound **191** (20 mg, 0.032 mmol) was dissolved in pyridine-Ac_2O (1:1, 3 mL) and stirred at room temperature for 6 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give **196** (24 mg, 93%) as a white solid; $[\alpha]_D$ +83.4° (c 0.003 in $CHCl_3$) IR (film) cm^{-1}: 2956, 2253, 1745, 1370, 1239, 1107, 903; ^1H NMR (500 MHz, $CDCl_3$) δ 7.70 (1H, s, triazole H), 5.53 – 5.48 (1H, m, H-4'), 5.37 (1H, dd, *J* 3.1, 1.5, H-4), 5.15 (1H, dd, *J* 9.2, 3.1, H-3'), 5.05 (1H, dd, *J* 9.7, 3.3, H-3), 4.68 (2H, q, *J* 12.5, OCH_2C=C), 4.55 (1H, dd, *J* 14.0, 1.0, H-6'a), 4.37 (1H, dd, *J* 14.1, 10.4, H-6'b), 4.28 – 4.21 (1H, m, H-1'), 4.17 (1H, d, *J* 10.4, H-5'), 4.15 – 4.10 (1H, m, H-1), 3.90 – 3.84 (1H, m, H-5), 3.82 – 3.72 (2H, overlapping signals, H-2 & H-2'), 3.70 – 3.59 (3H, overlapping signals, H-6 & OC*H*HCH_3), 3.58 – 3.50 (3H, overlapping signals, OCH*H*CH_3 & $OCH_2CH_2CH(CH_3)_2$) , 2.45 (3H, dt, *J* 16.1, 8.3), 2.36 (2H, ddd, *J* 20.2, 11.0, 7.0), 2.26 (1H, m), 2.19, 2.14, 2.07, 2.03 (each 3H, each s, each acetate CH_3), 1.70 – 1.60 (3H, m), 1.55 – 1.47 (2H, m), 1.44 – 1.37 (3H, m), 1.23 (2H, m, -NCH_2C*H_2*$CH(CH_3)_2$), 1.18 (3H, t, *J* 7.0, OCH_2C*H_3*), 0.88 (12H, d, *J* 6.7, each $OCH_2CH_2CH(C$*H_3*$)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 170.2, 170.0, 169.9 (each C=O), 146.2 (triazole *C*H=C), 123.4 (triazole CH=*C*), 74.2, 74.1 (C-2 & C-2'), 72.3, 71.8 (C-1 & C-1'), 70.2 (C-5), 70.1 (C-3), 69.6 (CH_2), 69.4 (CH_2), 69.2 (C-5'), 69.2 (C-3'), 68.8 (C-4), 66.9 (C-4'), 65.1 (CH_2), 51.6, 51.5 (each CH_2N), 50.2 (C-6), 49.5 (CH_2N), 38.8 (CH_2), 35.0 (CH), 26.5 (CH), 24.8 (CH), 23.2, 22.8 (CH_2), 22.7, 22.5 ($OCH_2CH_2CH(CH_3)_2$), 21.2 (CH_2), 20.8, 20.8 (2s), 20.7 (each *C*H_3), 15.5 (OCH_2*C*H_3); ESI-HRMS calcd for $C_{39}H_{65}N_4O_{13}$ 797.4548, found *m/z* 797.4541 $[M+H]^+$

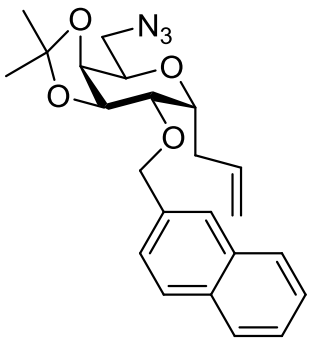


Macrocycle 197. Compound **192** (25 mg, 0.038 mmol) was dissolved in pyridine-Ac_2O (1:1, 1.5 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give **197** (29 mg, 92%) as a white solid; $[\alpha]_D$ +44.0° (c 0.007 in $CHCl_3$) IR (film) cm^{-1}: 2956, 2253, 1745, 1370, 1239, 1107, 903; ^1H NMR (500 MHz, $CDCl_3$) δ 7.63 (1H, s, triazole H), 7.35 – 7.21 (3H, m, Ar-H), 7.12 (2H, m, Ar-H), 5.42 (1H, apt t, *J* 3.0, H-4'), 5.30 (1H, dd, *J* 3.0, 2.3, H-4), 5.05 (1H, dd, *J* 8.8, 3.3, H-3'), 4.97 (1H, dd, *J* 9.4, 3.4, H-3), 4.65 (1H, d, *J* 12.7, OC*H*HC=C), 4.57 (1H, d, *J* 12.6, OCH*H*C=C), 4.48 (1H, dd, *J* 14.3, 1.3, H-6a'), 4.33 (1H, dd, *J* 13.9, 10.6, H-6b'), 4.16 – 4.08 (2H, overlapping signals, H-1' & H-5'), 4.01 – 3.96 (1H, m, H-1), 3.83 – 3.79 (1H, m, H-5), 3.63 – 3.56 (3H, overlapping signals, H-2, H-2' & H-6a), 3.46 (4H, overlapping signals, H-6b, NCH_2Ph & OC*H*HCH_3), 3.39 (1H, m, OC*H*$HCH_2CH(CH_3)_2$), 3.36–3.29 (2H, overlapping signals, OCH*H*CH_3 & OCH*H*$CH_2CH(CH_3)_2$), 2.49–2.32 (3H, m), 2.28–2.20 (1H, m) (each CH_2N), 2.11, 2.06, 2.00, 1.97 (each 3H, each s, each acetate CH_3), 1.64 (4H, overlapping signals, each CH_2) 1.53 (1H, td, *J* 13.4, 6.7, $OCH_2CH_2CH(CH_3)_2$), 1.27 (2H, dt, *J* 21.3, 6.9, $OCH_2CH_2CH(CH_3)_2$), 1.00 (3H, t, *J* 7.0, OCH_2CH_3), 0.77 (6H, t, *J* 6.4, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 170.0 (2s) (each C=O), 146.2 (triazole CH=*C*), 129.1 (Ar-C), 128.7, 128.4, 128.3, 126.5 (each Ar-CH), 123.5 (triazole *C*H=C), 74.3, 74.1 (C-2 & C-2'), 71.9, 71.6 (C-1 & C-1'), 70.2 (C-5), 70.0 (C-3), 69.6 (C-3'), 69.3 (2s) (CH_2 & C-5'), 69.2 (C-4), 69.0 (C-6), 68.8 (C-4)', 66.9 (CH_2), 65.2 (OCH_2C=C), 56.9, 52.0, 51.8, 50.8, 50.1 (each CH_2N), 38.8 (CH_2), 24.9 (CH), 22.7, 22.5, 21.0 (2s), 20.9, 20.8, 15.5 (each CH_3); ESI-HRMS calcd for $C_{41}H_{61}N_4O_{13}$ 817.4235, found *m/z* 817.4227 $[M+H]^+$

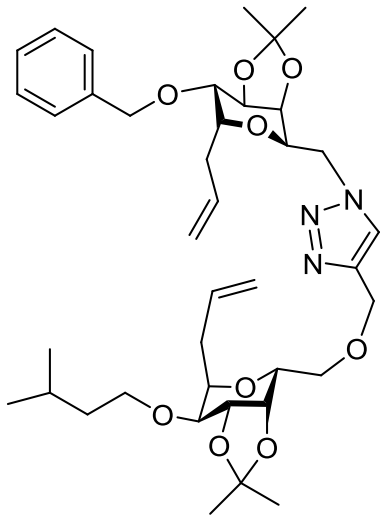
6.4 Chapter 4-Experimental



1-*C*-Allyl-1,6-dideoxy-2-*O*-benzyl-3,4-*O*-isopropylidene-6-azido-α-D-galactopyranoside (201). To a stirred suspension of **175** (1.5 g, 5.5 mmol) in DMF (15 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil, 290 mg, 7.2 mmol) slowly with vigorous stirring. After 15 min, benzyl bromide (1.65 mL, 13.9 mmol) was added and the reaction was allowed warm to room temperature and stirred for 14 h. EtOAc and H_2O were added, phases separated and the organic phase was washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography to give **201** (1.54 g, 77%) as a colourless oil; $[\alpha]_D$ + 7.0° (c 0.05 in $CHCl_3$); IR (film) cm^{-1}: 2981, 2927, 1711, 1380, 1210, 1059; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.28 (5H, m, PhH), 5.81 – 5.71 (1H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.06 (2H, ddd, *J* 10.3, 8.4, 3.3 CH_2CH=CH_2), 4.70 (1H, d, *J* 11.8, C*H*HPh), 4.55 (1H, d, *J* 11.8, CH*H*Ph), 4.41 (1H, dd, *J* 7.3, 3.5, H-3), 4.21 (1H, dd, *J* 7.3, 1.8, H-4), 4.09 (1H, ddd, *J* 7.4, 5.5, 1.8, H-5), 4.05 (1H, ddd, *J* 7.9, 6.7, 3.1, H-1), 3.55 (1H, t, *J* 3.3, H-2), 3.50 (1H, dd, *J* 12.5, 7.6, H-6a), 3.26 (1H, dd, *J* 12.5, 5.5, H-6b), 2.43 (1H, ddd, *J* 18.8, 7.9, 6.5, C*H*HCH=CH_2), 2.33 (1H, dt, *J* 14.3, 7.1, CH*H*CH=CH_2), 1.47 (3H, s, isopropylidene CH_3), 1.34 (3H, s, isopropylidene CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.9 (Ar-C), 134.5 (CH_2CH=CH_2), 128.5, 128.0, 127.8 (each Ar-CH), 117.3 (CH_2CH=CH_2), 109.9 (isopropylidene C), 75.3 (C-2), 73.0 (C-4), 72.7 (CH_2-Ph), 71.8 (C-3), 71.5 (C-1), 69.0 (C-5), 52.0 (C-6), 34.9 (CH_2CH=CH_2), 26.8 (isopropylidene CH_3), 24.8 (isopropylidene CH_3); ESI-HRMS calcd for $C_{19}H_{25}N_3O_4Na$ 382.1732 found *m/z* 382.1721 $[M+Na]^+$

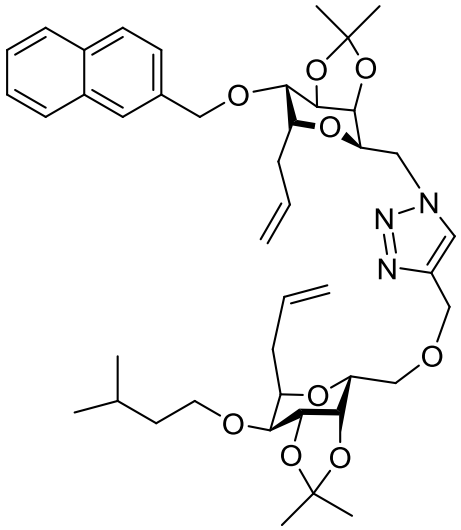


1-*C*-Allyl-1,6-dideoxy-2-*O*-naphthalen-2-ylmethyl-3,4-*O*-isopropylidene-6-azido-α-D-galactopyranoside (202). To a stirred suspension of **175** (1.4 g, 5.2 mmol) in DMF (10 mL) at 0 °C was added sodium hydride (60 % dispersion in mineral oil, 312 mg, 7.8 mmol) slowly with vigorous stirring. After 15 min, 2-(bromomethyl)-naphthalene (4 mL, 18.2 mmol) was added and the reaction was warmed to room temperature and stirred for 24 h. EtOAc and H_2O were added, phases were separated and the organic phase was washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (Petroleum ether-EtOAc 4:1) to give **202** (1.44g, 68%) as a colourless oil. $[\alpha]_D$ + 7.9° (c 0.07 in $CHCl_3$) IR (film) cm^{-1}: 2986, 2101 1641, 1375, 1059, 905; ^1H NMR (500 MHz, $CDCl_3$) δ 7.84 – 7.79 (3H, m, ArH), 7.75 (1H, s, ArH), 7.46 (3H, m, ArH), 5.77 (1H, ddt, *J* 17.1, 10.1, 7.0, CH_2CH=CH_2), 5.09 – 4.99 (2H, m, CH_2CH=CH_2), 4.83 (1H, d, *J* 11.9, CH_2Ar), 4.69 (1H, d, *J* 11.9, CH_2Ar), 4.42 (1H, dt, *J* 15.3, 7.7, H-3), 4.20 (1H, dd, *J* 7.3, 1.8, H-4), 4.11 (1H, ddd, *J* 7.3, 5.5, 1.7, H-5), 4.06 (1H, ddd, *J* 8.1, 6.5, 3.1, H-1), 3.59 (1H, t, *J* 3.3, H-2), 3.50 (1H, dd, *J* 12.5, 7.6, H-6a), 3.26 (1H, dd, *J* 12.5, 5.5, H-6b), 2.45 (1H, ddd, *J* 14.4, 7.9, 6.6, CH_2CH=C*H*H), 2.35 (1H, dt, *J* 14.2, 6.7, CH_2CH=CH*H*), 1.46 (3H, s, isopropylidene CH_3), 1.32 (3H, s, isopropylidene CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.3 (Ar-C), 134.4 (CH_2CH=CH_2), 133.2, 133.1 (each Ar-C), 128.3, 127.9, 127.7, 126.6, 126.3, 126.1, 125.7 (each Ar-CH), 117.3 (CH_2CH=CH_2), 109.8 (isopropylidene C), 75.3 (C-2), 73.0 (C-4), 72.8 (CH_2-Ar), 71.9 (C-3), 71.4 (C-1), 68.9 (C-5), 52.0 (C-6), 34.8 (CH_2CH=CH_2), 26.8 (isopropylidene CH_3), 24.7 (isopropylidene CH_3); ESI-HRMS calcd for $C_{23}H_{27}N_3O_4Na$ 432.2002 found *m/z* 432.2011 $[M+Na]^+$



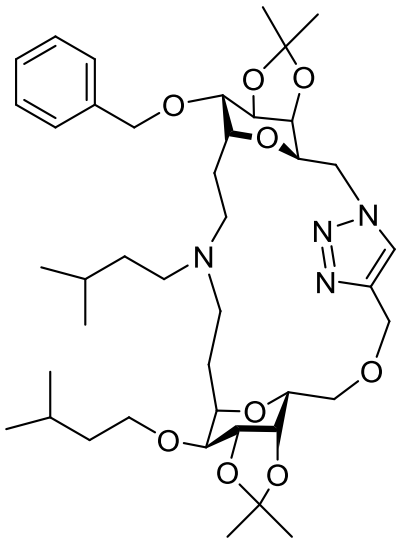
1-*C*-Allyl-1,6-dideoxy-6-(4-(((1-*C*-allyl-1,6-dideoxy-2-*O*-isopentyl-3,4-*O*-isopropylidene-α-D-galactopyranos-6-yl)oxymethyl)-1H-1,2,3-triazol-1-yl)-2-*O*-isopentyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (203). Alkyne **180** (135 mg, 0.383 mmol) and azide **201** (135 mg, 0.383 mmol) were dissolved in a mixture of acetonitrile-H_2O (4 mL, 1:1). To this was added CuI (66 mg, 0.345 mmol) and the reaction was heated at reflux for 24 h. Upon cooling the reaction was diluted with EtOAc, washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-Petroleum ether 1:1) to give compound **203** (209 mg, 77%) as a yellow oil. $[\alpha]_D$ +19.4° (c 0.05 in $CHCl_3$); IR (film) cm^{-1}: 2923, 1512, 1373, 1092, 908; ^1H NMR (500 MHz, $CDCl_3$) 7.70 (1H, s, triazole H), 7.36 – 7.31 (2H, m, Ar-H), 7.29 (3H, dd, *J* 12.5, 5.1, ArH), 5.79 (1H, ddt, *J* 16.9, 10.1, 7.0, CH_2CH=CH_2), 5.64 (1H, ddt, *J* 17.2, 10.3, 7.0, CH_2CH=CH_2), 5.13 – 4.93 (4H, overlapping signals, each CH_2CH=CH_2), 4.74 – 4.67 (2H, q, *J* 12.4, OCH_2C=C), 4.65 (1H, d, *J* 11.7, CHHPh), 4.61 (1H, dd, *J* 14.0, 4.1, H-6'a), 4.52 (1H, d, *J* 11.8, CHHPh), 4.44 – 4.36 (2H, overlapping signals, H-6'b, H-3'), 4.33 – 4.23 (3H, overlapping signals, H-4, H-3 & H-5'), 4.20 (1H, dd, *J* 7.4, 1.4, H-4'), 4.11 (1H, t, *J* 6.3, H-5), 4.09 – 4.03 (1H, m, H-1'), 4.01 (1H, td, *J* 7.3, 2.7, H-1), 3.74 – 3.61 (3H, overlapping signals, H-6 & $OCHHCH_2CH(CH_3)_2$), 3.56 (1H, t, *J* 3.4, H-2'), 3.46 – 3.39 (1H, m, $OCHHCH_2CH(CH_3)_2$), 3.33 (1H, t, *J* 2.7, H-2), 2.38 (2H, dt, *J* 16.1, 7.3, CH_2CH=CH_2), 2.34 – 2.27 (1H, m, CH_2CH=CH_2), 2.22 – 2.15 (1H, m, CH_2CH=CH_2), 1.70 (1H, tt, *J* 13.4, 6.7, $OCH_2CH_2CH(CH_3)_2$), 1.50 (3H, s, isopropylidene CH_3), 1.48 (3H, s, isopropylidene CH_3), 1.44 (2H, dd, *J* 13.5, 6.8, $OCH_2CH_2CH(CH_3)_2$), 1.35 (3H, s, isopropylidene CH_3), 1.33 (3H, s, isopropylidene CH_3), 0.89 (6H, d, *J* 6.7, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.9 (triazole C=C), 137.6, 134.5 (CH_2CH=CH_2), 134.3 (CH_2CH=CH_2),

128.5, 128.0, 127.9, 124.3 (each Ar-C), 117.5 ($CH_2CH=CH_2$), 116.8 ($CH_2CH=CH_2$), 110.2 (isopropylidene C), 109.4 (isopropylidene C), 76.1 (C-2), 74.9 (C-2'), 73.0 (CH_2-Ph), 72.9 (C-4), 72.9 (C-4'), 71.7 (C-3), 71.6 (C-3), 71.6 (C-1), 70.9 (C-1), 70.5 (C-6), 69.3 ($OCH_2CH_2CH(CH_3)_2$), 68.8 (C-5'), 68.5 (C-5), 65.0 ($OCH_2C=C$), 51.9 (C-6'), 39.0 ($OCH_2CH_2CH(CH_3)_2$) 35.2 ($CH_2CH=CH_2$), 34.9 ($CH_2CH=CH_2$), 26.9 (isopropylidene CH_3), 26.8 (isopropylidene CH_3), 24.9 (isopropylidene CH_3), 24.8 (isopropylidene CH_3), 24.7 ($OCH_2CH_2CH(CH_3)_2$), 22.7 ($OCH_2CH_2CH(CH_3)_2$); ESI-HRMS calcd for $C_{39}H_{58}N_3O_9$ 712.4173, found *m/z* 712.4171 $[M+H]^+$



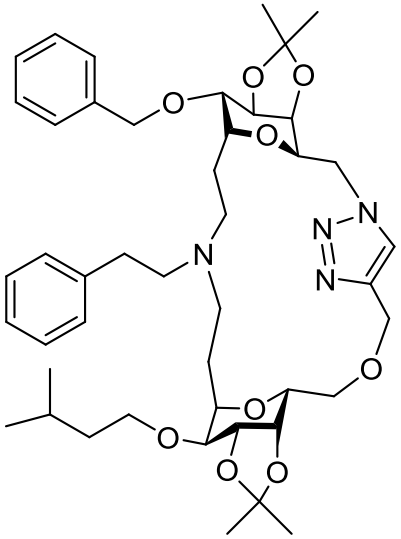
1-*C*-Allyl-1,6-dideoxy-6-(4-(((1-*C*-allyl-1,6-dideoxy-2-*O*-isopentyl-3,4-*O*-isopropylidene-α-D-galactopyranos-6-yl)oxymethyl)-1H-1,2,3-triazol-1-yl)-2-*O*-naphthalen-2-ylmethyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (204). Alkyne **180** (173 mg, 0.49 mmol) and azide **202** (201 mg, 0.49 mmol) were dissolved in acetonitrile-H_2O 1:1 (5 mL). To this was added CuI (75 mg, 0.39 mmol) and the reaction was heated at reflux for 24 h. Upon cooling the reaction was diluted with EtOAc, washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:1) to give compound **204** (280 mg, 75%) as a yellow oil; $[\alpha]_D$ +24.7° (c 0.06 in $CHCl_3$); IR (film) cm^{-1}: 2926, 1641, 1381, 1090, 907; ^1H NMR (500 MHz, $CDCl_3$) δ 7.81 (3H, m, ArH), 7.70 (2H, d, *J* 8.1, ArH), 7.46 (2H, dd, *J* 8.9, 5.2), 7.40 (1H, d, *J* 8.4, ArH), 5.79 (1H, ddt, *J* 16.9, 10.1, 7.0, $CH_2CH=CH_2$), 5.64 (1H, ddt, *J* 17.2, 10.3, 7.0, $CH_2CH=CH_2$), 5.13 – 4.93 (4H, overlapping signals, each $CH_2CH=CH_2$), 4.79 (1H, d, *J* 12.0, CHHAr), 4.68 (3H, overlapping signals, CHHAr & $OCH_2C=C$), 4.60 (1H, dd, *J* 14.0, 3.9, H-6'a), 4.43 (1H, dd, *J* 7.6, 3.7, H-3'), 4.41 – 4.36 (1H, m, H-6'b), 4.31

– 4.25 (3H, overlapping signals, H-4', H-5' & H-3), 4.19 (1H, d, *J* 7.4, H-4), 4.12 – 4.03 (2H, overlapping signals, H-1' & H-5), 3.99 (1H, td, *J* 7.2, 2.4, H-1), 3.72 – 3.57 (4H, overlapping signals, OCH*H*$CH_2CH(CH_3)_2$, H-2' & H-6), 3.41 (1H, dd, *J* 15.7, 6.7, OC*H*$HCH_2CH(CH_3)_2$), 3.32 (1H, t, *J* 2.7, H-2), 2.34 (3 H, m), 2.23 – 2.14 (1H, m) (each C*H*$_2$CH=CH_2), 1.68 (1H, td, *J* 13.3, 6.7 $OCH_2CH_2CH(CH_3)_2$), 1.48 (3H, s, isopropylidene CH_3), 1.46 (3H, s, isopropylidene CH_3), 1.43 (2H, dd, *J* 13.5, 6.7, $OCH_2CH_2CH(CH_3)_2$), 1.33 (3H, s, isopropylidene CH_3), 1.31 (3H, s, isopropylidene CH_3), 0.88 (6H, d, *J* 6.6, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.9 (triazole C=C), 135.0 (Ar-C), 134.7 ($CH_2CH=CH_2$), 134.3 ($CH_2CH=CH_2$), 133.2, 133.1 (each Ar-C), 128.4, 128.0, 127.8, 126.8, 126.3, 126.2, 125.9, 124.3 (each Ar-CH), 117.5 ($CH_2CH=CH_2$), 117.1 ($CH_2CH=CH_2$), 110.2 (isopropylidene C), 109.4 (isopropylidene C), 76.1 (C-2), 74.9 (C-2'), 73.1 (C-4), 73.0 (C-4'), 72.9 (CH_2Ph), 71.9 (C-3'), 71.6 (C-1'), 71.5 (C-1), 71.0 (C-3), 70.5 (C-6), 69.3 ($OCH_2CH_2CH(CH_3)_2$), 68.8 (C-5'), 68.4 (C-5), 65.0 (O-CH_2C=C), 51.8 (C-6'), 39.0 ($OCH_2CH_2CH(CH_3)_2$), 35.2 ($CH_2CH=CH_2$), 34.8 ($CH_2CH=CH_2$), 26.9 (isopropylidene CH_3), 26.8 (isopropylidene CH_3), 24.9 (isopropylidene CH_3), 24.9 ($OCH_2CH_2CH(CH_3)_2$), 24.7 (isopropylidene CH_3), 22.7 ($OCH_2CH_2CH(CH_3)_2$); ESI-HRMS calcd for $C_{43}H_{59}N_3O_9Na$ 784.4149, found *m*/*z* 784.4161 $[M+Na]^+$



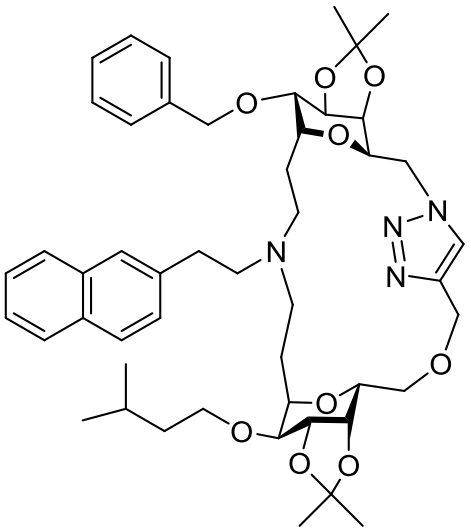
Macrocycle 205. To a stirred solution of **203** (99 mg, 0.139 mmol) in dioxane-H_2O (3:1, 2 mL) was added 2,6-lutidine (56 μL, 0.486 mmol), $NaIO_4$ (133 mg, 0.625 mmol) and a catalytic amount of OsO_4 (2 drops, 2.5% solution in *t*-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H_2O and CH_2Cl_2 were added, layers were separated and the aqueous layer was extracted into CH_2Cl_2. The combined organic phases were washed

with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 ml) and isopentylamine (16 µl, 0.139 mmol) was added. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (103 mg, 0.486 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **205** (60 mg, 54%) as an off white solid; $[\alpha]_D$ +47.2° (c 0.01 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (1H, s, triazole H), 7.36 – 7.26 (5H, m, ArH), 4.78 (1H, d, *J* 11.6, OC*H*HC=C), 4.74 – 4.61 (3H, overlapping signals, OCH*H*C=C, PhC*H*HO & H-6'a), 4.51 (1H, d, *J* 12.0, PhCH*H*O), 4.42 (1H, m, H-3), 4.40 – 4.32 (1H, m, H-6'b), 4.29 (1H, m, H-3'), 4.23 (1H, m, H-4'), 4.15 (2H, overlapping signals, H-4 & H-5), 4.07 (2H, overlapping signals, H-5 & H-1'), 3.95 (1H, m, H-1), 3.79 – 3.70 (2H, m, H-6), 3.69 – 3.62 (1H, m, OC*H*HCH$_2$CH(CH$_3$)$_2$), 3.56 – 3.44 (3H, overlapping signals, OC*H*HCH$_2$CH(CH$_3$)$_2$, H-2 & H-2'), 2.68 (2H, m), 2.59 (1H, m), 2.48 (2H, m), 2.29 (1H, m), (each CH_2N), 1.71 (4H, dd, *J* 13.4, 6.7), 1.51 (6H, s, 2 x isopropylidene CH_3), 1.47 (2H, d, *J* 4.7), 1.42 (1H, d, *J* 5.8), 1.37 (3H, s, isopropylidene CH_3), 1.34 (3H, s, isopropylidene CH_3), 1.27 (3H, overlapping signals, CH_2 and CH) 0.95 – 0.81 (12H, m, each OCH$_2$CH$_2$CH(*CH$_3$*)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.0 (triazole CH=*C*), 137.3 (Ar-C), 128.4, 128.1, 128.0 (each Ar-CH), 123.0 (triazole *C*H=C), 110.1 (isopropylidene C), 109.5 (isopropylidene C), 77.3 (C-2), 75.3 (C-2), 74.5 (C-4), 73.8 (C-4), 73.4 (C-3), 72.7 (OCH$_2$Ph), 71.6 (C-3), 71.2 (C-6), 71.1 (C-1), 70.2 (C-1), 69.3 (OCH$_2$CH$_2$CH(CH$_3$)$_2$), 69.0 (C-5), 68.6 (C-5), 66.0 (O*C*H$_2$C=C), 52.6 (C-6), 51.3 (-CH$_2$N-), 49.4 (CH_2N), 38.8 (CH_2), 31.6, 29.6, 29.6, 27.5, 26.6, 26.5, 25.7, 24.9, 24.5, 22.8, 22.6, 22.5; ESI-HRMS calcd for $C_{42}H_{67}N_4O_9$ 771.4908, found *m/z* 771.4901 $[M+H]^+$



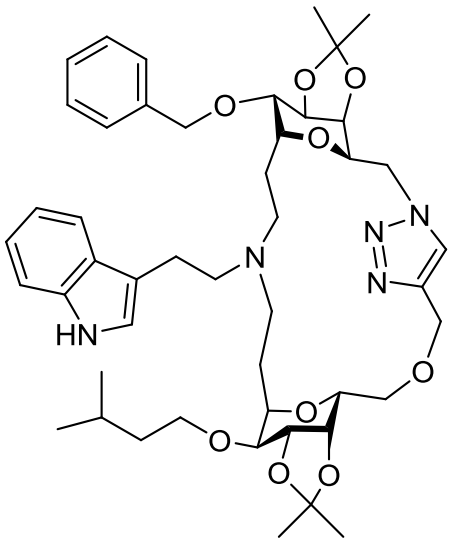
Macrocycle 206. To a stirred solution of **203** (111 mg, 0.155 mmol) in dioxane-H_2O (3:1, 2 mL) was added 2,6-lutidine (64 μL, 0.545 mmol), $NaIO_4$ (149 mg, 0.697 mmol) and a catalytic amount of OsO_4 (2 drops, 2.5% solution in *t*-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H_2O and CH_2Cl_2 were added, layers were separated and the aqueous layer was extracted into CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and 2-phenylethylamine hydrochloride (24 mg, 0.155 mmol) was added. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (115 mg, 0.542 mmol) was then added and the reaction mixture was stirred for 3 h. The reaction was quenched by the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **206** (75 mg, 60%) as an off white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (1H, s, triazole H), 7.36 – 7.08 (10H, m, PhH), 4.78 – 4.60 (4H, overlapping signals, OC*H*HPh, H-6a & OCH_2C=C), 4.49 (1H, d, *J* 11.8, OCH*H*Ph), 4.40 (2H, overlapping signals, H-3, H-6'b), 4.32 – 4.21 (2H, overlapping signals, H-3' & H-4'), 4.15 (2H, overlapping signals, H-5 & H-4), 4.04 (2H, overlapping signals, H-5 & H-1'), 3.94 (1H, m, H-1), 3.75 (2H, m, H-6), 3.70 – 3.60 (1H, m, OC*H*H$CH_2CH(CH_3)_2$), 3.50 (3H, overlapping signals, OCH*H*$CH_2CH(CH_3)_2$, H-2 & H-2'), 2.71 (8H, overlapping signals, each CH_2N), 1.74 – 1.63 (3H, m), 1.52 (6H, m, isopropylidene CH_3), 1.47 (4H, dd, *J* 13.3, 6.5), 1.37 (3H, s, isopropylidene CH_3), 1.34 (3H, s, isopropylidene CH_3), 0.90 (6H, dd, *J* 6.2, 3.5, $OCH_3CH_2CH(CH_3)_2$); ^{13}C NMR, (125 MHz, $CDCl_3$) δ 146.0 (triazole CH=*C*), 137.4 (Ar-C), 128.7, 128.5, 128.4, 128.3, 128.1 (each Ar-

CH), 123.2 (triazole *C*H=C), 110.2 (isopropylidene C), 109.7 (isopropylidene C), 77.3 (C-2), 75.3 (C-2), 74.6 (C-4), 73.9 (C-4), 73.5 (C-3), 72.7 (O*C*H_2Ph), 71.8 (C-3), 71.3 (C-6), 71.0 (C-1), 70.0 (C-1) 69.5 (O*C*$H_2CH_2CH(CH_3)_2$), 69.0 (C-5), 68.8 (C-5), 66.1 (OCH_2C=C), 52.6 (C-6), 51.8, 51.3 (each CH_2N), 38.8 (CH_2), 27.5 (isopropylidene CH_3), 26.7 (isopropylidene CH_3), 25.8, 25.0 (isopropylidene CH_3), 24.6 (isopropylidene CH_3), 22.7 ($OCH_2CH_2CH(CH_3)_2$; ESI-HRMS calcd for $C_{45}H_{65}N_4O_9$ 805.4751, found *m/z* 805.4745 $[M+H]^+$



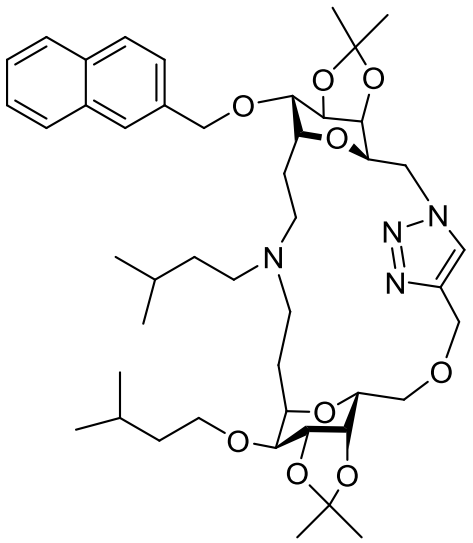
Macrocycle 207. To a stirred solution of **203** (118 mg, 0.154 mmol) in dioxane-H_2O (3:1, 2 mL) was added 2,6-lutidine (63 μL, 0.540 mmol), $NaIO_4$ (148 mg, 0.69 mmol) and a catalytic amount of OsO_4 (2 drops, 2.5% solution in *t*-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H_2O and CH_2Cl_2 were added, layers were separated and the aqueous layer was extracted into CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and 2-(2-Naphthyl)ethanamine hydrochloride (32 mg, 0.154 mmol) was added. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (114 mg, 0.539 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **207** (75 mg, 57%) as an off white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 – 7.71 (5H, m, ArH), 7.59 (1H, s, ArH), 7.49 – 7.37 (3H, m, ArH), 7.27 (4H, dd, *J* 13.0, 7.7, ArH), 4.80 – 4.55 (5H, overlapping signals, O*C*H_2C=C, O*C*HHPh & H-6'), 4.47 (1H, d, *J* 11.9, OCH*H*Ph), 4.39 (1H,

dd, *J* 7.2, 3.3, H-3), 4.24 (2H, overlapping signals, H-3' & H-4'), 4.06 (4H, overlapping signals, H-4, H-5, H-5' & H-1'), 3.96 (1H, m, H-1), 3.74 (2H, dd, *J* 19.0, 10.6, H-6), 3.65 (1H, m, OC*H*HCH$_2$CH(CH$_3$)$_2$), 3.54 – 3.47 (2H, overlapping signals, OCH*H*CH$_2$CH(CH$_3$)$_2$ & H-2), 3.45 (1H, m, H-2), 2.83 (6H, overlapping signals, each CH$_2$N), 1.74 – 1.65 (2H, m), 1.52 (3H, s, isopropylidene CH$_3$), 1.50 (3H, s, isopropylidene CH$_3$), 1.46 (5H, overlapping signals), 1.36 (3H, s, isopropylidene CH$_3$), 1.33 (3H, s, isopropylidene CH$_3$), 1.28 (2H, m), 0.88 (6H, dt, *J* 10.9, 5.4, OCH$_2$CH$_2$CH(C*H$_3$*)$_2$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 145.9 (triazole CH=*C*), 137.3, 133.5, 132.0 (each Ar-C), 128.4, 128.4, 128.2, 128.0, 127.6, 127.5, 127.3, 126.8, 126.0, 125.4 (each Ar-CH), 123.2 (triazole *C*H=C), 110.1 (isopropylidene C), 109.6 (isopropylidene C), 77.2 (C-2), 75.2 (C-2), 74.4 (C-4), 73.7 (C-4), 73.3 (C-3), 72.7 (OCH$_2$Ph), 71.7 (C-3), 71.2 (C-6), 70.9 (C-1), 70.0 (C-1), 69.5 (OCH$_2$CH$_2$CH(CH$_3$)$_2$, 68.9 (C-5), 68.8 (C-5), 65.9 (OCH$_2$C=C), 52.9 (C-6), 52.5 (CH$_2$N), 51.6 (CH$_2$N), 51.2 (CH$_2$N), 38.8 (CH$_2$), 27.4, 26.6, 25.6, 24.9, 24.8, 24.6, 22.6 (OCH$_2$CH$_2$CH(*C*H$_3$)$_2$; ESI-HRMS calcd for C$_{49}$H$_{67}$N$_4$O$_9$ 855.4908, found *m/z* 855.4901 [M+H]$^+$



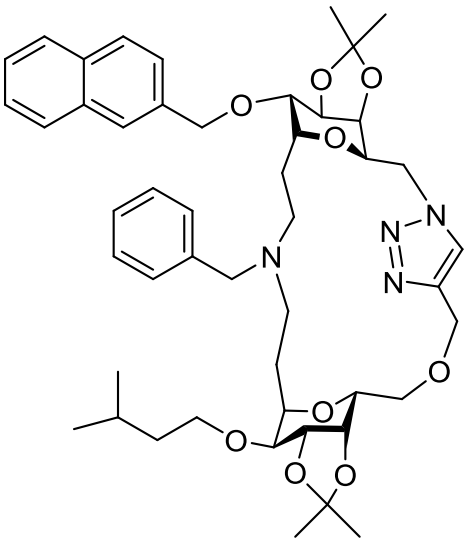
Macrocycle 208. To a stirred solution of **203** (116 mg, 0.162 mmol) in dioxane-H$_2$O (3:1, 2 mL) was added 2,6-lutidine (66 μL, 0.570 mmol), NaIO$_4$ (156 mg, 0.730 mmol) and a catalytic amount of OsO$_4$ (2 drops, 2.5% solution in *t*-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H$_2$O and CH$_2$Cl$_2$ were added, layers were separated and the aqueous layer was extracted into CH$_2$Cl$_2$. The combined organic phases were washed with brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and tryptamine hydrochloride (32 mg, 0.162 mmol) was added. The solution was stirred at room temperature

for 20 min. Sodium triacetoxyborohydride (120 mg, 0.567 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **208** (88 mg, 65%) as an off white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (1H, s, triazole H), 7.34 – 7.22 (5H, m, ArH), 7.23 – 7.15 (2H, m, ArH), 7.12 (2H, d, *J* 7.1), 6.94 (1H, d, *J* 7.6), 4.73 (2H, overlapping signals, OCH_2C=C), 4.66 (2H, overlapping signals, OC*H*HPh & H-6'a), 4.49 (1H, d, *J* 12.0, OCH*H*Ph), 4.42 (1H, dd, *J* 7.4, 3.4, H-3), 4.38 (1H, dd, *J* 14.3, 10.0, H-6'b), 4.31 – 4.24 (1H, overlapping signals, H-3'& H-4'), 4.15 (2H, overlapping signals, H-4 & H-5), 4.09 – 4.05 (1H, m, H-5'), 4.03 (1H, ddd, *J* 11.3, 4.2, 2.8, H-1'), 3.95 – 3.91 (1H, m, H-1), 3.78 – 3.73 (1H, m, CH*H*), 3.68 – 3.61 (2H, m, each C*H*H), 3.51 (2H, overlapping signals, H-2 & C*H*H), 3.47 – 3.44 (1H, m, H-2), 2.73 (8H, overlapping signals, each CH_2), 1.70 (3H, overlapping signals), 1.52 (1H, s, isopropylidene CH_3), 1.51 (3H, s, isopropylidene CH_3) 1.50 – 1.42 (4H, overlapping signals, each CH_2), 1.36 (3H, s, isopropylidene CH_3), 1.34 (3H, s, isopropylidene CH_3), 1.32 (2H, m, CH_2) 0.92 – 0.85 (6H, m, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.8, 138.2, 133.4, 130.9, 129.4 (each Ar-C), 129.2, 128.8, 128.6, 125.6, 123.1, 122.3, 119.6, 119.3, 112.3 (each Ar-CH), 110.1 (isopropylidene C), 109.6 (isopropylidene C) 78.4, 78.0, 74.3 (CH_2), 73.3, 73.2, 72.9, 72.7, 72.0, 71.3, 71.2, 70.9, 70.6 (CH), 70.5, 65.5, 60.7, 53.2, 52.9, 52.7, 52.5, 52.3, 40.2 (each CH_2), 26.9, 26.8, 24.9, 24.7 (each CH_3), 23.2 (CH), 22.8 (CH_2), 22.3 (CH_3); ESI-HRMS calcd for $C_{47}H_{66}N_5O_9$ 844.4860, found *m/z* 844.4855 $[M+H]^+$



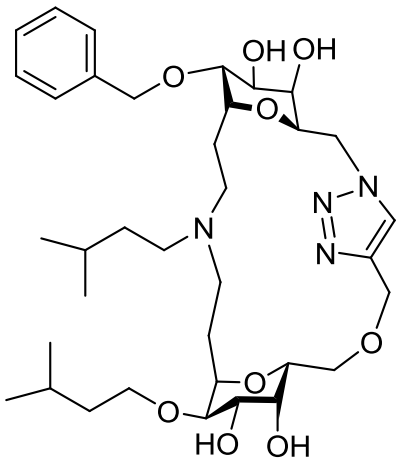
Macrocycle 209. To a stirred solution of **204** (105 mg, 0.137 mmol) in dioxane-H_2O (3:1, 2 mL) was added 2,6-lutidine (56 μL, 0.482 mmol), $NaIO_4$ (131 mg, 0.616 mmol) and a catalytic amount of OsO_4 (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H_2O and CH_2Cl_2 were added, layers were separated and the aqueous layer was extracted into CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and isopentylamine (16 μL, 0.137 mmol) was added. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **209** (66 mg, 59%) as an off white solid; $[\alpha]_D$ +21.3° (c 0.02 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 – 7.76 (3H, m, ArH), 7.74 (1H, s, ArH), 7.50 – 7.42 (4H, m, ArH), 4.79 (2H, overlapping signals, OC*H*HC=C & OC*H*HAr), 4.67 (3H, overlapping signals, OCH*H*C=C, OCH*H*Ar & H-6'a), 4.45 (1H, dd, *J* 7.0, 2.2, H-3), 4.37 (1H, m, H-6'b), 4.31 (1H, m, H-3'), 4.22 (1H, m, H-4'), 4.17 (1H, m H-5'), 4.12 (1H, m, H-4), 4.05 (2H, overlapping signals, H-5 & H-1'), 3.92 (1H, m, H-1), 3.79 – 3.63 (3H, overlapping signals, H-6 & OC*H*HCH_2CH$(CH_3)_2$), 3.51 (3H, overlapping signals, OC*H*HCH_2CH$(CH_3)_2$, H-2 & H-2'), 2.67 (2H, m), 2.53 (1H, m), 2.43 (1H, m,), 2.30 (1H, m), 2.08 (1H, m), (each CH_2N) 1.78 – 1.60 (5H, m), 1.51 (3H, s, isopropylidene CH_3), 1.50 (5H, overlapping signals, isopropylidene CH_3 & CH_2), 1.42 (1H, d, *J* 6.5), 1.36 (3H, s, isopropylidene CH_3), 1.33 (3H, s, isopropylidene CH_3), 1.17 (2H, m,

CH_2) 0.91 (6H, d, *J* 6.6, $CH_2CH_2CH(CH_3)_2$), 0.78 (6H, dd, *J* 26.9, 6.3, $CH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.2 (triazole CH=*C*), 134.9, 133.2, 133.2, 128.4, 128.0, 127.8, 126.4, 126.3, 126.2, 110.3 (isopropylidene C), 109.7 (isopropylidene C), 75.3 (C-2), 74.9 (C-2), 74.0 (C-4), 73.6 (C-4), 72.9 (C-3), 71.9 (OCH_2Ar), 71.4 (C-3), 71.3 (C-6), 71.2 (C-1), 70.3 (C-1), 69.4 ($OCH_2CH_2CH(CH_3)_2$), 69.2 (C-5), 68.7 (C-5) , 66.2 ($OCH_2C{=}C$), 52.8 (C-6), 51.8, 51.56, 49.3 (each CH_2N), 38.9 (CH_2), 36.3 (CH_2), 29.8, 27.7 (isopropylidene CH_3), 26.7 (isopropylidene CH_3), 26.6 (isopropylidene CH_3), 25.9 (isopropylidene CH_3), 25.1, 24.6, 22.9, 22.8; ESI-HRMS calcd for $C_{46}H_{69}N_4O_9$ 821.5065, found *m/z* 821.5063 $[M+H]^+$



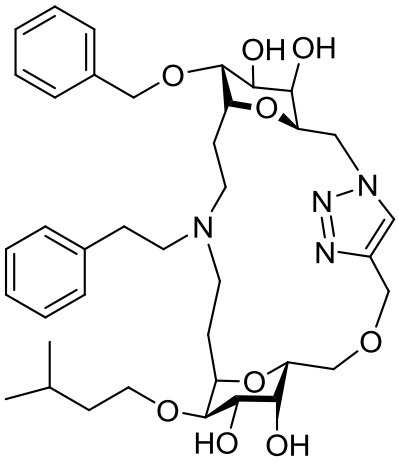
Macrocycle 210. To a stirred solution of **204** (115 mg, 0.151 mmol) in dioxane-H_2O (3:1, 2 mL) was added 2,6-lutidine (62 μL, 0.528 mmol), $NaIO_4$ (145 mg, 0.679 mmol) and a catalytic amount of OsO_4 (2 drops, 2.5% solution in *t*-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H_2O and CH_2Cl_2 were added, layers were separated and the aqueous layer was extracted into CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and benzylamine (16 μL, 0.151 mmol) was added. The solution was stirred at room temperature for 25 min. Sodium triacetoxyborohydride (112 mg, 0.528 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd $NaHCO_3$ and the product was extracted into EtOAc, dried with $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH_2Cl_2-EtOAc 1:9) to give compound **210** (74 mg, 58%) as an off white

solid; $[\alpha]_D$ +62.4° (c 0.03 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 – 7.78 (3H, m, ArH), 7.68 (1H, s, ArH), 7.52 – 7.44 (2H, m, ArH), 7.41 (1H, d, *J* 9.3, ArH), 7.37 – 7.29 (2H, m, ArH), 7.26 (1H, s, ArH), 7.24 – 7.16 (2H, m, ArH), 7.13 (1H, d, *J* 7.0, ArH), 4.75 (2H, q, 12.0, OC*H*$_2$C=C), 4.67 (2H, overlapping signals, H-6'a & OC*H*HAr), 4.52 (1H, d, *J* 12.1, OCH*H*Ar), 4.40 (1H, dd, *J* 7.5, 3.2, H-3), 4.33 (1H, dd, *J* 14.3, 9.9, H-6'b), 4.28 (1H, dd, *J* 7.5, 1.0, H-3'), 4.26 – 4.21 (1H, m, H-4'), 4.15 (2H, dd, overlapping signals, H-4 & H-5'), 4.09 (1H, d, *J* 7.6, H-5), 4.07 – 4.02 (1H, m, H-1'), 3.90 – 3.84 (1H, m, H-1), 3.73 (2H, dt, *J* 10.2, 9.0, H-6), 3.62 (1H, dt, *J* 9.2, 6.4, OC*H*HCH$_2$CH(CH$_3$)$_2$), 3.51 – 3.37 (4H, overlapping signals, OCH*H*CH$_2$CH(CH$_3$)$_2$, PhC*H*HN, H-2 & H-2'), 3.25 (1H, d, *J* 13.7, PhCH*H*N), 2.70 (1H, m), 2.55 (2H, d, *J* 8.5), 2.40 (1H, m), (each CH$_2$N), 1.71 (5H, overlapping signals, 2 x CH$_2$ & OCH$_2$CH$_2$C*H*(CH$_3$)$_2$), 1.50 (3H, s, isopropylidene CH$_3$), 1.45 (5H, overlapping signals, OCH$_2$C*H*$_2$CH(CH$_3$)$_2$ & isopropylidene CH$_3$), 1.33 (3H, isopropylidene CH$_3$), 1.27 (3H, isopropylidene CH$_3$), 0.90 (6H, dd, *J* 6.6, 0.9, OCH$_2$CH$_2$CH(C*H*$_3$)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.0 (triazole CH=*C*), 140.4, 134.9, 133.2 (each Ar-C), 128.6, 128.5, 128.3, 128.2, 128.0, 127.8, 127.4, 127.0, 126.4, 126.3, 126.2 (each Ar-CH), 123.4 (triazole *C*H=C), 110.2 (isopropylidene C), 109.6 (isopropylidene C), 77.4 (C-2), 75.0 (C-2), 74.2 (C-4), 73.8 (C-4), 73.6 (C-3), 73.0 (O*C*H$_2$Ar), 71.9 (C-4), 71.3 (C-6), 70.7 (C-1), 70.2 (C-1), 69.5 (OCH$_2$CH$_2$CH(CH$_3$)$_2$), 69.2 (C-5), 68.8 (C-5), 66.0 (O*C*H$_2$C=C), 56.8 (Ph*C*H$_2$N), 52.7 (C-6), 51.4 (CH$_2$N), 51.2 (CH$_2$N), 38.9 (CH$_2$), 29.8, 27.5 (isopropylidene CH$_3$), 26.6 (isopropylidene CH$_3$), 25.6, 25.0 (isopropylidene CH$_3$), 24.7 (isopropylidene CH$_3$), 22.7 (OCH$_2$CH$_2$CH(CH$_3$)$_2$); ESI-HRMS calcd for $C_{48}H_{65}N_4O_9$ 841.4752, found *m/z* 841.4751 $[M+H]^+$



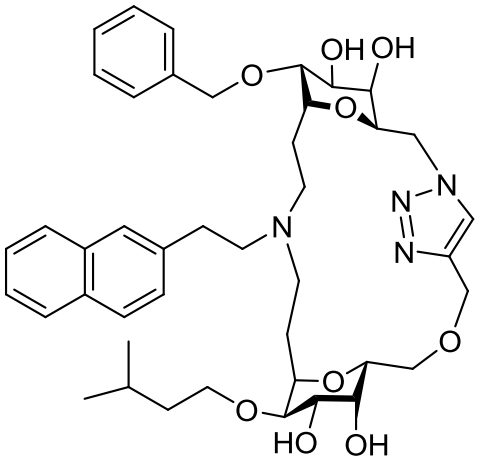
Macrocycle 211. Compound **205** (40 mg, 0.052 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure

and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and the solvents were concentrated under reduced pressure to give **211** (27 mg, 77%) as a white powder; ^{1}H NMR (500 MHz, CD_3OD) δ 7.84 (1H, s, triazoleH), 7.28 (1H, d, *J* 7.2, ArH), 7.23 (2H, t, *J* 7.3, ArH), 7.18 (3H, d, *J* 7.1, ArH), 4.65 (1H, d, *J* 11.8, OC*H*H), 4.63 – 4.55 (1H, m, C*H*H), 4.51 (4H, overlapping signals, each CH_2), 3.91 (3H, overlapping signals, each CH), 3.78 – 3.70 (3H, overlapping signals, each CH), 3.67 (1H, t, *J* 9.2, CH), 3.60 (1H, d, *J* 8.4, CH), 3.58 – 3.50 (2H, m, CH_2), 3.47 (1H, dd, *J* 16.2, 6.8, CH_2), 2.46 (2H, m), 2.36 (2H, m), 2.32 – 2.23 (1H, m), 1.99 (1H, s) (each CH_2N), 1.71 (1H, s), 1.66 – 1.56 (4H, m), 1.35 (4H, ddd, *J* 29.5, 14.7, 8.0, $CH_2CH_2CH(CH_3)_2$), 1.17 (4H, overlapping signals, each CH_2), 0.81 (6H, dt, *J* 8.8, 4.4, $OCH_2CH_2CH(CH_3)_2$), 0.77 (6H, t, *J* 6.2, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 145.6 (*C*=CH), 138.5 (Ar-C), 127.9, 127.6, 127.3 (each Ar-CH), 124.1 (C=*C*H), 76.9, 76.5 (each CH), 72.8 (OCH_2Ph), 71.5, 71.3, 70.5, 69.8, 69.4, 69.2 (each CH), 69.0, 64.0, 59.2, 51.4, 51.3, 50.9, 38.8 (each CH_2), 26.4, 24.7 (each CH), 21.8, 21.7, 21.5, 21.4 (each CH_3); ESI-HRMS calcd for $C_{36}H_{59}N_4O_9$ 691.4282, found *m/z* 691.4276 $[M+H]^+$



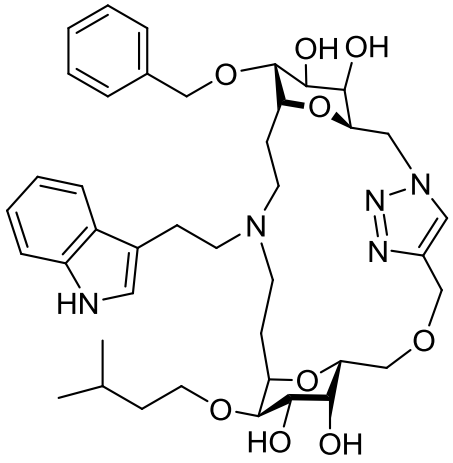
Macrocycle 212. Compound **206** (50 mg, 0.062 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give **212** (33 mg, 73%) as a white powder; ^{1}H NMR (500 MHz, CD_3OD) δ 7.85 (1H, s, triazole H), 7.25 (3H, d, *J* 6.4, ArH), 7.13 (6H, dd, *J* 12.0, 7.0, ArH), 7.10 – 7.04 (1H, m, ArH), 6.98 (1H, d, *J* 7.2, ArH), 4.65 (1H, d, *J* 11.6,

OC*H*HC=C), 4.63 – 4.40 (6H, overlapping signals, each CH_2), 3.97 (2H, overlapping signals, each CH), 3.92 (2H, overlapping signals, each CH), 3.77 (3H, overlapping signals, each CH), 3.68 (2H, dd, *J* 16.5, 9.0, each CH), 3.63 – 3.51 (4H, overlapping signals, each CH_2), 3.48 (2H, overlapping signals, each CH), 2.52 (6H, overlapping signals), 2.26 (1 H, m), 2.07 (1 H, m) (each CH*H*N), 1.67 – 1.57 (6H, overlapping signals, 2 x CH_2 & $OCH_2CH_2CH(CH_3)_2$), 1.45 – 1.29 (4 H, overlapping signals), 0.86 – 0.74 (6 H, m, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 145.6 (*C*=CH), 139.8, 138.5 (each Ar-C), 132.0, 129.5, 128.2, 128.1, 128.0, 127.7, 127.3, 125.7 (each Ar-CH), 124.1 (C=*C*H), 77.0, 76.7 (each CH), 72.9 (CH_2), 71.8, 71.5, 71.4, 71.3, 70.6, 70.0, 69.9, 69.5, 69.2 (each CH), 69.0 (CH_2), 64.1 (CH_2), 59.2, 52.2, 51.2, 51.2, 50.9, 38.8 (each CH_2), 31.1, 24.7 (CH), 21.8, 21.4 (each CH_3); ESI-HRMS calcd for $C_{41}H_{63}N_4O_9$ 755.4595, found *m/z* 755.4580 $[M+H]^+$



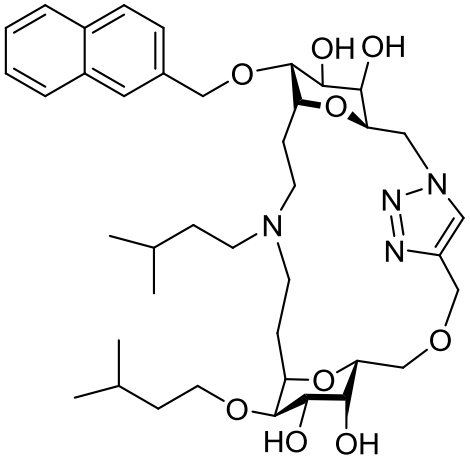
Macrocycle 213. Compound **207** (45 mg, 0.053 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give **213** (32 mg, 78%) as a white powder. ^{1}H NMR (500 MHz, CD_3OD) δ 7.82 (1H, s, triazole H), 7.70 – 7.64 (2H, m, ArH), 7.63 – 7.57 (2H, m, ArH), 7.43 (1H, s, ArH), 7.31 (2H, ddd, *J* 9.6, 8.2, 6.1, ArH), 7.21 (2H, d, *J* 6.3, ArH), 7.11 (1H, d, *J* 8.5, ArH), 7.09 – 7.02 (2H, m, ArH), 4.62 (2H, dd, *J* 18.0, 6.5, CH_2), 4.55 (2H, brs, CH_2), 4.45 (2H, m, CH_2), 4.02 – 3.95 (2H, overlapping signals, each CH), 3.88 (2H, overlapping signals, each CH), 3.75 (2H, overlapping signals, each CH), 3.70 (1H, apt d, *J* 9.6, CH), 3.64 (1H, apt d, *J* 8.3, CH), 3.58 (2H, m), 3.55 – 3.48 (2H, m, CH_2), 3.48 – 3.42 (1H, m, CH*H*), 2.71 – 2.57 (4H, overlapping signals, CH_2N), 2.49 (2H, m, CH_2N), 2.46 – 2.41 (1H, m, C*H*HN), 2.31 – 2.22 (1H, m, CH*H*N), 2.09 (1H, dd, *J* 19.9, 10.1), 1.76 – 1.55

(5H, overlapping signals), 1.42 – 1.28 (3H, m), 1.22 (1H, t, *J* 7.2), 0.81 – 0.69 (6H, dd, *J* 6.0, 4.2, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 145.7 (*C*=CH), 138.4, 137.6, 133.6, 132.1 (each Ar-C), 128.0, 127.6, 127.3, 127.2, 127.0, 126.9, 126.3, 125.6 (Ar-CH), 124.9, 124.1 (C=*C*H), 77.0, 76.7, 72.9 (CH_2), 71.8, 71.4, 71.4, 71.2, 70.6, 70.0, 69.9, 69.4, 69.2 (each CH), 69.0, 64.1 (each CH_2), 59.2, 52.1, 52.0, 51.2, 51.2, 50.9, 48.5, 38.8, 31.2 (each CH_2), 24.7 (CH), 21.9, 21.5 (CH_3); ESI-HRMS calcd for $C_{43}H_{59}N_4O_9$ 775.4282, found *m/z* 775.4277 $[M+H]^+$

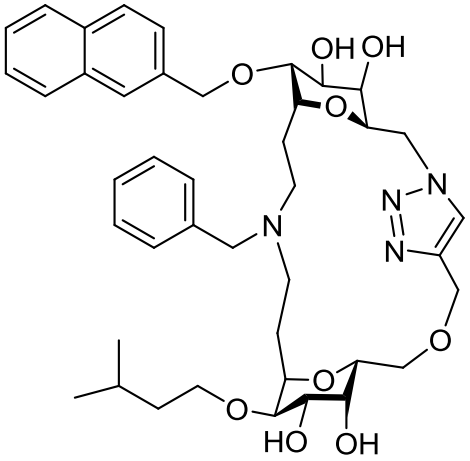


Macrocycle 214. Compound **208** (50 mg, 0.059 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give **214** (36 mg, 79%) as a white powder; ^1H NMR (500 MHz, CD_3OD) δ 7.84 (1H, s, triazole H), 7.40 (1H, d, *J* 7.9, ArH), 7.29 – 7.12 (4H, m, ArH), 7.08 – 7.02 (2H, m, ArH), 6.98 (1H, t, *J* 7.5, ArH), 6.86 (2H, dd, *J* 16.0, 8.9, ArH), 4.53 (6H, overlapping signals, each CH_2), 3.98 (1H, m, CH), 3.93 (1H, m, CH), 3.92 – 3.84 (2H, overlapping signals, each CH), 3.73 (2H, overlapping signals, each CH), 3.68 (1H, m, CH), 3.61 (1H, m, CH), 3.56 (1H, d, *J* 4.9, C*H*H), 3.55 – 3.45 (3H, overlapping signals, CH*H* & CH_2), 2.76 – 2.63 (3H, overlapping signals, CH_2 & C*H*H), 2.54 (1H, m, CH*H*), 2.30 (1H, m, C*H*H), 2.09 (1H, m, CH*H*), 1.60 (5H, overlapping signals, 2 x CH_2 & $OCH_2CH_2CH(CH_3)_2$), 1.42 – 1.28 (2H, m, $OCH_2CH_2CH(CH_3)_2$), 0.72 (6H, d, *J* 6.7, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 139.8 (*C*=CH), 138.2, 133.4, 130.9, 129.4, 129.2, 128.8, 128.6, 125.6, 123.1 (C=*C*H), 122.3, 119.6, 119.3, 112.3 (each Ar-C), 78.4, 78.0, 74.3 (CH_2), 73.3, 73.2, 72.9, 72.7, 72.0, 71.3, 71.2, 70.9, 70.6 (CH), 70.5, 65.5,

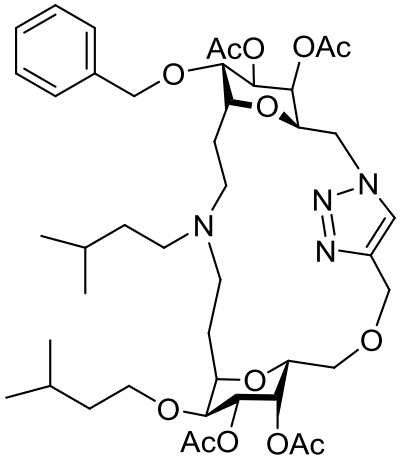
60.7, 53.2, 52.9, 52.7, 52.5, 52.3, 40.2 (each CH_2), 26.1, 23.2 (CH), 22.8 (CH_2), 22.3 (CH_3); ESI-HRMS calcd for $C_{41}H_{58}N_5O_9$ 763.4156, found *m/z* 763.4151 $[M+H]^+$



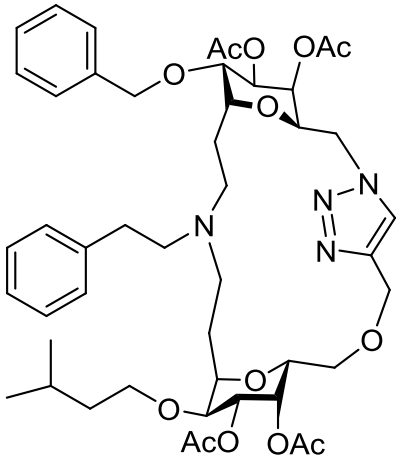
Macrocycle 215. Compound **209** (45 mg, 0.055 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give **215** (32 mg, 78%) as a white powder; ^{1}H NMR (500 MHz, CD_3OD) δ 7.81 (1H, s, triazole H), 7.77 – 7.70 (4H, m, ArH), 7.44 – 7.35 (3H, m, ArH), 4.84 (1H, d, *J* 11.9, OC*H*H), 4.63 (1H, d, *J* 11.9, OCH*H*), 4.57 (1H, d, *J* 10.2, C*H*H), 4.55 – 4.42 (3H, overlapping signals, CH_2 & CH*H*), 3.95 – 3.87 (4H, overlapping signals, each CH), 3.79 (2H, overlapping signals, each CH), 3.72 (1H, m, CH), 3.65 (1H, d, *J* 8.7, C*H*H), 3.59 (2H, overlapping signals, CHH & CH), 3.56 – 3.50 (3H, overlapping signals, C*H*H & 2 x CH), 3.47 (1H, t, *J* 8.0, CH*H*), 2.37 (2H, m, CH_2N), 2.21 (2H, m, CH_2N), 2.09 (1H, m, C*H*HN), 1.93 (1H, m, CH*H*N), 1.62 (3H, overlapping signals, CH_2 & CH), 1.54 – 1.46 (2 H, m, CH_2), 1.44 –1.30 (2H, m, CH_2), 1.30 – 1.21 (1H, m, CH), 1.08 (2H, m, CH_2), 0.84 – 0.79 (6H, m, $CH_2CH_2CH(CH_3)_2$), 0.68 (5H, t, *J* 6.3, $CH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 145.6 (*C*=CH), 136.0, 133.3, 133.1 (each Ar-C), 127.7, 127.5, 127.2, 126.4, 125.7, 125.6 (each Ar-CH), 124.1 (C=*C*H), 76.9, 76.7 (CH), 72.9 (CH_2), 71.3, 71.2 (each CH), 70.0 (CH_2), 69.9, 69.5, 69.2 (each CH), 69.0 (CH_2), 64.0 (CH_2), 51.4, 51.2, 50.9, 48.3, 38.8 (each CH_2), 33.8, 26.4 (CH), 24.7 (CH), 21.8, 21.7, 21.5 (CH_3), 21.4 (CH_3); ESI-HRMS calcd for $C_{40}H_{61}N_4O_9$ 741.4438, found *m/z* 741.4430 $[M+H]^+$



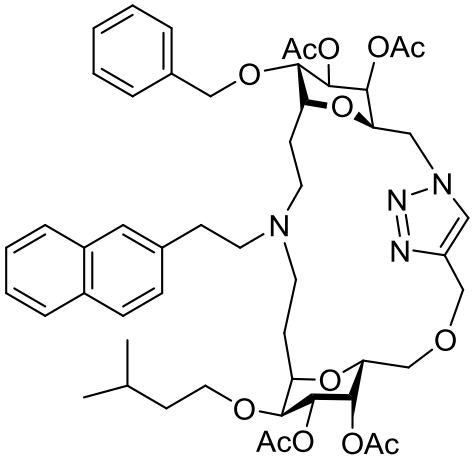
Macrocycle 216. Compound **210** (50 mg, 0.059 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give **216** (36 mg, 79%) as a white powder; ^{1}H NMR (500 MHz, CD_3OD) δ 7.81 (1H, s, triazole H), 7.70 (3H, dd, *J* 15.4, 8.6, ArH), 7.39 – 7.33 (2H, m, ArH), 7.23 (1H, d, *J* 4.2, ArH), 7.13 – 7.07 (3H, m, ArH), 4.73 (1H, d, *J* 12.1, OCH*H*), 4.66 – 4.59 (1H, m, CH*H*), 4.51 (4H, overlapping signals, each CH_2), 3.96 – 3.87 (2H, overlapping signals, each CH), 3.85 (1H, brs, CH), 3.78 (1H, m, CH), 3.72 (1H, brs, CH), 3.70 – 3.55 (3H, overlapping signals, CH & CH_2), 3.53 (1H, dd, *J* 9.4, 3.3, CH), 3.49 – 3.41 (2H, overlapping signals, CH & C*H*H), 3.34 (1H, dd, *J* 11.4, 4.7, CH*H*), 3.29 (2H, t, *J* 10.0, CH_2), 2.34 (2H, m, CH_2N), 2.18 – 2.11 (1H, m, C*H*HN), 1.99 (1H, m, CH*H*N), 1.59 (4H, overlapping signals, C*H*H, CH_2 & $OCH_2CH_2CH(CH_3)_2$), 1.49 (1H, m, CH*H*), 1.37 – 1.26 (2H, m, $OCH_2CH_2CH(CH_3)_2$), 0.78 (6H, dd, *J* 12.2, 6.7, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 145.6 (*C*=CH), 135.9, 133.2, 133.0, 128.5, 128.2, 128.0, 127.9, 127.7, 127.5, 127.2, 126.8, 126.6, 126.4, 125.8, 125.8, 125.6 (each Ar-C), 124.1 (C=*C*H), 76.9, 76.7 (each CH), 72.8 (CH_2), 71.6, 71.4, 70.4 (CH_2), 69.8, 69.7, 69.2, 69.0 (each CH), 68.9, 64.0, 59.2, 55.8, 52.1, 51.9, 51.5, 50.6, 38.7 (each CH_2), 24.6 (CH), 21.8, 21.5 (CH_3); ESI-HRMS calcd for $C_{42}H_{57}N_4O_9$ 761.4125, found *m/z* 761.4122 $[M+H]^+$



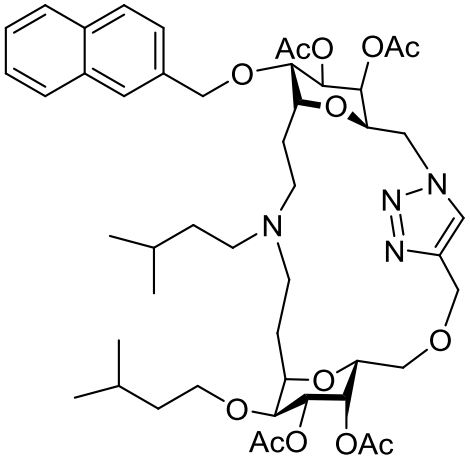
Macrocycle 217. Compound **211** (20 mg, 0.029 mmol) was dissolved in pyridine-Ac_2O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give **217** (20 mg, 80%) as a off-white foam; $[\alpha]_D$ +64.1° (c 0.08 in $CHCl_3$); IR (film) cm^{-1}: 2956, 2253, 1745, 1370, 1239, 1107, 903; ^1H NMR (500 MHz, $CDCl_3$) δ 7.76 (1H, s, triazole H), 7.37 – 7.28 (5H, m, ArH), 5.53 – 5.51 (1H, m, H-4'), 5.36 (1H, dd, *J* 3.2, 1.7, H-4), 5.24 (1H, dd, *J* 9.0, 3.2, H-3'), 5.06 (1H, dd, *J* 9.6, 3.2, H-3), 4.68 (3H, d, *J* 11.7, CH_2 & C*H*H), 4.59 (1H, d, *J* 11.9, CH*H*), 4.53 (1H, d, *J* 13.8, H-6'a), 4.45 – 4.36 (1H, m, H-6'b), 4.22 (2H, d, *J* 6.9, each CH), 4.14 – 4.07 (1H, m, each CH), 3.91 (2H, dd, *J* 8.7, 5.0, each CH), 3.75 (1 H, dd, *J* 9.2, 5.7, CH), 3.65 (2H, ddd, *J* 20.9, 10.5, 5.2), 3.55 (3H, dt, *J* 12.5, 4.5, H-6a & $OCH_2CH_2CH(CH_3)_2$), 2.43 (7H, m, each CH_2), 2.22 (2H, m, CH_2), 2.18, 2.14, 2.05, 2.04 (each 3H, each s, each acetate CH_3), 1.71 – 1.62 (2H, m, each $OCH_2CH_2CH(CH_3)_2$), 1.53 – 1.43 (2H, m, $OCH_2CH_2CH(CH_3)_2$), 1.44 – 1.36 (2H, m, $OCH_2CH_2CH(CH_3)_2$), 0.91 – 0.82 (12H, m, each $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 170.2, 169.9, 169.9 (C=O), 146.1 (triazole CH=*C*), 137.6 (Ar-C), 128.4, 128.0, 127.7 (each Ar-CH), 123.4 (triazole *C*H=C), 74.2 (C-2), 73.5 (C-2), 73.2 (OCH_2Ph), 72.1 (C-1), 71.7 (CH), 70.2 (CH), 70.0 (CH), 69.7 (CH_2), 69.4 (CH), 69.3 (CH), 69.1 (CH), 65.1 (CH_2), 53.4, 51.3 (CH_2N), 38.8 (CH_2), 26.4, 24.8 (CH), 22.7, 22.6, 22.5, 22.4, 20.8, 20.8, 20.7, 20.7 (each CH_3); ESI-HRMS calcd for $C_{44}H_{67}N_4O_{13}$ 859.4704, found *m/z* 859.4712 $[M+H]^+$



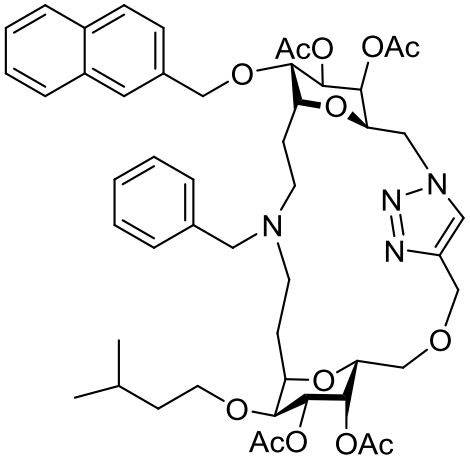
Macrocycle 218. Compound **212** (25 mg, 0.0344 mmol) was dissolved in pyridine-Ac_2O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give **218** (25 mg, 82%) as a brown foam; $[\alpha]_D$ +65.7° (c 0.07 in $CHCl_3$); IR (film) cm^{-1}: 2956, 2253, 1745, 1370, 1239, 1107, 903; ^1H NMR (500 MHz, $CDCl_3$) δ 7.73 (1H, s, triazole H), 7.33 – 7.26 (5H, m, ArH), 7.24 (3H, t, *J* 7.3, ArH), 7.18 (1H, t, *J* 7.3, ArH), 7.07 (1H, d, *J* 7.0, ArH), 5.54 – 5.50 (1H, m, H-4'), 5.36 (1H, dd, *J* 3.1, 1.4, H-4), 5.22 (1H, dd, *J* 9.2, 3.3, H-3'), 5.05 (1H, dd, *J* 9.7, 3.4, H-3), 4.67 (3H, d, *J* 12.0, overlapping signals, OCH_2C=C & OC*H*HPh), 4.58 (1H, d, *J* 11.8, OCH*H*Ph), 4.53 (1H, dd, *J* 14.3, 1.4, H-6'a), 4.38 (1H, dd, *J* 14.2, 10.3, H-6'b), 4.24 – 4.19 (1H, m, H-1'), 4.19 – 4.14 (1H, m, H-5'), 4.14 – 4.10 (1H, m, H-1), 3.92 (1H, dd, *J* 9.2, 5.2, H-2), 3.89 – 3.85 (1H, m, H-5), 3.77 (1H, dd, *J* 9.7, 5.7, H-2'), 3.69 – 3.60 (2H, m, H-6), 3.59 – 3.51 (2H, m, $OCH_2CH_2CH(CH_3)_2$), 2.75 – 2.56 (6H, overlapping signals, CH_2N), 2.44 – 2.37 (1H, m, CH_2), 2.33 – 2.26 (1H, m, CH_2), 2.18, 2.14, 2.04, 2.02 (each 3H , each s, each acetate CH_3), 1.86 – 1.75 (2H, m), 1.69 (3H, overlapping signals), 1.42 (3H, overlapping signals), 0.88 (6H, d, *J* 6.6, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 170.2, 170.0, 169.9 (each C=O), 146.1 (triazole CH=C), 139.9, 137.6 (each Ar-C), 128.6, 128.4, 128.4, 127.9, 127.7, 126.0 (each Ar-CH), 123.4 (triazole CH=*C*), 74.2 (C-2), 73.5 (C-2), 73.2 (OCH_2Ph), 72.2 (C-1), 71.7 (C-1), 70.2 (C-3), 70.1 (C-5), 69.7 (CH_2), 69.4 (CH_2), 69.3 (C-3), 69.2 (C-5), 69.2 (C-4), 68.8 (C-4), 65.1 (CH_2), 52.5, 51.3, 51.0 (each *C*H_2N), 50.2 (C-6), 38.8 (CH_2), 31.9 (CH), 24.8, 22.7 (each CH_2), 22.5 (CH_2), 22.4 (CH_3), 21.3 (CH_2), 20.8, 20.8, 20.7, 20.7 (each CH_3); ESI-HRMS calcd for $C_{47}H_{65}N_4O_{13}$ 845.4548, found *m*/*z* 845.4542 $[M+H]^+$



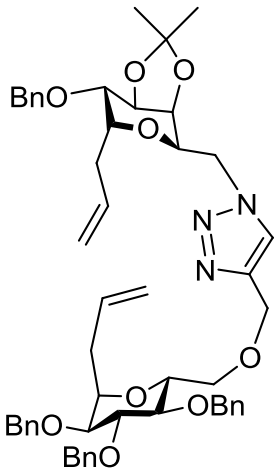
Macrocycle 219. Compound **213** (22 mg, 0.028 mmol) was dissolved in pyridine-Ac_2O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give **219** (22 mg, 83%) as a white foam; $[\alpha]_D$ +44.5° (c 0.06 in $CHCl_3$); IR (film) cm^{-1}: 2956, 2253, 1745, 1370, 1239, 1107, 903; ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 – 7.78 (1H, m, ArH), 7.74 (3H, d, *J* 5.7, ArH), 7.54 (1H, s, triazole H), 7.48 – 7.40 (2H, m, ArH), 7.30 – 7.21 (6H, m, ArH), 5.45 (1H, m, H-4'), 5.34 (1H, dd, *J* 3.2, 1.5, H-4), 5.21 (1H, dd, *J* 9.1, 3.3, H-3'), 5.06 (1H, dd, *J* 9.7, 3.4, H-3), 4.67 (2H, m, OCH_2C=C), 4.56 (1H, d, *J* 11.8, C*H*H), 4.49 (2H, overlapping signals, CH*H* & H-6'a), 4.38 (2H, overlapping signals, H-6'b & CH), 4.24 – 4.18 (2H, overlapping signals, each CH), 4.16 – 4.09 (2H, overlapping signals, each CH), 3.90 (1H, dd, *J* 9.1, 5.1, CH), 3.87 – 3.83 (1H, m, CH), 3.77 (1H, dd, *J* 9.7, 5.7, H-6a), 3.63 (1H, dd, *J* 10.2, 8.0, H-6b), 3.57 – 3.51 (3H, overlapping signals, CH_2 & CH), 2.80 (4H, overlapping signals, each CH_2N), 2.65 (2H, t, *J* 7.8, CH_2N), 2.51 – 2.44 (1H, m), 2.33 (2H, ddd, *J* 17.0, 9.4, 4.1, CH_2N), 2.17, 2.15, 2.05, 2.04 (each 3H, each s, each acetate CH_3), 1.91 – 1.79 (4H, overlapping signals, each CH_2), 1.79 – 1.70 (2H, m), 1.71 – 1.64 (1H, m, $OCH_2CH_2CH(CH_3)_2$), 1.43 (2H, ddd, *J* 14.7, 13.7, 6.8, $OCH_2CH_2CH(CH_3)_2$), 0.87 (6H, d, *J* 6.7, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0, 170.0, 169.9, 169.9 (each C=O), 160.7, 160.4, 160.1, 159.7, 144.1, 137.1, 133.5, 132.6, 132.1, 129.3, 128.7, 128.6, 128.4, 128.3, 127.8, 127.7, 127.6, 126.8, 126.4, 126.1, 125.0 (each Ar-C), 74.8, 74.5, 73.9, 73.6 (each CH), 73.3 (CH_2), 70.8, 70.2, 70.2, 70.1, 69.9, 68.6, 68.1, 68.1, 68.1, 68.0, 67.6, 67.3, 66.9, 66.7 (each CH), 63.3, 53.9, 52.6, 50.8, 50.7, 49.6, 38.7, 29.6 (each CH_2), 25.0 (CH), 22.5, 22.3, 20.8, 20.7, 20.6, 20.6 (each CH_3); ESI-HRMS calcd for $C_{51}H_{67}N_4O_{13}$ 943.4704, found *m/z* 943.4708 $[M+H]^+$



Macrocycle 220. Compound **215** (26 mg, 0.035 mmol) was dissolved in pyridine-Ac_2O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give **220** (26 mg, 82%) as a white foam; $[\alpha]_D$ +67.4° (c 0.1 in $CHCl_3$); IR (film) cm^{-1}: 2956, 2253, 1745, 1370, 1239, 1107, 903 cm^{-1} ^1H NMR (500 MHz, $CDCl_3$) δ 7.83 (3H, d, *J* 7.8, ArH), 7.75 (1H, s, ArH), 7.72 – 7.66 (1H, m, ArH), 7.52 – 7.47 (2H, m, ArH), 7.43 (1H, d, *J* 8.1, ArH), 5.54 (1H, m, H-4'), 5.36 (1H, m, H-4), 5.30 – 5.24 (1H, m, H-3'), 5.04 (1H, m, H-3), 4.82 (1H, d, *J* 12.2, OC*H*HPh), 4.76 (1H, d, *J* 11.7, OCH*H*Ph), 4.67 (2H, m, $OCH_2C{=}C$), 4.53 (1H, apt d, *J* 13.9, H-6'a), 4.41 – 4.32 (1H, m, H-6'b), 4.20 (1H, m, H-1'), 4.09 (1H, m, H-5'), 3.97 (1H, m, H-1), 3.90 – 3.82 (1H, m, H-5), 3.75 (2H, m, H-2 & H-2'), 3.67 (1H, m, H-6a), 3.61 (1H, m, H-6b), 3.53 (2H, m, $OCH_2CH_2CH(CH_3)_2$), 3.46 (1H, s), 2.46 (2H, m, CH_2), 2.37 (2H, m, CH_2), 2.33 – 2.22 (2H, m, CH_2), 2.15 (6H, m, each acetate CH_3), 2.04 (6H, s, each acetate CH_3), 1.84 (3H, s), 1.68 (6H, s), 1.40 (5H, dd, *J* 21.7, 15.4), 0.84 (12H, m, $CH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.23, 169.22, 168.96, 168.90 (each C=O), 146.2 (triazole CH=*C*), 134.9, 133.2, 133.2, 128.4, 128.0, 127.8, 126.4, 126.3, 126.2 (each Ar-C), 75.3 (CH), 74.9 (CH), 74.0 (CH), 73.6 (CH), 72.9 (CH), 71.9 (CH_2), 71.4 (CH), 71.3 (CH_2), 71.2 (CH), 70.3 (CH), 69.4 ($OCH_2CH_2CH(CH_3)_2$), 69.2 (CH), 68.7 (CH) , 66.2 ($OCH_2C{=}C$), 52.8 51.8, 51.56, 49.3, 38.9, 36.3, 29.8 (each CH_2), 25.1, 24.6 (CH), 22.9, 22.8 (CH_3); ESI-HRMS calcd for $C_{48}H_{69}N_4O_{13}$ 909.4861, found *m/z* 909.4855 $[M+H]^+$

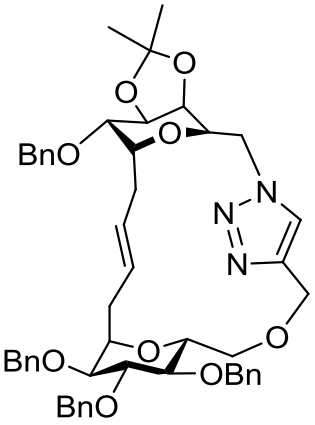


Macrocycle 221. Compound **216** (22 mg, 0.029 mmol) was dissolved in pyridine-Ac_2O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure to give **221** (23 mg, 84%) as a white solid; $[\alpha]_D$ +54.0° (c 0.06 in $CHCl_3$); IR (film) cm^{-1}: 2956, 2253, 1745, 1370, 1239, 1107, 903; ^1H NMR (500 MHz, $CDCl_3$) δ 7.85 – 7.75 (4H, m, ArH), 7.68 (3H, m, ArH), 7.52 – 7.45 (3H, m, ArH), 7.39 – 7.28 (4H, m, ArH), 7.26 – 7.14 (8H, m, ArH), 5.54 – 5.50 (1H, m, H-4'), 5.38 – 5.34 (1H, m, H-4), 5.24 (1H, dd, *J* 9.0, 3.3, H-3'), 5.03 (1H, dd, *J* 9.4, 3.3, H-3), 4.69 (2H, overlapping signals, each C*H*H), 4.64 (2H, d, *J* 12.5), 4.60 (3H, overlapping signals), 4.54 (1H, dd, *J* 14.3, 1.3), 4.42 – 4.36 (1H, m), 4.19 (3H, overlapping signals), 4.06 – 4.01 (1H, m), 3.90 – 3.83 (2H, m), 3.71 – 3.60 (2H, m, each CH), 3.55 (2H, overlapping signals, C*H*H & CH), 3.49 (2H, dd, *J* 9.8, 2.3, CH_2), 3.45 (1H, dd, *J* 10.9, 4.3, CH_2), 3.41 – 3.35 (1H, m, CH_2), 2.56 – 2.42 (3H, m, CH_2N), 2.41 – 2.33 (2H, m,CH_2N), 2.33 – 2.24 (2H, m, CH_2N), 2.14 (3H, s, acetate CH_3), 2.13 (3H, s, acetate CH_3), 2.04 (6H, s, each acetate CH_3), 1.65 – 1.56 (3H, m), 1.40 – 1.29 (5H, m), 0.85 (7H, t, *J* 6.5, $OCH_2CH_2CH(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.23, 169.22, 168.96, 168.90 (each C=O), 145.10, 133.99, 132.11, 132.02 (Ar-C), 127.95, 127.57, 127.54, 127.23, 126.83, 126.69, 125.63, 125.37, 125.26, 125.10, 124.64, 122.46 (each Ar-CH), 74.46, 73.57 (each CH), 73.24 (CH_2), 72.44, 72.01, 69.10 (CH_2), 68.94 (CH_2), 68.52, 68.45, 68.16, 67.93, 67.91, 67.71, 67.70 (each CH), 64.11 (CH_2), 50.84, 50.80, 49.75, 49.08, 37.72, 28.68 (each CH_2), 23.77 (CH), 21.56, 21.43, 19.88, 19.77, 19.68 (each CH_3); ESI-HRMS calcd for $C_{50}H_{65}N_4O_{13}$ 929.4548, found *m/z* 929.4555 $[M+H]^+$



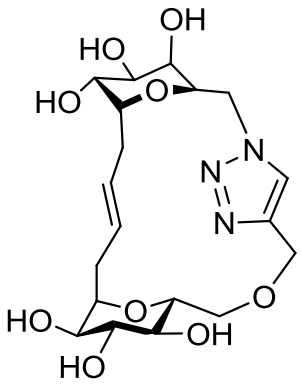
1-*C*-Allyl-1,6-dideoxy-6-(4-(((1-*C*-allyl-1,6-dideoxy-2,3,4-tri-*O*-benzyl-α-D-glucopyranos-6-yl)oxymethyl)-1H-1,2,3-triazol-1-yl)-2-*O*-benzyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (222). Alkyne **152** (200 mg, 0.390 mmol) and azide **201** (140 mg, 0.390 mmol) were dissolved in a mixture of acetonitrile-H_2O (5 mL, 1:1). To this was added CuI (75 mg, 0.39 mmol) and the reaction was heated at reflux for 24 h. Upon cooling the reaction was diluted with EtOAc, washed with H_2O, brine, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:1) to give compound **222** (289 mg, 85%) as a yellow oil; IR (film) cm^{-1}: 2915, 1640, 1381, 1092, 906; ^1H NMR (500 MHz, $CDCl_3$) δ 7.64 (1H, s, triazole H), 7.35 – 7.26 (20H, m, ArH), 5.86 – 5.72 (1H, m, CH_2CH=CH_2), 5.61 (1H, dd, *J* 17.1, 10.2, CH_2CH=CH_2), 5.07 (2H, dd, *J* 17.1, 13.8, CH_2CH=CH_2), 4.99 – 4.88 (3H, overlapping signals, CH_2CH=CH_2 & OC*H*HPh), 4.81 (1H, dd, *J* 10.9, 5.7, OCH_2Ph), 4.72 – 4.60 (5H, overlapping signals, OCH_2Ph & OCH_2C=C), 4.56 – 4.47 (3H, overlapping signals, H-6'a & OCH_2Ph), 4.40 – 4.33 (2H, overlapping signals, CH & H-6'b), 4.23 (1H, m, CH), 4.15 (1H, d, *J* 9.0, CH), 4.09 (1H, dd, *J* 10.3, 5.4, CH), 4.06 – 4.02 (1H, m, CH), 3.81 – 3.77 (1H, m, CH), 3.74 (2H, overlapping signals, H-6a and CH), 3.67 (1 H, d, *J* 10.6, H-6b), 3.60 (3 H, overlapping signals, each CH), 3.53 (1 H, t, *J* 3.4, CH), 2.52 – 2.44 (2 H, m, CH_2CH=CH_2), 2.31 – 2.24 (1H, m, C*H*HCH=CH_2), 2.18 (1H, dd, *J* 14.6, 6.6, CH*H*CH=CH_2), 1.49 (3H, s, isopropylidene CH_3), 1.33 (3H, s, isopropylidene CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.7 (triazole CH=*C*), 138.9, 138.3, 138.3, 137.6 (each Ar-C), 134.8 (CH_2CH=CH_2), 134.5 (CH_2CH=CH_2), 134.3, 133.9, 133.3, 128.6, 128.5, 128.5, 128.5, 128.4, 128.1, 128.1, 127.9, 127.9, 127.9, 127.9, 127.6 (each Ar-CH), 124.3 (triazole *C*H=C), 117.4 (CH_2CH=CH_2), 117.0 (CH_2CH=CH_2), 110.2 (isopropylidene C), 82.4 (CH), 80.1 (CH), 78.1 (CH), 75.5 (OCH_2Ph), 75.2 (OCH_2Ph), 74.9 (CH), 73.8 (CH), 73.2 (CH), 73.0 (OCH_2Ph),

72.8 (OCH_2Ph), 71.7 (CH), 71.6 (CH), 71.2 (CH), 69.2 (CH_2), 68.8 (CH), 65.0 ($OCH_2C{=}C$), 51.8 (C-6), 34.9 ($CH_2CH{=}CH_2$), 29.9 ($CH_2CH{=}CH_2$), 26.8 (isopropylidene CH_3), 24.8 (isopropylidene CH_3); ESI-HRMS calcd for $C_{52}H_{62}N_3O_9$ 872.4486, found *m/z* 872.4479 $[M+H]^+$



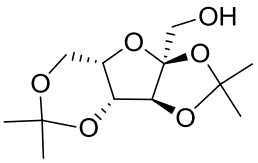
Macrocycle 223. Compound **222** (280 mg, 0.321 mmol) was dissolved in toluene (6 mL) and degassed at -78 °C. Upon warming to room temperature, Hoveyda-Grubbs II catalyst (30 mg, 0.048 mmol) was added and the reaction heated to 90 °C for 8 h. The reaction was cooled to room temperature and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:5) to give **223** (251 mg, 93%) as a colourless oil; IR (film) cm^{-1}: 2913, 1641, 1381, 1090, 905; ^1H NMR (500 MHz, $CDCl_3$) δ 7.85 (1H, s, triazole H), 7.37 – 7.22 (20H, m, ArH), 5.71 – 5.63 (1H, dt, C*H*=CH, *J* 15.0, 6.9), 5.48 – 5.39 (1H, dt, *J* 15.1, 7.1, CH=C*H*,), 4.92 (1H, d, *J* 11.0, OC*H*HPh), 4.88 (1H, d, *J* 11.0, OCH*H*Ph), 4.81 (2H, dd, *J* 11.3, 8.8, overlapping signals, OCH_2Ph, OC*H*HC=C), 4.70 (1H, d, *J* 11.6, OC*H*HPh), 4.66 – 4.54 (4H, overlapping signals, OCH_2Ph, OC*H*HPh and H-6a), 4.50 (2H, dd, *J* 13.8, 7.4, OCH_2Ph), 4.46 (1H, d, *J* 11.7, OCH_2Ph), 4.34 (1H, dd, *J* 14.4, 9.3, CH*H*), 4.27 (1H, d, *J* 7.8, CH), 4.10 (1H, d, *J* 9.2, CH), 4.03 (1H, dd, *J* 10.7, 3.3, CH), 3.97 (1H, d, *J* 11.7, CH), 3.86 – 3.80 (1H, m, CH), 3.73 (3H, overlapping signals, 2 x CH & C*H*H), 3.62 – 3.59 (1H, m, CH*H*), 3.58 (1H, t, *J* 3.3, CH), 3.30 (1H, ddd, *J* 9.7, 5.0, 3.3, CH), 2.56 – 2.46 (1H, m, C*H*HCH=CH), 2.45 – 2.39 (1H, m, C*H*HCH=CH), 2.36 (1H, dd, *J* 15.4, 6.0, CH*H*CH=CH), 1.80 (1H, dd, *J* 15.2, 7.9, CH*H*CH=CH), 1.48 (3H, s, isopropylidene CH_3), 1.35 (3H, s, isopropylidene CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.07 (triazole CH=*C*), 138.63, 138.21, 137.97, 137.36 (each Ar-C, 130.05 (CH=CH), 128.52 (CH=CH), 128.48, 128.46, 128.41, 128.04, 128.02, 127.94, 127.88, 127.85, 127.82, 127.80 (each Ar-CH), 123.91 (triazole *C*H=C), 110.61 (isopropylidene C), 82.58, 80.14, 78.85, 75.48 (CH), 75.34 (OCH_2Ph), 75.11 (OCH_2Ph), 74.04 (CH), 73.76 (CH),

73.30 (OCH_2Ph), 73.15 (OCH_2Ph), 72.63 (CH), 72.21 (CH), 71.38, 70.44 (CH_2), 69.62, 65.71 ($OCH_2C{=}C$), 52.05 (C-6), 35.18 (CH_2), 26.88 (CH_2), 26.41 (isopropylidene CH_3), 24.76 (isopropylidene CH_3); ESI-HRMS calcd for $C_{50}H_{58}N_3O_9$ 844.4173, found *m/z* 844.4171 $[M+H]^+$



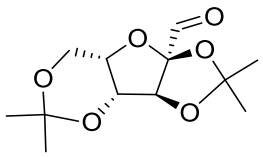
Macrocycle 224. Compound **223** (250 mg, 0.296 mmol) was dissolved in EtSH-BF_3.Et_2O (5 mL, 4:1) and stirred at room temperature for 24 h. Solvents were removed and the residue was purified via flash chromatography (CH_2Cl_2-MeOH 8:2) to give **224** (116 mg, 89%) as a white solid; ^{1}HNMR (D_2O, 500 MHz) δ 7.92 (1H, s, triazole H), 5.19 (1H, dt, C*H*=CH, *J* 15.0, 6.9), 5.01 (1H, dt, C*H*=CH, *J* 15.1, 7.0), 4.69 (1H, d, *J* 12.9, OC*H*HC=C), 4.52 (2H, dd, *J* 18.6, 11.0, H-6'), 4.45 (1H, d, *J* 12.8, OCH*H*C=C), 4.01 (1H, m, H-5'), 3.97 (1H, m, H-3'), 3.86 (2H, overlapping signals, H-4' & H-1'), 3.74 (2H, overlapping signals, H-5 & H-6a), 3.67 (1H, ddd, J 9.0, 5.7, 2.5, H-1) 3.61 (1H, brs, H-2'), 3.59 (1H, dd, *J* 11.4, 7.0, H-6b), 3.51 (1H, dd, *J* 9.6, 5.9, H-2), 3.40 (1H, t, *J* 9.1, H-3), 3.08 (1H, t, *J* 9.1-H-4), 2.10 (4H, overlapping signals, each C*H*$_2$C=C); ^{13}C NMR (125 MHz, D_2O) δ 142.1 (*C*=CH), 131.9, 131.6 (each *C*H=CH), 127.7 (C=*C*H), 78.9 (C-1), 78.1 (C-1') 75.6 (C-3), 74.1 (C-5'), 73.9 (C-5), 73.5 (C-6), 73.4 (C-4), 73.1 (C-2) 72.1 (C-3'), 72.0 (C-2'), 70.5 (H-4'), 63.4 ($OCH_2C{=}C$), 51.5 (C-6'), 26.2 (*C*H_2C=C); ESI-HRMS calcd for $C_{19}H_{30}N_3O_9$ 444.1982, found *m/z* 444.1978 $[M+H]^+$

6.5 Chapter 5-Experimental

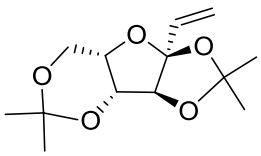


2,3:4,6-Di-*O*-isopropylidene-α-L-sorbofuranose[10] (242). Iodine (1.14 g, 4.5 mmol) was added to a solution of L-sorbose (5 g, 27.7 mmol) in dry acetone (350 mL). The solution was

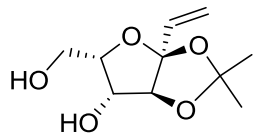
stirred at room temperature for 20 h. A 0.2 M solution of sodium sulfite was then added dropwise until the red colour dissipated. The acetone was removed under reduced pressure and the remaining aqueous layer was extracted with CH_2Cl_2. The organic layers were then combined, washed with H_2O, dried over $MgSO_4$ and the solvents were concentrated under reduced pressure. The residue was then purified via flash chromatography (EtOAc-cyclohexane 1:1 R_f 0.33) to give the desired product as a white solid (85%); IR (film) cm^{-1}: 3487, 2990, 2938, 1375, 1243, 1197, 1122, 1078; ^1H NMR (500MHz, $CDCl_3$) δ 4.49 (1H, s, H-3), 4.33 (1H, s, H-4), 4.10 (1H, s, H-5), 4.07(2H, m, H-6), 3.84 (2H, m, H-1), 1.44, 1.37, 1.31, 1.30 (each 3H, each s, each CH_3); ^{13}C NMR (125MHz, $CDCl_3$) δ 114.5 (C-2), 111.9 ($C(Me)_2$), 97.5 (($C(Me)_2$), 84.8 (C-3), 73.3 (C-5), 72.3 (C-4), 63.4 (C-1), 29.0, 27.5, 26.6, 18.8 (each CH_3); ESI-HRMS calcd for $C_{12}H_{20}O_6Na$ 283.1158, found *m/z* 283.1144 $[M+Na]^+$



2,3:4,6-Di-*O*-isopropylidene-α-L-xylo-hexos-2-ulo-2,5-furanose[11] To a stirring solution of oxalyl chloride (2.9 mL, 34.5 mmol) in CH_2Cl_2 at -78 °C was added anhydrous DMSO (3.2 mL, 46 mmol). After 15 min a solution of **242** (3 g, 11.5 mmol) in CH_2Cl_2 was added. Stirring was continued for a further 4 h before the temperature was increased to -50°C and anhydrous triethylamine was added. After 30 min the temperature was increased to room temperature and H_2O was added. The layers were separated and the aqueous layer was extracted into CH_2Cl_2. The organic layers were combined and washed with brine, dried over $MgSO_4$ and the solvents were concentrated under reduced pressure. The residue was purified via flash chromatography (EtOAc-cyclohexane 1:1 R_f 0.2) to give the title compound as an off-white solid (83%); ^1H NMR (500MHz, $CDCl_3$) δ 9.62 (1H, s, C*H*O), 4.55 (1H, s, H-3), 4.36 (1H, m, H-4), 4.23 (1H, m, H-5), 4.12 (2H, m, H-6), 1.50, 1.40, 1.35, 1.29 (each 3H, each s, each CH_3); ^{13}C NMR (125MHz, $CDCl_3$) δ 194.5 (CHO), 114.5 ($C(Me)_2$), 112.1 (C-2), 97.8 (($C(Me)_2$), 90.7 (C-3), 86.7 (C-5), 74.0 (C-4), 60.1 (C-6), 29.1, 27.3, 26.3, 18.9 (each CH_3)

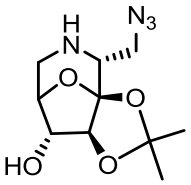


1,2-Dideoxy-4,5:6,8-di-*O*-isopropylidene-α-L-xylo-hept-1-ene-3-ulo-3,6-furanose (243). Ph_3PCH_3Br (4.15 g, 11.6 mmol) was taken up in THF (50 mL) and stirred at -78 °C. NaHMDS (11.6 mL, 1.0 M in THF) was then added dropwise. The reaction was stirred at -78 °C for 30 min and a solution of 2,3:4,6-Di-*O*-isopropylidene-α-L-xylo-hexos-2-ulo-2,5-furanose (2 g, 7.74 mmol) in THF (50 mL) was added via cannula. The reaction was stirred for a further 10 min at -78 °C and left to stir at room temperature overnight. The reaction was then quenched with satd NH_4Cl. The aqueous layer was extracted into EtOAc and the combined organic layers were washed with H_2O, brine, dried over $MgSO_4$ and the solvents were concentrated under reduced pressure. The compound was then purified via flash chromatography (EtOAc-cyclohexane 1:8 R_f 0.5) to yield compound **243** as a clear oil (63%); IR (film) cm^{-1}: 2991, 2933, 1650, 1453, 1409, 1373, 1195, 1120, 1074, 990, 912, 873, 831; ^1H NMR (500MHz, $CDCl_3$) δ 6.05 (1H, dd, *J* 10.5 , 17.2, H-2), 5.70 (1H, dd, *J* 1.6, 10.5, H-1a), 5.29 (1H, dd, *J* 1.6, 17.2, H-1b), 4.29 (1H, d, *J* 2.1, H-6), 4.28 (1H, s, H-4), 4.09 (1H, dd, *J* 2.0, 3.9, H-5), 4.04 (2H, m, H-7), 1.52, 1.43, 1.37, 1.35 (each 3H, each s, each CH_3); ^{13}C NMR (125MHz, $CDCl_3$) δ 136.3 (C-2), 117.4 (C-1), 113.2 (C-3), 111.7 ($C(Me)_2$), 97.5 (($C(Me)_2$), 88.0 (C-4), 73.8 (C-6), 72.6 (C-5), 60.5 (C-7), 29.1, 27.3, 26.3,18.9 (each CH_3); ESI-HRMS calcd for $C_{13}H_{21}O_5$ 257.1389, found *m/z* 257.1398 $[M+H]^+$

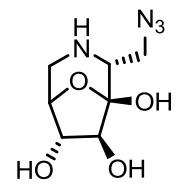


1,2-Dideoxy-4,5-*O*-isopropylidene-α-L-xylo-hept-1-ene-3-ulo-3,6-furanose (244). Compound **243** (1.55g, 6.05mmol) was dissolved in AcOH-H_2O (60% V/V) and heated to 60 °C for 2 h. It was then concentrated and columned (EtOAc-cyclohexane 1:1 R_f 0.28) to yield **244** (87%) as a clear oil; $[\alpha]_D$ +20.3° (*c* 1.0, $CHCl_3$); IR (film) cm^{-1}: 3495 (broad), 2988, 2935, 2895, 1649, 1456, 1411, 1384, 1175, 1078, 1045, 989, 912, 868, 832, 787, 737; ^1H NMR (500MHz, $CDCl_3$) δ 6.05 (1H, dd, *J* 10.5, 17.2, H-2), 5.70 (1H, dd, *J* 1.6, 10.5, H-1a), 5.29 (1H, dd, *J* 1.6, 17.2, H-1b), 4.20 (3H, m, H-3, H-4 & H-5 overlapping), 4.04 (2H, m, H-7), 1.46, 1.28 (each 3H, each s, each CH_3); ^{13}C NMR (125MHz, $CDCl_3$) δ 135.9 (C-2), 116.9

(C-1), 112.2 (C-3), 111.4 ($C(Me)_2$), 88.36 (C-4), 79.8 (C-6), 72.6 (C-5), 60.5 (C-7), 26.8, 25.9 (each CH_3); ESI-HRMS calcd for $C_{10}H_{17}O_5$ 217.1076; found *m/z* 217.1086 $[M+H]^+$

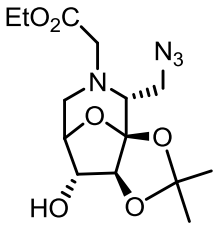


10-Azidomethyl-3,3-dimethyl-2,4,11-trioxa-9aza-tricyclo undecan-6-ol (245). To compound **244** (1.14 g, 5.3 mmol) dissolved in dry CH_2Cl_2 (70 mL) was added pyridine (1.02 mL, 12.7 mmol). The reaction was stirred at 0 °C and $SOCl_2$ (0.47 mL, 6.34 mmol) in dry CH_2Cl_2 (35 mL) was added dropwise. The reaction was then stirred for a further 2 h at 0 °C before being washed with H_2O, brine, dried over $MgSO_4$ and the solvents were concentrated under reduced pressure. The crude product was then dissolved in dry DMF and NaN_3 (1.42 g, 17.9 mmol) was added. The reaction was then stirred at 110 °C for 20 h. H_2O was then added and the resulting mixture was extracted into Et_2O. The organic layers were combined, washed with H_2O, dried over $MgSO_4$, filtered and the solvents were concentrated under reduced pressure. The residue was purified via flash chromatography (EtOAc-cyclohexane 1:1 R_f 0.18) to yield compound **245** (40%). $[\alpha]_D$ +24.9° (*c* 1.0, $CHCl_3$); IR (film) cm^{-1}: 3504 , 3319, 2853, 2099, 1453, 1375, 1288, 1234, 1199, 1134, 1109, 1051, 1014, 941, 870, 837; ^1H NMR (500MHz, $CDCl_3$) δ 4.52 (1H, d, *J* 6.7, H-6), 4.40 (1H, d, *J* 6.7, H-5), 4.38 (1H, s, H-4), 3.76 (1H, dd, *J* 3.0, 11.1, H-1a), 3.32 (1H, dd, *J* 3.1, 9.0, H-1b), 3.24 (1H, dd, *J* 9.4, 11.7, H-2), 3.04 (1H, dd, *J* 12.1, 1.0, H-7a) 2.99 (1H, dd, *J* 12.0, 2.1, H-7b), 1.55, 1.36 (each 3H, each s, Each CH_3); ^{13}C NMR (125MHz, $CDCl_3$) δ 116.4 (C-3), 110.0 (C(Me)2), 88.3 (C-4), 79.3 (C-6), 75.7 (C-5), 57.2 (C-2), 52.1 (C-1), 44.9 (C-7), 28.3 (CH3), 26.8 (CH3)[1]; ESI-HRMS calcd for $C_{10}H_{17}N_4O_4$ 257.1250, found *m/z* 257.1249 $[M+H]^+$

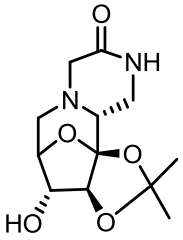


2-Azidomethyl-6-oxa-3-aza-bicyclo[3.2.2]nonane-1,8,9-triol (246). Compound **245** was taken up in methanolic HCl (3mL) and stirred overnight at room temperature. Solvents were

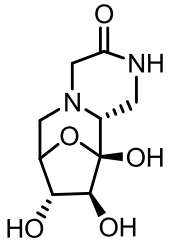
removed under reduced pressure and the resulting residue was taken up in H_2O and lyophilised. The product was obtained as a brown foam (60%); $[\alpha]_D$ +73.3° (*c* 1.0, H_2O); IR (film) cm^{-1}: 3398 (br), 2928, 2109, 1733, 1623, 1443; ^{1}NMR (600 MHz, D_2O) δ 4.26 (1H, d, *J* 7.63, H-6), 4.22 (1H, d, *J* 9.5, H-5), 4.03 (1H, s, H-4), 3.64 (1H, dd, *J* 13.0, 4.0, H-1a), 3.33-3.28 (1H, dd, *J* 7.86, 13.0, H-2), 3.07 (1H, dd, *J* 7.8, 4.0, H-1b), 2.93 (1H, d, *J* 13.5, H-7a), 2.83 (1H, dd, *J* 11.0, H-7b); ^{13}C NMR (150 MHz, D_2O) δ 100.0 (C-3), 78.3 (C-4), 75.4 (C-5), 75.0 (C-6), 59.4 (C-2), 51.0 (C-1), 42.8 (C-7); ESI-HRMS calcd for $C_7H_{13}N_4O_4$ 217.0937, found *m/z* 217.0933 $[M+H]^+$



(10-Azidomethyl-6-hydroxy-3,3-dimethyl-2,4,12-trioxa-9-aza-tricyclo[5.3.2.01,5]dodec-9-yl)-acetic acid ethyl ester (248). Compound **245** (235 mg, 0.9 mmol) was taken up in THF (50 mL) and triethylamine (435 uL, 3.1 mmol) was added. The solution was stirred at room temperature and ethyl bromoacetate (298 uL, 2.7 mmol) was added to the solution along with tetra-*n*-butylammonium iodide (299 mg, 0.8 mmol). The reaction was heated at reflux for 5 h, washed with H_2O, brine, dried over $MgSO_4$ and the solvents were concentrated under reduced pressure. The residue was purified via column chromatography (EtOAc-cyclohexane 1:1, R_f 0.38) to give the title compound as a clear oil (490 mg, 61%); $[\alpha]_D$ -26.2° (*c* 1.0, $CHCl_3$); IR (film) cm^{-1}: 3502, 3319, 2986, 2936, 2852, 2104, 1445, 1287, 1201, 864; ^{1}H NMR (500MHz, $CDCl_3$) δ 4.47 (2H, m, H-6 & H-4 overlapping), 4.36 (1H, dd, *J* 7.1, 13.0, H-5), 4.23 (2H, q, *J* 7.0, H-10), 3.90 (1H, dd, *J* 2.1, 13.4, H-1a), 3.7 (1H, d, *J* 18.0, H-8a), 3.45 (1H, dd, *J* 5.2, 14.0 H-1b) 3.15 (1H, d, *J* 18.2, H-8b), 3.09 (1H, dd, *J* 2.0, 5.3, H-2), 2.93 (1H, d, *J* 11.0, H-7a), 2.65 (1H, dd, *J* 2.1, 11.4, H-7b) 1.56, 1.40 (each 3H, each s, Each CH_3), 1.29 (t, 3H, J 7 Hz H-11); ^{13}C NMR (125MHz, $CDCl_3$) δ 171.51 (C=O), 116.4, 109.0, 88.9, 78.2, 76.2, 62.2, 61.5, 53.3, 52.2, 49.2, 28.4, 26.7, 14.1; ESI-HRMS calcd for $C_{14}H_{23}N_4O_6$ 343.1618, found *m/z* 343.1624



Tricyclic compound (249). Compound **248** (158 mg, 0.46 mmol) was dissolved in EtOAc (8 mL) and 10% palladium on carbon was added. The reaction was stirred overnight under an atmosphere of hydrogen. The solution was filtered through Celite® and the solvent was concentrated under reduced pressure to give compound as a white solid (108mg, 90%); mp 189-191˚C; $[\alpha]_D$ +15.8° (*c* 1.0, $CHCl_3$); R_f 0.13 EtOAc-cyclohexane 1:1; IR (film) cm^{-1}: 3331, 2943, 2247, 1668, 1213, 1048; ^1H NMR (500MHz, $CDCl_3$) δ 7.34 (1H, d, NHC=O), 4.46 (1H, d, *J* 7.0, H-6), 4.42 (1H, s, H-5), 4.34 (1H, d, *J* 4.1) 3.48 (1H, d, *J* 16.7), 3.42 (1H, dt, *J* 11.1, 4.0) , 3.21 (1H, t, *J* 11.4, H-7a), 3.00-2.93 (3 H, m), 2.41 (1H, dd, *J* 11.9, 2.0, H-7b), 1.49, 1.28 (each 3H, each s, each CH_3);^{13}C NMR (125 MHz, $CDCl_3$) δ 168.5, 116.57, 109.7, 87.6, 77.93, 75.9, 57.8, 56.6, 52.3, 41.85, 28.35, 27.1; ESI-HRMS calcd for $C_{12}H_{19}N_2O_5$ 271.1294, found *m/z* 271.1290 $[M+H]^+$



Tricyclic compound 250. Tricyclic compound **249** (97 mg, 0.36 mmol) was taken up in. methanolic HCl (13 mL) and stirred for 10 hours. Solvents were concentrated under reduced pressure and the residue was taken up in H_2O and lyophilized to give the product as a white foam (73%); $[\alpha]_D$ +106.6° (*c* 1.0,H_2O); IR (film) cm^{-1}: 3300 (br), 2492, 1671, 1377, 1095; ^1H NMR (500 MHz, D_2O) δ 4.55 (1H, d, *J* 7.0, H-6), 4.35 (1H, d, *J* 7.0 Hz, H-5), 4.03 (1H, s, H-4), 3.90 (1H, d, *J* 16.6, H-8a), 3.72 (1H, d, *J* 17.0, H-8b), 3.67 (1H, dd, *J* 4.1, 13.1, H-1a), 3.64 (1H, dd, *J* 4.1, 11.5, H-2), 3.52 (1H, d, *J* 12.7, H-7a), 3.38 (1H, t, *J* 12.21, H-1b), 3.05 (1H, d, *J* 12.7, H-7b); ^{13}C NMR (150 MHz, D_2O) δ 109.9 (C), 100 (C-4), 77.0 (C-5), 74.7 (C-4), 72.2 (C-6), 61.2 (C-2), 54.5 (C-8), 51.6 (C-7), 39.0 (C-1); ESI-HRMS calcd for $C_9H_{15}N_2O_5$ 231.0981, found *m/z* 231.0977 $[M+H]^+$

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