SYNTHESIS OF NOVEL CARBOHYDRATE BASED
ENZYME INHIBITOR LIBRARIES UTILISING CLICK
CHEMISTRY

BRENDAN LUKE WILKINSON
BSc (Hons)

Eskitis Institute for Cell and Molecular Therapies
School of Biomolecular and Physical Sciences
Griffith University

Submitted in fulfilment of the requirements of the degree of Doctor of
Philosophy

March 2007
Statement of Originality

This work has not been previously been submitted for a degree or diploma in any University. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

Brendan Luke Wilkinson

BSc (Hons).

March 2007.
Preface

Unless otherwise stated, the results presented in this thesis are those of the author. Parts of this work have appeared elsewhere in refereed journal publications which are presented with the dissertation in Appendix one on CD attached to the back of this thesis. $^1$H NMR and $^{13}$C{$^1$H} NMR spectra are presented in Appendix two on the same CD.

Refereed Journal Publications


**Book Chapters**


**Conference Posters**

‘Combinatorial Click Synthesis of Glycosyltriazole Arylsulfonamides: a New Class of Carbonic Anhydrase II Inhibitor’

Conference Lectures

‘Preparation of structurally diverse neoglycoconjugates by Click Chemistry’

**Table of Contents**

Statement of Originality i

Preface ii

List of Figures x

List of Schemes xii

List of Tables xv

List of Abbreviations xvii

Acknowledgements xxi

Synopsis xxii

**Chapter One: Click Chemistry in Carbohydrate based Drug Development and Glycobiology**

1.1 Introduction 1

1.1.1 Background and Significance 1

1.1.2 Glycomimetics as probes and drug leads 2

1.1.3 Click Chemistry: background and perspective 6

1.2 Small molecule probes and drug leads 9

1.2.1 General 9

1.2.2 Glycosidase and Glycosyltransferase inhibitors 10

1.2.3 Glycoamino acid and glycopeptide mimics 14

1.2.4 Bio-orthogonal chemical reporter strategies 20

1.3 Multivalent neoglycoconjugates 23

1.3.1 General 23

1.3.2 Glycopolymers and polysaccharide mimics 24
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3.3</td>
<td>Cyclodextrins and artificial receptors</td>
<td>30</td>
</tr>
<tr>
<td>1.3.4</td>
<td>Glycodendrimers, glycoclusters and lectin inhibitors</td>
<td>33</td>
</tr>
<tr>
<td>1.4</td>
<td>Carbohydrate microarrays and Self-Assembled Monolayers (SAMs)</td>
<td>40</td>
</tr>
<tr>
<td>1.4.1</td>
<td>General</td>
<td>40</td>
</tr>
<tr>
<td>1.4.2</td>
<td>Glycoarrays</td>
<td>41</td>
</tr>
<tr>
<td>1.4.3</td>
<td>Carbohydrate Self-assembled monolayers</td>
<td>43</td>
</tr>
<tr>
<td>1.5</td>
<td>Conclusions</td>
<td>45</td>
</tr>
<tr>
<td>1.6</td>
<td>References</td>
<td>46</td>
</tr>
</tbody>
</table>

**Chapter Two: Synthetic Utility of the Cu$^1$-catalysed cycloaddition reaction in Carbohydrate Chemistry**  

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Introduction</td>
<td>63</td>
</tr>
<tr>
<td>2.2</td>
<td>Results and Discussion</td>
<td>66</td>
</tr>
<tr>
<td>2.2.1</td>
<td>Rate of Triazole formation: influence of solvent and catalyst system</td>
<td>66</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Stability of glycosyl triazole linkage toward synthetic manipulation</td>
<td>70</td>
</tr>
<tr>
<td>2.2.3</td>
<td>Preparation of triazole-linked neoglycoconjugates</td>
<td>72</td>
</tr>
<tr>
<td>2.3</td>
<td>Conclusions</td>
<td>76</td>
</tr>
<tr>
<td>2.4</td>
<td>Experimental</td>
<td>77</td>
</tr>
<tr>
<td>2.4.1</td>
<td>General</td>
<td>77</td>
</tr>
<tr>
<td>2.4.2</td>
<td>Note on nomenclature</td>
<td>78</td>
</tr>
<tr>
<td>2.4.3</td>
<td>Preparation of compounds <strong>2.12-2.20</strong>: General procedure</td>
<td>79</td>
</tr>
<tr>
<td>2.4.4</td>
<td>Analytical data</td>
<td>79</td>
</tr>
<tr>
<td>2.5</td>
<td>References</td>
<td>99</td>
</tr>
</tbody>
</table>
Chapter Three: Inhibition of Carbonic Anhydrase with Glycoconjugate Benzene sulfonamides

3.1 Introduction

3.2 Results and Discussion
   3.2.1 Library design and synthesis
   3.2.2 In vitro inhibition of CA isozymes I, II, IX, XII and XIV.

3.3 Conclusions

3.4 Experimental
   3.4.1 General
   3.4.2 Synthesis of glycoconjugate benzene sulfonamides. General procedure 1.
   3.4.3 Deprotection of triazole sulfonamide glycoconjugates. General procedure 2.
   3.4.4 CA inhibition Assay
   3.4.5 Analytical data
      3.4.5.1 Benzene sulfonamide scaffolds
      3.4.5.2 Glycoconjugate benzene sulfonamides (library I)
      3.4.5.3 Glycoconjugate benzene sulfonamides (library II)
      3.4.5.4 Glycoconjugate benzene sulfonamides (library III)

3.5 References

Chapter Four: Parallel synthesis of N-alkylated azasugars using Click Chemistry

4.1 Introduction
   4.1.1 Background
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1.2</td>
<td>Glycosidases: Mechanism and structure</td>
<td>197</td>
</tr>
<tr>
<td>4.1.3</td>
<td>Azasugars: natural occurrence and biological activity</td>
<td>201</td>
</tr>
<tr>
<td>4.1.4</td>
<td>Therapeutic potential of azasugars as inhibitors of glycosidases</td>
<td>203</td>
</tr>
<tr>
<td>4.1.5</td>
<td>N-alkylated azasugars: biological activity and therapeutic applications</td>
<td>203</td>
</tr>
<tr>
<td>4.1.6</td>
<td>Seven-membered ring azasugars: a new class of glycosidase inhibitor</td>
<td>208</td>
</tr>
<tr>
<td>4.1.7</td>
<td>Synthesis of azasugar libraries using click chemistry</td>
<td>209</td>
</tr>
<tr>
<td>4.2</td>
<td>Results and discussion</td>
<td>211</td>
</tr>
<tr>
<td>4.2.1</td>
<td>Scaffold design and synthesis</td>
<td>211</td>
</tr>
<tr>
<td>4.2.2</td>
<td>Preparation of N-alkylated azasugar libraries</td>
<td>217</td>
</tr>
<tr>
<td>4.3</td>
<td>Conclusions</td>
<td>225</td>
</tr>
<tr>
<td>4.4</td>
<td>Experimental</td>
<td>227</td>
</tr>
<tr>
<td>4.4.1</td>
<td>General methods</td>
<td>227</td>
</tr>
<tr>
<td>4.4.2</td>
<td>Notes on nomenclature</td>
<td>227</td>
</tr>
<tr>
<td>4.4.3</td>
<td>Preparation of N-alkylated azasugar-triazoles: General procedure 1.</td>
<td>229</td>
</tr>
<tr>
<td>4.4.4</td>
<td>Deprotection of azasugar scaffolds, 4.28 and 4.29: General procedure 2.</td>
<td>229</td>
</tr>
<tr>
<td>4.4.5</td>
<td>Preparation of azasugar scaffolds, 4.30 and 4.32: General procedure 3.</td>
<td>230</td>
</tr>
<tr>
<td>4.4.6</td>
<td>Deprotection of azasugar library 4.32a-d and scaffolds 4.30 and 4.32</td>
<td>230</td>
</tr>
<tr>
<td>4.4.7</td>
<td>Analytical Data</td>
<td>230</td>
</tr>
<tr>
<td>4.4.7.1</td>
<td>N-propynyl azasugar scaffolds</td>
<td>230</td>
</tr>
<tr>
<td>4.4.7.2</td>
<td>Alkyl azide building block (c)</td>
<td>238</td>
</tr>
<tr>
<td>4.4.7.3</td>
<td>N-alkylated azasugar triazoles</td>
<td>240</td>
</tr>
<tr>
<td>4.5</td>
<td>References</td>
<td>255</td>
</tr>
</tbody>
</table>
Chapter Five: Concluding comments and future directions 265

5.1 General 265
5.2 Chapter Two 265
5.3 Chapter Three 268
5.4 Chapter Four 270
LIST OF FIGURES

Chapter 1:

Figure 1.1. 1-Glycosyl 4-phenyl triazoles 1.1 and 1.2 as a new class of aglycone-modified β-glycosidase inhibitor. 11

Figure 1.2. The C-4 modified 1,2,3-triazole analogue 1.5 of Relenza 1.3 generated by click chemistry of azide 1.4. 12

Figure 1.3. 1,4-Disubstituted 1,2,3-triazoles (B) can function as a potential mimics of glycopeptide bonds (A) due to similar geometric features. 16

Figure 1.4. Glycopeptide antibiotic analogues of tyrocidine (tyc), Tyc4PG-14 and Tyc4PG-15. 20

Chapter 2:

Figure 2.1 ORTEP-3 representation of model compound 2.2 69

Figure 2.2 Compound 2.20 76

Chapter 3:

Figure 3.1 Some clinically used carbonica anhydrase inhibitors 109

Figure 3.2 Benzene-sulfonamide scaffolds, methyl 4-sulfamoylbenzoate 1,2,3-triazole-4-benzene sulfonamide 3.16. 118

Figure 3.3 ORTEP-3 representation of N-(prop-2-ynyl)-4-sulfamoyl benzamide (3.11). 118
**Figure 3.4** Azido sugar building block panel: a-g (R = Ac), a’-g’ (R = H) h (R = Bz), h’ (R = H).

**Figure 3.5** Propargyl glycoside building blocks (i-m)

**Figure 3.6** ORTEP-3 representation of compound 3.14i.

**Chapter 4:**

**Figure 4.1** General mechanisms for the inverting (A) and retaining (B) glycosidases.

**Figure 4.2** Some naturally occurring azasugars.

**Figure 4.4** Synthetic N-Alkylated azasugars acting as potent glycosidase and glycosyltransferase inhibitors.

**Figure 4.5** Polyhydroxylated azepanes as a new class of glycosidase inhibitor.

**Figure 4.6** ORTEP -3 representation of 1,2:5,6-dianhydro-3,4-di-O-p-methoxybenzyl-L-iditol 4.27.

**Figure 4.7** Aliphatic and aryl azide building blocks for the construction of N-alkylated azasugar libraries
**LIST OF SCHEMES**

**Chapter 1:**

**Scheme 1.1** (A) The Cu$^1$-catalysed 1,3-dipolar cycloaddtion of an organic azide and terminal acetylene affords, exclusively the 1,4-disubstituted 1,2,3-triazole regioisomer. (B) The non-catalysed cycloaddition affords a regioisomeric mixture of 1,4- and 1,5-disubstituted-1,2,3-triazole regioisomers.

**Scheme 1.2** Proposed step-wise mechanism for the step-wise, Cu$^1$-catalysed 1,3-DCR of an azide and acetylene.

**Scheme 1.3** Hydrophobic donor-substrate analogue 1.6 identified by “in situ click chemistry” as a potent inhibitor $\alpha$-1,3-FucT VI ($K_i$ 62 nM).

**Scheme 1.4** Synthesis of glycosyl triazole-linked glycoamino acid and glycopeptide mimics.

**Scheme 1.5** Synthesis of glycopeptide mimics using a dual chemoselective ligation/click chemistry approach.

**Scheme 1.6** Metabolic engineering of human T-Lymphoma cell line Jurkat and labelling azido L-fucose derivatives with biotin reporter probe using click chemistry.

**Scheme 1.7** The bioconjugation of the fluorogenic naphthalene probe to a functionalised L-fucose derivative by click chemistry.

**Scheme 1.8** Click chemistry polymerisation of triazole functionalised, trehalose poly(glycoamidoamine) co-polymers as stabilised polyplex delivery agents.

**Scheme 1.9** Preparation of pendently attached glycopolymers through (A) the
simultaneous functionalisation of a water soluble terpolymer and (B) the
cascade functionalisation of an ester-functionalised polyacrylamide.

**Scheme 1.10** Iterative assembly by of a triazole linked α-1,6-oligomannose
analogue using click chemistry.

**Scheme 1.11.** Convergent synthesis of a manno-β-cyclodextrin using click
chemistry.

**Scheme 1.12** The synthesis of an unsymmetrical and bi-functionalised
dendrimer using click chemistry.

**Scheme 1.13.** The preparation of glycoclusters using click chemistry.

**Scheme 1.14.** The immobilisation of an azido ethyl galactoside to a non-
covalently attached polystyrene-supported acetylene linker using click
 chemistry.

**Scheme 1.15** Triazole linked glycoarrays formed by a Cu¹-catalysed 1,3-DCR
to a covalently supported propionamide linker.

**Scheme 1.16** Fabrication of carbohydrate SAM using click chemistry.

**Chapter 2:**

**Scheme 2.1** Cu¹-catalysed cycloaddition of an organic azide and terminal
acetylene affords a 1,4-disubstituted 1,2,3-triazole.

**Scheme 2.2** Synthetic manipulation of model triazole 2.2
Chapter 3:

**Scheme 3.1** Modular synthesis of glycoconjugate benzene sulfonamides by “click-tailing”: library format A (top) and library format B (bottom).

**Scheme 3.2** Preparation of glycoconjugate benzene sulfonamide library I.

**Scheme 3.3** Preparation of glycoconjugate benzene sulfonamide library II.

**Scheme 3.4** Preparation glycoconjugate benzene sulfonamide library III.

Chapter 4:

**Scheme 4.1** Preparation of N-alkylated azasugar libraries using click chemistry

**Scheme 4.2** Synthesis of 6 and 7-membered azasugar scaffolds 4.28–4.33.

**Scheme 4.3** Aminocyclisation of C₂-symmetric bis-epoxide 4.28 with propargyl amine to form the N-propynyl D-glucopiperidine 4.29 and N-propynyl L-ido azepane 4.30.

**Scheme 4.4** Parallel synthesis of N-methylene triazole azasugar libraries (4.30a–d, 4.31a’) and 4.3 from N-propynyl piperidine 4.28 and azepane 4.29 scaffolds.
LIST OF TABLES

Chapter 1:

Table 1.1 Clinically used carbohydrate based drugs and glycomimetic drugs 4

Chapter 2:

Table 2.1 Study of formation of glycosyl triazole 2.2 under variable solvent and catalyst conditions. 68

Table 2.2 Examples of 1,2,3-triazole glycoconjugates 73

Chapter 3:

Table 3.1 The relative catalytic activity, affinity for sulfonamides, tissue distributions and sub-cellular locations of presently known human CA isozymes. 107

Table 3.2 In vitro inhibition data for reference CA inhibitors 3.2–3.7, 3.9. 126

Table 3.3 Inhibition selectivity for the tumour-associated extracellular isozyme hCA IX for reference compounds 3.2-3.9. 127

Table 3.4 In vitro inhibition data for benzene sulfonamide scaffolds 3.11 and 3.12 and glycoconjugate benzene sulfonamides (library I) against CA isozymes hCA I, II, IX XII and XIV. 130

Table 3.5 In vitro inhibition data of parent scaffold 3.13 and glycoconjugate benzene sulfonamides (library II) of isozymes hCA I, II and IX. 136

Table 3.6 In vitro inhibition data for azidobenzene sulfonamide 3.14 and glycoconjugate sulfonamides (library III) of isozymes hCA I, II and IX. 139
Chapter 4:

Table 4.1 Therapeutic applications of \(N\)-alkylated azasugars. 205

Table 4.2 Examples for the nomenclature of \(N\)-alkylated azasugar triazoles. 227
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac</td>
<td>acetate</td>
</tr>
<tr>
<td>AcOH</td>
<td>acetic acid</td>
</tr>
<tr>
<td>AgOTf</td>
<td>silver trifluoromethanesulfonate</td>
</tr>
<tr>
<td>Ar</td>
<td>aryl</td>
</tr>
<tr>
<td>Ara</td>
<td>arabinose</td>
</tr>
<tr>
<td>Asn</td>
<td>asparagine</td>
</tr>
<tr>
<td>BF$_3$.Et$_2$O</td>
<td>boron trifluoride diethyl etherate</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butoxycarbonyl</td>
</tr>
<tr>
<td>br s</td>
<td>broad singlet</td>
</tr>
<tr>
<td>Bu</td>
<td>butyl</td>
</tr>
<tr>
<td>Bz</td>
<td>benzoyle</td>
</tr>
<tr>
<td>BzCl</td>
<td>benzoyle chloride</td>
</tr>
<tr>
<td>$^{13}$C{$^1$H} NMR</td>
<td>Proton decoupled Carbon-13 Nuclear Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>CAN</td>
<td>ceric ammonium nitrate</td>
</tr>
<tr>
<td>CDCl$_3$</td>
<td>deuterated chloroform</td>
</tr>
<tr>
<td>Cu$^{I}$</td>
<td>copper (I)</td>
</tr>
<tr>
<td>CuI</td>
<td>copper (I) iodide</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>dd</td>
<td>doublet of doublets</td>
</tr>
<tr>
<td>ddd</td>
<td>doublet of doublet of doublets</td>
</tr>
<tr>
<td>dt</td>
<td>doublet of triplets</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>1,2-DCE</td>
<td>1,2-dichloroethane</td>
</tr>
<tr>
<td>DDQ</td>
<td>2,3-dichloro-5,6-dicyano-p-benzoquinone</td>
</tr>
<tr>
<td>DIPEA</td>
<td>diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylamino pyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DMSO-(d_6)</td>
<td>deuterated dimethylsulfoxide</td>
</tr>
<tr>
<td>EDC</td>
<td>(N)-(3-dimethylaminopropyl)-(N')-ethylcarbodiimide</td>
</tr>
<tr>
<td>equiv.</td>
<td>equivalents</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic Reticulum</td>
</tr>
<tr>
<td>ESI-MS</td>
<td>Electrospray Ionisation Mass Spectrometry</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>FTICR-MS</td>
<td>Fourier Transform Ion Cyclotron Resonance Mass Spectrometry</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>Gal</td>
<td>galactose</td>
</tr>
<tr>
<td>gHSQC</td>
<td>gradient Heteronuclear Single Quantum Correlation Spectroscopy</td>
</tr>
<tr>
<td>gCOSY</td>
<td>gradient Correlation Spectroscopy</td>
</tr>
<tr>
<td>Glc</td>
<td>glucose</td>
</tr>
<tr>
<td>GlcNAc</td>
<td>2-acetamido-2-deoxy-D-glucose or (N)-acetyl D-glucosamine</td>
</tr>
<tr>
<td>(^1)H NMR</td>
<td>Proton Nuclear Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>hr</td>
<td>Hour</td>
</tr>
<tr>
<td>HRMS</td>
<td>High Resolution Mass Spectrometry</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>¹Pr</td>
<td>isopropyl</td>
</tr>
<tr>
<td>HBTU</td>
<td>O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate</td>
</tr>
<tr>
<td>HeLa</td>
<td>human cervical carcinoma cell-line</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid</td>
</tr>
<tr>
<td>HOBt</td>
<td>1-hydroxylbenzotriazole</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HPTLC</td>
<td>high performance thin layer chromatography</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>K</td>
<td>Kelvin</td>
</tr>
<tr>
<td>Kᵢ</td>
<td>Inhibition constant</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>M</td>
<td>molarity</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>Man</td>
<td>mannose</td>
</tr>
<tr>
<td>MHz</td>
<td>megahertz</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>mmol</td>
<td>millimole</td>
</tr>
<tr>
<td>mol</td>
<td>mole</td>
</tr>
<tr>
<td>MPM</td>
<td>p-methoxybenzyl</td>
</tr>
<tr>
<td>Ms</td>
<td>methanesulfonyl</td>
</tr>
<tr>
<td>MsCl</td>
<td>methanesulfonyl chloride</td>
</tr>
</tbody>
</table>
$N$  normality
{o/n} overnight
$p-$  para
Ph  phenyl
pDNA  plasmid DNA
Pro  proline
ppm  parts per million
q  quartet
RT  room temperature
Ser  serine
T  Tesla
t  triplet
TBAF  tetrabutylammonium fluoride
{tert-}  tertiary
TFA  trifluoroacetic acid
THF  tetrahydrofuran
Thr  threonine
TLC  thin layer chromatography
Tr  trityl
δ  chemical shift
TBDMS  {tert}-butyldimethylsilyl
TBDMS{Cl}  {tert}-butyldimethylsilyl chloride
UV  ultraviolet
{w/v}  weight per volume
µL  microlitre
°C  degrees Celsius
Acknowledgements

To complete this thesis, I had the help and support of many people. To my supervisors, Dr. Todd Houston, Dr. Sally-Ann Poulsen and Dr. Laurent Bornaghi, I thank you for your professional guidance and support throughout my PhD. Your patience and skill have helped shape me into a professional organic chemist and for that I am truly grateful. To my colleagues, who have shared much of my frustration and fortune and I thank them for their invaluable moral support and assistance. A special thanks to Dr. Sue Boyd for her assistance with NMR data acquisition and processing. Also, I’d like to express gratitude to Prof. Peter Healy and Mr. Alan White for their professionalism and enthusiasm with X-ray crystallographic data collection and interpretation. I’d also like to thank Alan for teaching me to play squash and also driving me to hospital after breaking my nose as a result of one of our many testosterone-fuelled squash games.

For financial support, I acknowledge the Griffith University Post Graduate Award (GUPRA) and the Eskitis Institute for cell and molecular therapies.

On a personal note, the completion of this thesis would not be possible without the support, love and encouragement of my family. There are really no words that can express my gratitude. Finally, to my wife and love of my life, Siew Huay, for always being there for me and sharing the experience. Her enduring patience, love and tenderness, especially during tumultuous times were a solid foundation, without which, this thesis would not be possible.
Synopsis

Within a short timeframe, the CuI-catalysed 1,3-dipolar cycloaddition (1,3-DCR) of an organic azide to a terminal acetylene to form a 1,4-disubstituted-1,2,3-triazole, has emerged as a powerful synthetic transformation in combinatorial chemistry, organic synthesis and bioconjugation research. This synthetic methodology, now known as click chemistry, has had an appreciable impact in the drug discovery and biotechnology sectors and has shown broad scope and compatibility with small molecule and polymeric substrates. The application of this powerful synthetic transformation, specifically in carbohydrate based drug discovery and glycobiology is a recent and emerging trend.

Chapter one of this thesis is a review of the current literature concerning the use of click chemistry in carbohydrate based drug discovery and glycobiology. Several examples have appeared within the literature highlighting the potential of click chemistry for rapidly generating structurally diverse neoglycoconjugates, ranging from small molecule drug leads to multivalent constructs, as well as a bioconjugation strategy for labelling cell-surface glycoconjugates. The review aims to be exhaustive in its coverage, with emphasis on future perspective. This thesis presents the investigation of click chemistry as a synthetic tool in carbohydrate chemistry, and its application for generating novel carbohydrate based enzyme inhibitor libraries for lead discovery and optimisation purposes.

Chapter two describes the utility of click chemistry and the glycosyl triazole moiety in synthetic carbohydrate chemistry. The reaction is well suited to the synthesis of mimetics of complex oligosaccharides and glycoconjugates, owing to the mild
ambient nature and remarkable regio- and stereo-selectivity. The transformation was therefore interrogated under conditions typically encountered in carbohydrate chemistry, including glycosylation reactions and protective group manipulations. The study represents the first exhaustive investigation into the stability of the triazole moiety under these conditions as well as the synthetic utility of the Cu$^1$-catalysed 1,3-DCR as a potential orthogonal transformation in carbohydrate chemistry and an adjunct to existing methods.

The first aspect of the study aimed to examine the stability of the glycosyl triazole moiety under conditions employed in protective group chemistry and the compatibility of the transformation with pre-installed functional groups. Using click chemistry, the triazole moiety could be introduced onto the carbohydrate scaffold in the presence of a wide range of protective functional groups. In addition, the 1,2,3-triazole moiety was indeed shown to be a robust entity that is compatible with essential protecting group manipulations and glycosylation chemistry - an important outcome with respect to its potential utility as an additional tool for the synthesis of oligosaccharide/glycoconjugate mimetics, which are often heavily reliant on orthogonal reaction sequences.

Next, the utility of the reaction with respect to solvent and catalyst conditions was examined. The reaction was performed in different organic and aqueous solvents in the presence of two different Cu$^1$-catalyst systems. It was shown that the reaction is reasonably insensitive to the nature of the solvent or aqueous co-solvent and the catalyst system. Reaction times and yields displayed little variation with respect to the solvent and catalyst system. In all cases, the 1,4-disubstituted 1,2,3-glycosyl
triazole model compound was isolated in high yields and required minimal purification. The work also amply demonstrated, in a proof-of-concept manner, the powerful scope of the reaction for preparing structurally diverse neoglycoconjugates in high yield and purity. Several artificial glycomimetics were prepared using a suite of glycosyl azides through the facile 1,3-DCR to a series of acetylenes.

Chapter three presents an extensive study into the preparation and biological activity of glycoconjugate benzene sulfonamides as a novel class of carbonic anhydrase (CA) inhibitor. The conjugation of carbohydrate “tails” to a benzene sulfonamide pharmacophore provides access to CA inhibitors which are neutral, water-soluble and features high chiral density and polyfunctionality that may be exploited for tissue delivery applications and to survey active site architectures in order to impart isozyme selectivity. Glycoconjugate benzene sulfonamides could also display compromised plasma membrane permeability allowing for the selective targeting of tumour associated isozymes with extracellular catalytic domains. Glycoconjugate benzene sulfonamides have received little attention as CA inhibitors, and this work represents the first comprehensive study in the area.

By utilising a novel “click-tailing” strategy developed in our laboratory, a panel of structurally diverse carbohydrate “tails” were appended to the primary arylsulfonamide (ArSO₂NH₂) pharmacophore. A panel of azido sugars and propargyl glycosides were reacted with acetylene- and azide-functionalised benzene sulfonamide scaffolds, respectively, and subsequently evaluated for their inhibition of human carbonic anhydrase (hCA) isozymes hCA I, II, IX, XII and XIV in vitro. In this manner, a total of 50 glycoconjugate benzene sulfonamides belonging to three
libraries were prepared and assessed for their inhibition of human cystolic isozymes hCA I, II and transmembrane isozymes hCA IX, XII and XIV. Selective inhibition among CA isozymes is challenging owing to conservation of active site topology within this enzyme family, yet the design of selective CA inhibitors is necessary for the development of efficacious and safe CA-based therapeutics which are void of side effects arising from systemic CA inhibition. Many of the glycoconjugate benzene sulfonamides exhibited a non-clustered in vitro inhibition profile, demonstrating that the carbohydrate tail was a powerful structural element able to distinguish isozyme selectivity. A significant outcome of this study was the discovery of several potent and selective CA inhibitors of the tumour-associated transmembrane isozyme, hCA IX, and the physiologically dominant cytosolic isozyme, hCA II.

Chapter four of this thesis explores the synthetic utility of click chemistry for the solution-phase synthesis of N-alkylated azasugar libraries. To date, click chemistry has seen limited application for the synthesis and screening of natural product-based libraries. To the best of my understanding, this work represents the first example of the use of click chemistry for the generation of azasugars containing structurally diverse N-alkyl substituents as potential glycosidase and glycosyltransferase inhibitors. By employing the click chemistry methodology, various synthetically accessible aliphatic and aromatic azides were conjugated to the acetylene-functionalised 6- and 7-membered ring N-propynyl azasugar scaffolds using click chemistry, thus providing expedient access to N-methylene triazole-substituted azasugars in a single, high yielding step. The work demonstrates the applicability of the reaction for generating not only the structural diversity deemed necessary for
distinguishing inhibitory potency and selectivity, but also a powerful means of tuning the physicochemical properties of the azasugar for *in vivo* targeting and lead optimisation purposes.
Click Chemistry in Carbohydrate-Based Drug Development & Glycobiology

1.1 Introduction

1.1.1 Background and significance

Protein and lipid glycosylation is an omnipresent and life-governing process. Oligosaccharides and polysaccharides, either free or anchored to proteins and lipids to form glycoconjugates, exert a multitude of biological effects ranging from nascent protein folding and stabilisation, energy storage, structural support and protection, and as molecular recognition motifs for cell-cell communication events underlying physiological processes including cellular differentiation and development, hormone trafficking and immune surveillance.\(^1\) Overwhelming evidence is now available to implicate both glycan binding and defective metabolic pathways in pathological processes including inflammation and microbial virulence, neurodegenerative conditions and tumour metastasis.\(^2\) The clinical intervention of a defective metabolic pathway, the inhibition of a glycan recognition or pathogen binding event and the elucidation of aberrant glycosylation patterns expressed on certain cancer cell surfaces, are each primary avenues for rational carbohydrate-based drug design and vaccine development.\(^3\)

The acquisition of a well defined, single glycoform is a prerequisite for correlating structure and function and ultimately developing carbohydrate-based therapeutics. Glycan biosynthesis occurs within the secretory pathway of the ER-Golgi complex and is not template driven nor under direct transcriptional control.\(^1a\) Naturally
occurring glycoproteins are therefore expressed as collections of glycoforms which contain the same underlying peptide sequence but display variable patterns of glycosylation. Due to this considerable microheterogeneity, the isolation of a single glycoform for structure-activity purposes from a biological extract and/or from genetic engineering methods alone is often exceedingly difficult. Whilst genetic engineering and “knock-out/knock-in” methods have contributed enormously to understanding the implications of protein glycosylation, arguably the most reliable means of procuring homogeneous glycoforms is via their de novo chemical synthesis.

Chemical methods have emerged as powerful tools for elucidating cellular mechanisms of health and pathology associated with glycosylation. Improvements in our understanding of glycan metabolism and structure-function relationships using molecular tools allow for the development novel drug leads for the treatment of carbohydrate-related disease processes. Two possible pathways can be envisaged, either through interrogation of cell surface glycoprotein/lipid binding events with artificial oligosaccharides, glycoconjugates and their analogues; or the perturbation of glycan metabolic pathways with small molecule enzyme inhibitors, leading to downstream changes in glycosylation patterns.

1.1.2 Glycomimetics as probes and drug leads

Although the knowledge base concerning the structure and function of glycans has increased dramatically in recent years, our ability to routinely apply this knowledge in a drug development context is still far from complete. The sheer numbers of potential carbohydrate-related drug targets and the inherent difficulties associated
with the synthesis of natural glycans have fuelled efforts to prepare carbohydrate mimics from natural or synthetic origins as novel drug leads or as biochemical tools.\textsuperscript{7} Natural and synthetic carbohydrates are often considered “privileged” combinatorial scaffolds because of the unrivalled structural diversity arising from a high chiral density, variable branching patterns and polyfunctionality.\textsuperscript{8} The synthetic modification and inherent reactivity of these powerful building blocks can in turn lead to a tremendous explosion in structural and stereochemical diversity.

Many of the carbohydrate-based drugs currently in clinical use are indeed mimics of endogenous carbohydrates and act as inhibitors of carbohydrate-protein binding events or modulators of glycan metabolism (Table 1.1).\textsuperscript{2,4,7,9} Carbohydrates linked to many natural products are often a prerequisite for biological activity and can thus heavily influence the pharmacokinetics, drug targeting and mechanism of action. Indeed, the literature is replete with examples of carbohydrate-containing natural products possessing anti-microbial and anti-cancer properties, such as the aminoglycosides, saponins and anthracyclines.\textsuperscript{10} Despite the plethora of disease states that involve carbohydrate-recognition phenomena, comparatively few carbohydrate-based drugs are in clinical use. The failure of many carbohydrate-based therapeutics during the 1980’s and 1990’s can however, be attributed to a poor understanding of their mechanism and pharmacokinetic profiles, rather than inherent flaws in carbohydrate-based drugs.\textsuperscript{11} When a detailed knowledge is applied in a drug design setting, excellent results have been achieved.\textsuperscript{12} Heparin, for example, a polysaccharide displaying potent anti-coagulant properties is amongst the worlds most widely used drug.\textsuperscript{3b,13} The aminoglycoside and glycopeptide antibiotics remain the frontline defence against a wide range of drug-resistant bacterial infections.\textsuperscript{3c}
Chapter One

Owing to their unique physicochemical and biochemical properties, glycoconjugates have also been employed as vehicles and prodrugs for cell-selective drug targeting.\textsuperscript{9,14}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Target</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acarbose, Voglibiose, Miglitol (e.g. Glyset\textsuperscript{®})</td>
<td>Intestinal $\alpha$-glucoamylase, $\alpha$-Glucosidase</td>
<td>Non-insulin dependent Diabetes mellitus (NIDDM)</td>
</tr>
<tr>
<td>N\textsuperscript{-}butyl deoxynojirimycin (Zavesca\textsuperscript{®})</td>
<td>Glucosylceramide synthase</td>
<td>Lysosomal storage diseases (Type 1 Gauchers disease)</td>
</tr>
<tr>
<td>Heparin analogues (e.g. Lovenox\textsuperscript{®})</td>
<td>Antithrombin III (factor Xa)</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>2-Deoxy-2,3-deanhydro-$N$-acetylanuramic acid derivatives (e.g. Tamiflu\textsuperscript{®})</td>
<td>Influenza sialidase</td>
<td>Anti-influenza</td>
</tr>
<tr>
<td>Vancomycin (Vancocyn\textsuperscript{®}, Lyphocin\textsuperscript{®})</td>
<td>Bacterial transpeptidase</td>
<td>Methicillin-resistant \textit{Staphylococcus aureus} (MRSA)</td>
</tr>
<tr>
<td>Mimetics or derivatives of Sialylated or sulfated Le\textsuperscript{a/x} epitopes.\textsuperscript{2b}</td>
<td>Selectins</td>
<td>Inflammation reactions and over-recruitment of cytokines etc.</td>
</tr>
<tr>
<td>Topirimate (Topamax\textsuperscript{®})</td>
<td>Brain carbonic anhydrase (CA) isozymes</td>
<td>Anti-convulsant, migraine</td>
</tr>
</tbody>
</table>
* Postulated anti-convulsant mechanism is inhibition of brain carbonic anhydrase (CA) isozymes.$^{15}$

Despite the widespread physiological and pathophysiological effects of carbohydrates, there are relatively few clinically used carbohydrate-based drugs. Carbohydrates possess less than ideal therapeutic profiles concerning solubility, \textit{in vivo} stability, target binding affinity and cell permeability (although notable exceptions do exist)$^{9}$ and as such have received little attention compared to other small-molecule drug classes. The synthetic modification of natural polyfunctional carbohydrate scaffolds can however lead to analogues with improved bioactivity and pharmacokinetic profiles. Non-natural functionalities can be introduced into the carbohydrate ring to exploit a specific structural element within the target receptor that is otherwise neglected in the native ligand-receptor binding event.$^{7,8}$ The substitution or elimination of ring hydroxyl groups often leads to improved solubility and \textit{in vivo} clearance. In addition, the bioavailability of carbohydrate-based therapeutics can be improved by the substitution of the natural, labile glycosidic linkage with a robust, artificial linkage that is impervious to digestive degradation and enzymatic catabolism. Importantly, the installation of artificial linkages can potentially simplify complex multi-step syntheses by exploiting chemistry which is facile, high yielding, orthogonal and stereoselective, whilst retaining as much as possible the geometry and electronics of the native linkage.$^{16}$

\section*{1.1.3 Click Chemistry: background and perspective}

The 1,3-dipolar cycloaddition reaction (1,3-DCR) of a 1,3-dipole to a dipolarophile (i.e. an acetylene or alkene) for the synthesis of five-membered heterocycles are ubiquitous transformations in synthetic organic chemistry.$^{17}$ Recently, the
Sharpless\textsuperscript{18a} and Meldal\textsuperscript{18b} groups have reported the dramatic rate enhancement (up to $10^7$ times) and improved regioselectivity of the Huisgen 1,3-DCRG of an organic azide to a terminal acetylene to afford, regiospecifically, the 1,4-disubstituted, 1,2,3-triazole in the presence of a Cu\textsuperscript{I} catalyst (Scheme 1.1). The Cu\textsuperscript{I}-catalysed 1,3-DCR has successfully fulfilled the requirements of “click chemistry” as prescribed by Sharpless and within the past few years has become a premier component of the click chemistry paradigm.\textsuperscript{19}

![Scheme 1.1](image)

The classical non-catalysed process proceeds by a concerted mechanism under thermal conditions to afford a mixture 1,4- and 1,5-disubstituted 1,2,3-triazole regioisomers. The relative proportion of regioisomers and the rate can be predicted form electronic and steric effects.\textsuperscript{20} Increased 1,4-regioselectivity is increasingly observed with electron-deficient acetylene dipolarophiles (increased dipolarophilicity) and electron rich azides. The Cu\textsuperscript{I}-catalysed (“click”) process has been postulated to occur by a stepwise mechanism on the basis of recent thermal and kinetic studies.\textsuperscript{21} The substantial rate increase of the Cu\textsuperscript{I}-catalysed process in aqueous solvents is rationalised in terms of a stepwise process which lowers the
activation barrier relative to that of the non-catalysed process by as much as 11.8 kcal mol\(^{-1}\).\(^{18a,21}\) The proposed catalytic cycle involves several postulated and transient Cu\(^{1}\)-acetylide complexes, starting with the complexation of the alkyne to the Cu\(^{1}\) metal centre to form a Cu\(^{1}\)-alkyne π-complex (A) (Scheme 1.2).\(^{21}\) The enhanced reaction rate in water relative to organic solvents can be rationalised in terms of the endothermic ligand disassociation in organic media, for example, acetonitrile (endothermic by 0.6 kcal mol\(^{-1}\)) relative to water (exothermic by 11.8 kcal mol\(^{-1}\)).\(^{21}\) The formation of the Cu\(^{1}\)-acetylide complexes is also water-assisted, since water lowers the pKa of the acetylene C-H by 9.8 pKa units.\(^{21}\) Formation of the Cu\(^{1}\)-acetylide species allows for subsequent ligand displacement with azide and results in a dimeric copper species (B). Complexation with azide activates it toward nucleophilic attack at the N-3 with the acetylide C-4 (The numbering is given according to triazole nomenclature). The resulting metallocycle (C) undergoes facile ring contraction via a transannular association of the N-1 lone pair with the C5-Cu π* orbital to give the copper-triazole complex (D). Protonation of the triazole species, possibly with water and disassociation of the labile copper complex affords the 1,4-disubstituted 1,2,3-triazole (E), thus regenerating the catalyst and ending the cycle.
Within a short time-frame, click chemistry has proven to be of remarkable utility and broad scope, not only in organic synthesis, but in chemical biology and drug discovery. Azides and acetylenes are by definition kinetically stable entities possessing high built-in energy and are tolerant to a wide range of synthetic conditions. Click chemistry is highly modular and simplifies difficult syntheses, thus enabling a more cost-effective and efficient surveillance of structural space. The biocompatibility of the reaction, tolerance towards a broad range of pH and relative inertness of acetylenes and azides within highly functionalised biological milieus has allowed click chemistry to become a viable bioconjugation strategy for labelling bio-
molecules and for *in situ* lead discovery applications.\textsuperscript{23} The 1,2,3-triazole moiety is a potential pharmacophore owing to its moderate dipole character and rigidity and can therefore be readily incorporated into a design strategy, rather than used as a passive linker between two respective fragments of structural space.\textsuperscript{24} Indeed, several examples exist within the literature which describe the biological activity of 1,2,3-triazoles, including anti-HIV-1,\textsuperscript{25} antibacterial,\textsuperscript{26} selective β3 adrenergic receptor inhibition,\textsuperscript{27} anti-platelet activity,\textsuperscript{28} and anti-inflammatory\textsuperscript{29} agents to name a few.

More recently, click chemistry has emerged as a powerful conjugation strategy for the preparation of structurally diverse neoglycoconjugates of biomedical interest. The following review aims to highlight recent developments appearing within the literature concerning the use of click chemistry in carbohydrate-based drug discovery and glycobiology. Topics range from small-molecule probes and drug leads, multivalent neoglycoconjugates acting as lectin inhibitors and potential vaccines, and as a bioconjugation strategy for labelling of engineered cell surface glycans. The review aims to be comprehensive with commentary on future perspective.

1.2 Small molecule probes and drug leads

1.2.1 General

Heterocyclic glycoconjugates are widespread in nature. Obvious examples are the nucleosides and nucleotides, the primary building blocks of the nucleic acids. The design of inhibitors of DNA replication based on the mimicry of nucleoside scaffolds has, for decades, been the cornerstone of rational anti-infective and anti-cancer drug research.\textsuperscript{30} 1,3-DCRs on carbohydrates, in this case, have proven to be of tremendous utility in such research programmes and have been the subject of
previous reviews. Certain heterocyclic carbohydrates from natural and non-natural origin containing exocyclic nitrogens act as strong inhibitors of certain sugar processing enzymes and are thus valuable antibiotic, anti-fungal and anti-parasitic lead compounds. Examples include Nagstatin, a natural product consisting of a fused tetrahydroimidazo[1,2-α]pyridine ring system and a potent inhibitor of β-hexosaminidases, the Nikkomycin nucleoside-peptide antibiotics isolated from *Streptomyces sp.* which are potent chitin synthase inhibitors. The trehazolins and allosamidines contain exocyclic nitrogen heterocycles and act as potent trehalase and chitinase inhibitors, respectively. Intramolecular 1,3-DCR’s between azides and alkynes have also been utilised to generate novel fused bicyclic polyhydroxylated alkaloids as potential analogues of bioactive analogues of the indole and indolizidine-type.

### 1.2.2 Glycosidase and Glycosyltransferase inhibitors

Heterocyclic glycoconjugates are potential mechanistic probes or drug leads owing to their hydrolytic stability towards glycosidases and glycosyltransferases. The strong dipole character and rigidity of the triazole ring can potentially mimic the charge build-up and flattening of the carbohydrate ring which is normally associated with the oxocarbenium-like ion transition state. In addition, the modular synthesis of carbohydrate triazole analogues by click chemistry lends itself particularly well to the parallel synthesis of small molecule carbohydrate-based libraries as potential drug leads.

Recently, Basu and Rossi have demonstrated the β-glycosidase inhibition of 1-β-D-glucosyl-4-phenyl triazole 1.1 and 1-β-D-galactosyl-4-phenyl triazole 1.2 as a new
Chapter One

class of aglycone-modified β-glycosidase inhibitor (Figure 1.1). Compounds were screened at 0.24 mM against sweet almond β-glucosidase, Escherichia coli β-galactosidase (ECG), and bovine liver β-galactosidase in a preliminary fashion using known glycosidase inhibitors deoxynojirimycin (DNJ) and deoxygalactonojirimycin (DGJ) as a comparative reference compounds. The phenyl triazoles were shown to be weak inhibitors of glycosidases but also importantly not substrates for enzyme hydrolysis. In a similar fashion, Périon et al. have employed a mild uncatalysed 1,3-DCR to generate a series of saccharidyl-1,4,5-trisubstituted 1,2,3-triazoles as heterocyclic analogues of the well known glucoamylase inhibitor and anti-diabetic drug, acarbose.

![Figure 1.1](image1)

**Figure 1.1**

Influenza virus A and B Sialidase (EC 3.2.1.18) is strongly inhibited by 2,3-dianhydro-\(N\)-acetylneuraminic derivatives such as the clinically used drug, Relenza 1.3 (Zanamivir®). Motivated by the emergence of avian flu strains (eg H5N1) and the need for new frontline anti-influenza agents, Jiang and colleagues reported the biological activity of a library of triazole analogues of Relenza as avian influenza inhibitors. Systemic modification of C-4 ring substituents with amino or guanidino has led to potent and selective inhibition of viral neuraminidase. Thus, the C-4 azido neuraminic acid derivative 1.4, which has previously served as a synthetic precursor to 1.3, was used as the scaffold for library design (Figure 1.2). A library of 16 triazole analogues modified in the C-4 position were prepared by click chemistry
and were assayed using a neutral red (NR) assay with the data reported as percent protection of MCDK infected cells at 50 µM inhibitor concentration. The protective rate of the triazole analogue 1.5 against the H5N1 strain was found to be comparable to that of Zanamivir (>64% cf. 86%).

![Chemical Structures](image)

**Figure 1.2**

There are at least 5 distinct human α-1,3-fucosyltransferases (α-1,3-FucT III to VIII, EC 2.4.1.152) known which catalyse the transfer of L-fucose from the β-configured GDP-fucose donor to a 3-OH group of an N-acetyllactosamine acceptor to give the Lewis X trisaccharide antigen (LeX). Alternatively, some fucosyl transferases (eg αFucT V) utilise the 3′-O-sialylated lactosamine acceptor to give the sialyl Lewis X antigen (sialyl LeX). Both antigenic oligosaccharides are key components implicated in oncogenesis and tumour metastasis, as well as inflammation. Inhibitors of α-1,3-FucT III are thus important lead compounds in the development of anti-inflammatory and anti-tumour agents.

Owing to the complex four-partner enzymatic transition state and the low catalytic proficiency of α-1,3-FucT, the successful design of potent inhibitors has been limited. Strongest inhibition lies in the design of substrate-donor analogues since most of the binding energy lies in the retention of the GDP moiety, yet the strongest inhibitors to date have mainly been in the µM range and too weak to constitute...
Chapter One

effective leads. Recently, Wong et al.\textsuperscript{45} identified a nanomolar inhibitor of $\alpha$-1,3-FucT from a library of hydrophobic aglycone donor-substrate analogues using a novel \textit{in situ} “click chemistry” screening approach. The library of 85 donor substrate analogues were prepared by click chemistry in microtitre plate format and screened \textit{in situ} within the same well in which they were synthesised (Scheme 1.3). The GPD donor analogue 1.6 was identified as the most potent FucT VI inhibitor known to date ($K_i$ 62 nM).

\begin{center}
\begin{tikzpicture}
\node[anchor=south west,inner sep=0] at (0,0) {\includegraphics[width=\textwidth]{image.png}};
\end{tikzpicture}
\end{center}

\textbf{1.6}

Scheme 1.3\textsuperscript{45}

\textit{Leishmania} sp. is the causative agent of Leishmaniasis infection which currently affects 12 million people worldwide.\textsuperscript{46} Leishmaniasis infection is implicated in a host of disease states ranging from subcutaneous lesions to lethal visceral infections. Current therapy of leishmaniasis is far from adequate owing to the emergence of drug resistant strains, as well as the low efficacy, severe side effects, and high toxicity and cost of currently used drugs. The primary carbohydrate reserve of
Chapter One

*Leishmania sp.* is a β-1,2 linked mannan (with an average degree of polymerisation of 4–40) which is biosynthesised by a specific β-1,2 mannosyltransferase. It has been shown from previous genetic studies that the β-1,2 linked mannan oligosaccharides are important for parasite survival during intracellular infectivity stages within host macrophages. A detailed investigation into the substrate specificity and active site structure of the β-1,2-mannnosyl transferase is necessary for the development of potent inhibitors of this enzyme and novel Leishmaniasis therapies. Williams and co-workers have recently reported the synthesis of a library of α-mannosides using click chemistry, the library members assessed as potential substrates for β-1,2-mannnosyl transferase (*L. Mexicana*). Thus, two libraries of 14 compounds were prepared by conjugating seven aliphatic and aromatic azides and acetylenes to the structurally-conserved α-propynyl mannoside and α-methyl 6-azido-6-deoxy mannoside scaffolds, respectively. Based on HPTLC and fluorography analysis of digest fractions, it was concluded that library members derived from the α-propynyl mannoside scaffold were tolerated as substrates, but not the library derived from the 6-azido mannoside. Also, the enzyme was found to tolerate a broad range of aromatic substituents, an important step in the development of β-1,2-mannnosyl transferase inhibitors and new Leishmania therapies.

*1.2.3 Glycoamino acid and glycopeptide mimics*

Glycoprotein-based therapeutics constitute more than one-third of approved biopharmaceuticals. Glycoproteins and peptides which contain glycans commonly *N*-linked to an asparagnine (belonging to the Asn-X-Ser/Thr consensus sequence) or *O*-linked to Ser or Thr residues play pivotal roles in biology. The branched glycan component (as an poly-, oligo-, or monosaccharide) are not only important for
protein folding, stability and specificity, but can determine the primary function of the mature glycoprotein as a modulator of intercellular communication. The \textit{de novo} synthesis is a viable method to accessing native glycoforms and artificial glycoproteins (neoglycoproteins) of biomedical interest.\textsuperscript{4,49,50} Furthermore, the conjugation of a naturally occurring antigenic glycan to a protein or peptide carrier often results in the enhancement of an immune response to a carbohydrate antigen or the \textit{in vivo} stability and biological activity of a peptide drug.\textsuperscript{50}

The theme strongly reverberated in the mimicry of endogenous glycopeptides is the substitution of labile \textit{N}- and \textit{O}-glycosidic bonds with robust linkages that also mimic the geometry and electronics of the native glycopeptide bond.\textsuperscript{51} Considerable interest has been directed toward facile syntheses of non-hydrolysable mimetics in aqueous solution through chemoselective ligation of preformed oligosaccharide and peptide fragments containing mutually reactive functional groups.\textsuperscript{16} The click chemistry between preformed carbohydrate and peptide fragments bearing azide and acetylene functionalities has numerous advantages over traditional glycosylation and peptide bond formation chemistry and has emerged as a promising chemoselective ligation strategy. It allows for the mild, chemoselective and stereospecific conjugation of preformed building blocks/fragments under aqueous conditions and is insensitive to global protecting group effects or synthetic conditions. Azide and acetylenes are also powerful functional groups since they are relatively inert to a wide range of synthetic transformations occurring on the peptide or oligosaccharide component (e.g. glycosylation, protecting group chemistry, peptide coupling etc). Click chemistry can therefore greatly simplify synthesis and can be introduced at virtually any stage of the glycoprotein/peptide synthesis (convergent or linear strategies). From a mimetic
point-of-view, 1,2,3-triazole linked glycoamino acid mimics share similar structural and electronic features to that of the native amide counterparts and are relatively impervious to enzyme or digestive hydrolysis.\textsuperscript{22a} 1,2,3-Triazoles have a larger dipole moment compared with amide bonds (\(\sim 5.0\) D by \textit{ab initio} calculation cf. \(3.8\) D of \(N\)-methylacetamide) and can potentially enhance amide mimicry due to their weak hydrogen bond acceptor properties.\textsuperscript{22a} The dipole character can also be fine tuned depending on the electronic effects of the neighbouring groups. For example, the C-5 bond can function as a potential hydrogen bond donor, much like an amide proton given suitable polarisation exists between the N-2 and N-3 bonds of the triazole ring. Distances between the carbons in the 1 and 4 position of the triazole ring are similar compared to the distance between the \(\alpha\) carbon (\(R\)) and the carbon attached to the amide (\(R', 5.0\) A cf. 3.8 A) (Figure 1.4).\textsuperscript{22a}

Since the pioneering work of Meldal and colleagues\textsuperscript{18b} who described the first use of click chemistry to generate glycopeptides on solid support, there has been a flurry of activity within the literature concerning the development of glycoamino acid and glycopeptide mimics using click chemistry. In a comprehensive study, Rutjes et al.\textsuperscript{52} have recently reported the expedient synthesis of \(N\)-glycosyl and \(C\)-glycosyl triazoles as glycoamino acid mimics from glycosyl azide and ethynyl glycosides, respectively (Scheme 1.4). Click chemistry between acetylene or azide-functionalised amino...
acids and the respective carbohydrate building blocks in solution resulted in the formation of triazole-glycoamino acids under mild conditions in moderate to good yields. The bifunctional glycoamino acids could then potentially undergo subsequent peptide couplings at either the carboxy or amino terminus. In a preliminary study, the triazole moiety was also shown to be stable to mineral acid and base treatment. Likewise, ethynyl glycosides have also been exploited by Massi and co-workers for the efficient generation of C-glycosyl isoxazole and C-glycosyl triazaole α-amino acids. In the latter case, the Cu¹-catalysed cycloaddition of the various ethynyl glycosides with a suite of azide-functionalised amino acids proceeded smoothly at room temperature in toluene in good yields.

In a simple and elegant study, Macmillan and Blanc have demonstrated the compatibility of click chemistry to a native chemical ligation strategy employing a chemoselective nucleophilic displacement of alkyl bromides with side chain cysteine thiols. The glycoamino acid mimics were based on N-acetyl glucosamine, N-acetyl lactosamine and chitobiose, all major constituents in endogenous N-linked glycoproteins. An important finding revealed the unimportance of the order in which the chemoselective ligation and click chemistry events took place in constructing the triazole modified neoglycopeptides. In this respect, the click reaction itself could
potentially function as the chemoselective ligation event, as demonstrated in the model reaction of benzyl mercaptan with 2-bromoacetyl propynyl amide or with the preformed glycosyl triazole glycoamino acid mimic containing the bromoacetamide moiety (Scheme 1.5).

**Scheme 1.5**

Click chemistry has recently been used as a glycosylation tool to modify the physicochemical properties of an azabicycloalkane amino acid peptidomimetic of a homoSer-Pro dipeptide. The click reaction between acetylated and free propynyl β-D-glucopyranoside with various azide-functionalised peptidomimetic scaffolds afforded the triazole conjugate in good yields (72-84%) under aqueous conditions. In a similar fashion, 1,2,3-triazole analogues of pyrazinone-based peptidomimetics have been generated by click chemistry of various glycosyl azides with substituted ethynyl pyrazinone scaffolds.

Many carbohydrate macrocycles of natural product origin, including glycosylated cyclic peptides, possess remarkable biological activities and diverse structural characteristics. Indeed, several are used clinically as antibiotics and anti-fungal
The macrocyclisation and glycosylation are two key structural modifications which occur late in the biosynthesis and confer much of the unique physicochemical properties and biological activity \textit{per se}. Westermann and colleagues\textsuperscript{57} have recently utilised click chemistry in combination with ring closing metathesis to prepare a library of macrocyclic glycolipid mimics. The synthetic strategy involved a consecutive click ligation and ring closing metathesis using dual functionalised carbohydrate building blocks incorporating azide and acetylene groups.

Motivated by the emergence of vancomycin-resistant strains of \textit{Enterococcus faecium} (VRE), Thorson and co-workers\textsuperscript{58} have reported the synthesis and biological activity of a library of 1,2,3-triazole-modified vancomycin analogues. From a library of 15 compounds, a carboxylic acid derivative was shown to be twice as effective as vancomycin against antibiotic resistant strains \textit{E. faecium} and \textit{S. aureus}.

Walsh and Lin\textsuperscript{59} have also exploited click chemistry as part of a chemoenzymatic approach to prepare carbohydrate-modified cyclic peptide tyrocidine (tyc) antibiotics with lowered toxicity. A library of 247 glycopeptides was prepared by employing a thioesterase mediated macrocyclisation followed by Cu\textsuperscript{1}-catalysed triazole formation in 96-well plate format. Following anti-bacterial and haemolytic assays, two glycopeptides, \textbf{Tye4PG-14} and \textbf{Tye4PG-15}, were identified as retaining the anti-bacterial potency of the native peptide, whilst increasing its therapeutic index by a factor of 6. (Figure 1.4).
1.2.4 Bioorthogonal chemical reporter strategies

The development of bioorthogonal chemical methods for profiling disease-related glycoproteins expressed on living cell surfaces is an important and recent development in glycomics research and carbohydrate-based drug development. In particular, the biosynthetic incorporation of unnatural saccharides into glycan epitopes expressed on living cell surfaces is a recent strategy described by the Bertozzi group and has provided considerable insight into glycosylation structure and function in vivo. The principle is called metabolic oligosaccharide engineering and involves the incorporation of synthetically modified saccharides containing an inert but mutually reactive functional group into a host glycoprotein by exploiting a particular metabolic pathway. The favourable size and inertness of azide and acetylene-functionalised substrates within living cellular environments as well as the promiscuity of certain glycosyltransferases enables these abiotic substrates to “slip through” the cellular catalytic machinery and become incorporated into cell surface glycans. The residues then can serve as chemoselective handles for bioconjugation to
a fluorescent marker for visualisation, or an affinity tag for proteomic enrichment. In this respect, click chemistry has been instrumental as a bioorthogonal tagging strategy, owing largely to the biocompatibility of the reaction and the inertness of azides and acetylenes within complex biological environments.

In an early example, Bertozzi and co-workers utilised a strain-promoted cycloaddition of a cyclooctane functionalised biotin probe with recombinant glycoproteins and cell surface glycoproteins displaying N-azidoacetyl sialic acid (Sia-NAz) residues. The 1,2,3-triazole products were detected in both recombinant systems and on the cell surface of human T-Lymphoma Jurkat cells by Western blot analysis and flow cytometry, respectively. In the latter case, fluorescence measurements were shown to be dose-dependent and proportional to the density of the Sia-NAz residues on the cell surface glycoproteins as well as the incubation time with the cyclooctane biotin probe.

L-Fucose is a ubiquitous component in sialyl Lewis\(^\text{y}\) (SLe\(^\text{y}\)) and sialyl Lewis\(^\text{x}\) (SLe\(^\text{x}\)) antigenic selectins found unregulated in certain cancers. The Bertozzi group recently has recently utilised click reaction as a key bioconjugation step in a fluorescent reporter strategy for the chemical profiling of fucosylated glycoproteins as new cancer biomarkers (Scheme 1.6). Utilising the fucose salvage pathway and the promiscuity of fucosyl transferases, three synthetic azido fucose analogues, functionalised with azide at the 2, 4 and 6 positions, respectively, were incorporated into cell surface glycans of human T-lymphoma cell line Jurkat. The azide markers were incorporated into the glycan and presented on the cell surface by culturing the cell culture with 125 μM of each of azide analogue. The cells were lysed with an NP-
40 detergent which is known to be compatible with click chemistry. The click bioconjugation of the cell lysates with acetylene biotin marker, followed by binding of triazole tagged glycoprotein to α-biotin-horse-radish peroxidise conjugate (HRP) allowed for Western blot analysis. Significant protein labelling (up to 40% of all available fucosylation sites) of only the 6-azido analogue was observed by Western blot analysis of the hydrolysates, although this analogue was deemed cytotoxic at elevated culture concentrations (100 μM). It was postulated that the azide analogues substituted at C-2 and C-4 were not acceptable substrates for the fucose salvage pathway.

Scheme 1.6

Very recently, Wong and co-workers have developed a click-activated fluorogenic labelling technique to analyse cell surface glycoprotein fucosylation in vivo (Scheme 1.7). In a similar manner to that reported by Bertozzi and colleagues, the artificial 6-azido and 6-ethynyl fucose substrates were incorporated into cell surface glycoproteins of human lymphoma Jurkat cell lines via the fucose salvage pathway. The extent of cell surface glycoprotein fucosylation was analysed in vivo by flow cytometry and fluorescent microscopy following the click bioconjugation of the
fluorogenic probe to the functionalised L-fucose analogues. Fluorescence was only detected for the 6-azido analogue, following fluorogenic bioconjugation of 4-ethynyl-N-ethyl-1,8-naphthalimide, indicating selective uptake and integration of this artificial substrate into cell surface glycans.

Scheme 1.7

1.3 Multivalent neoglycoconjugates

1.3.1 General

Nature compensates for the weak binding affinity between a monovalent carbohydrate ligand and cognate receptor protein ($K_a$ in the ranges of $10^{3-4}$ M$^{-1}$, although exceptions are known) by expressing multiple copies of the carbohydrate recognition epitope, or multiple receptor binding sites on a cell surface receptor - a concept known as the “multivalent” or “glycoside cluster effect”.$^{66}$ The overall effect is the increase in binding affinity to an extent which exceeds the sum of the constituent binding events in sufficient strength to effect or inhibit a cellular or physiological response. By exploiting one or more of these mechanisms, chemists have assembled a wide range of structurally diverse polymeric artificial carbohydrate polymers (glycopolymers) as effectors or inhibitors of cellular processes and analytical tools for investigating carbohydrate-protein binding events.$^{67}$ Artificial
glycopolymers hold great promise as inhibitors of bacterial adhesion\textsuperscript{68} and anti-cancer vaccines,\textsuperscript{69} but also sustainable and economic biomaterials with a wide range of biomedical and drug delivery applications, including biocompatible matrices for tissue engineering\textsuperscript{70} and sustained drug release and as polymeric vehicles for targeted drug- and gene delivery.\textsuperscript{71} Apart from their wide spread biomedical applications, glycopolymers can also function as mimics of naturally occurring polysaccharides and display anti-inflammatory, anti-coagulant and anti-tumour properties and are thus viable leads for drug development and vaccine development.\textsuperscript{72,73}

\textbf{1.3.2 Glycopolymers and polysaccharide mimics}

Novel synthetic methods are critical for the development of innovative glycopolymers of biomedical and pharmaceutical importance. Yet devising novel synthetic pathways for developing biocompatible glycopolymers that are well defined and tailored for a specific purpose is a continuing challenge faced by carbohydrate chemists.\textsuperscript{73} Subtle changes in polymer structure and pendant attachments can have profound effects on the mechanical properties and biological activity of the material. Motivated by the requirement for more expedient synthesis of multivalent glycopolymers, Haddleton and co-workers\textsuperscript{74} have recently reported the use of a novel, dual click chemistry/transition-metal-mediated-living-radical-polymerisation (TMM-LRP) strategy for the facile preparation of a linear glycopolymer library containing pendantly attached carbohydrates. The acetylene functionalised poly(methylacrylate) polymer was assembled through TMM-LRP and was used as a subsequent scaffold for combinatorial design. A series of glycosyl azides and azido ethyl glycosides were then grafted by click chemistry in a highly efficient manner to the acetylene-functionalised polymer backbone providing rapid
access to glycopolymer libraries with different bulk properties and potential protein binding affinity. The work demonstrates the powerful utility of click chemistry in preparing pendant-functionalised glycopolymers in a combinatorial fashion.

Reineke et al. have recently utilised click chemistry as a pivotal step in the preparation of glycopolymer-nucleic acid complexes (polyplexes) as gene-delivery agents. The synthetic poly(glycoamidoamine) co-polymers were chemically tailored to be non-toxic and biocompatible, whilst improving genetic transfection efficiency by stabilising plasmid DNA (pDNA) complexation, and preventing cellular disassociation, aggregation and biological degradation of the polyplex system prior to delivery at the therapeutic site. On the basis of previous studies implicating heterocyclic amide co-functionalised polymers as strong DNA binding agents, the triazole-amide moiety (two per repeat unit) was postulated as a strong DNA binding motif via hydrogen bonding and van der Waals interactions. Diazido trehalose units were efficiently polymerised by click chemistry to a variety of dialkyne-amide-terminated oligoamine monomers containing 1, 2 and 3 secondary amine(s) (Scheme 1.8). N-Boc deprotection of the ethyleneamine groups resulted a library of biocompatible and polycationic carbohydrate co-polymers with similar degrees of polymerisation, dispersity, molecular weights and polymer stiffness in aqueous solution. The highly cationic nature rendered the polymers capable of phosphate charge neutralisation and polyplex formation. To elucidate the effect of the 1,2,3-triazole ring and the relative number of ethyleneamine groups in the polymer backbone on pDNA complexation, a series of gel electrophoresis and fluorescent ethidium bromide assays were conducted. Cellular delivery efficacy into HeLa cells was also determined using fluorescent flow cytometry analysis.
Nature’s ability to perform myriad chemical transformations under exquisite regio- and stereo-control within complex, polyfunctional chemical environments has captured the imagination of synthetic organicchemists for many years. The ability to perform multiple transformations on a single substrate *in vitro* using orthogonal chemistries is a key challenge facing chemists in the quest for novel materials and drugs.\(^7^6\) Two possible routes have emerged; either conducting multiple transformations simultaneously on separate functional groups within the same molecule, or multiple transformations on the same functional group in a “cascade-type” fashion. Impressive examples have emerged thus far and these are primarily concerned with total synthesis of natural products and peptides.\(^7^7\) However its adaptation to polymer science has been limited, owing difficult nature of these polyfunctional macromolecules. The orthogonal functionalisation of polymeric macromolecules requires methods which operate under high fidelity, mild conditions and strong regio- and stereocontrol.\(^7^7\) Click chemistry provides great scope in glycopolymerychemistry, as it can be used as a polymerisation tool or to functionalise preformed polymers with equal fidelity under orthogonal reaction conditions.

Hawker and colleagues\(^7^8\) have recently exploited click chemistry as a mild, regio/stereoselective strategy for performing cascade functionalisations on linear
glycopolymers. In the simultaneous method, an acetylene-functionalised polystyrene co-polymer was reacted with 2,3,4,6-tetra-O-β-D-glucopyranosyl azide using click chemistry whilst a hydroxyl pendant side chain was reacted simultaneously with an N-hydroxysuccinimide (NHS) activated ester, affording the functionalised co-polymer containing a triazole and ester pendant sequence (Scheme 1.9A). On the other hand, the cascade mechanism proceeded via the sequential reaction of a dual ester functionalised polyacrylate with propargyl amine affording the propynyl amide which was subsequently grafted to the glucosyl azide in a one pot manner (Scheme 1.9B).

![Scheme 1.9](image_url)

Cellulose is an abundant natural biomaterial and its synthetic modification can lead to materials possessing bulk properties which are suitable for a wide range of industrial and biomedical applications. In the pharmaceutical industry, functionalised cellulose homopolymers (cellulosics) have been extensively used as biodegradable enteric coatings for controlled drug release formulations and more recently for bone, cartilage and brain tissue engineering owing to its excellent biocompatibility. The first example of the chemical modification of cellulose using
Chapter One

click chemistry was recently reported by Heinze and co-workers.\textsuperscript{79} Click chemistry of the 6-azido-6-deoxy cellulose scaffold afforded triazole spaced carboxyester, thiophene and aniline functionalised cellulosics displaying degree of substitution values up to 0.9 and good solubility in organic solvents (DMF, DMSO). The click methodology offers hydrolytically stable bonds between the polymer backbone and functional groups and overcomes the formation of polymer cross-linking by-products that are typically observed with conventional cellulose esterification.

The conjugation of multivalent antigenic oligosaccharides to preformed biomacromolecules, in particular polypeptides, often results in an increase in an immune response and has become a primary strategy in the development of anti-tumour vaccines.\textsuperscript{75} Research efforts in this area are now focused towards the preparation of multi-antigenic constructs with the aim of stimulating the immune system towards a broad range of malignant transformations rather than one (i.e. using monomeric antigenic constructs). In this respect, more sophisticated and complex synthetic strategies will be required and are being sought.\textsuperscript{80} In a proof-of-concept manner, the Danishefsky group has recently demonstrated the viability of click chemistry as a strategy for linking antigenic oligosaccharides to keyhole limpet hemocyanin (KLH) polypeptide carriers.\textsuperscript{81} The authors concluded click chemistry to be a practical and orthogonal linker strategy owing to its high degree of chemoselectivity and tolerance towards other synthetic transformations. In addition, the compatibility of the chemistry under aqueous conditions permits the use of a broad range of carrier proteins/peptides proteins which are otherwise unstable or insoluble within organic solvents.
Many (1→3)-β-D-glucopyranan polymers, such as Lentinan, possess immuno-stimulating and anti-cancer properties.\textsuperscript{82} Natural and functionalised (1→3)-β-D-glucopyranan polymers have been utilised as novel biomaterials possessing lectin and RNA binding affinity owing to their unique triple-stranded helical superstructure.\textsuperscript{83} In an effort to generate more regioselective methods for the preparation of functionalised glucan polymers, Shinkai and co-workers,\textsuperscript{84} have utilised click chemistry to chemically modify the (1→3)-β-D-glucan, 6-azido-6-deoxycurdlan. In a pilot study, the click reactions were performed with a variety of acetylene-terminated modules with potential lectin binding, redox-active, phytoactive, and fluorescent properties in order to develop novel polysaccharide-based materials.

Dondoni and co-workers\textsuperscript{85} have recently prepared a series of triazole linked (1,6)-oligomannose analogues as potential mimics of lipoglycan structures and high density oligomannose-type anti-microbial vaccines.\textsuperscript{86} The orthogonally substituted ethynyl α-C-mannoside building block was conjugated to the 6-azido-α-C mannoside platform to afford the triazole linked dimannoside coupling product. Using an iterative click strategy, involving the necessary installation of an azide group at C-6 and coupling to the ethynyl building block resulted in the facile construction of a structurally rigid triazole linked oligomannose over a total of 4 consecutive cycles (Scheme 1.10). Whilst the click coupling reactions required up to 30 hours for completion at room temperature, microwave irradiation at 100 °C dramatically shortened this to 5 minutes, whilst retaining good yields and regioselectivity.
The first reported example of the use of click chemistry in the modular synthesis of branched oligosaccharide mimics was demonstrated by the work of Field and colleagues. The facile synthesis of amyllopectin fragments occurred in a single step between dipropynylated maltoside core fragments and glycosyl azide building blocks based on \( \beta-D\)-glucose, maltose, maltotriose and maltoheptose, respectively. Employing propynyl functionalised maltose templates substituted at C-4/C-6’ and C-6/C-6’, respectively, allowed for the template assisted click synthesis of the respective regio-isomeric amyllopectin analogues.

### 1.3.3 Cyclodextrins and artificial receptors

Cyclodextrins (CDs) are water soluble, cyclic malto-oligosaccharides derived from starch which possess a hydrophilic outer surface and a relatively lipophilic central cavity. For quite some time, CDs have been used in drug and cosmetic formulations as solubilising carriers of otherwise hydrophobic and water-insoluble molecules through the reversible formation of water soluble inclusion complexes but recently have been gaining considerable attention as topical delivery agents to improve the bioavailability of drugs administered by this route. There has also been much interest in CDs as vehicles for the cell-specific delivery of hydrophobic drugs to target sites through multivalent interactions with carbohydrate receptors. CDs are also non-immunogenic and offer enhanced biocompatibility and water
solubility compared to other multivalent carbohydrate constructs based on rigid macrocyclic scaffolds, such as azacrown ethers, cyclic peptides, calixarenes and cyclophanes.88,90

The first example of an artificial CD analogue incorporating a heterocyclic motif within the binding cavity was demonstrated by Vasella and co-workers91 during ongoing structural and binding investigations of artificial CD analogues. The intramolecular, thermal Huisgen 1,3-DCR between a dual azide/acetylene functionalised amylose fragment proceeded under thermal conditions to afford the regioisomeric mixture of a heterocyclic β-CD mimic.

Click chemistry offers numerous advantages over preexisting cycloglycosylation and cyclooligomerisation methods, which are often impeded by long synthetic sequences, low cyclisation yields, as well as difficulties in resolving anomeric mixtures and/or macrocycles of different sizes. Click chemistry was originally proposed by Gin et al.92 as a high yielding, stereo- and regiospecific cyclodimerisation strategy for preparing well defined, modified β-CD analogues. The work reported the convergent click cyclodimerisation of a dual azide/acetylene-functionalised trimannoside using a CuI/DBU catalytic system, affording the cyclic dimer in good yields (80%) with the concomitant formation of cyclic trimer (15%) (Scheme 1.11). The mannose based macrocycle also showed similar propensity to form inclusion complexes with hydrophobic fluorogenic molecules compared to native β-CD, as determined by fluorescence emission spectroscopy. In a recent extension from this work, a series of $C_2$ and $C_3$-symmetric oligosaccharide macrocycles were prepared using click chemistry in a convergent manner from a suitably difunctionalised monosaccharide
Chapter One

and disaccharide, respectively. The investigation of macrocycle cavity shape and the potential for host-guest interactions was conducted using computational and NMR shift titration methods, revealing a functionalised and sterically congested cavity which may prove useful as novel molecular pores or receptors for studying host-guest interactions.

Scheme 1.11

Click chemistry has been used to prepare $C_2$-symmetric carbohydrate/amino acid hybrid macrocycles as potential water soluble inclusion complexes. The cyclodimerisation of bifunctional azido-acetylene glycopeptide building blocks proceeded in acetonitrile using a CuI/DIPEA catalyst system in moderate yields. Interestingly, the analogous reaction was attempted using aqueous conditions prescribed by Sharpless and showed minor product conversion at elevated temperatures (70°C). The novel carbohydrate/amino acid hybrid macrocycles were investigated as potential water soluble artificial receptors using computational methods. It was demonstrated that the 1,2,3-triazole motif imparted increased macrocycle rigidity and cavity size, relative to previously reported non-triazole analogues.
The synthetic modification of preformed CDs provides an alternative route to artificial CDs with particular properties. Exploiting differences in reactivity between primary and secondary hydroxyl groups orientated at the outer and inner faces, respectively, has allowed for the selective substitution of these groups. Schubert et al. \(^{95}\) have recently reported the use of click chemistry in the preparation of seven-armed star-shaped polymer, heptakis-p\(\varepsilon\)CL-\(\beta\)-cyclodextrin. Star shaped polymers have added advantages of over linear polymers since they have lower hydrodynamic volume and a larger number of functionalised end-groups for further modification. The acetylene functionalised poly(\(\varepsilon\)-caprolactone) (p\(\varepsilon\)CL) was prepared via a ring opening polymerisation of \(\varepsilon\)-caprolactone utilising 5-hexyn-1-ol initiator and tin(II)octanoate catalyst, which was in turn appended to heptakis-azido-\(\beta\)-cyclodextrin via click chemistry and microwave irradiation, affording poly(\(\varepsilon\)-caprolactone)-\(\beta\)-cyclodextrin in a single step.

### 1.3.4 Glycodendrimers, glycoclusters and lectin inhibitors

Glycodendrimers are highly globular, monodisperse, oligo- or polymeric carbohydrate-containing nanostructures. Carbohydrates are typically presented at the periphery of a dendritic core in high density with well defined, symmetrical branching patterns, and are prepared through a series of iterative steps via convergent or divergent synthetic strategies.\(^{96}\) The innate structural control, monodispersity and relative size of glycodendrimers - in between that of glycoclusters and polyvalent glycopolymers, endow numerous advantages which have been successfully exploited for a variety of biological and biomedical applications, including multivalent tools.
for investigating carbohydrate-protein interactions, microbial anti-adhesives, cancer vaccines, and as biocompatible scaffolds for drug and gene delivery purposes.\textsuperscript{96,97}

The successful synthesis of structurally well defined glycodenrimers and glycoclusters must employ chemistries which maximise atom efficiency. Such chemistries should display a high degree of tolerance towards polyfunctional macromolecules and provide access to structurally diverse architectures in the smallest number of possible steps. Click chemistry has allowed for flexible and rapid synthetic design of a wide range of sophisticated carbohydrate nanostructures of biomedical importance. The first example of the use of Cu\textsuperscript{I}-catalysis in generating triazole linked glycodenrimers was demonstrated by Santoyo-González and colleagues.\textsuperscript{98} The employment of organic-soluble copper catalysts ((Ph\textsubscript{3}P)\textsubscript{3}•CuBr and (EtO)\textsubscript{3}•CuI) enabled the facile construction of a diverse range of 1,4-disubstituted 1,2,3-triazole linked neoglycoconjugates and glycodendrimers.

Synthetic modifications of the original click chemistry conditions have recently appeared within the literature with the aim of improving the efficiency and scope of click chemistry in multivalent neoglycoconjugate design and synthesis. For example, Van der Eycken and colleagues\textsuperscript{99} have reported a highly efficient, one pot, microwave assisted click chemistry method based on a three-component system employing sodium azide, alkyl halide and acetylene. Pieters et al.\textsuperscript{100} have reported the facile synthesis of glycodendrimers containing up to nine peripheral carbohydrate units in high yields and shortened reaction times using microwave assisted click chemistry. Another recent example is provided by Moran and co-workers who have described microwave assisted click chemistry to rapidly label oligonucleotides on solid support with pendant carbohydrate attachments.\textsuperscript{101} Testament to the impressive
Chapter One

scope and compatibility with other synthetic tools, Chang and co-workers have recently explored the utility of sonication (sonochemistry) as a facile means for generating triazole linked neoglycoconjugates under mild, ambient conditions. In addition, Wang et al. have recently reported a one pot procedure for the synthesis of multivalent, triazole linked neoglycoconjugates in solution from glycosyl halide precursors.

Perhaps the greatest advantage of the click methodology in multivalent neoglycoconjugate synthesis is the ability to conduct convergent or divergent syntheses in aqueous solution. This effectively enables the anchoring of deprotected saccharides to the core in a single step, furnishing the target glycodendrimer in high yields and purity, whilst bypassing otherwise necessary activation and/or protection and deprotection steps. Click chemistry has been utilised by Sharpless, Hawker and colleagues as a general and facile strategy for the preparation of a series of difunctionalised and unsymmetrical dendritic co-polymers incorporating dual purpose binding/detection chain terminals (Scheme 1.12). Multi-functionalised dendrimers have potential recognition/detection/delivery applications and can be utilised as multivalent ligands for in situ monitoring or as novel drug delivery agents. The facile nature of the reaction between the appropriately functionalised dendrons with azide or acetylene functionalities allowed for the highly modular, flexible and divergent assembly of the difunctionalised glycodendrimer. The binding element was a polymannose-functionalised periphery capable of binding to Concanavalin A (Con A). The fluorescent coumarin-functionalised periphery was located at the opposite chain terminal and was employed for potential diagnostic purposes. Multi-functionalised glycodendrimers allow for the introduction of binding elements, reporter elements
and/or drug molecules at defined locations within the same nanostructure. The bi-functionalised glycodendrimer was assayed for binding with Con A in a standard hemagglutination assay. From this assay, a relative binding affinity of 240 was observed relative to free mannose, which equated to a per-unit affinity of 15.

\[\text{Scheme 1.12}^{104}\]

Riguera and colleagues\(^{105}\) have recently reported the facile preparation of unprotected glycodendrimers based on click chemistry. Several \(O\)-propynyl glycosides based on fucose, mannose, and lactose were appended to three generations of azido-terminated gallic acid-triethylene glycol dendrimers in a highly efficient and divergent manner under aqueous conditions and in the presence of catalytic quantities of \(\text{Cu}^1\) catalyst (1 mol%). Impressive atom efficiency and reported yields (up to 92%) were observed, with the incorporation of up to 27 free propynyl glycosides in a single step. In a separate study by the same group, the applicability of the click methodology was further investigated on other co-
polymeric core materials, such as azido-terminated PEG-dendritic block co-
polymers. Similar atom efficiency and fidelity was also achieved using this 
versatile and novel co-polymer.

Galectins are a ubiquitous family of cytosolic β-D-galactoside binding proteins, of 
which 14 are known in mammalian systems. They display broad and diffuse 
biological activities and their expression during cellular development and 
oncogenesis is highly variable. In particular, Galectin-1 (Gal-1) and Galectin-3 (Gal-
3) are implicated in a wide range of biological and pathological processes including 
cell apoptosis, innate immune function, oncogenesis, and HIV-1 infectivity. The 
chemical modulation of these ambiguous proteins using multivalent inhibitors and 
effectors is therefore of great interest to glycobiology and clinical research. Roy 
and colleagues have prepared a suite of β-D-galactose containing 
neoglycoconjugates as potential galectin inhibitors from a series of sugar and non-
sugar blocks containing azide or acetylene functionalities. The resulting triazole 
neoglycoconjugates were rapidly prepared using click chemistry and screened 
individually against three Galectin proteins; Gal-1, Gal-2 and Gal-3. A $C_3$-
symmetrical triazolo-lactose analogue was prepared by a CuI-catalysed cycloaddition 
in good yield (83%) and was identified as a strong inhibitor of Gal-1 (20 μM) and a 
moderate inhibitor of Gal-3 (250 μM). In particular, a 13.3-fold increase in potency 
for each lactose unit in the trivalent array was observed relative to free lactose 
against Gal-1, leading to a total relative potency of ~40.

In a similar study, Nilsson et al. have examined the binding affinity of a wide 
range of multivalent lactose-bearing neoglycoconjugates against cancer related
galectins, Gal-1, -3, -4N, 4C, -4, -7, -8N and -9N. The multivalent neoglycoconjugates were prepared by a Cu$^+$-catalysed cycloaddition of the readily available 2-azidoethyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside and various acetylene functionalised phenyl-bis-alanine and phenyl-tris-alanine building blocks, affording the di- and trivalent neoglycoconjugates, respectively. To investigate the potential effect of the aglycone on binding, the corresponding monovalent homologues were prepared and used as a reference for relative binding affinity along with methyl β-D-lactoside. From a total of 8 potential galectin ligands, the divalent carbamate species was found to have a binding affinity for Gal-1 of one order of magnitude stronger than all other ligands studied, with a $K_d$ of 3.2 μM and a cluster effect of 7.7 and 30 relative to the corresponding monomer and methyl β-D-lactoside, respectively. In contrast, the corresponding monomer displayed markedly weaker affinity for Gal-1, with a $K_d$ of 49 μM.

Deprotected 2-azidoethyl glycosides of lactose and N-acetyl glucosamine have been employed by Gao and co-workers$^{110}$ as water soluble building blocks in multivalent ligand design. The facile click reaction between the deprotected ω-azido glycosides and the uniformly propynyl alkylated methyl β-D-glucoside scaffold resulted in the rapid, modular generation of glycoclusters under aqueous conditions (Scheme 1.13). The two glycoclusters were assayed by a competitive ligand displacement assay (AGP-asialoglycan) using capillary electrophoresis. For both glycoclusters, an impressive 400-fold increase in binding affinity was observed to the plant lectin protein RCA$_{120}$ relative to free lactose, which is indicative of a strong multivalent effect.
Du and co-workers\textsuperscript{111} have previously reported the mild anti-tumour and increased white blood cell proliferation activity of linear \(\beta-D-(1\rightarrow6)-N\)-acetyl-\(\beta-D\)-glucosamine hexa- and nonasaccharides. Based on these findings, synthetic efforts were directed towards multivalent models in order to enhance \textit{in vivo} potency. The facile convergent synthesis of a \(C_3\)-symmetric \((1\rightarrow6)-N\)-acetyl-\(\beta-D\)-glucosamine octadecasaccharide and the corresponding monovalent hexasaccharide was achieved in good yields using click chemistry. In a preliminary \textit{in vivo} assay, the \(C_3\)-symmetric, tri-antennary octadecasaccharide showed moderate tumour growth inhibition relative to the linear oligosaccharides and the corresponding monovalent hexasaccharide.

### 1.4 Carbohydrate microarrays and Self-Assembled Monolayers (SAMs)

#### 1.4.1 General

Progress in glycomics research and carbohydrate-based drug discovery has arisen not only from an impressive array of synthetic and separation technologies, but also the
development of robust, high throughput screening tools for the identification of protein-carbohydrate receptor interactions.\textsuperscript{112} Carbohydrate microarrays (glycoarrays) have emerged as versatile, high throughput tools for the screening of potential lectin proteins and more recently for investigating the binding of pathogenic bacteria to carbohydrates.\textsuperscript{113} Carbohydrate microarrays consist of several hundred or thousands of carbohydrate ligands immobilised on a solid surface. The screening of test proteins for carbohydrate affinity occurs simultaneously in chip format using a variety of design strategies, whilst consuming only minute quantities of test compound and protein, an important prerequisite when using complex oligosaccharides and test compounds which have been painstakingly acquired. Self-assembled monolayers (SAMs) are also gaining attention and consist of homogeneous saccharides packed onto surfaces in a well defined and controllable manner. SAM technology allows for exquisite control over carbohydrate density and concentration, which allows for real time monitoring and quantitation of binding events.\textsuperscript{112c} Owing to the near quantitative product conversion and high fidelity, click chemistry is well poised to be the forerunner biomolecular immobilisation strategy for potential in situ screening of carbohydrate-protein interactions.

\textbf{1.4.2 Glycoarrays}

The earliest example of the construction of glycoarrays using click chemistry was demonstrated by Wong and co-workers,\textsuperscript{114} in an attempt to develop an accessible, high throughput screening method for the identification lectin-like proteins selective for galactose in microtitre plate format. An azidoethyl galactoside was used as the model carbohydrate for the study and was tethered via a Cu\textsuperscript{I}/DIPEA catalysed
cycloaddition to the hydrophobic propionamide which, in turn was attached non-covalently to the polystyrene support of the well surface (Scheme 1.14).

![Scheme 1.14](image)

The click reaction optimised to allow conjugation on micromolar scales (4-80 μM), and was found complete under all conditions by ESI-MS in excellent yields (up to 92%), according to a previously reported sulfuric acid-phenol (SAP) assay. The Ricin B lectin protein isolated from *Ricinus communus* (castor bean), which selectively binds β-linked D-galactosides, was screened against the galactolipid array. Using the same click chemistry immobilisation strategy, a suite of 11 oligosaccharides displaying variable terminal branching patterns and degrees of terminal sialylation and/or fucosylation were then screened against potential lectin proteins. The enzyme catalysed fucosylation of the immobilised trisaccharide allowed for the *in situ* generation of immobilised sialyl LeX ligands. The *in situ* generated sialyl LeX ligand bound selectively to a fucose specific lectin from *Tetragonolobus purpureas*, which did not show any affinity for the non-fucosylated trisaccharide precursor.

Wong and colleagues have also reported a similar non-covalent glycoarray utilising click chemistry as a key immobilisation strategy for the high throughput screening of
Fuc-T inhibitors.\textsuperscript{115} \textit{N}-Acetyllactosamine was immobilised to the solid support by click chemistry and was incubated with the triazole inhibitor, Fuc-T and the substrate, GDP-Fuc. The \textit{in situ} fucosylation of the displayed \textit{N}-acetyl lactosamine array was quantified by binding to peroxidise-bound \textit{T. purpureas} lectin protein. In this manner, four compounds were detected with nanomolar inhibition constants, three of which contained a bi-phenyl substituent which is consistent with earlier findings.\textsuperscript{45} Mei et al.\textsuperscript{116} have also employed click chemistry as an immobilisation strategy in conjunction with quantum dots (QDs) as a fluorescent label for the detection and quantitation of carbohydrate-protein binding.

In an effort to generate glycoarrays with enhanced stability and robustness towards high throughput screening protocols, Wong and co-workers\textsuperscript{117} have exploited click chemistry to immobilise carbohydrate arrays to \textit{covalently} attached solid supported linkers via thiourea or amide bonds. The immobilisation of azido ethyl glycosides to an alkyne-terminated propionamide linker proceeded with high fidelity and efficiency using click chemistry (Scheme 1.15). Analysis of the immobilised ligand by ESI-MS could be achieved by the mild, reductive cleavage of the disulfide linker from the covalent anchor and subsequent dissolution. In a similar approach to the previous study, two glycoarrays were screened against lectins selective for \(\alpha\)-fucose (\textit{Lotus tetragonolobus}, LTL) and galactose (\textit{Erythrina cristagalli}, EC) residues. The tetrasaccharide breast cancer antigenic determinant, Globo-H was also screened against LTL and was found to bind to this lectin in a concentration-dependent manner (\(K_d\ 22.8\ \mu M\)) which was comparable with previous findings. In the same study, the immobilisation of carbohydrate-ligands using click chemistry was also shown to be applicable to ELISA type formats. The covalently attached glycoarrays
were also assessed for their ability to bind a GP-120-antibody (2G12), which recognises dense oligomannose clusters presented on the surface of HIV-1 gp120 (Man₉GlcNHAc₂). Several triazole tethered oligomannose arrays were reported to display very strong affinity for the antibody ($K_d = 0.1-1.0 \, \mu\text{M}$).

**Scheme 1.15**

1.4.3 Carbohydrate Self-assembled monolayers

Click chemistry between azide-functionalised sugar linkers with solid-supported alkynes has recently been exploited by Wang and co-workers for the fabrication of carbohydrate-containing SAMs, using a similar approach developed by Chidsey and co-workers for the fabrication of oligonucleotide SAMs. In this case, traditional alkane thiol linkers were employed, owing to their clean and spontaneous adsorption to the solid gold surface. Further functionalisation at the linker terminus with an alkyne group allowed for the fabrication of the carbohydrate SAM by the chemoselective delivery of various azido(triethylene)glycol $O$-glycosides, based on $\alpha$-D-mannose, $\alpha$-D-galactose and $\beta$-D-lactose via click chemistry (Scheme 1.16). The
carbohydrate ligands were successively interspersed with azido(triethylene)glycol forming a stable, rigid and well-dispersed SAM. The relative binding affinities (reported as affinity constants, $K_A$) of various lectin proteins selective for these carbohydrate epitopes were determined by real-time analytical techniques, SPR and Quartz Crystal Microbalance (QCM). Relative binding affinities to the lectin proteins, in particular that of the triazole-mannose SAM with Con A lectin ($K_A 3.9 \pm 0.2 \times 10^6 \text{ M}^{-1}$), were found to be in good agreement with previously reported values.

Chaikof and co-workers\textsuperscript{120} have recently demonstrated the potential of click chemistry to immobilise carbohydrates to glass surfaces using a flexible, biocompatible polyethylene glycol (PEG) linkers. The attachment of the PEG linker to the maleimide-terminated solid support was achieved using a PEG chain functionalised at both termini with cyclopentadiene and an alkyne. Successive immobilisation of the azide-functionalised carbohydrate was then achieved using click chemistry under buffered aqueous conditions (PBS, pH 7.0) with impressive fidelity and homogeneity.
Chapter One

1.5 Conclusions

Click chemistry has had a considerable impact on drug discovery and biotechnology since its conception some 7 years ago. However, the application to glycomics research and carbohydrate based drug development is more recent, yet the sheer volume of literature which has appeared the past 3 to 4 years is testament to its broad scope and versatility. Click chemistry holds great promise as a premier bioconjugation strategy for a wide variety of preparative and analytical applications. It has become a powerful synthetic tool for preparing a wide range of structurally diverse neoglycoconjugates, either as probes for the elucidation of carbohydrate-protein binding and as drug leads. It has also shown potential as a bioorthogonal strategy for the labelling of chemically-engineered cell surface glycans. These tools serve to correlate glycan structure and function in physiological states of health and disease, leading to enhanced understanding of the nature of protein and lipid glycosylation and the identification of novel targets for clinical intervention and vaccine development. It has also become a robust immobilisation strategy for the fabrication of surface-based carbohydrate arrays and monolayers facilitating high throughput lectin-carbohydrate investigations for the identification of lectin proteins of particular relevance in pathology.
1.6 References


Chapter One


20. (a) Sustmann, R. Simple model for substituent effects in cycloaddition reactions. I. 1,3-Dipolar cycloadditions. Tetrahedron Lett. 1971, 29, 2717-20; (b) Bräse, S., Gil, C.,
Chapter One


Chapter One


31. For reviews concerning 1,3-dipolar cycloadditions on carbohydrates, see: (a) Gallos, J. K., Koumbis, A. E. 1,3-Dipolar cycloadditions in the Synthesis of Carbohydrate Mimics.
Chapter One


56. Ermolat'ev, D. Dehaen, L., ^an der Eycken, E. Indirect coupling of the 2(1H)-pyrazinone scaffold with various (oligo)-saccharides via "click chemistry": En route towards glycopeptidomimetics. QSAR & Comb. Sci. 2004, 23 (10), 915-918.


Chapter One


84. Hasegawa, T., Umeda, M., Numata, M., Li, C., Bae, A. -H., Fujisawa, T., Haraguchi, S., Sakurai, K., Shinkai, S. ‘Click chemistry’ on polysaccharides: a convenient, general, and


99. Appukkutan, P., Dehaen, W., Fokin, V. V., Van der Eycken, E. A Microwave assisted Click Chemistry Synthesis of 1,4-Disubstituted 1,2,3-Triazoles via a Copper(I)-Catalyzed Three-Component Reaction. *Org. Lett.* 2004, **6** (23), 4223-4225.


The Synthetic Utility of Glycosyl Triazoles in Carbohydrate Chemistry

This chapter is an expanded version of the article:

2.1 Introduction

Oligosaccharides linked to proteins and lipids via *N*- or *O*-glycosidic linkages (glycoconjugates) have been shown to govern crucial life processes and disease states.\(^1\),\(^2\),\(^3\),\(^4\),\(^5\),\(^6\) Glycosylation patterns for proteins and lipids are exquisitely controlled via mechanisms independent from that of the genomic transcription and as a result glycoconjugates from natural sources often exist as heterogeneous isoforms (glycoforms). The isolation of homogenous material from crude biological extracts in sufficient quantities for study can, therefore, be difficult. Medicinal chemistry, through provision of an impressive array of synthetic methodologies, offers a potentially more reliable route to homogenous glycoconjugates leading to either exact copies of naturally occurring glycoconjugates or, alternatively, incorporating unnatural glycosidic linkages. An appreciable benefit of the latter approach is that the ‘artificial’ glycoconjugate may retain the geometric and spatial characteristics of the native glycoform\(^7\),\(^8\) yet exhibit stability towards *N*- and *O*-glycosyl hydrolase activity and are able to inhibit these enzymes to some extent,\(^9\) so increasing the potential for a wide array of *in vivo* applications. In addition the artificial linkage may be fine
tuned as an inert functionality towards subsequent synthetic transformations necessary elsewhere within the target molecule synthesis.

In the past three years there has been a flourish of activity in the literature concerning the 1,3-dipolar cycloaddition reaction (1,3-DCR) of organic azides with terminal acetylenes (Huisgen reaction).\textsuperscript{10} This interest stems largely from the optimisation of 1,3-DCR by Sharpless\textsuperscript{11} and Meldal\textsuperscript{12} with respect to ease and efficiency of catalysis and regioselectivity of the triazole product. The reaction involves a step wise Cu(I)-catalysed dipolar cycloaddition of a terminal acetylene to an organic azide to form, exclusively, the 1,4-disubstituted 1,2,3-triazole (Scheme 2.1).\textsuperscript{12,13} The highly exothermic and kinetically controlled reaction is conducted favourably in water and is also tolerant to a wide variety of organic co-solvents and as such has become a premier component of “click chemistry”.\textsuperscript{11b}

\[ \begin{array}{c}
R^1-N_3 \\
(0.25 - 0.5 M) \\
\text{+} \\
R^2-\equiv \\
(1 \text{ eq}) \\
\text{Cu(I)} \\
\end{array} \rightarrow \begin{array}{c}
R^1-N_2-N_3 \\
(\text{5 eq}) \\
R^2 \\
\end{array} \]

\textbf{Scheme 2.1}

The modified 1,3-DCR has shown great versatility and utility on non-saccharide substrates in medicinal chemistry, chemical biology and materials science.\textsuperscript{13,14} The application to “traditional” carbohydrate chemistry\textsuperscript{15} has been somewhat slower, but presents as an attractive reaction for several reasons. Glycosyl azides are generally stable, crystalline solids, inert towards a wide range of reaction conditions and are
available diastereomically pure.\textsuperscript{16} In traditional carbohydrate synthesis they serve primarily as stable precursors of glycosyl amines (for glycopeptide synthesis) and are also less commonly precursors to glycosyl fluoride donors.\textsuperscript{17} Glycosyl azides are readily synthesised through the stereoselective bimolecular displacement of a glycosyl halide with an azide nucleophile\textsuperscript{17} and 2-azido sugars are obtained through the triflyl azide-mediated diazotransfer of amino sugars.\textsuperscript{18} Preparation of carbohydrates containing a terminal acetylene moiety is also readily achieved either by the alkylation of carbohydrates (to synthesize propargyl ethers) or Lewis-acid-catalysed glycosylation (to synthesize $O$-propargyl glycosides).\textsuperscript{19} The 1,3-DCR utilises low-cost and relatively non-toxic reagents and solvents so alleviating the need to use often expensive and highly toxic glycosylation and peptide coupling reagents, the risk and costs of which are acute on bulk scales. Finally, product purification is simple, in many cases chromatography is not necessary with either precipitation\textsuperscript{20} or liquid-liquid extraction sufficient to obtain pure product.

The incorporation of an azide and/or an alkyne moiety on a carbohydrate scaffold unleashes the potential to access a new dimension of structural diversity\textsuperscript{21} to complement that already inherent to carbohydrates and so it is anticipated that interest in the 1,3-DCR with carbohydrate substrates will grow. A detailed synthetic analysis concerning the utility of this reaction and the stability of the formed glycosyl triazole linkage towards typical carbohydrate reaction sequences would be beneficial to assess the viability of the glycosyl triazole linker as a tool for carbohydrate chemistry, and was the inspiration for the study described herein. Specifically, we report (i) an investigation of the rate of formation of the glycosyl triazole linkage with variable solvent, Cu\textsuperscript{1} catalyst and temperature parameters, (ii) the stability of
Chapter Two

this linkage towards conditions commonly used in carbohydrate reaction sequences, including hydroxyl group protection/deprotection, O-glycosylations and nucleophilic displacement, (iii) an examination of the retention of anomeric stereochemistry and the rates of triazole formation with respect to the glycosyl azide precursor configuration (α/β), and (iv) the versatility of the 1,3-DCR of a various sugar azides with a diverse array of acetylene partners.

2.2 Results and discussion

2.2.1 Rate of triazole formation: influence of solvent and catalyst system

The effect of solvent and the type Cu₁ catalyst upon the rate of triazole formation and the regio- and stereoselectivity was examined using a model 1,3-dipolar cycloaddition reaction (Table 2.1). The model glycosyl triazole 2.2.15f,22 was prepared by the 1,3-DCR of propargyl alcohol with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl azide 2.1 using procedures adapted from the literature (Scheme 2.2).12,13,20 Homogeneous mixtures were observed using organic solvents (entries 1-4) at the designated temperatures. Triazole formation proceeded smoothly and complete retention of anomeric configuration and exclusive 1,4-regiochemistry was observed in all cases. In terms of product conversion and isolated yield of the triazole product, variable results were achieved. The reaction in THF at 40°C (entry 3) is complete within 1.5 hours and lowering the temperature to 25°C (entry 4) does not substantially affect the rate, with reaction completion evident by TLC within 4 hours. In both cases, the yields were excellent (94% and 95%, respectively). The analogous reaction in toluene and acetonitrile (entries 1 and 2, respectively) resulted in slightly diminished yields (73% and 82%) and slower product conversion (12 and 8 hours, respectively).
The 1,3-DCR of propargyl alcohol with \textbf{2.1} was conducted using “aqueous click chemistry” in the presence of a CuSO$_4$/ascorbate mixture in aqueous alcohol (entries 5-10). The regiospecific and stereoselective formation of the \( \beta \)-D-glucosyl triazole \textbf{2.2} was observed and has been confirmed by X-ray crystallography (Figure 2.1).\textsuperscript{23} Irrespective of the alcohol co-solvent employed, the reaction to form \textbf{2.2} was complete within 2 hours (as evidenced by TLC) when the reaction was carried out at a slightly elevated temperature (40\textdegree C) (Table 2.1, entries 5-10). Noteworthy is that when the coupling partner propargyl alcohol was employed also as bulk co-solvent this did not substantially increase the rate of reaction. An increased reaction time (8 hours) was necessary to afford complete conversion when the reaction is conducted at room temperature (Table 2.1, compare entries 5 and 10); although the yields were similar (90 \textit{cf.} 92\%). The reactants were initially insoluble in all solvent media, but as the reaction progressed, a deep yellow, homogeneous mixture was observed. In all cases, the yield (following liquid–liquid extraction into CH$_2$Cl$_2$) was high and exhibited minimal variability (79–92\%). This simple work up provided material sufficiently pure for most ensuing synthetic purposes, however a final solid-phase extraction (SPE) purification step was performed to remove trace paramagnetic Cu(II) salts for the purpose of NMR analysis and the provision of analytically pure material. Reaction conditions were simple, requiring only vigorous stirring at the designated temperature within capped scintillation vials.
Table 2.1 Study of formation of glycosyl triazole 2.2 under variable solvent and catalyst conditions.$^{a,b}$

![Diagram of chemical reaction](image)

<table>
<thead>
<tr>
<th>Entry$^{a,b}$</th>
<th>solvent</th>
<th>temp (°C)</th>
<th>time (h)</th>
<th>yield$^c$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Toluene</td>
<td>40</td>
<td>~12</td>
<td>73</td>
</tr>
<tr>
<td>2</td>
<td>Acetonitrile</td>
<td>40</td>
<td>8</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>THF</td>
<td>40</td>
<td>1.5</td>
<td>94</td>
</tr>
<tr>
<td>4</td>
<td>THF</td>
<td>25</td>
<td>4</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>methanol-H$_2$O (1:1)</td>
<td>40</td>
<td>2</td>
<td>79</td>
</tr>
<tr>
<td>6</td>
<td>ethanol-H$_2$O (1:1)</td>
<td>40</td>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td>7</td>
<td>$i$-propanol-H$_2$O (1:1)</td>
<td>40</td>
<td>2</td>
<td>91</td>
</tr>
<tr>
<td>8</td>
<td>tert-butanol-H$_2$O (1:1)</td>
<td>40</td>
<td>2</td>
<td>92</td>
</tr>
<tr>
<td>9</td>
<td>tert-butanol-H$_2$O (1:1)</td>
<td>25</td>
<td>8</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>propargyl alcohol-H$_2$O (1:1)</td>
<td>40</td>
<td>2</td>
<td>85</td>
</tr>
</tbody>
</table>

$^a$Entries 1-4: Reactions were carried out using 2.1 (0.2 M), propargyl alcohol (0.5 M), 20 mol% CuI and DIPEA (0.2 M).

$^b$Entries 5-10: reactions were carried out using 2.1 (0.5 M), propargyl alcohol (0.5 M), 20 mol% CuSO$_4$, 40 mol% sodium ascorbate (relative to substrate) in 1:1 water/alcohol (with the exception of entry 10).

$^c$Yield calculated following liquid-liquid extraction into CH$_2$Cl$_2$. 
The crystal structure of $2.2$ indicates retention of anomeric stereochemistry as well as the regiospecific formation of the 1,4-disubstituted 1,2,3-glucosyl triazole. In the solid state, model triazole $2.2$ adopts a $^4C_1$ chair conformation typical of a D-gluco configuration.\textsuperscript{22} Interestingly, the $^1H$ NMR spectra in CDCl$_3$ at 298 K shows significant second order splitting for protons at positions C-1, C-2 and C-3 indicating a significant degree of ring distortion in solution.

The regioselectivity of the reaction is also verified by $^1H$ NMR and $^{13}C$ NMR chemical shift values of the obtained triazole products. The chemical shift for the H-5 triazole proton in $2.2$ (δ 7.78 ppm) and indeed all triazoles prepared in this thesis, are in good agreement with literature values.\textsuperscript{11a,12,15f,20,23} Chemical shift values for $^1H$ resonance signals in CDCl$_3$ are observed between δ 7.62–8.65 ppm in all cases. Similarly, the C-5 triazole resonance values are observed between δ 120–125 ppm.
and those attributable to the C-4 triazole are observed between \( \delta 142–145 \) ppm in the \(^{13}\text{C}\) NMR spectra in all cases. These values are consistent with those previously observed for 1,4-disubstituted 1,2,3-triazoles.\(^{11a,12}\) A gHSQC correlation between the C-5 carbon resonance signal to that of the triazole H-5 resonance signal is also consistent with this 1,4-regiochemistry assignment.

### 2.2.2 Stability of glycosyl triazole linkage toward synthetic manipulation

Next, the stability of the glycosyl triazole linkage towards commonly used carbohydrate chemistry reaction conditions was investigated (Scheme 2.2). Silyl, trityl, acetyl, benzoyl and benzyl alcohol protecting groups were all smoothly introduced on to the alcohol moiety of 2.2 to generate compounds 2.3–2.7, respectively, with the triazole linkage remaining intact. Reaction conditions to remove these protecting groups were also compatible with the triazole linkage, either regenerating 2.2 or the globally deprotected analogue 2.11. Protection and deprotection yields were excellent with no baseline decomposition observed by TLC. Interestingly, the hydrogenation of the benzylated compound 2.7 was sluggish using typical conditions (\( \text{H}_2, 5\% \text{ Pd/C} \)), necessitating basic hydrogenation conditions (i.e. \( \text{H}_2, 30\% \text{ Pd(OH}_2\text{)/C} \)). Removal of the benzyl ether in the C-2 position of the sugar was potentially problematic, quite possibly due to steric hindrance imposed by the glucosyl triazole. Despite this, the free glucosyl triazole 2.11 was recovered in good yield (78\%) after stirring for 24 hours at room temperature. Glycosylation of the triazole acceptor 2.2 using either typical Lewis acid-catalysis (BF\(_3\).Et\(_2\)O) or Koenigs-Knorr conditions (AgOTf) successfully generated 2.8 and 2.9, respectively, although yields were not high (53\% and 41\%, respectively). This was possibly due to the weak glycosyl acceptor nature of the triazole alcohol. Alternatively, the triazole may
interfere with activation of the glycosyl donor through interaction with the Lewis acid; nonetheless, this provides rapid entry into disaccharide mimics. Finally, the azide displacement of the crude mesylated intermediate from 2.2 to form the triazole azide 2.10 provides a system for carrying out iterative click reactions.

\[ \text{Scheme 2.2}^a \]

\*Conditions: a. TBDMSCl, imidazole, CH\(_2\)Cl\(_2\), rt., 1 h, 88%. b. TBAF, THF, rt., 15 min, 82%. c. TrCl, pyridine, 40\(^\circ\)C, o/n, 90%. d. camphor sulfonic acid, CH\(_2\)Cl\(_2\), rt., 4 h, 85%. e. BzCl, Et\(_3\)N, CH\(_2\)Cl\(_2\), o/n, 82%. f. Ac\(_2\)O/pyridine, rt., 2 h, 90%. g. NaH, BnBr, DMF, rt., o/n, 68%. h. 2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-glucopyranosyl bromide (1.0 equiv.), AgOTf, CH\(_2\)Cl\(_2\), 52% of 2.8. i. \(\beta\)-D-galactose pentaacetate (1.0 equiv.), BF\(_3\).Et\(_2\)O, CH\(_2\)Cl\(_2\), rt., o/n, 43% of 2.9. j. i) MsCl, Et\(_3\)N, CH\(_2\)Cl\(_2\), rt., 30 min; then ii) NaN\(_3\), DMF, o/n, rt., 53%. k. NaOCH\(_3\), CH\(_3\)OH, rt., 12 h, 92%. l. NaOCH\(_3\), CH\(_3\)OH, rt., 30 min, 90%. m. H\(_2\), 30% Pd(OH)\(_2\)/C, CH\(_3\)OH/CH\(_2\)Cl\(_2\), rt., 24 h, 78%.
2.2.3 Preparation of triazole-linked neoglycoconjugates

Having established some of the chemical modifications available, we then sought to prepare of a variety of glycoconjugates with diversity in both the carbohydrate and aglycone moieties. Derivatives of hydrophobic bioactive compounds and synthetic precursors to more complex glycoconjugates were sought as part of our general focus on carbohydrate-based therapeutics. Table 2.2 shows compounds synthesised from our model glucosyl azide 2.1 (compounds 2.12–2.15), methyl 2,3,4-tri-O-acetyl-1-azido-1-deoxy-β-D-glucopyranuronate24 (compound 2.16), 2,3,4-tri-O-acetyl-α-D-arabinopyranosyl azide (compound 2.17), 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (compound 2.1815f) and 2,3,4,6-tetra-O-benzyl-α-D-glucosyl azide (compound 2.19). Minimal or no chromatography is required in all cases to obtain samples of sufficient purity for further synthetic transformation. Reaction rates and yields varied somewhat depending on the acetylene dipolarophilicity and solubility. While the reaction is reported as reasonably insensitive to substrate solubility, more hydrophobic substrates generally reacted more sluggishly, as is shown for the steroidal glucosyl triazole 2.15. Interestingly, while the reaction for 2.15 took 20 hours to reach completion, the estradiol analogue 2.14 required only 30 minutes for completion under identical reaction conditions. Furthermore, both compounds were isolated by precipitation from cold water, although a final chromatographic purification step was required for these two compounds in order to remove traces of starting materials and Cu salts.
Table 2.2 Examples of 1,2,3-triazole glycoconjugates.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time (yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="" /></td>
<td>30 min (92%)</td>
</tr>
<tr>
<td><img src="image2" alt="" /></td>
<td>20 min (77%)</td>
</tr>
<tr>
<td><img src="image3" alt="" /></td>
<td>30 min (71%)</td>
</tr>
<tr>
<td><img src="image4" alt="" /></td>
<td>20 h (64%)</td>
</tr>
</tbody>
</table>
Our attention then shifted to examining the rate dependence on anomeric configuration and the nature of the saccharide protecting groups. The reaction of

\[ \text{H}_3\text{CO}_2\text{O} - \text{OAc} - \text{OAc} - \text{N}^\text{N} - \text{N}^\text{N} - \text{SO}_2\text{NH}_2 \]

30 min (85%)$^c$

2.16

\[ \text{AcO} - \text{OAc} - \text{OAc} \]

10 min (94%)

2.17

\[ \text{AcO} - \text{AcNH} - \text{N}^\text{N} - \text{OH} \]

3 h (62%)

2.18

\[ \text{BnO} - \text{BnO} - \text{BnO} - \text{BnO} - \text{N}^\text{N} - \text{OH} \]

48 h (73%)

2.19

\(^a\)General reaction conditions: azide (0.5 M), acetylene (0.5 M), CuSO\(_4\cdot5\)H\(_2\)O (0.2 equiv.), sodium ascorbate (0.4 equiv.), 1:1 tert-BuOH/H\(_2\)O, 40°C, with the exception of 19, which used acetylene (4.2 equiv.), CuSO\(_4\cdot5\)H\(_2\)O (0.4 equiv.), sodium ascorbate (0.8 equiv.) at 60°C. \(^b\)Standard purification as described in the Experimental. \(^c\)Molar yield calculated after precipitation and filtration.
Chapter Two

2,3,4,6-tetra-O-benzyl-α-D-glucosyl azide to the α-glucosyl triazole 2.19 proceeded with complete α-retention of anomeric stereochemistry. The reaction was slow, requiring a total of ca. 48 hours at 60°C to reach completion. Doubling of the CuSO₄ and sodium ascorbate catalyst load (i.e. 0.4 equiv. CuSO₄, 0.8 equiv. ascorbate) was also required since gradual oxidation of the Cu⁺-complex intermediate was observed using standard conditions with no product converse evident after 8 hours by either TLC or ESI-MS. The sluggish nature of this reaction was rationalized as resulting from increased steric bulk surrounding the anomeric centre upon transformation from the linear azide to the triazole, increased hydrophobicity imparted by the benzyl groups, and stabilization of the azide dipole by the anomeric effect.

The scope of the reaction can be expanded further by installing the azide/acetylene functionality into other positions on the carbohydrate ring and/or on to different carbohydrate partners to serve as a convenient and direct route to triazole-tethered disaccharide mimics. The triazole-tethered disaccharide 2.20 was formed in 78% yield from the 1,3-DCR of a 6-azido glucose analogue and a glucose-derived propynyl ether. This yield is an improvement over the glycosylations on 2.2 to synthesize the structurally related triazole-tethered disaccharides 2.8 (52%) and 2.9 (43%). 1,3-DCR can therefore serve as a useful means of rapidly generating artificial cyclodextrins, glycodendrimers and glycopolymers under kinetically controlled conditions.
Chapter Two

2.20

Figure 2.2

2.3 Conclusions

The facile Cu¹-catalysed 1,3-dipolar cycloaddition reaction has emerged of late as a potent tool in accessing diverse molecular architectures. Its versatility and potential is now starting to be realized in glycochemistry, providing expedient access to an interesting and relatively new family of pharmacologically relevant heterocyclic carbohydrates. Owing to the inherent complex nature of carbohydrate chemistry, especially concerning anomeric stereochemistry and stability, the reaction has not been sufficiently scrutinized as a viable alternative or addition to classical methods. We have successfully explored the utility of the reaction by examining the compatibility and tolerance of the conditions to pre-installed saccharide protecting groups, but also the stability of the installed triazole linkage under protection/deprotection sequences and glycosylations. The reaction is forgiving, mild, high yielding, simple to purify, and stereo- and regiospecific. Owing to such impressive versatility, we envisage the reaction to be useful addition to the arsenal of traditional solution-based reactions within carbohydrate chemistry. Further exploration of the scope of this chemistry will also include modification of other hydrophobic drug molecules to improve their water solubility. Furthermore, due to the inherent robustness of the glycosyl triazole linkage, it is reasonable to expect the
reaction to be compatible with existing solution- and solid-phase, convergent (block) and linear glycoprotein/peptide strategies. Finally, recent regioselective access to the 1,5-disubstituted triazole from azide and alkyne precursors will expand the structural diversity available from the chemistry presented here.29

2.4 Experimental

2.4.1 General
Glycosyl azide precursors were prepared by phase transfer nucleophilic displacement of corresponding peracetylated α-glycosylbromide.16 All reagents were purchased from commercial sources and were used without further purification. All solvents were available commercially dried or freshly dried and distilled prior to use. THF was freshly distilled from sodium benzophenone ketal. Toluene, pyridine and dichloromethane were distilled from calcium hydride and stored over activated 4Å sieves. Methanol was refluxed over magnesium and a catalytic quantity of iodine prior to distillation and storage over activated 3Å sieves. Reaction progress was monitored by TLC using Silica gel-60 F254 plates with detection by short wave UV fluorescence (λ = 254 nm) and staining with 10% (v/v) H2SO4 in ethanol char. Flash chromatography was conducted using Merk flash silica gel 60 (60–240 mesh). Solid phase extraction (SPE) was performed using Phenomenex Strata® cartridges prepacked with silica (SI-1).

1H NMR (400 MHz), 13C{1H} NMR (100 MHz), 2D gCOSY and gHSQC spectra were recorded on a Varian Unity 400 MHz spectrometer with chemical shift values given in ppm (δ) using deuterated solvent as specified. 13C NMR spectra were recorded at 100 MHz and referenced to either δ 77 ppm (CDCl3) or δ 39.5 ppm
Chapter Two

(DMSO-\textit{d}_\textit{6}). Melting points were recorded on a GallenKamp Variable Temperature Apparatus by capillary method and are reported as uncorrected. Mass spectra were recorded on a Fisons VG platform II and a Waters Micromass ZQ4000 spectrometer employing a single quad dual source and using electrospray as the ionisation technique in positive and negative ion modes. A waters HPLC system 600 (controller and pump) delivered isocratic streams for direct injections (CH\textsubscript{3}CN–H\textsubscript{2}O eluents). High resolution electrospray ionisation mass spectra were recorded on a Bruker Daltonix 4.7T Fourier transform ion cyclotron resonance mass spectrometer (FTICRMS) fitted with an Apollo ESI source in positive ion or negative ion as stated. Elemental analyses were conducted by the University of Queensland Microanalytical service. X-ray diffraction data collection and data refinement was conducted on a Rigaku AFC-7R diffractometer using MSC/AF7 Diffractometer Control Software. Data collections and refinements were performed by Prof. Peter Healy and Mr. Alan White at the Eskitis Institute and school of Biomolecular and Physical Sciences, Griffith University.

2.4.2 Note on nomenclature

Glycosyl triazoles are named according to IUPAC-IUBMB “Nomenclature of Carbohydrates” ([www.chem.qmw.ac.uk/iupac/]	extsuperscript{30} and the literature.\textsuperscript{15f,20} Compound names and NMR assignments are given with preference to the triazole ring. Compound 2.2 is given as an example.
2.4.3 Preparation of compounds 2.12-2.20: General procedure

To a vigorously stirring suspension or solution of the azide (0.5 M) and acetylene (0.5 M) in the selected co-solvent, was added a solution of CuSO$_4$.5H$_2$O (0.2 equiv.) and sodium ascorbate (0.4 equiv.) in distilled H$_2$O. The deep yellow mixture was stirred vigorously at 40 °C and the reaction progress was monitored by TLC. Once complete, the crude mixture was extracted with 3 equal amounts of CH$_2$Cl$_2$, combined, dried (MgSO$_4$), filtered and evaporated to afford the glycosyl triazole as off-white to white solid. A portion or the entire crude solid was purified by SPE, flash chromatography or recrystallization from hot absolute ethanol to afford analytically pure compound.

2.4.4 Analytical data

4-Hydroxymethyl-1-(2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (2.2).

To a vigorously stirring suspension of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl azide (2.1, 2.0 g, 5.35 mmol) in tert-butyl alcohol (11 mL) was added propargyl alcohol (1.3 mL, 22.43 mmol, 4.2 equiv.). The reaction was initiated by the addition of a solution of CuSO$_4$.5H$_2$O (270 mg, 1.08 mmol, 0.2 equiv.) and sodium ascorbate (424.5 mg, 2.14 mmol, 0.4 equiv.) in distilled H$_2$O (11 mL). The deep yellow suspension was stirred vigorously at 40°C for 2 hours when found complete by TLC. Distilled H$_2$O (20 mL) was added and the aqueous layer extracted with CH$_2$Cl$_2$ (2 × 50 mL). The combined organic extracts were dried (MgSO$_4$), filtered and evaporated to afford a crude yellow solid. Recrystallisation from hot absolute ethanol afforded the title compound as colourless crystals (2.1 g, 92%). $R_f$ 0.22 (1:4 hexanes–EtOAc);
Chapter Two

Mp 150-151°C (Lit. 15f 148–150°C as a mixture of 4- and 5-hydroxymethyl regioisomers). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.87 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.06 (s, 3H, OAc), 3.99 (ddd, $^3$J$_{5'-4'} = 10.2$ Hz, $^3$J$_{5'-6'} = 5.2$ Hz, $^3$J$_{5'-6'} = 2.4$ Hz, 1H, H$_5'$), 4.13 (dd, $^2$J$_{6'-6''} = 12.4$ Hz, $^3$J$_{6'-5'} = 2.4$ Hz, 1H, H$_6'$), 4.28 (dd, $^2$J$_{6'-6''} = 12.4$ Hz, $^3$J$_{6'-5'} = 5.2$ Hz, 1H, H$_6''$), 4.79 (s, 2H, CH$_2$OH), 5.20–5.25 (m, 1H, H$_4'$), 5.38–5.45 (m, 2H, H$_3$/H$_2'$), 5.86–5.88 (m, 1H, H$_1'$), 7.78 (s, 1H, triazole H); $^{13}$C{$^1$H} NMR (100 MHz, CDCl$_3$) $\delta$ 20.4 (OAc), 20.7 (OAc), 20.9 (OAc), 56.6 (CH$_2$OH), 61.8 (C$_6$), 67.9 (C$_2$), 70.6 (C$_3'$), 72.9 (C$_4'$), 75.3 (C$_5'$), 86.1 (C$_1'$), 120.9 (triazole CH), 148.5 (triazole C), 169.3 (OAc C=O), 169.6 (OAc C=O), 170.2 (OAc C=O), 170.7 (OAc C=O). HRMS (ESI) calcd for C$_{17}$H$_{23}$N$_3$O$_{10}$Na$: 452.12756$. Found: 452.12695. Anal. calcd for C$_{17}$H$_{23}$N$_3$O$_{10}$: C, 47.55; H, 5.40; N, 9.79. Found: C, 47.33; H, 5.39; N, 9.51.

Colourless crystals of 2.2 (C$_{17}$H$_{23}$N$_3$O$_{10}$) suitable for X-ray crystallographic analysis was obtained from hot absolute ethanol: $M = 429.38$, monoclinic, space group P2$_1$, $a = 14.8103$ (19), $b = 9.3896$ (16), $c = 7.6195$ (11)Å, $\beta = 104.504$ (10)$^0$, $V = 104.504$ (10)Å$^3$, $T = 295.2$ K, $Z = 2$, $D_x = 1.390$ Mg m$^{-3}$, $\mu$(Mo-K$\alpha$) = 0.116 mm$^{-1}$, 2399 reflections measured, 1922 unique ($R_{int} = 0.0181$). Refinement converged to $R_F =$ 0.0379, $wR(F^2) = 0.1096$.

4-tert-Butyldimethylsilyloxymethyl-1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (2.3).

A solution of the alcohol 2.2 (200 mg, 0.47 mmol) and imidazole (95 mg, 1.40 mmol, 3 equiv.) was prepared in anhydrous CH$_2$Cl$_2$ (5 mL) under nitrogen and tert-
butyldimethylsilyl chloride (85 mg, 0.56 mmol, 1.2 equiv.) was added in a single portion. The solution was stirred at room temperature under nitrogen for 2 hours when found complete by TLC. CH₂Cl₂ (5 mL) was then added and the solution and the organic layer washed with 1N HCl (5 mL), saturated NaHCO₃ (5 mL) and brine (5 mL). The organic layer was then dried (MgSO₄), filtered and evaporated to a crude oil, which was purified by flash silica chromatography (2:3 EtOAc-hexanes) to afford the title compound as white solid (225 mg, 0.41 mmol, 88%). Rf 0.74 (1:4 hexanes–EtOAc); Mp 100–101°C. ¹H NMR (400 MHz, CDCl₃) δ 0.08 (s, 6H, 2 × SiCH₃), 0.90 (s, 9H, tert-butyl), 1.85 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.06 (s, 3H, OAc), 3.98 (ddd, 3J₅'-₆' = 10.4 Hz, 3J₅'-₆'' = 5.2 Hz, 3J₅'-₆'''' = 2.4 Hz, 1H, H₅'), 4.14 (dd, 2J₆'-₆'' = 12.8 Hz, 3J₆'-₅'' = 2.4 Hz, 1H, H₆'), 4.23 (dd, 2J₆''-₆'''' = 12.8 Hz, 3J₆''-₅'''' = 5.2 Hz, 1H, H₆'''), 4.82 (s, 2H, CH₂OSi), 5.21–5.26 (m, 1H, H₄'), 5.37–5.41 (m, 1H, H₃'), 5.44–5.48 (m, 1H, H₂'), 5.85 (d, 3J₁'-₂' = 9.2 Hz, 1H, H₁'), 7.67 (s, 1H, triazole CH), ¹³C(¹H) NMR (100 MHz, CDCl₃) δ –5.2 (SiCH₃), –5.1 (SiCH₃), 18.5 (tert-butyl C), 20.4 (OAc), 20.71 (OAc), 20.74 (OAc), 20.9 (OAc), 26.1 (tert-butyl CH₃), 57.9 (CH₂OSi), 61.8 (C₆'), 67.9 (C₄'), 70.4 (C₃'), 73.0 (C₂'), 75.3 (C₅'), 85.8 (C₁'), 120.1 (triazole CH), 149.6 (triazole C), 169.1 (OAc), 169.6 (OAc), 170.1 (OAc), 170.7 (OAc). HRMS (ESI) caleed for C₂₃H₃₇N₃O₁₀SiNa⁺: 566.214042. Found: 566.215711. Anal. caleed for C₂₃H₃₇N₃O₁₀Si: C, 50.81; H, 6.86; N, 7.73; Found: C, 50.89; H, 7.07; N, 7.61.

De-protection of silyl ether (2.3 → 2.2). A portion of the silyl ether 2.3 (25 mg, 0.05 mmol) was dissolved in anhydrous THF (1.0 mL) and a 1.0 M solution of tetrabutylammonium fluoride (500 µL, 0.5 mmol, 10 equiv.) was added. The solution was stirred for 15 minutes at room temperature when found complete by TLC.
Evaporation and purification by flash silica chromatography (1:4 hexanes–EtOAc) afforded the alcohol 2.2 as white solid (18 mg, 0.04 mmol, 82%).

4-Trityloxymethyl-1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (2.4).

To a solution of the alcohol 2.2 (200 mg, 0.47 mmol) in anhydrous pyridine (5 mL) was added trityl chloride (230 mg, 0.84 mmol, 1.8 equiv.). The solution was warmed to 40°C and stirred for 24 hours. The solvent was evaporated under reduced pressure and the residue purified by flash silica chromatography to afford a white solid. A final recrystallization from hot absolute ethanol to remove trace trityl alcohol afforded the title compound as a colourless crystalline solid (283 mg, 90%). $R_f$ 0.63 (3:7 hexanes–EtOAc); Mp 192–193°C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.87 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.09 (s, 3H, OAc), 4.00 (ddd, $^3$J$_{5\cdot4'}=10.0$ Hz, $^3$J$_{5\cdot6'}=4.8$ Hz, $^3$J$_{5\cdot6''}=2$ Hz, 1H, H$_5'$) 4.16 (dd, $^2$J$_{6\cdot6''}=12.8$ Hz, $3$J$_{6\cdot5'}=2.4$ Hz, 1H, H$_6'$), 4.28 (s, 2H, CH$_2$OTr), 4.31 (dd, $^2$J$_{6\cdot6''}=12.8$ Hz, $3$J$_{6\cdot5'}=5.2$ Hz, 1H, H$_6'$) 5.23–5.28 (m, 1H, H$_4'$), 5.39–5.44 (m, 1H, H$_3'$), 5.47–5.51 (m, 1H, H$_2'$), 5.87 (d, $^3$J$_{1\cdot2'}=9.6$ Hz, 1H, H$_1$), 7.22–7.32 (m, 10H, $Ar$ H), 7.46–7.49 (m, 5H, $Ar$ H), 7.75 (s, 1H, triazole CH); $^{13}$C{1H} NMR (100 MHz, CDCl$_3$) $\delta$ 20.4 (OAc), 20.76 (OAc), 20.77 (OAc), 20.9 (OAc), 58.8 (C$_5$'), 61.8 (C$_6$'), 68.0 (C$_4$'), 70.4 (C$_2$'), 73.0 (C$_3$'), 75.4 (CH$_2$OTr), 85.9 (C$_1$'), 87.6 (Tr C), 120.5 (triazole CH), 127.4 ($Ar$ CH), 128.2 ($Ar$ CH), 128.9 ($Ar$ CH), 143.8 (triazole C), 147.1 ($Ar$ C), 169.1 (OAc), 169.6 (OAc), 170.2 (OAc), 170.7 (OAc). HRMS (ESI) calec for C$_{36}$H$_{37}$N$_3$O$_{10}$Na$: 694.237116$. Found 694.237058. Anal. calec for C$_{36}$H$_{37}$N$_3$O$_{10}$: C, 64.37; H, 5.55; N, 6.26. Found: C, 64.12; H, 5.38; N, 6.35.
Chapter Two

**Deprotection of trityl ether (2.4 → 2.2).** A portion of the trityl ether 2.4 (42 mg, 0.06 mmol) was dissolved in 1:1 CH₂Cl₂–CH₃OH (3 mL). A catalytic amount of camphorsulfonic acid was then added and the solution was stirred vigorously at 40°C for 1 hour when found complete by TLC. The solution was neutralized by the addition of triethylamine (10 μL, 0.07 mmol) and extracted with CH₂Cl₂ (2 × 2 mL). The combined organic extracts were washed with distilled H₂O (4 mL) and the organic layer was dried (MgSO₄), filtered and evaporated. The resulting crude residue was purified by gradient flash silica chromatography (1:4 EtOAc–hexanes to neat EtOAc) to afford the alcohol 2.2 as white solid (22 mg, 0.05 mmol 85%).

4-Benzoyloxymethyl-1-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (2.5).

To a solution of the alcohol 2.2 (200 mg, 0.47 mmol) in anhydrous CH₂Cl₂ (5 ml) and triethylamine (195 μL, 1.41 mmol, 3 equiv.) was added benzoyl chloride (52 μL, 0.71 mmol, 1.5 equiv.) drop-wise. The reaction stirred at room temperature for 2 hours when found complete by TLC. CH₂Cl₂ (5 mL) was then added and the organic layer washed consecutively with 1N HCl (2 × 5 mL), saturated NaHCO₃ (2 × 5 mL) and brine (5 mL). The organic layer was dried (MgSO₄), filtered and evaporated to afford crude, off-white solid. Recrystallisation from hot absolute ethanol afforded the title compound as an off-white solid (220 mg, 82%). Rₛ 0.53 (3:7 hexanes–EtOAc); Mp 180–181°C. ¹H NMR (CDCl₃, 400 MHz) δ 1.82 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.06 (s, 3H, OAc), 3.99 (ddd, 3J₅′-₆′ = 10.2 Hz, 3J₅′-₆″ = 5.2 Hz, 3J₅′-₆‴ = 2.0 Hz, 1H, H₅′), 4.13 (dd, 2J₆‴-₆‴ = 12.8 Hz, 3J₆‴-₅‴ = 2.0 Hz, 1H, H₆‴), 4.28 (dd, 2J₆‴-₆‴ = 12.8 Hz, 3J₆‴-₅‴ = 4.8 Hz, 1H, H₆‴), 5.20–5.25 (m, 1H, H₄‴), 5.37–5.41
Deprotection of benzoyl ester (2.5 → 2.11). A portion of the benzoyl ester 2.6 (25 mg, 0.047 mmol) was suspended in anhydrous methanol (2 mL) and a 1.0 M solution of sodium methoxide (10 μL, 0.01 mmol) in anhydrous methanol was added. The pale yellow solution was stirred overnight at 40°C to effect complete benzoyl deprotection. TLC indicated reaction completion after 12 hours (1:4 hexanes–EtOAc and 1:9 H2O–CH3CN). The solution was neutralized by the addition of Amberlite IR-120 acidic ion-exchange resin, filtered and evaporated to dryness under reduced pressure affording the pentol 2.11 as a white solid (10 mg, 0.04 mmol, 92%).
solvent under reduced pressure afforded the crude off-white solid. Recrystallization from hot absolute ethanol afforded the title compound as an off-white amorphous solid (198 mg, 90%). $R_f$ 0.48 (3:7 hexanes–EtOAc); Mp 143–144°C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.86 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.08 (s, 3H, OAc), 3.98 (ddd, $^3$J$\gamma$,$\gamma'$ = 10.0 Hz, $^3$J$\gamma$,$\gamma''$ = 4.8 Hz, $^3$J$\gamma$,$\gamma'''$ = 2.0 Hz, 1H, H$\gamma$), 4.14 (dd, $^2$J$\gamma$,$\gamma''$ = 12.8 Hz, $^3$J$\gamma$,$\gamma'''$ = 2.0 Hz, 1H, H$\gamma''$), 4.29 (dd, $^2$J$\gamma$,$\gamma'''$ = 12.8 Hz, $^3$J$\gamma$,$\gamma''''$ = 5.2 Hz, 1H, H$\gamma'''$), 5.20 (s, 2H, CH$_2$OAc), 5.21–5.24 (m, 1H, H$\gamma$), 5.39–5.41 (m, 2H, H$_2$$\gamma$, H$_3$), 5.84–5.86 (m, 1H, H$\gamma$), 7.82 (s, 1H, triazole CH); $^{13}$C {$^1$H} NMR (100 MHz, CDCl$_3$) $\delta$ 20.3 (OAc), 20.69 (OAc), 20.72 (OAc), 20.9 (OAc), 21.0 (OAc), 57.6 (C$_5$), 61.7 (C$_6$), 67.9 (C$_4$), 70.5 (CH$_2$OAc), 72.8 (C$_3$), 75.4 (C$_2$), 86.0 (C$_1$), 122.3 (triazole CH), 143.9 (triazole C), 169.1 (OAc), 169.5 (OAc), 170.1 (OAc), 170.7 (OAc), 171.0 (OAc). HRMS (ESI) calcd for C$_{19}$H$_{25}$N$_3$O$_{11}$Na$: 494.13810. Found: 494.137458. Anal. calcd for C$_{19}$H$_{25}$N$_3$O$_{11}$: C, 54.03; H, 5.10; N, 7.88. Found: C, 53.91; H, 5.12; N, 7.74.

Deprotection of peracetate (2.6 → 2.11). A portion of the per-O-acetate (100 mg, 0.21 mmol) was suspended in anhydrous methanol (3 mL) and a 1.0 M solution of sodium methoxide in anhydrous methanol (30 μL, 0.03 mmol) was added. The resulting pale yellow solution was stirred at room temperature for 15 minutes before reaction completion. The solution was neutralized by the addition of Amberlite IR-120 acidic ion exchange resin, filtered and evaporated to dryness under reduced pressure to afford the pentol 2.11 as white solid (49 mg, 0.19 mmol, 90%). See compound 2.11 for analytical details.
To a solution of the pentol 11 (140 mg, 0.54 mmol) in anhydrous DMF (5 mL) was cooled to 0°C under nitrogen. Sodium hydride (60% w/w mineral oil dispersion) [130 mg, 3.24 mmol, 6 equiv.] was then added in a single portion. The mixture was allowed to warm to room temperature and stirred for an additional 30 minutes before benzyl bromide (380 μL, 3.24 mmol, 6.0 equiv.) was added. The deep yellow solution was stirred at room temperature for 8 hours. The solution was then cooled again to 0°C and methanol was added gradually. The mixture was concentrated in vacuo and CH₂Cl₂ (20 mL) was added. The organic extract was washed consecutively with 1N HCl (2 × 5 mL), saturated NaHCO₃ (2 × 5 mL) and brine (5 mL). The organic layer was dried (MgSO₄), filtered and evaporated under reduced pressure to afford a pale yellow oil. Purification by flash silica chromatography (1:4 EtOAc–hexanes) afforded the title compound as white crystalline solid (330 mg, 0.46 mmol, 86%). Rf 0.43 (2:3 hexanes–EtOAc); Mp 98–99°C. \(^1\)H NMR (400 MHz, CDCl₃) δ 3.65–3.76 (m, 3 H, H₅', H₆', H₆'') 3.78–3.89 (m, 2H, H₃', H₄'), 4.02–4.06 (m, 2H, H₂'), 4.07–4.09 (m, 2H, CH₂OBn), 4.47–4.56 (m, 4H, CH₂Ph), 4.59–4.67 (m, 2H, CH₂Ph), 4.67–4.73 (m, 2H, CH₂Ph), 4.88–4.95 (m, 2H, CH₂Ph), 5.96 (d, \(^3\)J₁₋₂' = 9.6 Hz, 1H, H₁'), 6.93–6.97 (m, 2H, OBN CH), 7.16–7.19 (m, 4H, OBN CH), 7.27–7.34 (m, 19H, OBN CH), 7.66 (s, 1H, triazole CH); \(^{13}\)C{\(^1\)H} NMR (100 MHz, CDCl₃) δ 63.8 (CH₂Ph), 68.6 (C₆'), 72.2 (CH₂Ph), 73.8 (CH₂Ph), 75.1 (CH₂OBn), 75.4 (CH₂Ph), 76.0 (CH₂Ph), 77.5 (C₃' or C₄'), 78.2 (C₅'), 81.0 (C₂'), 85.7 (C₃' or C₄'), 87.8 (C₁'), 122.2 (triazole CH), 127.94 (Ph C), 127.99, 128.01, 128.03, 128.06, 128.09, 128.17, 128.18, 128.23, 128.46, 128.59, 128.64, 128.67, 128.72, 137.2,
Chapter Two


Deprotection of perbenzyl ether 2.7 → 2.11. The perbenzyl ether 2.6 (20 mg, 0.03 mmol) was dissolved anhydrous 1:2 CH₂Cl₂–CH₃OH (~ 2 mL) and a catalytic amount of activated 30% Pd(OH)₂/C was added. The vessel was placed under nitrogen, evacuated and then placed under an atmosphere of hydrogen and stirred at room temperature for 24 hours. TLC indicated near reaction completion after ca. 24 hours (1:4 hexanes–EtOAc and 1:9 H₂O–CH₃CN). The mixture was filtered on celite and eluted several times with methanol. Evaporation of the filtrate afforded the pentol 11 as clear gum (6 mg, 0.02 mmol, 78%).

[1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]methyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (2.8). A solution of the alcohol 2.2 (100 mg, 0.23 mmol) and 2,3,4,6-tetra-O-acetyl-α-D-glucosyl bromide (172 mg, 0.42 mmol, 1.8 equiv.) in anhydrous CH₂Cl₂ (5 mL) was prepared under nitrogen. Silver trifluoromethansulfonate (66 mg, 0.26 mmol, 1.1 equiv.) was then added and the mixture stirred at room temperature for 4 hours when found complete by TLC (3:7 hexanes–EtOAc). The solution was neutralized by the addition of DIPEA (100 μL, 0.57 mmol). Insoluble silver salts were removed by filtration on celite and the crude
product was eluted with CH\textsubscript{2}Cl\textsubscript{2} (~20 mL). Concentration of the filtrate under reduced pressure and flash silica chromatography of the crude residue (2:3 EtOAc–hexanes) afforded the title compound as white foam (91 mg, 0.12 mmol, 52%). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 1.88 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.97 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.10 (s, 3H, OAc), 3.74 (dd, 1H, \(^{3}J_{5-4} = 10.2\) Hz, \(^{3}J_{5-6} = 4.4\) Hz \(^{3}J_{5-6'} = 2.4\) Hz, 1H, GlcO H\textsubscript{5}), 3.99 (ddd, \(^{3}J_{5'-4'} = 10.4\) Hz, \(^{3}J_{5'-6'} = 4.8\) Hz, \(^{3}J_{5'-6''} = 2.0\) Hz, 1H, GlcN H\textsubscript{5'}), 4.13 (dd, \(^{2}J_{6'-6} = 12.4\) Hz, \(^{3}J_{6'-5'} = 2.0\) Hz, 1H, GlcO H\textsubscript{6'}), 4.26–4.32 (m, 2H, GlcN H\textsubscript{6'}, GlcO H\textsubscript{6}), 4.54 (d, \(^{3}J_{1'-2'} = 8.0\) Hz, 1H, GlcO H\textsubscript{1}), 4.78–4.92 (m, 2H, \(\text{CH}_2\text{O}\)), 4.98 (dd, \(^{3}J_{2-3} = 9.6\) Hz, \(^{3}J_{2-1} = 8.4\) Hz, 1H, GlcO H\textsubscript{2}), 5.06–5.11 (m, 1H, GlcO H\textsubscript{4}), 5.15–5.20 (m, 1H, GlcO H\textsubscript{3}), 5.21–5.26 (m, 1H, GlcN H\textsubscript{4'}), 5.39–5.42 (m, 2H, GlcN H\textsubscript{2', H3'}), 5.84–5.86 (m, 1H, GlcN H\textsubscript{1'}), 7.80 (s, 1H, triazole H); \textsuperscript{13}C \{\textsuperscript{1}H\} NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\) 20.3 (OAc), 20.72 (2 × OAc), 20.79 (3 × OAc), 20.86 (OAc), 20.89 (OAc), 61.7 (GlcN or GlcO C\textsubscript{6'/6'}), 62.0 (GlcN or GlcO C\textsubscript{6'/6'}), 62.1 (\(\text{CH}_2\text{O}\)), 67.9 (GlcN C\textsubscript{4'}), 68.6 (GlcO C\textsubscript{4}), 70.6 (GlcN C\textsubscript{2'}, or C\textsubscript{3'}), 71.3 (GlcO C\textsubscript{2}), 72.0 (GlcO C\textsubscript{5'}), 72.6 (GlcN C\textsubscript{2'} or C\textsubscript{3'}), 72.8 (GlcO C\textsubscript{3}), 75.5 (GlcN C\textsubscript{5'}), 86.1 (GlcN C\textsubscript{1'}), 99.0 (GlcO C\textsubscript{1}), 122.3 (triazole CH), 169.55 (OAc), 169.57 (OAc), 169.62 (OAc), 170.05 (OAc), 170.1 (OAc), 170.4 (OAc), 170.6 (OAc), 170.9 (OAc). HRMS (ESI) calcd for C\textsubscript{31}H\textsubscript{41}N\textsubscript{3}O\textsubscript{19}Na\textsuperscript{+}: 782.22267. Found: 782.223136. Anal. calcd for C\textsubscript{31}H\textsubscript{41}N\textsubscript{3}O\textsubscript{19}: C, 49.01; H, 5.44; N, 5.53; O, 40.02. Found: C, 48.93; H, 5.61; N, 5.17.
[1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (2.9). A solution of the alcohol 2.2 (157 mg, 0.37 mmol) and β-D-galactose pentaacetate (143 mg, 0.37 mmol, 1 equiv.) in anhydrous CH₂Cl₂ (5 mL) was prepared under nitrogen and cooled to 0°C. BF₃·Et₂O (113 μL, 0.92 mmol, 2.5 equiv.) was then added dropwise and the reaction was allowed to warm to room temperature. The reaction was stirred over night under nitrogen when found complete by TLC. CH₂Cl₂ (5 ml) was added and washed with saturated NaHCO₃ (2 × 5 mL) and brine (5 mL). The organic layer was dried (MgSO₄), filtered and evaporated to a pale yellow oil. Purification by flash silica chromatography afforded the title compound as a clear gum which slowly crystallized on standing in the refrigerator (121 mg, 0.16 mmol, 43%). Mp106–107°C. ¹H NMR (400 MHz, CDCl₃) δ 1.85 (s, 3H, OAc), 1.92 (s, 3H, OAc), 1.93 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.12 (s, 3H, OAc), 3.92–3.96 (m, 1H, Gal H₅'), 4.00 (ddd, 3J₅'-₄' = 7.2 Hz, 3J₅'-₆'' = 2 Hz, 1H, Glc H₅'), 4.07–4.13 (m, 2H, Glc H₆'', Gal H₆'), 4.21 (dd, 2J₆-₆'' = 11.6 Hz, 3J₆-₅ = 6.4 Hz, 1H, Gal H₆), 4.27 (dd, 2J₆'-₆'' = 12.8 Hz, 3J₆'-₅' = 5.2 Hz, 1H, Glc H₆'), 4.46 (d, 3J₁-₂ = 8.0 Hz, 1H, Gal H₁), 4.76–4.92 (m, 2H, CH₂O), 5.17 (dd, 3J₂-₃ = 10.8 Hz, 3J₂-₁ = 8.0 Hz, 1H, Gal H₂), 4.98 (dd, 3J₃-₂ = 10.4 Hz, 3J₃-₄ = 3.2 Hz, 1H, Gal H₃), 5.21–5.26 (m, 1H, Glc H₄), 5.35–5.43 (m, 3H, Glc H₂', Glc H₃', Gal H₄), 5.85 (d, 3J₁-₂ = 8.8 Hz, 1H, Glc H₁'), 7.80 (s, 1H, triazole H); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 20.3 (OAc), 20.69 (OAc), 20.71 (OAc), 20.75 (OAc), 20.83 (2 × OAc), 20.87 (OAc), 20.9 (OAc), 61.5 (CH₂O), 61.6 (Gal C₆), 61.7 (Glc C₆'), 67.3
4-Azidomethyl-1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (2.10).

To a solution of the alcohol 2.2 (200 mg, 0.47 mmol) in anhydrous CH₂Cl₂ (5 mL) was added triethylamine (195 μL, 1.41 mmol, 3 equiv.) and methanesulfonyl chloride (55 μL, 0.71 mmol, 1.5 equiv.). The solution was stirred at room temperature under nitrogen for 30 minutes when found complete by TLC. CH₂Cl₂ (5 mL) was then added and the solution was washed consecutively with 1N HCl (2 × 5 mL), saturated NaHCO₃ (5 mL) and brine (5 mL). The organic layer was dried (MgSO₄), filtered and evaporated to afford the mesylate as a pale yellow oil. The crude mesylate was immediately dissolved in anhydrous DMF (2 mL) and NaN₃ (153 mg, 2.35 mmol, 5 equiv.) was added. The mixture was warmed to 40°C and stirred for 24 hours. The mixture was concentrated in vacuo and CH₂Cl₂ (5 mL) was added. The extract was washed with distilled H₂O (2 mL) and brine (2 mL). The organic layer was dried (MgSO₄), filtered and evaporated to afford a crude off white solid. Recrystallization from hot absolute ethanol afforded the title compound as an amorphous white solid (102 mg, 0.25 mmol, 53%). Rₜ 0.39 (1:4 hexanes–EtOAc); Mp 110–111°C. ¹H NMR (400 MHz, CDCl₃) δ 1.87 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.07 (s, 3H, OAc), 4.00 (ddd, 3J₅'-₆' = 10.4 Hz,
3J5′-6′ = 5.2 Hz, 3J5′-6″ = 2.4 Hz, 1H, H5′), 4.14 (dd, 2J6′-6″ = 12.8 Hz, 3J6″-5′ = 2.4 Hz, 1H, H6″), 4.30 (dd, 2J6′-6″ = 12.4 Hz, 3J6″-5′ = 5.2 Hz, 1H, H6″), 4.48 (s, 2H, CH2N3), 5.20–5.25 (m, 1H, H4′), 5.38–5.44 (m, 2H, H-2, H3′), 5.84–5.90 (m, 1H, H1′), 7.79 (s, 1H, triazole CH); 13C{1H} NMR (100 MHz, CDCl3) δ 20.3 (OAc), 20.7 (OAc), 20.8 (OAc), 20.9 (OAc), 45.7 (N3CH2), 61.7 (C6′), 67.9 (C4′), 70.5 (C3′), 72.7 (C2′), 75.5 (C5′), 86.1 (C1′), 121.0 (triazole CH), 169.2 (OAc), 169.6 (OAc), 170.1 (OAc), 170.7 (OAc). HRMS (ESI) calcd for C17H22N6O9Na+: 477.134047. Found 477.134673.

Anal. cacld for C17H22N6O9: C, 44.94; H, 4.88; N, 18.50. Found: C, 44.72; H, 4.90; N, 18.27.

1-(β-D-Glucopyanosyl)-4-hydroxymethyl-1,2,3-triazole (2.11). White solid (various yields: see text). Rf 0.15 (1:9 H2O–CH3CN); Mp 162–163°C. 1H NMR (400 MHz, D2O) δ 3.46–3.50 (m, 1H, H4′), 3.55–3.67 (m, 3H, H3′, H5′, H6′), 3.75–3.78 (m, 1H, H6″), 3.84–3.89 (m, 1H, H2′), 4.60 (s, 2H, C-H2OH), 5.61 (d, 3J1′-2′ = 9.2 Hz, 1H, H1′), 8.06 (s, 1H, triazole CH); 13C{1H} NMR (100 MHz, D2O) δ 54.7 (CH2OH), 60.6 (C6′), 69.1 (C4′), 72.4 (C2′), 76.1 (C3′), 79.0 (C5′), 87.6 (C1′), 123.5 (triazole CH). HRMS (ESI) calcd for C9H15N3O6Na+: 284.085310. Found: 284.085457.

4-Pyridin-2-yl-1-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (2.12). White amorphous solid (92%). Rf 0.42 (1:4 hexanes–EtOAc); Mp 195–196°C. 1H NMR (400 MHz, CDCl3) δ 1.87 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.08
Chapter Two

(3H, OAc), 4.01 (ddd, \( J_{5'\cdot A'\cdot 4'} = 10.4\) Hz, \( J_{5'\cdot 6'} = 4.8\) Hz, \( J_{5'\cdot 6''} = 2.0\) Hz, 1H, H5'), 4.15 (dd, \( J_{6'\cdot 6''} = 12.8\) Hz, \( J_{6'\cdot 5'} = 2.0\) Hz, 1H, H6'), 4.29 (dd, \( J_{6''\cdot 6'} = 12.4\) Hz, \( J_{6''\cdot 5'\cdot 6'} = 4.8\) Hz, H6'), 5.23–5.28 (m, 1H, H4'), 5.40–5.45 (m, 1H, H3'), 5.50–5.55 (m, 1H, H2'), 5.90 (d, \( J_{1'\cdot 2'} = 9.2\) Hz, 1H, H1'), 7.30–7.33 (m, 1H, Ar H), 7.85–7.88 (m, 1H, Ar H), 8.21–8.23 (m, 1H, Ar H), 8.65 (s, 1H, triazole H); \(^{13}\)C\{\(^{1}\)H\} NMR (100 MHz, CDCl\(_3\)) \( \delta \) 20.4 (OAc), 20.73 (s, 3H, OAc), 20.74 (s, 3H, OAc), 20.9 (s, OAc), 61.7 (C6'), 67.9 (C4'), 70.7 (C3'), 72.9 (C2'), 75.3 (C5'), 86.1 (C1'), 120.7 (triazole CH), 121.0 (Ar C), 123.5 (Ar CH), 137.3 (Ar CH), 148.9 (triazole C), 149.5 (Ar CH), 149.6 (Ar C), 169.0 (OAc), 169.5 (OAc), 170.2 (OAc), 170.7 (OAc). HRMS (ESI) calcd for C\(_{21}\)H\(_{24}\)N\(_4\)O\(_9\)Na+: 499.143549. Found: 499.142640. Anal. calcd for C\(_{21}\)H\(_{24}\)N\(_4\)O\(_9\): C, 52.44; H, 5.08; N, 11.76. Found: C, 52.17; H, 5.04; N, 11.36.

### 4-Amidomethyl-L-\( \text{N} \)BocVal-1-(2',3',4',6'-tetra-O-acetyl-\( \beta \)-D-glucopyranosyl)-1,2,3-triazole

(2.13). White foam (77%). \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 0.85 (d, \( J_{CH-CH} = 6.4\) Hz, 3H, \(^{3}\)Pr CH3), 0.91 (d, \( J_{CH-CH} = 7.2\) Hz, \(^{3}\)Pr CH3), 1.41 (s, 9H, 3 \( \times \) tert-butyl CH3), 1.84 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.06 (s, OAc), 2.13 (m, 1H, \(^{3}\)Pr CH), 3.92 (m, 1H, Boc NH), 3.98 (ddd, \( J_{5'\cdot A'\cdot 4'} = 10.2\) Hz, \( J_{5'\cdot 6'} = 4.4\), \( J_{5'\cdot 6''} = 2.0\) Hz, 1H, H5'), 4.12 (dd, \( J_{6''\cdot 6'} = 12.8\) Hz, \( J_{6''\cdot 5'\cdot 6'} = 4.8\) Hz, 1H, H6'), 4.26 (br s, 2H, CH\(_2\)CONH), 5.06–5.10 (m, 1H, \(^{3}\)Pr CH), 5.20–5.25 (m, 1H, Boc NH), 5.37–5.43 (m, 1H, H2'), 5.83–5.85 (m, 1H, H1'), 6.73 (br s, 1H, CONH), 7.78 (s, triazole CH); \(^{13}\)C\{\(^{1}\)H\} NMR (100 MHz, CDCl\(_3\)) \( \delta \) 17.8 (\(^{3}\)Pr CH3), 19.5 (\(^{3}\)Pr CH3), 20.3 (OAc), 20.7 (OAc), 20.7 (OAc), 20.9 (OAc), 28.5 (\( \text{tert} \)-butyl C), 31.0 (\(^{3}\)Pr CH),
35.0 (CH$_2$CONH), 60.0 ([Pr C), 61.70 (C$_5$’), 67.9 (C$_6$’), 70.5 (C$_2$’), 72.8 (C$_3$’), 75.3 (C$_4$’), 86.0 (C$_1$’), 156.1 (Boc C=O), 169.0 (OAc), 169.5 (OAc), 170.2 (OAc), 170.7 (OAc), 170.6 (CONH). HRMS (ESI) calcd for C$_{27}$H$_{41}$N$_5$O$_{12}$Na$: 650.26439. Found: 650.26406.

4-(17α-Estradiol)-1-(2’,3’,4’,6’-tetra-O-acetyl-β-D-glucopyranosyl]-1,2,3-triazole (2.14). Pale yellow solid (71%). Mp 133–134°C. $^1$H NMR (400 MHz, CDCl$_3$) δ 0.45–0.60 (m, 1H, estradiol H$_{18}$), 1.02 (s, 3H, estradiol CH$_3$), 1.20–1.60 (m, 6H, estradiol), 1.83 (s, 3H, OAc), 1.85–1.91 (m, 2H, estradiol CH$_2$), 1.96–2.12 (m, 6H, estradiol H), 2.02 (s, 3H, OAc), 2.05 (s, 6H, 2 × OAc), 2.46–2.54 (m, 2H, estradiol CH$_2$), 2.70 (br s, 1H, OH), 2.72–2.81 (m, 2H, estradiol), 3.99 (ddd, $^3$J$_{5’-4’}$ = 10.0 Hz, $^3$J$_{5’-6’}$ = 4.8, $^3$J$_{5’-6’’}$ = 2.0 Hz, 1H, H$_{5’}$), 4.14 (dd, $^2$J$_{6’-6’}$ = 12.8 Hz, $^3$J$_{6’-5’}$ = 2.0 Hz, 1H, H$_{6’}$), 5.22–5.27 (m, 1H, H$_{4’}$), 5.37–5.42 (m, 1H, H$_{3’}$), 5.44–5.48 (m, 1H, H$_{2’}$), 5.86 (d, $^3$J$_{1’-2’}$ = 8.0 Hz, 1H, H$_{1’}$), 6.52–6.58 (m, 3H, Ar CH), 7.02–7.04 (m, 1H, Ar CH), 7.70 (s, 1H, triazole CH); $^{13}$C($^1$H) NMR (100 MHz, CDCl$_3$) δ 14.4 (Estradiol C$_{18}$), 20.3 (OAc), 20.7 (OAc), 20.8 (OAc), 20.9 (OAc), 23.9 (estradiol), 26.5 (estradiol), 27.5 (estradiol), 29.8 (estradiol), 33.0 (estradiol), 38.20 (estradiol), 39.6 (estradiol), 43.6 (estradiol), 47.6 (estradiol), 48.6 (estradiol), 61.8 (C$_6$’), 68.0 (C$_4$’), 70.5 (C$_2$’), 72.9 (C$_3$’), 75.3 (C$_5$’), 82.8 (estradiol C$_{17}$), 85.9 (C$_1$’), 112.9 (Ar CH), 115.5 (Ar CH), 120.3 (triazole CH), 126.5 (Ar CH), 132.5 (Ar C), 138.4 (Ar C), 153.9 (triazole C), 154.8 (phenol...
C), 169.1 (OAc), 169.7 (OAc), 170.2 (OAc), 170.9 (OAc). HRMS (ESI) calcd for 
C\textsubscript{34}H\textsubscript{43}N\textsubscript{3}O\textsubscript{11}Na\textsuperscript{+}: 692.27898. Found: 692.279186.

4-Ethisterone-1-(2',3',4',6'-tetra-O-acetyl-\beta-D-glucopyranosyl)-1,2,3-triazole

(2.15). White crystalline solid (64%). Mp 124–125\textdegree{}C. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \) 
0.32–0.396 (m, 1H, ethisterone CH), 0.65–0.71 (m, 1H, ethisterone CH), 1.04 (s, 3H, 
ethisterone CH\textsubscript{3}), 1.16 (s, 3H, ethisterone CH\textsubscript{3}), 1.33–1.66 (m, 7H, ethisterone), 2.01 
(s, 3H, OAc), 2.02–2.04 (m, 4H, ethisterone), 2.05 (s, 3H, OAc), 2.06 (s, 6H, 2 \times 
OAc), 2.22–2.41 (m, 5H, ethisterone), 2.44–2.52 (m, 1H, ethisterone CH), 3.99 (ddd, 
\( ^3J_{5'\cdot 4'} = 10.0 \text{ Hz}, ^3J_{5'\cdot 6'} = 4.8 \text{ Hz}, ^3J_{5'\cdot 6''} = 2.0 \text{ Hz}, 1H, H_5' \)), 4.14 (dd, \( ^2J_{6'\cdot 5'} = 12.8 \text{ Hz}, 
^3J_{6'\cdot 6''} = 2.0 \text{ Hz}, 1H, H_6' \)), 4.30 (dd, \( ^2J_{6'\cdot 5'} = 12.8 \text{ Hz}, ^3J_{6'\cdot 6''} = 4.8 \text{ Hz}, 1H, H_6' \)), 5.20– 
5.25 (m, 1H, H\textsubscript{4'}), 5.36–5.44 (m, 2H, H\textsubscript{2'} and H\textsubscript{3'}), 5.68 (s, 1H, ethisterone C\textsuperscript{=}CH), 
5.83–5.85 (m, 1H, H\textsubscript{1'}), 7.66 (s, 1H, triazole CH); \textsuperscript{13}C\{\textsuperscript{1}H\} NMR (100 MHz, CDCl\textsubscript{3}) 
\( \delta \) 14.4 (ethisterone CH\textsubscript{3}), 17.6 (ethisterone CH\textsubscript{3}), 20.3 (OAc), 20.7 (OAc), 20.8 
(OAc), 20.9 (OAc), 24.1, 31.8, 32.7, 33.0, 34.1, 35.9, 36.5, 38.0, 38.8, 47.1, 49.0, 
53.6 (ethisterone), 61.3 (C\textsubscript{6'}), 68.0 (C\textsubscript{4'}), 70.5 (C\textsubscript{2'} or C\textsubscript{3'}), 72.8 (C\textsubscript{2'} or C\textsubscript{3'}), 75.4 (C\textsubscript{5'}) 
82.5 (ethisterone C\textsubscript{17}), 86.0 (C\textsubscript{1'}), 119.9 (triazole CH), 124.0 (ethisterone C\textsuperscript{=}CH), 
154.5 (triazole C), 168.9 (OAc), 169.6 (OAc), 170.1 (OAc), 170.7 (ethisterone 
C\textsuperscript{=}CH), 171.4 (OAc), 199.7 (ethisterone C\textsuperscript{=}O). HRMS (ESI) calcd for 
C\textsubscript{35}H\textsubscript{47}N\textsubscript{3}O\textsubscript{11}Na\textsuperscript{+}: 708.31028. Found: 708.310775. Anal. calcd for C\textsubscript{35}H\textsubscript{47}N\textsubscript{3}O\textsubscript{11}: C, 
61.30; H, 6.91; N, 6.13. Found: C, 61.31; H, 6.99; N, 5.98.
4-(4-Sulfamoylamidomethyl)-1-(methyl 2',3',4'-tri-O-acetyl-β-D-glucuronate)-1,2,3-triazole (2.16).

Off-white amorphous solid (85%). $R_f$ 0.08 (1:4 hexanes–EtOAc); Mp 221–222°C (decomp). $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 1.76 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.98 (s, 3H, OAc), 3.59 (s, 3H, CO$_2$CH$_3$), 4.51 (d, $^3J_{\text{CH2-NH}}$ = 5.2 Hz, 2H, NHCH$_2$), 4.76 (d, $^3J_{5^\prime-4^\prime}$ = 10.0 Hz, 1H, H$_5^\prime$), 5.19–5.24 (m, 1H, H$_4^\prime$), 5.44–5.59 (m, 1H, H$_3^\prime$), 5.72–5.76 (m, 1H, H$_2^\prime$), 6.34 (d, $^3J_{1^\prime-2^\prime}$ = 9.6 Hz, 1H, H$_1^\prime$), 7.45 (br s, 2H, SO$_2$NH$_2$), 7.86–8.01 (m, 4H, Ar CH), 8.35 (s, 1H, triazole H), 9.23 (t, $^3J_{\text{NH-CH2}}$ = 5.6 Hz, 1H, CONH); $^{13}$C$_1$($^1$H) NMR (DMSO-$d_6$, 100 MHz) $\delta$ 20.6 (OAc CH$_3$), 20.9 (OAc CH$_3$), 21.0 (OAc CH$_3$), 35.5 (CH$_2$NH), 53.3 (CO$_2$CH$_3$), 69.0 (C$_4$), 70.4 (C$_2$), 72.3 (C$_3$), 73.5 (C$_5$), 84.4 (C$_1$), 122.9 (triazole CH), 126.3 (Ar CH), 128.7 (Ar CH), 137.6 (Ar C), 146.4 (Ar C), 147.1 (triazole C), 165.9 (C=O), 167.3 (C=O), 169.1 (C=O), 170.0 (C=O), 170.2 (C=O). HRMS (neg ion) calcd for C$_{23}$H$_{26}$N$_5$O$_{12}$S$: 596.130416. Found: 596.132499. Anal. calcd for C$_{23}$H$_{27}$N$_5$O$_{12}$S$: C, 46.23; H, 4.55; N, 11.72. Found: C, 46.10; H, 4.66; N, 11.51.

4-Methylenebenzotriazole-1-(2’,3’,4’-tri-O-acetyl-α-D-arabinosyl)-1,2,3-triazole (2.17).

White solid (94%). $R_f$ 0.21 (1:4 hexanes–EtOAc); Mp 215–216°C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.80 (s, 3H, OAc), 1.98 (s, 3H, OAc), 2.17 (s, 3H, OAc), 3.87 (dd, $^2J_{5^\prime-5''}$ = 13.6, $^3J_{5^\prime-4^\prime}$ = 1.2 Hz, 1H, H$_5^\prime$), 4.11 (dd, $^2J_{5''-5'}$ = 13.6 Hz, $^3J_{5''-4'}$ = 2.0 Hz,
Chapter Two

1H, H$_{5'}$), 5.19 (dd, $^3J_{3',2'} = 10.0$ Hz, $^3J_{3',4'} = 3.2$ Hz, 1H, H$_{3'}$), 3.58 (ddd, $^3J_{4',3'} = 3.6$ Hz, $^3J_{4',5'} = 2.0$ Hz, $^3J_{4',5'} = 1.2$ Hz, 1H, H$_{4'}$), 5.43–5.48 (m, 1H, H$_2$), 5.66 (d, $^3J_{1',2'} = 11.2$ Hz, 1H, H$_{1'}$), 5.92–6.03 (m, 2H, NCH$_2$), 7.33–7.37 (m, 1H, Ar H), 7.43–7.47 (m, 1H, Ar H), 7.64–7.66 (m, 1H Ar H), 7.81 (s, triazole CH), 8.03–8.05 (m, 1H, Ar H); $^{13}$C $^1$H NMR (100 MHz, CDCl$_3$) δ 20.3 (OAc), 20.7 (OAc), 21.1 (OAc), 43.9 (NCH$_2$), 67.5 (C$_5$), 67.8 (C$_4$), 68.4 (C$_2$), 70.5 (C$_3$), 87.0 (C$_1$), 110.2 (Ar CH), 120.1 (Ar CH), 121.9 (triazole CH), 124.3 (Ar CH), 127.9 (Ar CH), 143.8 (triazole C), 169.1 (OAc), 170.0 (OAc), 170.3 (OAc); HRMS (ESI) calcd for C$_{20}$H$_{22}$N$_6$O$_7$Na+: 481.144218. Found: 481.14411. Anal. calcd for C$_{20}$H$_{22}$N$_6$O$_7$: C, 52.40; H, 4.84; N, 18.33. Found: C, 52.39; H, 4.94; N, 18.05.

1-(2'-Deoxy-2'-acetamido-3',4',6'-tri-O-acetyl-β-D-glucopyranosyl)-4-[(hydroxymethyl)-1,2,3-triazole (2.18).$^{15f}$

Off-white foam (62%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.57 (s, 3H, NHAc), 1.92 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.98 (s, 3H, OAc), 3.99–4.03 (dd, $^3J_{6',5'} = 12.4$ Hz, $^3J_{6',4'} = 2.4$ Hz, 1H, H$_{5'}$), 4.09–4.13 (dd, $^3J_{6',5'} = 12.4$ Hz, $^3J_{6',4'} = 5.2$ Hz, 1H, H$_{6'}$), 4.19 (ddd, $^3J_{5',4'} = 10.0$ Hz, $^3J_{5',6'} = 4.8$ Hz, $^3J_{5',6'} = 2.4$ Hz, 1H, H$_{5'}$), 4.47 (d, $^3J_{CH2-OH} = 4.4$ Hz, 2H, CH$_2$OH), 5.04–5.09 (m, 1H, H$_{3'}$), 5.20–5.21 (m, 1H, CH$_2$OH), 5.29–5.34 (m, 1H, H$_{4'}$), 6.06 (d, $^3J_{1',2'} = 10.0$ Hz, 1H, H$_{1'}$), 8.04 (d, $^3J_{NH-2'} = 9.6$ Hz, 1H, NHAc NH), 8.09 (s, 1H, triazole CH); $^{13}$C $^1$H NMR (100 MHz, $d_6$ DMSO) δ 25.7 (OAc), 25.9 (OAc), 26.0 (OAc), 27.8 (NHAc), 57.4 (C$_2$), 60.0 (CH$_2$OH), 67.3 (C$_4$), 73.4 (C$_5$), 77.9 (C$_3$), 78.7 (C$_6$), 90.0 (C$_1$), 126.8 (triazole CH), 153.6 (triazole C), 174.8 (C=O), 174.9 (C=O), 175.0 (C=O), 175.5 (C=O). HRMS (ESI) calcd for C$_{17}$H$_{24}$N$_4$O$_9$Na$: 541.143549. Found: 451.142863.
**4-Hydroxymethyl-1-(2’,3’,4’,6’-tetra-O-benzyl-α-D-glucopyranosyl)-1,2,3-triazole (2.19).** To a vigorously stirring solution of 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl azide (100 mg, 0.18 mmol) in hot tert-butyl alcohol (500 μL at ca. 60°C), was added propargyl alcohol (45 μL, 0.74 mmol, 4.2 equiv.) and a solution of CuSO₄·5H₂O (18 mg, 0.08 mmol, 0.4 equiv.) and sodium ascorbate (30 mg, 0.15 mmol, 0.8 equiv.) in distilled H₂O (500 μL). The deep yellow suspension was stirred at 60°C for 2 days, at which time TLC indicated reaction completion (1:1 EtOAc–hexanes). The aqueous phase was then extracted with CH₂Cl₂ (2 × 10 mL), and the combined organic extracts were dried (MgSO₄), filtered and evaporated to afford a pale yellow syrup, which crystallized on standing to afford a white amorphous solid. (79 mg, 0.13 mmol, 73%). Rᶠ 0.19 (1:1 EtOAc–hexanes); Mp 82–83°C. ¹H NMR (400 MHz, CDCl₃) δ 3.51 (dd, 2J₆’–₆” = 10.8 Hz, 3J₆’–₅’ = 2.0 Hz, 1H, H₆’), 3.67 (dd, 2J₆”–₆’ = 10.8 Hz, 3J₆”–₅” = 2.8 Hz, 1H, H₆”), 3.81 (dd, 3J₄’–₅’ = 10.0 Hz, 3J₄’–₃’ = 8.4 Hz, 1H, H₄’), 3.89–3.93 (m, 1H, H₅’), 4.02 (dd, 3J₂’–₃’ = 9.6 Hz, 3J₂’–₁’ = 6.0 Hz, 1H, H₂’), 4.39–4.53 (m, 2H, CH₂Ph), 4.49–4.73 (m, 2H, CH₂Ph), 4.52–4.85 (m, 2H, CH₂Ph), 4.60–4.64 (m, 1H, H₅’), 4.80 (s, 2H, OCH₂), 4.84–4.94 (m, 2H, CH₂Ph), 5.80 (d, 3J₁’–₂’ = 5.6 Hz, 1H, H₁’), 7.13–7.16 (m, 4H, Ar CH), 7.25–7.33 (m, 16H, Ar CH), 7.51 (s, triazole CH); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 56.8 (OCH₂), 68.3 (C₆’), 73.7 (C₅’), 73.9 (CH₂Ph), 74.4 (CH₂Ph), 75.0 (CH₂Ph), 75.8 (CH₂Ph), 77.4 (C₄’), 78.8 (C₂’), 82.0 (C₃’), 84.3 (C₁’), 124.0 (triazole CH), 127.9, 127.93, 127.97, 128.1, 128.2, 128.4, 128.5, 128.59, 128.62, 128.7, 128.9 (11 × Ar CH) 137.7, 137.8, 138.3, 138.7 (4 × Ar C). HRMS cale for C₃₇H₃₉N₃O₆Na⁺: 644.273116. Found: 644.272986.
Methyl 2,3,4-tri-O-acetyl-6-deoxy-6-(4-\{(2,3,4,6-tetra-O-acetyl-\beta-D-glucopyranosyl)oxy\} methyl)-1H-1,2,3-triazol-1-yl)-\beta-D-glucopyranoside (2.20).

White foam (78%). \( R_f \) 0.43 (2:3 hexanes–EtOAc). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 1.96 (s, 3H, OAc), 1.99 (s, 6H, 2 × OAc), 2.00 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.08 (s, 6H, 2 × OAc), 3.14 (s, 3H, OCH\(_3\)), 3.72 (ddd, \( ^3J_{5.4} = 10.0 \) Hz, \( ^3J_{5.6} = 5.2 \) Hz, \( ^3J_{5.6'} = 2.8 \) Hz, 1H, \( \beta\) Glc H\(_5\)), 4.14 (dd, \( ^2J_{6.6'} = 12.4 \) Hz, \( ^3J_{6.5} = 2.4 \) Hz, 1H \( \beta\) Glc H\(_6\)), 4.17–4.20 (m, 1H, \( \alpha\) Glc H\(_5\)), 4.26 (dd, \( ^2J_{6'.6} = 12.4 \) Hz, \( ^3J_{6'.5} = 4.62 \), 1H, \( \beta\) Glc H\(_6\)), 4.39 (dd, \( ^2J_{6'.6} = 14.4 \) Hz, \( ^3J_{6.5} = 8.4 \) Hz, 1H, \( \alpha\) Glc H\(_6\)), 4.56 (dd, \( ^2J_{6'.6} = 14.6 \) Hz, \( ^3J_{6'.5} = 2.4 \) Hz, 1H, \( \alpha\) Glc H\(_6\)), 4.52 (d, \( ^3J_{1.2} = 8.4 \) Hz, 1H, \( \beta\) Glc H\(_1\)), 4.73–4.82 (m, 3H, \( \alpha\) Glc H\(_3, \alpha\) Glc H\(_4, CH_2O\)), 4.89 (d, \( ^3J_{1.2} = 3.6 \) Hz, 1H, \( \alpha\) Glc H\(_1\)), 4.99 (dd, \( ^3J_{2.3} = 9.2 \) Hz, \( ^3J_{2.1} = 8.0 \) Hz, 1H, \( \beta\) Glc H\(_2\)), 5.05–5.09 (m, 1H, \( \beta\) Glc H\(_4\)), 5.14–5.19 (m, 1H, \( \alpha\) Glc H\(_5\)), 7.62 (s, 1H, triazole H); \(^{13}\)C\(_{\{1\}H}\) NMR (100 MHz, CDCl\(_3\)) \( \delta \) 50.9 (\( \beta\) Glc C\(_5\)) 55.8 (OCH\(_3\)), 62.0 (\( \beta\) Glc C\(_6\)), 62.9 (CH\(_2O\)), 67.8 (\( \alpha\) Glc C\(_3\)), 68.5 (\( \beta\) Glc C\(_4\)), 69.9 (\( \alpha\) Glc C\(_3\)), 70.0 (\( \alpha\) Glc C\(_2\)), 70.9 (\( \alpha\) Glc C\(_4\)), 71.3 (\( \beta\) Glc C\(_2\)), 72.1 (\( \beta\) Glc H\(_3\)), 72.9 (\( \alpha\) Glc C\(_3\)), 96.9 (\( \alpha\) Glc C\(_1\)), 99.7 (\( \beta\) Glc C\(_1\)), 124.6 (triazole CH), 144.4 (triazole C), 169.5 (OAc), 169.6 (OAc), 170.0 (OAc), 170.2 (OAc), 170.4 (OAc), 170.5 (OAc), 170.9 (OAc). HRMS (ESI) called for C\(_{30}H_{41}N_3O_{18}\)Na\(^+\): 754.227733. Found 754.226711. Anal. calcd for C\(_{30}H_{41}N_3O_{18}\): C, 49.25; H, 5.65; N, 5.74. Found: C, 48.92; H, 5.71; N, 5.49.
2.5 References


7. (a) Herzner, H., Reipen, T., Schultz, M., Kunz, H. Synthesis of glycopeptides containing carbohydrate and peptide recognition motifs. *Chem. Rev.* 2000, 100, 4495-4537; (b) Kiefel,
Chapter Two


12. TornØe, C. W., Christensen, C., Meldal, M. Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. *J. Org. Chem.* 2002, 67 (9), 3057-3064.


Chapter Two


23. (a) Elguegro, J. Gonzáles, E., Jacquier, R. Research in theazole series. XXVI. Nuclear magnetic resonance study and the reaction of 1,2,3-triazole with halo nitrobenzenes. *Bull.*
Chapter Two


25. Bodine, K. D., Gin, D. Y., Gin, M. S. Highly Convergent Synthesis of C3- or C2-Symmetric Carbohydrate Macrocycles. Org. Lett. 2005, 7 (20), 4479-4482. Note the α-azides in the mannose derivatives used here also react sluggishly as we observed in the synthesis of 2.19.


3 Inhibition of Carbonic Anhydrase by Glycoconjugate Benzene Sulfonamides

This chapter is an expanded version of the following articles:


3.1 Introduction

The carbonic anhydrase (CA, EC 4.2.1.1) zinc metalloenzymes are ubiquitous in all kingdoms and are encoded by four distinct and evolutionary unrelated gene families: the α-CAs (present in vertebrates, bacteria, algae and the cytoplasm of green plants), the β-CAs (predominantly found in bacteria, algae, chloroplasts of mono- and
dicotyledons), the γ-CAs (mainly in *Archaea* and some bacteria) and the δ-CAs, which are present in marine diatoms. These ancient enzymes are very efficient catalysts for the reversible hydration of carbon dioxide (CO$_2$) to bicarbonate anion (HCO$_3^-$) and a proton (H$^+$); a fundamental physiological reaction that underpins many regulatory processes associated with respiration and transport of CO$_2$/HCO$_3^-$ between metabolising tissues and the lungs, photosynthesis in higher plants, provision of HCO$_3^-$ for biosynthetic pathways (gluconeogenesis, lipogenesis and ureagenesis), pH regulation and CO$_2$ homeostasis, electrolyte and fluid secretion, as well as bone resorption and calcification. In mammals 16 different α-CA isozymes and CA related proteins (CARP) have been identified and characterised to date, 15 of which are present in humans (designated hCA; CA XV is absent in humans and primates but is present in rodents and other higher mammals). They exhibit variable enzyme kinetics, tissue distribution, expression levels and subcellular locations (Table 3.1). Several hCA isozymes are cytosolic (CA I–III, CA VII, CA XIII), four are membrane bound or transmembrane proteins (CA IV, CA IX, CA XII, CA XIV), 2 are mitochondrial (CA VA and CA VB) and one is secreted into the saliva and milk (CA VI). Many of these isozymes are quite recent discoveries compared with the physiologically abundant and widely distributed isozymes hCA I and hCA II (known since the 1930s, with isozymes acknowledged in the 1960s) and many have shown promise as druggable targets through inhibition of their enzymatic activity.
Table 3.1 The relative catalytic activity, affinity for sulfonamides, tissue distributions and sub-cellular locations of presently known human CA isozymes$^{1-5}$

<table>
<thead>
<tr>
<th>Isozyme</th>
<th>Catalytic activity</th>
<th>Affinity for sulfonamides$^a$</th>
<th>Subcellular localisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA I</td>
<td>Low (10% of that of CA II)</td>
<td>Medium</td>
<td>Cytosol</td>
</tr>
<tr>
<td>CA II</td>
<td>High</td>
<td>Very high</td>
<td>Cytosol</td>
</tr>
<tr>
<td>CA III</td>
<td>Very Low (0.3% of that of hCA II)</td>
<td>Very low</td>
<td>Cytosol</td>
</tr>
<tr>
<td>CA IV</td>
<td>High</td>
<td>High</td>
<td>Membrane-bound (GPI-anchored)</td>
</tr>
<tr>
<td>CA VA</td>
<td>Moderate-high$^*$</td>
<td>High</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>CA VB</td>
<td>High</td>
<td>High</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>CA VII</td>
<td>High</td>
<td>Very high</td>
<td>Cytosol</td>
</tr>
<tr>
<td>CARP VIII</td>
<td>Acatalytic</td>
<td>‡</td>
<td>Cytosol</td>
</tr>
<tr>
<td>CA IX</td>
<td>Moderate</td>
<td>High</td>
<td>Transmembrane</td>
</tr>
<tr>
<td>CARP X</td>
<td>Acatalytic</td>
<td>‡</td>
<td>Cytosol</td>
</tr>
<tr>
<td>CARP XI</td>
<td>Acatalytic</td>
<td>‡</td>
<td>Cytosol</td>
</tr>
<tr>
<td>CA XII</td>
<td>Low</td>
<td>High</td>
<td>Transmembrane</td>
</tr>
<tr>
<td>CA XIII</td>
<td>Moderate</td>
<td>Medium-high</td>
<td>Cytosol</td>
</tr>
<tr>
<td>CA XIV</td>
<td>Low</td>
<td>High</td>
<td>Transmembrane</td>
</tr>
<tr>
<td>CA XV</td>
<td>Low</td>
<td>Unknown</td>
<td>Membrane-bound (GPI-anchored)</td>
</tr>
</tbody>
</table>

$^a$ The native CARP isozymes do not possess catalytic activity owing to absence of Zn (II) active site. Their affinity for sulfonamides has not been measured.

$^*$ Moderate at pH 7.4, high at pH 8.2 and above.
Chapter Three

The dysregulation of human CA isozyme expression is implicated in a host of pathophysiological processes such as oncogenesis, elevated intraocular pressure, obesity, memory loss, depression, arteriosclerosis and renal pathology and the modulation of CA activity through inhibition or activation is a potential avenue for therapeutic intervention. Since the original report by Mann and Keilin concerning the inhibition of CA with sulfanilamide (3.1), primary aryl and heteroaryl sulfonamide CA inhibitors (CAIs) have become a clinical mainstay as antihypertensive, antiglaucoma, antithyroid and hypoglycemic drugs. The aryl- and heteroaryl sulfonamides acetazolamide (3.2), methazolamide (3.3) and bis-sulfonamide, dichlorophenamide (3.4) have been used clinically for over 40 years as systemic CA inhibitors. Acetazolamide 3.2 is the first non-mercurial diuretic used clinically.

The systemic administration of clinically used drugs 3.2–3.4 often causes serious side effects owing to the inhibition of CAs in other tissues, thus prompting the development of isozyme specific inhibitors as well tissue specific delivery systems. A major breakthrough in tissue specific delivery of CA-based therapeutics came with the advent of topically acting sulfonamide dorzolamide (3.5, Trusopt® 1995 Merk & Co) and soon thereafter the structurally related brinzolamide (3.6, Azopt® 1998 Alcon Laboratories) as potent inhibitors of hCA II and hCA XII within the ciliary processes of the eye. Both drugs have been in clinical use for the treatment of open-angle glaucoma since 1995.1-5,10
The classical recognition fragment necessary for CA inhibition, the primary arylsulfonamide (ArSO$_2$NH$_2$), has been identified by the pioneering work of Krebs.$^{11}$ The mechanism of catalysis and inhibition with primary arylsulfonamides is understood in great detail.$^{1-6}$ The sulfonamide anion (ArSO$_2$NH$^-$) coordinates to the CA active site Zn(II) in a tetrahedral geometry thereby inhibiting the catalytic ability of the enzyme. Early structure activity and optimisation studies of the aryl sulfonamide pharmacophore were primarily concerned with modifying the ring size, heteroatom positioning and substitution patterns, a strategy coined the “ring approach”. This strategy proved enormously successful, leading to the development of clinically used drugs and benchmark CA inhibitors, 3.2-3.7.$^{1,12}$
Important discoveries have emerged within the CA-based disciplines that have provided new opportunities and challenges for drug discovery. The inhibition of previously characterised human isozymes, hCA IV, VA, VB, VII, IX, XII and XIII, with a wide range of sulfonamides, sulfamates and sulfamides has provided valuable structure-activity relationship (SAR) data allowing for a much closer insight into the roles of these intriguing and ubiquitous enzymes in cellular mechanisms of pathology with the ultimate aim of designing isozyme specific inhibitors. In addition, recent X-ray diffractional studies of inhibitors bound to isozymes hCA I and II have provided valuable insights into the structural requirements of their inhibition, which is of invaluable assistance in the design isozyme-selective inhibitors.

Various representatives of the α-, β-, γ-, and δ-CA classes have recently been isolated from pathogenic bacteria, protozoa and fungi. Similar to animal and plant CA isozymes, these CAs are deemed critical for the survival of the organism and more significantly, exhibit catalytic activities quite distinct from the human isozymes. The elucidation of microbial isozyme structure and activity could ultimately lead to the development of selective CA inhibitors as novel anti-infective drugs for the treatment of pandemic, drug-resistant infections caused by *Helicobacter pylori*, *Mycobacterium tuberculosis*, *Plasmodium falciparum* to name a few.

With respect to inhibition of hCA isozymes, the therapeutic potential of aryl sulfonamides, sulfamates and sulfamides is becoming increasingly realised for treating a wide range of disease states, including epilepsy and related convulsive disorders, obesity and cancer. Selective inhibitors of the cytosolic and extracellular brain isozymes, including the newly cloned cytosolic isozyme hCA VII,
have potential in psychiatric medicine as pharmacological tools and as much needed therapeutic agents.\textsuperscript{18,19,20} Topiramate (3,9, TPM, Topimax\textsuperscript{®}),\textsuperscript{16,21} a fructopyranose sulfamate derivative and potent hCA VII inhibitor ($K_i$ 0.87 nM) and hCA II inhibitor ($K_i$ 5.0 nM)$^{16,22}$ is clinically used as an anticonvulsant drug for the treatment of epilepsy and Lennox-Gastaut syndrome and is also a potential indication for depression and migraine, possibly due in part to the potent inhibition of brain CA isozymes. In addition, activation of hCA catalytic activity in the CNS also holds promise as a treatment for a host of memory and learning disorders where synaptic efficacy needs to be enhanced, including depression, Alzheimer’s disease and ageing.\textsuperscript{23,24}

The extracellular hCA isozymes IX, XII, and XIV are transmembrane proteins possessing a single transmembrane domain and a catalytic site outside the cell. Similar to their cytosolic counterparts, the extracellular CA isozymes are essential regulators of extracellular pH and CO\textsubscript{2} concentrations. The maintenance of extracellular pH has been demonstrated to be closely associated with the formation of metabolon complexes (multiple membrane-bound proteins which have close physiological and functional contact) with anion exchangers and bicarbonate transporters. Unlike other hCA isozymes, hCA IX is a multi-domain transmembrane protein with a more complex tertiary structure and organisation. It is composed of four domains; an $N$-terminal proteoglycan domain implicated in cellular adhesion and proliferation, a CA catalytic domain which is efficiently inhibited by primary aryl sulfonamides, a transmembrane region and a short cytoplasmic tail.\textsuperscript{6,25,26} hCA IX is also expressed in healthy epithelial cells of the gastric mucosa where it is believed to play important roles in morphogenesis and cellular differentiation.\textsuperscript{6,24,26}
Recent investigations by Parkkila and colleagues have suggested that membrane bound isozymes hCA IX and XII are important for embryonic development and organogenesis owing to their expression and activity within several tissues of the mouse embryo including the brain, lung, pancreas and liver.\textsuperscript{26}

Of increasing interest and relevance in cancer chemotherapy is the association and overexpression of transmembrane isozymes, hCA IX and hCA XII on certain tumour cells. Of particular interest is the hCA IX isozyme which is found ectopically expressed in many hypoxic tissues through the transcriptional activation of hypoxia-induced factor 1 (HIF-1).\textsuperscript{6} hCA IX is therefore frequently found overexpressed on tumour cells (primarily carcinomas) within tissues not normally known to express hCA IX, including carcinomas of the lung, breast, colon, oesophagus and cervix.\textsuperscript{6} On the other hand, hCA IX expression is down-regulated during carcinogenesis within tissues that normally express hCA IX (e.g. gastric mucosa). The differential expression patterns of hCA IX has enabled it to function as a potential cancer biomarker for the early detection of gastric cancer and certain carcinomas.\textsuperscript{6,27} It has been confirmed that hCA IX is a high activity CA isozyme responsible for the extracellular acidification of the tumour microenvironment. Multiple downstream effects of reduced extracellular pH are associated with tumour progression and poor prognosis.\textsuperscript{6,28,29,30} The chemoresistance to the weakly basic drugs paclitaxel, topotecan and mitoxantrone is also an effect of lowered extracellular pH since they exist in a protonated form, hence impairing their ability to permeate the plasma membrane.\textsuperscript{6}
Research on the involvement of these isozymes in cancer has progressed considerably in recent years, particularly for hCA IX. The development of selective inhibitors of transmembrane hCA IX and XII isozymes could potentially lead to a new class of cancer chemotherapeutics and diagnostics.\textsuperscript{6,8,31} For example, Indisulam (3.8) is a potent hCA IX inhibitor \textit{in vitro} and \textit{in vivo} ($K_{i}$, 24 nM) and is currently in phase II clinical trials as an anticancer agent for the treatment solid tumors.\textsuperscript{6,16}

The transmembrane hCA XIV isozyme is predominantly expressed in the brain and CNS but is also expressed to a lesser degree in the kidney, colon, small intestine and urinary bladder.\textsuperscript{32,33} hCA XIV shares a similar kinetic profile (medium-low activity at physiological pH) and general topology with the other transmembrane isozymes, hCA IX and hCA XII. Unlike the latter isozymes, hCA XIV is not implicated in cancer and has a broader tissue distribution and hence physiological functions.\textsuperscript{32,33} Downstream physiological effects of hCA XIV mediated pH regulation and CO$_2$ homeostasis are not entirely apparent, although evidence exists to suggest a role in modulating excitatory synaptic transmissions within the CNS.\textsuperscript{31,32} A closer investigation into the implications of the inhibition of this relatively poorly understood CA isozyme will provide insight into its physiological function and its potential to molecular medicine will be realised.

Considerable achievements have been made with regard to inhibitor design and synthesis, as well as the isolation and structural characterisation of new isozymes of pathological relevance.\textsuperscript{1-6} Therefore, a main emphasis of current CA research is to develop selective tissue delivery systems of CA inhibitors and/or inhibitors which are selective at the isozyme, tissue and/or organism level.
Aryl and heteroaryl sulfonamide pharmacophores have become reliable scaffolds upon which medicinal chemists have appended tails to modify the physicochemical characteristics in order to improve isozyme selectivity, solubility, tolerability and delivery to diseased tissues. This conventional, fragment-based approach has been developed by Supuran and co-workers and is termed the “tail approach”.

The initial motivation for the development of the tail approach was the provision of water soluble sulfonamides and hCA II inhibitors as neutral and topically-active drugs for the treatment of glaucoma.

The preparation of aromatic sulfonamide inhibitors with polar or charged tails with an impaired ability to diffuse through lipid membranes is one possible strategy to selectively differentiate the extracellular isozymes from the physiologically dominant cytosolic isozymes hCA I and II. This could potentially lead to effective therapeutics with lowered toxicity and side effects arising from systemic hCA inhibition. To date, only relatively simple tail groups tethered to aryl sulfonamide scaffolds via amino, imino, hydroxy and hydrazino functionalities have been prepared and assessed as inhibitors of various extracellular isozymes. It is apparent that an exhaustive and more innovative study with regard to ligand structure is required to survey the active site architectures of these clinically relevant isozymes leading to the provision of isozyme selective CA inhibitors and diagnostics.

An alternative approach to equip drugs with improved solubility and tolerability is to attach carbohydrate moieties to generate glycoconjugates. However, this carbohydrate based strategy has not received significant attention by the pharmaceutical industry. In terms of their pharmacokinetic profile, carbohydrates
generally exist outside the industry-accepted parameters, leaving more traditional small organic molecules to be pursued for drug discovery.\textsuperscript{43}

It is, however important to note that there are several routes of drug administration apart from the oral delivery. In addition to their ability to modify the physicochemical properties of a drug, carbohydrates are in their own right powerful and flexible scaffolds for combinatorial library design and there are several examples which have appeared within the literature (and indeed the clinic) concerning the use of carbohydrates as either drug molecules, glycosylated pro-drugs and delivery systems, or as formulations for solubilising of poorly soluble chemotherapeutic agents.\textsuperscript{39-42}

Owing to the success of the tail approach to generate potent CA inhibitors and the extracellular location of the hCA IX active site, we explore the potential of tethering carbohydrate “tails” to the high affinity pharmacophore to prepare sulfonamide glycoconjugates. We reason that tethering carbohydrate tails to sulfonamides could, in addition to limiting membrane permeability, impart improved water solubility and tolerability to the CA inhibitor as well as offering the added advantage and opportunity to survey regions of biological space removed from the aryl sulfonamide core.

Despite the synthesis of a multitude of CA inhibitors prepared according to the tail strategy and the appreciable benefits of appending carbohydrate moieties to drug molecules, the combination of these two strategies to generate CA inhibitors is essentially unexplored. Prior to this work, only a single example existed in literature
Chapter Three

(nine compounds, which have not been evaluated against cancer relevant CA isozymes).44 This relatively untouched medicinal chemistry landscape has served as the primary inspiration for the compounds presented in the current study.

Within a short timeframe, the Cu\(^{1}\)-catalysed 1,3-DCR of an organic azide and acetylene (“click chemistry”) in aqueous solvents has emerged as a premier bioconjugation strategy and synthetic tool for the facile syntheses of small molecule libraries and as such, has had an appreciable impact on the drug discovery and biotechnology sectors.45 Motivated by the recent successful applications of click chemistry in drug discovery, we aim to apply this methodology to prepare glycoconjugate benzene sulfonamide libraries. We have developed a modular synthesis of three glycoconjugate benzene sulfonamide libraries using a novel “click-tailing” approach as a dual solubilising and isozyme differentiating strategy, and investigated their \textit{in vitro} inhibition at cytosolic isozymes hCA I and II and transmembrane extracellular isozymes hCA IX, XII and XIV.

3.2 Results and Discussion

3.2.1 Library design and synthesis

To facilitate the synthetic strategy it was necessary to synthesise CA recognition scaffolds that possessed dual functionality; both (i) an anchor for reliable CA affinity (i.e. an aryl sulfonamide), and (ii) either a terminal alkyne or azide moiety to act as the partner for click chemistry with our panel of sugar building blocks. Thus two complementary library formats were envisaged; A) reaction of the acetylene containing sulfonamide scaffold with glycosyl azide and/or deoxy sugar azide
compounds and B) reaction of an azide containing benzene sulfonamide scaffold with sugar acetylene compounds (Scheme 3.1).

\[ \text{Sulfonamide} + \text{N}_3\text{Sugar} \xrightarrow{\text{Cu}^+} \text{Sulfonamide} \]

\[ \text{Sulfonamide} + \equiv\text{Sugar} \xrightarrow{\text{Cu}^+} \text{Sulfonamide} \]

**Scheme 3.1**

Many ester and amide derivatives of 4-carboxybenzene sulfonamide (3.10) are potent (low nanomolar) inhibitors of hCA II.\(^{31}\) The amide derivatives also display good *in vivo* activity and prolonged duration as antiglaucoma agents.\(^{46}\) The N-propynyl carboxybenzamide derivative 3.11 and O-propynyl benzoyl ester derivative 3.12 were thus designed as the scaffolds for click chemistry with a panel of azido sugars according to library format A (library I). Scaffold 3.11 was prepared by HBTU-mediated amide coupling of 3.10 with propargyl amine. The molecular structure of 3.11 was resolved by X-ray crystallography and is in good agreement with spectroscopic and spectrometric analysis (Figure 3.3). The carboxy ester analogue (3.12) was prepared by the carbodiimide esterification of 3.10 using EDC in the presence of excess propargyl alcohol. A scaffold lacking the carboxy moiety, including 4-ethynyl benzene sulfonamide (3.13) was also prepared to determine the effect on CA inhibition and selectivity. The 4-ethynyl benzene sulfonamide (3.13) scaffold was prepared by a modified Sonagashira coupling\(^{47}\) of the corresponding phenyl iodide with trimethylsilyl acetylene in neat triethylamine, following desilylation using tetrabutylammonium fluoride in THF.
Figure 3.2

Figure 3.3
Thus, the acetylene containing scaffolds 3.11–3.13 were reacted with a panel of azide-functionalised sugars according to library format A to afford library I (from scaffolds 3.11 and 3.12) and library II (from scaffold 3.13). A panel of seven glycosyl azides (a–f, h) and an azido sugar (g) were investigated as carbohydrate tails for conjugation to scaffolds 3.11–3.13 (Figure 3.4). Glycosyl azides were prepared by a bimolecular nucleophilic displacement of the corresponding glycosyl halide with sodium azide under phase-transfer conditions.\textsuperscript{48} Methyl (2,3,4-tri-O-acetyl-\(\beta\)-D-glucopyranosyl azide)uronate (e) was prepared by a TEMPO-catalysed sodium hypochlorite oxidation of \(\beta\)-D-glucopyranosyl azide\textsuperscript{49} or via nucleophilic displacement of the corresponding \(\alpha\)-glycosylbromide.\textsuperscript{48} Methyl 6-azido-6-deoxy-2,3,4-tri-O-acetyl \(\alpha\)-D-glucopyranoside (g) was prepared from methyl-\(\alpha\)-D-
glucopyranoside according to the literature procedure. 2,3,5-Tri-O-benzoyl β-D-ribofuranosyl azide (h) was prepared by the Lewis acid-promoted azidolysis of the corresponding β-D-ribofuranosyl acetate with trimethylsilyl azide in the presence of stannic chloride promoter (5 mol%).

**Library I**

The N-carboxyamide scaffold 3.11 was reacted with each member of the azido sugars a–g to generate the amide series of peracetylated glycoconjugates according to format A (library I, Scheme 3.2).

![Scheme 3.2](image)

**Scheme 3.2**

Reagents and conditions: (i) azide (a–g) (1.0 equiv.), sodium ascorbate (0.2 equiv.), CuSO$_4$·5H$_2$O (0.1 equiv.), 1:1 tert-butanol–water, 40°C, 30 min–1h, 58-96% (ii) NaOCH$_3$, CH$_3$OH, rt, 2 h, quantitative.

Reactions were generally complete following 30 minutes of vigorous stirring at 40°C (as evidenced by TLC). Purification was straightforward in all cases, involving evaporation/concentration of the crude mixture followed by flash silica chromatography/filtration in order to remove trace starting materials and paramagnetic CuI salts. The amide series 3.11a–g was insoluble in most organic
solvents, although successful dissolution in methanolic dichloromethane or chloroform resulted in homogeneous mixtures that aided purification by flash chromatography or silica filtration. Mild de-O-acetylation of the chromatographed material employing methanolic sodium methoxide (pH 8-10) afforded the deprotected glycoconjugates 3.11a’–g’ in near quantitative yields.

With respect to the ester series of library I (3.12a–g and 3.12a’–g’), the deprotection of glycosylazide panel a–f and the methyl glucoside g was required prior to conjugation with the acetylene scaffold 3.12, since the ester linkage within the carboxy ester glycoconjugates 3.12a–g were labile under the deprotection conditions. Analysis of the crude mixture by TLC and ESI-MS suggests undesired cleavage of the sulfamoyl benzyl ester with concomitant formation of the methyl ester transesterification side-by product (3.15). In the case of the ester series, yields were somewhat lower (58–72%) compared to their protected counterparts owing to their high polarity and poor solubility in organic solvents.

**Library II**

In order to establish the effect of the carboxy ester and amide linker regions in protein binding as well as the provision of hydrolytically stable benzene sulfonamide glycoconjugates as CA inhibitors, the phenylacetylene scaffold 3.13 was reacted with glycosyl azides a,b,d–f and h according to library format A. In this manner, the per-O-acylated 1,2,3-triazole glycoconjugates (3.13a, 3.13b, 3.13d–f, and 3.13h) were prepared in excellent yields following simple flash chromatography/silica filtration (library II, Scheme 3.3). The effect of carbohydrate ring size and CA inhibition is also of interest and as such, the β-D-ribosyl azide h was included in this library.
Scheme 3.3

Reagents and conditions: (i) azide (a, b, d–f, h) (1.0 equiv.), sodium ascorbate (0.2 equiv.), CuSO$_4$.5H$_2$O (0.1 equiv.), tert-butanol–water, 40°C, 30 min–1h, 69-88% (ii) NaOCH$_3$, CH$_3$OH, rt, 15 min–2 h, near quantitative.

Deprotection of the per-O-acylated glycosyl triazoles 3.13a,b,d–f under identical conditions to that of library I furnishes the deprotected glycosyl triazoles 3.13a',b' 3.13d'–f' in near quantitative yields. Deprotection of the tri-O-benzoylated β-D-ribofuranosyl triazole 3.13h required a longer reaction time (2 hours) under slightly more forcing conditions (pH 10–11) employing methanolic sodium methoxide and was recovered in near quantitative yields.

Library III

There now exists ample evidence to suggest the therapeutic potential of O-glycosides both as pro-drugs (unmasked in vivo by glycosidases) and for drug targeting (lectin mediated drug targeting). Several complex O-glycosides of natural origin act as potent antitumour agents and antibiotics, and include the clinically used anthracyclines and bleomycins. Compelling spectroscopic and crystallographic evidence suggests the glycone moieties of these drugs to be essential structural motifs for the underlying DNA-binding and/or cleavage mechanisms.

Motivated in part by this unique biological activity, we sought to prepare an O-glycoside benzene sulfonamide library. In order to reverse the functionalities of
library building blocks, thereby enhancing the flexibility of library design and diversity, 4-azidobenzene sulfonamide 3.14 was selected as an azide-functionalised aryl sulfonamide for its conjugation to acetylene-functionalised sugar building blocks to assemble a library of traizaole-tethered O-glycoside benzene sulfonamides according library format B (library III, Scheme 3.4). Scaffold 3.14 was prepared using a mild, neutral diazo transfer procedure from sulfanilamide employing tert-butyl nitrite and sodium azide in aqueous tert-butanol.

Scheme 3.4

Reagents and conditions: (i) acetylene (i–m) (1.0 equiv.), sodium ascorbate (0.2 equiv.), CuSO$_4$.5H$_2$O (0.1 equiv.), 1:1 tert-butanol–water, 40°C, 30 min–1h, 77–92% (ii) NaOCH$_3$, CH$_3$OH, rt, 15 min–2 h, quantitative.

Figure 3.5
A panel of $O$-glycosides with and without corresponding endogenous glycosidases was included in the study (Figure 3.5), such that this second generation of glycoconjugate sulfonamides may have the potential to act as prodrugs for the sulfonamide pharmacophore in the \textit{in vivo} environment. Propynyl glycoside building blocks ($i$–$j$, 1 and $m$) were prepared by the lewis acid glycosylation of the $\beta$-$D$-glycosyl acetates with propargyl alcohol acceptor in the presence of stannic chloride promoter.\cite{57} The propynyl arabinopyranoside ($k$) was prepared as a 2:1 $\alpha/\beta$ mixture using AgOTf-promoted glycosylation (Koenigs-Knorr) of 2,3,4-tri-$O$-acetyl $\beta$-$D$-arabinopyranosyl bromide with propargyl alcohol.

Thus, the reaction of 4-azidobenzene sulfonamide scaffold 3.14 with a panel of per-$O$-acylated propynyl glycosides $i$–$m$ furnished a library of $O$-glycosides containing a 1,2,3-triazole-linked benzene sulfonamide pharmacophore (3.14i–m). The enhanced solubility of per-$O$-acylated triazoles 3.14i–m in organic solvents (e.g. dichloromethane) facilitated purification by simple aqueous workup followed by flash chromatography/silica filtration. The D-\textit{gluco} configured representative of this library (3.14i) crystallised from absolute ethanol and its structure was solved by X-ray crystallography (Figure 3.6). From the crystal structure, full retention of anomeric configuration as well as regiospecific formation of the 1,4-disubstituted 1,2,3-triazole is confirmed. In an identical fashion to the previous libraries, de-$O$-acylation using methanolic sodium methoxide afforded the deprotected glycoconjugates in near quantitative yields (3.14i’–m’).

Chapter Three
The members of this glycoconjugate library represent a novel structural class with no reported examples of 1,4-disubstituted 1,2,3-triazoles wherein the triazole N-1 substituent was a benzene sulfonamide. In order to investigate the effect of the carbohydrate component on CA inhibition, as well the potential for the activity to be influenced by endogenous glycosidase expression, a ‘clipped’ triazole benzene sulfonamide, bearing a 4-CH$_2$OH alcohol substituent (3.16) was prepared and its inhibition of isozymes hCA I, II and IX compared with that of its glycosylated counterparts. Compound 3.16, along with scaffolds 3.11, 3.12 and 3.14 were recently investigated by our group as a new class of potential anti-mycobacterial agents.$^{58}$
3.2.2 In vitro inhibition of CA isozymes I, II, IX, XII and XIV.

CA inhibition ($K_i$) data was determined for all compounds by assaying the CA catalysed hydration of CO$_2$.\textsuperscript{59} The in vitro inhibition data of the clinically used inhibitors 3.2–3.8 against isozymes hCA I, II, IX, XII, and XIV, as well as their selectivity for hCA IX versus hCA I and II are presented for comparison within Tables 3.2 and 3.3, respectively.

**Table 3.2 In vitro inhibition data for reference CA inhibitors 3.2–3.7, 3.8.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$ (nM)$^a$</th>
<th>hCA I$^b$</th>
<th>hCA II$^b$</th>
<th>hCA IX$^c$</th>
<th>hCA XII$^c$</th>
<th>hCA XIV$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZA 3.2</td>
<td>900</td>
<td>12</td>
<td>25</td>
<td>5.7</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>MZA 3.3</td>
<td>780</td>
<td>14</td>
<td>27</td>
<td>3.4</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>EZA 3.4</td>
<td>25</td>
<td>8</td>
<td>34</td>
<td>22</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>DRZ 3.5</td>
<td>50,000</td>
<td>9</td>
<td>52</td>
<td>3.5</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>BRZ 3.6</td>
<td>15</td>
<td>3</td>
<td>37</td>
<td>3.0</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>DCP 3.7</td>
<td>1200</td>
<td>38</td>
<td>50</td>
<td>50</td>
<td>345</td>
<td></td>
</tr>
<tr>
<td>IND 3.8</td>
<td>31</td>
<td>15</td>
<td>24</td>
<td>nt</td>
<td>Nt</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Errors in the range of ±5–10% of the reported value, from three determinations, by the CO$_2$ hydration method.\textsuperscript{59}

$^b$ Full-length human (cloned) isozymes.\textsuperscript{30-32}

$^c$ Catalytic domain of human (cloned) isozymes.\textsuperscript{30-32}

nt = not tested
Chapter Three

Table 3.3 Inhibition selectivity for the tumour-associated extracellular isozyme hCA IX for reference compounds 3.2-3.8.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Selectivity ratios$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_i$(hCA I)/$K_i$(hCA IX)</td>
</tr>
<tr>
<td>AZA 3.2</td>
<td>36</td>
</tr>
<tr>
<td>MZA 3.3</td>
<td>28.9</td>
</tr>
<tr>
<td>EZA 3.4</td>
<td>0.74</td>
</tr>
<tr>
<td>DRZ 3.5</td>
<td>962</td>
</tr>
<tr>
<td>BRZ 3.6</td>
<td>0.36</td>
</tr>
<tr>
<td>DCP 3.7</td>
<td>24</td>
</tr>
<tr>
<td>IND 3.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

$^a$ The $K_i$ ratios are indicative of isozyme selectivity.

**Benzene sulfonamide scaffolds**

The parent propynyl amide 3.11 and ester 3.12 benzene sulfonamides were assayed against isozymes hCA I, II, IX XII and XIV. For comparison with their glycosylated counterpartes, *in vitro* inhibition data of 3.11 and 3.12 are presented in Table 3.4. The acetylene 3.13 and azide scaffold 3.14 were assayed as inhibitors of isozymes hCA I, II and IX *in vitro*. For comparison, *in vitro* inhibition data of 3.13 is presented in Table 3.5 alongside that of the glycoconjugate benzene sulfonamide library II. Likewise, the *in vitro* inhibition data for 3.14 is presented in Table 3.6 alongside inhibition data for glycoconjugatebenzene sulfonamide library III.

The parent amide scaffold 3.11 and ester scaffold 3.12 exhibit similar inhibition potency and isozyme selectivity to each other against all hCA isozymes investigated.
Notable exceptions include the inhibition of extracellular isozymes, hCA XII and XIV by the amide scaffold 3.11 which exhibit an approximately 8–fold increase inhibition of both isozymes compared with 3.12. Noteworthy, the amide 3.11 is amongst the most potent and selective inhibitor of hCA XII discovered to date ($K_i$ 1.0 nM). At hCA IX the parent compounds 3.11 and 3.12 of library I had $K_i$s of 113 and 104 nM, respectively, approximately 2–fold weaker than inhibition at hCA II and 40-50-fold selective with respect to inhibition of hCA I.

At hCA XIV the parent compounds 3.11 and 3.12 have $K_i$s of 11.3 and 83 nM, respectively. Compound 3.11 is mildly selective for hCA XIV over hCA II (4.2–fold), while compound 3.12 is slightly selective for hCA II over hCA XIV. Thus, the replacement of the amide NH with the ester results in lowered selectivity for the transmembrane hCA XIV isozyme over the dominant cytosolic hCA II isozyme, a reversal in trend previously not observed against the other four isozymes (I, II, IX, and XII). This behaviour may emphasise a region in the hCA XIV active site that warrants further investigation into hCA XIV isozyme selectivity.

Removal of the carboxyamide or ester functionalities in 3.11 and 3.12, respectively and replacement with an acetylene group (as in 3.13) results in strong inhibition of isozymes hCA II and IX ($K_i$ 5.1 nM and $K_i$ 8.1 nM, respectively) and increased selectivity for isozyme hCA IX with respect to hCA I. Incorporation of an azide group results in an inhibitor with similar potency against isozymes hCA I, II and IX to that of the ester scaffold 3.12. The phenylazide 3.14 and hydroxymethyl phenyl-1,2,3-triazole 3.16 are both moderate inhibitors of hCA IX with comparable $K_i$s (105 nM and 134 nM respectively).
Library I

Glycoconjugate benzene sulfonamides of library I comprising the ester and amide-functionalised triazoles (3.11a/3.12a-g, 3.11a’/3.12a’-g’) were assayed for their in vitro inhibition of isozymes hCA I, II, IX, XII and XIV. Inhibition data is presented in Table 3.4.

\[
\begin{align*}
3.11a & \quad X = NH, R = Ac, 3.11a’ X = NH, R = H \\
3.12a & \quad X = O, R = Ac, 3.12a’ X = O, R = H \\
3.11b & \quad X = NH, R = Ac, 3.11b’ X = NH, R = H \\
3.12b & \quad X = O, R = Ac, 3.12b’ X = O, R = H \\
3.11c & \quad X = NH, R = Ac, 3.11c’ X = NH, R = H \\
3.12c & \quad X = O, R = Ac, 3.12c’ X = O, R = H \\
3.11d & \quad X = NH, R = Ac, 3.11d’ X = NH, R = H \\
3.12d & \quad X = O, R = Ac, 3.12d’ X = O, R = H \\
3.11e & \quad X = NH, R = Ac, 3.11e’ X = NH, R = H \\
3.12e & \quad X = O, R = Ac, 3.12e’ X = O, R = H \\
3.11f & \quad X = NH, R = Ac, 3.11f’ X = NH, R = H \\
3.12f & \quad X = O, R = Ac, 3.12f’ X = O, R = H \\
3.11g & \quad X = NH, R = Ac, 3.11g’ X = NH, R = H \\
3.12g & \quad X = O, R = Ac, 3.12g’ X = O, R = H
\end{align*}
\]
Table 3.4 *In vitro* inhibition data for benzene sulfonamide scaffolds 3.11 and 3.12 and glycoconjugate benzene sulfonamides (library I) against CA isozymes hCA I, II, IX XII and XIV.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$ (nM)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hCA I$^b$</td>
</tr>
<tr>
<td>3.8</td>
<td>31</td>
</tr>
<tr>
<td>3.11</td>
<td>6100</td>
</tr>
<tr>
<td>3.12</td>
<td>4000</td>
</tr>
<tr>
<td>3.11a</td>
<td>5600</td>
</tr>
<tr>
<td>3.11a'</td>
<td>2000</td>
</tr>
<tr>
<td>3.12a</td>
<td>2300</td>
</tr>
<tr>
<td>3.12a'</td>
<td>4400</td>
</tr>
<tr>
<td>3.11b</td>
<td>8700</td>
</tr>
<tr>
<td>3.11b'</td>
<td>6600</td>
</tr>
<tr>
<td>3.12b</td>
<td>3600</td>
</tr>
<tr>
<td>3.12b'</td>
<td>5800</td>
</tr>
<tr>
<td>3.11c</td>
<td>2400</td>
</tr>
<tr>
<td>3.11c'</td>
<td>2700</td>
</tr>
<tr>
<td>3.12c</td>
<td>4900</td>
</tr>
<tr>
<td>3.12c'</td>
<td>7.7</td>
</tr>
<tr>
<td>3.11d</td>
<td>1200</td>
</tr>
<tr>
<td>3.11d'</td>
<td>9100</td>
</tr>
<tr>
<td>3.12d</td>
<td>5100</td>
</tr>
<tr>
<td>3.12d'</td>
<td>4800</td>
</tr>
<tr>
<td>3.11e</td>
<td>5800</td>
</tr>
<tr>
<td>3.11e'</td>
<td>2400</td>
</tr>
</tbody>
</table>
Chapter Three

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3.12e</td>
<td>5400</td>
<td>7.0</td>
<td>125</td>
<td>6.1</td>
<td>155</td>
</tr>
<tr>
<td>3.12e’</td>
<td>9.6</td>
<td>7.2</td>
<td>241</td>
<td>7.4</td>
<td>10.8</td>
</tr>
<tr>
<td>3.11f</td>
<td>9200</td>
<td>7.5</td>
<td>221</td>
<td>7.9</td>
<td>134</td>
</tr>
<tr>
<td>3.11f’</td>
<td>9500</td>
<td>267</td>
<td>1100</td>
<td>3.2</td>
<td>64</td>
</tr>
<tr>
<td>3.12f</td>
<td>3900</td>
<td>423</td>
<td>130</td>
<td>7.7</td>
<td>73</td>
</tr>
<tr>
<td>3.12f’</td>
<td>3500</td>
<td>7.3</td>
<td>39</td>
<td>1.0</td>
<td>10.8</td>
</tr>
<tr>
<td>3.11g</td>
<td>10400</td>
<td>44</td>
<td>135</td>
<td>8.0</td>
<td>76</td>
</tr>
<tr>
<td>3.11g’</td>
<td>9300</td>
<td>90</td>
<td>204</td>
<td>3.8</td>
<td>78</td>
</tr>
<tr>
<td>3.12g</td>
<td>3400</td>
<td>50</td>
<td>54</td>
<td>8.1</td>
<td>74</td>
</tr>
<tr>
<td>3.12g’</td>
<td>3100</td>
<td>8.6</td>
<td>67</td>
<td>7.3</td>
<td>10.6</td>
</tr>
</tbody>
</table>

*a* Errors in the range of ±5–10% of the reported value, from three determinations, by the CO₂ hydration method.59

*b* Full-length human (cloned) isozyme.30-32

*c* Catalytic domain of human (cloned) isozymes.30-32

*d* The *K*_ᵢ ratios are indicative of isozyme selectivity.

nt = not tested

With respect to inhibition of isozyme hCAI, irrespective of the presence of global hydroxyl protective groups, inhibition is generally in the low μM range, similar to the parent scaffolds 3.11 and 3.12 (*K*_ᵢ 6.1 μM and 4.0 μM, respectively). Notable exceptions are the deprotected glycosyl triazoles belonging to the ester series; the D-arabino configured analogue (3.12c’) and the methyl D-glucuronate derivative (3.12e’) which both exhibit potent inhibition of hCA I (*K*_ᵢ 7.7 nM and 9.6 nM respectively).

At hCA II the sugar triazole tail has a variable effect on inhibition – with some derivatives displaying improved potency while others displaying weaker potency compared to the acetylene scaffolds 3.11 and 3.12. The deprotected ester series
(3.12a’-g’) of library I show a potent and tightly clustered inhibition profile for hCA II, ranging from 5.8–8.6 nM, approximately 5.5 to 8.1–fold potent than the ester parent 3.12. In contrast, the analogous amides (3.11a’-g’) exhibit variable and non-clustered inhibition at hCA II, with the D-glucosyl (3.11a’) and D-galactosyl (3.11b’) configured glycosyl triazoles the only compounds within this group to display improved inhibitory potencies (8.2 and 7.4 nM, respectively) parallel to the ester analogues (3.12a’ and 3.12b’). For the remaining members of the deprotected amides (3.11c’-g’), a considerable loss of inhibitory potency is observed, with Kₜₛ ranging from 90–378 nM. The protected esters 3.12a–g display non-clustered inhibition compared to their deprotected counterparts. Of the compounds with improved potency relative to the parent amide 3.12 are the per-O-acetylated D-galacto- (3.12b) and D-arabino- (3.12c) configured analogues and the D-glucuronic acid methyl ester derivative (3.12e), with Kₜₛ ranging from 6.8–7.3 nM. The per-O-acetylated amides (3.11a-g) also exhibit non-clustered inhibition at hCA II and variable inhibition compared with their deprotected counterparts. The methyl α-D-glucoside (3.11a) has 2–fold stronger inhibition than the triol (3.11a’) and similar inhibition to the parent amide 3.11. The per-O-acetylated glycoconjugates 3.11d, 3.11e and 3.11f all display 6–, 50–, and 36–fold stronger inhibition, respectively, than their deprotected counterparts, while the per-O-acetylated D-glucosyl- (3.11a) and D-galactosyl triazole (3.11b) are 8.2–fold and 2.6–fold weaker inhibitors of hCA II, respectively, compared to the parent acetylene scaffolds.

With respect inhibition of hCA IX, there is at least one compound within each series (amide, ester, protected or deprotected) that exhibits improved hCA IX inhibition over the parent scaffolds. Notable exceptions included the D-glucosyl- configured
compounds (3.11a, 3.11a’, 3.12a and 3.12a’) and the N-acetyl glucosamine derivatives (3.11d, 3.11d’, 3.12d and 3.12d’) that always result in reduced inhibition compared with the corresponding acetylene scaffold. The acetylated amide series (3.11a-g) are moderate inhibitors of hCA IX ($K_i$ 26–471 nM) and generally 2 to 3–fold potent than their protected counterparts (3.11a-g’). One notable exception is the deprotected methyl D-glucuronate (3.11e’) which is a more potent inhibitor of hCA IX ($K_i$ 23 nM) than its acylated precursor 3.11 ($K_i$ 471 nM). Remarkably, 3.11e’ display similar inhibition at hCA IX to that of Indisulam (3.8) which is in phase II clinical trials for the treatment of solid tumours; however unlike 3.8 which is not selective for hCA IX with respect to either hCA I or II, the methyl D-glucuronate 3.11e’ is selective for hCA IX with respect to hCA I (140-fold) and hCA II (16.4-fold). The selectivity of compound 3.11e’ for hCA IX with respect to hCA II is an impressive 26–fold higher than 3.8 (Table 3.3).

Glycoconjugates of 3.11 and 3.12 result in inhibitors displaying variable and non-clustered in vitro inhibition data at hCA XII with $K_i$s ranging from 1.0–388 nM. In contrast to its potent and selective inhibition of hCA IX, compound 3.11e’ is a weak inhibitor of hCA XII ($K_i$ 388 nM) and when excluding this compound from that data set, the inhibition profile becomes far more clustered. ($K_i$ 10–19.7 nM). The ester series are generally stronger inhibitors of hCA XII ($K_i$s < 10 nM) and are also selective for this isozyme with respect to the other extracellular isozymes hCA IX and XIV (although some were non-selective with respect to hCA II).

Compounds which display low nanomolar inhibition and selectivity for hCA XII include the per-O-acetylated D-glucosyl triazole 3.11a ($K_i$ of 4.3 nM) which is 89–
fold selective over hCA II and 100-fold selective over hCA IX, respectively. The N-acetyl glucosamine ester derivative 3.12d is also a potent and selective inhibitor of hCA XII ($K_i$ 3.8 nM) with selectivity ratios of 56 against hCA II and 307 against hCA IX. The maltose amide derivative 3.11f also exhibits potent inhibition of hCA XII ($K_i$ 3.2 nM) and was also selective for this isozyme with respect to inhibition at hCA II (83-fold) and hCA IX (344-fold). Glycoconjugates also had some selectivity for hCA XII compared to XIV—the only exception being the glucosyl triazole, 3.11a’ which was approximately equipotent at these isozymes.

This trend was found to be reversed for the ester series, with the protected counterparts (3.12a-g) showing weaker inhibition than the deprotected analogues (3.12a’-g’). Interestingly, the protected methyl D-glucuronate (3.12e) is approximately 2-fold potent than the triol (3.12e’) and is a moderate inhibitor of hCA IX ($K_i$ 125 nM). These results demonstrate that by tethering a sugar triazole tail onto the CA anchor pharmacophore it is possible to reverse the CA isozyme selectivity trends observed in the nonglycoconjugate parent compounds 3.11 and 3.12 and also all standard inhibitors, neither of which are hCA IX selective.

With respect to inhibition of hCA XIV, installation of the triazole sugar tail onto the amide scaffold 3.11 generally results in a slight reduction of inhibitor potency, with the exception of the D-glucosyl triazoles 3.11a and 3.11a’ which are approximately equipotent to the parent amide 3.11. Glycoconjugates 3.11a, 3.11a’, 3.12a’, 3.12c’, 3.12e, 3.12f’, and 3.12g’ exhibit potent and clustered inhibition of hCA XIV with $K_i$s in the range of 10.0–11.0 nM. Esters 3.12c’, 3.12e’, 3.12f’, and 3.12g’ comprising free sugar tails are approximately 8-fold stronger inhibitors of hCA XIV than the non-
Chapter Three

glycoconjugate parent ester 3.12, although selectivity of the glycoconjugates for hCA II over hCA XIV closely parallel the parent.

A slight reduction in hCA XIV inhibitor potency is observed for the remaining glycoconjugates compared to the amide parent amide 3.11, although some glycoconjugates of the amide series exhibit similar selectivity for hCA XIV over hCA II, a relationship which parallels that of the parent amide 3.11. A noteworthy exception is the D-gluco configured amide 3.11a (hCA XIV $K_i$ 11.0 nM) which is much more selective for hCA XIV over hCA II (35–fold) and hCA IX (39–fold). In summary, the amide parent 3.11 is a potent and mildly selective inhibitor of hCA XIV cf. hCA II and the conjugation of carbohydrate tails to this scaffold resulted in weaker inhibitors which generally retained the selectivity profile of 3.11. Likewise, the conjugation of deprotected carbohydrate tails to the ester scaffold 3.12 generally resulted in increased inhibition of hCA XIV which retained much of the selectivity for this isozyme with respect to hCA II. Interestingly, the deprotected esters 3.12c’, 3.12e’, 3.12f’ and 3.12g’ are potent inhibitors of hCA XIV ($K_i$ ~10 nM), approximately 8–fold potent than the parent ester 3.12. This result demonstrates that a polar carbohydrate tail group may be significant for delivering potent and selective hCA XIV inhibitors.

**Library II**

Glycoconjugate benzene sulfonamides of library II lacking a carboxy ester or amide function spacer group were assayed for inhibition of isozymes hCA I, II, IX. *In vitro* inhibition data is presented in Table 3.5.
Chapter Three

Table 3.5 *In vitro* inhibition data of parent scaffold 3.13 and glycoconjugate benzene sulfonamides (library II) of isozymes hCA I, II and IX.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( K_i ) (nM) ( ^a )</th>
<th>Selectivity ratios ( ^d )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hCA I ( ^b )</td>
<td>hCA II ( ^b )</td>
</tr>
<tr>
<td>3.13</td>
<td>1080</td>
<td>5.1</td>
</tr>
<tr>
<td>3.13a</td>
<td>4400</td>
<td>9.1</td>
</tr>
<tr>
<td>3.13a'</td>
<td>9.4</td>
<td>380</td>
</tr>
<tr>
<td>3.13b</td>
<td>4300</td>
<td>8.7</td>
</tr>
<tr>
<td>3.13b'</td>
<td>9.3</td>
<td>8.8</td>
</tr>
<tr>
<td>3.13d</td>
<td>5000</td>
<td>440</td>
</tr>
<tr>
<td>3.13d'</td>
<td>4400</td>
<td>460</td>
</tr>
<tr>
<td>3.13e</td>
<td>48</td>
<td>13</td>
</tr>
<tr>
<td>3.13e'</td>
<td>560</td>
<td>13</td>
</tr>
<tr>
<td>3.13f</td>
<td>2500</td>
<td>235</td>
</tr>
</tbody>
</table>
Chapter Three

<table>
<thead>
<tr>
<th>3.13f'</th>
<th>5000</th>
<th>432</th>
<th>130</th>
<th>38.5</th>
<th>3.32</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.13h</td>
<td>5300</td>
<td>427</td>
<td>100</td>
<td>53.0</td>
<td>4.27</td>
</tr>
<tr>
<td>3.13h’</td>
<td>4300</td>
<td>7.5</td>
<td>121</td>
<td>35.5</td>
<td>0.06</td>
</tr>
</tbody>
</table>

\(a\) Errors in the range of ±5–10% of the reported value, from three determinations, by the \(\text{CO}_2\) hydration method.39

\(b\) Human (cloned) isoymes.30-32

\(c\) Catalytic domain of human (cloned) isoymes.30-32

\(d\) \(K_i\) ratios are indicative of isozyme selectivity.

Glycosyl triazoles of library II generally exhibit weak to moderate inhibition of hCA I (low \(\mu\)M), comparable to the parent acetylene scaffold 3.13 (1.1 \(\mu\)M). The deprotected \(D\)-gluco (3.13a’) and the \(D\)-galacto (3.13b’) configured glycosyl triazoles are remarkable, however and showed strong hCAI inhibition (\(K_i\) 9.4 nM and 9.3 nM, respectively). Interestingly, these compounds are roughly 450-fold potent inhibitors of hCA I than their protected congeners, 3.13a and 3.13b (4.4 \(\mu\)M and 4.3 \(\mu\)M, respectively). The \(D\)-glucuronic acid methyl ester derivatives, 3.13e and 3.13e’, are also sub-micromolar inhibitors of hCA I (\(K_i\) 48 and 560 nM).

The conjugation of the acetylene scaffold 3.13, already a potent inhibitor \textit{in vitro} of hCA II (\(K_i\) 5.1 nM), with glycosyl azides a, b, d-f, h generally results in weaker inhibition of this isozyme with \(K_i\)s ranging from 460 nM to 7.5 nM. Of the glycosyl triazoles investigated, neither the protected nor deprotected analogues show any consistency with respect to hCA II inhibition. Outstanding compounds within this library exhibiting similar inhibitiory potency to the parent acetylene 3.13 are the deprotected \(D\)-glucosyl triazole (3.13a’), the analogous \(D\)-galactosyl triazole (3.13b’), the methyl \(D\)-glucuronate (3.13e’), and the \(D\)-ribofuranosyl triazole 3.13’.
analogue with $K_s$ in the range of 7.5 nM–9.1 nM. The resulting loss of inhibition at hCA II could thus be partly viewed a result of the removal of the carboxy ester or amide moiety, which indicates their relative importance as structural components for the generation of potent hCA II inhibitors.

Unlike the analogous carboxyester and amide-functionalised derivatives of library I, the conjugation of carbohydrate tails to the benzene sulfonamide scaffold 3.13 lacking such functional groups results in a clustered inhibition profile and approximately 10-fold loss in inhibitory potency at hCA IX. The methyl D-glucuronates (3.13e and 3.13e’) are exceptional, however, exhibiting potent inhibition of hCA IX ($K_i$ 9.9 nM and 8.4 nM, respectively). Compounds 3.13e and 3.13e’ show some selectivity for hCA IX with respect to hCA I and II, although not as pronounced as the amide derivative 3.11e’. Although a moderate inhibitor of hCA IX ($K_i$ 85 nM), the deprotected $N$-acetyl glucosamine derivative (3.13d’) is remarkably selective for hCA IX with respect to isozymes I (50-fold) and II (4.4 to 5.4-fold). This is in contrast to other compounds within this series, in particular the parent scaffold 3.13 which display no selectivity for hCA IX with respect to hCA II.
Chapter Three

Library III

Glycoconjugate benzene sulfonamides of library III comprising O-glycoside tails (3.14i-m, 3.14i’-m’) were assayed for the inhibition of isozymes hCA I, II and IX. *In vitro* inhibition data is presented in Table 3.6.

![Chemical structures of compounds](image)

Table 3.6 *In vitro* inhibition data for 4-azidobenzene sulfonamide 3.14 and glycoconjugate sulfonamides (library III) of isozymes hCA I, II and IX.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$ (nM)$^a$</th>
<th>Selectivity ratios$^d$</th>
<th>$K_i$(hCA I)/ $K_i$(hCA IX)</th>
<th>$K_i$(hCA II)/ $K_i$(hCA IX)</th>
</tr>
</thead>
</table>
|          | hCA I$^b$   | hCA II$^b$  | hCA IX$^c$ | \[
| 3.14     | 3900        | 47          | 105          | 37.1                       | 0.45          |
| 3.16     | 6200        | 48          | 134          | 46.3                       | 0.36          |
| 3.14i    | 1500        | 46          | 107          | 14.0                       | 0.43          |
| 3.14i’   | 7.0         | 8.7         | 101          | 0.07                       | 0.09          |
| 3.14j    | 6.9         | 6.8         | 9.7          | 0.71                       | 0.70          |
| 3.14j’   | 4700        | 53          | 120          | 39.2                       | 0.44          |
| 3.14k    | 5800        | 9.3         | 72           | 80.6                       | 0.13          |
Interestingly, the incorporation of an O-glycoside moiety has a variable influence on hCA I inhibition. O-Glycosides \(3.14i-m, 3.14i', 3.14m'\) generally exhibit stronger and more non-clustered inhibition of hCA I than did the glycosyl triazole analogues. Glycosides \(3.14j', 3.14k, 3.14l\) and \(3.14m'\) exhibit \(K_i\)s typically observed for primary aryl sulfonamides at hCA I (\(K_i\) 4.4–5.8 μM). The remaining compounds \(3.14i', 3.14j, 3.14k', 3.14l',\) and \(3.14m\) show much stronger inhibition of hCA I (\(K_i\) 6.9–85 nM) and are 481 to 565-fold potent inhibitors compared with the azide scaffold \(3.14\). In particular, the D-galactoside \(3.14j\) (6.9 nM) and D-glucoside \(3.14i'\) (7.0 nM) are amongst the most potent inhibitors of hCA I discovered to date. The deprotected D-maltoside \(3.14l'\) was 6.5-fold selective for hCA I with respect to hCA II, the only hCA I–selective compound detected within this library. The hydroxymethyl 1,2,3 triazole \(3.16\) is found to be the weakest inhibitor amongst all sulfonamides evaluated against hCA I (6.2 μM). Thus, the removal of the glycone component thus generating \(3.16\) results in a 1.1 to 881-fold reduction in inhibitor potency, suggesting the significance of the carbohydrate tail as an important
structural component for the selective inhibition of this ambiguous yet abundant isozyme.

The members of library III \(3.14i',\ 3.14j,\ 3.14k,\ 3.14k',\ 3.14l,\ 3.14m,\ 3.14m'\) are potent inhibitors of hCA II (\(K_i\)s ranged from 4.2–9.3 nM), while compounds \(3.14i,\ 3.14j'\) and \(3.14l'\) have \(K_i\) values similar to the azide parent \(3.14\) (\(K_i\)s ranged from 46–53 nM). Some of the compounds (\(3.14i',\ 3.14j,\ 3.14k'\) and \(3.14l\)) exhibit similar inhibition at both hCA I and II, while others (\(3.14i,\ 3.14j',\ 3.14k,\) and \(3.14l',\ 3.14m,\ 3.14m'\)) are selective for hCA II. In particular, the deprotected D-ribofuranoside \(3.14m'\), is more than 1000–fold selective for hCA II cf. hCA I (hCA II \(K_i\) 4.2 nM). Compound \(3.16\) exhibits inhibition similar to the phenyl azide scaffold \(3.14\) (\(K_i\) 47 nM) and glycoconjugates \(3.14i,\ 3.14j'\) and \(3.14l'\). It is apparent that the \(O\)-glycoside tail groups can strongly influence the selectivity between the physiologically dominant hCA I and II isozymes, demonstrating that the structural diversity of even simple sugars allows for the discrimination of subtle differences in the active site topology of these isozymes.

Interestingly, \(O\)-glycoside tails impart a relatively clustered pattern of inhibition at hCA IX in contrast to isozymes I and II, with eight of the glycoconjugates exhibiting \(K_i\)s in the range of 69–120 nM. Exceptions include the per-\(O\)-acetylated D-maltoside \(3.14m\) which is a weak inhibitor of hCA IX (\(K_i\) 345 nM) and the per-\(O\)-acetylated galactoside (\(3.14j\)) which exhibits potent inhibition of all isozymes investigated, in particular hCA IX (\(K_i\) 9.7 nM). The weak inhibition of \(3.14m\) could be rationalised in terms steric bulk of the acetylated disaccharide tail thus impeding sufficient access to the enzyme active site. The galactoside \(3.14j\) is approximately 11-fold potent than
the parent azide $3.14$ and is amongst the most potent sulfonamide based inhibitors of hCA IX discovered thus far. In terms of structure-activity relationships, the potency of $3.14j$ is quite remarkable given that it, and the C-4 epimer, $3.14i$ ($K_i$ of 107 nM), share only a single stereochemical difference far removed from the sulfonamide CA zinc binding moiety. It may be drawn from these results that subtle differences in the $O$-glycoside tails can deliver compounds capable of differentially inhibiting CA isozymes. With the exception of $3.14j$ the new sulfonamides are weaker inhibitors at hCA IX compared to hCA II.

### 3.3 Conclusions

To maximise the benefits and safety of future CA therapies the systemic and indiscriminate inhibition of CAs present in multiple tissue sites is a primary motivation for the development of isozyme-specific inhibitors and/or drug-delivery systems. However, the strong conservation of amino acid sequence and three-dimensional architecture of the catalytic site among the human isozymes has made it a challenging process to target subtle isozyme differences. Herein, we have prepared of a library of benzene sulfonamides with triazole tethered carbohydrate tails as a dual isozyme-differentiating and solubilising strategy. The evaluation of \textit{in vitro} inhibition at cytosolic isozymes hCA I, II, and transmembrane isozymes hCA IX, XII, and XIV indicated a qualitative structure–activity relationship effectively demonstrating the effective interrogation of CA active site topology by the carbohydrate tail. Within the study, many potent inhibitors with variable isozyme selectivity were generated—an important outcome in the quest for potential therapeutic applications. The work demonstrates the feasibility of the “click tailing” approach for developing isozyme selective CA inhibitors. In particular, the
conjugation of carbohydrate tails to a primary aryl sulfonamide scaffold by “click-tailing” is a novel and powerful means of differentiating isozyme selectivity, developing neutral, water soluble inhibitors for drug delivery applications, and as a means of targeting extracellular and clinically relevant isozymes due to impaired permeation of the plasma membrane.

The methyl D-glucuronate derivative 3.11e’ from library I is an outstanding compound which has remarkable potency and selectivity for the extracellular hCA IX isozyme cf. hCA II, 16-fold compound more so than that of Indisulam 3.8. Also, the deprotected D-ribofuranoside 3.14m’ from library III is a potent and selective inhibitor of hCA II. The analogous β-D-ribofuranosyl triazole 3.13h’ of library II shows similar potency and selectivity for hCA II. Collectively, these compounds constitute important leads for the generation of potent and selective CA inhibitors and CA-based therapeutics. Future endeavours will focus on X-ray diffractional studies on inhibitor-protein complexes and the establishment of structure-activity relationships for promising compounds.
Chapter Three

3.4 Experimental section

3.4.1 General

For general materials and methods concerning reagents, purification and analysis, please refer to section 2.4.1.

3.4.2 Synthesis of glycoconjugate benzene sulfonamides (Library I–III). General procedure 1.

A mixture of the azide (1.0 equiv.) and acetylene (1.0 equiv.) was suspended in a tert-butyl alcohol and distilled water (1:1, 0.2–0.5 M final concentration). A solution of sodium ascorbate (0.2 equiv.) in water, followed by a solution of CuSO$_4$•5H$_2$O (0.1 equiv.) in water was successively added. The bright yellow heterogeneous mixture was stirred vigorously at 40°C until TLC indicated reaction completion (generally within 2 hours). The mixture was evaporated under reduced pressure and the resulting residue was purified by flash chromatography to yield analytically pure material. Molar yields were calculated following flash chromatography and drying under high vacuum at ambient temperature. The 1,4-regioselectivity of the reaction was verified by $^1$H and $^{13}$C NMR chemical shifts of the triazole moiety in products obtained using DMSO-$d_6$ and D$_2$O, and are in agreement with literature values (see Chapter two, references 11a, 12, 15f, 20, and 23).

3.4.3 Deprotection of benzene sulfonamide glycoconjugates. General procedure 2.

Compounds and were prepared by the treating the per-O-acetate and (final concentration of ~0.1–0.2 M) with anhydrous methanolic sodium methoxide (final pH 9–12) at room temperature. Reactions were found to be complete within 30 minutes by TLC. Neutralisation of the methoxide by Amberlite IR-120 acidic ion
exchange resin, followed by filtration and evaporation of the filtrate afforded pure material by $^1$H NMR spectroscopy. Likewise, the analogous esters 3.12a’–3.12g’ were prepared by deprotecting the per-O-acetylated glycosyl azides a–f and the 6-deoxy-6-azido glucoside g in the same way as described above, prior to the cycloaddition reaction with 3.12 (according to general procedure 2; see scheme 3.2). Special precaution was required for the methyl D-glucuronates 3.11e, 3.12e, 3.13e in order to prevent saponification of the methyl ester using sodium methoxide in methanol. In this case, the pH was kept as near as possible to 8–9 with a prolonged reaction time (~1 hour) in order to retain the methyl ester.

3.4.4 CA inhibition Assay

The CA inhibition assay was performed by the Supuran group at the University of Milan, Italy. Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalyzed CO$_2$ hydration activity.$^{59}$ Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM HEPES (pH 7.5) as buffer, 0.1 M Na$_2$SO$_4$ (for maintaining constant the ionic strength), at 25°C, following the CO$_2$ hydration reaction for a period of 10–100 s (the non-catalyzed reaction needs around 60–100 s in the assay conditions, whereas the catalyzed reactions are of around 6–10 s). The CO$_2$ concentrations ranged from 1.7 to 17 mM for the determination of kinetic parameters. For each inhibitor, tested in the concentration range between 0.01 nm and 100 μM, at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The non-catalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (1 mM) were prepared in distilled water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations) and dilutions up to 0.01 nm were done thereafter with distilled water. Inhibitor and
Chapter Three

enzyme solutions were pre-incubated together for 15 minutes at room temperature prior to assay, in order to allow for the formation of the E–I complex. The inhibition constants were obtained by nonlinear least squares methods using PRISM 3® software. The curve-fitting algorithm allowed us to obtain the IC$_{50}$ values, working at the lowest concentration of substrate of 1.7 mM, from which $K_i$ values were calculated by using the Cheng-Prusoff equation.$^{29,30}$ The catalytic activity (in the absence of inhibitors) of these enzymes was calculated from Lineweaver-Burk plots and represent the mean from at least three different determinations. Enzyme concentrations in the assay system were 9.2 nM for hCA I, 7.3 nM for hCA II, and 8.5 nM for hCA IX, 0.11 μM for hCA XII and 0.13 μM for hCA XIV. Enzymes used here were recombinant ones, prepared and purified as described earlier.$^{29,30}$

3.4.5 Analytical Data

3.4.5.1 Benzene sulfonamide scaffolds

![Structure of N-(prop-2-ynyl)-4-sulfamoylbenzamide (3.11).](image)

A solution of 4-carboxybenzene sulfonamide (2.0 g, 9.9 mmol) and propargyl amine (637 μL, 9.9 mmol, 1.0 equiv.) in anhydrous DMF (40 mL) was prepared under nitrogen. N-hydroxybenzotriazole monohydrate (940 mg, 6.6 mmol, 0.6 equiv.), DIPEA (1.7 mL, 9.9 mmol, 1.0 equiv.) and HBTU (3.8 g, 9.9 mmol, 1.0 equiv.) was then consecutively added. The deep yellow solution was stirred at room temperature under nitrogen for 1 hour when found complete by TLC. The mixture was concentrated under reduced pressure and ethyl acetate (40 mL) was added. The organic phase was washed with water (40 mL) and the water layer back-extracted with ethyl acetate (3 × 40 mL). The combined organic layers were washed with
brine, dried (MgSO$_4$), filtered and evaporated to a crude white solid. Recrystallisation from hot methanol–water (9:1) afforded the title compound as white crystalline solid (1.9 g, 8.2 mmol, 82%). $R_f$ 0.65 (100% EtOAc); Mp 216–217°C (decomp). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.12 (t, $^4$J$_{CH-CH}$ = 2.4 Hz, 1H, acetylene CH), 4.05 (dd, $^2$J$_{CH-NH}$ = 5.6 Hz, $^4$J$_{CH-CH}$ = 2.8 Hz, 2H, CH$_2$), 7.45 (br s, 2H, SO$_2$NH$_2$), 7.86–7.98 (m, 4H, Ar H), 9.09 (t, $^3$J = 10.4 Hz, 1H, CONH); $^{13}$C ($^1$H) NMR (100 MHz, d$_6$-DMSO) $\delta$ 29.03 (NCH$_2$), 73.81 (C=CH), 81.67 (C=CH), 126.37 (Ar CH), 128.64 (Ar CH), 137.34 (Ar C), 147.14 (Ar C), 165.62 (C=O). HRMS (ESI) caled for C$_{10}$H$_9$N$_2$O$_3$S: 237.033936; found: 237.034388. Anal. caled for C$_{10}$H$_{10}$N$_2$O$_3$S: C, 50.41; H, 4.23; N, 11.76; S, 13.46. Found: C, 50.15; H, 4.11; N, 11.52; S, 13.38.

**Prop-2-ylnyl 4-sulfamoylbenzoate (3.12).**

A solution of 4-carboxybenzene sulfonamide (2.0 g, 9.9 mmol) in dry DMF (40 mL) was prepared under nitrogen. Propargyl alcohol (1.17 mL, 19.8 mmol, 2.0 equiv.), triethylamine (2.8 mL, 19.9 mmol, 2.0 equiv.) and EDC (as dihydrochloride salt, 1.9 g, 9.9 mmol, 1.0 equiv.) was then added consecutively. The solution was stirred at room temperature under nitrogen for an additional 4 hours. The mixture was then concentrated under reduced pressure and ethyl acetate (40 mL) was added. The organic extract was washed with saturated aqueous NaHCO$_3$ (40 mL) and back extracted with ethyl acetate (2 × 40 mL). The organic layers were combined and washed with brine (40 mL), dried (MgSO$_4$), filtered and evaporated. The crude oil was purified by flash silica chromatography (1:1 EtOAc–hexanes) to afford the title compound as white crystalline solid (912 mg, 3.8 mmol, 38%). $R_f$ 0.38 (2:3 hexanes–
Chapter Three

EtOAc); Mp 110–111°C. ¹H NMR (400 MHz, DMSO-d₆) δ 3.63 (t, ²J₂H₂ = 2.0 Hz, 1H, C=CH), 4.97 (d, ³J₂H₂ = 2.8 Hz, 2H, CH₂), 7.55 (br s, 2H, SO₂NH₂), 7.93–8.13 (m, 4H, Ar H); ¹³C{¹H} NMR (100 MHz, DMSO-d₆) δ 53.00 (OCH₂), 78.12 (C=CH), 78.28 (C=CH), 126.16 (Ar CH), 130.03 (Ar CH), 131.68 (Ar C), 148.32 (Ar C), 164.05 (C=O). Anal. calcd for C₁₀H₉NO₄S: C, 50.20; H, 3.79; N, 5.85; S, 13.40. Found: C, 50.18; H, 3.83; N, 5.83; S, 13.43.

4-[(Trimethylsilyl)ethynyl]-benzene sulfonamide. To a stirred solution of 4-iodobenzene sulfonamide (5.0 g, 17.7 mmol) in anhydrous Et₃N (60 mL) was added trimethylsilyl acetylene (3.0 mL, 21.2 mmol, 1.2 equiv.), CuI (180 mg, 0.71 mmol, 0.04 equiv.) and PdCl₂(PPh₃)₂ (250 mg, 0.35 mmol, 0.02 equiv.) under an atmosphere of nitrogen. A deep green suspension had formed after several minutes at room temperature. After 1 hour of stirring at this temperature, the insoluble salts were filtered on celite and eluted with ethyl acetate. The filtrate was collected and concentrated under reduced pressure. Ethyl acetate (100 mL) was added and the crude extract was washed with distilled water (100 mL) and brine (50 mL), dried (MgSO₄), filtered and evaporated under reduced pressure. The resulting crude residue was purified by flash silica chromatography (1:4 EtOAc–hexanes then 1:1 EtOAc–hexanes) to afford the title compound as a pale yellow solid (3.7 g, 14.6 mmol, 82%). Rₛ 0.26 (3:7 EtOAc–hexanes); Mp 131–132°C. ¹H NMR (400 MHz, DMSO-d₆) δ 0.217 (s, 9H, (CH₃)₃Si), 7.42 (br s, 2H, SO₂NH₂), 7.60–7.78 (m, 4H, Ar CH).
4-Ethynylbenzene sulfonamide (3.13). To a stirring solution of 4-((Trimethylsilyl)ethynyl)-benzene sulfonamide (1.5 g, 5.9 mmol) in THF (40 mL) was added a 1M solution of tetrabutyl ammonium fluoride in THF (25 mL, 25 mmol, 4.2 equiv.). The deep yellow solution was stirred vigorously at room temperature for 30 min, at which time TLC had indicated reaction completion. The mixture concentrated under reduced pressure, diluted with CH$_2$Cl$_2$ (100 ml) and washed with water (50 mL) and brine (50 mL). The organic layer was then dried (MgSO$_4$), filtered, and evaporated to afford a yellow residue which was purified by filtration on silica (1:1 EtOAc–hexanes) to remove trace ammonium salts yielding the title compound as pale yellow, crystalline solid (950 mg, 5.3 mmol, 89%). $R_f$ 0.37 (1:1 EtOAc–hexanes); Mp 174–175°C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.41 (s, 1H, C≡CH), 7.41 (br s, 2H, SO$_2$NH$_2$), 7.63–7.79 (m, 4H, $Ar$ H); $^{13}$C{$^1$H} NMR (100 MHz, DMSO-$d_6$) $\delta$ 82.98 (C≡CH), 84.05 (C≡CH), 125.72 ($Ar$ C), 126.63 ($Ar$ CH), 132.92 ($Ar$ CH), 144.78 ($Ar$ C).

4-Azidobenzene sulfonamide (3.14). A slurry of NaN$_3$ (2.3 g, 34.9 mmol, 3.0 equiv.) in distilled H$_2$O (2 mL) was prepared followed by the addition of tert-butyl alcohol (16 mL). Sulfanilamide (2.0 g, 11.6 mmol) was then added followed by the drop-wise addition of tert-butyl nitrite (16 mL). The deep yellow solution was stirred at room temperature over night when found complete by TLC (1:1 EtOAc–hexanes). The crude mixture was diluted with ethyl acetate (50 mL) and washed with distilled water (50 mL). The aqueous layer was back extracted with EtOAc (3 × 40 mL). The combined organic extracts were washed with brine (100 mL), dried (MgSO$_4$), filtered and evaporated to afford a crude oil. Precipitation from 3:7 EtOAc–hexanes (~100 mL) and washing
with cold hexanes afforded the title compound as pale yellow crystalline solid (1.7 g, 74%); Mp 117–118°C (decomp; Lit.\textsuperscript{55} 119–122°C); \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6) δ 7.26–7.81 (m, 4H, Ar H), 7.34 (br s, 2H, SO\textsubscript{2}NH\textsubscript{2}); \textsuperscript{13}C\textsubscript{\textit{i}}\textsuperscript{1}H NMR (100 MHz, DMSO-\textit{d}_6) δ 120.16 (Ar CH), 128.30 (Ar CH), 141.20 (Ar C), 143.60 (Ar C).

3.4.5.2 Glycoconjugate benzene sulfonamides (library I)

4-([4-(Aminosulfonyl)benzoyl]amino)methyl-1-(2',3',4',6'-tetra-O-acetyl-\beta-D-glucopyranosyl)-1-H-1,2,3-triazole (3.11a). The title compound was prepared according to the general procedure 1 and isolated as white solid (172 mg, 0.28 mmol, 86%). \textit{R}_f 0.15 (100% EtOAc); Mp 198–199°C. \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6) δ 1.75 (s, 3H, OAc), 1.92 (s, 3H, OAc), 1.95 (s, 3H OAc), 1.99 (s, 3H OAc), 4.01–4.10 (m, 2H, H\textsubscript{6}'', H\textsubscript{6}'''), 4.27–4.33 (m, 1H, H\textsubscript{5}''), 4.50 (d, \textsuperscript{3}J\textsubscript{CH-NH} = 6.0 Hz, 2H, CH\textsubscript{2}NH), 5.11–5.16 (m, 1H, H\textsubscript{4}''), 5.47–5.52 (m, 1H, H\textsubscript{3}''), 5.61–5.66 (m, 1H, H\textsubscript{2}''), 6.28 (d, \textsuperscript{3}J\textsubscript{CH-NH} = 9.2 Hz, 1H, H\textsubscript{1}''), 7.44 (s, 2H, SO\textsubscript{2}NH\textsubscript{2}), 7.86–7.99 (m, 4H, Ar H), 8.26 (s, 1H, triazole H), 9.22 (t, \textsuperscript{3}J\textsubscript{NH-CH} = 5.2 Hz, 1H, CH\textsubscript{2}NH); \textsuperscript{13}C\textsubscript{\textit{i}}\textsuperscript{1}H NMR (100 MHz, DMSO-\textit{d}_6) δ 20.55 (OAc), 20.87 (OAc), 21.00 (OAc), 21.14 (OAc), 35.46 (CH\textsubscript{2}NH), 62.38 (C\textsubscript{6}''), 68.13 (C\textsubscript{4}''), 70.69 (C\textsubscript{3}''), 72.91 (C\textsubscript{2}''), 73.85 (C\textsubscript{5}''), 84.46 (C\textsubscript{1}''), 122.82 (triazole CH), 126.31 (Ar CH), 128.64 (Ar CH), 137.63 (Ar C), 146.24 (triazole C or Ar C), 146.96 (triazole C or Ar C), 166.05 (C=O), 169.23 (C=O), 170.11 (C=O), 170.34 (C=O), 170.83 (C=O). HRMS (ESI) calcd for C\textsubscript{24}H\textsubscript{28}N\textsubscript{5}O\textsubscript{12}S\textsubscript{9} 610.146066.
Chapter Three

Found: 610.147077. Anal. calcd for C$_{24}$H$_{29}$N$_{5}$O$_{12}$S•0.5 H$_{2}$O: C, 46.45; H, 4.87; N, 11.29. Found: C, 46.48; H, 4.70; N, 10.98.

4-((4-(Aminosulfonyl)benzoyl)amino)methyl-1-(β-D-glucopyranosyl)-1-H-1,2,3-triazole (3.11a'). Title compound was prepared according to general procedure 2 and isolated as white solid (71 mg, 0.16 mmol, ~100%); Mp 205–210°C (decomp). $R_f$ 0.15 (1:9 H$_2$O–CH$_3$CN). $^1$H NMR (400 MHz, D$_2$O) δ 3.46–3.51 (m, 1H, H$_4$), 3.55–3.60 (m, 1H, H$_3'$), 3.58–3.66 (m, 2H, H$_5$, H$_6'$), 3.74–3.79 (m, 1H, H$_6''$), 3.84–3.88 (m, 1H, H$_2$), 4.59 (s, 2H, CH$_2$NH), 5.61 (d, $^3J_{1-2'}$ = 8.8 Hz, 1H, H$_1$), 7.80–7.88 (m, 4H, Ar), 8.07 (s, 1H, triazole CH); $^{13}$C{$^1$H} NMR (100 MHz, 2% D$_2$O in DMSO-d$_6$) δ 35.19 (CH$_2$NH), 60.53 (C$_6$-), 69.07 (C$_4$), 72.41 (C$_2$-), 76.02 (C$_3$-), 79.04 (C$_5$-), 87.59 (C$_1$-), 123.36 (triazole CH), 126.44 (Ar CH), 128.44 (Ar CH), 137.71 (Ar C), 144.35 (triazole C or Ar C), 144.94 (triazole C or Ar C), 169.54 (C=O). HRMS (ESI) calcd for C$_{16}$H$_{20}$N$_{5}$O$_{8}$S$: 442.10387. Found: 442.103042.

4-((4-(Aminosulfonyl)benzoyl)amino)methyl-1-(2’,3’,4’,6’-tetra-O-acetyl-β-D-galactopyranosyl)-1-H-1,2,3-triazole (3.11b). The title compound was prepared according to the general procedure 1 and isolated as white solid (174 mg, 0.28 mmol,
Chapter Three

87%). R<sub>f</sub> 0.11 (1:4 hexanes–EtOAc); Mp 161–162°C (decomp). <sup>1</sup>H NMR (400 MHz, DMSO-<em>d</em><sub>6</sub>) δ 1.78 (s, 3H, OAc), 1.91 (s, 3H, OAc), 1.95 (s, 3H, OAc), 2.15 (s, 3H, OAc), 3.88 (dd, <sup>2</sup>J<sub>6′-S'</sub> = 11.6 Hz, <sup>3</sup>J<sub>S'-S</sub> = 7.6 Hz, 1H, H<sub>6′</sub>), 4.10 (dd, <sup>2</sup>J<sub>6'-S</sub> = 11.6 Hz, <sup>3</sup>J<sub>S'-S</sub> = 5.2 Hz, 1H, H<sub>6</sub>), 4.51 (d, <sup>3</sup>J<sub>CH-NH</sub> = 5.5 Hz, 2H, CH<sub>2</sub>NH), 4.52–4.55 (m, 1H, H<sub>5</sub>), 5.38–5.43 (m, 2H, H<sub>3</sub>, H<sub>4</sub>), 5.55–5.60 (m, 2H, H<sub>2</sub>), 6.21 (d, <sup>3</sup>J<sub>1′-2′</sub> = 9.2 Hz, 1H, H<sub>1′</sub>), 7.45 (s, 2H, SO<sub>2</sub>NH<sub>2</sub>), 7.86–7.99 (m, 4H, Ar H), 8.16 (s, 1H, triazole H), 9.19 (t, <sup>3</sup>J<sub>NH-CH</sub> = 5.1 Hz, 1H, CH<sub>2</sub>NH); <sup>13</sup>C NMR (100 MHz, DMSO-<em>d</em><sub>6</sub>) δ <br>20.69 (OAc), 20.98 (OAc), 21.10 (OAc), 21.17 (OAc), 35.46 (CH<sub>2</sub>NH), 62.25 (C<sub>6</sub>N), 68.01 (C<sub>4</sub>), 68.30 (C<sub>3</sub>), 71.14 (C<sub>2</sub>), 73.63 (C<sub>5</sub>), 84.85 (C<sub>1</sub>), 123.13 (triazole CH), 126.29 (Ar CH), 128.66 (Ar CH), 137.63 (Ar C), 145.97 (triazole C or Ar C), 147.05 (triazole C or Ar C), 165.89 (C=O), 169.18 (C=O), 170.12 (C=O), 170.59 (C=O), 170.66 (C=O). HRMS (ESI) calcd for C<sub>24</sub>H<sub>28</sub>N<sub>5</sub>O<sub>12</sub>S: 610.146066. Found: 610.147458. Anal. calcd for C<sub>24</sub>H<sub>29</sub>N<sub>5</sub>O<sub>12</sub>S·0.5H<sub>2</sub>O: C, 46.45; H, 4.87; N, 11.29. Found: C, 46.40; H, 4.77; N, 10.96.

4-(4-Sulfamoylbenzamido)methyl-1-(β-D-galactopyranosyl)-1-H-1,2,3-triazole (3.11b’). The title compound was prepared according to general procedure 2 and isolated as white foam (72 mg, 0.16 mmol, ~100%). R<sub>f</sub> 0.13 (1:9 H<sub>2</sub>O–CH<sub>3</sub>CN). <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ 3.64–3.65 (m, 2H, H<sub>6′</sub>, H<sub>6</sub>), 3.74 (dd, <sup>3</sup>J<sub>3′-2′</sub> = 9.6 Hz, <sup>3</sup>J<sub>3′-4′</sub> = 3.2 Hz, 1H, H<sub>3′</sub>), 3.87 (m, 1H, H<sub>5</sub>), 3.85–3.86 (dd, <sup>3</sup>J<sub>4′-3′</sub> = 3.4 Hz, <sup>3</sup>J<sub>4′-5′</sub> = 0.8 Hz, 1H, H<sub>4′</sub>), 4.05–4.10 (m, 1H, H<sub>2</sub>), 4.59 (s, 2H, CH<sub>2</sub>NH), 5.55 (d, 1H, <sup>3</sup>J<sub>1′-2′</sub> = 8.8 Hz, 1H, H<sub>1′</sub>), 6.21 (d, 1H, H<sub>1′</sub>), 7.80–7.88 (m, 4H, Ar CH), 8.11 (s, 1H, triazole CH); <sup>13</sup>C NMR
(100 MHz, D$_2$O) $\delta$ 35.24 (CH$_2$NH), 60.99 (C$_6$-), 68.71 (C$_4$), 69.91 (C$_2$-), 73.06 (C$_3$-), 78.45 (C$_5$-), 88.20 (C$_1$-), 123.05 (triazole CH), 126.44 (Ar H), 128.44 (Ar H), 137.72 (Ar C), 144.32 (Ar C or triazole C), 145.63 (triazole C or Ar C), 169.53 (C=O).

HRMS (ESI) calcd for C$_{16}$H$_{20}$N$_5$O$_8$S$: 442.103807. Found: 442.103051.

4-([4-(Aminosulfonyl)benzoyl]amino)methyl-1-(2’,3’,4’-tri-O-acetyl-α-D-arabinopyranosyl)-1-H-1,2,3-triazole (3.11c). The title compound was prepared according to the general procedure 1 and isolated as an off-white solid (147 mg, 0.27 mmol, 82%). $R_f$ 0.10 (1:4 hexanes–EtOAc); Mp 125–126$^\circ$C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.78 (s, 3H, OAc), 1.92 (s, 3H, OAc), 2.12 (s, 3H, OAc), 3.89 (dd, $^2$J$_{5’,5’}$ = 13.2 Hz, $^3$J$_{5’,4’}$ = 2.0 Hz, 1H, H$_{5’}$), 4.13 (dd, 1H, $^2$J$_{5’,5’}$ = 13.4 Hz, $^3$J$_{5’,4’}$ = 1.3 Hz, 1H, H$_{5’}$), 4.51 (d, $^3$J$_{CH-NH}$ = 5.6 Hz, 1H, CH$_2$NH), 5.27–5.28 (m, 1H, H$_4$), 5.36 (dd, $^3$J$_{5’,2’}$ = 10.4 Hz, $^3$J$_{5’,4’}$ = 5.2 Hz, 1H, H$_3’$), 5.55–5.59 (m, 1H, H$_2’$), 6.07 (d, $^3$J$_{1’,2’}$ = 9.2 Hz, 1H, H$_1$), 7.45 (br s, 2H, SO$_2$NH$_2$), 7.86–7.99 (m, 4H, Ar H), 8.21 (s, 1H, triazole H), 9.18 (t, $^3$J$_{NH-CH}$ = 5.2 Hz, 1H, CH$_2$NH); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 20.70 (OAc), 21.06 (OAc), 21.40 (OAc), 67.12 (C$_5$-), 68.57 (C$_4$-), 68.60 (C$_3$-), 71.05 (C$_2$-), 85.40 (C$_1$-), 123.04 (triazole CH), 126.29 (Ar CH), 128.65 (Ar CH), 137.65 (Ar C), 145.92 (triazole C or Ar C), 147.04 (triazole C or Ar C), 165.89 (C=O), 169.22 (C=O), 170.20 (C=O), 170.55 (C=O). HRMS (ESI) calcd for C$_{21}$H$_{24}$N$_5$O$_8$S$: 538.124936. Found: 538.125762. Anal. calcd for C$_{21}$H$_{27}$N$_5$O$_{11}$S•0.5H$_2$O: C, 45.98; H, 4.78; N, 12.77. Found: C, 45.73; H, 4.17; N, 12.40.
Chapter Three

4-((4-(Aminosulfonyl)benzoyl)amino)methyl-1-(α-D-Arabinopyranosyl)-1-H-1,2,3-triazole (3.11c'). The title compound was prepared according to general procedure 2 and isolated as pale yellow solid (77 mg, 0.19 mmol, ~100%). Rf 0.26 (1:9 CH₃OH–EtOAc); Mp 210–212°C (decomp). ¹H NMR (400 MHz, 2% D₂O in DMSO-d₆) δ 3.74 (dd, 3J₃'-2' = 10.0 Hz, 3J₃'-4' = 3.6 Hz, 1H, H₃'), 3.80–3.83 (m, 1H, H₅'), 3.83–3.86 (m, 2H, H₄', H₅'-'), 4.06–4.11 (m, 1H, H₂'), 4.59 (s, 2H, CH₂NH), 5.48 (d, 1H, 3J₁'-₂' = 9.2 Hz, 1H, H₁'), 7.83–7.91 (m, 4H, Ar H), 8.12 (s, 1H, triazole H); ¹³C {¹H} NMR (100 MHz, 2% D₂O in DMSO-d₆) δ 35.20 (CH₂NH), 68.55 (C₄'), 69.68 (C₅'), 69.84 (C₂'), 72.77 (C₃'), 88.55 (C₁'), 123.02 (triazole CH), 125.51 (Ar CH), 128.53 (Ar CH), 137.67 (Ar C), 144.64 (triazole C or Ar C), 145.83 (triazole C or Ar C), 169.18 (C=O). HRMS (ESI) calcd for C₁₅H₁₈N₅O₇S⁻: 412.093242. Found: 412.092448.

4-((4-(Aminosulfonyl)benzoyl)amino)methyl-1-(2’-acetamido-2’-deoxy-3’4’,6’-tri-O-acetyl-β-D-glucopyranosyl)-1-H-1,2,3-triazole (3.11d). The title compound was prepared according to general procedure 1 and isolated as white solid (149 mg, 0.24 mmol, 91%). Rf 0.32 (1:9 CH₃OH–EtOAc); Mp 231–232°C (decomp). ¹H NMR
Chapter Three

(400 MHz, DMSO-d$_6$) $\delta$ 1.55 (s, 3H, NHAc), 1.91 (s, 3H, OAc), 1.95 (s, 3H, OAc, CH$_3$), 1.97 (s, 3H, OAc), 4.00 (dd, $^2J_{\text{H-H}} = 12.4$ Hz, $^3J_{\text{H-H}} = 2.0$ Hz, 1H, H$_6$'), 4.10 (dd, $^2J_{\text{H-H}} = 12$ Hz, $^3J_{\text{H-H}} = 4.8$ Hz, 1H, H$_6$'), 4.18 (ddcd, $^3J_{\text{H-H}} = 7.2$ Hz, $^3J_{\text{H-H}} = 4.8$ Hz, 1H, H$_6$'), 4.50 (d, $^3J_{\text{H-H}} = 6.0$ Hz, 2H, C$_9$), 4.51–4.58 (m, 1H, H$_2$), 5.02–5.08 (m, 1H, H$_4$), 5.29–5.34 (m, 1H, H$_3$'), 6.07 (d, $^3J_{\text{H-H}} = 9.2$ Hz, 1H, NHAc CONH), 8.13 (s, triazole H), 9.20 (t, $^3J_{\text{H-H}} = 6.0$ Hz, 1H, CH$_2$NH); $^{13}$C $\{^1$H$\}$ NMR (100 MHz, DMSO-d$_6$) $\delta$ 20.96 (OAc), 21.09 (OAc), 21.20 (OAc), 23.02 (OAc), 35.49 (CH$_2$NH), 52.69 (C$_2$), 62.50 (C$_6$), 68.67 (C$_3$), 73.07 (C$_4$), 74.05 (C$_5$), 85.29 (C$_1$), 122.45 (triazole CH), 126.70 (Ar CH), 128.67 (Ar CH), 137.70 (Ar C), 145.80 (triazole C or Ar C) 147.02 (triazole C or Ar C), 165.87 (C=O), 170.01 (C=O), 170.11 (C=O), 170.27 (C=O), 170.72 (C=O). HRMS (ESI) calcd. for C$_{24}$H$_{30}$N$_6$O$_{11}$S: 609.16205. Found: 609.162988. Anal. calcd for C$_{24}$H$_{30}$N$_6$O$_{11}$S: C, 47.21; H, 4.95; N, 13.76. Found: C, 47.03; H, 5.19; N, 12.87.

4-([(4-(Aminosulfonyl)benzoyl]amino)methyl-1-(2'-acetamido-2'-deoxy-$\beta$-D-glucopyranosyl)-1-H-1,2,3-triazole (3.11d'). The title compound was prepared to general procedure 2 and isolated as white solid (79 mg, 0.16 mmol, ~100%). $R_f$ 0.09 (1:9 H$_2$O:CH$_3$CN); Mp 214–215°C. $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 1.62 (s, 3H, NHAc CH$_3$), 3.47–3.52 (m, 1H, H$_3$'), 3.64–3.68 (m, 1H, H$_4$'), 3.87–4.05 (m, 1H, H$_2$'), 4.48 (d, $^3J_{\text{H-H}} = 5.8$ Hz, 2H, CH$_2$NH), 4.58–4.61 (m, 1H, H$_6$'), 5.21–5.24 (m, 2H, H$_3$, H$_4$'), 5.66 (d, 1H, $^3J_{\text{H-H}} = 9.6$ Hz, 1H, H$_1$'), 7.44 (br s, 2H, SO$_2$NH$_2$), 7.82 (d,
3 \( J_{NH,2'} \) = 8.8 Hz, 1H, NHAc NH), 7.85–8.00 (m, 4H, Ar), 7.97 (s, 1H, triazole 1H), 9.19 (t, \( J_{NH,CH} = 6.0 \) Hz, 1H, CH\(_2\)NH); \( \textsuperscript{13}C\{\textsuperscript{1}H\} \) NMR (100 MHz, 2% D\(_2\)O in DMSO-\(d_6\)) \( \delta \) 23.30 (NHAc CH\(_3\)), 35.40 (CH\(_2\)NH), 55.00 (C\(_2\'\)), 61.81 (C\(_6\'\)), 70.44 (C\(_4\'\)), 74.46 (C\(_3\)), 80.63 (C\(_5\')), 86.59 (C\(_1\)), 122.22 (triazole CH), 126.32 (Ar CH), 128.66 (Ar CH), 137.68 (Ar C), 145.26 (triazole C or Ar C), 146.87 (triazole C or Ar C), 166.10 (O=C), 169.982 (C=O). HRMS (ESI) calcd for C\(_{18}H_{23}N_6O_8S^-\) : 483.130356. Found: 483.129389.

4-((4-(Aminosulfonyl)benzoyl)amino)methyl-1-(2',3',4'-tri-O-acetyl-\( \beta \)-d-glucuronic acid methyl ester)-1-H-1,2,3-triazole (3.11e). See compound 2.16 for analytical data.

4-((4-(Aminosulfonyl)benzoyl)amino)methyl-1-(\( \beta \)-d-glucuronic acid methyl ester)-1-H-1,2,3-triazole (3.11e’). The title compound was prepared according to general procedure 2 and isolated as a clear gum (79 mg, 0.17 mmol, \( \sim \)100%). R\(_f\) 0.13 (1:9 CH\(_3\)OH–EtOAc). \( ^1\)H NMR (400 MHz, 2% D\(_2\)O in DMSO-\(d_6\)) \( \delta \) 3.40–3.49 (m, 2H, H\(_3\), H\(_4\)), 3.61 (s, 3H, COCH\(_3\)), 3.82–3.86 (m, 1H, H\(_2\)), 4.08 (d, \( J_{5'-4'} = 9.6 \) Hz, 1H, H\(_5\)), 4.51 (s, 2H, CH\(_2\)NH), 5.66 (d, \( J_{1'-2'} = 9.6 \) Hz, 1H, H\(_1\')), 7.85–8.00 (m, 4H,
Chapter Three

157

4-(4-(Aminosulfonyl)benzoyl]amino)methyl-1-(hepta-O-acetyl-β-D-maltosyl)-1H-1,2,3-triazole (3.11f). The title compound was prepared according to general procedure 1 and isolated as pale yellow foam (424 mg, 0.47 mmol, 78%). \( R_f \) 0.23 (1:4 hexanes–EtOAc). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \( \delta \) 1.72 (s, 3H, OAc), 1.92 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.99 (s, 3H, OAc), 2.00 (s, 3H, OAc), 3.88–4.01 (m, 2H, Glc\( \alpha \) H\(_5\), Glc\( \beta \) H\(_6\)), 4.05–4.16 (m, 3H, Glc\( \alpha \) H\(_6\), Glc\( \beta \) H\(_4\), Glc\( \beta \) H\(_6\)), 4.29 (ddd, \( ^3J_{5\'\cdot5}\) = 9.6 Hz, \( ^3J_{5\'\cdot6}\) = 5.6 Hz, \( ^3J_{5\'\cdot6}\) = 2.4 Hz, 1H, Glc\( \beta \) H\(_5\)), 4.40 (dd, \( ^2J_{6\'\cdot6}\) = 12.4 Hz, \( ^3J_{6\'\cdot5}\) = 2.4 Hz, 1H, Glc\( \beta \) H\(_6\)), 4.50 (d, \( ^3J_{\text{CH-NH}} = 5.6 \text{ Hz}, 2\text{H, CH}_2\text{NH})\), 4.88 (dd, \( ^3J_{2\'\cdot2}\) = 10.8 Hz, \( ^3J_{2\'\cdot1}\) = 4.0 Hz, 1H, Glc\( \alpha \) H\(_2\)), 4.95–5.00 (m, 1H, Glc\( \alpha \) H\(_4\)), 5.18–5.23 (m, 1H, Glc\( \alpha \) H\(_3\)), 5.32 (d, \( ^2J_{1\cdot2}\) = 4.0 Hz, 1H, Glc\( \alpha \) H\(_1\)), 5.47–5.57 (m, 2H, Glc\( \beta \) H\(_2\), Glc\( \beta \) H\(_3\)), 6.25 (d, \( ^3J_{1\cdot2}\) = 9.2 Hz, 1H, Glc\( \beta \) H\(_1\)), 7.45 (br s, 2H, SO\(_2\)NH\(_2\)), 7.86–7.99 (m, 4H, \( Ar \)), 8.16 (s, 1H, triazole CH), 9.21 (t, \( ^3J_{\text{NH-CH}} = 5.6 \text{ Hz}, 1\text{H, CH}_2\text{NH})\); \(^{13}\)C \({^1}\)H NMR (100 MHz, DMSO-\(d_6\)) \( \delta \) 20.63 (OAc), 20.94 (OAc), 21.00 (OAc), 21.10 (OAc), 21.20 (OAc), 21.24 (OAc), 35.51 (CH\(_2\)NH), 62.06 (Glc\( \beta \) C\(_6\)), 63.52 (Glc\( \alpha \) C\(_6\)), 68.36 (Glc\( \alpha \) C\(_4\)), 68.83 (Glc\( \alpha \) C\(_2\))
Chapter Three

C_5^\text{\textprime}), 69.59 (Glc\alpha C_3^\text{\textprime}), 70.09 (Glc\alpha C_2^\text{\textprime}), 71.30 (Glc\beta C_3^\text{\textprime}), 73.88 (Glc\beta C_4^\text{\textprime}), 74.50 (Glc\beta C_5^\text{\textprime}), 75.13 (Glc\beta C_2^\text{\textprime}), 84.07 (Glc\beta C_1^\text{\textprime}), 96.40 (Glc\alpha C_1^\text{\textprime}), 122.74 (triazole CH), 126.29 (Ar CH), 128.64 (Ar CH), 137.64 (Ar C), 146.19 (triazole C or 6r C), 147.05 (triazole C or 6r C), 165.87 (C=O). HeMS (ESI) calcd for C_{36}H_{44}N_{5}O_{20}S:\ 898.230583. Found: 898.228045. Anal. calcd for C_{36}H_{45}N_{5}O_{20}S: C, 48.05\%; H, 5.04\%; N, 7.78\%; S, 3.56. Found: C, 45.26\%; H, 4.99\%; N, 7.36\%; S, 3.24.

4-([4-(Aminosulfonyl)benzoyl]amino)methyl-1-(\beta-D-maltosyl)-1-H-1,2,3-triazole (3.11f'). The title compound was prepared according to general procedure 2 and isolated as pale yellow foam (134 mg, 0.22 mmol ~100%). R_t 0.10 (1:9 H_2O:CH_3CN). 1H NMR (400 MHz, D_2O) \delta 3.27–3.32 (m, 1H, Glc\alpha H_4^\prime or Glc\beta H_4^\prime), 3.46 (dd, \_^3J_{2-3^\prime} = 10.0 Hz, \_^3J_{2-1^\prime} = 4.0 Hz, Glc\alpha H_2^\prime), 3.55–3.79 (m, 8 H, Glc\alpha H_3^\prime, Glc\alpha H_5^\prime, Glc\alpha H_6^\primeH_6^\prime, and Glc\beta H_3^\prime, Glc\beta H_5^\prime, Glc\beta H_6^\prime/H_6^\prime), 3.83–3.87 (m, 1H, Glc\beta H_3^\prime), 3.88–3.82 (m, 1H, Glc\beta H_2^\prime), 4.58 (s, 2H, CH_2NH), 5.33 (d, \_^3J_{1-2^\prime} = 4.0 Hz, 1H, Glc\beta H_1^\prime), 5.62 (d, \_^3J_{1-2^\prime} = 9.2 Hz 1H, Glc\beta H_1^\prime), 7.77–7.86 (m, 4H, Ar), 8.07 (s, 1H, triazole CH); 13C \{^1H\} NMR (100 MHz, D_2O) \delta 35.18 (CH_2NH), 60.58 (Glc\alpha C_6^\prime or Glc\beta C_6^\prime), 60.59 (Glc\alpha C_6^\prime or Glc\beta C_6^\prime), 69.47, 71.84, 72.27, 72.91, 72.99, 75.97, 76.50, 77.63 (Glc\alpha C_2^\prime, Glc\alpha C_3^\prime, Glc\alpha C_4^\prime, Glc\alpha C_5^\prime, and Glc\beta C_2^\prime, Glc\beta C_3^\prime, Glc\beta C_4^\prime Glc\beta C_5^\prime), 87.42 (Glc\beta C_1^\prime), 99.83 (Glc\alpha C_1^\prime), 123.32 (triazole CH), 126.33 (Ar CH), 128.36 (Ar CH), 137.39 (Ar C), 144.70 (triazole C or Ar C), 144.97.
(triazole C or Ar C), 169.32 (C=O). HRMS (ESI) calcd for C_{22}H_{30}N_{5}O_{13}S^{-}: 604.15663. Found: 604.155698.

**Methyl 2’,3’,4’-tri-O-acetyl-6-[4-([[aminosulfonyl]benzoyl]amino)methyl]-1H-1,2,3-triazol-1-yl]-6-deoxy-β-D-glucopyranoside (3.11g).** The title compound was prepared according to general procedure 1 and isolated as white foam (278 mg, 0.48 mmol, 82%). R_{f} 0.12 (100% EtOAc). \(^1^H\) NMR (400 MHz, DMSO-d<sub>6</sub>) \(\delta\) 1.92 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.98 (s, 3H, OAc), 3.03 (s, 3H, OCH<sub>3</sub>), 4.11 (m, 1H, H<sub>5</sub>), 4.44–4.49 (m, 2H, H<sub>6</sub>, CH<sub>2</sub>NH), 4.55 (dd, \(^3J_{6-N}=14.4\) Hz, \(^3J_{6-N}=2.8\) Hz, 1H, H<sub>6</sub>), 4.78–4.82 (m, 2H, H<sub>1’,2’</sub>), 4.83–4.88 (m, 1H, H<sub>4’</sub>), 5.21–5.26 (m, 1H, H<sub>3’</sub>), 7.44 (br s, 2H, SO<sub>2</sub>NH<sub>2</sub>), 7.85–7.98 (m, 4H, Ar), 7.97 (s, 1H, triazole CH), 9.19 (t, \(^3J_{NH-CH}=5.6\) Hz, 1H, CH<sub>2</sub>NH); \(^{13}\)C NMR \(\{\{^1\}^\text{H}\}\) (100 MHz, DMSO-d<sub>6</sub>) \(\delta\) 20.96 (OAc), 21.03 (OAc), 21.13 (OAc), 35.55 (CH<sub>2</sub>NH), 50.40 (C<sub>6</sub>), 55.28 (OCH<sub>3</sub>), 68.00 (C<sub>5</sub>), 70.12 (C<sub>1’</sub> or C<sub>2’</sub>), 70.17 (C<sub>1’</sub> or C<sub>2’</sub>), 70.33 (C<sub>3’</sub>), 96.57 (C<sub>1’</sub>), 124.54 (triazole CH), 126.31 (Ar CH), 128.58 (Ar CH), 137.68 (Ar C), 145.52 (triazole C or Ar C) 146.95 (triazole C or Ar C), 165.89 (C=O), 170.07 (C=O), 170.34 (C=O), 170.41 (C=O). HRMS (ESI) calcd for C<sub>23</sub>H<sub>27</sub>N<sub>4</sub>O<sub>12</sub>S<sub>2</sub>: 582.151151. Found: 582.150947. Anal. calcd for C<sub>23</sub>H<sub>29</sub>N<sub>5</sub>O<sub>11</sub>S: C, 47.34; H, 5.01; N, 12.00. Found: C, 46.31; H, 4.96; N, 11.66.
Methyl 6-[4-(([aminosulfonyl]benzoyl)amino)methyl]-1H-1,2,3-triazol-1-yl]-6-deoxy-β-D-glucopyranoside (3.11g'). The title compound was prepared according to general procedure 1 and isolated as pale yellow foam (76 mg, 0.17 mmol, 97%). $R_f$ 0.21 (1:9 CH$_3$OH–EtOAc). $^1$H NMR (400 MHz, 2% D$_2$O in DMSO-$d_6$) δ 2.87 (s, 3H, OCH$_3$), 2.96 (dd, $^3$J$_{4\cdot 5\cdot}$ = 10.4 Hz, $^3$J$_{4\cdot 3\cdot}$ = 8.8 Hz, 1H, H$_4$), 3.14 (dd, $^3$J$_{2\cdot 3\cdot}$ = 9.6 Hz, $^3$J$_{2\cdot 1\cdot}$ = 3.6 Hz, 1H, H$_2$), 3.31- 3.36 (m, 1H, H$_3$), 3.59 – 3.65 (m, 1H, H$_5$), 4.33 (dd, $^2$J$_{6\cdot 6\cdot}$ = 14.0 Hz, $^2$J$_{6\cdot 5\cdot}$ = 9.2 Hz, 1H, H$_6$), 4.42 (d, 1H, $^3$J$_{1\cdot 2\cdot}$ = 3.6 Hz, 1H, H$_1$), 4.47–4.48 (m, 2H, CH$_2$NH), 4.65 (dd, $^2$J$_{6\cdot 6\cdot}$ = 14.0 Hz, 1H, H$_6$), 7.85 (s, 1H, triazole CH), 7.86–7.97 (m, 4H, Ar), 9.18 (t, $^3$J$_{NH-CH}$ = 5.2 Hz, 1H CH$_2$NH); $^{13}$C NMR $^1$H (100 MHz, 2% D$_2$O in DMSO-$d_6$) δ 35.45 (CH$_2$NH), 51.50 (C$_6$), 54.87 (OCH$_3$), 71.07 (C$_5$), 72.12 (C$_4\cdot$ or C$_2$), 72.21 (C$_4\cdot$ or C$_2$), 73.53 (C$_3$), 100.31 (C$_1$), 124.50 (triazole CH), 126.33 (Ar CH), 128.57 (Ar CH), 137.67 (Ar C), 145.33 (triazole C or Ar C), 146.87 (triazole C or Ar C), 165.74 (C=O). HRMS (ESI) calcd for C$_{17}$H$_{21}$N$_4$O$_9$S: 456.119457. Found: 456.118334.
4-((4-(Aminosulfonyl)benzoyl)oxy)methyl-1-(2’-3’-4’-6’-tetra-O-acetyl-β-D-glucopyranosyl)-1-H-1,2,3-triazole (3.12a).

The title compound was prepared according to general procedure 1 and isolated as a white solid (142 mg, 0.23 mmol, 86%). R<sub>f</sub> 0.43 (1:4 hexanes–EtOAc); Mp 196–197°C. <sup>1</sup>H NMR (400 MHz, DMSO-<sup>d6</sup>) δ 1.75 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.97 (s, 3H, OAc), 2.00 (s, 3H, OAc), 4.05 (dd, 2J<sub>6</sub>=6 = 12.8 Hz, 3J<sub>6</sub>=5 = 2.4 Hz, 1H, H<sub>6</sub>′), 4.10 (dd, 2J<sub>6</sub>=6 = 12.8 Hz, 3J<sub>6</sub>=5 = 5.2 Hz, 1H, H<sub>6</sub>′′), 4.35 (dd, 3J<sub>5</sub>=4 = 10 Hz, 3J<sub>5</sub>=6 = 5.2 Hz, 1H, H<sub>5</sub>′), 5.13–5.18 (m, 1H, H<sub>4</sub>′), 5.40–5.46 (m, 2H, CH<sub>2</sub>O), 5.51–5.55 (m, 1H, H<sub>3</sub>′), 5.63–5.67 (m, 1H, H<sub>2</sub>′), 6.35 (d, 3J<sub>1</sub>=2 = 9.2 Hz, 1H, H<sub>1</sub>′), 7.54 (s, 2H, SO<sub>2</sub>NH<sub>2</sub>), 7.92–8.11 (m, 4H, <i>Ar H</i>), 8.57 (s, 1H, triazole H); <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, DMSO-<sup>d6</sup>) δ 20.56 (OAc), 20.92 (OAc), 21.06 (OAc), 21.19 (OAc), 58.91 (CH<sub>2</sub>O), 62.46 (C<sub>6</sub>′), 68.19 (C<sub>4</sub>′), 70.84 (C<sub>3</sub>′), 72.76 (C<sub>2</sub>′), 73.87 (C<sub>5</sub>′), 84.56 (C<sub>1</sub>′), 124.70 (triazole CH), 126.76 (<i>Ar CH</i>) 130.68 (<i>Ar CH</i>) 132.69 (<i>Ar C</i>) 143.16 (triazole C or <i>Ar C</i>) 148.92 (triazole C or <i>Ar C</i>) 165.09 (C=O), 169.16 (C=O), 170.04 (C=O), 170.22 (C=O), 170.70 (C=O). HRMS (ESI) calcd for C<sub>24</sub>H<sub>27</sub>N<sub>4</sub>O<sub>13</sub>S: 611.130081. Found: 611.127932. Anal. calcd for C<sub>24</sub>H<sub>28</sub>N<sub>4</sub>O<sub>13</sub>S: C, 47.06; H, 4.61; N, 9.15; O, 33.85; S, 5.23. Found: C, 46.80; H, 4.66; N, 8.96; S, 4.95.
4-([4-(Aminosulfonyl)benzoyl]oxy)methyl-1-(β-D-glucopyranosyl)-1-H-1,2,3-triazole (3.12a'). The title compound was prepared according to general procedure 1 and isolated as white foam (82 mg, 0.19 mmol, 72%). Rf 0.10 (3:7 EtOH–CHCl₃). ¹H NMR (400 MHz, D₂O) δ 3.47 – 3.53 (m, 2H, H₄, H₅), 3.56–3.66 (m, 2H, H₃, H₆), 3.74 – 3.79 (m, 1H, H₆), 3.86 – 3.80 (m, 1H, H₂), 5.65 (d, ³J₁⁻₂ = 8 Hz, 1H, H₁), 7.81 – 8.00 (Ar H), 8.25 (s, triazole CH); ¹³C (¹H) NMR (100 MHz, D₂O) δ 58.33 (CH₂O), 60.55 (C₆), 69.09 (C₇), 72.44 (C₈), 76.04 (C₉), 79.04 (C₁₀), 87.66 (C₁₁), 125.21 (triazole CH), 126.24 (Ar CH), 130.62 (Ar CH), 133.21 (Ar C), 142.80 (triazole C or Ar C), 145.67 (triazole Ar C), 166.64 (C=O). HRMS (ESI) called for C₁₆H₁₉N₄O₉S⁻: 443.087822. Found: 443.087455.

4-([4-(Aminosulfonyl)benzoyl]oxy)methyl-1-(2’-3’-4’-6’-tetra-O-acetyl-β-D-galactopyranosyl)-1-H-1,2,3-triazole (3.12b).

The title compound was prepared according to general procedure 1 and isolated as white foam (184 mg, 0.30 mmol, 92%). Rf 0.41 (1:4 hexanes–EtOAc). ¹H NMR (400 MHz, DMSO-d₆) δ 1.78 (s, 3H, OAc), 1.92 (s, 3H, OAc), 1.96 (s, OAc), 2.16 (s, OAc), 4.00 (dd, ²J₆⁺₆⁻ = 11.6 Hz, ³J₆⁻₅⁺ = 1.2 Hz, 1H, H₆), 4.11 (dd, ²J₆⁻₆⁺ = 11.6 Hz, ³J₆⁻₅⁺ = 4.8 Hz, 1H, H₆⁻), 4.55 – 4.58 (m, 1H, H₅), 5.40 (dd, ³J₄⁻₃⁺ = 3.6 Hz,
Chapter Three

$3J_{4',5'} = 1.2 \text{ Hz, } H_4'$), 5.42 (s, 2H, CH$_2$O), 5.44–5.45 (m, 1H, H$_3$), 5.56–5.61 (m, 1H, H$_2$), 6.27 (d, $3J_{1',2'} = 9.2 \text{ Hz, } 1H, H_{1'}$), 7.53 (s, 2H, SO$_2$NH$_2$), 7.91–8.11 (m, 4H, $Ar$ H), 8.50 (s, 1H, triazole H); $^{13}C\{^{1}H\}$ NMR (100 MHz, DMSO-$d_6$) $\delta$ 20.65 (OAc), 20.99 (OAc), 21.09 (OAc), 21.17 (OAc), 58.78 (CH$_2$O), 62.25 (C$_6$), 67.98 (C$_4$), 68.43 (C$_3$), 71.04 (C$_2$), 73.70 (C$_5$), 84.96 (C$_1$), 125.23 (triazole CH), 126.77 ($Ar$ CH), 130.68 ($Ar$ CH), 132.70 ($Ar$ C), 142.92 ($Ar$ C), 148.91 (triazole C), 165.13 (C=O), 169.20 (C=O), 170.13 (C=O), 170.66 (C=O). HRMS (ESI) calculated for C$_{24}$H$_{27}$N$_4$O$_{13}$S$: 611.130081. Found: 611.129142. Anal. calcd for C$_{24}$H$_{28}$N$_4$O$_{13}$S: C, 47.06; H, 4.61; N, 9.15; S, 5.23. Found: C, 46.45; H, 4.76; N, 8.75; S, 4.86.

4-((4-(Aminosulfonyl)benzoyl)oxy)methyl-1-($\beta$-D-galactopyranosyl)-1-$H$-1,2,3-triazole (3.12b'). The title compound was prepared according to general procedure 1 and isolated as white foam (136 mg, 0.31 mmol, 63%). $R_f$ 0.12 (3:7 EtOH–CHCl$_3$). $^1H$ NMR (400 MHz, 2% D$_2$O in DMSO-$d_6$) $\delta$ 3.41–3.48 (m, 3H, H$_5$, H$_6$, H$_6'$), 3.51 (dd, $3J_{3',2'} = 9.2 \text{ Hz, } 3J_{3',4'} = 3.2 \text{ Hz, } 1H, H_3$), 3.67–3.73 (m, 1H, H$_4$), 3.88–4.03 (m, 1H, H$_2$), 5.43 (s, 2H, CH$_2$O), 5.48 (d, $3J_{1',2'} = 8.8 \text{ Hz, } 1H, H_{1'}$), 7.91–8.12 (m, 4H, $Ar$), 8.37 (s, 1H, triazole CH); $^{13}C\{^{1}H\}$ NMR (100 MHz, 2% D$_2$O in DMSO-$d_6$) $\delta$ 58.42 (CH$_2$O), 60.38 (C$_6$), 68.39 (C$_4$), 69.24 (C$_2$), 73.52 (C$_5$), 78.46 (C$_3$), 88.19 (C$_1$), 124.01 (triazole CH), 126.21 ($Ar$ CH), 130.10 ($Ar$ CH), 132.16 ($Ar$ C), 141.69 ($Ar$ C), 148.13 (triazole C), 164.62 (C=O). HRMS (ESI) calcd for C$_{16}$H$_{19}$N$_4$O$_9$S$: 443.087822. Found: 443.087859.
4-([4-(Aminosulfonyl)benzoyl]oxy)methyl-1-(2’-3’-4’tri-O-acetyl-β-D-Arabino.pyranosyl)-1H-1,2,3-triazole (3.12c’).

The title compound was prepared according to general procedure 1 and isolated as white foam (228 mg, 0.42 mmol, 88%). $R_f$ 0.55 (1:4 hexanes–EtOAc). $^1$H NMR (400 MHz, DMSO-$d_6$) 1.78 (s, 3H, OAc), 1.93 (s, 3H, OAc), 2.13 (s, 3H, OAc), 4.02 (dd, $^2J_{5',5''} = 13.2$ Hz, $^3J_{5',4'} = 1.6$ Hz, 1H, H$_5'$), 4.14–4.18 (m, 1H, H$_5'$), 5.28–5.30 (m, 1H, H$_4'$), 5.38 (dd, $^3J_{3',2'} = 10.0$ Hz, $^3J_{3',4'} = 3.6$ Hz, 1H, H$_3'$), 5.42 (s, 2H, CH$_2$O), 5.55–5.60 (m, 1H, H$_2''$), 6.13 (d, $^3J_{1',2'} = 9.2$ Hz, 1H, H$_1'$), 7.53 (br s, 2H, SO$_2$NH$_2$), 7.91–8.10 (m, 4H, Ar CH), 8.46 (s, 1H, triazole H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 20.66 (OAc), 21.07 (OAc), 21.40 (OAc), 58.81 (CH$_2$O), 67.18 (C$_5'$), 68.57 (C$_4'$), 68.69 (C$_2'$), 70.95 (C$_3'$), 85.52 (C$_1'$), 125.11 (triazole CH), 126.76 (Ar CH), 130.67 (Ar CH), 132.71 (Ar C), 142.86 (Ar C), 148.91 (triazole CH), 165.11 (C=O), 169.24 (C=O), 170.20 (C=O), 170.55 (C=O). HRMS (ESI) calculated for C$_{21}$H$_{23}$N$_4$O$_{11}$S: 539.108952. Found: 539.107401. Anal. calcd for C$_{21}$H$_{24}$N$_4$O$_{11}$S: C, 46.67; H, 4.48; N, 10.37; S, 5.93. Found: C, 44.29; H, 4.38; N, 9.66; S, 5.52.

4-([4-(Aminosulfonyl)benzoyl]oxy)methyl-1-(β-D-Arabino.pyranosyl)-1H-1,2,3-triazole (3.12c’). The title compound was prepared according to procedure 1 and
isolated as white solid (105 mg, 0.25 mmol, 84%). $R_f$ 0.26 (3:7 EtOH–CHCl₃); Mp 184–189°C. $^1$H NMR (400 MHz, 2% D₂O in DMSO-$d_6$) $\delta$ 3.53 (dd, $^3J_{3\cdots,2\cdots} = 9.6$ Hz, $^3J_{3\cdots,4\cdots} = 3.6$ Hz, 1H, H₃), 3.72–3.74 (m, 2H, H₄, H₅), 3.78 (dd, $^2J_{5\cdots,5\cdots} = 12.4$ Hz, $^3J_{5\cdots,4\cdots} = 2.0$ Hz, 1H, H₅), 3.88–4.03 (m, 1H, H₆), 5.41 (d, $^3J_{1\cdots,2\cdots} = 10.0$ Hz, 1H, H₁), 5.43 (s, 2H, CH₂O), 8.36 (s, 1H, triazole CH), 7.91–8.12 (m, 4H, Ar), $^{13}$C $^1$H NMR (100 MHz, 2% D₂O in DMSO-$d_6$) $\delta$ 59.06 (C₇H₂O), 69.03 (C₄), 69.96 (C₅, C₂'), 73.86 (C₃'), 89.10 (C₁'), 124.59 (triazole CH), 126.79 (Ar CH), 130.70 (Ar CH), 132.74 (Ar C), 142.25 (triazole C or Ar C), 148.81 (triazole C or Ar C), 165.21 (C=O). HRMS (ESI) caled for C₁₅H₁₇N₄O₈S₈: 413.077258. Found: 413.076552.

4-(([4-(Aminosulfonyl)benzoyl]oxy)methyl-1-(2’-acetamido-2’-deoxy-3’,4’,6’-tri-O-acetyl-β-D-glucopyranosyl)-1-$H$-1,2,3-triazole (3.12d). The title compound was prepared according to the general procedure 1 and isolated as white solid (292 mg, 0.60 mmol, 89%). $R_f$ 0.45 (100% EtOAc); Mp 217–218°C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.55 (s, 3H, NHAc CH₃), 1.92 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.98 (s, OAc), 4.03 (dd, $^2J_{6\cdots,6\cdots} = 12.4$ Hz, $^3J_{6\cdots,5\cdots} = 2$ Hz, 1H, H₆), 4.12 (dd, $^2J_{6\cdots,5\cdots} = 12.8$ Hz, $^3J_{6\cdots,5\cdots} = 5.6$ Hz, 1H, H₆), 4.23 (dd, $^3J_{5\cdots,4\cdots} = 10.0$ Hz, $^3J_{5\cdots,6\cdots} = 5.6$ Hz, $^3J_{5\cdots,6\cdots} = 2.0$ Hz, 1H, H₅), 4.56–4.64 (m, 1H, H₂), 5.06–5.10 (m, 1H, H₄), 5.30–5.35 (m, 1H, H₃'), 5.38–5.45 (m, 2H, CH₂O), 6.11 (d, $^3J_{1\cdots,2\cdots} = 9.6$ Hz, 1H, H₁'), 7.54 (s, 2H, SO₂NH₂), 7.91–8.12 (m, 4H, Ar H), 8.05 (d, $^3J_{NH-2} = 9.6$ Hz, 1H, NHAc CONH), 8.44 (s, triazole H); $^{13}$C $^1$H NMR (100 MHz, DMSO-$d_6$) $\delta$ 20.96 (OAc), 21.10 (OAc), 21.20 (OAc), 22.97 (NHAc CH₃), 52.74 (C₂), 58.96 (CH₂O), 62.45 (C₆'), 68.66
Chapter Three

(C₃ⁿ), 72.99 (C₄ⁿ), 74.10 (C₅ⁿ), 85.45 (C₁ⁿ), 124.49 (triazole CH), 126.75 (Ar CH), 130.70 (Ar CH), 132.71 (Ar CH), 142.64 (Ar C), 148.91 (triazole C), 165.10 (C=O), 170.02 (C=O), 170.10 (C=O), 170.27 (C=O), 170.71 (C=O). HRMS (ESI) calcd for C₂₄H₂₈N₅O₁₂S⁻: 610.146066. Found: 610.145354. Anal. calcd for C₂₄H₂₉N₅O₁₂S: C, 47.13; H, 4.78; N, 11.45; O, 31.39; S, 5.24. Found: C, 46.60; H, 4.76; N, 11.27; S, 5.01.

![Chemical Structure](image)

4-({[4-(Aminosulfonyl)benzoyl]oxy}methyl-1-(2'-acetamido-2'-deoxy-β-D-glucopyranosyl)-1H-1,2,3-triazole (3.12d'). The title compound was prepared according to the general procedure 1 and isolated as an off-white solid (180 mg, 0.38 mmol, 68%). Rₛ 0.09 (3:7 EtOH–CHCl₃); Mp 205–208°C (decomp). ¹H NMR (400 MHz, 2% D₂O in DMSO-¢) δ 1.57 (s, 3H, NHAc CH₃), 3.22–3.27 (m, 1H, H₄'), 3.68–3.46 (m, 2H, H₅', H₆'), 3.49–3.54 (m, 1H, H₃'), 3.65–3.69 (m, 1H, H₆'''), 4.01–4.08 (m, 1H, H₂'), 5.39 (s, 2H, CH₂O), 5.71 (d, ³J₁'-₂' = 9.6 Hz, 1H, H₁'), 7.85 (d, ³J₉⁻₂₁ = 9.2 Hz, 1H, NHAc CONH), 7.91–8.10 (m, 4H, Ar), 8.27 (s, 1H, triazole CH); ¹³C {¹H} NMR (100 MHz, DMSO-¢) δ 23.36 (NHAc CH₃), 55.20 (C₂'), 58.99 (CH₂O), 61.35 (C₆'), 70.61 (C₄'), 74.57 (C₃'), 80.81 (C₅'), 86.74 (C₁'), 124.34 (triazole CH), 126.76 (Ar CH), 130.69 (Ar CH), 132.76 (Ar C), 142.10 (triazole C or Ar C), 148.87 (triazole C), 165.13 (C=O), 169.82 (C=O). HRMS (ESI) calcd for C₁₈H₂₉N₅O₉S⁻: 484.114372. Found: 484.113226.
4-((4-(Aminosulfonyl)benzoyl[oxy]methyl-1-(2',3',4'-tri-O-acetyl-\(\beta\)-D-glucuronic acid methyl ester)-1-H-1,2,3-triazole (3.12e). The title compound was prepared according to general procedure 1 and isolated as white foam (307 mg, 92\%). \(R_f\) 0.58 (1:4 hexanes–EtOAc). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 1.76 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.99 (s, 3H, OAc), 3.60 (s, 3H, COCH\(_3\)), 4.79 (d, \(^3\)J\(_{3,4}\) = 10.0 Hz, 1H, H\(_5\)), 5.21–5.26 (m, 1H, H\(_4\)), 5.43 (s, 2H, CH\(_2\)O), 5.57–5.62 (m, 1H, H\(_3\)), 5.73–5.78 (m, 1H, H\(_2\)), 6.40 (d, \(^3\)J\(_{1,2}\) = 9.2 Hz, 1H, H\(_1\)), 7.54 (br s, 2H, SO\(_2\)NH\(_2\)), 7.91–8.12 (m, 4H, \(Ar\) H), 8.65 (s, 1H, triazole H); \(^{13}\)C\(^{\{1\}H}\) NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 20.56 (OAc), 20.90 (OAc), 20.95 (OAc), 53.30 (COCH\(_3\)), 58.90 (CH\(_2\)O), 69.05 (C\(_4\)), 70.48 (C\(_3\)), 72.13 (C\(_2\)), 73.56 (C\(_5\)), 84.48 (C\(_1\)), 124.90 (triazole CH), 126.77 (\(Ar\) CH), 130.69 (\(Ar\) CH), 132.67 (\(Ar\) C), 143.28 (triazole C or \(Ar\) C), 148.92 (triazole C or \(Ar\) C), 165.09 (C=O), 167.24 (C=O), 169.10 (C=O), 169.98 (C=O), 170.19 (C=O). HRMS (ESI) calculated for C\(_{23}\)H\(_{23}\)N\(_4\)O\(_{13}\)S\(_2\): 633.091109. Found: 633.091128. Anal. calcd for C\(_{23}\)H\(_{26}\)N\(_4\)O\(_{13}\)S\(_2\)O: C, 44.81; H, 4.58; N, 9.09; S, 5.20. Found: C, 44.01; H, 4.30; N, 8.81; S, 4.89.
Chapter Three

4-((4-(Aminosulfonyl)benzoyl)oxy)methyl-1-(β-D-glucuronic acid methyl ester)-1-H-1,2,3-triazole (3.12e'). The title compound was prepared according to general procedure 1 and isolated as hygroscopic white foam (42 mg, 0.08 mmol, 72%). $R_f$ 0.32 (1:9 CH$_3$OH–EtOAc). $^1$H NMR (400 MHz, 2% D$_2$O in DMSO-$d_6$) $\delta$ 3.27–3.32 (m, 1H, H$_3$), 3.86–3.01 (m, 1H, H$_4$), 3.65 (s, 3H, COCH$_3$), 3.84–3.88 (m, 1H, H$_2$), 4.63 (d, $^3$J$_{5'4}$ = 9.6 Hz, 1H, H$_5$), 5.42 (s, 2H, CH$_2$O), 5.72 (d, $^3$J$_{1'2'}$ = 9.6 Hz, 1H, H$_1$), 7.91–8.12 (m, 4H, Ar H), 8.46 (s, 1H, triazole CH); $^{13}$C ($^1$H) NMR (100 MHz, 2% D$_2$O in DMSO-$d_6$) $\delta$ 52.81 (COCH$_3$), 58.92 (CH$_2$O), 71.56 (C$_3$), 71.89 (C$_4$), 76.68 (C$_2$), 78.00 (C$_5$), 87.79 (C$_1$), 125.09 (triazole CH), 126.80 (Ar CH), 130.73 (Ar CH), 136.85 (Ar C), 142.40 (triazole C or Ar C), 148.49 (triazole C or Ar C), 165.20 (C=O), 169.35 (C=O). HRMS (ESI) calcd for C$_{17}$H$_{19}$N$_4$O$_{10}$S$^{-}$: 471.082737. Found: 471.082075.

4-((4-(Aminosulfonyl)benzoyl)oxy)methyl-1-(hepta-O-acetyl-β-D-maltosyl)-1-H-1,2,3-triazole (3.12f). The title compound was prepared according to general procedure 1 and isolated as white foam (185 mg, 0.21 mmol, 91%). $R_f$ 0.46 (1:4 hexanes–EtOAc). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.71 (s, 3H, OAc), 1.93 (s, 6H,)
Chapter Three

2 × OAc), 1.96 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.99 (s, 3H, OAc), 2.02 (s, 3H, OAc), 3.87–4.02 (m, 2H, Glcα H₅, Glcβ H₆), 4.07–4.17 (m, 3H, Glcα H₆, Glcβ H₄, Glcβ H₆), 4.32 (ddd, 3J₅₋₄ = 10.0 Hz, 3J₅₋₆ = 5.6 Hz, 3J₅₋₆ = 2.4 Hz, 1H, Glcβ H₅), 4.42 (dd, 3J₆₋₅ = 12.4 Hz, 3J₆₋₅ = 2 Hz, 1H, Glcβ H₆), 4.89 (dd, 1H, 3J₂₋₃ = 10.4 Hz, 3J₂₋₃ = 3.6 Hz, 1H, Glcα H₂), 4.96–5.01 (m, 1H, Glcα H₄), 5.19–5.24 (m, 1H, Glcα H₃), 5.33 (d, 3J₁₋₂ = 4.0 Hz, 1H, Glcα H₁), 5.38–5.45 (m, 2H, CH₂O), 5.49–5.53 (m, 1H, Glcβ H₃), 5.55–5.60 (m, 1H, Glcβ H₃), 6.31 (d, 3J₁₋₂ = 8.8 Hz, 1H, Glcβ H₁), 7.54 (s, 2H, SO₂NH₂), 7.91–8.10 (m, 4H, Ar CH), 8.47 (s, 1H, triazole H);

¹³C{¹H} NMR (100 MHz, DMSO-d₆) δ 20.57 (OAc), 20.95 (OAc), 21.01 (OAc), 21.02 (OAc), 21.12 (OAc), 21.19 (OAc), 21.25 (OAc), 58.91 (CH₂O), 62.06 (Glcα C₆), 63.45 (Glcβ C₆), 68.37 (Glcα C₄), 68.85 (Glcβ C₄), 69.58 (Glcα C₃), 70.11 (Glcα C₂), 71.38 (Glcβ C₃), 73.84 (Glcα C₅), 74.58 (Glcβ C₅), 74.96 (Glcβ C₅), 79.85 (Glcβ C₂), 84.16 (Glcβ C₁), 96.41 (Glcα C₁), 124.74 (triazole CH), 126.76 (Ar CH), 130.67 (Ar CH), 132.68 (Ar C), 143.06 (Ar C), 148.91 (triazole C), 165.09 (C=O), 169.36 (C=O), 169.84 (C=O), 170.19 (C=O), 170.35 (C=O), 170.53 (C=O), 170.67 (C=O), 170.78 (C=O). HRMS (ESI) calced for C₃₆H₄₃N₄O₂₁S: 899.214599. Found: 899.211425. Anal. calced for C₃₆H₄₄N₄O₂₁S: C, 48.00; H, 4.92; N, 6.22; S, 3.56. Found: C, 46.45; H, 4.91; N, 5.96; S, 3.27.

4-[(4-(Aminosulfonyl)benzoyl)oxy]methyl-1-(β-D-maltosyl)-1-H-1,2,3-triazole (3.12f). The title compound was prepared according to general procedure 1 and
isolated as white foam (161 mg, 0.26 mmol, 58%). $R_f$ 0.39 (1:4 CH$_3$OH–EtOAc). $^1$H NMR (400 MHz, D$_2$O) $\delta$ 3.27–3.32 (m, 1H, Glc$\alpha$ H$_4$ or Glc$\beta$ H$_4$), 3.55 – 3.79 (m, 8 H, Glc$\alpha$ H$_3$, Glc$\alpha$ H$_5'$, Glc$\alpha$ H$_6$H$_6''$, and Glc$\beta$ H$_3$, Glc$\beta$ H$_5'$, Glc$\beta$ H$_6$/H$_6''$), 3.46 (dd, $^3$J$_{2'-3'}$ = 10.0 Hz, $^3$J$_{2'-1'}$ = 3.6 Hz, 1H, Glc$\alpha$ H$_2$), 3.84–3.84 (m, 2H, Glc$\beta$ H$_2$), 3.84–3.84 (Glc$\beta$ H$_2$ and Glc$\beta$ H$_3$), 5.34 (d, $^3$J$_{1'-2'}$ = 3.6 Hz, 1H, Glc$\alpha$ H$_1$), 5.39 (s, 2H, CH$_2$O), 5.66 (d, $^3$J$_{1'-2'}$ = 8.8 Hz, 1H, Glc$\beta$ H$_1$), 7.78–7.97 (m, 4H, Ar H), 8.25 (s, 1H, triazole CH); $^{13}$C{$^1$H} NMR (100 MHz, D$_2$O) $\delta$ 58.33 (CH$_2$O), 60.54 (Glc$\alpha$ C$_6$ or Glc$\beta$ C$_6$), 69.47, 71.84, 72.31, 72.92, 72.99, 75.93, 76.52, 77.66 (Glc$\alpha$ C$_2'$, Glc$\alpha$ C$_3'$, Glc$\alpha$ C$_4'$, Glc$\alpha$ C$_5'$ and Glc$\beta$ C$_2'$, Glc$\beta$ C$_3'$, Glc$\beta$ C$_4'$ Glc$\beta$ C$_5'$), 87.49 (Glc$\beta$ C$_1'$), 99.81 (Glc$\alpha$ C$_1'$), 125.16 (triazole CH), 126.23 (Ar CH), 130.69 (Ar CH), 133.57 (Ar CH), 142.82 (triazole C or Ar C), 145.68 (triazole C or Ar C), 166.59 (C=O). HRMS (ESI) calcld for C$_{22}$H$_{29}$N$_4$O$_{14}$S$^-$: 605.140646. Found: 605.138433.

Methyl 2',3',4'-tri-O-acetyl-6-[4-([4-(aminosulfonyl)benzoyl]oxy)methyl]-1H-triazol-1-yl]-6-deoxy-β-d-glucopyranoside (3.12g). The title compound was prepared according to general procedure 1 and isolated as white foam (123 mg, 0.21 mmol, 89%). $R_f$ 0.42 (1:4 hexanes–EtOAc). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.92 (OAc), 1.96 (OAc), 1.99 (OAc), 2.99 (OAc), 4.13 (ddd, $^3$J$_{5'-4'}$ = 10.8 Hz, $^3$J$_{5'-6'}$ = 8.4 Hz, $^3$J$_{5'-6''}$ = 2.8 Hz, 1H, H$_4$), 4.52 (dd, $^2$J$_{6-5'}$ = 14.4 Hz, $^3$J$_{6-5}$ = 8.4 Hz, 1H, H$_6$), 4.62 (dd, $^2$J$_{6'-5'}$ = 14.4 Hz, $^3$J$_{6'-5}$ = 3.0 Hz, 1H, H$_6''$), 4.80–4.84 (m, 2H, H$_1$, H$_2$), 4.87–
Chapter Three

4.91 (m, 1H, H₄), 5.22–5.23 (m, 1H, H₃), 5.41 (s, 2H, CH₂O), 7.53 (br s, 2H, SO₂NH₂), 7.91–8.08 (m, 4H, Ar CH), 8.27 (s, triazole H); ¹³C{¹H} NMR (100 MHz, DMSO-d₆) δ 20.99 (OAc), 21.05 (OAc), 21.17 (OAc), 50.59 (C₆), 55.17 (OCH₃), 59.06 (CH₂O), 68.03 (C₅), 70.08 (C₁₉ or C₂₊), 70.20 (C₁₉ or C₂₊), 70.35 (C₅), 96.57 (C₁₉), 126.55 (triazole CH), 126.77 (Ar CH), 130.58 (Ar CH), 132.75 (Ar C), 142.34 (triazole C or Ar C), 148.87 (triazole C or Ar C), 165.11 (C=O), 170.03 (C=O), 170.27 (C=O), 170.34 (C=O). HRMS (ESI) calcd for C₂₃H₂₇N₄O₁₂S⁻: 583.135167. Found: 583.133906. Anal. calcd for C₂₃H₂₈N₄O₁₂S: C, 47.26; H, 4.83; N, 9.58; S, 5.49. Found: C, 46.72; H, 4.65; N, 8.04; S, 4.95.

Methyl 6-deoxy-6-(4-{4-Sulfamoylbenzoyloxy}methyl-1H-1,2,3-triazol-1-yl)-α-D-glucopyranoside (3g’). The title compound was prepared according to procedure 1 and isolated as white solid (75 mg, 0.16 mmol, 72%). Rf 0.52 (3:7 EtOH–CHCl₃); Mp 121–122°C. ¹H NMR (400 MHz, 2% D₂O in DMSO-d₆) δ 2.85 (s, 3H, OCH₃), 2.98 (dd, 3J₄₅₋₅ = 10.0 Hz, 3J₄₋₃ = 8.8 Hz, 1H, H₄′), 3.16 (dd, 3J₂₋₃ = 9.6 Hz, 3J₂₋₁ = 3.6 Hz, 1H, H₂′), 3.32–3.37 (m, 1H, H₃′), 3.61–3.66 (m, 1H, H₅′), 4.38 (dd, 3J₆₋₆ = 14.0, 3J₆₋₅ = 9.2 Hz, 1H, H₆′), 4.43 (d, 3J₁₋₂ = 4.0 Hz, 1H, H₁′), 4.70 (dd, 3J₆₋₆ = 14.0 Hz, 3J₆₋₅ = 2.4 Hz, 1H, H₆′), 5.40 (s, 2H, CH₂O), 7.90–8.07 (m, 4H, Ar), 8.16 (s, 1H, triazole CH); ¹³C{¹H} NMR (100 MHz, 2% D₂O in DMSO-d₆) δ 51.01 (C₆), 54.04 (OCH₃), 58.39 (CH₂O), 70.41 (C₅′), 71.66 (C₄′, C₂′), 73.06 (C₃′), 99.67 (C₁′), 171.
125.89 (triazole CH), 126.08 (Ar CH), 129.89 (Ar CH), 132.08 (Ar C), 141.41 (triazole C or Ar C), 148.75 (triazole C or Ar C), 164.43 (C=O). HRMS (ESI) calcd for \( \text{C}_{17}\text{H}_{21}\text{N}_{4}\text{O}_{9}\text{S} \): 457.10347. Found: 457.10341.

**3.4.5.3 Glycoconjugate benzene sulfonamides (library II)**

![Diagram of 4-(Aminosulfonyl)phenyl]-1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazole](image)

The title compound was prepared according to general procedure 1 and isolated as white solid (102 mg, 0.18 mmol, 69%). \( R_f \) 0.29 (3:7 hexanes–EtOAc); Mp 218-219°C. \( ^1\text{H} \) NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 1.78 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.98 (s, 3H, OAc), 2.01 (s, 3H, OAc), 4.07 (dd, 1H, \( ^2J_{6',6''} = 12.8 \) Hz, \( ^3J_{6',5'} = 2.4 \) Hz, 1H, H\( \ce{O} \)), 4.14 (dd, \( ^2J_{6',6''} = 12.8 \) Hz, \( ^3J_{6',5'} = 5.6 \) Hz, 1H, H\( \ce{O} \)), 4.41 (ddd, \( ^3J_{5',4'} = 10 \) Hz, \( ^3J_{5',6''} = 5.6 \) Hz, \( ^3J_{5',6'} = 2.4 \) Hz, 1H, H\( _2 \)), 5.10–5.16 (m, 1H, H\( _4 \)), 5.56–5.61 (m, 1H, H\( _3 \)), 5.62–5.67 (m, 1H, H\( _2 \)), 6.41 (d, \( ^3J_{1',2'} = 8.8 \) Hz, 1H, H\( _1 \)), 7.37 (br s, 2H, SO\( _2\text{NH}_2 \)), 7.87–8.01 (m, Ar), 8.68 (s, 1H, triazole CH); \( ^{13}\text{C}\{^1\text{H}\} \) NMR (100 MHz, DMSO-\( d_6 \)) \( \delta \) 20.56 (OAc), 20.92 (OAc), 21.07 (OAc), 21.20 (OAc), 62.43 (C\( _6 \)), 68.21 (C\( _4 \)), 70.99 (C\( _3 \)), 72.68 (C\( _2 \)), 73.88 (C\( _3 \)), 84.69 (C\( _1 \)), 122.34 (triazole CH), 126.21 (Ar CH), 127.18 (Ar CH), 133.81 (Ar C), 144.27 (triazole C or Ar C), 146.40 (triazole C or Ar C), 169.30 (OAc), 170.07 (OAc), 170.24 (OAc), 170.70 (OAc). HRMS (ESI) calcd for \( \text{C}_{22}\text{H}_{25}\text{N}_{4}\text{O}_{11}\text{S} \): 553.124602. Found: 553.123679. Anal. calcd for \( \text{C}_{22}\text{H}_{26}\text{N}_{4}\text{O}_{11}\text{S}.2\text{H}_{2}\text{O} \): C, 44.74; H, 5.12; N, 9.49; S, 5.43. Found: C, 44.79; H, 4.64; N, 9.28; S, 5.16.
4-[4-(Aminosulfonyl)phenyl]-1-(β-D-glucopyranosyl)-1H-1,2,3-triazole (3.13a').

The title compound was prepared from 3.13a according to general procedure 2 and isolated as white crystalline solid (32 mg, 0.08 mmol, ~100%). Mp 243–244°C (decomp). \(^1\)H NMR (400 MHz, 2% D₂O in DMSO-\(d_6\)) \(\delta\) 3.20–3.25 (m, 1H, \(H_4\)), 3.38–3.49 (m, 3H, \(H_3, H_5, H_6\)), 3.67–3.69 (m, 1H, \(H_6^-\)), 3.74–3.78 (m, 1H, \(H_2\)), 5.57 (d, \(^3J_{1',2'} = 9.2\) Hz, 1H, \(H_1\)), 7.86–8.05 (m, 4H, \(Ar\) CH), 8.94 (s, 1H, triazole CH); \(^13\)C \({}^1\)H NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 61.28 (C\(_6\)), 70.10 (C\(_4\)), 72.81 (C\(_2\)), 77.23 (C\(_3\)), 80.53 (C\(_5\)), 88.37 (C\(_1\)), 122.38 (triazole CH), 126.11 (Ar CH), 127.15 (Ar CH), 134.41 (Ar C), 143.76 (triazole C or Ar C), 145.83 (triazole C or Ar C). HRMS (ESI) calcd for C\(_{14}\)H\(_{17}\)N\(_4\)O\(_7\)S\(_9\) 387.096894. Found: 387.097523.

4-[4-(Aminosulfonyl)phenyl]-1-(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl)-1H-1,2,3-triazole (3.13b).

The title compound was prepared according to general procedure 1 and isolated as white solid after flash chromatography (255 mg, 0.46 mmol, 86%). \(R_f\) 0.21 (1:4 hexanes–EtOAc); Mp 219–220°C (decomp). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 1.81 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.97 (s, 3H, OAc), 2.19 (s, 3H, OAc), 4.03 (dd, \(^2J_{6'-5'} = 11.6\) Hz, \(^3J_{6'-5'} = 7.2\) Hz, 1H, \(H_6^-\)), 4.13 (dd, \(^2J_{6'-5'} = 11.2\) Hz, \(^3J_{6'-5'} = 4.8\) Hz, 1H, \(H_5\)), 4.61–4.64 (m, 1H, \(H_5^-\)), 5.42–5.53 (m, 1H, \(H_4\)), 5.49 (dd, \(^3J_{3'-2'} = 10.4\) Hz, \(^3J_{3'-2'} = 3.6\) Hz, 1H, \(H_3^-\)), 5.58–5.63 (m, 1H, \(H_2\)), 6.32 (d, \(^3J_{1'-2'} = 9.2\) Hz, 1H, \(H_1\)), 7.37
Chapter Three

(br s, 2H, SO₂NH₂), 7.87–8.10 (m, 4H, Ar), 8.75 (s, 1H, triazole CH); ¹³C{¹H} NMR (100 MHz, DMSO-d₆) δ 20.67 (OAc), 20.99 (OAc), 21.11 (OAc), 21.15 (OAc), 62.27 (C₆), 67.95 (C₄), 68.59 (C₃'), 71.00 (C₂'), 73.72 (C₅'), 85.23 (C₁'), 122.51 (triazole CH), 126.33 (Ar CH), 127.05 (Ar CH), 133.85 (Ar C), 144.23 (Ar C), 146.39 (triazole C), 169.36 (OAc), 170.16 (OAc), 170.62 (OAc), 170.68 (OAc).

HRMS (ESI) calcd for C₂₂H₂₆N₄O₁₁SNa⁺: 577.121102. Found: 577.122218. Anal. calcd for C₂₂H₂₆N₄O₁₁S: C, 47.65; H, 4.73; N, 10.10; S, 5.78. Found: C, 47.27; H, 4.72; N, 9.67; S, 5.51.

4-[(4-(Aminosulfon|phenyl)-1-(β-D-galactopyranosyl)-1H-1,2,3-triazole (3.13b').

The title compound was prepared from 3.13b according to general procedure 2 and isolated as pale yellow crystalline solid (140 mg, 0.36 mmol, ~100%). Rₚ 0.12 (1:9 H₂O–CH₃CN); Mp 171–172°C (decomp).

¹H NMR (400 MHz, ~1% D₂O in DMSO-d₆) δ 3.45–3.52 (m, 2H, H₆, H₆'), 3.56 (dd, ³J₃',₂' = 9.2 Hz, ³J₃',₄' = 3.2 Hz, 1H, H₃'), 3.72–3.75 (m, 1H, H₅), 3.76–3.77 (m, 1H, H₄'), 4.04–4.09 (m, 1H, H₂'), 5.52 (d, ³J₁',₂' = 9.2 Hz, 1H, H₁'), 7.36 (br s, 2H, SO₂NH₂), 7.86–8.08 (m, 4H, Ar H), 8.90 (s, 1H, triazole CH); ¹³C{¹H} NMR (100 MHz, 1% D₂O in DMSO-d₆) δ 61.04 (C₆), 68.97 (C₅'), 69.97 (C₂'), 74.11 (C₃'), 79.14 (C₄'), 88.03 (C₁'), 122.17 (triazole CH), 126.13 (Ar CH), 127.10 (Ar CH), 143.76 (triazole C or Ar C), 145.84 (triazole C or Ar C). HRMS (ESI) calcd for C₁₄H₁₈N₄O₇S⁺: 409.079016. Found: 409.078171.
The title compound was prepared according to general procedure 1 and isolated as white solid (195 mg, 3.52 mmol, 88%). Rf 0.42 (1:9 CH₃OH–EtOAc); Mp 249.6°C (decomp). ¹H NMR (400 MHz, DMSO-⁶d) δ 1.57 (s, 3H, NHAc), 1.93 (s, 3H, OAc), 1.98 (s, 3H, OAc), 2.00 (s, 3H, OAc), 4.06 (dd, ²J₆-v.₆-v' = 12.4 Hz, ³J₆-v.₅ = 2.0 Hz, 1H, H₆'), 4.15 (dd, ²J₆-v.₆-v' = 12.5 Hz, ³J₆-v.₅ = 5.2 Hz, 1H, H₆'), 4.28 (ddd, ³J₅-v.₄-v = 10.4 Hz, ³J₅-v.₆ = 5.2 Hz, ³J₅-v.₅-v = 2.4 Hz, 1H, H₅'), 4.58–4.67 (m, 1H, H₂'), 5.06–5.11 (m, 1H, H₄'), 5.34–5.39 (m, 1H, H₃'), 6.14 (d, 1H, ³Jₑ-v.₂ = 10.0 Hz, 1H, H₁'), 7.37 (br s, 2H, SO₂NH₂), 7.87–8.00 (m, 4H, Ar), 8.10 (d, ³J₁NH₂ = 9.2 Hz, 1H, NHAc NH), 8.98 (s, 1H, triazole H); ¹³C (¹H) NMR (100 MHz, DMSO-⁶d) δ 20.96 (OAc), 21.10 (OAc), 21.20 (OAc), 22.98 (NHAc), 53.05 (C₂'), 62.43 (C₆'), 68.70 (C₄'), 72.91 (C₃'), 74.10 (C₅'), 85.68 (C₇'), 122.81 (triazole CH), 126.14 (Ar CH), 127.17 (Ar CH), 134.01 (Ar C), 144.16 (triazole CH or Ar C), 145.98 (triazole CH or Ar C), 170.06 (C=O), 170.21 (C=O), 170.27 (C=O), 170.71 (C=O). HRMS (ESI) calcd for C₂₂H₆₆N₅O₁₀S: 577.121102. Found: 577.122218. Anal. calcd for C₂₂H₂₇N₅O₁₀S: C, 47.74; H, 4.92; N, 12.65; S, 5.79. Found: C, 47.82; H, 4.97; N, 12.18; S, 5.45.
The title compound was prepared from \textit{3.13d} according to general procedure 2 and isolated as white solid (116 mg, 0.27 mmol, \textasciitilde100\%). Mp 215–217° C. \textit{\textit{1}}H NMR (400 MHz, 1\% D\textsubscript{2}O in DMSO-\textit{d}\textsubscript{6}) \( \delta \) 1.59 (s, 3H, NHAc), 3.24–3.23 (m, 1H, H\textsubscript{4}), 3.43–3.49 (m, 2H, H\textsubscript{6\textprime}, H\textsubscript{6\textprime\prime}), 3.55–3.59 (m, 1H, H\textsubscript{3}), 3.68–3.73 (m, 1H, H\textsubscript{3\textprime}), 4.04–4.09 (m, 1H, H\textsubscript{2}), 5.73 (d, \( J\textsubscript{1\textprime-2\textprime} = 10.0 \) Hz, 1H, H\textsubscript{1\textprime}), 7.85–8.02 (m, 4H, H\textsubscript{6}), 8.81 (s, 1H, triazole CH); \textit{\textit{13}}C\{\textit{\textit{1}}H\} NMR (100 MHz, DMSO-\textit{d}\textsubscript{6}) \( \delta \) 23.28 (NHAc CH\textsubscript{3}), 55.46 (C\textsubscript{2}), 61.32 (C\textsubscript{6}), 70.55 (C\textsubscript{4}), 74.25 (C\textsubscript{5}), 80.74 (C\textsubscript{3}), 86.99 (C\textsubscript{1\textprime}), 122.02 (triazole CH), 126.10 (Ar CH), 127.10 (Ar CH), 134.35 (Ar C), 143.86 (triazole C or Ar C), 145.59 (triazole C or Ar C), 169.92 (NHAc C=O). HRMS (ESI) calcd for C\textsubscript{16}H\textsubscript{20}N\textsubscript{5}O\textsubscript{7}S\textsubscript{9} 426.108893. Found: 426.107678.

The title compound was prepared according to general procedure 1 and isolated as white solid (145 mg, 0.27 mmol, 87\%). \textit{R}_{f} 0.63 (1:9 CH\textsubscript{3}OH–CH\textsubscript{2}Cl\textsubscript{2}); Mp 249–251° (decomp); \textit{\textit{1}}H NMR (400 MHz, DMSO-\textit{d}\textsubscript{6}) \( \delta \) 1.79 (s, 3H, OAc), 1.98 (s, 3H, OAc), 2.01 (s, 3H, OAc), 3.61 (s, 3H, COCH\textsubscript{3}), 4.84 (d, \( J\textsubscript{5\textprime-4\textprime} = 10.4 \) Hz, 1H, H\textsubscript{5\textprime}), 5.19–5.24 (m, 1H, H\textsubscript{4}), 5.63–5.74 (m, H\textsubscript{2\textprime} and H\textsubscript{3}), 6.46 (d, \( J\textsubscript{1\textprime-2\textprime} = 8.8 \) Hz, 1H, H\textsubscript{1\textprime}),
Chapter Three

7.37 (br s, 2H, SO2NH2), 7.88–8.01 (m, 4H, Ar CH), 9.15 (s, 1H, triazole CH);

13C\{1H\} NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 20.57 (OAc), 20.90 (OAc), 20.95 (OAc), 53.39 (OCH3), 69.13 (C4), 70.65 (C2'), 72.03 (C3'), 73.59 (C5'), 84.58 (C1'), 122.52 (triazole CH), 126.22 (Ar CH), 127.21 (Ar CH), 133.73 (Ar C), 144.32 (Ar C), 156.52 (triazole C), 167.22 (C=O), 164.24 (OAc), 170.02 (OAc), 170.20 (OAc). HRMS (ESI) calcd for C21H24N4O11SNa+: 563.103756. Found: 563.103756.

---

\[ 
\text{4-[4-(Aminosulfonyl)phenyl]-1-(\beta-D-glucuronic acid methyl ester)-1H-1,2,3-triazole} \ (3.13e'). \]

The title compound was prepared according to general procedure 2 and isolated as pale yellow foam (22 mg, 0.05 mmol, ~100%).

1H NMR (400 MHz, 2% D2O in DMSO-\(d_6\)) \(\delta\) 3.45–3.54 (m, 2H, H3· and H4·), 3.63 (s, 3H, OCH3), 3.85–3.89 (m, 1H, H2·), 4.19 (d, \(\jmath_{5',4'}\) = 8.8 Hz, 1H, H5·), 5.79 (d, \(\jmath_{1',2'}\) = 0.06 (m, 4H, Ar CH), 8.98 (s, 1H, triazole CH); 13C\{1H\} NMR (100 MHz, 2% D2O in DMSO-\(d_6\)) \(\delta\) 52.78 (OCH3), 71.80 (C3), 72.31 (C4), 76.39 (C2), 78.02 (C5'), 87.95 (C1'), 122.51 (triazole CH), 126.16 (Ar CH), 127.14 (Ar CH), 134.28 (Ar C), 143.86 (Ar C), 145.97 (triazole C), 169.39 (C=O). HRMS (ESI) calcd for C15H18N4O8S+: 437.073756. Found: 437.073966.
4-[4-(Aminosulfonyl)phenyl]-1-(hepta-O-acetyl-β-D-galactopyranosyl)-1H-1,2,3-triazole (3.13f). The title compound was prepared according to the general procedure 1 and isolated as white solid (210 mg, 0.25 mmol, 83%). Rf 0.26 (3:7 hexanes–EtOAc); Mp 241–242°C (decomp). 1H NMR (400 MHz, DMSO-d6) δ 1.75 (s, 3H, OAc), 1.94 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.99 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.03 (s, 3H, OAc), 3.89–4.03 (m, 2H, Glc ᵃH₅, Glc ᵃH₆⁻), 4.09–4.18 (m, 3H, Glc ᵇH₄⁻, Glc ᵇH₆⁻, Glc ᵇH₆⁻), 4.38 (ddd, ᵃJ₃,₄⁻ = 9.6 Hz, ᵃJ₅,₆⁻ = 5.6 Hz, ᵃJ₅,₆⁻ = 2.0 Hz, 1H, Glc ᵇH₅⁻), 4.44 (dd, ᵃJ₆⁻,₆⁻ = 12.4 Hz, ᵃJ₆⁻,₅⁻ = 2.4 Hz, 1H, Glc ᵇH₆⁻), 4.90 (dd, ᵃJ₂⁻,₃⁻ = 10 Hz ᵃJ₂⁻,₁⁻ = 4.0 Hz, 1H, Glc ᵇH₃⁻), 5.23 (dd, ᵃJ₂⁻,₂⁻ = 10 Hz ᵃJ₃⁻,₄⁻ = 9.6 Hz, 1H, Glc ᵇH₄⁻), 5.36 (d, ᵃJ₁⁻,₂⁻ = 4.0 Hz, 1H, Glc ᵇH₁⁻), 5.50–5.54 (m, 1H, Glc ᵇH₂⁻), 5.61–5.66 (m, 1H, Glc ᵇH₃⁻), 6.37 (d, ᵃJ₁⁻,₂⁻ = 9.2 Hz, 1H, Glc ᵇH₁⁻), 7.37 (br s, 2H, SO₂NH₂), 7.87–8.02 (m, 4H, Ar), 9.87 (s, 1H, triazole CH); 13C{¹H} NMR (100 MHz, DMSO-d6) δ 20.61 (OAc), 20.95 (OAc), 21.03 (2 × OAc), 21.12 (OAc), 21.20 (OAc), 21.25 (OAc), 62.08 (Glc ᵇC₆⁻), 63.48 (Glc ᵇC₆⁻), 68.38 (Glc ᵇC₄⁻), 68.87 (Glc ᵇC₄⁻), 69.59 (Glc ᵇC₃⁻), 70.12 (Glc ᵇC₂⁻), 71.47 (Glc ᵇC₂⁻), 74.13 (Glc ᵇC₅⁻), 74.59 (Glc ᵇC₅⁻), 74.92 (Glc ᵇC₃⁻), 84.27 (Glc ᵇC₁⁻), 96.44 (Glc ᵇC₁⁻), 122.42 (triazole CH), 126.23 (Ar CH), 127.15 (Ar CH), 133.79 (Ar C), 144.26 (triazole C or Ar C), 146.31 (triazole C or Ar C), 169.48 (OAc), 169.85 (OAc), 170.20 (OAc), 170.37 (OAc), 170.55 (OAc), 170.68 (OAc), 170.80 (OAc). HRMS (ESI) calcd for C₃₄H₄₂N₄O₁₉S₁Na⁺: 865.205623. Found:
Chapter Three

865.206114. Anal. calcd for C$_{34}$H$_{42}$N$_4$O$_{19}$S$_9$: C, 48.45; H, 5.02; N, 6.65; S, 3.80.
Found: C, 48.21; H, 4.93; N, 6.64; S, 3.40.

4-[4-(Aminosulfonyl)phenyl]-1-(β-d-galactopyranosyl)-1H-1,2,3-triazole (3.13f').

The title compound was prepared from 3.13b according to general procedure 2 and isolated as white solid (45 mg, 0.08 mmol, 92%). $R_f$ 0.14 (1:9 H$_2$O–CH$_3$CN); Mp 228–230°C (decomp). $^1$H NMR (400 MHz, D$_2$O) δ 3.28–3.33 (m, 1H, Glcα H$_3$/H$_4^-$ or Glcβ H$_3$/H$_4^-$), 3.48 (dd, $^3$J$_{2^-3^-}$ = 10.0 Hz, $^3$J$_{2^-1^-}$ = 3.6 Hz, 1H, Glcα H$_2$), 3.57–3.85 (m, 8H, Glcα H$_3$/H$_4^-$, Glcα H$_5^-$, Glcα H$_6^-$, Glcα H$_6^-$, Glcβ H$_3$/H$_4^-$, Glcβ H$_5^-$, Glcβ H$_6^-$ and Glcβ H$_6^-$), 3.87–3.86 (m, 2H, Glcβ H$_2^-$, Glcβ H$_3^-$), 3.35 (d, 1H, Glcα H$_1^-$), 5.68 (d, $^3$J$_{1^-2^-}$ = 8.0 Hz, 1H, Glcβ H$_1^-$), 7.72–7.79 (m, 4H, Ar), 8.46 (s, 1H, triazole CH); $^{13}$C$_{^{1}}$H NMR (100 MHz, D$_2$O) δ 60.65 (Glcα C$_6^-$ and Glcβ C$_6^-$), 69.49, 71.86, 72.40, 72.95, 73.06, 76.01, 76.54, 77.72 (Glcα C$_2^-$, Glcα C$_3^-$, Glcα C$_4^-$, Glcα C$_5^-$ and Glcβ C$_2^-$, Glcβ C$_3^-$, Glcβ C$_4^-$, Glcβ C$_5^-$), 87.59 (Glcβ C$_1^-$), 99.85 (Glcα C$_1^-$), 122.51 (triazole CH), 126.49 (Ar CH), 126.77 (Ar CH), 133.74 (Ar C), 141.07 (triazole C or Ar C), 146.25 (triazole C or Ar C). HRMS (ESI) calcd for C$_{20}$H$_{28}$N$_4$O$_{12}$S$_1$Na$: 571.131664. Found: 571.131272.
4-[4-(Aminosulfonyl)phenyl]-1-(2',3',4'-tri-O-benzoyl-β-D-ribofuranosyl)-1H-1,2,3-triazole (3.13h).

The title compound was prepared according to general procedure 1 and isolated as white solid (343 mg, 0.51 mmol, 88%). $R_f$ 0.52 (2:3 hexanes-EtOAc); Mp 168–169°C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.58 (dd, $^2J_{5',5''} = 12.0$ Hz, $^3J_{5',4'} = 4.4$ Hz, 1H, $H_{5''}$), 4.70 (dd, $^2J_{5',5''} = 12.4$ Hz, $^3J_{5',4'} = 3.6$ Hz, 1H, $H_{5''}$), 4.96–5.00 (m, 1H, $H_1$), 6.43 (dd, $^3J_{3',4'} = 6.0$ Hz, $^3J_{3',2'} = 5.2$ Hz, 1H, $H_{3'}$,), 6.33 (dd, $^3J_{2',3'} = 5.2$ Hz, $^3J_{2',1'} = 2.8$ Hz, 1H, $H_{2'}$), 6.78 (d, $^3J_{1',2'} = 2.8$ Hz, 1H, $H_{1'}$), 7.39 (br s, 2H, SO$_2$NH$_2$), 7.40–7.50 (m, 6H, Ar), 7.56–7.68 (m, 3H, Ar), 7.87–8.01 (m, 10H, Ar), 8.97 (s, 1H, triazole CH); $^{13}$C ($^1$H) NMR (100 MHz, DMSO-$d_6$) $\delta$ 63.86 (C$_{5''}$), 71.69 (C$_{3''}$), 75.19 (C$_{2''}$), 80.61 (C$_4$), 90.25 (C$_1$), 123.24 (triazole CH), 126.29, 127.09, 129.31, 129.43, 129.45, 129.78, 129.93, 130.07, 130.15, 133.88, 134.18, 134.61, 134.77 (Ar CH and Ar C), 144.20 (triazole C or Ar C), 146.33 (triazole C or Ar C), 165.15 (C=O), 165.37 (C=O), 166.08 (C=O). HRMS (ESI) calcd for C$_{34}$H$_{28}$N$_4$O$_9$SNa$: 691.146922$. Found: 691.146135. Anal. calcd for C$_{34}$H$_{28}$N$_4$O: C, 61.07; H, 4.22; N, 8.38; S, 4.80. Found: C, 60.61; H, 4.11; N, 8.47; S, 4.65.

4-[4-(Aminosulfonyl)phenyl]-1-(β-D-ribofuranosyl)-1H-1,2,3-triazole (3.13h’).

The title compound prepared from 3.13h according to general procedure 2 and isolated as white solid (84 mg, 0.24 mmol, ~100%). $R_f$ 0.16 (1:9 CH$_3$OH–EtOAc); Mp 219–220°C. $^1$H NMR (400 MHz, 1%
Chapter Three

$D_2O$ in DMSO-$d_6$ $\delta$ 3.51 (dd, $^2J_{5',5''} = 12.0$ Hz, $^3J_{5',4'} = 4.4$ Hz, 1H, $H_5'$), 3.62 (dd, $^2J_{5',5''} = 12.4$ Hz, $^3J_{5',4'} = 4.0$ Hz, 1H, $H_5''$), 3.87–4.00 (m, 1H, $H_4$), 4.12–4.15 (m, 1H, $H_3$), 4.40–4.43 (m, 1H, $H_2$), 5.96 (d, $^3J_{1',2} = 4.4$ Hz, 1H, $H_1'$), 3.73 (br s, 2H, SO$_2$NH$_2$), 7.87–8.03 (m, 4H, $Ar$), 8.88 (s, 1H, triazole CH); $^{13}$C NMR (100 MHz, 1% $D_2O$ in DMSO-$d_6$) $\delta$ 61.88 (C$_{5n}$), 70.86 (C$_{3n}$), 75.63 (C$_{2n}$), 86.57 (C$_{4n}$), 92.90 (C$_{1n}$), 121.72 (triazole CH), 126.15 ($Ar$ CH), 127.14 ($Ar$ CH), 134.34 ($Ar$ C), 143.89 (triazole C), 146.06 ($Ar$ C). HRMS (ESI) calcd for C$_{13}$H$_{17}$N$_4$O$_6$S$^+$: 357.086501. Found: 357.082990.

3.4.5.4 Glycoconjugate benzene sulfonamides (library III)

4-[[2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl]oxymethyl]-1H-1,2,3-triazol-1-yl]benzene sulfonamide (3.14i). The title compound was prepared according to general procedure 1 and isolated as white foam (175 mg, 0.3 mmol, 77%). $R_f$ 0.63 (100% EtOAc). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.87 (s, 3H, OAc), 1.90 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.99 (s, 3H, OAc), 3.89–4.07 (m, 3H, H$_6$–), 4.19 (dd, $^2J_{6''-6'} = Hz$, 12.4 Hz, $^3J_{6''-5''} = 5.2$ Hz, 1H, $H_6$–), 4.77 (dd, $^3J_{2'-1'} = 9.6$ Hz, $^3J_{2'-3'} = 8.0$ Hz, 1H, $H_2'$), 4.80 (AB q, $^2J_{AB} = 12.4$ Hz, 2H, OCH$_2$), 4.89 (d, $^3J_{1'-2'} = 9.6$ Hz, 1H, $H_1'$), 4.91–4.95 (m, 1H, $H_4$), 5.25 (m, 1H, $H_3$), 7.50 (br s, 2H, SO$_2$NH$_2$), 7.99–8.11 (m, 4H, $Ar$), 8.87 (s, 1H, triazole CH); $^{13}$C{$_1^1$H} NMR (100 MHz, DMSO-$d_6$) $\delta$ 20.94 (OAc), 21.01 (OAc), 21.06 (OAc), 21.18 (OAc), 62.24 (C$_6$), 62.36 (OCH$_2$), 68.79 (C$_4$), 71.36 (C$_5$), 71.53 (C$_2$), 72.73 (C$_3$), 99.21 (C$_1$), 121.02 ($Ar$ CH), 123.72
Chapter Three

(triazole CH), 128.20 (Ar CH), 139.22 (Ar C), 144.58 (triazole C or Ar C), 145.02 (triazole C or Ar C), 169.69 (C=O), 169.53 (C=O), 170.21 (C=O), 170.75 (C=O).

HRMS (ESI) calcd for C$_{23}$H$_{27}$N$_{4}$O$_{12}$S$^{-}$: 583.135392. Found: 583.134630. Anal. calcd for C$_{23}$H$_{28}$N$_{4}$O$_{12}$S: C, 45.84; H, 5.02; N, 9.30; S, 5.32. Found: C, 46.54; H, 4.91; N, 9.12; S, 5.18.

Colourless crystals of 3.14i (C$_{23}$H$_{28}$N$_{4}$O$_{12}$S.CH$_{4}$O) suitable for X-ray crystallographic analysis was obtained from absolute ethanol: $M = 616.61$, monoclinic, space group P2$_1$, $a = 17.784$ (3), $b = 7.349$ (5), $c = 10.977$ (2) Å, $\beta = 104.446$ (15), $V = 1389.3$ (10) A$^3$, $T = 295$ K, $Z = 2$, $D_x = 1.474$ Mg m$^{-3}$, $\mu$(Mo-K$\alpha$) = 0.191 mm$^{-1}$, 3889 reflections measured, 3458 unique ($R_{int} = 0.0800$). Refinement converged to $R_p = 0.0707$, $wR(F^2) = 0.2166$.

![Chemical Structure](image)

4-(4-[[β-D-glucopyranosyl]oxymethyl]-1H-1,2,3-triazol-1-yl)benzene sulphonamide (3.14i’). The title compound was prepared from 3.14i according to general procedure 2 and isolated as white solid (107 mg, 0.26 mmol, ~100%). $R_f$ 0.12 (1:9 H$_2$O–CH$_3$CN); Mp 244–246°C (decomp). $^1$H NMR (400 MHz, 2% D$_2$O in DMSO-$d_6$) $\delta$ 3.16–3.20 (m, 1H, H$_2$), 3.23–3.28 (m, 1H, H$_4$), 3.33–3.38 (m, 2H, H$_3$, H$_5$), 3.58 (dd, $^2$J$_{6\cdot6\cdot6}$ = 12.8 Hz, $^3$J$_{6\cdot5\cdot6}$ = 5.6 Hz, 1H, H$_6$), 3.78 (dd, $^2$J$_{6\cdot6\cdot6}$ = 12.4 Hz, $^3$J$_{6\cdot5\cdot6}$ = 2.0 Hz, 1H, H$_6$), 4.47 (d, $^3$J$_{1\cdot2\cdot1}$ = 8.0 Hz, 1H, H$_1$), 4.89 (AB q, $^2$J$_{AB} = 12.8$ Hz, 2H, OCH$_2$), 7.86–7.99 (m, 4H, Ar), 8.49 (s, 1H, triazole CH); $^{13}$C{${^1}$H} NMR
Chapter Three

(100 MHz, 2% D2O in DMSO-d6) δ 60.96 (C6), 62.09 (OCH2), 69.84 (C4), 73.23 (C3), 75.92 (C2), 76.21 (C5), 101.84 (C1), 121.75 (Ar CH), 124.00 (triazole CH), 128.08 (Ar CH), 139.54 (Ar C), 142.24 (triazole C or Ar C), 144.92 (triazole C or Ar C). HRMS (ESI) calcd for C15H19N4O8S-9 415.02908. Found: 415.093029.

4-(4-[[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl]oxymethyl]-1H-1,2,3-triazol-1-yl)benzene sulphonamide (3.14j).

The title compound was prepared according to general procedure 1 and isolated as pale yellow foam (156 mg, 0.38 mmol, 82%). Rf 0.23 (1:4 hexanes–EtOAc). 1H NMR (400 MHz, DMSO-d6) δ 1.87 (s, 3H, OAc), 1.91 (s, 3H, OAc), 1.98 (s, 3H, OAc), 2.09 (s, 3H, OAc), 4.01–4.10 (m, 2H, H6), 4.20–4.23 (m, 1H, H5), 4.72 (d, 3J1-2 = 10.0 Hz, 1H, H1), 4.80 (AB q, 2JAB = 12.4 Hz, 2H, OCH2), 4.84 (d, 3J1-2 = 8.0 Hz, 1H, H1), 4.93 (dd, 3J2-3 = 10.0 Hz, 3J2-1 = 8.0 Hz, 1H, H2), 5.14 (dd, 3J5-6 = 10.0 Hz, 3J5-4 = 3.6 Hz, 1H, H3), 5.23–4.25 (m, 1H, H4), 7.49 (br s, 2H, SO3NH2), 7.98–8.11 (m, 4H, Ar), 8.88 (s, 1H, triazole CH); 13C{1H} NMR δ 21.00 (OAc), 21.07 (OAc), 21.11 (OAc), 21.18 (OAc), 61.96 (OCH2), 62.18 (C6), 67.99 (C4), 69.26 (C3), 70.69 (C2), 70.88 (C5), 99.71 (C1), 121.00 (Ar CH), 123.65 (triazole CH), 128.22 (Ar C), 139.20 (Ar C), 144.60 (triazole C or Ar C), 145.09 (triazole C or Ar C) 169.79 (OAc), 170.15 (OAc), 170.60 (OAc), 170.62 (OAc). HRMS (ESI) calcd for C23H22N4O12S: 582.135392. Found: 583.134423. Anal. calcd for C23H28N4O12S: C, 47.26; H, 4.83; N, 9.58; S, 5.49. Found: C. 46.91; H, 4.84; N, 9.57; S, 5.31.
The title compound was prepared according to general procedure 2 and isolated as white solid (48 mg, 0.12 mmol, ~100%). \( R_f \) 0.13 (1:9 H\(_2\)O–CH\(_3\)CN); Mp 241–244°C (decomp). \(^1\)H NMR (400 MHz, D\(_2\)O) \( \delta \) 3.42 (dd, \(^3\)J\(_{2'3'}\) = 9.6 Hz, \(^3\)J\(_{2'1'}\) = 7.6 Hz, 1H, H\(_2\)), 3.50 (dd, \(^3\)J\(_{3'2'}\) = 10.0 Hz, \(^3\)J\(_{3'4'}\) = 3.6 Hz, 1H, H\(_3\)), 3.56–3.69 (m, 3H, H\(_5\), H\(_6\), H\(_6'\)), 3.78–3.79 (m, 1H, H\(_4\)), 4.41 (d, \(^3\)J\(_{1'2'}\) = 7.6 Hz, 1H, H\(_1\)), 4.91 (AB q, \(^2\)J\(_{\text{AB}}\) = 12.4 Hz, 2H, OCH\(_2\)), 4.89–8.01 (m, 4H, \( \alpha \)r), 8.53 (s, 1H, triazole CH); \(^13\)C\(_{\text{\( ^1\)H}}\) NMR (100 MHz, DMSO-\(d_6\)) \( \delta \) 61.19 (C\(_6\)), 62.01 (OCH\(_2\)), 68.79 (C\(_4\)), 71.10 (C\(_3\)), 73.89 (C\(_2\)), 76.0 (C\(_5\)), 103.59 (C\(_1\)), 120.92 (Ar CH), 123.50 (triazole CH), 128.25 (Ar CH), 139.29 (Ar C), 144.44 (triazole C or Ar C), 146.21 (triazole C or Ar C). HRMS (ESI) calcd for C\(_{15}\)H\(_{19}\)N\(_4\)O\(_8\)-\(S\): 415.092908. Found: 415.093072.

\( 4-(4-\{[\beta-D-galactopyranosyl]oxymethyl\}-1H-1,2,3-triazol-1-yl)benzene sulfonamide \) (3.14j'). The title compound was prepared according to general procedure 1 and isolated as white foam (291 \( \mu \)l mixture by \(^1\)H NMR; 145 mg, 0.28 mmol, 89%); \( R_f \) 0.30 (1:4 hexanes–EtOAc); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \( \delta \) 1.90 (s, 3H, \( \alpha \)OAc CH\(_3\)), 1.91 (s, 6H, \( \alpha \) and \( \beta \)OAc), 1.93 (s, 3H, \( \beta \)OAc), 2.07 (s, 3H, \( \alpha \)OAc), 2.08 (s, 3H, \( \beta \)OAc), 3.67 (dd, \(^2\)J\(_{5'5''}\) = 13.2 Hz, \(^3\)J\(_{5'4'}\) = 2.0 Hz, 1H, \( \beta \)H\(_5\)), 3.83 (dd, \(^2\)J\(_{5'5''}\) = 13.2 Hz, \(^3\)J\(_{5'4'}\) = 2.0 Hz, 1H, \( \alpha \)H\(_5\)), 3.80 (dd,
\[ {\text{^2}J_{5',5}^n} = 13.2 \text{ Hz}, \ {\text{^3}J_{5',4}^n} = 2.4 \text{ Hz, } \alpha H_{5'} \), \ 4.02 \ (\text{dd, } {\text{^2}J_{5',5}^n} = 13.2 \text{ Hz, } {\text{^3}J_{5',4}^n} = 2.4 \text{ Hz, } 1H, \beta H_{5'} \), \ 4.72 \ (d, \ {\text{^3}J_{1',2}^n} = 8.0 \text{ Hz, } 1H, \alpha H_{1'} \), \ 4.73 \ (d, \ {\text{^3}J_{1',2}^n} = 2.8 \text{ Hz, } 1H, \beta H_{1'} \), \ 4.74 \ (\text{AB q, } {\text{^2}J_{AB}^n} = 12.8 \text{ Hz, } 2H, \beta OCH_{2} \), \ 4.78 \ (\text{AB q, } {\text{^2}J_{AB}^n} = 12.8 \text{ Hz, } 2H, \alpha OCH_{2} \), \ 4.93 \ (\text{dd, } {\text{^3}J_{2'-3}^n} = 10.0 \text{ Hz, } {\text{^3}J_{2',1}^n} = 8.0 \text{ Hz, } 1H, \alpha H_{2'} \), \ 4.97 \ (\text{dd, } {\text{^3}J_{2'-3}^n} = 10.4 \text{ Hz, } {\text{^3}J_{2',1}^n} = 3.2 \text{ Hz, } 1H, \beta H_{2'} \), \ 5.08 \ (\text{dd, } {\text{^3}J_{3'-2}^n} = 9.6 \text{ Hz, } {\text{^3}J_{3',4}^n} = 3.2 \text{ Hz, } 1H, \beta H_{3'} \), \ 5.12–5.14 \ (m, 1H, \alpha H_{4'} \), \ 5.18 \ (\text{dd, } {\text{^3}J_{3',2}^n} = 11.2 \text{ Hz, } {\text{^3}J_{3',4}^n} = 3.6 \text{ Hz, } 1H, \alpha H_{3'} \), \ 5.23–5.25 \ (m, 1H, \beta H_{4'} \), \ 7.49 \ (\text{br s, } 2H, \text{SO}_2 \text{NH}_2 \), \ 7.98–8.11 \ (m, 4H, Ar CH), \ 8.89 \ (s, 1H, \alpha triazole CH), \ 8.96 \ (s, 1H, \beta triazole CH); \ ^{13}C \{^1H\} \ NMR (100 MHz, DMSO-\text{d}_6) \delta 21.07 \ (\text{OAc} \), \ 21.13 \ (\text{OAc} \), \ 21.37 \ (\text{OAc} \), \ 61.84 \ (\text{OCH} \), \ 63.89 \ (C_5 \), \ 68.60 \ (C_4 \), \ 69.51 \ (C_2 \), \ 70.63 \ (C_3 \), \ 99.87 \ (C_1 \), \ 120.99 \ (Ar CH), \ 123.56 \ (triazole CH), \ 128.21 \ (Ar CH), \ 139.21 \ (Ph C), \ 145.56 \ (triazole C or Ar C), \ 145.13 \ (triazole C or Ar C), \ 169.82 \ (C=O), \ 170.23 \ (C=O), \ 170.51 \ (C=O). \ HRMS (ESI) calcd. for \text{C}_{20}\text{H}_{23}\text{N}_{4}\text{O}_{10}\text{S}^+ \text{; } 511.114249. \ Found: 511.113207. \ Anal. calcd for \text{C}_{20}\text{H}_{24}\text{N}_{4}\text{O}_{10}\text{S} \cdot 0.5 \text{H}_2\text{O}: \text{C}, \ 46.06; \text{H}, \ 4.83; \text{N}, \ 10.74; \text{S}, \ 6.15. \ Found: \text{C}, \ 45.81; \text{H}, \ 4.76; \text{N}, \ 10.72.

4-(4-\{\alpha/\beta-d-Arabinopyranosyl\}oxyethyl]-1-H-1,2,3-triazol-1-yl)benzensulfonamide (3.14k'). The title compound was prepared from 3.12k according to general procedure 2 and isolated as white solid (2:1 \alpha/\beta mixture by \text{^1}H \ NMR; \ 75 \text{ mg, } 0.19 \text{ mmol, } \sim \text{100\%}); \text{Rf} \ 0.23 \ (1:9 \text{ CH}_3\text{OH–EtOAc}); \text{^1}H \ NMR (400 MHz, 2\% \text{D}_2\text{O in DMSO-\text{d}_6}) \delta 3.31–3.47 \ (m, 4H, \alpha H_2', \alpha H_5', \beta H_5', \beta H_3'), 3.56–3.58 \ (m, 1H, \beta H_2'), 3.60–3.62 \ (m, 1H, \alpha H_4'), 3.67–3.73 \ (m, 4H, \alpha H_3', \alpha H_4', \beta H_4'), 3.71 \ (\text{dd, } {\text{^3}J_{3'-2}^n} = 12.0 \text{ Hz, } {\text{^3}J_{3',4}^n} = 3.6 \text{ Hz, } 1H, \beta H_3'), \ 4.23 \ (d, \ {\text{^3}J_{1',2}^n} = 6.0 \text{ Hz, }
Chapter Three

1H, αH1\(\cdot\)), 4.66 (AB q, \(^2J_{AB} = 12.0 \text{ Hz, } 1\text{H, } \beta\text{OCH}_2\)), 4.75 (AB q, \(^2J_{AB} = 12.8 \text{ Hz, } 2\text{H, } \alpha\text{OCH}_2\)), 4.80 (d, \(^3J_{1\cdot,2\cdot} = 2.4 \text{ Hz, } 1\text{H, } \beta\text{H1}\cdot\)), 7.98–8.10 (m, 4H, \(\text{Ar CH}\)), 8.87 (s, 1H, \(\alpha\text{ triazole CH}\)), 8.89 (s, 1H, \(\beta\text{ triazole CH}\)); \(^{13}\text{C}\{1\text{H}\} \text{ NMR (100 MHz, DMSO-d}_6\}) \(\delta\) 60.73 (\(\beta\text{OCH}_2\)), 61.44 (\(\alpha\text{OCH}_2\)), 63.85, 65.87, 67.99, 68.79, 69.06, 69.49, 71.03, 72.95, (\(\alpha\text{C}_2\), \(\alpha\text{C}_3\), \(\alpha\text{C}_4\), \(\alpha\text{C}_5\), \(\beta\text{C}_2\), \(\beta\text{C}_3\), \(\beta\text{C}_4\), \(\beta\text{C}_5\)), 99.60 (\(\beta\text{C}_1\cdot\)), 120.93 (\(\beta\text{Ar CH}\)), 120.65 (\(\alpha\text{Ar CH}\)), 123.18 (\(\alpha\text{ triazole CH}\)), 123.32 (\(\beta\text{ triazole CH}\)), 128.24 (\(\text{Ar CH}\)), 139.30 (\(\text{Ar C}\)), 144.36 (\(\text{Ar C}\)), 146.05 (\(\alpha\text{ triazole C}\)), 146.15 (\(\beta\text{ triazole C}\)).

HRMS (ESI) calcd. for C\(_{14}\)H\(_{17}\)N\(_4\)O\(_7\)S: 385.0823. Found: 385.0825.

4-(4-{[hepta-\(\text{O}-\text{acetyl-}\beta\text{-d-maltopyranosyl}]-\text{oxymethyl}]\})-1H-1,2,3-triazol-1-yl)benzene sulfonamide (3.14I). The title compound was prepared according to general procedure 1 and isolated as pale yellow foam (166 mg, 0.19 mmol, 86%). \(R_f\) 0.22 (1:1 EtOAc–hexanes). \(^1\text{H NMR (400 MHz, DMSO-d}_6\}) \(\delta\) 1.86 (OAc), 1.90 (OAc), 1.92 (OAc), 1.94 (OAc), 1.96 (OAc), 1.99 (OAc), 2.05 (OAc), 3.83–4.03 (m, 4H, Glcα H4\(\cdot\)), Glcα H5\(\cdot\), Gleβ H5\(\cdot\), Glc α or Gleβ H6\(\cdot\)), 4.14 (dd, \(^2J_{6\cdot,6\cdot} = 12.0 \text{ Hz,}\) \(^3J_{6\cdot,5\cdot} = 4.4 \text{ Hz, } 1\text{H, Glec α or Glec β H6\(\cdot\)}}\)), 4.18 (dd, \(^2J_{6\cdot,6\cdot} = 12.4 \text{ Hz,}\) \(^3J_{6\cdot,5\cdot} = 4.8 \text{ Hz,}\) 1H, Glcα or Glec β H6\(\cdot\)), 4.39 (dd, \(^2J_{6\cdot,6\cdot} = 12.0 \text{ Hz,}\) \(^3J_{6\cdot,5\cdot} = 2.4 \text{ Hz, } 1\text{H, Glec α or Glec β H6\(\cdot\)}}\)), 4.67 (dd, \(^3J_{2\cdot,3\cdot} = 9.6 \text{ Hz,}\) \(^3J_{2\cdot,1\cdot} = 8.0 \text{ Hz, } 1\text{H, Glec β H2\(\cdot\)}}\)), 4.78 (AB q, \(^2J_{AB} = 12.8 \text{ Hz, } 2\text{H, } \text{OCH}_2\)), 4.84 (dd, \(^3J_{2\cdot,3\cdot} = 9.6 \text{ Hz,}\) \(^3J_{2\cdot,1\cdot} = 3.6 \text{ Hz, } 1\text{H, Glcα H2\(\cdot\)}}\)), 4.91 (d, \(^3J_{1\cdot,2\cdot} = 8.0 \text{ Hz, } 1\text{H, Glec β H1\(\cdot\)}}\)), 5.17–5.22 (m, 1H, Glcα H3\(\cdot\)), 5.25 (d, \(^3J_{1\cdot,2\cdot} = 3.6 \text{ Hz, } 1\text{H, Glcα H1\(\cdot\)}}\)), 5.25–5.30 (m, 1H, Glec β H3\(\cdot\)), 7.50
Chapter Three

(1H, triazole CH); $^{13}$C {$^{1}$H}

NMR (100 MHz, DMSO-$d_6$) δ 21.95 (OAc), 21.02 (2 × OAc), 21.11 (OAc), 21.21 (OAc), 21.26 (OAc), 21.44 (OAc), 60.43 (Glc C$_6$'), 62.07 (Glc C$_6$), 63.54 (OCH$_2$), 68.42 (Glc C$_4$'), 68.68 (Glc C$_4$), 69.53 (Glc C$_3$), 70.11 (Glc C$_3$'), 72.02 (Glc C$_2$'), 72.20 (Glc C$_2$), 74.30 (Glc C$_5$'), 74.99 (Glc C$_5$'), 96.05 (Glcβ H$_1$), 98.75 (Glcα H$_1$), 121.03 (Ar CH), 123.73 (triazole CH), 128.19 (Ar CH), 139.22 (Ar C), 144.57 (triazole C or 6r C), 144.99 (triazole C or Ar C), 169.83 (OAc), 169.88 (OAc), 170.27 (OAc), 170.54 (OAc), 170.69 (OAc), 170.85 (OAc). HRMS (ESI) calcd for C$_{35}$H$_{43}$N$_4$O$_{20}$S: 871.219684. Found: 871.220422. Anal. calcd for C$_{35}$H$_{44}$N$_4$O$_{20}$SH$_2$: C, 47.19; H, 5.20; N, 6.29; S, 3.60. Found: C, 47.10; H, 5.14; N, 5.94; S, 3.55.

4-(4-[[β-D-Maltopyranosyl]oxymethyl]-1H-1,2,3-triazol-1-yl)benzene sulfonamide (3.14f'). The title compound was prepared from 3.14m according to general procedure 2 and isolated as an off-white solid (99 mg, 0.17 mmol, ~100%); Mp 192–196°C (decomp). $^1$H NMR (400 MHz, D$_2$O) δ 3.22 (dd, $^3$J$_{2,3}$ = 9.2 Hz, $^3$J$_{2,1}$ = 8.0 Hz, 1H, Glcβ H$_2$), 3.25–3.30 (m, 1H, Glcβ H$_4$), 3.43 (dd, $^3$J$_{2,3}$ = 10.0 Hz, $^3$J$_{2,1}$ = 4.0 Hz, Glcα H$_1$), 3.49–3.66 (m, 7H, Glc α H$_3$, H$_4$, H$_5$, H$_6$ and Glc β H$_3$, H$_5$, H$_6$), 3.71 (dd, $^3$J$_{6,6}$ = 12.0 Hz, $^3$J$_{6,5}$ = 2.0 Hz, 1H, Glc α or Glcβ H$_6$), 3.80 (dd, $^3$J$_{6,6}$ = 12.4 Hz, $^3$J$_{6,5}$ = 1.6 Hz, 1H, Glc α or Glcβ H$_6$), 4.49 (d, $^3$J$_{1,2}$ = 8.0 Hz, 1H, 1H, Glcβ H$_1$), 4.90 (AB q, $^2$J$_{AB}$ = 12.4 Hz, 2H, OCH$_2$), 5.26 (d, $^3$J$_{1,2}$ = 3.6 Hz, 1H, Glcα H$_1$), 7.87–
7.99 (m, 4H, Ar), 8.49 (s, 1H, triazole CH); $^1^3^C\{t^1^H\}$ NMR (100 MHz, D$_2$O) δ 60.23 (OCH$_2$), 60.87, 62.10, 69.48, 71.79, 72.85, 72.97, 73.07, 74.78, 76.33, 76.81 (Glca H$_2$, H$_3$, H$_4$, H$_5$, H$_6$ and Glcβ H$_2$, H$_3$, H$_4$, H$_5$, H$_6$), 99.70 (Glca H$_1$), 101.59 (Glcβ H$_1$), 121.75 (Ar CH), 124.06 (triazole CH), 128.02 (Ar CH), 139.58 (Ar C), 141.93 (triazole C or Ar C), 144.74 (triazole C or Ar C). HRMS (ESI) calcd for C$_{21}$H$_{29}$N$_4$O$_3$S: 577.145731. Found: 577.145862.

4-(4-[[2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl]oxymethyl]-1H-1,2,3-triazol-1-yl)benzene sulfonamide

(3.14m).

The title compound was prepared according to general procedure 1 and isolated as pale yellow foam (675 mg, 0.97 mmol, 91%). $R_f$ 0.53 (2:3 hexanes–EtOAc). $^1^H$ NMR (400 MHz, DMSO-$d_6$) δ 4.51 (dd, $^2$J$_{5',5''}$ = 12.0 Hz, $^3$J$_{5',4}$ = 5.2 Hz, 1H, H$_5'$), 4.66 (dd, $^2$J$_{5'',5''}$ = 12.0 Hz, $^3$J$_{5'',4}$ = 4.0 Hz, 1H, H$_5''$), 4.78–4.82 (m, 1H, H$_4$), 4.81 (AB q, $^2$J$_{AB}$ = 12.4 Hz, 2H, OCH$_2$), 5.52 (s, 1H, H$_1$), 5.60 (d, $^3$J$_{2',3'}$ = 4.8 Hz, 1H, H$_2'$), 5.77 (d, $^3$J$_{3',4'}$ = 6.8 Hz, $^3$J$_{3',2'}$ = 4.8 Hz, 1H, H$_3'$), 7.34–8.07 (m, 21 Hz, Ar and SO$_2$NH$_2$), 8.84 (s, 1H, triazole CH); $^{13}$C\{t$^1$H\} NMR (100 MHz, DMSO-$d_6$) δ 61.05 (OCH$_2$), 65.06 (C$_5'$), 72.59 (C$_3'$), 75.68 (C$_2'$), 79.17 (C$_4'$), 104.67 (C$_1'$), 120.93 (Ar CH), 123.35 (triazole CH), 128.14 (Ar CH), 129.26, 129.31, 129.35, 129.55, 129.87, 129.90, 130.04, (Ar CH), 134.056, 134.47, 134.62, 139.21 (Ar C), 144.48 (triazole C or Ar C), 145.21 (triazole C or Ar C), 165.32 (C=O), 165.49 (C=O), 165.11 (C=O). HRMS (ESI) calcd for C$_{35}$H$_{31}$N$_4$O$_{10}$SNa$^+$: 969.175539. Found: 969.174859. Anal. calcd for
Chapter Three

C₃₅H₃₅N₄O₁₀S.H₂O: C, 58.65; H, 4.50; N, 7.82; S, 4.47. Found: C, 58.26; H, 4.30; N, 7.54; S, 4.34.

4-(4-[[β-D-ribofuranosyl]oxymethyl]-1H-1,2,3-triazol-1-yl)benzene sulfonamide (3.14m').

The title compound was prepared from 3.14m according to general procedure 2 and isolated as pale yellow solid (112 mg, 0.29 mmol, ~100%). Rf 0.12 (1:9 CH₃OH–EtOAc); Mp 146–147⁰C. ¹H NMR (400 MHz, DMSO-ｄ₆)  δ 3.37 (dd, 2J₅-₅‴ = 12.0 Hz, 3J₅‴-₄‴ = 6.4 Hz, 1H, H₅‴), 3.54 (dd, 2J₅‴-₅‴ = 11.6 Hz, 3J₅‴-₄‴ = 3.72, 1H, H₅‴), 3.73 (d, 3J₂-₃‴ = Hz, 1H, H₂‴), 3.79 (ddd, 3J₄‴-₃‴ = 10.4 Hz, 3J₄‴-₅‴ = 6.4 Hz, 3J₄‴-₅‴ = 4.0 Hz, 1H, H₄‴), 3.87 (dd, 1H, H₅‴), 4.67 (ABq, 2JAB = 12.4 Hz, 2H, CH₂O), 4.88 (s, 1H, H₁‴), 7.97–8.11 (m, 4H, Ar), 8.85 (s, 1H, triazole CH); ¹³C NMR (100 MHz, DMSO-ｄ₆)  δ 60.10 (C₅‴), 63.62 (CH₂O), 71.43 (C₃‴), 74.93 (C₂‴), 84.43 (C₄‴), 106.83 (C₁‴), 120.98 (Ar CH), 123.21 (triazole CH), 128.20 (Ar CH), 139.31 (Ar C), 144.38 (triazole C or Ar C), 146.04 (triazole C or Ar C). HRMS (ESI) calcd for C₁₄H₁₈N₄O₇SNa⁺: 409.078838. Found: 409.078059.
3.5 References


191


Chapter Four

4 Synthesis of N-Alkylated Azasugar libraries using Click Chemistry

4.1 Introduction

4.1.1 Background

Carbohydrates and their naturally occurring polymers account for two-thirds of the carbon in the biosphere - a reflection of their widespread distribution and biological function.\(^1\) Carbohydrates in the form of oligosaccharides, polysaccharides and their glycoconjugates are essential for the survival of an organism, functioning either as inert scaffolds for structural support, as energy reservoirs or as multivalent architectures required for intra- and intercellular recognition events.\(^1-3\) In the absence of acid, the O-glycosidic linkage is among the most stable bond encountered in natural polymers, being up to 100 times more stable than the phosphodiester bond of DNA, which in turn is 1000 times more stable than a native peptide bond.\(^2\) Nevertheless, the catalytic cleavage and formation of these bonds occurs with remarkable efficiency by a group of carbohydrate active enzymes (CAZymes) which includes the glycosidases and transglycosidases (EC 3.2.1-), glycosyltransferases (EC 2.4.x.y), and the polysaccharide lyases (EC 4.2.2-). These enzymes are classified into over 200 sequence related families which are archived and frequently updated in the CAZy database (http://afmb.cnrs-mrs.fr/CAZY/).\(^3\) Their biological activities range from the intestinal digestion of carbohydrates and glucose metabolism, glycogen phosphorylation,\(^4,5\) and the co- and post-translational processing of N- and O-linked glycoproteins.\(^6\) Transition state analogues acting as reversible and competitive inhibitors of digestive, lysosomal and N- and O-linked
oligosaccharide processing glycosidases and glycosyltransferases are useful biochemical tools for elucidating glycan structure and function. Transition state analogue inhibitors of glycosidases and glycosyltransferases are also important drug leads for a host of carbohydrate-based pathological processes, such as microbial and viral infection, cancer metastasis, and a range of metabolic disorders including diabetes and lysosomal storage diseases. The potential therapeutic applications of glycosidase and glycosyltransferase inhibitors of natural and non-natural origin has been the subject of previous reviews.7,8

4.1.2 Glycosidases: Mechanism and structure

Glycosidases are classified according to substrate selectivity but owing to the relatively broad and poorly defined substrate specificity, a more pragmatic system has been devised based on the similarity in amino acid sequence.9 To date, 108 sequence related families have been identified with a further division according to similarity in protein fold (clans). The glycosidases are remarkably efficient enzymes (rate constants up to 1000 s⁻¹) with rate enhancements in the order of 10^{17} and 10^{22} – fold relative to the spontaneous hydrolysis of the glycoside.11 Glycosidases are also classified mechanistically as either retaining or inverting, depending on the overall stereochemical outcome of the hydrolysed bond, with the exception of the Family 4 glycosidases (e.g. 6-Phospho-β-D-glucosidase) and a recently discovered phosphoglucosidase from Bacillus subtilis and Thermotoga maritima, both of which use a completely unrelated mechanism that requires an NAD⁺ cofactor and a divalent Mn²⁺ ion for catalysis.10,11 A further level of classification is given to glycosidases depending on the locale for cleavage within the glycan chain. Thus, exo-glycosidases cleave terminal sugar units, while endo-glycosidases cleave glycosidic linkages
within the chain. Glycosidase mechanism is the subject of recent reviews \(^1-3,12\) and the widely accepted mechanism for both retaining and inverting glycosidases proposed by Koshland\(^{12a}\) is depicted in Figure 4.1.

![Diagram of glycosidase mechanism](image)

**Figure 4.1**

The inverting glycosidases (Figure 4.1A) employ a double displacement \(S_N2\)-like mechanism. Two carboxyl groups are located within the active site and are separated
by 7–11 Å, one acting as a general acid that protonates the exocyclic glycosidic oxygen thus activating the anomic centre for nucleophilic attack, and a second catalytic base carboxylate which activates the incoming nucleophilic water for attack on the anomic centre. The reaction proceeds via an oxocarbenium-like ion transition state which is remarkably stabilised (up to $K_d 10^{-20}$ M) and fleeting, and more than likely represents numerous discrete species through the reaction trajectory. The oxocarbenium ion-like transition state is also typified by a flattening of the sugar ring and a growing rehybridisation of the C-1 carbon from sp$^3$ to sp$^2$. The retaining glycosidases employ a double displacement mechanism involving a covalently bound intermediate.$^{13}$ The catalytic carboxylate diad is separated by 5 Å with one acting as a general acid/base catalyst while the other acts as the nucleophile. As with the inverting mechanism, the reaction proceeds through an oxocarbenium-like ion transition state. Much of our understanding of retaining glycosidase mechanisms stems from the isolation of covalent intermediates and analysis using X-ray crystallography and mass spectrometry,$^{14}$ as well as primary and secondary kinetic isotope effects.$^{10,11,15}$

The formation of native glycosidic linkages is catalysed by the glycosyltransferases (EC 2.4.x.y). These ubiquitous enzymes are frequently found to employ retaining and inverting mechanisms similar to glycosidases, and also involve an oxocarbenium ion-like transition state.$^{16}$ The rational design of transition state analogues and inhibitors of glycosyltransferases employs, in many cases, similar principles.$^8,16$
Chapter Four

4.1.3 Azasugars: natural occurrence and biological activity

Polyhydroxylated alkaloids resembling the structure and conformation of carbohydrates are widespread in plants and microorganisms.\textsuperscript{17,18} They are named azasugars (or iminosugars) and can be regarded as monosaccharide derivatives with the endocyclic oxygen atom replaced with a nitrogen atom, thus mimicking the structure, conformation and chirality of their carbohydrate counterparts. Azasugars have been found as secondary metabolites in nearly all levels of the phylogenetic tree, ranging plants, microorganisms, fungi and more recently in insects and sea sponges, indicating common biosynthetic and evolutionary pathways or important biological roles distinct from other alkaloid classes.

Since the discovery in 1966 of the D-gluco configured piperidine and antibiotic, nojirimycin (NJ) 4.1a from the fermentation broths of \textit{Streptomyces sp.} and some time later that of the C-1 deoxy derivative, 1-deoxynojirimycin (1-DNJ) 4.1b from the roots of the mulberry tree (\textit{Morus sp.}),\textsuperscript{19} a vast body of literature has appeared concerning the isolation, total synthesis and biological activity of mono- and bicyclic polyhydroxylated alkaloids.\textsuperscript{20,26} More than 25 additional piperidine analogues of 4.1a and 4.1b have been obtained from natural sources and a plethora of semi-synthetic and synthetic derivatives have been prepared and evaluated for biological activity.\textsuperscript{21} Azasugars are broadly classified according to five structural classes: the monocyclic polyhydroxylated pyrrolidines (e.g. 2,5-dideoxy-2,5-imino-D-mannitol, DMDP 4.3) and piperidines (e.g. nojirimycin 4.1a, 1-deoxynojirimycin 4.1b, and 1,2-dideoxy DNJ, fagomine 4.2), the bicyclic indolizidines (e.g. Swainsonine 4.4 and Castanospermine 4.5), pyrrolizidines (e.g. Australine 4.6 and Casuarine 4.7) and nortropanes (the Calystegines such as calysegine B\textsubscript{1} 4.8).\textsuperscript{23-25} In addition to these
compounds, various glycosides, epimers (e.g., the D-manno and D-galacto configured derivatives of 4.1a and 4.1b) and regioisomers have been isolated and reported from natural sources. The first naturally occurring N-alkylated derivative of 4.1b, N-methyl DNJ (4.9) was isolated from the leaves and roots of the Mulberry tree (*Morus sp.*), along with a various O-glucosides and O-galactosides of 4.1b. Sometime later, Nagstatin 4.10, an N-acetylgalactosamine configured azasugar analogue fused with a substituted imidazole was isolated from the fermentation broths of *Streptomyces amakusaensis* MG846 fF3 and was shown to be a potent inhibitor various β-hexosamidases. Recently, several amphiphilic C-1 alkylated derivatives of 4.1b have been isolated from the Madagascar sponge *Batzella sp.* (the batzellasides A 4.11a, B 4.11b and C 4.11c).
4.1.4 Therapeutic potential of azasugars as inhibitors of glycosidases

The biological activity of azasugars can in many cases be attributed to their ability to act as competitive, reversible inhibitors of glycosidases and glycosyltransterases.\textsuperscript{7,24} Azasugars mimic the positive build-up associated with the oxocerbenium-like ion transition state, since they become protonated at physiological pH within the active site and form strong electrostatic interactions with the catalytic carboxylates.\textsuperscript{7} However, naturally occurring piperidines and strong glycosidase inhibitors such as \textbf{4.1a} and \textbf{4.1b} are known to adopt an unexpected chair conformation rather than the flattened half-chair typically associated with the transition state.\textsuperscript{7} This has prompted the synthesis of analogues that more closely mimic the transition state, both structurally and electronically, with the aim of developing more potent and selective inhibitors.\textsuperscript{7a}

Owing to their ability to inhibit various digestive, lysosomal and \textit{N}- and \textit{O}-linked oligosaccharide processing glycosidases and glycosyltransferases, azasugars have emerged as useful biochemical tools for establishing glycan structure-function relationships and serve as viable drug leads for the treatment of a host of acquired and inherited diseases, including diabetes mellitus,\textsuperscript{25} lysosomal storage diseases including the often fatal group of glycosphingolipid storage (GSL) diseases,\textsuperscript{26} retroviral infections including HIV/AIDS\textsuperscript{27} and cancer.\textsuperscript{28,29}

4.1.5 \textit{N}-alkylated azasugars: biological activity and therapeutic applications

The preparation and evaluation of \textit{N}-alkylated azasugars is an active area of research, as it has been shown that these compounds possess markedly different inhibition profiles than their non-alkylated derivatives and are valuable tools for elucidating
biochemical pathways associated with protein and lipid glycosylation. $^{6,30,31,35}$ Naturally occurring $N$-alkylated azasugars, such as the indolizidines alkaloids swainsonine 4.4 castanosperine 4.5, and the tetrahydroxypyrrolizidine australine 4.6 have been shown to be potent inhibitors of ER-oligosaccharide processing glycosidases, such ER $\alpha$-glucosidase I and II and the golgi-resident $\alpha$-mannosidases (IA, IB and II).$^{6,7c}$ These enzymes are involved in crucial quality control mechanisms during the early stages of $N$- and $O$- linked glycoprotein biosynthesis within the ER-golgi pathway and assist in the correct folding and sequencing of nascent glycoproteins.$^{6}$ The selective inhibition in many cases leads to aberrant glycosylation patterns and/or reduced secretion rates of mature glycoproteins, thus leading to a potential downstream modulation of cell-cell and cell-pathogen binding processes. Based on these observations, various synthetic $N$-alkylated polyhydroxylated piperidines,$^{30}$ such as $N$-butyl DNJ 4.12 (Zavesca®) were found to be potent and selective inhibitors of ER $\alpha$-glucosidase I (IC$_{50}$ 0.57 $\mu$M),$^{29}$ and reduce the formation of HIV-1gp120 viral envelope protein and syncytium formation in vivo.$^{31}$ $N$-butyl DNJ 4.12 went to clinical trials as a monotherapy as well as in combination with AZT for the treatment of HIV/AIDS in 1994/1995.$^{31}$ Although unsuccessful, the trial provided valuable mechanistic insight into the relationship of ER $\alpha$-glucosidase I inhibition and anti-retroviral activity, thus prompting the evaluation of novel analogues as inhibitors of related viruses, including hepatitis B and C (HBV and HCV).$^{32,33,32}$ Table 4.1 summarises the biological activity and therapeutic applications of some naturally occurring and synthetic $N$-alkylated azasugars.
Table 4.1 Therapeutic applications of \(N\)-alkylated azasugars.

<table>
<thead>
<tr>
<th>Azasugar</th>
<th>Compound name</th>
<th>Target Glycosidase/Glycosyltransferase</th>
<th>Clinical uses and potential uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.13</td>
<td>(N)-hydroxy-ethyl-1-DNJ (Miglitol, Glyset( ^{\text{\textregistered}} ))</td>
<td>Intestinal disaccharidases (sucrase, trehalase, maltase etc.)</td>
<td>Type 2 diabetes mellitus (NIDDM)</td>
</tr>
<tr>
<td>4.12</td>
<td>(N)-butyl-DNJ (Zavesca( ^{\text{\textregistered}} ))</td>
<td>Ceramide (\beta)-glucosyltransferase, ER (\alpha)-Glucosidase I</td>
<td>GSL storage disorders (Gauchers disease), Anti-retroviral (eg HIV-1, HBV)</td>
</tr>
<tr>
<td>4.16</td>
<td>(N)-butyl-DGJ (OGT923)</td>
<td>Ceramide (\beta)-glucosyltransferase</td>
<td>GSL-lysosomal disorders (Gauchers disease)</td>
</tr>
<tr>
<td>4.17</td>
<td>(N)-nonyl-DNJ</td>
<td>Ceramide (\beta)-glucosyltransferase, ER (\alpha)-glucosidase I</td>
<td>GSL-lysosomal disorders (Gauchers disease), Anti-retroviral (HIV-1, HBV, HCV)</td>
</tr>
</tbody>
</table>
Various $N$-alkylated derivatives of DNJ 4.1b have also been prepared as potential antidiabetic agents. Inhibition of various disaccharidases located within the brush border region of the small intestine result in a reduction of postprandial blood glucose levels and is hence a viable strategy for the development of antidiabetic agents. In order to improve the *in vivo* activity of DNJ 4.1b and inhibitor potency, several $N$-alkylated derivatives have been prepared and evaluated as inhibitors of digestional glycosidases with equal or greater potency than 4.1b, whilst exhibiting improved *in vivo* activity. Promising compounds include the $N$-hydroxyethyl analogue and clinically used anti-diabetic drug, Miglitol 4.13 (Glyset®), and lead compounds emligliate (BAY o 1248) 4.14, and the disaccharide mimic MDL 73945 4.15.
In addition to their anti-proliferative and anti-viral properties arising from their inhibition of ER-processing glycosidases, many synthetic N-alkylated azasugars have been reported to act as donor, acceptor and bisubstrate transition state analogues of some clinically relevant glycosyltransferases.\textsuperscript{8d} Thus, various epimers of castanospermine 4.4 have been shown to act as potent inhibitors of α-1,6-fucosyltransferase and the bisubstrate N-alkylated fuconojoirimycin analogue 4.18, is a strong inhibitor of α-1-3 fucosyltransferase IV (IC\textsubscript{50} 81μM) \textit{in vitro}.\textsuperscript{33}

Several amphiphilic N-alkylated DNJ derivatives, such as N-nonyl DNJ 4.16 and its C-4 epimer, N-butyl DGJ 4.17 are acceptor substrate analogues and potent inhibitors of ceramide β-glucosyltransferase (glucosylceramide synthetase, EC 2.4.1.80) \textit{in vivo}.\textsuperscript{31} This enzyme is responsible for the biosynthesis of glucosylceramide, a common metabolite required for the biosynthesis of complex glycosphingolipids.
Chapter Four

The catalytic impairment of lysosomal glycosidases responsible for the catabolism of glucosylceramide leads to its accumulation in many tissues (neural, cardiac, kidney and phagocytes) to levels which become deleterious or toxic and is the pathological basis for the inherited and frequently fatal GSL storage diseases, such as Tay-Sachs, Sandhoff, Fabry and Gaucher disease. Inhibition of ceramide β-glucosyltransferase (glucosylceramide synthase) reduces glucosylceramide accumulation and as such is called substrate reduction therapy (SRT). N-butyl DNJ 4.12 ($K_i$ 7.4 μM) is used clinically for the treatment of type I Gaucher disease. Importantly, 4.12 has become a valuable platform upon which the development of lead compounds possessing anti-lysosomal storage disease and antiviral properties with improved bioavailability, inhibitor potency and safety can be achieved. 26,27,31

4.1.6 Seven-membered ring azasugars: a new class of glycosidase inhibitor

A recently identified and relatively unexplored class of carbohydrate-processing enzyme inhibitor include the seven-membered ring azasugars; the 1,6-dideoxy-1,6-iminoheptitols (the α-D-glucos configured analogue, 4.19), and the tri- and teterahydroxyazepanes (e.g. C₇-symmetric L-ido configured azepanes 4.20 and 4.21). Compound 4.19 is a good inhibitor of α-galactosidase from coffee bean ($K_i$ 2.2 μM) but is also a poor inhibitor of α- and β-glucosidase. On the other hand, the L-ido-configured C₇-symmetric tetrahydroxyazepane 4.20 is a good inhibitor of α-glucosidase ($K_i$ 4.8 μM) and β-glucosidase ($K_i$ 17 μM), and more recently, various N-alkylated derivatives, such as the N-hydroxy ethyl analogue 4.21 has also been shown to inhibit the growth of various cancer cell lines (GI₅₀ values in the range of 2 to $9 \times 10^{-5}$ M) and display weak inhibition of HIV-1 protease. Symmetrical N-alkylated tetrahydroxyazepanes dimers, such as 4.22 have recently been shown to
act as DNA minor groove binders, and also display *in vitro* anti-cancer properties presumably because of their inhibition of metabolic and processing glycosidases.\textsuperscript{38}

\begin{align*}
\text{Figure 4.5} & ^{34-39} \\
\text{4.19} & \quad \text{4.20} & \quad \text{4.21} & \quad \text{4.22}
\end{align*}

\textbf{4.1.7 Synthesis of azasugar libraries using click chemistry}

With the aim of improving the glycosidase/glycosyltransferase inhibitory potency and selectivity, considerable effort has been invested into the combinatorial and parallel synthesis of azasugar libraries.\textsuperscript{39} The multistep synthesis and isolation of a structurally conserved azasugar scaffold containing one or more reactive functionalities enable the conjugation of structurally diverse substituents at one or more positions. This decorative approach is a powerful means of accessing compounds for lead optimisation or discovery purposes.\textsuperscript{43} Various groups have reported the synthesis of azasugar libraries using the “scaffold decoration” strategy and more notable examples have included the preparation of an \(N\)-alkylated fucojirimycin library in microtitre format by Wong et al.,\textsuperscript{40} an \(N\)-alkylated azafagomine library containing a variable tri-peptide sequence in solid phase by Bols et al.,\textsuperscript{41} and a C(2)-substituted tetrahydropyridoimidazole library reported by Vasella and co-workers.\textsuperscript{42}

The Cu\textsuperscript{I}-catalysed 1,3-dipolar cycloaddition of an organic azide and a terminal acetylene (“click chemistry”) using aqueous solvents has in recent years emerged as
a premier bioconjugation strategy as well as a modular synthetic approach for
fragment-based drug discovery. Wong and colleagues have recently utilised click
chemistry to screen and identify potent and selective fucosyltransferase inhibitors in
microtitre format in situ. More recently, the parallel synthesis of an influenza
neuraminidase inhibitor library using click chemistry has been reported. Click
chemistry has also been exploited as a powerful conjugation strategy for the
preparation of natural product based libraries; with two examples including the
preparation and lead optimisation of vancomycin and glycopeptide antibiotics.

Based on these encouraging results, we are interested in applying the Cu¹-catalysed
1,3-DCR as a facile strategy for the preparation of N-alkylated azasugar libraries
(Scheme 4.1). The modular assembly of N-alkylated azasugars using click chemistry
is a flexible and dynamic approach for expedient access to inhibitors which can be
used to probe potential binding elements removed from the catalytic active site. By
incorporating the appropriate N-propynyl group into the structurally conserved six-
membered ring azasugar scaffold, various alkyl and aliphatic azides can be rapidly
grafted to provide inhibitors with variable physicochemical and inhibitory properties
- an important step in the discovery and optimisation of potent and selective
inhibitors of glycosidase/glycosyltransferases. A further degree of diversity can be
achieved with the use of a seven-membered ring azasugar scaffold, such as the N-
propynyl azepane, and a direct comparison of inhibitory activity with the piperidine
counterparts would thus provide a greater surveillance and correlation of biological
and chemical spaces with respect to carbohydrate-processing enzymes. Furthermore,
the polyhydroxylated azepanes are a relatively unexplored class of
glycosidase/glycosyltransferase inhibitor and the combinatorial or parallel synthesis
of azepane libraries is thus a viable approach for establishing valuable structure-activity relationships.

\[
\begin{align*}
\text{Scheme 4.1} \\
\end{align*}
\]

R = Protecting group or H
X = Alkyl/Aryl substituent

4.2 Results and discussion

4.2.1 Scaffold design and synthesis

The pyridoimidazole azasugars, including the natural product, Nagstatin 4.10, are potent inhibitors of lysosomal \(\beta\)-glucosidases and have received significant interest owing to their anticancer properties.\(^{7e,48,49}\) Considerable research has thus been directed toward the preparation of fused six and seven-membered ring azole derivatives which mimic more closely the flattened half chair conformation (and sp\(^2\) character of the pseudo-anomeric carbon) associated with the transition state.\(^{48}\)

Various fused pyridotriazoles and pyridotetrazoles have thus been reported which are generally weaker inhibitors of \(\beta\)-glycosidases than the pyridoimidazole counterparts,\(^{49}\) owing in part to the reduced basicity of the endocyclic nitrogen. However, the pyridotetrazole derivatives were found to be useful probes for determining the mechanism of the retaining \(\beta\)-glycosidases.\(^{50}\) Various 1,2,3-triazole containing compounds have been shown to possess a range of biological activities, and recently the 1,4-disubstituted 1-\(\beta\)-D-glycosyl and galactosyl 1,2,3-triazoles have

211
been assessed as inhibitors of $\beta$-glucosidase and $\beta$-galactosidase.\textsuperscript{51} We are therefore interested in incorporating an alkyl linker between the endocyclic nitrogen atom and the 1,4-disubstituted 1,2,3-triazole ring, thus potentially allowing for favourable binding interactions from the triazole ring whilst retaining the basicity of the endocyclic nitrogen necessary for good inhibition.

Previously, Le Merrer and colleagues examined the nucleophilic ring opening of homochiral $C_2$-symmetric bis-epoxides with benzyl amine as a key step in the regiospecific synthesis of DNJ \textbf{4.1b} and L-ido-tetrahydroxyazepane \textbf{4.20}.\textsuperscript{36a,52} By adopting a similar strategy, the installation of the required $N$-propynyl group could be achieved by the nucleophilic ring opening of the structurally rigid and crystalline $C_2$-symmetric bis-epoxide \textbf{4.27} (Figure 4.6) with propargyl amine to form the $N$-propynyl D-gluco-piperidine \textbf{4.28} and the L-ido-azepane \textbf{4.29} scaffolds (Scheme 4.2).
The nucleophilic ring opening and aminocyclisation of distereomeric, \( C_2 \)-symmetric bis-epoxides with benzyl amine has been examined under different experimental conditions.\(^{36a} \) The reaction conditions as well as steric influences can be modified to favour one of the two reaction pathways: the 6-\textit{exo-tet} process which favours the formation of the \( D \)-\textit{gluco}-piperidine; or the 7-\textit{endo-tet} process which favours the formation of the \( C_2 \)-symmetric \( L \)-\textit{id-o-azepane} (Scheme 4.3).\(^{36a,52,53} \) In particular, the nature of the hydroxyl protecting group at C-3 and C-4 are shown to influence the relative distribution of the piperidine and azepane products. The addition of an acid
catalyst and the use of conformationally rigid protecting groups, such as a trans-acetonide, favours the 7-endo-tet process and azepane formation.\textsuperscript{36a}

We are thus interested using a protecting group that imparts a reasonable degree of structural rigidity leading to approximately equal product distributions, but which can also be removed under mild and selective conditions as the final step in the library synthesis. The \( p \)-methoxybenzyl ether protecting group (MPM) was selected since it can be removed under orthogonal and mild oxidative conditions, using ceric ammonium nitrate (CAN)\textsuperscript{54} or dichlorodicyanoquinone (DDQ),\textsuperscript{55} as well as providing potentially crystalline synthetic intermediates to facilitate their purification by non-chromatographic methods.

Alkylation of the optically pure and commercially available 1,2:5,6-di-O-isopropylidene-D-mannitol with sodium hydride in DMF afforded the 3,4-di-O-\( p \)-methoxybenzyl ether 4.23 in good yield. Cleavage of the bis-acetonide was achieved

\[ \text{Scheme 4.3}^{36a,52,53} \]
Chapter Four

by stirring 4.23 in aqueous acetic acid (70% w/v) at room temperature for 20 hours affording the crude tetrol 4.24 which was used in the next step without further purification. Stirring at room temperature was required to avoid premature cleavage of the p-methoxybenzyl ether groups. Following evaporation at room temperature under reduced pressure, the resulting hygroscopic tetrol 4.24 was azeotropically dried by co-evaporation several times with a 1:1 mixture of anhydrous acetonitrile and toluene prior to use in the subsequent step. Selective silylation of the hydroxyl groups at C-1 and C-6 position was achieved by immediately treating the crude tetrol 4.24 with tert-butyldimethylsilyl chloride (2.2 equiv.) in anhydrous DMF at 0°C, furnishing the bis-silylether 4.25 in good yield (68%). Treatment of the 4.25 with methanesulfonyl chloride in anhydrous dichloromethane at 0°C facilitated the activation of C-3 and C-4 towards the ensuing S_N2 nucleophilic attack. Thus, the crude mesylate 4.26 was treated with an aqueous solution of 1N HCl in methanol at 0°C for 1 hour to effect cleavage of the silyl ether groups. This was promptly followed by a base-promoted (addition of 20% w/v solution of potassium hydroxide) intramolecular S_N2, leading to the inversion of stereochemistry at C-2 and C-4, thus the stereospecific formation of the L-ido-configured C_2-symmetric bis-epoxide 4.27. Pure bis-epoxide 4.27 precipitated from the reaction mixture and is obtained pure by vacuum filtration and recrysallisation from hot absolute ethanol. The molecular structure has been solved by X-ray crystallography and confirms the expected stereochemical configuration (Figure 4.6).
With optically pure, crystalline $C_2$-symmetric bis-epoxide 4.27 in hand and available in gram quantities, we sought to optimise the experimental conditions for the aminocyclisation with propargyl amine. However, reproduction of the literature method$^{36a}$ which involved treating the substrate with excess propargyl amine (5.0 equiv.) in refluxing chloroform proved to be unsuccessful. The reaction was found to be sluggish under these conditions and partial decomposition of the substrate was observed by TLC after 48 hours at reflux, even in the presence of 10 equiv. of propargyl amine. Microwave irradiation (300 W, 150°C, up to 30 minutes) in the presence of 5 and 10 equiv. of propargyl amine using either 1,2-DCE, dimethylformamide, and acetone as solvents did not lead to product formation by TLC. In an attempt to assist epoxide opening and subsequent cyclisation, the reaction was performed in a mildly protic solvent at slightly elevated temperature. The bis-epoxide 4.27 was thus treated with excess propargyl amine (5.0 equiv.) in warm methanol (45°C). The mixture was initially insoluble at this temperature, although dissolution was achieved after 8 hours of vigorous stirring. Smooth product conversion is detected by TLC after 12 hours, with the disappearance of the starting material and the presence of two product bands which stained positive to acidic
cerium ammonium molybdite char. The formation of the 3,4-di-\textit{O}-\textit{p}-methoxybenzyl D-\textit{gluco}-piperidine 4.28 and the structural isomer, \textit{C}_2-symmetric L-\textit{ido}-azepane 4.29, were readily separated by flash chromatography (dichloromethane–EtOAc gradient eluents) and isolated in good yields (4.28, 42%; and 4.29, 40%, respectively).

**4.2.2 Preparation of N-alkylated azasugar libraries**

Having obtained both the \textit{N}-propynyl piperidine 4.28 and azepane 4.29 scaffolds in sufficient quantities, we proceeded to prepare \textit{N}-alkylated azasugar libraries containing a structurally conserved \textit{N}-methylene 1,2,3-triazole moiety. In this respect, our aim was two-fold: firstly, the generation of structurally diverse \textit{N}-alkylated azasugars as potential inhibitors of target enzymes - \textit{\alpha}- and \textit{\beta}-glucosidase, ER \textit{\alpha}-glucosidase I, or ceramide \textit{\beta}-\textit{D}-glucosyltransferase; and secondly to profile the synthetic utility of click chemistry in generating azasugar libraries for lead discovery and optimisation purposes.

To date, a plethora of synthetic procedures and Cu\textsuperscript{I} catalyst systems have been reported in the literature concerning the 1,3-DCR of an organic azide and terminal acetylene. Owing to the ease of purification, the use of aqueous, non-toxic solvents and reagents, as well as the ability to perform the reaction in the absence of an inert atmosphere, the original method prescribed by Sharpless and colleagues\textsuperscript{56} is still the method of choice for many practitioners. Due to our recent investigation into the synthetic utility of the reaction in carbohydrate chemistry,\textsuperscript{57} we have applied similar methodology to prepare \textit{N}-alkylated azasugar libraries (Scheme 4.4).

A variety of aliphatic and aryl azides were selected on the basis of previously established structure-activity relationships with respect to the selectivity and potency
of the inhibition at target glycosidases (Figure 4.7). Thus, the grafting of a terminal D-gluco ring, such as glucopyranosyl azide a, would result in a disaccharide mimic as a potential inhibitor of various intestinal disaccharidases, thus providing novel and rapid entry into azasugar-based anti-diabetic agents. The conjugation of aryl azides, such as building block b resulted in a structurally rigid N-alkylated azasugar and a relatively unexplored class of glycosidase inhibitor.

\[\text{Figure 4.7}\]

On the other hand, the grafting of hydrophobic alkyl substituents, such as c and d, would result in inhibitors more suited for targeting ER-oligosaccharide-processing glucosidase I or ceramide β-glucosyltransferase. In the case of ceramide β-glucosyltransferase, the conjugation of aliphatic alkyl azides c and d, allows for the generation of an amphiphilic acceptor substrate analogue as a potential, competitive inhibitor (with respect to ceramide; non-competitive with respect to UDP-glucose) of ceramide β-glucosyltransferase. Using alkyl azides c and d as building blocks in the library design thus allows for the generation of N-alkylated azasugars comprising no more than ten carbons between the terminus and the endocyclic nitrogen. A considerable reduction in inhibitory potency has been observed when exceeding this limit. In addition to the hydrophobic alkyl chain, azide e also contains a polar pyrimidine terminal ring imparting improved solubility, thus facilitating purification.
by ion-exchange chromatography as well as potentially reducing the high cytotoxicity frequently observed for amphiphilic piperdines, such as N-nonyl-DNJ 4.17. The rigidity and the presence of the multiple hydrogen bonding sites within the terminal pyrimidine ring could also be exploited to detect binding interactions far removed from the catalytic site, thus augmenting the total number of interactions for the identification of more potent and selective inhibitors.

![Chemical structures](image-url)
The Cu¹-catalysed 1,3-DCR used to prepare azasugars libraries were performed within capped scintillation vials in aqueous tert-butyl alcohol (1:1 v/v, 0.1–0.5 M concentration) in the presence of sub-stoichiometric quantities of sodium ascorbate, CuSO₄ and 1.0 equiv. of the corresponding azide (Scheme 4.4). Slightly elevated temperatures (40°C) were employed to enhance the rate of triazole formation. Under
these conditions, TLC and ESI-MS detected formation of the triazole within 30 minutes. The \( p \)-methoxybenzyl groups at positions C-3 and C-4 on the azasugar ring ensured a reasonable degree of organic solubility, thus facilitating purification by flash silica chromatography or solid phase extraction on silica. The purification of the library members were achieved by organic extraction and aqueous workup followed by purification on silica in moderate to good yields (62-78%).

Having successfully obtained the protected azasugar library, we proceeded to deprotect the \( p \)-methoxybenzyl ether groups by mild and selective oxidative cleavage to afford target library compounds. Initial treatment of library compounds and scaffolds 4.28 and 4.29 with DDQ (1.2 equiv.) in aqueous dichloromethane (1:9 \( \text{H}_2\text{O–CH}_2\text{Cl}_2 \)), however, did not result in the smooth cleavage of the benzyl ether as expected.\(^{55}\) A dark red-brown precipitate was immediately observed, which indicated the formation of the DDQ-ammonium salt. After stirring for 2 hours at room temperature, analysis of the crude mixtures by TLC and ESI-MS revealed only minor product conversion. Furthermore, multiple product bands on TLC indicated the presence of significant quantities of starting material, mono-protected species and by-products arising from side reactions. Such side reactions may have arisen from the oxidation of the tertiary amine, concomitant formation of undesired methoxybenzyl acetal and potential oxidative cleavage of the \( N \)-methylene triazole bond.\(^{60}\) These conditions proved to be less than ideal and the oxidative cleavage method employing DDQ was hastily abandoned.

An alternative method for the mild and selective oxidative cleavage of substituted benzyl ethers involves the use of ceric ammonium nitrate in aqueous acetonitrile (1:9
When applied to our substrates, the reaction was initially found to produce less of a complex mixture than DDQ by TLC. The reaction was sluggish at room temperature, even in the presence of an acid catalyst (TFA 5–10 mol%). Significant amounts of starting material as well as the mono-protected species are present after stirring for several hours at 40°C under these conditions. However, the presence of a separate band on TLC did not help to distinguish starting material or product. This intriguing observation led to the subsequent analysis of the crude mixture by ESI-MS, which provided evidence for the cleavage of the N-methylene triazole bond. Furthermore, the analogous treatment of the propynyl scaffolds 4.28 and 4.29 fails to show any evidence of N-propynyl cleavage by ESI-MS and TLC. Given the timeframe, a closer examination into the nature of this side reaction was not possible, and we thus sought an alternative protecting group strategy for accessing the deprotected N-alkylated azasugars.

Substituted benzyl ethers have long been known to be labile to Lewis or mineral acids and a variety of conditions have been reported in the literature for their removal under acidic conditions. Since no other acid-labile functional groups are present on the parent scaffolds 4.28 and 4.29, a simple solution to accessing our target libraries involved the acidic cleavage of the p-methoxybenzyl ethers, acylation of the resulting tetrol and conjugation of the per-O-acetylated scaffolds 4.30 and 4.32 to the azide building blocks (a-d). It was envisaged that a final methoxide deprotection of the per-O-acetylated triazoles should furnish our target compounds in quantitative or near quantitative yields. If necessary, a final purification by acidic ion exchange chromatography would afford pure material (Amberlite IR-120 acidic ion exchange resin, 1-5% aqueous NH₄OH eluent). Thus, the substituted benzyl ethers 4.28 and
4.29 were treated with aqueous TFA (1:4 TFA–H₂O) at slightly elevated temperature (40°C). After stirring overnight at this temperature, the reaction was found complete by TLC and ESI-MS. The mixtures were lyophilised and immediately acetylated using a mixture of anhydrous pyridine and acetic anhydride (1:1 v/v). Somewhat disappointingly, the tetra-O-acetates 4.30 and 4.32 were isolated in poor yields (42% from the p-methoxybenzyl ethers 4.28 and 4.29, respectively) following evaporation and purification by flash chromatography. The presence of piperidine or azepane acetate salts would have resulted in their high retention on silica, thus reducing overall isolated yield. The inclusion of a final desalting step in the workup procedure (aqueous NaHCO₃ wash) prior to purification by chromatography would potentially result in higher yields. Although it was possible to purify a portion of the crude lyophilised mixtures by ion exchange chromatography to obtain pure tetrols 4.31 and 4.33, we prefer the indirect but reliable method of acylating the crude mixtures (Pyr/Ac₂O) followed by a mild methoxide deprotection to obtain pure compound for assay and analysis.

Using an identical method for the preparation of the p-methoxybenzyl-protected triazole libraries, the per-O-acylated triazole library 4.32a-d, was prepared in an efficient manner from the L-ido-azepane scaffold 4.32 and azide building blocks a-d (Scheme 4.4). The analogous piperidine library could not be prepared due to time constraints and insufficient material. However given the successful synthesis of the analogous azepane library 4.32a-d and the successful isolation and deprotection of the per-O-acetylated piperidine scaffold 4.30, it can be reasonably envisaged that a successful synthesis of the piperidine library could be achieved given sufficient time for synthetic scale-up.
To illustrate the applicability of this strategy to the piperidine scaffold 4.30, conjugation of 4.30 with alkyl azide building block (d) was carried out with sufficient remaining material to furnish the triazole conjugate 4.30. The O-acetylated L-ido-azepane library 4.32a-d was isolated in good yields (65–75%) following purification by flash chromatography. Deprotection of the per-O-acetates 4.32a-d was achieved by employing anhydrous methanolic sodium methoxide to afford the target compounds 4.32a’-d’ in quantitative yields following the neutralisation with acidic Amberlite IR-120 (Scheme 4.4, step d). The polarity and hydrogen bonding properties of the 1,2,3-triazole moiety contributed to the good water solubility of target library compounds. Noteworthy was the successful isolation of the deprotected compounds 4.32c’ and 4.32d’ consisting of N-alkyl chain lengths similar to the previously described cytotoxic and highly amphiphilic ER α-glucosidase I inhibitor, N-nonyl DNJ 4.17. The development of soluble of N-alkylated azasugars, such as 4.32c’ and 4.32d’ therefore provides access to compounds with potentially useful inhibition profiles and physicochemical properties but significantly lowered cytotoxicity. It is our intention to screen the library compounds for their inhibition of glucosidases mentioned vide supra. To this end, a collaborative research effort has been established at the University of Oxford for screening against ER α-glucosidase I and ceramide β-glucosyltransferase. No in vitro data has yet been determined but this campaign is currently underway.

4.3 Conclusions
The glycosidases and glycosyltransferases play important roles in key biological processes. Many of these ubiquitous enzymes are involved in defective metabolic pathways as well as the biosynthesis of cell-surface glycans involved in cell-pathogen events. Azasugars are a group of polyhydroxylated alkaloids and are potent inhibitors of glycosidases and glycosyltransferases since they mimic the stereochemical arrangement, structure and transition state of the natural substrates. Naturally occurring and synthetic azasugars are therefore valuable biosynthetic probes. Moreover, the selective inhibition of disease related glycosidases and glycosyltransferases is a viable strategy for the clinical intervention of a variety of pathological and disease states such as viral infection, cancer metastasis, diabetes and a host of lysosomal storage diseases. The vast body of research that has arisen within the last three decades concerning catalytic mechanism and structure activity relationships has provided a sound basis for rational inhibitor design. However, concerns regarding enzyme selectivity, potency and in vivo stability and clearance of azasugar based drugs have prompted the use of more combinatorial and parallel synthetic strategies for lead discovery and optimisation purposes.

Click chemistry has become a powerful synthetic tool in high throughput screening and a drug discovery regime that has, to date, seen limited application in natural product based libraries. We have successfully illustrated the utility and application of click chemistry in preparing N-alkylated azasugar libraries using both N-propynyl piperidine and N-propynyl azepane scaffolds and various synthetically accessible azide building blocks. We believe that the technique is a powerful approach to accessing the structural diversity necessary for distinguishing enzyme selectivity but also tuning the physicochemical properties of the azasugar in a single, high-yielding
Chapter Four

step thus leading to the development of potent and selective inhibitors with potential anti-viral, anti-cancer and anti-lysosomal storage disease applications.
4.4 Experimental section

4.4.1 General methods

Please consult section 2.4.1 for general methods concerning reagents, purification and analysis. Please see section 4.4.2 for TLC visualisation of azasugar libraries.

4.4.2 Notes on nomenclature

Azasugars are named according to IUPAC-IUBMB “Nomenclature of Carbohydrates”, section 2-CARB-34 (http://www.chem.qmul.ac.uk/iupac/2carb/34.html)\(^{62}\) and according to the literature.\(^{36a}\) Compound names and NMR assignments are given with preference to the triazole ring. Examples are given below for compounds 4.28\(^a\) and 4.32\(^c\)\(^\prime\) in Table 4.2.
### Table 4.2 Examples for the nomenclature of \( N \)-alkylated azasugar triazoles.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Chemical Structure" /></td>
<td>1-(1,3-Dimethyl-5-((propylamino)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione)-4-(( N )-methylene-1,6-dideoxy-1,6-imino-L-iditol)-1,2,3-triazole</td>
</tr>
<tr>
<td><img src="image" alt="Chemical Structure" /></td>
<td>1-(2',3',4',6'-Tetra-O-acetyl-( \beta )-D-glucopyranosyl)-4-(( N )-methylene-3,4-di-( O )-p-methoxybenzyl-1,6-dideoxy-1,6-imino-D-glucitol)-1,2,3-triazole</td>
</tr>
</tbody>
</table>

4.32c'  
4.28a
4.4.3 Preparation of N-alkylated azasugar-triazoles (4.28a-d, 4.29a-d, and 4.32a-d):

General procedure 1.

A mixture of the azide (1.0 equiv.) and acetylene (1.0 equiv.) was prepared in tert-butyl alcohol and water (1:1, 0.2–0.5 M final concentration) and placed within a capped scintillation vial. Sodium ascorbate (0.2 equiv.) and CuSO₄·5H₂O (0.1 equiv.) was then added. The mixture was stirred vigorously at 40°C and monitored by TLC. Reactions were generally complete within 30 minutes at this temperature. Visualisation of libraries by TLC was performed by UV fluorescence and by charring plates with a mixture of 10% ammonium molybdite (w/v) in 10% aqueous H₂SO₄ containing 0.8% cerium sulphate. Conventional aqueous workup using CH₂Cl₂ or EtOAc organic phases followed by purification of the crude residue by silica filtration/flash chromatography afforded pure compound. The 1,4-regioselectivity of the reaction was verified by ¹H and ¹³C NMR chemical shifts of the triazole moiety in products obtained using CDCl₃, DMSO-d₆ and D₂O, and are in agreement with literature values (see Chapter two, references 11a, 12, 15f, 20, and 23).

4.4.4 Deprotection of azasugar scaffolds, 4.28 and 4.29: General procedure 2. A solution of the benzyl ether, 4.28 or 4.29 (−0.1 M) was prepared in mixture of TFA and distilled water (1:1 v/v) and stirred at slightly elevated temperature. The reaction was found complete after overnight stirring by TLC (C-18 reverse phase employing 1:1 CH₃CN–H₂O eluent) and ESI-MS. Once complete, the solvent was evaporated under reduced pressure and lyophilised to dryness. Mixtures were used in the next step without further purification (See section 4.4.5).
4.4.5 Preparation of azasugar scaffolds, 4.30 and 4.32: General procedure 3. A solution of the crude lyophilised reaction mixture (section 4.4.4) was prepared in pyridine and an equal volume of acetic anhydride was then added (~0.1 M final concentration). The reaction was stirred at room temperature until found complete by TLC. The solvent was removed under reduced pressure and the resulting residue purified by flash silica chromatography (1:2 EtOAc–CH₂Cl₂). A portion of the resulting pure material was retained for deprotection according to general method 4 (section 4.4.5) to obtain tetrols 4.31 and 4.33.

4.4.6 Preparation of azasugar library 4.32a’-d’ and scaffolds 4.31 and 4.33: General procedure 4. Compounds were prepared by the treating the per-O-acetate and (final concentration of ~0.1–0.2 M) with anhydrous methanolic sodium methoxide (final pH 9–12). Full deprotection was evident by TLC within 30 minutes of stirring at room temperature. The solution was neutralised by the addition of acidic ion exchange resin (Amberlite IR-120 [H⁺]), filtered and washed several times with methanol. Evaporation of the filtrates afforded the deprotected compounds 4.31, 4.33, 4.32a’-d’, all of which were shown to be pure by ¹H NMR.

4.4.7 Analytical Data

4.4.7.1 N-Propynyl azasugar scaffolds

![Diagram of 1,2:5,6-Di-O-isopropylidene-3-4-di-O-p-methoxylbenzyl-D-mannitol (4.23).]

A solution of 1,2:5,6-diisopropylidene-D-mannitol (25.0 g, 49.7 mmol) in anhydrous DMF at 0°C under nitrogen was
prepared and a 60% mineral oil dispersion of sodium hydride (5.0 g, 124 mmol, 2.5 equiv.) was added in three portions over 15 minutes. The suspension was stirred at 0°C for 30 minutes and then p-methoxybenzyl chloride (16.9 mL, 124.3 mmol, 2.5 equiv.) was added dropwise. The suspension was then brought to room temperature and stirred for 4 hours. The reaction was quenched by the careful addition of methanol at 0°C. Concentration of the mixture under reduced pressure afforded a crude oil which was purified by flash chromatography to afford clear oil which slowly crystallised on standing to white crystalline solid (18.6 g, 36.78 mmol, 74%).

$R_f$ 0.22 (1:4 EtOAc–hexanes); Mp 43–44°C. $^1$H NMR (400 MHz, CDCl$_3$) δ 1.32 (s, 6H, $^3$Pr CH$_3$), 1.40 (s, 6H, $^3$Pr CH$_3$), 3.73–3.75 (m, 2H, H$_3$), 3.78 (s, 6H, PhOCH$_3$), 3.80 (dd, $^3$J$_{1-1'}$ = 8.4 Hz, $^3$J$_{1-2}$ = 6.4 Hz, 2H, H$_1$), 3.97 (dd, $^2$J$_{1-1'}$ = 8.4 Hz, $^3$J$_{1-2}$ = 6.4 Hz, 2H, H$_1$), 4.16–4.21 (m, 2H, H$_2$), 4.60 (s, 4H, OCH$_2$Ph), 6.80–7.20 (m, 10H, Ar); $^{13}$C($^1$H) NMR (100 MHz, CDCl$_3$) δ 25.49 ($^3$Pr CH$_3$), 26.91 ($^3$Pr CH$_3$), 55.47 (PhOCH$_3$), 67.06 (C$_1$), 74.38 (OCH$_2$Ph), 76.13 (C$_2$), 79.78 (C$_3$), 108.73 ($^3$Pr C), 113.95 (Ar CH), 129.72 (Ar CH), 130.66 (Ar C), 159.52 (Ar C). Anal. calcd for C$_{28}$H$_{38}$O$_8$: C, 66.91; H, 7.62. Found: C, 66.77; H, 7.62. HRMS (ESI) calcd for C$_{28}$H$_{38}$O$_8$Na$_1^+$: 525.245893. Found: 525.24463.

3,4-Di-O-p-methoxbenzyl-1,6-di-O-tert-butyl dimethylsilyl-D-mannitol (4.25).

A portion of pure 4.23 (24 g, 47.8 mmol) was dissolved in aqueous glacial acetic acid (70% v/v, 500 mL) and stirred at room temperature for 24 hours. At this time, TLC indicated full acetonide deprotection. The mixture was evaporated at room temperature under reduced pressure to afford the tetrol 4.24 as pale yellow oil which, due to the highly hygroscopic nature, was
used in the following step without further purification or analysis. The resulting crude tetrol (12.5 g, 29.6 mmol) was azeotropically dried with CH$_3$CN (3 × 50 mL) and its solution in anhydrous DMF (30 mL) was immediately prepared under an atmosphere of nitrogen. The solution was cooled to 0°C and imidazole (8.1 g, 118.3 mmol, 4.0 equiv.) was added, followed by tert-butyl(dimethyl)silyl chloride (11.1 g, 73.9 mmol, 2.5 equiv.) in three equal portions. The thick suspension was stirred at 0°C for 2 hours until found complete by TLC. The reaction was quenched with saturated, aqueous ammonium chloride (60 mL) and extracted with CH$_2$Cl$_2$ (3 × 60 mL). The organic fractions were combined, washed with brine (100 mL), dried over MgSO$_4$ and concentrated under reduced pressure to afford crude yellow oil. Flash chromatography (15% EtOAc in hexanes) afforded clear oil that slowly crystallised upon refrigeration to a white solid. $R_f$ 0.28 (15% EtOAc in hexanes); Mp 58–59°C.

$^1$H NMR (400 MHz, CDCl$_3$) δ 0.04 (s, 6H, Me$_2$Si), 0.05 (s, 6H, Me$_2$Si), 0.89 (s, 18H, tert-butyl CH$_3$), 3.57 (dd, 2H, $^2$$J_{1,1}$ = 10 Hz, $^3$$J_{1,2}$ = 5.2 Hz, H$_1$), 3.72 (dd, 2H, $^2$$J_{1',1}$ = 10.0 Hz, $^3$$J_{1',2}$ = 3.2 Hz, 2H, H$_1'$), 3.77 (s, 6H, PhOCH$_3$), 3.79–3.87 (m, 4H, H$_2$, H$_3$), 4.58 (AB q, $^2$$J_{AB}$ = 11.2 Hz, 4H, OCH$_2$Ph), 6.82–7.25 (m, 10H, Ar); $^{13}$C{${^1}$H} NMR (100 MHz, CDCl$_3$) δ-5.10 (Me$_2$Si), -5.09 (Me$_2$Si), 18.51 (tert-butyl C), 26.17 (tert-butyl CH$_3$), 55.42 (PhOCH$_3$), 64.60 (C$_1$), 70.96 (C$_2$ or C$_3$), 73.70 (OCH$_2$Ph), 77.85 (C$_2$ or C$_3$), 113.96 (Ar CH), 130.21 (Ar CH), 130.67 (Ar C), 159.53 (Ar C). HRMS (ESI) calcd for C$_{34}$H$_{58}$O$_8$Si$_2$Na$^+$: 673.356235. Found: 673.357531. Anal. calcd for C$_{34}$H$_{58}$O$_8$Si$_2$: C, 62.73; H, 8.98. Found: C, 62.15; H, 8.96.

2,5-Di-O-Mesyl-3,4-Di-O-p-methoxybenzyl-1,6-di-O-tert-butyl dimethylsilyl-D-mannitol (4.26).
To a solution of 4.25 (9.8 g, 15.1 mmol) in anhydrous CH$_2$Cl$_2$ (25 mL) at 0°C under nitrogen, was added triethylamine (8.67 mL, 61.9 mmol, 4.1 equiv.) followed by the dropwise addition methanesulfonyl chloride (3.52 mL, 45.2 mmol, 2.0 equiv.). The thick suspension was stirred at 0°C for 15 minutes, at which time TLC indicated reaction completion. The reaction was quenched by the careful addition of water (20 mL) and extracted into CH$_2$Cl$_2$ (30 mL). The aqueous layer was then further extracted with CH$_2$Cl$_2$ (3 × 40 mL) and the combined organic layers were washed with brine (50 mL), dried (MgSO$_4$) and evaporated under reduced pressure to afford the crude mesylate as orange oil. Crystallisation from anhydrous methanol at 0°C afforded the mesylate 4.26 as (9.5 g, 19.4 mmol, 78%) as an unstable white crystalline solid. $R_f$ 0.22 (15% EtOAc in hexanes); Mp 81–82°C. $^1$H NMR (400 MHz, CDCl$_3$) δ 0.07 (s, 6H, Me$_2$Si), 0.08 (s, 6H, Me$_2$Si), 0.90 (s, 18H, tert-butyl CH$_3$), 2.97 (s, 6H, OMs CH$_3$), 3.78 (s, 6H, PhOCH$_3$), 3.90 (dd, $^3$J$_{1-11}$ = 11.6 Hz, $^3$J$_{1-12}$ = 6.4 Hz, 2H, H$_1$), 3.95–3.96 (m, 2H, H$_2$), 3.99 (dd, $^3$J$_{1-11}$ = 11.6 Hz, $^3$J$_{1-12}$ = 3.6 Hz, 2H, H$_1$), 4.62 (AB q, $^2$J$_{AB}$, 10.4 Hz, 4H, CH$_2$Ph), 4.77–4.80 (m, 2H, H$_2$), 6.83–7.27 (m, 8H, Ar); $^{13}$C$^1$H NMR (100 MHz, CDCl$_3$) δ −5.19 (SiCH$_3$), −5.16 (SiCH$_3$), 18.51 (tert-butyl C), 26.10 (tert-butyl CH$_3$), 38.78 (OMs CH$_3$), 55.47 (PhOCH$_3$), 62.17 (C$_1$), 74.14 (CH$_2$Ph), 78.03 (C$_3$), 83.48 (C$_2$), 114.05 (Ar CH), 129.75 (Ar C), 130.33 (Ar CH), 159.65 (Ar CH).

![Diagram](image)

**1,2:5,6-Dianhydro-3,4-di-O-methoxybenzyl-L-iditol (4.27).**

A solution of the mesylate 4.26 (13.2 g, 16.4 mmol) in methanol was cooled to 0°C and concentrated aqueous HCl (1N, 3.47 mL, 39.2 mmol, 2.4 equiv.) was added dropwise. The solution was stirred for an additional 1
hour to effect full silyl deprotection. At this time, an aqueous solution of potassium hydroxide (20% w/v, 28 mL, 98 mmol, 6.0 equiv.) was added drop-wise. A thick white precipitate had formed following stirring overnight at 20°C and was collected by vacuum filtration and washed with cold methanol–H₂O (3:7, 2 × 20 mL). A final recrystallisation of the crude white solid from hot absolute ethanol afforded the bis-epoxide as fine colourless needles (3.52 g, 9.11 mmol, 55.4%). R₆ 0.50 (1:1 EtOAc–hexanes); Mp 108–109°C. ¹H NMR (400 MHz, CDCl₃) δ 2.48 (dd, ²J₁₋₁ = 4.8 Hz, ³J₁₋₂ = 2.8 Hz, 2H, H₁), 2.69 (dd, ²J₁₋₁ = 5.2 Hz, ³J₁₋₂ = 4.4 Hz, 2H, H₁), 3.15–3.17 (m, 2H, H₂), 3.22–3.25 (m, 2H, H₃), 3.79 (s, 6H, PhOCH₃), 4.62 (AB q, ²JAB = 11.6 Hz, 4H, OCH₂Ph), 6.83–7.24 (m, 8H, Ar); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 43.3 (C₁), 52.67 (PhOCH₃), 55.48 (C₂), 72.09 (OCH₂Ph), 80.28 (C₃), 113.93 (Ar CH), 129.83 (Ar CH), 130.20 (Ar C), 159.48 (Ar C). HRMS (ESI) calcd for C₂₂H₂₆O₆Na⁺: 409.162161. Found: 409.162031. Anal. calcd for C₂₂H₂₆O₆: C, 68.38; H, 6.78. Found: C, 68.37; H, 6.91.

Colourless needles of 4.27 (C₂₂H₂₆O₆) suitable for X-ray crystallographic analysis was obtained by the slow evaporation of absolute ethanol–CHCl₃ (1:1 v/v): M = 386.43, monoclinic, space group P2₁, a = 5.1245 (5), b = 10.6190 (9), c = 18.513 (7)Å, β = 94.320 (1)°, V = 1004.6 (4)Å³, T = 293.0 K, Z = 2, D₀ = 1.278 Mg m⁻³, μ(Mo-Kα) = 0.092 mm⁻¹, 1860 reflections measured, 1219 unique (Rint = 0.1159). Refinement converged to R = 0.0729, wR(F²) = 0.2512.
N-Propynyl-3,4-di-O-p-methoxybenzyl-1,5-dideoxy-1,5-imino-D-glucitol (4.28).

The bis-epoxide 4.27 (1.0 g, 2.6 mmol) was suspended in methanol (25 mL) and propargyl amine (3.4 mL, 5.2 mmol, 2.0 equiv.) was then added. Dissolution was achieved within 4 hours by vigorous stirring at 45°C. The deep yellow solution was then stirred o/n at this temperature, at which time TLC indicated reaction completion. The mixture was concentrated under reduced pressure and the resulting orange oil purified by flash chromatography (1:1 EtOAc–CH₂Cl₂ then 100% EtOAc) to afford the piperidine 4.28 (520 mg, 1.2 mmol, 45%). R₉ 0.21 (2:3 EtOAc–CH₂Cl₂); Mp 139–140°C. ¹H NMR (400 MHz, CDCl₃) δ 2.23 (t, ⁴JCH·CH = 2.4 Hz, 1H, C=CH), 2.42–2.45 (m, 1H, H₅), 2.53–2.58 (m, 1H, H₁), 2.89 (dd, ²J₁·-₁ = 11.2 Hz, ³J₁·-₂ = 4.8 Hz, 1H, H₁'), 3.27–3.32 (m, 1H, H₃), 3.36 (dd, ²JCH·CH = 18.0 Hz, ⁴JCH·CH = 2.4 Hz, 1H, NCH), 3.57–3.62 (m, 1H, H₄), 3.63–3.68 (m, 2H, H₂, H₆), 3.73 (dd, ²JCH·CH = 18.4 Hz, ⁴JCH·CH = 2.4 Hz, 1H, NCH), 3.79 (s, 3H, PhOCH₃), 3.80 (s, 3H, PhOCH₃), 3.81–3.84 (m, 1H, H₆'), 4.76 (AB q, ²JAB = 11.2 Hz, 2H, 10.8 Hz, CH₂OPh), 4.80 (AB q, ²JAB = 10.8 Hz, 2H, CH₂OPh), 6.86–7.30 (m, 8H, Ar); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 42.49 (NCH₂), 55.49 (PhOCH₃), 55.52 (PhOCH₃), 56.29 (C₁), 57.40 (C₀), 63.46 (C₅), 69.35 (C₂), 74.98 (OCH₂Ph), 75.11 (OCH₂Ph), 75.13 (C=CH), 76.55 (C=CH), 77.34 (C₄), 86.95 (C₃), 114.21 (Ar CH), 114.33 (Ar CH), 129.77 (Ar CH), 129.95 (Ar CH), 130.45 (Ar C), 130.89 (Ar C), 159.62 (Ar C), 159.63 (Ar C). HRMS (ESI) calcd for C₂₅H₃₁N₅O₆⁺: 442.222413. Found: 442.220925; Anal. calcd for C₂₅H₃₁NO₆: C, 68.01; H, 7.08; N, 3.17. Found: C, 67.67; H, 7.05; N, 3.20.
Chapter Four

\[ \text{N-Propynyl-3,4-di-\(O-p\)-methoxybenzyl-1,6-dideoxy-1,6-imino-L-iditol (4.29).} \]

Title compound was isolated using the identical procedure for 4.28 and was isolated as pale yellow solid (47 mg, 41%).

\( R_f \) 0.12 (1:4 EtOAc–CH\(_2\)Cl\(_2\)); Mp 65–66\( ^\circ \)C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 2.31 (t, \( 3J_{CH-CH} = 2.4 \) Hz, 1H, C=CH), 2.53 (dd, \( 3J_{1-1'} = 12.8 \) Hz, \( 3J_{1-2} = 8.0 \) Hz, 2H, H\(_1\)), 3.07 (d, \( 3J_{1'-2} = 12.4 \) Hz, 2H, H\(_1\)), 3.54 (dd, \( 2J_{CH-CH} = 16.4 \) Hz, \( 4J_{CH-C=CH} = 1.6 \) Hz, 2H, NCH\(_2\)), 3.98–3.91 (m, 2H, H\(_3\), H\(_4\)), 4.58 (AB q, \( 2J_{AB} = 11.2 \) Hz, 4H, OCH\(_2\)Ph), 7.03 (m, 8H, Ar); \(^{13}\)C \( \{^1\}^1\)H NMR (100 MHz, CDCl\(_3\)) \( \delta \) 49.01 (NCH\(_2\)), 55.50 (PhOCH\(_3\)), 56.79 (C\(_1\)), 68.14 (C\(_2\)), 73.37 (OCH\(_2\)Ph), 75.36 (C=CH), 77.6 (C=CH), 85.12 (C\(_3\)), 114.17 (Ar CH), 129.84 (Ar CH), 130.19 (Ar C), 159.63 (Ar C). HRMS (ESI) calcd for C\(_{25}\)H\(_{32}\)N\(_1\)O\(_6\): 442.22413. Found: 442.221071. Anal. calcd for C\(_{25}\)H\(_{31}\)NO\(_6\): C, 68.01; H, 7.08; N, 3.17. Found: C, 68.01; H, 7.14; N, 3.02.

\[ \text{N-Propynyl-2,3,4,6-tetra-\(O\)-acetyl-1,5-dideoxy-1,5-imino-d-glucitol (4.30).} \]

The title compound was prepared according to general procedure 3 and was isolated as clear oil (85 mg, 0.23 mmol, 42%). \( R_f \) 0.43 (1:1 EtOAc–CH\(_2\)Cl\(_2\)). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 1.98 (s, 3H, OAc), 1.99 (s, 3H, OAc), 2.00 (s, 3H, OAc) 2.05 (s, 3H, OAc), 2.28 (t, \( 4J_{CH-CH} = 2.4 \) Hz, 1H C=CH), 2.57–2.62 (m, 1H, H\(_1\)), 2.71 (m, 1H, H\(_5\)), 3.00 (dd, \( 2J_{1-1'} = 11.2 \) Hz, \( 3J_{1'-2} = 5.2 \) Hz, 1H, H\(_1\)), 3.39 (dd, \( 2J_{CH-CH} = 18.4 \) Hz, \( 4J_{CH-CH} = 2.4 \) Hz, 1H, NCH\(_2\)), 3.73 (dd, \( 2J_{CH-CH} = 18.4 \) Hz, \( 4J_{CH-CH} = 2.0 \) Hz, 1H, NCH), 4.10–4.20 (m, 2H, H\(_3\), H\(_4\)), 4.94–5.00 (m, 1H, H\(_2\)), 5.00–5.10 (m, 2H, H\(_6\)); \(^{13}\)C \( \{^1\}^1\)H NMR (100 MHz,
Chapter Four

CDCl$_3$ $\delta$ 20.79 (OAc), 20.87 (OAc), 20.91 (OAc), 42.58 (NCH$_2$), 53.88 (C$_1$), 58.86 (C$_4$), 60.02 (C$_5$), 69.20 (C$_6$ or C=CH), 69.40 (C$_6$ or C=CH), 74.48 (C$_2$), 75.26 (C$_3$), 76.10 (C=CH), 169.83 (OAc), 170.13 (OAc), 170.47 (OAc), 171.06 (OAc).

**N-Propynyl-1,5-dideoxy-1,5-imino-D-glucitol (4.31).** The title compound was prepared from 4.30 according to general procedure 4 and isolated as white solid (25 mg, $\sim$100%). $^1$H NMR (400 MHz, D$_2$O) $\delta$ 2.21–2.24 (m, 1H, H$_5$), 2.41 (m, 1H, H$_1$), 2.58 (t, $^4$J$_{CH-CH}$ = 2.4 Hz, 1H, C=CH), 2.82 (dd, $^2$J$_{1',1}$ = 11.2 Hz, $^3$J$_{1',2}$ = 5.2 Hz, 1H, H$_1'$), 3.11–3.15 (m, 1H, H$_3$), 3.27–3.31 (m, 1H, H$_4$), 3.35 (dd, $^2$J$_{CH-CH}$ = 18.0 Hz, $^4$J$_{CH-CH}$ = 2.4 Hz, 1H, NCH), 3.44 (dd, $^3$J$_{2',1}$ = 10.4 Hz, $^3$J$_{2',3}$ = 9.2 Hz, $^3$J$_{2',1'}$ = 4.8 Hz, 1H, H$_2$), 3.73 (m, 2H, H$_6$ and H$_6'$); $^{13}$C NMR (100 MHz, D$_2$O) $\delta$ 41.79 (NCH$_2$), 55.99 (C$_1$), 56.88 (C$_6$), 63.76 (C$_3$), 68.89 (C$_2$), 69.68 (C$_4$), 75.86 (C$_3$), 76.89 (C=CH), 78.25 (C=CH). HRMS (ESI) calcd for C$_9$H$_{16}$NO$_4^+$: 202.107384. Found: 202.107345.

**N-Propynyl-2,3,4,5-tetra-O-acetyl-1,6-dideoxy-1,6-imino-L-iditol (4.32).** The title compound was prepared according to general procedure 3 and isolated as clear oil (220 mg, 0.60 mmol, 43%). $R_f$ 0.45 (1:1 EtOAc–CH$_2$Cl$_2$). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 2.01 (s, 6H, 2×OAc), 2.02 (s, 6H, 2×OAc), 2.26 (t, 1H, $^4$J$_{CH-CH}$ = 2.4 Hz, 1H, C=CH), 2.83 (dd, $^2$J$_{1',1}$ = 14.0 Hz, $^3$J$_{1',2}$ = 7.2 Hz, 1H, H$_1$), 3.00 (dd, $^2$J$_{1',1}$ = 13.6 Hz, $^3$J$_{1',2}$ = 4.0 Hz, 1H, H$_1'$), 3.41 (dd, $^2$J$_{CH-CH}$ = 17.2 Hz, $^4$J$_{CH-CH}$ = 2.0 Hz, 1H, NCH), 3.49 (dd, $^2$J$_{CH-CH}$ = 17.2 Hz, $^4$J$_{CH-CH}$ = 2.4 Hz, 1H, NCH), 5.08–5.13 (m, 2H, H$_2$), 5.26 (dd, $^3$J$_{3',2}$ = 6.0
Hz, $^3J_{3,4} = 2.4$ Hz, 2H, H$_3$; $^{13}$C$^{1}$H NMR (100 MHz, CDCl$_3$) δ 20.73 (OAc), 21.02 (OAc), 48.55 (C$_1$), 55.66 (NCH$_2$), 72.01 (C$_2$ and C$_3$), 73.89 (C≡CH), 78.00 (C≡CH), 169.47 (OAc), 169.92 (OAc).

**N-Propynyl-1,6-dideoxy-1,6-imino-L-iditol (4.33).** The title compound was prepared from 4.32 (48 mg, 0.13 mmol) according to general procedure 4 and isolated as a white gum (26 mg, 0.13 mmol, ~100%). $^1$H NMR (400 MHz, D$_2$O) δ 2.58 (t, $^4J_{CH-CH} = 2.4$ Hz, 1H, C≡H), 2.62 (dd, $^2J_{1,1'} = 13.2$ Hz, $^3J_{1,2} = 2$ Hz, H$_1$), 2.85 (dd, $^2J_{1,1'} = 13.6$ Hz, $^3J_{1,2} = 4.0$ Hz, 2H H$_1$), 3.31 (d, $^4J_{CH-CH} = 2.4$ Hz, 2H, NCH$_2$), 3.33 (dd, $^3J_{3,2} = 6.0$ Hz, $^3J_{3,4} = 2.4$ Hz, 2H, H$_3$), 3.59–3.64 (m, 2H, H$_2$); $^{13}$C$^{1}$H NMR (100 MHz, D$_2$O) δ 47.84 (NCH$_2$), 57.92 (C$_1$), 71.01 (C$_2$), 74.96 (C≡CH), 75.53 (C$_3$), 78.43 (C≡CH). HRMS (ESI) calcd for C$_9$H$_{16}$NO$_4^+$: 202.107384. Found: 202.107231.

### 4.4.7.2 Alkyl azide building block (c)

5-((3-hydroxypropylamino)methylene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione.

A solution of 5-((diethylamino)methylene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (5.62 g, 26.6 mmol) in methanol (15 mL) was cooled to 0°C followed by the addition of 3-amino-1-propanol (2.1 mL, 26.7 mmol). A white precipitate was formed within 2 minutes and the suspension was stirred at 0°C for an additional 15 minutes. The precipitate was filtered and washed with cold
methanol (~ 20 mL). The crude, off-white solid was recrystallised from hot, absolute 
ethanol to afford the title compound as colourless needles (4.6 g, 72%). \( R_f \) 0.22 
(100% EtOAc); \( \text{Mp} \) 129–130°C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 1.89 (pentet, \(^3\)\( J_{\text{CH-CH}} \), 6.0 Hz, 2H, \( \beta \text{CH}_2 \)) 2.19 (br s, 1H, \( \text{OH} \)) 3.28 (s, 3H, NCH\(_3\)) 3.29 (s, 3H, NCH\(_3\)) 3.63 (m, 2H, NHCH\(_2\)) 3.78 (t, \(^3\)\( J_{\text{CH-CH}} = 5.6 \) Hz, 2H, \( \text{CH}_2\text{OH} \)) 8.17 (d, \(^3\)\( J_{\text{CH-NH}} = 14.4 \) Hz, 1H C=CH) 10.40 (br s, \( \text{NH} \)); \(^13\)C\{\(^1\)H\} NMR (100 MHz, CDCl\(_3\)) \( \delta \) 27.32 (NCH\(_3\)), 28.03 (NCH\(_3\)), 32.27 (\( \beta \text{CH}_2 \)), 48.17 (CH\(_2\)NH), 59.61 (CH\(_2\)OH), 90.75 (C=CH), 152.67 (C=O), 159.88 (C=CH), 163.88 (C=O), 165.12 (C=O). Anal. calcd for 
C\(_{10}\)H\(_{15}\)N\(_3\)O\(_4\): C, 49.79; H, 6.27; N, 17.42. Found: C, 49.86 H, 6.28; N, 17.23.

5-((3-azidopropylamino)methylene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (building 
block c).

A solution of 5-((3-hydroxypropylamino)methylene)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (1.0 g, 4.2 mmol) and triethylamine (690 \( \mu \text{L}, 5.0 \) mmol, 1.2 equiv.) in anhydrous CH\(_2\)Cl\(_2\) (10 mL) was 
prepared under an atmosphere of nitrogen and cooled to 0°C. Methanesulphonyl 
chloride (390 \( \mu \text{L}, 5.0 \) mmol, 1.2 equiv.) was then added drop-wise. The solution was 
gradually brought to room temperature and the solution was stirred for 30 minutes 
until TLC had indicated reaction completion. The solution was then diluted with 
CH\(_2\)Cl\(_2\) (10 mL) and washed with distilled H\(_2\)O (10 mL) and brine (10 mL). The 
organic layer was dried (MgSO\(_4\)), filtered, and evaporated to afford a crude orange 
oil which was used in the following step without further purification. The crude 
mesylate was immediately dissolved in anhydrous DMF (15 mL) and sodium azide 
(810 mg, 12.5 mmol, 3.0 equiv.) was added in a single portion. The resulting deep
pink solution was stirred o/n at room temperature. At this time, TLC analysis indicated reaction completion. The solvent was concentrated \textit{in vacuo} and CH$_2$Cl$_2$ (20 mL) was added. The organic layer was washed with distilled H$_2$O (10 mL) and brine (10 mL), dried (MgSO$_4$), filtered and evaporated. The residue was purified by flash chromatography (2:3 hexanes–EtOAc) and recrystallisation from hot absolute ethanol to afford the azide as white solid (830 mg, 3.1 mmol, 74%). $R_f$ 0.67 (100% EtOAc); Mp 114–115$^\circ$C. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.19 (pentet, $^3J_{CH-CH}$ = 6.5 Hz, 2H, $\beta$CH$_2$), 3.29 (s, 3H, NCH$_3$), 3.30 (s, 3H, NCH$_3$), 3.43 (t, $^3J_{CH-CH}$ = 6.4 Hz, 2H, N$_3$CH$_2$), 3.56 (m, 2H, NHCH$_2$), 8.16 (d, $^3J_{CH-NH}$ = 14.4 Hz, 1H, C=CH/NH), 10.28 (br s, 1H, NH); $^{13}$C $^1$H NMR (100 MHz, CDCl$_3$) $\delta$ 27.35 (NCH$_3$), 28.06 (NCH$_3$), 29.71 ($\beta$CH$_2$), 47.77 (CH$_2$N$_3$), 48.35 (CH$_2$NH), 91.33 (C=CH), 152.29 (C=O), 159.85 (C=CH), 163.07 (C=O), 165.30 (C=O). Anal. calcd for C$_{10}$H$_{14}$N$_6$O$_3$: C, 45.11; H, 5.30; N, 31.56. Found: C, 45.17; H, 5.38; N, 31.42.

4.4.7.3 \textit{N-Alkylated azasugar triazoles}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{diagram.png}
\caption{1-(2',3',4',6'-Tetra-\textit{O-acetyl}-\textit{\beta}-\textit{D}-glucopyranosyl)-4-(\textit{N-methylene}-3,4-di-\textit{O-p-methoxybenzyl}-1,6-dideoxy-1,6-imino-\textit{D-glucitol})-1,2,3-triazole (4.28a).
}
\end{figure}

The title compound was prepared according to general procedure 1 and isolated as pale yellow foam (120 mg, 0.15 mmol, 68%). $R_f$ 0.22 (100% EtOAc); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.83 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.21–2.60 (m, 1H, piperidine H$_1$), 2.53–2.55 (m,
Chapter Four

1H, piperidine H5), 3.00 (dd, $^2J_{1\text{-}1} = 11.2$ Hz, $^3J_{1\text{-}2} = 4.4$ Hz, 1H, piperidine H1\text{'}),
3.27–3.31 (m, 1H, piperidine H3), 3.57–3.63 (m, 1H, piperidine H4), 3.63–3.67 (m, 1H, piperidine H2), 3.74 (s, 3H, OCH3Ph), 3.75 (s, 3H, OCH3Ph), 3.93–3.97 (m, 1H, NCH), 4.00 (ddd, $^2J_{6\text{-}6'} = 12.8$ Hz, $^3J_{6\text{-}5} = 2.0$ Hz, 1H, Glc H6\text{'}),
4.12–4.15 (m, 1H, NCH), 4.15 (dd, $^2J_{6\text{-}6'} = 12.8$ Hz, $^3J_{6\text{-}5} = 2.0$ Hz, 1H, Glc H6\text{'}),
4.31 (dd, $^2J_{6\text{-}6'} = 12.8$ Hz, $^3J_{6\text{-}5} = 5.2$ Hz, 1H, Glc H6\text{'}), 4.72 (AB q, $^2J_{AB} = 11.6$
Hz, 2H OCH2Ph), 4.73 (AB q, $^2J_{AB} = 10.4$ Hz, 2H, OCH2Ph) 5.21–5.26 (m, 1H, Glc
H4\text{'}), 5.33–5.38 (m, 1H, Glc H3\text{'}), 5.39–5.43 (m, 1H, Glc H2\text{'}), 5.83 (d, $^3J_{1\text{-}2'} = 8.8$
Hz, 1H, Glc H1\text{'}), 6.83–7.26 (m, 8H, Ar), 7.86 (br s, 1H, triazole CH); $^{13}$C {1H} NMR (100 MHz, CDCl3) δ 20.32 (OAc), 20.70 (OAc), 20.74 (OAc), 20.92 (OAc), 47.47 (NCH2), 55.44 (PhOCH3), 55.47 (PhOCH3), 55.72 (C1), 58.12 (NCH2), 60.33 (piperidine C6), 61.72 (Glc C6\text{'}), 64.34 (piperidine C5), 67.93 (Glc C2\text{'}), 69.35 (piperidine C2), 70.64 (Glc C3\text{'}), 72.69 (Glc C4\text{'}), 74.63 (Glc C5\text{'}), 74.83 (OCH2Ph), 75.38 (OCH2Ph), 77.71 (piperidine C4), 85.94 (Glc C1\text{'}), 86.14 (Glc C1\text{'}), 114.09 (Ar
CH), 114.20 (Ar CH), 121.77 (triazole CH), 129.67 (Ar CH), 129.78 (Ar CH), 130.59 (Ar C), 130.94 (Ar C), 143.42 (triazole C), 159.48 (Ar C), 159.50 (Ar C), 169.25 (C=O), 169.59 (C=O), 170.09 (C=O), 170.77 (C=O). HRMS (ESI) calced for
Chapter Four

1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-4-(N-methylene-3,4-di-O-p-methoxybenzyl-1,6-dideoxy-1,6-imino-L-iditol)-1,2,3-triazole (4.29a).

The title compound was prepared according to general procedure 1 and isolated as white solid (189 mg, 0.23 mmol, 83%). $R_f$ 0.14 (1:1 EtOAc–CH$_2$Cl$_2$); Mp 120–121°C. $^1$H NMR (400 MHz, CDCl$_3$) δ 1.86 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.72 (dd, $^2J_{1t-1t}$ = 12.4 Hz, $^3J_{1t-2t}$ = 7.6 Hz, 2H, azepane H$_1$), 2.93 (d, $^3J_{1t-2t}$ = 12.4 Hz, 2H, azepane H$_1$), 3.65–3.66 (m, 2H, azepane H$_1$), 3.79 (s, 6H, PhOCH$_3$), 4.04 (ddd, $^3J_{5t-4t}$ = 10.0 Hz, $^3J_{5t-6t}$ = 4.8 Hz, $^3J_{5t-6t}$ = 2.0 Hz, 1H, Glc H$_{5t}$), 4.18 (dd, $^2J_{6t-6t}$ = 12.8 Hz, $^3J_{6t-5t}$ = 2.0 Hz, 1H, Glc H$_{6t}$), 4.19 (br s, 2H, azepane H$_3$), 4.27 (br s, 2H, azepane H$_2$), 4.32 (dd, $^2J_{6t-6t}$ = 12.8 Hz, $^3J_{6t-5t}$ = 4.8 Hz, 1H, Glc H$_{6t}$), 4.45 (AB q, $^2J_{AB}$ = 11.2 Hz, 4H, OCH$_2$Ph), 5.25–5.30 (m, 1H, Glc H$_4$), 5.41–5.58 (m, 2H, Glc H$_2$, Glc H$_3$), 5.71 (d, $^3J_{1t-2t}$ = 8.4 Hz, 1H, Glc H$_1$), 6.84–7.21 (m, 8H, Ar), 8.21 (br s, 1H, triazole CH); $^{13}$C {$^1$H} NMR (100 MHz, CDCl$_3$) δ 20.32 (OAc), 20.71 (OAc), 20.74 (OAc), 20.93 (OAc), 54.11 (NCH$_2$), 55.47 (PhOCH$_3$), 57.48 (azepane C$_1$), 61.71 (Glc C$_6$), 67.93 (Glc C$_4$), 68.63 (azepane C$_2$), 70.67 (Glc C$_2$ or Glc C$_3$), 72.67 (Glc C$_2$ or Glc C$_3$), 73.54 (OCH$_2$Ph), 75.42 (Glc C$_5$), 85.94 (azepane C$_3$), 86.00 (Glc C$_1$), 114.13 (Ar CH), 121.59 (triazole CH), 129.78 (Ar CH), 130.33 (Ar C), 144.62 (triazole C), 159.56 (Ar C), 169.28 (OAc), 169.57 (OAc), 170.08 (OAc), 170.75 (OAc). HRMS (ESI) caleed for C$_{39}$H$_{51}$N$_4$O$_{15}$+: 815.334535. Found: 815.332035. Anal. caleed for C$_{39}$H$_{50}$N$_4$O$_{15}$: C, 57.49; H, 6.18; N, 6.88. Found: C, 57.53; H, 6.17; N, 6.71.
The title compound was prepared according to the general procedure 1 and isolated as clear oil (87 mg, 0.11 mmol, 78%). $R_f$ 0.32 (1:1 EtOAc–hexanes); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.85 (s, 3H, OAc), 1.99 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.81 (br s, 2H, azepane H$_1$), 3.00–3.03 (m, 2H, azepane H$_3$), 3.90–4.01 (br s, 2H, NCH$_2$), 3.98 (ddd, $^3$J$_{5',4'}$= 10.0 Hz, $^3$J$_{5',6'}$= 5.2 Hz, $^3$J$_{5'-6'}$= 2.0 Hz, 1H, Glc H$_5$), 4.14 (dd, $^2$J$_{6',6'}$= 12.8 Hz, $^3$J$_{6',5'}$= 2.0 Hz, 1H, Glc H$_6$), 4.29 (dd, $^2$J$_{6',6'}$= 12.4 Hz, $^3$J$_{6',5'}$= 5.2 Hz, 1H, Glc H$_6'$), 5.10 (br s, 2H, azepane H$_2$), 5.20–5.27 (m, 3H, Glc H$_4'$, azepane H$_3$), 5.37–5.43 (m, 2H, Glc H$_2'$ and Glc H$_3'$), 5.81–5.83 (m, 1H, Glc H$_1'$), 7.79 (br s, 1H, triazole CH); $^{13}$C {$^1$H} NMR (100 MHz, CDCl$_3$) $\delta$ 20.27 (OAc), 20.69 (OAc), 20.72 (OAc), 20.77 (OAc), 20.87 (2 × OAc), 21.04 (2 × OAc), 53.52 (NCH$_2$), 55.54 (azepane C$_1$), 61.78 (Glc C$_6$), 67.94 (Glc C$_4$), 70.61 (Glc C$_2'$ or Glc C$_3'$), 71.95 (azepane C$_2$), 72.25 (azepane C$_3$), 72.68 (Glc C$_2'$ or Glc C$_3'$), 75.35 (Glc C$_5$), 85.97 (Glc C$_1$), 121.01 (triazole CH), 143.45 (triazole C), 169.03 (OAc), 169.45 (2 × OAc), 169.54 (OAc), 169.83 (2 × OAc), 170.08 (OAc), 170.68 (OAc).
1-(β-D-glucopyranosyl)-4-(1,6-dideoxy-1,6-imino-L-iditol)-1,2,3-triazole (4.32a').

The title compound was prepared from 4.32a according to general procedure 4 and isolated as a white foam (28 mg, 0.07 mmol, ~100%). $^1$H NMR (400 MHz, D$_2$O) δ 2.51 (dd, $^2J_{1',1} = 13.6$ Hz, $^3J_{1',2} = 7.6$ Hz, 2H, azepane H$_1$), 2.76 (dd, $^2J_{1'} = 2.0$ Hz, 2H, azepane H$_3$), 3.30 (dd, $^3J_{3',2'} = 5.6$ Hz, $^3J_{3',4'} = 2.0$ Hz, 2H, azepane H$_3$), 3.47–3.67 (m, 7H, Glc H$_3$, Glc H$_4$, Glc H$_5$, Glc H$_6$, Glc H$_6'$ and azepane H$_2$), 3.76 (s, 2H, NCH$_2$), 3.84–3.89 (m, 1H, Glc H$_2$), 5.61 (d, $^3J_{1',2'} = 9.2$ Hz, 1H, Glc H$_1$), 8.10 (s, 1H, triazole CH); $^{13}$C{1H} NMR (100 MHz, D$_2$O) δ 52.75 (NCH$_2$), 58.37 (azepane C$_1$), 60.57 (Glc C$_6$), 69.12 (Glc C$_4$), 71.32 (azepane C$_2$), 72.54 (Glc C$_2$), 75.96 (azepane C$_3$), 76.45 (Glc C$_3$), 79.14 (Glc C$_5$), 87.69 (Glc H$_1$), 123.5 (triazole CH), 145.83 (triazole C). HRMS (ESI) calcd for C$_{15}$H$_{27}$N$_4$O$_9$+: 407.177256. Found: 407.176543.

1-(p-Sulfamoylphenyl)-4-(N-methylene-3,4-di- O-p-methoxybenzyl-1,6-dideoxy-1,6-imino-D-glucitol)-1,2,3-triazole (4.28b).

The title compound was prepared according to the general procedure 1 and isolated as pale yellow solid (140 mg, 0.22 mmol, 82%). $R_f$ 0.11 (1:9 CH$_3$OH–EtOAc); Mp 168–172°C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.97–2.01 (m, 1H, piperidine H$_5$), 2.01–2.06 (m, 1H, piperidine H$_4$), 2.83 (dd, $^2J_{1',1} = 11.2$ Hz, $^3J_{1',2} = 4.8$ Hz, 1H, piperidine H$_1$), 3.05–3.09 (m, 1H,
piperidine H₄), 3.33–3.37 (m, 1H, piperidine H₃), 3.45 (dd, ³J₂₃ = 10.0 Hz, ³J₂₁ = 9.2 Hz, ³J₂₋₁ = 4.8 Hz, 1H, piperidine H₂), 3.65 (dd, ²J₆₋₆ = 12.4 Hz, ³J₆₋₅ = 2.8 Hz, 2H, azepane H₁), 3.69 (s, 3H, PhOCH₃), 3.70 (s, 3H, PhOCH₃), 3.94–4.06 (m, 3H, NCH₂, piperidine H₆), 4.58 (AB q, ²J₉₋₂ = 10.4 Hz, 2H, OCH₂Ph), 4.67 (AB q, ²J₉₋₁ = 11.2 Hz, 2H, OCH₂Ph), 6.80–6.85 (m, 4H, Ar), 7.13–7.24 (m, 4H, Ar), 7.48 (br s, 2H, SO₂NH₂), 7.97–8.12 (m, 4H, Ar), 8.77 (s, 1H, triazole CH); ¹³C{¹H} NMR (100 MHz, DMSO-d₆) δ 47.19 (NCH₂), 55.68 (PhOCH₃), 55.73 (PhOCH₃), 57.93 (piperidine C₁), 58.05 (piperidine C₆), 64.92 (piperidine C₅), 70.51 (piperidine C₂), 74.26 (OCH₂Ph), 74.47 (OCH₂Ph), 79.47 (piperidine C₄), 87.89 (piperidine C₃), 114.14 (Ar CH), 114.21 (Ar CH), 120.83 (Ar CH), 122.85 (triazole CH), 128.17 (Ar CH), 129.76 (Ar CH), 129.78 (Ar CH), 131.61 (Ar C), 132.02 (Ar C), 139.38 (Ar C), 144.20 (triazole C or Ar C), 144.33 (triazole C or Ar C), 159.16 (Ar C), 159.26 (Ar C). HRMS (ESI) calcld for C₃₁H₃₈N₅O₈S⁺: 640.243567. Found: 640.241586. Anal. calcld for C₃₁H₃₇N₅O₈S: C, 58.20; H, 5.83; N, 10.95; S, 5.01. Found: C, 56.78; H, 5.75; N, 10.50; S, 4.65.

1-(p-Sulfamoylphenyl)-4-((N-methylene-3,4-di-O-p-methoxybenzyl-1,6-dideoxy-1,6-imino-L-iditol)-1,2,3-triazole (4.29b).

The title compound was prepared according to general procedure 1 and isolated as pale yellow foam (102 mg, 0.16 mmol, 70.4%). Rf 0.57 (1:9 CH₃OH–CH₂Cl₂). ¹H NMR (400 MHz, DMSO-d₆) δ 2.55 (dd, ²J₁₋₁ = 12.8 Hz, ³J₁₋₂ = 7.6 Hz, 2H, azepane H₁), 2.73 (dd, ²J₁₋₁ = 12.8 Hz, ³J₁₋₂ = 2.8 Hz, 2H, azepane H₁), 3.38 (dd, ³J₃₋₂ = 5.6 Hz, ³J₃₋₄ = 1.6 Hz, 2H, azepane H₃), 3.69
(s, 6H, PhOCH₃), 3.72–3.75 (m, 2H, azepane H₂), 3.78 (AB q, \(^2J_{AB} = 14.8\) Hz, 2H, NCH₂), 4.51 (s, 4H, OCH₂Ph), 4.55 (d, 2H, \(^3J_{OH-2} = 5.2\) Hz, 2H, azepane H₂ OH), 6.81–7.21 (m, 8H, Ar), 7.49 (br s, 2H, SO₂NH₂), 7.98–8.12 (m, 4H, Ar), 8.84 (s, 1H, triazole CH); \(^{13}\)C\(^{1}H\) NMR (100 MHz, DMSO-d₆) δ 55.70 (C₂), 58.56 (OCH₂Ph), 70.92 (C₁), 73.19 (NCH₂), 85.53 (C₃), 114.09 (Ar CH), 120.74 (Ar CH), 122.75 (triazole CH), 128.16 (Ar CH), 129.90 (Ar CH), 131.68 (Ar C), 139.39 (Ar C), 144.30 (Ar C or triazole C), 146.37 (Ar C or triazole C), 159.22 (Ar C). HRMS (ESI) calcd for C\(_{31}\)H\(_{38}\)N\(_{5}\)O\(_{8}\)S\(^{+}\): 640.243567. Found: 640.243875. Anal. calcd for C\(_{31}\)H\(_{37}\)N\(_{5}\)O\(_{8}\): C, 58.20; H, 5.83; N, 10.95; S, 5.01. Found: C, 56.72; H, 5.87; N, 10.25; S, 4.59.

1-(\(p\)-Sulfamoylphenyl)-4-((\(N\)-methylene-2,3,4,5-tetra-O-acetyl-1,6-imino-L-iditol)-1,2,3-triazole (4.32b).

The title compound was prepared according to the general procedure 1 and isolated as pale yellow foam (88 mg, 0.16 mmol, 74%). \(R_f\) 0.18 (1:4 CH\(_2\)Cl\(_2\)-EtOAc). \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ 2.02 (s, 6H 2 × OAc), 2.02 (s, 6H, OAc), 2.92 (br s, 2H, azepane H₁), 3.10 (br s, 2H, azepane H₁), 4.03 (br s, 2H, NCH₂), 5.12 (br s, 2H, azepane H₂), 5.30 (dd, \(^3J_{3-2} = 6.0\) Hz, \(^3J_{3-4} = 2.0\) Hz, azepane H₃), 7.90–8.06 (m, 4H, Ar) 8.21 (br s, 1H, triazole CH); \(^{13}\)C\(^{1}H\) NMR (100 MHz, CDCl\(_3\)) δ 20.82 (OAc), 21.08 (OAc), 53.58 (azepane C₁), 60.66 (NCH₂), 72.00 (azepane C₂ and C₃), 120.67 (Ar CH), 121.8 (triazole CH), 128.49 (Ar CH), 139.65 (Ar C), 142.61 (Ar C), 145.21 (triazole C), 169.59 (OAc), 170.08 (OAc).
1-(p-Sulfamoylphenyl)-4-(N-methylene-1,6-imino-L-iditol)-1,2,3-triazole (4.32b’).

The title compound was prepared according to general procedure 4 and isolated as pale yellow oil (38 mg, ~100%). $^1$H NMR (400 MHz, D$_2$O) $\delta$ 2.54 (dd, $^2$J$_{1\cdash1}$ = 13.6 Hz, $^3$J$_{1\cdash2}$ = 7.6 Hz, 2H, H$_1$), 2.79 (dd, $^2$J$_{1\cdash1}$ = 13.6 Hz, $^3$J$_{1\cdash2}$ = 4.4 Hz, 2H, H$_1$), 3.29 (dd, $^3$J$_{3\cdash2}$ = 5.6 Hz, $^3$J$_{3\cdash4}$ = 2.0 Hz, 2H, azepane H$_3$), 3.54–3.58 (m, 2H, azepane H$_2$), 3.77 (s, 2H, NCH$_2$), 7.71–7.86 (m, 4H, Ar CH), 8.34 (s, 1H, triazole CH); $^{13}$C($^1$H) NMR (100 MHz, 1:9 DMSO-d$_6$–D$_2$O) $\delta$ 52.81 (NCH$_2$), 58.26 (azepane C$_1$), 71.30 (azepane C$_2$), 75.71 (azepane C$_3$), 121.33 (Ar CH), 123.55 (triazole CH), 128.04 (Ar CH), 139.41 (Ar C), 142.06 (Ar C or triazole C), 144.23 (Ar C or triazole C). HRMS (ESI) calcd for C$_{15}$H$_{22}$N$_5$O$_6$S$_1^+$: 400.128537. Found: 400.12692.

1-(1,3-dimethyl-5-((propylamino)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione)-4-(N-methylene-1,6-dideoxy-1,6-imino-D-glucitol)-1,2,3-triazole (4.28c).

The title compound was prepared according to the general procedure 1 and isolated as white foam (124 mg, 0.18 mmol, 77%). $R_f$ 0.09 (1:9 CH$_3$OH–EtOAc). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 2.24 (pentet $^3$J$_{CH\cdashCH}$ = 6.4 Hz, 2H, $\beta$CH$_2$), 2.32–2.37 (piperidine H$_1$), 2.44–2.46 (m, 1H, piperidine H$_5$), 3.10–3.14 (dd, $^2$J$_{1\cdash1}$ = 11.6 Hz, $^3$J$_{1\cdash2}$ = 1H, piperidine H$_2$), 3.25 (s, 3H, NCH$_3$), 3.26 (s, 3H, NCH$_3$), 3.28–3.32 (m, 1H, piperidine H$_3$), 3.39–3.49 (m, 2H, $\gamma$CH$_2$), 3.46 (s, 3H, NCH$_3$), 3.57 (m, 2H, $\beta$CH$_2$), 3.63 (s, 3H, NCH$_3$), 3.71 (m, 1H, piperidine H$_4$), 7.77 (m, 4H, Ar CH), 8.32 (s, 1H, triazole CH).
Chapter Four

3.61–3.65 (m, 1H, piperidine H₄), 3.75 (s, 3H, PhOCH₃), 3.76 (s, 3H, PhOCH₃), 3.90–3.93 (m, 1H, piperidine H₆), 4.04–4.13 (m, 3H, piperidine H₆, NCH₂), 4.39 (t, 3J_{CH-CH} = 6.8 Hz, 2H, aCH₂), 4.72 (AB q, 2J_{AB} = 11.0 Hz, 2H, OCH₂Ph), 4.70 (AB q, 2J_{AB} = 10.6 Hz, 2H, OCH₂Ph), 6.80–7.25 (m, 8H, Gr), 7.63 (s, 1H, triazole CH), 8.07 (d, 3J_{CH-NH} = 14.4 Hz, 1H, CH/NH), 10.22–10.28 (m, 1H, NH), 13C {¹H} NMR (100 MHz, CDCl₃) δ 27.38 (NCH₃), 28.09 (NCH₃), 30.68 (CH₂), 46.88 (CH₂), 47.24 (aCH₂), 47.66 (NCH₂), 55.47 (PhOCH₃), 55.50 (PhOCH₃), 57.87 (piperidine C₁), 57.90 (piperidine C₂), 64.84 (piperidine C₃), 69.10 (piperidine C₄), 74.69 (OCH₂Ph), 74.93 (OCH₂Ph), 85.70 (piperidine C₅), 91.43 (C=CH), 114.13 (Ar CH), 114.16 (Ar CH), 124.25 (tribazole CH), 129.72 (Ar CH), 129.79 (Ar CH), 130.37 (Ar C), 130.81 (Ar C), 143.85 (tribazole C), 152.62 (C=CH), 159.54 (Ar C), 159.57 (Ar C), 160.01 (C=O), 163.11 (C=O), 165.16 (C=O). HRMS (ESI) calcd for C₃₅H₄₆N₇O₉+: 708.335148. Found: 708.336962. Anal. calcd for C₃₅H₄₅N₇O₉·5H₂O: C, 52.69; H, 6.95; N, 12.29. Found: C, 52.72; H, 6.34; N, 12.40.

1-(1,3-dimethyl-5-((propylamino)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione)-4-((N-methylene-3,4-di-O-p-methoxybenzyl-1,6-dideoxy-1,6-imino-L-iditol)-1,2,3-triazole (4.29c).

The title compound was prepared according to general procedure 1 and isolated as white foam (140 mg, 0.20 mmol, 87%). Rf 0.63 (1:9 CH₃OH–CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃) δ 2.27 (pentet, 3J_{CH-CH} = 6.8 Hz, 2H βCH₂), 2.69 (dd, 2J_{1,-1} = 12.4 Hz, 3J_{1,-2} = 7.6 Hz, 2H, azepane H₁), 2.91 (dd, 2J_{1,-1} = 12.8 Hz, 3J_{1,-2} = 1.6 Hz, 2H, azepane H₁), 3.27 (s, 3H, NCH₃), 3.28
(s, 3H, NCH$_3$), 3.44–3.49 (m, 2H, $\gamma$CH$_2$), 3.58 (dd, $^3$J$_{3,2}$ = 4.4 Hz, $^3$J$_{3,4}$ = 1.6 Hz, 2H, azepane H$_3$), 3.77 (s, 6H, PhOCH$_3$), 3.79–3.82 (m, 2H, azepane H$_2$), 4.39–4.43 (m, 2H, $\alpha$CH$_2$), 4.60 (AB q, $^2$J$_{AB}$ = 10.8 Hz, OCH$_2$Ph), 6.83–7.21 (m, 8H, Gr CH), 7.51 (s, 1H, triazole CH), 8.13 (d, $^3$J$_{NH-CH}$ = 14.4 Hz, 1H, C=CH), 10.27–10.32 (m, 1H, NH; $^{13}$C$_{\{\}}^1$H NMR (100 MHz, CDCl$_3$) $\delta$ 28.38 (NCH$_3$), 28.08 (NCH$_3$), 30.91 ($\beta$CH$_2$), 46.91 ($\alpha$CH$_2$), 47.24 ($\gamma$CH$_2$), 54.36 (NCH$_2$), 55.50 (PhOCH$_3$), 57.86 (azepane C$_1$), 68.80 (azepane C$_2$), 73.63 (OCH$_2$Ph), 85.89 (azepane C$_3$), 91.55 (C=CH), 114.16 (Ar CH), 123.14 (triazole CH), 129.81 (Ar CH), 130.27 (Ar C), 144.92 (triazole C), 152.24 (C=CH), 159.61 (Ar C) 159.98 (C=O), 163.02 (C=O), 165.25 (C=O). HRMS (ESI) calcd for C$_{35}$H$_{46}$N$_7$O$_9$+: 708.335148. Found: 708.338005. Anal. calcd for C$_{35}$H$_{45}$N$_7$O$_9$: C, 59.39; H, 6.41; N, 13.85. Found: C, 58.09; H, 6.45; N, 13.15.

1-(1,3-dimethyl-5-((propylamino)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione)-4-(N-methylene-1,6-dideoxy-1,6-imino-2,3,4,5-tetra-O-acetyl-L-iditol)-1,2,3-triazole (4.32c).

The title compound was prepared according to general procedure 1 and isolated as clear oil (98 mg, 0.15 mmol 84%). $R_f$ 0.28 (2:3 CH$_2$Cl$_2$–EtOAc). $^1$H NMR (400 MHz, CDCl$_3$) 2.01 (s, 6H, 2\times OAc), 2.02 (s, 6H, 2\times OAc), 2.30 (pentet, $^3$J$_{CH-CH}$ = 7.2 Hz, 2H, $\beta$CH$_2$), 2.83 (s, 2H, azepane H$_1$), 3.02 (s, 2H, azepane H$_1$), 3.23 (s, 3H, NCH$_3$), 3.24 (s, 3H, NCH$_3$), 3.46–3.51 (m, 2H, $\alpha$CH$_2$), 3.87 (s, 2H, NCH$_2$), 4.38–4.51 (m, 2H, $\gamma$CH$_2$), 4.98–5.03 (m, 2H, azepane H$_2$), 5.24 (dd, $^3$J$_{2,3}$ = 5.6 Hz, $^3$J$_{3,4}$ = 1.6 Hz, 2H, azepane H$_3$), 7.66 (s, 1H, triazole CH), 8.14 (d, $^3$J$_{CH-NH}$ = 14.0 Hz, 1H, C=CH), 10.24–10.30 (m, 1H, NH);
Chapter Four

$^{13}$C{\H}NMR (100 MHz, CDCl$_3$) $\delta$ 20.77 (OAc), 21.03 (OAc), 27.30 (NCH$_3$), 28.01 (NCH$_3$), 30.98 (BCH$_2$), 47.16 ($\alpha$CH$_2$), 47.33 ($\gamma$CH$_2$), 53.70 (azepane C$_1$), 55.32 (NCH$_3$), 71.81 (C$_2$ or C$_3$), 72.04 (C$_2$ or C$_3$), 91.37 (C=CH), 123.44 (triazole CH), 152.27 (C=CH), 159.99 (C=O), 163.00 (C=O), 165.11 (C=O), 169.49 (OAc), 170.00 (OAc).

1-(1,3-dimethyl-5-((propylamino)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione)-4-(N-methylene-1,6-dideoxy-1,6-imino-L-iditol)-1,2,3-triazole (4.32c’).

The title compound was prepared from 4.32c according to method 4 and isolated as clear gum (28 mg, 0.06 mmol, ~100%). $^1$H NMR (400 MHz, D$_2$O) $\delta$ 2.21 (pentet, $^3J_{CH-CH}$ = 6.4 Hz, 2H, $\beta$-CH$_2$), 2.46 (dd, $^2J_{1,1'}$ = 13.6 Hz, $^3J_{1,2}$ = 8.0 Hz, 2H, azepane H$_1$), 2.71 (dd, $^2J_{1,1'}$ = 13.6 Hz, $^3J_{1,2}$ = 4.0 Hz, 2H, azepane H$_1'$). 3.03 (s, 6H, 2 $\times$ NCH$_3$), 3.28 (dd, $^3J_{2,3}$ = 6.0 Hz, $^3J_{3,4}$ = 2.8 Hz, 2H, azepane H$_3$), 3.46–3.53 (m, 4H, azepane H$_2$, $\gamma$CH$_2$), 3.63 (br s, 2H, NCH$_2$), 4.43 (t, $^3J_{CH-CH}$ = 6.0 Hz, 2H, $\alpha$CH$_2$), 7.90 (s, 1H, C=CH), 7.95 (s, 1H, triazole CH); $^{13}$C{$^1$H} NMR (100 MHz, D$_2$O) $\delta$ 27.28 (BCH$_2$), 29.41 (NCH$_3$), 48.04 ($\alpha$CH$_2$), 48.26 ($\gamma$CH$_2$), 52.72 (NCH$_2$), 58.21 (azepane C$_1$), 71.26 (azepane C$_2$), 75.62 (azepane C$_3$), 90.21 (C=CH), 125.19 (triazole CH), 143.47 (triazole C), 153.20 (C=CH), 160.28 (2xC=O), 164.69 (C=O). HRMS (ESI) calcd for C$_{19}$H$_{30}$N$_7$O$_7$+: 468.220119. Found: 468.221857.
Chapter Four

1-(2,3-dimethyl-N-propylbutanamide)-4-(N-methylene-3,4-di-O-methoxybenzyl-1,6-dideoxy-1,6-imino-D-glucitol)-1,2,3-triazole (4.28d).

The title compound was prepared according to the general procedure 1 and isolated as clear oil (120 mg, 0.19 mmol, 72%). Rf 0.21 (1:4 CH2Cl2–EtOAc). \(^1\)H NMR (400 MHz, CDCl3) \(\delta\) 0.71 (d, \(^3\)J_{CH-CH} = 6.4 Hz, 3H, sec-butyl CH), 0.85 (t, \(^3\)J_{CH-CH} = 7.6 Hz, 3H, n-Pr CH), 1.03 (d, \(^3\)J_{CH-CH} = 6.4 Hz, 3H, sec-butyl CH), 1.27 (pentet, \(^3\)J_{CH-CH} = 7.2 Hz, 3H, n-pr CH), 2.37–2.49 (m, 3H, H1, H5, sec-butyl CH), 3.12–3.30 (m, 5H, n-Pr CH, piperidine H1', H2 and H3), 3.75 (s, 3H, PhOCH), 3.69–3.74 (m, 1H, piperidine H2), 3.76 (s, 3H, PhOCH), 3.89–3.92 (m, 1H, piperidine H6) 4.05–4.12 (m, 3H, NCH2 piperidine H6'), 4.16–4.68 (m, 2H, PhOCH), 4.77–4.83 (m, 3H, PhOCH, sec-butyl CH), 6.82–7.25 (m, 8H, Ar), 7.92 (triazole CH); \(^1^3\)C \{1H\} NMR (100 MHz, CDCl3) \(\delta\) 11.57 (n-Pr CH), 19.02 (sec-butyl CH), 19.49 (sec-butyl CH), 22.67 (n-Pr CH), 32.49 (sec-butyl CH), 41.74 (n-Pr CH), 55.47 (NCH2), 55.46 (PhOCH), 55.49 (PhOCH), 57.88 (piperidine C1), 60.63 (piperidine C6), 64.83 (piperidine C5), 69.16 (piperidine C2), 71.23 (sec-butyl CH), 74.71 (OCH2Ph), 74.99 (OCH2Ph), 77.43 (piperidine C4), 81.57 (piperidine C3), 114.14 (Ar CH), 114.20 (Ar CH), 123.95 (triazole CH), 129.69 (Ar CH), 129.88 (Ar CH), 130.42 (Ar C), 130.89 (Ar C), 142.23 (triazole C), 159.52 (Ar C), 159.55 (Ar C), 167.85 (C=O).
1-(2,3-dimethyl-N-propylbutanamide)-4-(N-methylene-1,6-dideoxy-1,6-imino-D-glucitol)-1,2,3-triazole (4.30d’).

The title compound was prepared from 4.30d according to general method 4 and isolated as pale yellow oil (12 mg, 0.03 mmol, ~100%). $^1$H NMR (400 MHz, D$_2$O) $\delta$ 0.64 (d, $^3$J$_{CH-CH} = 6.4$ Hz, 3H, sec-butyl CH$_3$), 0.70 (t, $^3$J$_{CH-CH} = 6.4$ Hz, 3H, sec-butyl CH$_3$), 1.33–1.42 (m, 2H, n-Pr CH$_2$), 2.06–2.16 (piperidine H$_1$ and H$_3$), 2.37–2.47 (sec-butyl CH), 2.93 (dd, $^2$J$_{\cdot-1}$ = 11.6 Hz, $^3$J$_{\cdot-2}$ = 4.8 Hz, 1H, piperidine H$_1$), 3.02–3.12 (m, 3H, n-pr CH$_2$, piperidine H$_3$), 3.32–3.37 (m, 1H, piperidine H$_4$), 3.42–3.48 (m, piperidine H$_2$), 3.82–3.87 (m, 1H, piperidine H$_6$), 3.96–4.07 (m, 3H, NCH$_2$, piperidine H$_6$’), 4.43 (d, $^3$J$_{CH-CH} = 10.4$ Hz, 1H, sec-butyl CH), 8.09 (s, 1H, triazole CH); $^{13}$C {$^1$H} NMR (100 MHz, D$_2$O) $\delta$ 10.74 (n-Pr CH$_3$), 17.99 (sec-butyl CH$_3$), 18.38 (sec- butyl CH$_3$), 21.75 (n-Pr CH$_2$), 30.93 (sec-butyl CH), 41.49 (n-Pr CH$_2$), 46.15 (NCH$_2$), 55.27 (piperidine C$_1$), 56.72 (piperidine C$_6$), 64.37 (piperidine C$_5$), 68.46 (piperidine C$_2$), 69.50 (piperidine C$_4$), 70.76 (sec- butyl CH), 77.91 (piperidine C$_3$), 125.18 (triazole CH), 140.56 (triazole C), 169.49 (C=O). HRMS (ESI) calcd for C$_{17}$H$_{32}$N$_5$O$_5$: 386.239794. Found: 386.241521.
The title compound was prepared according to general procedure 1 and isolated as clear oil (213 mg, 0.34 mmol, 75%). $R_f$ 0.21 (1:4 CH$_2$Cl$_2$–EtOAc). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.72 (d, $^3$$J_{CH-CH} = 6.4$ Hz, 3H, sec-butyl CH$_3$), 0.86 (t, $^3$$J_{CH-CH} = 7.2$ Hz, 3H n-Pr CH$_3$), 1.02 (d, $^3$$J_{CH-CH} = 6.4$ Hz, 3H, sec- butyl CH$_3$), 1.50 (pentet, $^3$$J_{CH-CH} = 7.2$ Hz, 2H, n-Pr CH$_2$), 2.45–2.55 (m, 1H, sec-butyl CH), 2.72 (dd, $^2$$J_{J_{1,1'}} = 12.0$ Hz, $^3$$J_{1,2} = 8.0$ Hz, 1H, H$_1$), 2.93 (d, $^3$$J_{1',2} = 8.0$ Hz, H$_1$), 3.11–3.22 (m, 2H, n-Pr CH$_2$), 3.50–3.64 (m, 2H, azepane H$_3$), 3.75 (s, 6H, PhOCH$_3$), 3.79–3.86 (m, 2H, azepane H$_2$), 3.89–3.96 (m, 2H, NCH$_2$), 4.57 (AB q, $^2$$J_{AB} = 10.8$ Hz, 4H, CH$_2$OPh), 4.85 (d, $^3$$J_{CH-CH} = 10.0$ Hz, 1H, sec-butyl CH), 7.00 (m, 8H, Ar), 7.25 (br s, CONH), 7.92 (s, 1H, triazole CH); $^{13}$C ($^1$H) NMR (100 MHz, CDCl$_3$) $\delta$ 11.57 (n-Pr CH$_3$), 18.95 (sec-butyl CH$_3$), 19.51 (sec-butyl CH$_3$), 22.67 (n-Pr CH$_2$), 32.51 (sec-butyl CH), 41.71 (n-Pr CH$_2$), 54.20 (NCH$_2$), 55.47 (PhOCH$_3$), 57.57 (azeape C$_1$), 68.64 (azeape C$_2$), 71.10 (aCH), 73.52 (OCH$_2$Ph), 85.89 (azeape C$_3$), 114.13 (Ar CH), 123.72 (triazole CH), 129.79 (Ar CH), 130.31 (Ar C), 143.83 (triazole C), 159.57 (Ar C), 167.89 (C=O).
1-(2,3-dimethyl-N-propylbutanamide)-4-(N-methylene-2,3,4,5-tetra-O-acetyl-1,6-dideoxy-1,6-imino-1-iditol)-1,2,3-triazole (4.32d).

The title compound was prepared according to the general procedure 1 and isolated as clear oil (89 mg, 0.16 mmol, ~100%). \( R_f \) 0.31 (2:3 EtOAc–hexanes); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 2.01 (s, 6H, OAc \( \text{CH}_3 \)), 2.00 (s, 6H, OAc \( \text{CH}_3 \)), 2.97 (pentet, \( ^3 J_{\text{CH-CH}} = 6.8 \) Hz, 2H, \( \text{CH}_2 \)), 2.78 (br s, 2H, \( \text{H}_1 \)), 2.97-3.00 (m, 2H, \( \text{H}_1 \)), 3.23 (s, 3H, NCH\(_3\)), 3.24 (s, 3H, NCH\(_3\)), 3.48 (dt, \( ^3 J_{\text{CH-CH}} = 13.6 \) Hz, \( ^3 J_{\text{CH-CH}} = 6.8 \) Hz, 2H, \( \text{CH}_2 \)), 3.86 (br s, 2H, NCH\(_2\)), 4.38-5.11 (m, 2H, \( \text{CH}_2 \)), 4.96-5.03 (m, 2H, azepane \( \text{H}_2 \)), 5.22-5.27 (m, 2H, azepane \( \text{H}_3 \)), 7.66 (br s, 1H, triazole \( \text{CH} \)), 8.14 (d, 1H, \( ^3 J_{\text{CH-NH}} = 14.0 \) Hz, 1H C=CH), 10.24-10.28 (m, 1H, C=CH\( \text{NH} \)).

1-(2,3-dimethyl-N-propylbutanamide)-4-(N-methylene-1,6-dideoxy-1,6-imino-1-iditol)-1,2,3-triazole (4.32d').

The title compound was prepared from 4.32d according to general procedure 4 and isolated as off-white foam (22 mg, 0.06 mmol, ~100%). \(^1\)H NMR (400 MHz, D\(_2\)O) \( \delta \) 0.64 (d, \( ^3 J_{\text{CH-CH}} = 6.4 \) Hz, 3H, sec-Bu \( \text{CH}_3 \)), 0.70 (t, \( ^3 J_{\text{CH-CH}} = 7.6 \) Hz, 3H, \( n-\text{Pr} \) \( \text{CH}_3 \)), 0.91 (d, \( ^3 J_{\text{CH-CH}} = 6.8 \) Hz, 3H, sec-butyl \( \text{CH}_3 \)), 1.37 (sextet, \( ^3 J_{\text{CH-CH}} = 7.2 \) Hz, 2H, \( n-\text{Pr} \) \( \text{CH}_2 \)), 2.37–2.46 (m, 2H, sec-Bu \( \text{CH} \)), 2.57 (dd, \( ^2 J_{1.1'} = 13.2 \) Hz, \( ^3 J_{1.2} = 7.6 \) Hz, 2H, azepane \( \text{H}_1 \)), 2.80 (dd, \( ^2 J_{1.1'} = 13.6 \) Hz, \( ^3 J_{1.2} = 3.6 \) Hz, 2H, azepane \( \text{H}_1 \)), 2.98–3.12 (m, 2H, \( n-\text{Pr} \) \( \text{CH}_2 \)), 3.33 (dd, \( ^3 J_{2.3} = 13.2 \) Hz, 2H, azepane \( \text{H}_1 \)).
5.6 Hz, $^3J_{3-4} = 1.6$ Hz, 2H, azepane H$_3$, 3.54–3.60 (m, 2H, azepane H$_2$), 3.82 (br s, NCH$_2$), 4.78 (d, $^3J_{CH-CH} = 10.8$ Hz, 1H, sec-butyl CH), 8.09 (s, 1H, triazole CH); $^{13}$C NMR (100 MHz, D$_2$O) δ 10.73 (n-Pr CH$_3$), 17.98 (sec-butyl CH$_3$), 18.38 (sec-butyl CH$_3$), 21.75 (n-Pr CH$_2$), 30.98 (sec-butyl CH), 41.50 (n-Pr CH$_2$), 52.73 (NCH$_2$), 57.90 (azepane C$_1$), 70.74 (sec-butyl CH), 70.87 (azepane C$_2$), 75.70 (azepane C$_3$), 124.70 (triazole CH), 142.58 (triazole C), 169.51 (C=O). HRMS (ESI) calcd for C$_{17}$H$_{32}$N$_5$O$_5$: 386.239794. Found: 386.239233.

### 4.5 References


Chapter Four


Chapter Four


59. For structure activity relationships concerning ER-glucosidase I inhibition, see references 7a and 27, and references cited therein.


5 Concluding comments and future directions

5.1 General
Since its simultaneous disclosure in 2002, the Cu\(^1\)-catalysed cycloaddition of an organic azide to a terminal acetylene has emerged as a powerful synthetic transformation, displaying broad scope and versatility in synthetic organic chemistry, chemical biology and combinatorial chemistry and high-throughput screening. Whilst the reaction has shown impressive utility to these disciplines, its full potential in traditional carbohydrate chemistry, glycobiology and carbohydrate-based drug discovery is yet to be realised. The limited applications presented thus far have prompted the research within this thesis. Importantly, the work has successfully demonstrated the utility of the reaction as a legitimate, orthogonal transformation in traditional carbohydrate chemistry and also as tool for the expedient modular synthesis of heterocyclic glycoconjugates as potential biochemical probes and drug leads.

5.2 Chapter two
Chapter two explored the potential of the Cu\(^1\)-catalysed cycloaddition reaction as a legitimate orthogonal transformation in synthetic carbohydrate chemistry. Owing to the mild, ambient nature and remarkable regio- and stereoselectivity, the reaction is amenable to the synthesis of mimetics of complex oligosaccharides and glycoconjugates. The reaction was therefore interrogated under conditions typically encountered in synthetic carbohydrate chemistry such as protective group manipulations and glycosylation reactions. The triazole moiety was investigated for
its stability under these conditions in order to assess the compatibility of the transformation and the triazole moiety to carbohydrate chemistry.

The first aspect of this study investigated the effect of solvent and catalyst on yield and rate of triazole formation. The study established that the chemistry is equally applicable to either aqueous or organic solvents using inexpensive and commercially available sources of Cu$^1$. The formation of the model β-D-glucosyl triazole 2.2 proceeded smoothly in all cases in good yields (73–95%) although the rate of formation showed was dependent on solvent and catalyst. In all cases, the reaction was shown to be regiospecific for the 1,4-disubstituted regioisomer and is in good agreement with observations using non-carbohydrate substrates previously reported in the literature. Importantly, the study demonstrated the feasibility of the click chemistry methodology as a powerful and robust synthetic strategy for expedient access to glycosyl triazoles acting as stable $N$-linked glycomimetics. Using the click chemistry methodology, our interests are now directed toward the preparation of heterocyclic-linked, 1,4-disubstituted glycosyl triazoles (e.g the 2-pyridyl substituted β-D-glucosyl 1,2,3-triazole 2.12) as potential donors for one-pot glycosylation reactions from stable glycosyl azide precursors.

The second aspect of the study aimed to demonstrate the compatibility of the reaction and the triazole moiety with conventional carbohydrate protective group chemistry. The triazole moiety could be installed in the presence of a wide range of important and well-established protecting groups, including acetates, benzoates, and benzyl, silyl and trityl ethers. It is shown that these protective groups could be easily introduced and removed in the presence of the triazole moiety, demonstrating the
true orthogonal capability of the reaction. In addition, the triazole moiety was shown to be stable towards strong nucleophiles such as azide; further augmenting the scope and utility of the chemistry in carbohydrate synthesis.

Finally, the work demonstrates the powerful utility of the reaction in generating a wide range of structurally diverse neoglycoconjugates. Using the click chemistry methodology, various commercially available and synthetically accessible acetylenes were efficiently grafted onto the carbohydrate scaffold in high yields. Several compounds are sufficiently insoluble in the aqueous media to facilitate purification by precipitation, but are equally amenable to purification by conventional liquid-liquid extraction and chromatographic purification. In general, the reaction is insensitive to the nature of the glycosyl azide configuration or the physicochemical and electronic properties of the acetylene dipolarophile. An interesting result is the synthesis of the benzylated α-D-glucosyl triazole 2.19 which required a longer reaction time at elevated temperature. In all cases, product purification was minimal, requiring either precipitation or solid-phase extraction. An impressive example included the cycloaddition of ethynyl functionalised steroids, 17α-ethynyl estradiol and ethisterone to tetra-O-acetyl-β-D-glucopyranosyl azide in tert-butanol–water (1:1 v/v) to form the triazole linked steroidal glycoconjugates 2.14 and 2.15, respectively in good yields (71 and 64 %, respectively). The work demonstrates the scope of the chemistry for generating molecular diversity using carbohydrate scaffolds, and thus the potential in a combinatorial and drug design setting.
5.3 Chapter 3

Chapter three was an exhaustive investigation into the application of the click methodology for synthesising libraries of glycoconjugate benzene sulfonamides as novel inhibitors of hCA I, II, IX, XII and XIV. Despite the numerous benefits associated with tethering carbohydrates to sulfonamide based CA inhibitors, only one example has been reported in the literature prior to this work. The carbohydrate moiety possesses a high chiral density, polarity and water solubility thus facilitating our dual isozyme differentiating solubilising strategy for a wide range of potential in vivo applications. The study therefore aimed to generate glycoconjugate benzene sulfonamides in order to develop CA inhibitors which show improved water solubility for tissue-selective delivery applications, an impaired membrane permeability allowing for the selective targeting of tumour associated isozymes hCA IX and XII with extracellular catalytic domains, and finally to probe the catalytic site architectures for the development of isozyme selective CA inhibitors.

Using a novel “click-tailing” fragment based design strategy, carbohydrate building blocks of variable ring size and conformation were efficiently conjugated to benzene sulfonamide pharmacophore scaffolds and evaluated as inhibitors of cytosolic isozymes hCA I and II, and transmembrane hCA IX, XII and XIV in vitro. This work constitutes the first comprehensive investigation into the inhibition of CA and the first example of the inhibition of tumour associated isozymes hCA IX and XII as well as the relatively unknown hCA XIV using glycoconjugate benzene sulfonamides as a novel class of CA inhibitor.
The non-clustered in vitro inhibition data strongly reflects the ability of the carbohydrate tail to impart isozyme selectivity, a significant outcome for the development of safe CA therapies void of systemic side effects arising from indiscriminate CA inhibition. Importantly, the study identified a potent and selective hCA IX inhibitor 3.11e’ (K_i 23 nM). In addition to the strong in vitro potency, 3.11e’ displayed strong selectivity for the hCA IX isozyme and is approximately 16-fold more selective cf. hCA II than indisulam 3.8 which is in phase II clinical trials for the treatment of solid tumours. The β-D-ribofuranosyl configured triazoles of library II and III, namely the deprotected triol analogues 3.13h’ and 3.14m’, were identified as potent and selective inhibitors of hCA II (K_i 7.5 nM and 4.2 nM, respectively). Collectively, these compounds constitute important leads for the development of safe and well-tolerated CA-based therapeutics. Research efforts are ongoing into establishing structure activity relationships and investigating other chemical methods for efficiently generating glycoconjugate benzene sulfonamides as potent and selective CA inhibitors.

The modular synthesis of heterocyclic glycoconjugates can be applied in variety of medicinal chemistry settings, not merely associated with the inhibition of carbohydrate processing enzymes or recognising proteins. This inhibition of CA, itself a non-carbohydrate recognising protein, with triazole linked glycoconjugate benzene sulfonamides demonstrates the potential of click chemistry as a tool for fragment based drug discovery using carbohydrate scaffolds.
Chapter four of this thesis explored the application of click chemistry in preparing \( N \)-alkylated azasugar libraries as novel glycosidase and glycosyltransferase inhibitors. \( N \)-Alkylated azasugars exhibit a broad range of biological activity and the chemical modification of the \( N \)-alkyl substituent within the azasugar scaffold can have a profound effect on inhibition potency and selectivity, as well as cytotoxicity and solubility. This study successfully demonstrates the utility of the click chemistry reaction as a powerful synthetic tool for the chemical elaboration of azasugar scaffolds that may be applied to lead discovery and optimisation. Using the click chemistry methodology, chemically diverse azide building blocks were grafted onto six and seven membered ring azasugar scaffolds to generate respective \( N \)-alkylated azasugar libraries. Importantly, the chemistry allowed for the direct functionalisation of the azasugar scaffold with a diverse range of azide building blocks, thereby facilitating the synthesis of enzyme-selective inhibitors and improving drug tolerance and pharmacokinetic profiles, in a single, high yielding step.

This work represents the first application of click chemistry for the generation of \( N \)-alkylated azasugar libraries. A significant aspect of the work involved the generation of a novel \( C_2 \)-symmetric, \( N \)-alkylated polyhydroxylated azepane library. These compounds possess interesting biological properties and constitute a relatively unexplored class of glycosidase inhibitor. As such, the click chemistry methodology serves as a powerful chemical approach for defining much needed structure activity relationships. Using the reaction, the inhibition and physicochemical properties of the \( N \)-alkylated azasugar can be fine-tuned to suit a design strategy and is a powerful tool for lead discovery and optimisation. Future work would be directed toward
acquiring inhibition data and also applying the methodology into microtitre format
for the rapid parallel synthesis of $N$-alkylated azasugar libraries.