CHEMICAL SYNTHESIS OF HEPARAN SULFATE OLIGOSACCHARIDES FOR USE IN SINGLE MOLECULE FLUORESCENCE ANALYSIS

A thesis submitted to The University of Manchester for the degree of Doctor of Philosophy in the Faculty of Engineering and Physical Sciences

2016

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SCHOOL OF CHEMISTRY
LIST OF CONTENTS

LIST OF FIGURES ........................................................................................................... 6
LIST OF TABLES .............................................................................................................. 7
LIST OF ABBREVIATIONS .............................................................................................. 8
ABSTRACT .......................................................................................................................... 12
DECLARATION .................................................................................................................... 12
COPYRIGHT STATEMENT ................................................................................................. 13
ACKNOWLEDGEMENTS .................................................................................................... 14

1. OUTLINE AND AIMS OF PROJECT ........................................................................... 15

2. BIOLOGY OF HEPARAN SULFATE ............................................................................ 19
   2.1 Heparan Sulfate Proteoglycans (HSPGs) ................................................................. 19
   2.2 Biosynthesis of HS .................................................................................................... 20
   2.3 Importance of HSPGs in Development and in Healthy Tissue ............................... 22
   2.4 Heparan Sulfate in Cancers ..................................................................................... 22
   2.5 Heparan Sulfate in HIV Infection ............................................................................ 23
   2.6 Heparan Sulfate in Alzheimer’s Disease ................................................................. 25

3. SYNTHESIS OF HS OLIGOSACCHARIDES ................................................................. 27
   3.1 Digestion of Native HS/heparin ................................................................................ 27
   3.2 Chemoenzymatic Synthesis of HS Oligosaccharides .............................................. 28
   3.3 Chemical Synthesis of HS Oligosaccharides ............................................................ 30
      3.3.1 Synthesis of L-Ido Building Blocks ................................................................ 30
      3.3.2 Modular Synthesis of HS Oligosaccharides ...................................................... 36
   3.4 Functionalisation/Labelling of Synthetic HS Oligosaccharides .............................. 43

4. ANALYSIS OF HS-PROTEIN INTERACTIONS ........................................................... 47
   4.1 Factors of Interest in HS-Protein Binding ............................................................... 47
   4.2 NMR for Investigation of HS-Protein Binding ......................................................... 48
   4.3 X-Ray Crystallography for Investigation of HS-Protein Binding ............................ 49

5. SINGLE MOLECULE FLUORESCENCE .................................................................... 51
   5.1 Outline of Single Molecule FRET ......................................................................... 51
   5.2 Use of smFRET for Study of Biological Interactions .............................................. 53

6. FLUORESCENT LABELLING METHOD FOR HS OLIGOSACCHARIDES ... 55
   6.1 Fluorescent Labelling via Aldehyde Tag .................................................................. 55
      6.1.1 Synthesis of HS Disaccharide with Aldehyde Tag ............................................ 55
      6.1.2 Attempted Labelling with Aldehyde-reactive Dye .......................................... 58
6.1.3 Attempted Labelling via Reductive Amination ........................................... 59
6.2 Fluorescent Labelling via Amine Tag ............................................................ 62
6.2.1 Attachment of Amine Tag to HS Disaccharide ........................................... 62
6.2.2 Alternative Synthesis of Amine-tagged Disaccharide ............................... 63
6.2.2.1 Synthesis of Glucoazide Donor .......................................................... 63
6.2.2.2 Attempted Synthesis using Mono-protected Amine Tag ....................... 65
6.2.2.3 Synthesis of Disaccharide using Doubly-protected Amine Tag ............... 67
6.2.3 Conjugation of Fluorophore to Amine-tagged Disaccharide ...................... 69

7. SINGLE MOLECULE FLUORESCENCE MEASUREMENTS OF LABELLED HS DISACCHARIDE .................................................................................................................. 74
7.1 Solution Measurements of Fluorescent Disaccharide ..................................... 74
7.2 TIRF Microscopy of Vesicle-encapsulated Fluorescent HS Disaccharide ...... 78
7.3 FCS of Fluorescently-labelled HS Disaccharide with FGF-1 ......................... 82

8. METHOD FOR 13C LABELLING OF HS OLIGOSACCHARIDES ...................... 85
8.1 Current Methods to Access 13C Labelled HS ............................................... 85
8.2 Synthesis of 13C Labelled HS Disaccharide ............................................... 85

9. ORTHOGONAL THIOGLYCOSIDE-BASED GLYCOSYLATION STRATEGY FOR HS OLIGOSACCHARIDE SYNTHESIS ................................................................. 89
9.1 Thioglycosides in Orthogonal Glycosylation ................................................. 89
9.2 Comparison to Current Glycosylation Strategies .......................................... 93
9.3 Synthesis of Glucoazide SBox and STaz Derivatives .................................... 94
9.4 Synthesis of Iduronate SBox Glycoside ...................................................... 95
9.4.1 Attempted Synthesis from Methyl Glycosides ........................................... 95
9.4.2 Synthesis from Glycosyl Acetate ............................................................. 96
9.5 Orthogonal Glycosylations to form HS Disaccharides ................................... 98
9.5.1 Glucoazide SBox Donors with Iduronate SPh Acceptor ............................ 98
9.5.2 Glucoazide STaz Donors with Iduronate SPh Acceptor ............................ 99
9.5.3 Glucoazide STaz Donors with Iduronate SBox Acceptor ......................... 100
9.5.4 Glucoazide SPh Donors with Iduronate SBox Acceptor .......................... 101
9.5.5 Iduronate SBox Donor with Glucoazide SPh Acceptor ........................... 102
9.6 Synthesis of Glucoazide SBox Derivatives from D-Glucosamine .................. 103

10. CHEMOSELECTIVE GLYCOSYLATION STRATEGY FOR HS OLIGOSACCHARIDE SYNTHESIS ................................................................................................. 105
10.1 Synthetic Strategy .......................................................................................... 105
10.2 Synthesis of Monosaccharide Building Blocks ........................................ 109
10.2.1 Synthesis of L-Ido Lactone ............................................................... 109
10.2.2 Synthesis of Glucoazide Donors ....................................................... 109
10.3 Synthesis of 4-para-Methoxybenzyl HS Disaccharides ......................... 111
10.3.1 Selective Activation of Glucoazide SPh Donors over Lactone ............. 111
10.3.2 Activation of Glucoazide Trichloroacetimidates over Lactone .......... 113
10.3.3 Methanolysis of L-Iduronic Lactone with Et₃N/MeOH ....................... 114
10.4 Chemoselective Glycosylation Strategy with Non-reducing End Cap .... 115
10.4.1 Synthesis of Tri-benzylated Donor Disaccharide ............................... 115
10.4.2 Synthesis of Lactone-containing Acceptor Disaccharide .................. 117
10.4.3 Synthesis of Tetrasaccharide via Chemoselective Glycosylation ....... 118
11. CONCLUSIONS ...................................................................................... 121
12. FURTHER WORK .................................................................................. 124
13. EXPERIMENTAL .................................................................................. 125
13.1 Synthesis ............................................................................................. 125
13.1.1 General Experimental Detail for Synthesis ........................................ 125
13.1.2 Synthesis of Disaccharide with Aldehyde Tag 135 ............................. 126
13.1.3 Attempted Conjugations to Disaccharide Aldehyde Tag ................... 134
13.1.4 Synthesis of Amine-tagged Disaccharide 153 .................................... 136
13.1.5 Synthesis of Glucoazide Donor 162 from D-Glucosamine .............. 142
13.1.6 Attachment of Mono-protected Amine Tag to 166 ......................... 148
13.1.7 Synthesis of Disaccharides with Doubly-protected Amine Tag ........ 150
13.1.8 Labelling of Disaccharide 153 with Alexa Fluor 488 SDP Ester .......... 157
13.1.9 Synthesis of 13C Labelled Disaccharide ......................................... 158
13.1.10 Synthesis of Glucosamine Trichloroacetimidates 219, 228–330 ........ 165
13.1.11 Glucoazide SBox Derivatives from Trichloroacetimidates ............. 172
13.1.12 Glucoazide STaz Derivatives from Trichloroacetimidates .............. 176
13.1.13 Synthesis of Iduronate Methyl Glycosides .................................... 179
13.1.14 Iduronate SBox Glycoside from Glycosyl Acetate 249 ................... 181
13.1.15 Synthesis of Disaccharides using Glucoazide SBox/STaz Donors ..... 186
13.1.16 Preparation of 4-Hydroxyl Disaccharide 220 ................................. 190
13.1.17 Synthesis of Glucoazide Donor 258 .............................................. 192
13.1.18 Synthesis of Glucoazide SBox Derivatives from D-Glucosamine ..... 194
13.1.19 Synthesis of L-Ido Lactone ............................................................. 196
13.1.20 Synthesis of 4-OPMB Lactone-containing Disaccharide 271 .......... 199
13.1.21 Synthesis of Glucoazide Donors 281, 284 and 286 ......................... 201
13.1.22 Synthesis of Lactone-containing Disaccharides 287, 289 and 290 .. 205
13.1.23 Preparation of Trichloroacetimidate Donor 292 .......................... 209
13.1.24 Hydrolysis of 4-OTBDMS Lactone 294 with Et₃N/MeOH ............. 211
13.1.25 Synthesis of Donor Disaccharide 300 ........................................ 213
13.1.26 Synthesis of Acceptor 301 and 2-OAc Tetrasaccharide 303 .......... 217
13.1.27 Synthesis of Tetrasaccharide 304 from Donor Disaccharide 254 ...... 220
13.2 Fluorescence Measurements ................................................................ 222
13.2.1 Steady-state Ensemble Spectroscopy .............................................. 222
13.2.2 Fluorescence Correlation Spectroscopy .......................................... 223
13.2.3 Multi-parameter Confocal Fluorescence Spectroscopy ....................... 223
13.2.4 Encapsulation of Disaccharide in Lipid Vesicles ............................... 224
13.2.5 TIRF Microscopy ............................................................................. 225
14. REFERENCES ......................................................................................... 227

Word count 68,297
LIST OF FIGURES

Figure 1. NS and NA domains of heparan sulfate ............................................................... 16
Figure 2. Schematic of inter- and intramolecular FRET ....................................................... 17
Figure 3. Structure of syndecans and glypicans ................................................................. 19
Figure 4. Biosynthesis of HS from heparosan precursor ....................................................... 21
Figure 5. Mechanism of CD4 peptide mimic-HS dodecasaccharide conjugate blocking HIV cell entry .................................................................................................................. 25
Figure 6. $^{1}C_{4}$, $^{4}C_{1}$ and $^{2}S_{0}$ conformations of 2-O-sulfated IdoA residues ............... 47
Figure 7. Jablonski diagram depicting the FRET process ..................................................... 51
Figure 8. Comparison of ensemble and single molecule methods for the analysis of a heterogeneous static or dynamic molecular population ................................................. 52
Figure 9. $^{1}H$ NMR of labelled/unlabelled disaccharide $178/153$ mixture and unlabelled disaccharide starting material $153$ ........................................................................... 71
Figure 10. Ensemble absorption spectra of: free dye, disaccharide $178$ and Alexa Fluor 488 labelled ssDNA ........................................................................................................... 74
Figure 11. Normalised fluorescence emission spectra of: free dye, disaccharide $178$ and Alexa Fluor 488 labelled ssDNA ...................................................................................... 75
Figure 12. Fluorescence correlation spectra of disaccharide $178$ and free dye .............. 76
Figure 13. 2D histogram plot of fluorescence lifetime ($\tau$) vs ratio of signal in the green detection channel ($S_G$) to that in the red detection channel ($S_R$) and the fluorescence anisotropy ($r$). ........................................................................................................ 78
Figure 14. Schematic of immobilisation of vesicle-encapsulated disaccharide $178$ .... 79
Figure 15. Representative TIRF images of immobilised vesicle-encapsulated disaccharide $178$ at varying concentrations .................................................................................... 80
Figure 16. Representative 1-step, 2-step and 3-step photobleaching traces for vesicle-encapsulated disaccharide $178$ (500 nM) obtained using TIRF microscopy .......... 81
Figure 17. Representative photobleaching traces for vesicle-encapsulated disaccharide $178$ (10 $\mu$M) obtained using TIRF microscopy ......................................................... 81
Figure 18. Example of FCS curve when two fluorescent diffusing species are present, one with diffusion coefficient $10^{-5}$ cm$^2$ s$^{-1}$ and one with diffusion coefficient $10^{-7}$ cm$^2$ s$^{-1}$ ................................................................................................................................. 82
Figure 19. (a) Compounds locked into the same conformation as lactone $270$ ($B_{2,5}$) that function as glycosyl donors; (b) Conformationally-locked uronic acids that act as glycosyl donors ........................................................................................................... 107
LIST OF TABLES

Table 1. Comparison of H-1 NMR signals for $\alpha$ and $\beta$ anomers of iduronate monosaccharides synthesised within the Gardiner group .............................. 66

Table 2. Diffusion times obtained for disaccharide 178 in the presence of unlabelled FGF-1 by FCS. .................................................................................................................. 83

Table 3. Dissociation constants ($K_d$) for disaccharides 182–185 determined by Hung et al. for FGF-1 and FGF-2 by ITC ................................................................. 84

Table 4. Glycosylations of glucoazide STaz donors with iduronate SPh acceptor. .... 100

Table 5. Glycosylations of glucoazide STaz donors with iduronate SBox acceptor... 101

Table 6. Attempted glycosylations of iduronate SBox donor with glucoazide SPh acceptors. .................................................................................................................. 103

Table 7. Benzylaion of O-6 of 283. .................................................................................. 111

Table 8. Glycosylation of 3-OBn, 4-OPMB glucoazide donors with lactone 270 ..... 112

Table 9. Glycosylation of 3-OBz, 4-OPMB glucoazide donors with lactone 270 ..... 113

Table 10. Glycosylation of 2-OAc disaccharide donor 300 with lactone-containing disaccharide 301 ........................................................................................................... 119
### LIST OF ABBREVIATIONS

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>1D</td>
<td>One dimensional</td>
</tr>
<tr>
<td>2D</td>
<td>Two dimensional</td>
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<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>a.u.</td>
<td>Arbitrary units</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
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<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
<tr>
<td>APP</td>
<td>Amyloid precursor protein</td>
</tr>
<tr>
<td>aq.</td>
<td>Aqueous</td>
</tr>
<tr>
<td>Ar</td>
<td>Aromatic (any aromatic group)</td>
</tr>
<tr>
<td>AT III</td>
<td>Antithrombin III</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated total reflectance</td>
</tr>
<tr>
<td>Aβ</td>
<td>Amyloid beta</td>
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<tr>
<td>BACE1</td>
<td>Beta-secretase 1 (also known as memapsin-2, aspartyl protease 2/ASP2)</td>
</tr>
<tr>
<td>BAIB</td>
<td>(Diacetoxyiodo)benzene</td>
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<tr>
<td>BBN</td>
<td>Borabicyclo[3.3.1]nonane</td>
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<td>Biotinyl-PE</td>
<td>Sodium 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotinyl)</td>
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<td>Boc</td>
<td>tert-Butoxycarbonyl</td>
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<td>BSA</td>
<td>Bis(trimethylsilyl)-acetamide</td>
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<td>1-Benzenesulfonyl piperidine</td>
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<td>C</td>
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<td>CXC4</td>
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<td>DBU</td>
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<td>2,3-Dichloro-5,6-dicyano-1,4-benzoquinone</td>
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<td>DEA</td>
<td>Diethyamine</td>
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<tr>
<td>DEPT</td>
<td>Distortionless enhancement by polarisation transfer</td>
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<td>DIPC</td>
<td>N,N'-Diisopropylcarbodiimide</td>
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<tr>
<td>DIPEA</td>
<td>Diisopropylethylamine</td>
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<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
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<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
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<td>DMDO</td>
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<td>DMF</td>
<td>N,N-Dimethylformamide</td>
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</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>dsDNA</td>
<td>Double stranded deoxyribonucleic acid</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>Egg-PC</td>
<td>L-α-Phosphatidylcholine</td>
</tr>
<tr>
<td>EMCCD</td>
<td>Electron multiplying charge coupled device</td>
</tr>
<tr>
<td>ES(I)</td>
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<tr>
<td>EXT1</td>
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</tr>
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<td>EXT2</td>
<td>Exostosin-2</td>
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<td>------------</td>
</tr>
<tr>
<td>FCS</td>
<td>Fluorescence correlation spectroscopy</td>
</tr>
<tr>
<td>FGF</td>
<td>Fibroblast growth factor</td>
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<tr>
<td>FGFR</td>
<td>Fibroblast growth factor receptor</td>
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<td>Fmoc</td>
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<td>Hedgehog (<em>Drosophila</em> polypeptide)</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<tr>
<td>HMBC</td>
<td>Heteronuclear multiple bond coherence</td>
</tr>
<tr>
<td>HMQC</td>
<td>Heteronuclear multiple quantum coherence</td>
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<td>HPLC</td>
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<td>2-Mercaptobenzoxazole</td>
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<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
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<td>L-Iduronic acid</td>
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<td>Interleukin</td>
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<td>K&lt;sub&gt;d&lt;/sub&gt;</td>
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<td>LCMS</td>
<td>Liquid chromatography mass spectrometry</td>
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<td>NBS</td>
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<td>NDST</td>
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<td>NMR</td>
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<tr>
<td>NOE(SY)</td>
<td>Nuclear Overhauser effect (spectroscopy)</td>
</tr>
<tr>
<td>Norm.</td>
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<td>para-Methoxyphenyl</td>
</tr>
<tr>
<td>Q-TOF</td>
<td>Quadrupole-time of flight</td>
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<tr>
<td>quant.</td>
<td>Quantitative</td>
</tr>
<tr>
<td>RRV</td>
<td>Relative reactivity value</td>
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<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>S/N</td>
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<tr>
<td>SDF1α</td>
<td>Stromal cell-derived factor 1 (also known as CXCL12)</td>
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<td>Single photon counting module</td>
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<td>Surface plasmon resonance</td>
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<td>Single stranded deoxyribonucleic acid</td>
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<tr>
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<td>S-Thiazolyl</td>
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<tr>
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<td>Tetrabutylammonium fluoride</td>
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<td>tert-Butyldiphenylsilyl</td>
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<td>Trifluoroacetic acid</td>
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<td>THF</td>
<td>Tetrahydrofuran</td>
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<td>Total internal reflection</td>
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<tr>
<td>TIRF</td>
<td>Total internal reflection fluorescence</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>Thin layer chromatography</td>
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<td>Ultraviolet-visible</td>
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<td>Wingless (<em>Drosophila</em> polypeptide)</td>
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<tr>
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<td>Wingless-related integration site</td>
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<tr>
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<td>Weight percentage</td>
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<td>d-Xylose</td>
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<tr>
<td>Z</td>
<td>Carboxybenzyl (Cbz)</td>
</tr>
<tr>
<td>ZMW</td>
<td>Zero mode waveguide</td>
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ABSTRACT

Chemical synthesis of heparan sulfate oligosaccharides for use in single molecule fluorescence analysis

Charlotte Dalton
The University of Manchester
Doctor of Philosophy
15/07/2016

Heparan sulfate (HS) is a cell-surface sulfated polysaccharide that binds to multiple proteins and has been implicated in cancer, viral infection and Alzheimer’s disease. Due to the heterogeneity of HS, the structural requirements for protein binding are ill-defined. Chemical synthesis of structurally-defined HS oligosaccharides, which are tunable in terms of length, order of monosaccharides and sulfation pattern, is required for the investigation of HS-protein binding.

Single molecule methods have been utilised in biophysics to study dynamic processes and can allow observation of rare events which would be ‘averaged out’ in ensemble measurements. Access to fluorescently labelled HS oligosaccharides should allow investigation of interactions with proteins at the single molecule level using methods such as single molecule FRET, providing a method complementary to NMR studies (ensemble) and X-ray crystallography (non-dynamic).

This thesis presents the development of a method for the fluorescent labelling of a chemically synthesised HS disaccharide utilising a reducing-end amine tag. Analysis of the fluorescence properties of the labelled disaccharide at ensemble and single molecule level indicated no perturbation of the fluorophore when attached to the sugar. Fluorescence correlation spectroscopy measurements of the fluorescent HS disaccharide with the protein FGF-1 showed no binding, which is attributed to the low concentration (1 nM) of disaccharide required in the experiment.

Additional work is presented in this thesis on the development of a method for atom-specific $^{13}$C labelling of HS oligosaccharides, which has been initiated by synthesis of a $^{13}$C labelled L-iduronate monosaccharide and a $^{13}$C labelled disaccharide. New strategies for the synthesis of HS oligosaccharides based on orthogonal thioglycoside-based glycosylations employing S-benzoazolyl and S-thiazolyl donors have been investigated. Development of a chemoselective glycosylation strategy for HS oligosaccharide synthesis utilising a ‘super-disarmed’ [2.2.2] L-iduronic lactone is presented.
DECLARATION

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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ACKNOWLEDGEMENTS

I would like to thank the supervisors of this project, Dr John Gardiner and Dr Steven Magennis. Thank you to PDRAs Dr Gavin Miller (Manchester) and Dr Steven Quinn (Glasgow) for their invaluable assistance, and to Dr Michael Morton and Aiden Rafferty (Glasgow) for carrying out the vesicles work. Dr Matthew Cliff and Mrs Rehana Sung are thanked for their assistance in NMR data collection and LCMS respectively. The EPSRC National Mass Spectrometry Facility at Swansea University is thanked for mass spectroscopy measurements. The project was funded via a BBSRC DTP studentship and the President’s Doctoral Scholar Award from the University of Manchester.

Thank you to the members of the Gardiner group for being an endless source of entertainment (except for Jinesh who is just a source of admin work). In particular I would like to thank the summer students and MChem students I have supervised for all their hard work and dedication. Thanks to my friends in MIB and beyond, and to my family, for their unwavering support. Finally, last and most certainly least (way behind Jinesh) thanks to my ‘partner in crime’ Robin.
1. OUTLINE AND AIMS OF PROJECT

Saccharides are ubiquitous in living systems and are essential for a wide variety of biological processes. This is exemplified by the sulfated glycosaminoglycan (GAG) heparan sulfate (HS), a heterogeneous cell-surface/extracellular matrix polysaccharide which binds to a wide range of proteins including chemokines and growth factors.\textsuperscript{1,2} As a result of interaction with these proteins, HS facilitates a number of cell signalling pathways and has been implicated in a variety of conditions including cancer,\textsuperscript{3} HIV\textsuperscript{4} and Alzheimer’s disease.\textsuperscript{5}

Heparan sulfate consists of variably sulfated disaccharide units composed of either L-iduronic (IdoA) or D-glucuronic (GlcA) acid 1,4 linked to D-glucosamine (GlcN). The exact composition of HS varies depending on cell type and organism.\textsuperscript{6} In general, there are two main regions within the polysaccharide chain, known as the N-sulfated (NS) and N-acetylated (NA) domains, although there is overlap between these (Figure 1). The NA domains consist largely of D-GlcA linked to N-acetyl glucosamine (GlcNAc) with little sulfation present. The NS domains have a higher proportion of IdoA and increased sulfation, with the major disaccharide being 2-O-sulfated L-iduronic acid 1,4 linked to N-sulfated glucosamine (GlcNS). The related GAG heparin is mostly composed of this disaccharide, hence heparin has been used as a model for the NS domains of HS,\textsuperscript{5} although heparin typically has more glucosamine 6-O-sulfate groups than HS.\textsuperscript{5} NS domains are regarded as the functional part of HS for protein binding. This is attributed to two factors: firstly, increased sulfation which allows more electrostatic interactions between HS and proteins. Crucially, there is variation in the sulfation pattern, which may allow for specific protein-binding sequences. Of particular importance is glucosamine O-6, which exhibits variable sulfation, but rare modifications such as glucosamine O-3 sulfate have been shown to play a key role in the interaction of HS/heparin with some proteins e.g. antithrombin III.\textsuperscript{9} Secondly, NS domains are more flexible than the rest of the structure due to the presence of 2-O-sulfated L-iduronic acid, which is more conformationally flexible than D-glucosamine or D-glucuronic acid.\textsuperscript{10}
Knowledge of the interactions between proteins and HS is desired both to increase understanding of processes occurring during development and disease, and as a potential tool for carbohydrate drug discovery. Due to the structural heterogeneity of native HS, chemical or enzymatic digestion of heparin/HS is often used to provide simpler oligosaccharides for study. These oligosaccharides can be separated using electrophoresis or chromatography techniques and characterised using NMR or mass spectrometry. Digestion products of heparan sulfate have been utilised to study HS-protein interactions, but determination of the purity and structure of digest products is not trivial and development of new analytical methods for this is on-going. In contrast, chemical synthesis of structurally defined HS oligosaccharides allows complete control and design of the structure, in particular allowing specific sulfation patterns and sequence length. Therefore, chemically synthesised HS oligosaccharides are preferable to oligosaccharides from HS digestion for studies of HS-protein interactions at a molecular level. Despite the potential difficulties of achieving stereoselective formation of α-glycosidic linkages and synthesis of rare L-idomonomosaccharides, chemical syntheses of HS oligosaccharides have been developed by multiple research groups. Although currently available methods allow variation of sequence length and sulfation pattern, development of more efficient syntheses which allow access to a diverse range of target structures is still required.

The binding of HS oligosaccharides to proteins at a molecular level has been studied using a number of methods, including NMR, X-ray crystallography and molecular dynamics simulations. However, these methods each have limitations, and there is a requirement for additional methods that can be used to interrogate HS-protein interactions at a molecular level.
binding at a molecular level. Single molecule FRET (Förster resonance energy transfer) is a technique that can be used to investigate molecular conformation and/or binding interactions. In this method, a donor fluorophore is irradiated in the presence of an acceptor fluorophore (which may be on the same or a different molecule). If the distance between the two fluorophores is in the range 1–10 nm, energy is transferred non-radiatively (FRET) with an efficiency which is inversely proportional to the sixth power of the distance between the fluorophores (Figure 2). At single molecule level, this distance can be accurately measured and molecular dynamics simulations can be used to model an overall structure based on acquired distances. Performing single molecule measurements also allows observation of rare or transients states which would usually be ‘averaged out’. Although smFRET has been applied to proteins\textsuperscript{24} and DNA\textsuperscript{25} relatively widely, the only studies reported involving saccharides thus far have been based on super-resolution imaging of cells containing fluorescently labelled glycans\textsuperscript{26,27}. It is therefore proposed that smFRET could be utilised as a new method for study of interactions between HS oligosaccharides and biologically important proteins.

![Diagram of FRET](image)

**Figure 2.** Schematic of inter- and intramolecular FRET; fluorophores represented by star shapes, green = donor, red = acceptor.
The overall aim of this research project is to develop smFRET into a tool for analysis of HS-protein interactions. Therefore, the initial aim is to develop a protocol for attaching a fluorophore to chemically synthesised NS domain-like (heparin-like) GlcNS-IdoA HS oligosaccharides (hereafter referred to as HS oligosaccharides) using a HS disaccharide as a model. The protocol will be designed such that it could be applied to a chemically synthesised HS oligosaccharide of any length and/or sulfation pattern. The resulting fluorescent conjugate will be analysed using an MFD (multiparameter detection) system suitable for single molecule fluorescence measurements in order to determine if the labelled disaccharide is suitable for study with proteins. The labelling protocol will then be applied to longer oligosaccharides for further study. The research project will also aim to develop new synthetic methodology to increase the efficiency of HS oligosaccharide synthesis, specifically to apply orthogonal and/or chemoselective strategies to the synthesis of HS oligosaccharides.
2. BIOLOGY OF HEPARAN SULFATE

2.1 Heparan Sulfate Proteoglycans (HSPGs)

Endogenous HS is mainly found at the cell surface in the form of proteoglycans (heavily glycosylated proteins) which exist in two major forms, the transmembrane syndecans and the membrane-anchored glypicans (Figure 3). There are four known syndecans (Sdc1-Sdc4), two of which (Sdc1 and Sdc3) display the related GAG chondroitin sulfate (CS; containing galactosamine as opposed to glucosamine in HS) in addition to HS. There are six members of the glypican family (GPC1-GPC6), lack of GPC3 is believed to cause the overgrowth disease Simpson-Golabi-Behmel syndrome and lack of GPC6 the skeletal condition omodysplasia. Additionally, there are three other membrane-bound HSPGs found in certain tissues: betaglycan and neuropilin-1 (both of which are considered ‘part time’ HSPGs as they can exist without HS decoration, also they can be decorated with CS instead of/in addition to HS), and CD44v3 which is found on lymphocytes. The HSPGs agrin, perlecan and collagen XVIII are found in the extracellular matrix. HSPGs typically have between one and five HS chains attached to the core protein, and are able to interact with a wide range of ligands hundreds of nanometres from the cell membrane due to the flexibility of HS. HSPGs typically have a half-life of 4–24 hours before they are internalised and degraded by lysosomes; complete turnover of the HSPGs of a cell typically occurs every 48 hours.

Figure 3. Structure of syndecans and glypicans (not to scale).
2.2 Biosynthesis of HS

The structural heterogeneity in HS (and to a lesser extent heparin) arises from multiple incomplete transformations that occurring during its biosynthesis. Biosynthesis takes place in the Golgi apparatus, firstly a $\beta$-GlcA-(1,3)-$\beta$-Gal-(1,3)-$\beta$-Gal-(1,4)-$\beta$-Xyl tetrasaccharide linker is built up on a serine residue of the HSPG core protein. Subsequent addition of GlcNAc and GlcA monosaccharides, catalysed by a complex of the glycosyltransferases EXT1 and EXT2, $^{33}$ forms a linear 1,4-linked NA domain sequence known as heparosan, which is then modified by a number of enzymes (Figure 4). Firstly, GlcNAc $N$-deacetylase/$N$-sulfotransferase (NDST) catalyses removal of some $N$-acetyl groups and sulfation of the resulting amines to give the overall NS- and NA-domain structure to the HS chain. Although these steps are coupled and performed by a single polypeptide, unsubstituted amines have been found to exist in HS from some tissues. $^{34}$ This step is a prerequisite for the subsequent modifications, $^{35}$ hence there is very little elaboration of the remaining NA domains. Uronyl C-5 epimerase then acts to invert the stereochemistry at C-5 of some GlcA residues (those positioned between two GlcNS residues) $^{36}$ to give IdoA. This change of configuration is reversible, but sulfation at either O-2 of IdoA or O-6 of adjacent GlcNS residues prevents both the reverse and forward reactions. $^{37}$ The crystal structure of the enzyme complexed with heparin hexasaccharide indicates that the presence of these sulfate groups increases the distance between C-5 and the tyrosine residues essential for catalytic activity. $^{38}$ It should also be noted that this inversion at C-5 formally changes the designation of the uronic acid-glucosamine glycosidic bond from $\beta$ to $\alpha$ as C-5 is the anomic reference atom. $^{39}$ After the inversion step, 2-$O$-sulfotransferase (2-OST) can catalyse sulfation of O-2 on IdoA, and to a lesser extent GlcA residues (<5%). $^{40}$ This is followed by sulfation of O-6 on some GlcNS residues, and a small amount of GlcN O-3 sulfation (1% for endothelial cell HS), $^{41}$ resulting in the final heterogeneous, variably sulfated structure.
Figure 4. Biosynthesis of HS from heparan precursor.
2.3 Importance of HSPGs in Development and in Healthy Tissue

Due to the wide range of proteins that interact with HS, HSPGs play a number of important roles in healthy tissue. During development, a number of key signalling molecules control formation of complex structures and tissues, including the Wnt/Wingless (Wg), Hedgehog (Hh) and fibroblast growth factor (FGF) families. HSPGs are required for the proper function of these signalling pathways, although the roles played appear to be varied and in some cases are not fully known. HSPGs are believed to stabilise signalling molecules such as Wg and Hh (in *Drosophila*) and are also involved in the transport of such molecules through cells during development, and possibly the formation of signalling complexes in these pathways. The interaction of HSPGs with FGFs has been most widely studied; in the mutant *Drosophila* species *sugarless* (in which HS biosynthesis is impaired) and *sulfateless* (in which sulfation of HS is impaired) the FGF signalling pathways required for migration of tracheal and mesodermal cells are defective. Knockdown of the gene encoding for *Drosophila* 6-OST results in perturbed tracheal development indicating O-6 sulfation of HS is required in FGF signalling.

In mature tissue, HSPGs continue to play a role in cell proliferation, differentiation and migration controlled by these signalling pathways. HSPGs can be shed from the cell surface to reduce interaction of the cell with ligands, which provides a means of regulating HS-protein interactions, and can direct ligands into the cell for degradation. HSPGs are essential for tissue development in embryos and to maintain healthy tissue in adults.

2.4 Heparan Sulfate in Cancers

The roles played by HSPGs in cancer are complex due to the wide range of signalling pathways they facilitate in healthy cells. Typically, when cells become cancerous, the HSPGs present are altered. For example, the expression of the HSPG glypican-1 is higher in cancerous pancreatic cells than in healthy ones. This may aid mitosis by increasing interaction with the growth factor FGF-2, making pancreatic cancer particularly aggressive. Conversely, the expression of glypican-3 is silenced in breast cancer cells, preventing inhibition of metastasis. Cancer cells may also have an increased rate of shedding of some HSPGs; pancreatic cancer cells shed the HSPG syndecan-1 at an increased rate, allowing continued mitosis by interaction of glypican-1 with FGF-2. Recently, it has been shown that breast cancer cells which are resistant to
the monoclonal antibody trastuzumab have an increased rate of HS shedding, and resistance can be initiated by addition of heparin to the cell culture.\textsuperscript{54}

In order to grow beyond 2 mm, tumours require their own blood supply. HSPGs can promote angiogenesis via interaction with FGF-2,\textsuperscript{55} however HSPGs on the endothelial cell-surface have also been shown to bind endostatin,\textsuperscript{56} an anti-angiogenic factor. Possibly, this allows endothelial cells to regulate angiogenic potential by changing the HSPGs present on the cell surface.

Metastasis is the process in which cancer spreads from one area to another not directly connected to the original area, often via the lymph or blood system. HSPGs mediate cell-cell adhesion of cancer cells to platelets and capillary endothelium by acting as ligands for the adhesion protein p-selectin,\textsuperscript{57} allowing cancer cells to enter the bloodstream. HSPGs in the extracellular matrix (ECM) form a physical barrier against tumour metastasis. However, tumour cells secrete heparanase which catalyses degradation of HS resulting in re-arrangement of the ECM and allowing metastasis to occur more easily.\textsuperscript{58}

Low molecular weight heparins (LMWHs) and unfractionated heparin have been used in cancer patients to treat or prevent thrombosis, and it has been noted that they confer anti-cancer properties.\textsuperscript{59} This has been attributed to multiple factors: interference with the formation of the fibrin coat which protects tumours,\textsuperscript{60} assistance of the immune response to circulating tumour cells,\textsuperscript{57} and blocking of cell adhesion.\textsuperscript{61} An alternative therapeutic angle into which research is on-going is the design of heparanase inhibitors.\textsuperscript{62} As the interactions and effects of HSPGs in cancers are varied and complex, further investigation is required to elucidate the roles of HSPGs during cancers fully and to identify therapeutic targets based on this knowledge.\textsuperscript{63}

2.5 Heparan Sulfate in HIV Infection

In order to infect cells, most pathogens must adhere themselves in some way to the cell surface. Multiple pathogens have been found to be capable of using HS for cell adheremnt. These include parasites such as \textit{Plasmodium falciparum},\textsuperscript{64} which causes malaria, bacteria such as \textit{Mycobacterium tuberculosis},\textsuperscript{65} and a number of viruses including HIV,\textsuperscript{66} herpes simplex viruses,\textsuperscript{67} hepatitis C,\textsuperscript{68} human papilloma virus\textsuperscript{69} and dengue virus.\textsuperscript{70}

HIV infects cells of the immune system, predominantly T cells with the cell-surface glycoprotein CD4 and at least one of the two co-factors CCR5 or CXCR4.\textsuperscript{71} To
do so, the virus must first cross the epithelial cells at the site of infection. The HSPGs on the surface of these cells can sequester HIV, and HIV particles attached to the cell surface have been found to remain infectious for >6 days.\textsuperscript{72} Syndecan-3 has also been found to trap HIV, which can then be transferred to passing T cells.\textsuperscript{73} HSPGs are also involved in the transport of HIV across the blood-brain barrier before infection.\textsuperscript{74}

The involvement of HSPGs in HIV infection of T cells was proposed in the late 1980s following the observation that heparin could inhibit HIV infection \textit{in vitro}.\textsuperscript{75} It was subsequently shown that cleavage of cell surface HS with heparitinase reduced the binding of HIV to T cells.\textsuperscript{76} The mechanism of entry involves interaction of the HIV envelope glycoprotein gp120 firstly with CD4 on the T cell, which results in conformational change in gp120 and generation/exposure of a new gp120 epitope that can then bind to the co-receptor. HS is capable of binding to gp120, and Lortat-Jacob \textit{et al.} have reported that the affinity is increased after interaction of gp120 with CD4.\textsuperscript{77} HS oligosaccharides ranging from 10 to 18 saccharide residues in length were found to interact with gp120 at the same site as the viral co-receptors, suggesting a potential therapeutic application. Lortat-Jacob and co-workers therefore synthesised a glycoconjugate consisting of a CD4 peptide mimic linked to a HS dodecasaccharide that acts to block HIV entry to cells (Figure 5).\textsuperscript{78} The specific HS saccharide sequence required for binding to gp120 is not yet fully known, but work by Matos \textit{et al.} suggests a binding sequence of >16 residues is required.\textsuperscript{79}
Figure 5. Mechanism of CD4 peptide mimic-HS dodecasaccharide conjugate blocking HIV cell entry; (a) normal interactions for HIV cell entry: gp120 binds to CD4 and conformational change is induced resulting in the ability to bind to co-receptor; (b) CD4-HS conjugate blocks these interactions by inducing conformational change in gp120 (CD4 peptide) and binding to the new epitope (HS dodecasaccharide). Re-drawn from ref. 78.

2.6 Roles Played by Heparan Sulfate in Alzheimer’s Disease

Alzheimer’s disease (AD) is a neurodegenerative disorder in which plaques form in the brain and impair neuron function; the plaques mainly consist of amyloid-β peptide (Aβ) which is derived from the larger amyloid precursor protein (APP). The accumulation of sulfated glycosaminoglycans in AD brain lesions was first identified in 1987 by Snow et al.\textsuperscript{80} Since this discovery, agrin, syndecans and glypicans have been found in AD amyloid plaques,\textsuperscript{81} although there is some debate over the presence of perlecan, with some studies unable to identify perlecan in plaques.\textsuperscript{82,83} This could be due to the fact that perlecan appears in plaques in some areas of the brain e.g. hippocampus, but not others (such as the cerebellum).\textsuperscript{84} The level of HS in AD brain has been found to be greater than that of healthy brain, with a 9.3-fold increase in HS in the hippocampus.\textsuperscript{85} The exact reason for this is unconfirmed, but treatment of glial cell culture (cells which surround and support/insulate neurons) with Aβ increased the expression of glypican-1 and syndecan-3,\textsuperscript{86} indicating that the greater amount of HS in AD brain could be caused by the presence of Aβ.
HSPGs have been shown to bind to Aβ peptides, which has resulted in a number of studies of the effect of HS on Aβ. Heparan sulfate was found to trigger aggregation when added to Aβ; interestingly the effect of inorganic sulfate was similar. The binding of GAGs to Aβ is pH dependant, increasing with decreasing pH, which is attributed to the requirement for protonated histidines in the Aβ binding site. Heparin was found to bind to fibrillar Aβ but not non-fibrillar Aβ, indicating that the binding is not just dependant on the primary peptide sequence. HSPGs (and CSPGs) can inhibit the degradation of Aβ in fibrillar form, but not non-fibrillar form, by proteases in vitro.

Neurofibrillary tangles (NFTs) are composed of paired helical filaments (PHFs) of a phosphorylated version of the protein tau, and also contribute to Alzheimer’s disease. HSPGs can bind to tau and heparin can promote assembly of recombinant tau into helical-like filaments in vitro. HS has also been found to promote phosphorylation of tau, and to prevent tau from binding to microtubules. The effect of other GAGs on tau was also studied and these effects were shown to be sulfation-dependant, with more highly-sulfated GAGs being more potent. It is believed that inability to bind to microtubules precedes PHF formation, and HS has been observed to co-localise with hyperphosphorylated tau in nerve cells of AD brain, suggesting a role for HS in NFT formation.

A promising therapeutic target for AD is the β-secretase BACE1, which initiates production of Aβ from APP. Initially, it was believed that heparin/HS could promote BACE1 cleavage of APP, however other studies found an inhibitory effect. Subsequently, low concentrations (1 µg mL⁻¹) of heparin were found to stimulate recombinant BACE1, whereas higher concentrations (10 or 100 µg mL⁻¹) inhibited it; also, the prodomain of BACE1 was found to be the binding region for heparin. Heparin had the highest inhibitory effect of the GAGs and lack of sulfation greatly reduced inhibition of BACE1. Synthetic HS oligosaccharides have been investigated to determine the structural requirements for BACE1 inhibition, although these have not yet been determined fully, this presents a drug discovery opportunity for AD.
3. SYNTHESIS OF HS OLIGOSACCHARIDES

3.1 Digestion of Native HS/heparin

Chemical synthesis is widely used where size-defined HS oligosaccharides with known sulfation patterns are required. However, there are two main alternative methods for the preparation of these compounds: HS/heparin digestion and chemoenzymatic synthesis.

Purification of HS or heparin from natural sources was the first method used to obtain HS oligosaccharides for study. In order to obtain size-defined HS fragments, either chemical or enzymatic digestion of native HS or heparin can be used. Chemical digestion often uses nitrous acid,\(^{103}\) which cleaves the polysaccharide at N-sulfated glucosamine residues, resulting in conversion to a 2,5-anhydromannose residue (Scheme 1).\(^ {104}\) This gives an aldehyde moiety at the reducing end (i.e. the terminus of the molecule bearing an anomeric carbon centre that is not part of a glycosidic bond).

Enzymatic digestion typically employs lyases from bacteria, and results in a 4,5-unsaturated bond in the non-reducing end uronic acid,\(^{105}\) which provides a chromophore that may facilitate separation e.g. by HPLC with UV-vis detection. However, formation of this bond results in a loss of stereochemical information in the uronic acid. It should be noted that neither method allows access to HS oligosaccharides with uronic acid residues at the reducing end.

\[
\begin{align*}
R^1 & = H \text{ or } SO_3^- \\
\text{Nitrous acid digestion} & \quad \text{Enzymatic digestion}
\end{align*}
\]

**Scheme 1.** Example of enzymatic and nitrous acid cleavage of native heparan sulfate.

After digestion analysis is performed to determine the composition of the fractions isolated. This allows HS fragments of known sequence and sulfation pattern to be used in protein binding studies and other experiments such as *in vivo* studies. Various mass spectrometry techniques have been utilised for HS analysis. For example, a MALDI method was developed in which HS fragments are complexed to a basic peptide to alleviate the issue of sulfate loss in the mass spectrometer.\(^ {106}\) However, this
complexation of the HS oligosaccharides prevents further analysis by tandem MS. Negative mode ESI has also been found to be a suitable ionisation method for HS oligosaccharides, allowing sulfate group retention. Ion mobility mass spectrometry has recently been used for the analysis of HS and other GAGs to separate isobaric ions and distinguish between different sulfation patterns, and has also provided information on conformation. NMR can also be employed for the characterisation of HS oligosaccharides, including $^1$H-$^{13}$C and $^1$H-$^{15}$N HSQC experiments.

### 3.2 Chemoenzymatic Synthesis of HS Oligosaccharides

Alternatively, HS oligosaccharides may be produced chemoenzymatically, either from monosaccharide building blocks (often chemically modified) or from polysaccharide precursors such as heparosan (derived from bacterial sources). Chemoenzymatic generation of IdoA-GlcN oligosaccharides from monosaccharides has been achieved in a number of reports. However, this is currently limited by the availability of some recombinant enzymes, mainly NDST, the $N$-deacetylase domain of which cannot be expressed effectively in *E.coli*. The solution to this is to use $N$-trifluoroacetyl derivatives of glucosamine, which are good substrates for the bacterial $N$-acetylglucosaminyItransferases KfiA and PmHS2 used to catalyse formation of the oligosaccharide chain. Later in the synthesis, the trifluoroacetyl group is removed with base to allow $N$-sulfation by the $N$-sulfotransferase. This is exemplified in the work of Liu et al., who chemoenzymatically synthesised IdoA containing heptasaccharide (Scheme 2). Monosaccharides with a UDP group (GlcN-trifluoroacetyl-UDP and GlcA-UDP) were used as substrates for the $N$-acetylglucosaminyItransferases allowing construction of heptasaccharide from disaccharide (which was itself prepared by nitrous acid digestion of heparosan). Treatment of with mild base (Et$_3$N in MeOH/H$_2$O) removed the trifluoroacetyl protecting group, allowing $N$-sulfation by NST. In order to selectively epimerise only the central glucuronic acid to iduronic acid, an additional GlcN-trifluoroacetyl residue was added to the non-reducing end, as C-5 epimerase will only act on GlcA residues that are positioned between two GlcNS moieties.
Scheme 2. Reagents: (i) 2, KfiA; (ii) 4, PmHS2; (iii) NST, Adenosine 3’-phosphate 5’-phosphosulfate (PAPS, sulfate donor), MeOH/Et3N/H2O; (iv) C-5 epimerase/2-OST enzymes, PAPS.

Chemoenzymatic production of HS/heparin from polysaccharides has largely been used to develop therapeutic alternatives to native heparin for anticoagulation. Heparosan, the initial NA domain polysaccharide formed in HS biosynthesis, can be obtained from the capsule of the E.coli K5 strain\textsuperscript{122} and has been converted to ‘neoheparin’ in six steps, although only the C-5 epimerisation is enzymatic.\textsuperscript{123}

The scale of enzymatic synthesis of HS oligosaccharides has increased considerably over the past decade from \textasciitilde10 \mu g\textsuperscript{124} to milligram scale\textsuperscript{121} due to a greater
understanding of the enzyme specificities and better enzyme expression. However, there are still limited reports of the use of chemoenzymatically synthesised HS oligosaccharides in biological and binding studies.\textsuperscript{125}

Digestion of HS and chemoenzymatic synthesis can be utilised to provide HS oligosaccharides. However, the difficulty of characterising digest products and the limited availability of recombinant enzymes means neither of these methods have dominated. In addition, enzyme specificities may limit the number of structures accessible via chemoenzymatic synthesis.\textsuperscript{22} Chemical synthesis remains a viable alternative to allow access to size-defined HS oligosaccharides with specific sulfation patterns.

3.3 Chemical Synthesis of HS Oligosaccharides

3.3.1 Synthesis of L-Ido Building Blocks

In order to chemically synthesise HS oligosaccharides, generation of L-ido pyranose building blocks is required on multi-gram scale. However, this is not trivial, as neither IdoA nor L-idose (Ido) are readily available as starting materials. Multiple approaches to the synthesis of these building blocks have been developed, but not all have been suitable for providing L-ido building blocks on the required scale for HS oligosaccharide synthesis.\textsuperscript{126}

Conceptually, the simplest method of producing IdoA would be to epimerise the C-5 position of readily available GlcA in its pyranose form (Scheme 3). This epimerisation was reported by Fischer and Schmidt,\textsuperscript{127} but subsequent work by Carlsson et al. showed that the mixture of products obtained did not include IdoA (9).\textsuperscript{128} In addition, differentiation between the hydroxyl groups of 9 would be anticipated to be difficult. Strategies devised to access L-ido building blocks typically involve installation of some protecting groups before generation of the L-ido centre at C-5 and conversion to the pyranose.

\begin{center}
\begin{tikzpicture}
% TikZ code for Scheme 3
\end{tikzpicture}
\end{center}

\textbf{Scheme 3.} Proposed synthesis of L-iduronic acid (9) by epimerisation at C-5 of D-glucuronic acid (8).
Following reports by Thiem and Ossowski\textsuperscript{129} that glycal 10 can be epimerised under basic conditions to preferentially give L-ido glycal 13 (4:1 ido/gluco), Seeberger et al. used this method to furnish L-ido building blocks.\textsuperscript{130} Glycals can be transformed into a range of glycosyl donors\textsuperscript{131} (sugars with a suitable leaving group at C-1 which can be reacted with a glycosyl acceptor to form a new glycosidic linkage). Seeberger and co-workers prepared several protected D-glucal glycals including 11 and 12 from diacetone glucose. Glycals 11 and 12 were subjected to base-catalysed epimerisation to give 1:1 mixtures of the D-gluco and L-ido glycals (Scheme 4). Conversion of the L-ido glycals to O-pentenyl glycosides was attempted, but was generally low-yielding (<30%) and resulted in L-guluronate by-products from inversion at C-2 (21–23). Acetyl protection of the glycal substrate at O-4 (17) resulted in preferential formation of desired L-ido product 20.

\textbf{Scheme 4. Reagents:} (i) NaOMe, MeOH, RT, 80%; (ii) Ac₂O, pyridine, 86%; (iii) TBDMSCl, imidazole, CH₂Cl₂, 85%; (iv) DMDO, Me₂CO, 0 °C, then 4-penten-1-ol, ZnCl₂, CH₂Cl₂, 25–30%.

Recently, Salamone et al. reported direct inversion at C-5 of a glucuronic acid derivative using a radical approach to afford iduronate derivative 28 (Scheme 5).\textsuperscript{132} Glucurionate methyl glycoside 24 was derivatised to place an alkyne group in the required position for the subsequent radical cyclisation. Hydrolysis of the methyl ester afforded carboxylic acid 25 which was converted to Barton ester intermediate 26 and subjected to radical cyclisation to afford bicyclic ido compound 27. Further elaboration afforded iduronate 28 which would be suitable as a glycosyl acceptor.
Scheme 5. Reagents: (i) Propargylaldehyde diethylacetal, P₂O₅, CHCl₃, 60 °C, 67%; (ii) NaOH, EtOH/H₂O, RT, 95%; (iii) Isobutyl chloroformate, N-methyl morpholine, THF, 0 °C, then 2-mercaptopyridine N-oxide sodium salt, then t-BuSH, hν, 48%; (iv) O₃, Me₂S, CH₂Cl₂, –78 °C, 80%; (v) mCPBA, NaHCO₃, CH₂Cl₂, 0 °C; (vi) TsOH, MeOH, Δ; (vii) MeI, Et₃N, DMF, 56% over three steps.

Chemoenzymatic methods may provide more efficient routes to L-ido derivatives by direct epimerisation. Although recombinant uronyl C-5 epimerase is not yet widely available, there is increasing interest in the biochemistry and synthetic applications of this enzyme.¹³³

Inversion at C-5 on furanose derivatives, mostly derived from readily available protected D-glucuronic acid γ-lactone or diacetone-D-glucose, has also been used to provide L-ido building blocks. For instance, Suda and co-workers converted D-glucuronic acid γ-lactone derivative 29 to L-iduronate pyranose derivative 33. The inversion was performed by installing a triflate at C-5 followed by nucleophilic displacement with sodium levulinate (Scheme 6).¹³⁴ L-Iduronate building block 33 was protected and utilised in the synthesis of HS trisaccharides. Similarly, Jacquinet et al. obtained 30 in six steps from diacetone-D-glucose and performed inversion by displacement of a C-5 triflate to afford L-ido furanose 32.¹³⁵
**Scheme 6.** Reagents: (i) TBDMSCl, imidazole, CH$_2$Cl$_2$; (ii) NaOMe, MeOH, 0 °C; (iii) Benzyl 2,2,2-trichloroacetimidate, TBDMSOTf, CH$_2$Cl$_2$, 0 °C; (iv) TBAF, AcOH, THF, −20 °C, 43% over four steps; (v) Tf$_2$O, pyridine, CH$_2$Cl$_2$, −20 °C; (vi) LevONa, DMF; (vii) H$_2$NNH$_2$·H$_2$O, pyridine/AcOH (3:2), 56% over three steps; (viii) TFA/H$_2$O (9:1), 95%.

L-Ido epoxide 35 was synthesised from diacetteone-d-glucose (34) in five steps by van Boeckel, subsequent treatment with dilute H$_2$SO$_4$ resulted in removal of the acetal protecting group and epoxide opening to give pyranose 36 (Scheme 7). Hung et al. and Tatai and co-workers independently reported conversion of epoxide 35 into 1,6-anhydrosugar derivative 37. After protection, this anhydrosugar was used as an acceptor for disaccharide formation (Hung) and for conversion to an Ido thioglycoside donor (Tatai).

**Scheme 7.** Reagents: (i) BnBr, NaH, DMF; (ii) AcOH (aq., 60%); (iii) MsCl, pyridine; (iv) KOAc, 18-crown-6; (v) KOr-Bu, t-BuOH, CH$_2$Cl$_2$; (vi) H$_2$SO$_4$ (aq., 0.1 M); (vii) Ac$_2$O, pyridine; (viii) H$_2$SO$_4$ (aq., 1 M), dioxane, Δ, 65%.

An alternative method for generation of L-ido building blocks is the reaction of pentodialdoses with organometallic reagents, which in limited cases gives selectivity for the L-ido product. Corina and Casiraghi reported a selectivity of 97:3 for formation of L-ido product 39 from aldehyde 38 (Scheme 8). It is believed that the magnesium atom chelates to the aldehyde and pyran oxygen atoms in the reaction, favouring attack on the si-face of C-5. However, this reaction is not synthetically useful for access to L-ido building blocks given the nature of group added at C-5.
Scheme 8. Reagents: (i) (4-Hydroxyphenyl)magnesium bromide, CH₂Cl₂, 0 °C, 65%.

Bonnaffé et al. achieved total L-ido selectivity from a pentodialdose reaction by addition of the bulky nucleophile tris-(phenylthio)methylthilium to aldehyde 41 (Scheme 9). Crucially, conversion of orthothioester 42 to its methyl ester derivative followed by acidic hydrolysis allowed formation of 33 which can be protected to form various iduronate donors and acceptors.

Diastereoselective hydroboration has also been used to access L-ido sugars from exo-glycals. Rochepeau-Jobron and Jacquinet reported conversion of exo-glycal 46, synthesised in six steps from commercially available glucose derivative 43, to L-ido derivative 47 by hydroboration with 9:1 L-ido/D-glucu selectivity (Scheme 10).
A de novo approach to L-ido synthons in which pyranoses are generated from linear precursors has been developed by Seeberger et al. Synthesis of D-glucuronate and L-iduronate monosaccharides 52 and 53 was achieved from divergent intermediate 48, itself derived from L-arabinose, using a Mukaiyama-type aldol reaction (Scheme 11). However, L-iduronate product 53 was obtained in only 6% overall yield. This de novo strategy was modified so the key L-ido forming step was a diastereoselective cyanohydrin reaction of aldehyde 55, which was synthesised from readily available D-xylose (Scheme 12). Different protecting groups such as pivaloyl (Piv) and levulinoyl (Lev) were also shown to be tolerated. Subsequently, the method was used to synthesise differently protected L-ido thioglycosides suitable for HS oligosaccharide synthesis.

\[
\begin{aligned}
\text{Scheme 11. Reagents:} & \quad \text{(i) BF}_3\cdot\text{Et}_2\text{O, 49, CH}_2\text{Cl}_2, 0 \degree \text{C, 95\% (50/51 = 3:2); (ii) FmocCl, pyridine, then HF\cdot\text{pyridine, THF; (iii) NIS, CH}_2\text{Cl}_2, \text{quant.}} \\
\end{aligned}
\]

\[
\begin{aligned}
\text{Scheme 12. Reagents:} & \quad \text{(i) SO}_3\cdot\text{pyridine, DMSO, DIPEA, 94\%; (ii) TMSCN, MgBr}_2\cdot\text{OEt}_2, \text{CH}_2\text{Cl}_2; (iii) H}_2\text{NNH}_2, \text{AcOH, CH}_2\text{Cl}_2, 82\% \text{ over two steps, 56/d-glucodervative (not shown) 8:1; (iv) AcCl, MeOH, toluene, 70\%; (v) LevOH, DIPC, DMAP, CH}_2\text{Cl}_2; (vi) NIS, CH}_2\text{Cl}_2, 80\% \text{ over two steps (1:1 } \alpha/\beta).}
\end{aligned}
\]
Considerable development has been made in the synthesis of L-ido building blocks in recent years. However, there is still further development required to allow the increasingly facile synthesis of HS oligosaccharides which will aid in investigation of structure-specific properties. In particular, these methods will need to be scalable and use readily available precursors.

3.3.2 Modular Synthesis of HS Oligosaccharides

In order to efficiently build long chains, modular syntheses using an iterative elongation process such as successive addition of disaccharide units are typically used to make HS oligosaccharides. Martín-Lomas et al. reported the synthesis of octasaccharide 64 by an n+2 modular approach (Scheme 13). In this strategy, the oligosaccharide chain is built from the reducing end upwards, with the anomeric O-isopropyl serving as a ‘reducing end cap.’ The benzylidene protecting group donors 61–63 served as temporary protection for O-4. Multiple steps were therefore required between glycosylations to remove the benzylidene group and re-protect O-6 with a benzoyl group. The use of esters was employed to mask positions where sulfation is desired in the final product, and ethers where it is not. This protection strategy has been widely employed in the synthesis of HS oligosaccharides. To cap the non-reducing end of the octasaccharide, O-4 benzyl protected disaccharide 60 was added as the final residue. Ester hydrolysis, O-sulfation, hydrogenation and N-sulfation furnished deprotected octasaccharide 64. Martín-Lomas and co-workers subsequently used this strategy to provide HS oligosaccharides of varying sequence length and sulfation pattern for biological study; the method was also adapted to solid phase Merrifield-type synthesis.
Seeberger et al. reported a modular synthesis of ‘mixed sequence’ HS oligosaccharides (i.e. containing both IdoA and GlcA residues) using di- and trisaccharide building blocks. Tetrasaccharides such as 67 were synthesised by coupling of disaccharide units (e.g. 65 and 66) by activation of a trichloroacetimidate donor over an O-pentenyl glycoside using catalytic TMSOTf (Scheme 14). This synthesis theoretically requires only two steps to add a disaccharide unit: deprotection of the O-4 silyl group to reveal the acceptor hydroxyl group followed by glycosylation. However, the C-4 hydroxyl group of GlcN-IdoA tetrasaccharide acceptors such as 67 was unexpectedly unreactive, preventing formation of hexasaccharides. This could not be attributed to the
conformation of the sequence, and no other reason was apparent. Hexasaccharide 69 was therefore synthesised by coupling of trisaccharide units 70 and 71. Subsequently, this modular synthesis approach was used to generate HS oligosaccharides for microarray experiments to investigate binding to FGF-1, FGF-2 and FGF-4 (Section 3.4).153,154

Scheme 14. Reagents: (i) TMSOTf, CH₂Cl₂, −25 °C, 86% for 67, 62% for 69 (from glycosylation of 70 and 71); (ii) HF/pyridine, AcOH, THF, 75%.
In an example of a 4+4 glycosylation strategy, Bonnaffé et al. reported modular synthesis of IdoA-GlcN octasaccharide 75 (Scheme 15). Tetrasaccharide donor 74 and acceptor 73 were prepared from divergent tetrasaccharide intermediate 72. The octasaccharide was obtained in good yield with only the α-linked product formed. As the allyl moiety is not a donor group, it was used to protect the anomeric position of the glycosyl acceptor, but could also be removed to form trichloroacetimidate donors such as 74, and used for functionalisation (Section 3.4).

Scheme 15. Reagents: (i) H₂-activated [IrI(C₈H₁₄)(MePh₂P)₂]PF₆, THF, RT, then HgO/HgCl₂, acetone/H₂O (9:1), RT; (ii) Cl₃CCN, K₂CO₃, CH₂Cl₂, RT, 87% over two steps; (iii) DDQ, CH₂Cl₂, RT, 81%; (iv) 73, TBDMSOTf, CH₂Cl₂, –40 to 0 °C, 93%.

Hung et al. reported a synthesis that furnished odd-numbered HS sequences up to nonasaccharide length by successive addition of disaccharide 77 to GlcN-Ido-GlcN trisaccharide 76, which bears a reducing end anomeric methoxy cap (Scheme 16). Idose-containing oligosaccharides 78–80 were subjected to oxidation with TEMPO/NaOCl to give iduronic acid residues before the deprotection steps. The resulting HS oligosaccharides were used to study interaction with eosinophil derived neurotoxin.
Scheme 16. Reagents: (i) 77, TMSOTf, CH$_2$Cl$_2$, –40 °C, 74% for 78, 57% over two steps for 79, 49% over two steps for 80; (ii) DDQ, CH$_2$Cl$_2$/H$_2$O (18:1).

Hung and co-workers utilised a different approach for synthesis of HS oligosaccharides used in studies with herpes simplex virus type 1 entry receptor, and hemagglutinin from Mycobacterium tuberculosis (Scheme 17).$^{157-159}$ In this strategy, the reducing end residue is a 1,6-anhydrosugar (prepared from L-ido epoxide 37) which serves as protection for the anomeric centre. Opening of anhydrosugar disaccharide 81 allows access to trichloroacetimidate donor 83 which can be glycosylated with 82 to generate anhydrosugar-capped tetrasaccharide 84. Conversion of this tetrasaccharide into trichloroacetimidate donor 85 allows glycosylation with either disaccharide acceptor 82 or tetrasaccharide acceptor 84. Crucially, the resulting HS oligosaccharides 86 and 87 have the anhydrosugar present at the reducing end, allowing late-state functionalisation after the iterative glycosylation steps, although the authors did not report this. The p-bromobenzyl (PBB) protecting group at O-3 of GlcN offers the potential to access 3-O-sulfated oligosaccharides as it can be removed in the presence of benzyl groups by selective reduction.$^{160}$ However, Hung et al. selectively removed the PBB group at the disaccharide level (81) and re-protected O-3 with an ester to allow access to oligosaccharides with 3-O-sulfate. Use of building blocks such as 81 with silyl protection at O-6 of GlcN allowed access to both per-O-6-sulfated and per-O-6-non-sulfated oligosaccharides.
Codée et al. used monomeric building blocks to synthesise pentasaccharide 96, avoiding the need to use precious disaccharide building blocks (Scheme 18). Hemiacetals such as 88 were used as glycosyl donors, as 1-hydroxyl donors with non-participating groups at C-2 had previously shown a high degree of $\alpha$-selectivity with relatively unreactive glycosyl acceptors. Hemiacetals 88 and 92 were selectively activated over the thiglycoside moieties in acceptors 89 and 91 using the 1-benzenesulfanyl piperidine (BSP)/$\text{TiO}_2$ activation system developed by Crich for thiglycoside activation. Codée and co-workers proposed that the method could also allow for addition of ‘unnatural’ synthons (such as conformationally restricted monosaccharides) more easily than a modular synthesis using disaccharide units, but this was not reported.
Polat and Wong reported a one-pot method for the synthesis of HS oligosaccharides, generating sequences up to pentasaccharide length by chemoselective activation of S-para-tolyl (STol) donors (i.e. selective activation of the STol group on the donor in the presence of an STol group on the glycosyl acceptor, Scheme 19). To enable design of one-pot syntheses of oligosaccharides, Wong et al. had previously devised a HPLC method for determining the relative reactivity value (RRV) of saccharide building blocks. Measurement of the RRVs of the chosen HS mono- and disaccharides allowed tuning of reactivity, where the most reactive donor is chemoselectively activated over, and the pentasaccharide is capped by addition of methyl glycoside in one pot. Huang and co-workers subsequently reported a modified one-pot synthesis of HS hexasaccharides in which a donor thioglycoside disaccharide is preactivated using STolOTf (formed in situ from STolCl and AgOTf) and the thioglycoside acceptor is then added to the activated donor. This avoids the requirement for reactivity tuning of the disaccharides.
Scheme 19. Reagents: (i) 98, NIS, TfOH, CH₂Cl₂, −45 °C to RT, then 99, NIS, TfOH, CH₂Cl₂, −45 °C to RT, one pot, 20% over two steps.

Modular synthesis strategies have been widely used for synthesis of HS for biological study. However, further development is still required to allow late-stage functionalisation of the oligosaccharides (at either reducing or non-reducing end), develop more efficient syntheses, and allow access to all sulfation patterns of interest from common intermediates.

3.4 Functionalisation/Labelling of Synthetic HS Oligosaccharides

In order to use synthetic HS oligosaccharides for biological investigation, functionalisation may be required e.g. for glycoconjugate formation, surface attachment or fluorescent labelling. A reactive ‘handle’ can be attached to the HS oligosaccharide to allow functionalisation. This should have minimal effect on the polarity, reactivity and shape of the molecule, to avoid hindrance to the synthesis and to maintain the HS-like nature of the oligosaccharide for biological study. For example, Seeberger et al. used reducing end amine moieties, generated from O-pentenyl glycosides via the thiolene reaction or by glycosylation of trichloroacetimidate donors, to attach HS
oligosaccharides to \(N\)-hydroxysuccinimide (NHS) ester coated slides for the generation of microarrays (Scheme 20).\(^\text{154}\) The reducing end amine tag was also used for the preparation of poly(amideamine) (PAMAM) type dendrimers, which were found to bind to FGF-1 in competition assays using immobilised heparin, with IC\(_{50}\) values in the micromolar range.\(^\text{169}\)

Similarly, Bonnaffé and co-workers used a reducing end allyl moiety in a thiol-ene reaction to generate glycoconjugates consisting of GlcN-IdoA tetra-, hexa- and octasaccharides linked by a PEG chain ‘spacer’ to mimic the NA domain (Scheme 21).\(^\text{155}\) During the deprotection steps, simultaneous \(N,O\)-sulfation (converting 105–107 to 108–109) was carried out, in contrast to the separate \(N\)- and \(O\)-sulfation steps often employed in HS oligosaccharide synthesis. The glycoconjugates were tested for their ability to inhibit the interferon-\(\gamma\)-heparin interaction using surface plasmon resonance (SPR). Only octasaccharide-containing conjugates 120–122 were found to inhibit the interaction; compound 121 was the most potent with an IC\(_{50}\) value of \(\sim 35\) nM.

\[\text{Scheme 20. Reagents: (i) HSCH}_2\text{CH}_2\text{NHZ, AIBN, THF, 75 °C; (ii) LiOH, H}_2\text{O}_2, \text{then KOH, MeOH; (iii) SO}_3\cdot\text{NEt}_3, \text{DMF, 55 °C; (iv) PMe}_3, \text{THF, NaOH, (v) SO}_3\cdot\text{pyridine, pyridine, then H}_2, \text{Pd/C.}\]
Scheme 21. Reagents: (i) K₂CO₃, MeOH, RT, 88–93%; (ii) HS(CH₂)₃SH, Et₃N, MeOH, RT, 84–92%; (iii) SO₃·pyridine, pyridine, RT then 55 °C, 73–96%; (iv) LiOH, H₂O₂, 73–91%; (v) 111, 112 or 113, hv (360 nm); (vi) Oxone® (KHSO₅·½KHSO₄·½K₂SO₄), K₂HPO₄, pH 7, 23–31% over two steps; (vii) Pd(OH)₂/C, H₂, pH 7.0 phosphate buffer, RT, 62% to quant.
Recent work by Dollé et al. utilised a reducing end alkyne for Huisgen cycloaddition between octasaccharide 123 and bi-functional linker 124 containing a protected thiol (Scheme 22). The thiol was then unmasked and used to add an $^{18}$F label for positron emission tomography (PET); the resulting conjugate was injected into rats to study the organ distribution of the compound. Interestingly, benzylated compound 127 was utilised in the study without the usual global deprotection step to reveal the free hydroxyls, although the reason for this was not specified.

Scheme 22. Reagents: (i) 124, CuSO$_4$, ascorbic acid, $t$-BuOH/H$_2$O, RT, 92%; (ii) Hydroxylamine (50 mM) in PBS (100 mM, pH 7.4), RT, 66%; (v) 126, DMSO, PBS (100 mM, pH 7.4), RT, 63%.

Overall, these examples of functionalisation show how diverse applications can be achieved through use of ‘handles’ bearing suitable functional groups. In particular amines and thiols are useful as they are widely used for protein labelling hence there are many suitable commercially available reagents for conjugation to these moieties.
4. ANALYSIS OF HS-PROTEIN INTERACTIONS

4.1 Factors of Interest in HS-Protein Binding

In the analysis of HS-protein binding using structure-defined oligosaccharides, the first question to be addressed is usually which oligosaccharides bind to the target protein. Knowledge of the minimum binding saccharide sequence is desired, particularly for use in therapeutic applications. For instance, determination of the minimum binding sequence required for heparin to bind to antithrombin III (AT III) has led to common use of heparin pentasaccharide (fondaparinux) as a synthetic antithrombotic agent.\textsuperscript{171, 172}

Of particular interest is the effect of common NS-domain sulfate groups (IdoA 2-O, GlcNS and 6-O) and the rarer GlcN 3-O sulfate modification on protein binding. The thermodynamics of binding can be quantified by the use of methods such as ITC, exemplified by the investigation of the binding of 48 synthetic HS disaccharides to FGF-1 and FGF-2 by Hung \textit{et al}.\textsuperscript{173, 174} The kinetics of binding can be studied using SPR.\textsuperscript{175, 176}

However, study of HS-protein complexes at molecular level is required to further understand the details of these interactions. For instance, 2-O-sulfated IdoA can adopt a $^1C_4$, $^4C_1$ or $^2S_0$ conformation, leading to overall conformational flexibility in the NS domain sequences (Figure 6).\textsuperscript{177} The conformation of the protein-bound saccharide is therefore an important factor in binding. Stoichiometry and dynamics of the binding interaction is also of interest; for instance some proteins (particularly chemokines) have been observed to dimerise or oligomerise in the presence of HS.\textsuperscript{178} The location of the binding site on the protein, which amino acids are present and how these might interact with the saccharide sequence are also of interest.

![Figure 6](image)

$^1C_4$, $^4C_1$ and $^2S_0$ conformations of 2-O-sulfated IdoA residues.

The ideal approach for investigation of HS-protein binding would allow study of all these factors. The most widely used methods to date have been NMR and X-ray crystallography, often in conjunction with methods such as ITC and SPR. There is an important caveat to HS-protein binding studies, as some HS sequences have been found to bind to proteins but not result in the expected biological response. For example, although GlcN 6-O-sulfation has been found to not be essential for FGF-2 binding,\textsuperscript{179, 180}
it is essential for FGF-2 mediated signalling. Therefore knowledge of HS-protein interactions must be taken in conjunction with signalling and biological function assays to give a better understanding of which structural features of HS are required for a particular cellular event.

4.2 NMR for Investigation of HS-Protein Binding

NMR has been widely used to investigate various aspects of HS-protein binding. Evidence of binding interactions may be obtained from perturbation of the chemical shift of the saccharide sequence in the presence of the protein, observation of transferred NOE effects, or by diffusion experiments. NMR can be used to investigate the minimum binding sequence for a protein. Casu et al. investigated the ability of HS tetrasaccharides to bind and induce dimerisation of FGF-1 using NMR in conjunction with SPR (to confirm if binding occurred) and MALDI mass spectrometry (to investigate stoichiometry of complex formed). Differences in chemical shift of particular tetrasaccharide signals after FGF-1 addition indicated both 6-O-sulfate groups interacted with the protein, and line broadening indicated fast ligand exchange. Casu and co-workers subsequently used NOESY experiments to investigate tetrasaccharide-FGF-2 binding and determine the minimum binding sequence for FGF-2.

NMR has been widely applied for study of HS sequence conformation in solution, and has also been used for conformational analysis of protein-bound HS oligosaccharides. In the first solution study of heparin pentasaccharide-AT III complex, Hricovini et al. showed the iduronate residue of bound heparin pentasaccharide preferentially adopted the $^2S_0$ conformation. This was determined by NOESY experiments, as the $^2S_0$ conformer displays a characteristic NOE between H-2 and H-5. Canales et al. used NOESY spectra to show that iduronate residues in a HS hexasaccharide display an equilibrium between $^1C_4$ and $^2S_0$ when bound to FGF-1. The authors suggested that this flexibility, combined with flexibility of the lysine and arginine side chains which interact with the sulfate groups of the hexasaccharide, may be able to alleviate the entropic penalty of binding. The $^1C_4$/2$S_0$ equilibrium has also been observed for an iduronate residue in a HS tetrasaccharide binding to interleukin-10 (IL-10).

The HS binding sites of proteins can also be studied using NMR to identify the amino acid residues involved and the saccharide-protein interactions occurring. $^1$H-$^15$N HSQC experiments have been used to probe the HS binding sites of multiple proteins,
including SDF1α, interleukin-8, human-β-defensin-2, heparanase and interferon-γ. NMR has also been used to study more complex biological systems, such as the interactions between FGF-2, FGF receptor and a HS pentasaccharide, allowing a 3D structure for the complex to be modelled.

Overall, NMR has proved to be a useful technique for investigation of HS-protein binding, giving information on minimum binding sequences, conformation of the saccharide when bound, and the protein binding site, and the potential to model the 3D structure of the complex.

4.3 X-Ray Crystallography for Investigation of HS-Protein Binding

X-ray crystallography has also been widely utilised to investigate the binding of HS to proteins. Proteins may be co-crystallised with HS oligosaccharides, or more often the crystalline protein may be immersed in a solution of the saccharide to form a complex. X-ray crystallography can be used in conjunction with binding assays to investigate the minimum binding sequence for a protein. Hung et al. synthesised all 48 of the theoretical HS disaccharides and used ITC to assess binding to FGF-1. Four disaccharides that were found to bind were then co-crystallised with FGF-1 and the complexes investigated using X-ray crystallography, which showed no contact between 6-O-sulfate or carboxylate groups of HS with the protein. When the same procedure was applied for FGF-2, three disaccharides were observed to bind, again 6-O-sulfate did not make contact with the binding site but did show an intermolecular interaction with a second FGF-2 molecule.

The conformation of protein-bound HS oligosaccharides may be studied using X-ray crystallography. Capila et al. used X-ray crystallography to investigate the binding of a heparin tetrasaccharide to the anticoagulant protein annexin V, and observed that the IdoA residue interacting with the protein adopted the $^{2}S_{0}$ conformer, but the non-interacting IdoA residue adopted the $^{1}C_{4}$ conformation. Annexin V displayed two HS binding sites on opposite surfaces. Lietha et al. also noted differences in the conformation of IdoA residues in a tetradecasaccharide heparin fragment when bound to the growth factor neurokinin 1 (NK1).

The focus of many HS-protein binding studies using X-ray crystallography has been to identify the HS binding site of the protein and characterise the interactions between the amino acids present and the saccharide. The HS binding sites of multiple proteins have been investigated using X-ray crystallography including SDF1α
(combined with NMR to investigate solution binding), FGF-2, heparinase, and heparanase. More complex interactions between HS, protein and protein receptor can also be investigated using X-ray crystallography. Pellegrini et al. used X-ray crystallography to study the interactions between protein FGF-1, FGF receptor (specifically FGFR2, one of the four FGF receptors in vertebrates) and a heparin decasaccharide. The heparin decasaccharide bridges two FGF-1 molecules to form a dimer. Each FGF-1 molecule also binds a receptor, resulting in an overall 2:2:1 FGFR2-FGF-1-heparin decasaccharide stoichiometry. Schlessinger et al. carried out a similar study for FGF-2 using FGFR1 receptor. In this case, each heparin decasaccharide molecule interacts with one FGF-2 molecule and one FGFR1 molecule. The heparin decasaccharide also makes hydrogen bond contacts to another FGF-2-FGFR1-heparin complex, resulting in an overall stoichiometry of 2:2:2.

X-ray crystallography may be considered better suited to more complex ternary systems such as these than NMR. However, the crystallisation of proteins remains a challenge, and could be a barrier to the use of X-ray crystallography to investigate the interaction of HS with less well-studied proteins. In addition, X-ray crystallography can only provide a static, solid-state picture of the interaction being studied.
5. SINGLE MOLECULE FLUORESCENCE

5.1 Outline of Single Molecule FRET

The phenomenon known as Förster Resonance Energy Transfer (FRET) occurs when two fluorophores are in close proximity, typically 2–10 nm apart.\textsuperscript{206} Excitation of one fluorophore, termed the donor, causes non-radiative energy transfer to the second fluorophore (acceptor, Figure 7). The energy transfer operates via a dipole-dipole coupling, which results in FRET efficiency being proportional to \(1/r^6\) (where \(r\) is the distance between the two fluorophores, Equation 1).\textsuperscript{207} FRET efficiency may be determined from the difference in fluorescence intensity, anisotropy, lifetime or photobleaching rate of the donor in the absence and presence of the acceptor.\textsuperscript{208}

\[
E = \frac{1}{1 + \left(\frac{r}{R_0}\right)^6}
\]

\textbf{Equation 1.} Relationship between FRET efficiency (\(E\)) and distance between chromophores (\(r\)), \(R_0\) is the Förster distance of the donor-acceptor pair (distance at which energy transfer is 50% efficient).\textsuperscript{209}

FRET is most commonly used in ensemble form (i.e. experiments involving multiple molecules), both \textit{in vivo} and \textit{in vitro}. Applications of ensemble FRET include investigation of ligand binding,\textsuperscript{210} enzyme activity assays,\textsuperscript{211} and investigation of the distribution, assembly and transport of biomolecules.\textsuperscript{212,213} Ensemble FRET has wide applicability due to the range of suitable fluorophores, including fluorescent proteins such as green fluorescent protein (GFP),\textsuperscript{214} small molecule derived moieties such as the
dansyl group or cyanine dyes, and intrinsic fluorophores such as tryptophan in proteins.\textsuperscript{215}

Ha \textit{et al.} reported the first example of smFRET between a donor and an acceptor fluorophore attached to a DNA strand in 1996.\textsuperscript{216} In smFRET a single FRET event is analysed independently of all others. Therefore, the value of $R_0$ is not averaged in the same way it would be in ensemble FRET, in which different orientations of the dyes and fluorescence quantum yields lead to variation of $R_0$. This means that smFRET is much more suitable for reliable measurement of distances on the nanometre scale, whereas ensemble FRET cannot usually give accurately measured distances.\textsuperscript{217,218}

Additionally, as each FRET event is independently analysed, smFRET is more suited than ensemble FRET to the study of dynamic processes e.g. protein folding,\textsuperscript{219} as rare events or states with a low population of molecules will not be ‘averaged out’ which may occur in ensemble measurements (Figure 8).\textsuperscript{220-222} smFRET measurements may be carried out on molecules diffusing through a confocal microscope at picomolar concentrations,\textsuperscript{223} or immobilised on a surface using total internal reflection (TIRF) microscopy.\textsuperscript{224}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Molecular population & \textit{Ensemble measurements} & \textit{Single molecule measurements} \\
\hline
Homogenous view of the overall population & Parameters determined for entire population (averaging) & Parameters determined for one single molecule over time \\
\hline
Molecular population & & \\
\hline
Identification of individual states & Parameters determined for large number of randomly selected single molecules & Parameters determined for one single molecule over time \\
\hline
Static or dynamic heterogeneous description of population & Parameters determined for large number of randomly selected single molecules & Parameters determined for one single molecule over time \\
\hline
\end{tabular}
\end{table}

\textbf{Figure 8.} Comparison of ensemble (top) and single molecule (bottom) methods for the analysis of a heterogeneous static (left) or dynamic (right) molecular population. Redrawn from ref. 222.
5.2 Use of smFRET for Study of Biological Interactions

Single molecule FRET has been applied for the study of a range of biological systems. Following the first smFRET example, Ha and co-workers applied the technique to analysis of a number of unusual DNA motifs. Holliday junctions are composed of four double-stranded DNA (dsDNA) strands and can exist in multiple conformations. These structures are an intermediate in the repair of dsDNA breaks and in genetic recombination, and are also used in DNA nanotechnology. Previously, study of the conformational kinetics of Holliday junctions was precluded by the inability to synchronize a single conformer in solution, meaning ensemble techniques could not be used. Ha et al. therefore applied smFRET to investigate the dynamics of the junction, determining the rates for conformer transitions and the effect of counterion (e.g. Mg$^{2+}$) concentration. smFRET was also used by Ha and co-workers to study the conformational dynamics of G-quadruplexes, which are stacks of square planar tetrads composed of guanine-rich DNA sequences. Telomeric DNA, a target for anticancer drugs, often forms G-quadruplexes; a better understanding of conformational dynamics of these structures is proposed to assist in drug design. A number of other DNA motifs have been studied using smFRET, including hairpins, triplexes and bends/kinks.

smFRET has also been employed for analysis of protein folding, which is typically a heterogeneous process. Initially, smFRET was used for study of small proteins typically displaying two-state folding (i.e. folding without intermediates). Haran et al. recently used adenylate kinase as a model system for large, multi-domain proteins, identifying six metastable states using smFRET. Intrinsically disordered proteins (IDPs), which partly or completely lack a 3D structure and have been implicated in cancer and neurodegenerative disease, have also been studied using smFRET. $\alpha$-Synuclein is an IDP implicated in Parkinson’s disease (PD) due to the presence of aggregates in PD brain and mutations in the $\alpha$-synuclein gene being linked to familial PD. Deniz et al. observed two helical conformations for micelle-bound $\alpha$-synuclein using smFRET. Subsequent work showed a PD-related mutation in the protein caused the protein to favour one structure over another. Klelmerman et al. used smFRET between donor dye-labelled (Alexa Fluor® 488) $\alpha$-synuclein and acceptor dye-labelled (Alexa Fluor 594) $\alpha$-synuclein to determine the distribution of oligomer sizes for the wild type and mutant forms, and to show a conformational change can occur from the initially formed oligomers to more stable structures.
Despite the increasingly widespread use of smFRET for investigation of biological systems, carbohydrate interactions have rarely been explored. Kaback et al. labelled lactose permease (LacY) with donor and acceptor dyes and studied the FRET response in the presence of an unlabelled binding galactose derivative, observing conformational change upon binding of the sugar. Kim and co-workers performed similar experiments with maltose-binding protein, but expanded the method to include a labelled maltose ligand for three-colour smFRET and observed that the ligand preferentially binds to the ‘open’ conformation of the protein. Although examples of smFRET with carbohydrates are currently limited, the extensive application of the method to study of DNA and proteins paves the way for application to other classes of biomolecules.
6. FLUORESCENT LABELLING METHOD FOR HS OLIGOSACCHARIDES

6.1 Fluorescent Labelling via Aldehyde Tag

6.1.1 Synthesis of HS Disaccharide with Aldehyde Tag

In order to access fluorescently labelled HS oligosaccharides for single molecule fluorescence studies, a protocol for fluorophore conjugation is required. Methodology for addition of a latent aldehyde tag at the reducing end of HS oligosaccharides and deprotection to give a reactive aldehyde tag has been developed within the Gardiner group.\textsuperscript{246} Disaccharide 135 has previously been synthesised and the aldehyde tag reduced with tritiated sodium borohydride. The radiolabelling of disaccharide 135 was used as a model reaction for radiolabelling of a HS dodecasaccharide.\textsuperscript{247} This aldehyde tag was chosen as a starting point for development of a fluorophore conjugation protocol, as the aldehyde moiety is often used in the labelling of open-chain monosaccharides by reactions such as reductive amination.\textsuperscript{248} In addition, genetically-encoded aldehydes have been utilised for fluorescent labelling of proteins,\textsuperscript{249} hence aldehyde-reactive dyes are commercially available. Disaccharide 135 was selected as an initial substrate for fluorophore conjugation. Once a labelling method using the aldehyde tag had been developed, it could be applied to longer oligosaccharides bearing the aldehyde tag which were already available from previous work in the group.\textsuperscript{246,250}

Firstly, glycosylation of protected thioglycoside 128\textsuperscript{a} with (S)-(−)-2,3-dibenzylxyloxy-1-propanol was performed using the NIS/AgOTf activating system\textsuperscript{251} to afford 129 (82\%, Scheme 23). The mechanism of activation of thioglycosides with NIS/Lewis acid is not fully known; NIS/AgOTf is believed to be a source of I\textsuperscript{+} which iodinates the sulfur atom and allows ISPh to act as a leaving group, which subsequently undergoes disproportionation to form iodine and PhSSPh.\textsuperscript{252} Formation of the 1,2-trans product (α anomer) is expected due to neighbouring group participation by the benzoyl group at C-2 which results in attack by the glycosyl acceptor from under the plane (as drawn).

\textsuperscript{a} Synthesis of 128 was performed by Dr Steen U. Hansen, Gardiner group, University of Manchester.
Scheme 23. Reagents: (i) (S)-(−)-2,3-dibenzylxyloxy-1-propanol, NIS, AgOTf, CH₂Cl₂, 4 Å mol. sieves, 0 °C, 82%.

Deprotection steps to furnish the required disaccharide were then carried out. The PMB group of 129 was removed by treatment with ceric ammonium nitrate (CAN) to afford 130 in 82% yield (Scheme 24). This deprotection is selective for the PMB group over the benzyl groups, as PMB is more readily oxidised than benzyl (oxidation potentials +1.65 V and +2.00 V respectively). Treatment of 130 with LiOH hydrolysed the methyl ester and removed the benzoyl protecting group to give acid 131 in 88% yield. Sulfation of the free hydroxyl groups was performed using sulfur trioxide-pyridine complex to afford 132 in 86% yield. HRMS confirmed the desired product had been obtained with both sulfate groups installed. Sulfation of O-4 of the glucosamine residue was required to ensure that the O-4/O-3 hydroxyls did not exist as a diol, as this diol could be cleaved in the presence of sodium periodate used in the final step to reveal the aldehyde handle. Hydrogenation of 132 was performed using Pd(OH)₂/C as catalyst to reduce the azido group and remove the benzyl protecting groups (therefore unmasking the protected diol) affording disaccharide 133 quantitatively. N-Sulfation of 133 furnished disaccharide 134 in 74% yield after size exclusion chromatography (Sephadex® LH-20 resin).
The diol moiety of 134 was cleaved with sodium periodate to afford aldehyde 135 (52%, Scheme 25). The mechanism of periodate cleavage involves formation of a five membered intermediate with both oxygen atoms bonded to iodine, which then collapses to yield desired aldehyde product 135, formaldehyde and NaIO₃.²⁵⁴

Scheme 24. Reagents: (i) CAN, MeCN/H₂O, RT, 85%; (ii) LiOH, THF/MeOH/H₂O, RT, 88%; (iii) SO₃·pyridine, pyridine, RT, 86%; (iv) H₂, Pd(OH)₂/C, EtOH, 40 °C, quant.; (v) SO₃·pyridine, NaHCO₃, H₂O, RT, 72%.

Scheme 25. Reagents: (i) NaIO₄, H₂O, RT, 54%.
The proton NMR spectrum of 135 did not exhibit the expected aldehyde proton signal in the δ 9.5–10.5 ppm region, however an additional \( \text{CH} \) signal was observed at ~5.16 ppm. \(^{13}\text{C} \) NMR also showed no signal in the aldehyde region for 135 (δ 180–220 ppm). These data suggest that hydrate 136 or ring closed species 137 are the dominant form in D\(_2\)O at ambient temperature (Scheme 26). Linhardt et al. reported a similar observation for aldehyde 138, which was found by NMR and mass spectrometry to exist as hydrate 139.\(^{255}\) High resolution mass spectrometry measurements for 135 gave an isotope pattern consistent with the theoretical model for [M–2Na]\(^2^-\), indicating the desired product was formed.

Scheme 26. Proposed equilibria between aldehyde 135, hydrate 136 and ring closed form 137, comparable equilibrium between 138 and 139 reported by Linhardt et al.\(^{255}\)

6.1.2 Attempted Labelling with Aldehyde-reactive Dye

Aldehyde-reactive dyes such as hydroxylamines (aminoxyacetamides) and hydrazides are commercially available for numerous fluorophores. Alexa Fluor 488 was selected as a suitable fluorophore (FRET donor) for conjugation to disaccharide 135, having previously been utilised for single molecule measurements on DNA in the Magennis laboratory.\(^{256}\) Fluorophores for single molecule studies are required to have high brightness (extinction coefficient >50,000 M\(^{-1}\) cm\(^{-1}\) and quantum yield >0.1) and good photostability.\(^{257}\) Alexa Fluor 488 has an extinction coefficient of 73,000 M\(^{-1}\) cm\(^{-1}\) and quantum yield of 0.92 (manufacturer data).

Direct labelling of aldehyde-tagged disaccharide 135 was attempted using Alexa Fluor 488 C\(_5\) hydroxylamine dye at pH 4.65 with aniline as catalyst following similar labelling procedures for proteins (Scheme 27).\(^{258}\) Analysis of the reaction by \(^1\text{H} \) NMR
after 24 hours at room temperature showed complete consumption of the starting material, none of the desired conjugate was isolated and the unreacted dye was recovered. An alternative strategy was therefore proposed which involved reaction of the aldehyde moiety with a mono-protected diamine, followed by deprotection, to afford a disaccharide with a reactive amine group for labelling, as amine moieties have been previously used for the conjugation of HS oligosaccharides (Section 3.4).

**Scheme 27.** Reagents: (i) Alexa Fluor 488 C₅ hydroxylamine, aniline, pH 4.65 buffer, RT, 24 h.

### 6.1.3 Attempted Labelling via Reductive Amination

Reductive amination has been employed for the conjugation of saccharides to fluorophores²⁵⁹ and biomolecules²⁶⁰ bearing an amine moiety. Although the open chain aldehyde form of the sugar is usually only present in low concentration and the formation of an imine is an equilibrium process, the reaction can be driven forward by reduction of the imine. Sodium cyanoborohydride reduces imines at pH 6–8, but only reduces aldehydes below pH 4, and is therefore commonly used as a selective reducing agent for reductive amination.²⁶¹

A procedure for the labelling of HS oligosaccharides bearing an aldehyde tag using reductive amination was proposed (Scheme 28). Reductive amination between the aldehyde moiety and Cbz-protected ethylenediamine, followed by removal of the Cbz group by hydrogenation should allow conjugation to an amine-reactive fluorophore.
Chapter Two: Results and Discussion

Scheme 28. Reagents: (i) N-Z-ethylenediamine hydrochloride, NaCNBH$_3$, H$_2$O, RT; (ii) H$_2$, Pd(OH)$_2$/C, H$_2$O, RT.

As an initial test of this methodology, D/L-glyceraldehyde (143) was used in place of disaccharide 135. Reductive amination of D/L-glyceraldehyde at pH 4.65 with N-Z-ethylenediamine afforded expected product 144 but no reaction was observed at pH 8 (Scheme 29). Formation of 144 was confirmed by mass spectrometry of the crude reaction mixture, as the remaining diamine reagent and sodium cyanoborohydride were not easily removed by size exclusion or flash chromatography. The crude product was subjected to hydrogenation conditions to yield 145, again confirmed by mass spectrometry.

Scheme 29. Reagents: (i) N-Z-ethylenediamine hydrochloride, NaCNBH$_3$, pH 4.65 buffer, RT; (ii) H$_2$, Pd(OH)$_2$/C, H$_2$O, RT.

Following these results, reductive amination using the same conditions was attempted on disaccharide 135. No reaction was observed at pH 7 over 24 h. When the reaction was carried out at pH 4.65 for 24 h, $^1$H NMR of the reaction mixture showed both the disaccharide and diamine starting materials remaining. However, HRMS showed the product ion [M–4Na+2H]$^2$ at m/z 406.5453 (calculated m/z 406.5443), indicating a small amount of the expected product had formed. The reductive amination may be slow because sodium cyanoborohydride is unable to reduce the imine intermediate at the required rate to drive the hydrate-aldehyde and aldehyde-imine equilibria toward the product.
Aniline has been used as a catalyst for the reductive amination between the open-chain aldehyde moiety of chitooligosaccharides and propargylamine in the presence of sodium cyanoborohydride. The mechanism for the reaction is proposed to involve formation of an imine with aniline, which then undergoes transimination with propargylamine followed by reduction to give the desired product. This procedure was applied to the reductive amination of disaccharide 135 and N-Boc-ethylenediamine, ensuring that the ratio of N-Boc-ethylenediamine to aniline was 10:1, as this had been found to minimise formation of the aniline derivative of the starting material (Scheme 30). However, 1H NMR of the reaction mixture showed a number of compounds were present. LCMS of the product mixture after removal of salts by size exclusion chromatography (Sephadex® G-25) showed aniline derivative 147 (m/z 779, [M–Na]) was formed, and formation of 146 was not observed. Formation of the aniline derivative was also observed when the reaction was performed with a HS dodecasaccharide substrate. This indicates that the transimination reaction is slower than the reduction of the aniline imine intermediate, and aniline is not a suitable catalyst for this reaction.

Scheme 30. Reagents: (i) N-Boc-ethylenediamine, aniline, NaCNBH3, pH 5.0 buffer, RT.

Overall, the aldehyde tag could not be utilised for labelling in these experiments, either via direct reaction with Alexa Fluor 488 hydroxylamine dye or using amine linkers in reductive amination. It is possible that this is due to aldehyde 135 existing as the hydrate or ring-closed form in solution, as observed by NMR, meaning there is little free aldehyde to react and drive the equilibria forward to the product. Therefore, an alternative ‘handle’ for addition of a fluorophore to HS oligosaccharides was investigated.

b This reaction was performed by Dr Gavin J. Miller, Gardiner group, University of Manchester.
6.2 Fluorescent Labelling via Amine Tag

6.2.1 Attachment of Amine Tag to HS Disaccharide

Due to the difficulty experienced in functionalising disaccharide 135 via the aldehyde tag, an alternative ‘handle’ for fluorophore conjugation to HS oligosaccharides was desired. Based on the reported use of amines for the conjugation of HS oligosaccharides post-deprotection (Section 3.4), and a number of examples in the literature of the use of Cbz-protected amine handles on HS oligosaccharides, a Cbz-protected amine was selected as a handle. A 2-carbon linker was selected in order to try and reduce though-space interactions between the sugar and fluorophore; it was perceived that a longer handle would result in additional flexibility of the dye.

Disaccharide 128 was reacted with N-Z-ethanolamine under NIS/AgOTf activating conditions to afford disaccharide 148 (81%, Scheme 31). The PMB removal step was not required as there is no disadvantage to having the glucosamine 3,4-diol present in the deprotected product in the absence of the periodate cleavage step. Disaccharide 148 was subjected to ester hydrolysis with LiOH (94%) and sulfation using sulfur trioxide pyridine complex (81%) to afford 150.

Selective reduction of the azido group of 150 over the benzyl and Cbz protecting groups was required to allow subsequent N-sulfation. The Staudinger reaction using trimethylphosphine was chosen, as this has been successfully applied previously in HS oligosaccharide synthesis. Disaccharide 151 was obtained in 75% yield after purification by size exclusion chromatography (Sephadex LH-20). The mechanism of the Staudinger reaction is believed to involve formation of a phosphazide intermediate followed by conversion to an iminophosphorane; attack by water then hydrolys this intermediate to the amine plus phosphine oxide. Disaccharide 153 bearing the desired primary amine handle was synthesised by N-sulfation of 151 (40%) followed by hydrogenation in the presence of Pd/C at 40 °C for 2 days (47%).
Scheme 31. **Reagents:** (i) N-Z-ethanolamine, NIS, AgOTf, CH$_2$Cl$_2$, 4 Å mol. sieves, 0 °C, 81%; (ii) LiOH, THF/MeOH/ H$_2$O, 0 °C, 94% (iii) SO$_3$·pyridine, pyridine, RT, 81%; (iv) PMe$_3$ in THF, NaOH, THF, RT, 75%; (v) SO$_3$·pyridine, Et$_3$N, pyridine, RT, 40%; (vi) H$_2$, Pd/C, EtOH/H$_2$O, 40 °C, 47%.

6.2.2 Alternative Synthesis of Amine-tagged Disaccharide

6.2.2.1 Synthesis of Glucoazide Donor

An alternative synthesis of disaccharide 153 was attempted in which N-Z-ethanolamine was glycosylated to a protected iduronate monosaccharide, followed by selective deprotection at C-4 and glycosylation with a glucosamine monosaccharide.

Glucoazide donor 162 was prepared from D-glucosamine hydrochloride (154), following procedures previously developed in the Gardiner group (Scheme 32). Firstly, the amine of 154 was protected with the trichloroacetyl group and the free hydroxyls were acetylated to give glycosyl acetate 155. In a modification to the previously used procedure, the acetylation was achieved with Ac$_2$O and catalytic TsOH with acetonitrile...
as a co-solvent,\textsuperscript{266} avoiding the use of pyridine as solvent. In addition, \textbf{155} was used crude in the next step to afford thioglycoside \textbf{156}, avoiding the need for crystallisation as previously reported, with no impact on yield (both 50\% over three steps). Removal of the acetyl and trichloroacetyl groups with potassium carbonate followed by diazo transfer with the reagent imidazole-1-sulfonyl azide\textsuperscript{267–269} (\textbf{157}) gave triol \textbf{158} in 73\%.

Protection of the amino group as an azide has been widely employed in HS oligosaccharide synthesis due to its non-participating nature, encouraging formation of 1,2-cis glycosidic linkages (\(\alpha\) anomer).\textsuperscript{270} Triol \textbf{158} was then protected with the \(p\)-methoxybenzylidene group, followed by benzyl protection of O-3 to give \textbf{160}.

\begin{align*}
\text{Scheme 32. Reagents:} & \quad \text{(i) } \text{Cl}_3\text{COCl, Et}_3\text{N, MeOH, RT; (ii) } \text{Ac}_2\text{O, TsOH, MeCN, 60 °C;} \\
& \quad \text{(iii) PhSH, TMSOTf, CH}_2\text{Cl}_2, \text{RT, 50% over three steps; (iv) } \text{K}_2\text{CO}_3, \text{MeOH/H}_2\text{O, 60 °C, then imidazole-1-sulfonyl azide (157), } \text{K}_2\text{CO}_3, \text{MeOH/H}_2\text{O, 73%; (v) } \text{p-Anisaldehyde dimethyl acetal, camphorsulfonic acid, 4 Å mol. sieves, MeCN, RT, 61%; (vi) } \text{BnBr, NaH, DMF, RT, 93%;} \\
& \end{align*}

Regioselective opening of the \(p\)-methoxybenzylidene was then required to place the PMB group at O-4. This has previously been achieved within the Gardiner group for \(\alpha\)-\textbf{160} using dibutylboron triflate and BH\(_3\)·THF complex.\textsuperscript{318} However, when this was attempted using \(\beta\)-\textbf{160}, a mixture of products was obtained indicating a lack of selectivity (Scheme 33). Other Lewis acids previously employed for regioselective opening of benzylidenes to O-4 ethers were also tested, such as Cu(OTf)\(_2\) and TMSOTf,\textsuperscript{271,272} but the reaction was again observed to be unselective. A by-product which was inseparable with the product was formed, believed to be 6-OPMB, 4-OH derivative \textbf{163}, and diol \textbf{164} was also observed. Synthesis of \textbf{163} was attempted by treatment of \(\beta\)-\textbf{160} with Et\(_3\)SiH and BF\(_3\)·OEt\(_2\), which should afford the 4-hydroxyl product.\textsuperscript{273} However, only formation of diol \textbf{164} was observed. Use of cyanuric chloride and NaCNBH\(_3\), which has also been reported to selectively open benzylidenes to the 4-hydroxyl product,\textsuperscript{274} afforded mainly diol \textbf{164} at 55 °C and a mixture of products at RT.
from which desired product 163 was not obtained. Recently, Kim et al. reported the synthesis of 163 by selective opening of β-160 using NaCNBH₃ and TFA,²⁷⁵ but noted the product was too unstable to collect any spectroscopic data (the compound was identified by mass spectrometry only).²⁷⁶ Therefore, it is possible that decomposition of 163 occurred in these reactions and hence the product was not isolated.

Following the method reported by Tatina et al.,²⁷⁷ use of cyanuric chloride and NaBH₄ for selective opening of β-160 to 161 was successful, affording the product in 84% yield, with no formation of 163 or 164 observed. Finally, benzylation of O-6 furnished required glycosyl donor 162 (72%).

Scheme 33. Reagents: (i) Cyanuric chloride, NaBH₄, MeCN, RT, 84%; (ii) BnBr, NaH, THF, 60 °C, 72%; (iii) Et₃SiH, BF₃·OEt₂, RT; (iv) Cyanuric chloride, NaCNBH₃, RT or 55 °C.

6.2.2.2 Attempted Synthesis using Mono-protected Amine Tag

To access the required acceptor bearing the amine handle, thioglycoside 165, which was available from previous work in the Gardiner group, was protected at O-4 with the chloroacetyl (ClAc) group (>99%, Scheme 34). Iduronate thioglycoside 166 was glycosylated with N-Z-ethanolamine to afford 167 (46%). Formation of the α anomer at this stage could not be confirmed due to overlap of H-1 and H-2 in the ¹H NMR spectrum. The chloroacetyl group of 167 was selectively removed in the presence of the benzoyl group using thiourea to afford 168 (81%). The mechanism of this deprotection involves S_N2 displacement of the chlorine atom by the sulfur atom of thiourea, followed by intramolecular displacement of the product alcohol by the NH₂ group of the intermediate.²⁷⁸

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²⁷⁸ This compound was synthesised by Dr Steen U. Hansen, Gardiner group, University of Manchester.
Scheme 34. Reagents: (i) Chloroacetyl chloride, pyridine, CH$_2$Cl$_2$, RT, >99%; (ii) N-Z-ethanolamine, NIS, AgOTf, CH$_2$Cl$_2$, 4 Å mol. sieves, 0 °C, 46%; (iii) Thiourea, EtOH, 70 °C, 81%.

Analysis of the $^1$H NMR spectrum of 168 showed a singlet for H-1, indicating the desired α anomer had been formed in the glycosylation by comparison with other iduronate monosaccharides synthesised in the Gardiner group (Table 1).  

Table 1. Comparison of H-1 NMR signals for α and β anomers of iduronate monosaccharides synthesised within the Gardiner group.  

<table>
<thead>
<tr>
<th>Number</th>
<th>R$^1$</th>
<th>H-1 NMR signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-169</td>
<td>α-OMe</td>
<td>singlet</td>
</tr>
<tr>
<td>β-169</td>
<td>β-OMe</td>
<td>doublet, $J = 2.0$ Hz</td>
</tr>
<tr>
<td>α-165</td>
<td>α-SPh</td>
<td>singlet</td>
</tr>
<tr>
<td>β-165</td>
<td>β-SPh</td>
<td>doublet, $J = 0.9$ Hz</td>
</tr>
</tbody>
</table>

Glycosylation of iduronate acceptor 168 with glucoazide donor 162 was performed using NIS/AgOTf as activator (Scheme 35). However, expected disaccharide product 148 was not isolated from the reaction mixture; $^1$H NMR showed complete consumption of donor and acceptor and multiple products were present which could not be separated by column chromatography.
Seeberger et al. have reported low-yielding glycosylations of iduronate acceptors bearing $N$-benzyloxycarbonyl-5-amino-pentane linkers, suggesting this was due to the presence of a free NH group in the protected amine tag on the iduronate acceptor, which has a deactivating effect.\(^{153}\) This effect is attributed to the hydrogen-bonding capability of the NH moiety and is manifested in the poor reactivity of GlcNAc glycosyl acceptors.\(^{280}\) Seeberger and co-workers reported that removal of the NH group in the linker by use of a (benzyl)benzyloxycarbonyl-amino linker allowed higher yields to be obtained.\(^{153}\) Therefore, it was proposed that the attachment of a doubly-protected amine tag to the iduronate monosaccharide would allow the glycosylation to proceed successfully. The outcome of this glycosylation also suggests that the mono-protected amine tag would not be suitable as a ‘reducing end cap’ for the synthesis of longer oligosaccharides (unlike the protected aldehyde handle\(^{246}\)) as all subsequent glycosylations may be impaired by the presence of the NH group.

### 6.2.2.3 Synthesis of Disaccharide using Doubly-protected Amine Tag

Glycosylation of iduronate monosaccharide 166 with $N$-benzyl-$N$-(benzyloxycarbonyl)ethanolamine (170, prepared in 68% yield by reaction of $N$-benzyl-ethanolamine with benzyl chloroformate\(^{281}\)) was performed to give 171 in 77% yield (Scheme 36). Subsequent removal of the chloroacetyl protecting group with thiourea gave acceptor 172 in 80% yield. Both 171 and 172 displayed additional signals in their NMR spectra, which were attributed to the presence of rotamers as observed by Seeberger and co-workers for iduronate monosaccharides and oligosaccharides with this residue at the reducing end.\(^{153}\)
Scheme 36. Reagents: (i) Benzyl chloroformate, EtOAc/saturated aq. NaHCO₃, RT, 68%; (ii) 170, NIS, AgOTf, CH₂Cl₂, 4 Å mol. sieves, 0 °C, 77%; (iii) Thiourea, EtOH, 70 °C, 80%.

Glycosylation of 172 with 4-OPMB glucoazide donor 162 afforded desired disaccharide 176 in 27% yield (Scheme 37). TLC and NMR analysis showed a number of by-products had formed which could not be separated by column chromatography. Additionally, HRMS indicated there had been partial iodination of the PMB group on the product, [M–H+I+NH₄]⁺ was observed at m/z 1300.3980 (calculated m/z 1300.3986). This has previously been observed in the Gardiner group when PMB has been used as a protecting group in glycosylations. Wacowich-Sgarbi and Bundle also observed this and reported that the iodinated PMB group could not be removed by DDQ. Mehta and Whitfield observed PMB iodination and reported that the iodinated PMB group could be removed with aqueous TFA.

Glycosyl donor 162 has a high number of electron donating protecting groups such as benzyl and PMB (so-called ‘armed’ donor). Iodine has been used as an activating system for armed thioglycoside donors originally by Field et al. who proposed the increased nucleophilicity of the sulfur should allow attack on molecular iodine, subsequently allowing oxocarbenium ion formation. When glycosylation of 172 with 162 was performed with iodine as promoter, consumption of the starting materials was observed by ¹H NMR and none of the desired product was isolated.

Based on the outcome of these glycosylations, 4-O-chloroacetyl glucosamine derivative 175 was proposed as a less armed donor for glycosylation with 172. Regioselective reductive ring-opening of 173 with triethylsilane afforded 174 in 84% yield, which was protected to give donor 175. Subsequent glycosylation with acceptor 172 afforded desired disaccharide 177 in 30% yield, along with 50% recovery of acceptor.

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¹ Compound 173 was synthesised by Garrett T. Potter, Gardiner group, University of Manchester.
Overall, glycosylations of glucoside donors with iduronate monosaccharides bearing an amine handle were poor. Although removal of the NH by use of a (benzyl)benzyloxy carbonyl-amino linker improved the glycosylation outcome, the yields were still low. Changing the O-4 protecting group on the donor from PMB to chloroacetyl, thus changing the electronics of the donor, did not considerably improve the yield. It is possible that iduronate monosaccharides bearing protected amine handles are reduced in reactivity relative to other iduronate acceptors e.g. methyl glycosides, previously used in the Gardiner group in glycosylations with glucoside SPh donors. Based on the results of these glycosylations, attachment of the amine tag at the disaccharide level is preferable.

### 6.2.3 Conjugation of Fluorophore to Amine-tagged Disaccharide

With amine-tagged disaccharide 153 in hand, conjugation to a fluorescent dye was then carried out to provide an initial analyte for single molecule spectroscopy (Scheme 38). Alexa Fluor 488 SDP ester was mixed in a shaker with 153 in 0.1 M aqueous NaHCO₃ buffer at room temperature for 96 h. To solubilise the dye, DMSO was used as a cosolvent. The reaction mixture was lyophilised to afford desired fluorescent conjugate 178 and was not further purified. Only 0.6 equivalents of the dye were used in order to ensure no free dye was left after the reaction, it was expected that any unreacted dye would be difficult to separate from the disaccharide due to similar molecular weights.
Formation of labelled disaccharide 178 was confirmed by mass spectrometry and $^1$H NMR, which as expected showed a mixture of unlabelled (153) and labelled (178) disaccharide but crucially no free dye (Figure 9).

Scheme 38. Reagents: (i) Alexa Fluor 488 SDP ester, 0.1 M aqueous NaHCO₃, DMSO.
Figure 9. $^1$H NMR of labelled/unlabelled disaccharide 178/153 mixture (blue) and unlabelled disaccharide starting material 153 (green).
Reaction of disaccharide 153 with Cyanine3 dye in NHS form was also carried out (Scheme 39). The Cyanine3 dye is available in two forms, one which contains sulfate groups and one which does not. Preparation of the conjugates of disaccharide 153 with both the sulfated and non-sulfated dyes could be useful, as it is possible the presence of additional sulfate groups on the dye may have an effect on HS-protein binding. However, reactions of both Cyanine3-NHS ester dyes under the same conditions as for Alexa Fluor 488 SDP ester were unsuccessful. $^1$H NMR after five days showed only disaccharide 153 with no product observed. This may be due to a slower reaction rate between the amine and NHS ester, resulting in the dye hydrolysing over time in solution. The reaction was also attempted using only DMSO as solvent, but again no reaction was observed. Cyanine3 labelling of disaccharide 153 was also attempted using the maleimide form of the dye at both pH 8 (0.1 M NaHCO$_3$) and pH 9 (0.1 M NaHCO$_3$/0.1 M Na$_2$CO$_3$), but no reaction was observed in either case by $^1$H NMR.

Overall, Alexa Fluor 488 SDP reacted smoothly with amine tagged disaccharide 153, and this method should be directly applicable to any HS oligosaccharides bearing the amine tag. The analogous conjugations with Cyanine3 dyes were unsuccessful, this is attributed to the hydrolysis side-reaction which is more rapid for NHS esters than SDP esters. Typically this is compensated for in protein labelling by use of an excess of dye, but in the case of 153 this was not used due to the expected difficulties in separating the free dye from the disaccharide.
Scheme 39. Reagents: (i) Cyanine3-NHS dye, 0.1 M NaHCO₃ buffer/DMSO or DMSO; (ii) Cyanine3-maleimide dye, 0.1 M NaHCO₃ buffer/DMSO or 0.1 M NaHCO₃/0.1 M Na₂CO₃ buffer/DMSO.
7. SINGLE MOLECULE FLUORESCENCE MEASUREMENTS OF LABELLED HS DISACCHARIDE

7.1 Solution Measurements of Fluorescent Disaccharide

In order to determine the suitability of Alexa Fluor 488-labelled HS oligosaccharides such as disaccharide 178 for study of protein binding using single molecule fluorescence, initial measurements were made on the disaccharide to investigate dye behaviour and characteristics. Firstly, the ensemble absorption and emission spectra of 178 were recorded (N.B. the obtained unlabelled/labelled mixture of 153 and 178 was used in all fluorescence measurements but is referred to as 178 for simplicity). The spectra were compared to those of the free dye (Alexa Fluor 488 SDP ester) and single-stranded DNA (ssDNA) labelled with Alexa Fluor 488 dye. Whilst the absorption spectra of disaccharide 178 and free dye were very similar (Figure 10), the emission spectrum of 178 showed a blue shift relative to the free dye (Figure 11). The absorption and emission spectra of disaccharide 178 are almost identical to those of Alexa Fluor 488 labelled single stranded DNA, with the exception of the 200–350 nm region of the absorption spectrum where nucleic acids absorb.

![Graph showing absorption spectra](image)

**Figure 10.** Ensemble absorption spectra of: free dye (Alexa Fluor 488 SDP ester, black line), disaccharide 178 (red line) and Alexa Fluor 488 labelled ssDNA (blue line). *Inset:* comparison of free dye and disaccharide 178 absorption spectra, 450–550 nm.

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* Single molecule solution measurements detailed in this section were performed by the author and Dr Steven D. Quinn, Magennis group, University of Glasgow.
Figure 11. Normalised fluorescence emission spectra of: free dye (Alexa Fluor 488 SDP ester, black line), disaccharide 178 (red line) and Alexa Fluor 488 labelled ssDNA (blue line).

Disaccharide 178 was analysed using fluorescence correlation spectroscopy (FCS) using confocal microscopy. FCS is a technique in which the fluctuations in fluorescence intensity from a sample are analysed over time. The correlation between fluorescence at time \( t \) and at a subsequent time \( (t + \tau) \) is calculated for a large number of \( \tau \) values to give an autocorrelation function \( G(\tau) \). The fluorescence intensity of the sample depends on the number of fluorophores in the observation volume at a given time, which in turn depends on the diffusion characteristics of the fluorescent species. Therefore, FCS can be used to measure the diffusion constant and hydrodynamic radius of the fluorescent species. Although not formally a single-molecule technique, FCS requires dilute samples so that the number of molecules in the observation volume at a given time is low and each molecule makes a substantial contribution to the observed signal, reducing the effect of averaging and allowing fluorescence fluctuations to be observed.

FCS analysis of disaccharide 178 and free dye could be fitted to the same model, indicating a lack of perturbation on the dye by the disaccharide (Figure 12). The measured diffusion time (time spent in the observation volume) for 178 was 307±4 \( \mu s \), comparable to that of the free dye (254±3 \( \mu s \)). Diffusion time is related to hydrodynamic radius (i.e. the radius of a sphere having the same diffusion
characteristics as the molecule) by Equations 2 and 3, hence the hydrodynamic radii for 178 and free dye were calculated to be 1.18 nm and 0.98 nm respectively. From the average intensity recorded over the measurement time, and the number of fluorophores present in the detection volume (which is inversely proportional to the y-intercept of \( G(t) \) vs \( \tau \)) the counts per molecule were calculated. For disaccharide 178 this was 42 kHz/molecule and for the free dye 40 kHz/molecule; this similarity indicates a lack of perturbation in the fluorophore in disaccharide 178.

\[
\tau_D = \frac{s^2}{4D}
\]

**Equation 2.** Relationship between diffusion time (\( \tau_D \)) and translational diffusion coefficient (\( D \)) where \( s \) is radius of the confocal detection volume.\(^{291}\)

\[
D = \frac{kT}{6\pi\eta R}
\]

**Equation 3.** Relationship between translational diffusion coefficient (\( D \)) and hydrodynamic radius (\( R \)) where \( k \) is Boltzmann’s constant, \( T \) is temperature and \( \eta \) is viscosity of the solvent.\(^{291}\)

![Figure 12](image.png)

**Figure 12.** Fluorescence correlation spectra of disaccharide 178 (red) and free dye (black); points show fit, line shows correlation curve.
Disaccharide 178 was then analysed at single molecule level using multiparameter fluorescence detection (MFD) on a confocal microscope. MFD allows photon-counting detection in four channels, two red and two green, each colour having one detector for parallel polarised photons and one for perpendicular polarised photons. The MFD set-up allows simultaneous measurement of the fluorescence intensity, colour, lifetime and polarisation. When disaccharide 178 was analysed by MFD, the majority of the dye emission was collected in the green channels as expected, with the tail of the emission spectrum overlapping into the red channels. Only one population of fluorescent molecules was observed, with a lifetime centred on 4.1 ns, very similar to the lifetime for the same dye attached to dsDNA, indicating an unquenched dye (Figure 13). The free dye was observed to have a longer lifetime of 4.3 ns, this is attributed to the presence of the additional aromatic SDP group in the free dye which can alter the properties of the fluorophore. The anisotropy observed for disaccharide 178 was close to zero (Figure 13). Anisotropy values can be used to determine if the fluorophore can rotate freely, which is crucial to know for distance calculation in smFRET as the Förster radius $R_0$ is dependant on the orientation factor of the fluorophores’ dipoles ($\kappa^2$, Equation 4). When the donor and acceptor fluorophores freely diffuse and rotate in solution $\kappa^2$ can be approximated to 2/3. Fluorescence anisotropy of $<0.2$ is usually assigned to a $\kappa^2$ value of 2/3, hence the fluorophore in disaccharide 178 can be considered to be freely rotating. However, this does not necessarily mean that this would continue to be the case in the presence of another molecule, either labelled or unlabelled, as the disaccharide may have additional interactions which reduce free rotation of the dye.
Figure 13. 2D histogram plot of fluorescence lifetime ($\tau$) vs ratio of signal in the green detection channel ($S_G$) to that in the red detection channel ($S_R$) and the fluorescence anisotropy ($r$).

$$R_0 = 9780 \left( n^{-4} \kappa^2 \Phi_D J \right)^{\frac{1}{5}} \text{ Å}$$

Equation 4. Definition of the Förster radius ($R_0$) for FRET, where $n$ is refractive index of the buffer, $\kappa^2$ is the orientation factor of the fluorophores’ dipoles, $\Phi_D$ is the quantum yield of the donor in the absence of acceptor and $J$ is the overlap integral between donor emission and acceptor excitation spectra.\(^{292}\)

7.2 TIRF Microscopy of Vesicle-encapsulated Fluorescent HS Disaccharide

In order to study the behaviour of labelled disaccharide 178 over longer time periods, the molecules were encapsulated into small unilamellar vesicles (SUVs) which could then be immobilised onto a surface.\(^6\) Vesicle encapsulation has been widely used for single molecule studies as it avoids the need to functionalise the molecule of interest to allow direct attachment to a surface and allows mimicking of cellular entrapment.\(^{236,293,294}\) The SUVs used were composed of 98% L-α-phosphatidylcholine

\(^6\) Experiments described in this section were carried out by Dr Michael J. Morton and Aiden Rafferty, Magennis group, University of Glasgow.
(Egg-PC) and 2% of a biotinylated lipid (biotinyl-PE) and were formed by the extrusion method, in which the lipid is passed repeatedly through a membrane with holes of defined size. Dynamic light scattering (DLS) of SUVs formed in the presence of disaccharide 178 showed an average diameter of 120 nm. The presence of biotin in the SUVs allows for binding to the protein neutravidin, which in turn binds to a biotin-PEG linker on the glass coverslide surface (Figure 14).

![Diagram of vesicle encapsulation](image)

**Figure 14.** Schematic of immobilisation of vesicle-encapsulated disaccharide 178 (not to scale).

The resulting surface was imaged using total internal fluorescence reflection (TIRF) microscopy. In this technique, the sample is illuminated by an evanescent wave (non-propagating) generated by total internal reflection; as a result only fluorophores in a thin layer of the sample (<200 nm) are excited. Background fluorescence from molecules which are out of focus is reduced, leading to the improved signal-to-noise (S/N) ratio required to view single fluorophores. TIRF microscopy has been widely applied to
image single molecules in vivo which are close to the cell surface, for example interactions occurring at the cell membrane.  

Imaging of vesicle-encapsulated disaccharide 178 using TIRF microscopy allowed investigation of the number of disaccharide molecules in a given vesicle. Firstly, to check that the observed fluorescence was due to encapsulated disaccharide 178, the concentration of vesicles added to the slide was varied, resulting in a change in the number of fluorescent spots as expected (Figure 15). Observation of the fluorescence of single spots in the image over time shows fluorescence at a constant intensity, with no blinking (switching of fluorophores between fluorescent and non-fluorescent state) observed, followed by stepwise bleaching (irreversible destruction of the fluorophore in its excited state) of the fluorophore(s) contributing to that particular spot.

![Figure 15](image_url)

**Figure 15.** Representative TIRF images of immobilised vesicle-encapsulated disaccharide 178 at varying concentrations; left to right: 0 pM, 1 pM, 3 pM, 10 pM.

When vesicles were prepared using 500 nM disaccharide 178, predominantly single-step bleaching events were seen, indicating only one disaccharide was encapsulated per vesicle, with a smaller number of two- and three-step events (Figure 16). When the vesicles were prepared in the presence of 1 µM disaccharide 178, predominantly two-step bleaching events were seen; four- and five-step bleaching events were also observed. Vesicles prepared in the presence of 10 µM disaccharide 178 displayed exponential-like photobleaching decays, indicating a large number (>10) of encapsulated disaccharide molecules (Figure 17).
Figure 16. Representative 1-step (top), 2-step (middle) and 3-step (bottom) photobleaching traces for vesicle-encapsulated disaccharide 178 (500 nM) obtained using TIRF microscopy; coloured lines represent raw data and black line represents fits acquired using a 1D edge-detection algorithm.

Figure 17. Representative photobleaching traces for vesicle-encapsulated disaccharide 178 (10 µM) obtained using TIRF microscopy.
7.3 FCS of Fluorescently-labelled HS Disaccharide with FGF-1
Following initial single molecule measurements of labelled disaccharide 178 which indicated little perturbation of the Alexa Fluor 488 dye, an initial test of the suitability of FCS to study HS-protein binding was carried out. FCS can be used to determine if a ligand binds to a receptor by change in diffusion time. If a complex is formed between a fluorescent and non-fluorescent species, the resulting higher molecular weight fluorescent species will display a longer diffusion time in FCS measurements. If both bound and unbound fluorescent ligand are present in solution, and the brightness of the fluorophore is the same in both states, the autocorrelation function is given by Equation 5. Treatment of FCS data to determine the number of fluorophores in the bound and unbound states allows calculation of dissociation constant $K_d$. The presence of two fluorescent diffusing species results in a change in shape of the autocorrelation function, which can be matched to autocorrelation functions calculated from postulated diffusion coefficients (Figure 18). FCS has been applied to a number of different types of interactions including protein-protein and DNA-protein binding.

\[
G(\tau) = \frac{1}{N^2} [N_1 D_1(\tau) + N_2 D_2(\tau)]
\]

**Equation 5.** Autocorrelation function for FCS measurement with two diffusing fluorescent species (e.g. bound and unbound fluorescent ligand) where $N_1$ and $N_2$ are the number of fluorophores in each of state, and $D_1$ and $D_2$ are the diffusion coefficients of the two species.

**Figure 18.** Example of FCS curve when two fluorescent diffusing species are present (blue line) one with diffusion coefficient $10^{-5}$ cm$^2$ s$^{-1}$ (red line represents original curve) and one with diffusion coefficient $10^{-7}$ cm$^2$ s$^{-1}$ (green line represents original curve). Fluorophores in both species are assumed to be equally bright and number of particles observed for each species is assumed to be equal ($N_1 = N_2 = 1$). Re-drawn from ref. 291.
FCS analysis of disaccharide 178 in the presence of varying concentrations of the HS-binding protein FGF-1 in unlabelled (i.e. native) form was performed. Assuming a molecular weight of 15,967 for FGF-1 (determined using the amino acid sequence provided by the manufacturer) a 14.5-fold molecular weight increase is expected upon binding of disaccharide 178 (MW = 1185). From the relationships between diffusion time, diffusion coefficient, hydrodynamic radius and molecular weight, this corresponds to an expected theoretical 2.4-fold increase in diffusion time for the binding complex, assuming there is no unbound ligand (Equations 2, 3 and 6).

\[ R = \left(\frac{3 \cdot MW \cdot \varrho}{4\pi}\right)^{\frac{1}{3}} \]

**Equation 6.** Relationship between hydrodynamic radius \((R)\) and molecular weight \((MW)\) where \(\varrho\) is specific gravity. \(^{291}\)

However, the FCS data collected did not show an increase in the diffusion time indicating no binding occurred between FGF-1 and disaccharide 178 under these conditions (Table 2).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Correlation fit</th>
<th>Diffusion time / s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1nM disaccharide 178</td>
<td>1.00</td>
<td>0.300</td>
</tr>
<tr>
<td>1 nm 178 + 1 nM FGF-1</td>
<td>1.00</td>
<td>0.295</td>
</tr>
<tr>
<td>1 nm 178 + 10 nM FGF-1</td>
<td>1.00</td>
<td>0.295</td>
</tr>
<tr>
<td>1 nm 178 + 100 nM FGF-1</td>
<td>1.00</td>
<td>0.296</td>
</tr>
</tbody>
</table>

HS disaccharides have been shown to bind to FGF-1 by Hung et al.\(^{173,174}\) A set of 48 possible HS disaccharides was chemically synthesised, comprising both GlcN-IdoA and GlcN-GlcA disaccharides with variable \(N\)-substitution (sulfated, acetylated or unsubstituted) and varying sulfation patterns. Four disaccharides were found to bind to FGF-1, all with IdoA O-2 sulfation, with dissociation constants in the micromolar range (Table 3). Therefore, it is likely the low concentration required (1 nM used in this case) for FCS is the cause of no binding being observed, rather than the disaccharide being unable to bind to FGF-1.
Table 3. Dissociation constants (K\textsubscript{d}) for disaccharides 182–185 determined by Hung \textit{et al.} for FGF-1 and FGF-2 by ITC;\textsuperscript{173,174} n.b. = no binding observed.

<table>
<thead>
<tr>
<th>Number</th>
<th>R\textsuperscript{1}</th>
<th>R\textsuperscript{2}</th>
<th>FGF-1 K\textsubscript{d} / µM</th>
<th>FGF-2 K\textsubscript{d} / µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>182</td>
<td>H</td>
<td>H</td>
<td>18.1</td>
<td>n.b.</td>
</tr>
<tr>
<td>183</td>
<td>H</td>
<td>SO\textsubscript{3}Na</td>
<td>4.13</td>
<td>1.73</td>
</tr>
<tr>
<td>184</td>
<td>SO\textsubscript{3}Na</td>
<td>H</td>
<td>21.2</td>
<td>17.5</td>
</tr>
<tr>
<td>185</td>
<td>SO\textsubscript{3}Na</td>
<td>SO\textsubscript{3}Na</td>
<td>9.71</td>
<td>9.01</td>
</tr>
</tbody>
</table>

There are a number of potential solutions to this issue. Firstly, zero mode waveguides (ZMWs) could be used in the FCS experiments. ZMWs are nanostructures with volumes as small as a zeptolitre (10\textsuperscript{-21}) which allow observation of single molecules at higher concentrations (up to micromolar).\textsuperscript{305} This method has been applied to the study of protein-protein interactions\textsuperscript{306} and enzymatic activity,\textsuperscript{307} and is currently being implemented in the Magennis laboratory.

Alternatively, the FCS measurements could be performed with labelled FGF-1 and unlabelled HS disaccharide, allowing for a much higher concentration of disaccharide to be used. However, this may be problematic as the difference in molecular weight between the labelled protein and the labelled protein-disaccharide complex would be greatly reduced (1.07-fold increase); resulting in a small increase in diffusion time (1.02-fold). This issue is particularly relevant in FCS-based immunoassays due to the high molecular weight of many antibodies, and has been remediated by use of nanoparticle-bound ligands which result in a higher molecular weight difference upon binding.\textsuperscript{308}

Longer HS oligosaccharides would be expected to bind more strongly than disaccharides; however, K\textsubscript{d} values for longer heparin/HS fragments have been shown to be in the tens of nanomolar to tens of micromolar range for a number of proteins.\textsuperscript{309–312} Therefore, it is probable that FCS studies using longer labelled HS oligosaccharides with unlabelled protein would suffer from the same issue observed in this experiment, and ZMWs would need to be used in order to observe binding.
8. METHOD FOR $^{13}$C LABELLING OF HS OLIGOSACCHARIDES

8.1 Current Methods to Access $^{13}$C Labelled HS

Although proton NMR spectroscopy has been widely employed for the investigation of HS-protein binding, there are only a few reports of the use of $^{13}$C NMR for such studies.\(^{187,313}\) In part, this can be attributed to the need to access structure-defined $^{13}$C-enriched HS oligosaccharides. Although $^{13}$C-enriched substances are theoretically not required for $^{13}$C NMR studies, they are practically required due to the great improvement in S/N ratio and resulting reduction of spectrometer time and cost.\(^{314}\)

Current methods of obtaining $^{13}$C labelled HS oligosaccharides are typically based on the modification of native HS. For instance, native heparin has been de-$N$-sulfated and $N$-acetylated using $^{13}$C-enriched acetic anhydride to place a $^{13}$CH$_3$ moiety in the structure.\(^{313}\) However, this approach removes $N$-sulfate groups which is likely to alter protein binding. Uniformly $^{13}$C labelled HS oligosaccharides can be produced from heparosan produced by *E.coli* in media enriched with uniformly $^{13}$C labelled glucose via chemoenzymatic methods as shown by Linhardt *et al.*\(^{315}\) This method has subsequently been adapted to use glucose labelled only at the anomeric carbon, resulting in atom-specific $^{13}$C labelling and simplified NMR spectra due to the absence of $^{13}$C-$^{13}$C coupling.\(^{316}\) However, the isotopic purity of the resulting oligosaccharide was found to be only ~70%, and the requirement for atom-specific $^{13}$C labelled glucose increases the cost of the process. Crucially, as chemoenzymatic modification of $^{13}$C-heparosan generates long $^{13}$C enriched HS sequences, digestion would be required in order to form shorter oligosaccharides for binding investigations. This approach therefore suffers the disadvantage of requiring extensive characterisation to determine the structure of the resulting oligosaccharides. There have been no reports of the chemical synthesis of $^{13}$C labelled HS oligosaccharides. Therefore, a synthetic strategy was planned to allow access to structure defined, atom-specific $^{13}$C labelled (as opposed to uniformly labelled) HS oligosaccharides by chemical synthesis.

8.2 Synthesis of $^{13}$C Labelled HS Disaccharide

For synthesis of atom-specific $^{13}$C labelled HS oligosaccharides, a building block was required that could be prepared using a relatively low-cost $^{13}$C source. Use of $^{13}$C labelled monosaccharides e.g. glucose as starting materials was dismissed due to the large number of steps that would be required to convert these expensive materials into useful HS monosaccharide units. Synthesis of iduronate monosaccharides in the
Chapter Two: Results and Discussion

Gardiner group utilises a diastereoselective cyanohydrin formation for inversion of C-5 on a glucofuranose derivative; replacement of the potassium cyanide reagent with K\textsuperscript{13}CN would allow facile access to a \textsuperscript{13}C labelled cyanohydrin which can then be elaborated to different iduronate donors or acceptors. This approach is particularly attractive as IdoA residues of HS can adopt varying conformations (Section 4.1), so incorporation of a \textsuperscript{13}C label into IdoA could be used to report on conformational changes by NMR. The carboxylate group is believed to contribute to HS-protein binding via interaction with positively charged amino acids, hence the \textsuperscript{13}C label should be well placed to act as a reporter for these interactions.

Atom-specific \textsuperscript{13}C labelled cyanohydrin 187 was therefore synthesised from diacetone glucose 34 in 38\% yield over four steps (Scheme 40). In the reaction of aldehyde 41 with KCN the L-ido product precipitates and re-equilibration by epimerisation of the D-gluco product remaining in solution under the slightly basic conditions results in a high d.e. of the L-ido cyanohydrin after 5 days. However, in the reaction with K\textsuperscript{13}CN, \textsuperscript{1}H NMR of the reaction mixture after 5 days showed only a 1:1 L-ido/D-gluco ratio; addition of water to the reaction mixture resulted in an improved ratio of 9:1 after 24 hours. Presence of the \textsuperscript{13}C label in cyanohydrin 187 could be clearly seen by the increased intensity of the carbonyl signal in \textsuperscript{13}C NMR.

Following successful incorporation of the \textsuperscript{13}C label, cyanohydrin 187 then had to be converted to an appropriate iduronate monosaccharide for HS oligosaccharide synthesis. Ideally, the \textsuperscript{13}C labelled building block should be added as late as possible in the synthesis to minimise the loss of precious \textsuperscript{13}C-enriched material. A strategy was envisioned in which a \textsuperscript{13}C labelled GlcN-IdoA disaccharide could be glycosylated to the reducing end of a protected HS oligosaccharide as the final step prior to deprotection/sulfation reactions. Methyl glycoside disaccharide 192 was identified as

![Scheme 40. Reagents: (i) BnCl, Bu\textsubscript{4}NH\textsubscript{2}SO\textsubscript{4}, NaOH, THF/H\textsubscript{2}O, 60 °C; (ii) AcOH, H\textsubscript{2}O, 60 °C; (iii) NaIO\textsubscript{4}, EtOH/H\textsubscript{2}O, RT; (iv) K\textsuperscript{13}CN, MgCl\textsubscript{2}·6H\textsubscript{2}O, H\textsubscript{2}O/EtOH, RT (38% over four steps).](attachment://Scheme_40.png)
being suitable for this purpose; in addition required iduronate methyl glycoside 190 could be obtained in four steps from cyanohydrin 187 using a route previously developed in the Gardiner group.\textsuperscript{320}

Reaction of cyanohydrin 187 with HCl (produced from reaction of acetyl chloride and methanol \textit{in situ}) afforded a mixture of pyranoside and furanoside methyl glycosides bearing the required methyl ester at C-5 (Scheme 41). This reaction is believed to proceed by a Pinner reaction of the nitrile group with HCl and methanol to give the methyl ester, plus deprotection of the isopropylidene group under acidic conditions. A mixture of pyranose and furanose products is formed due to the equilibration between the closed and open chain forms of the sugar, reaction of the anomeric hydroxyl with methanol results in pyranose and furanose methyl glycosides which are then unable to equilibrate.

Acetylation of the mixture was required to facilitate separation of the pyranose (26\%) and furanose (32\%) products by column chromatography. In the \(^1\)H NMR spectrum of pyranoside 188 \(^{13}\)C-\(^1\)H coupling was observed for the CO\(_2\)CH\(_3\) resonance (\(^3\)\(J_{CH} = 3.8\) Hz). In the \(^{13}\)C NMR spectrum of 188 coupling was observed from the \(^{13}\)C nuclide to C-5 (\(^1\)\(J_{CC} = 68.3\) Hz for \(\alpha\), 68.8 Hz for \(\beta\)) and to CO\(_2\)CH\(_3\) (\(^2\)\(J_{CC} = 2.7\) Hz for both anomers). Similar coupling was observed for all subsequent \(^{13}\)C-labelled mono- and disaccharides. Deacetylation of pyranoside 188 under Zemplén conditions\textsuperscript{321} furnished diol 189 (57\%).

To selectively install the desired benzoyl protecting group at O-2 and leave O-4 free, stannylene acetal chemistry was used. Regioselective protection of carbohydrates and diols using stannylene acetal chemistry has been widely reported.\textsuperscript{322} The diol first reacts with dibutyltin oxide to afford the stannylene acetal, which for carbohydrate substrates often exists as a trigonal bipyramidal dimer.\textsuperscript{323} Although the regioselectivity resulting from attack on the reagent (in this case BzCl) by the stannylene acetal is not fully understood, it is believed that the less electronegative hydroxyl oxygen is co-ordinated to two tin atoms, whereas the more electronegative oxygen is co-ordinated to only one. The more electronegative oxygen is therefore placed in the apical position of the complex, resulting in increased reactivity.\textsuperscript{324} Using this approach, \(\alpha\)-methyl glycoside acceptor \(\alpha\)-190 was prepared in 33\% yield (with 21\% of \(\beta\)-190).

Glycosylation of acceptor \(\alpha\)-190 with 4-OPMB glucoazide donor 162 provided disaccharide 191. However, a number of impurities were formed in the glycosylation and purification of disaccharide 191 could not be achieved using column
chromatography. Subsequent problems observed in the glycosylations of 4-OPMB donors including 162 to form lactone-containing disaccharides suggest that the PMB group is the cause of the impurity formation in this glycosylation (Section 10.3). Removal of the PMB group of disaccharide 191 using CAN-mediated oxidation afforded disaccharide 192 (24% over two steps), which can be used as a glycosyl acceptor.

**Scheme 41.** Reagents: (i) AcCl, MeOH, 60 °C; (ii) Ac₂O, DMAP, CH₂Cl₂, pyridine, RT, 26% (over 2 steps, plus 32% furanoside); (iii) NaOMe, MeOH, RT, 57%; (iv) Bu₂SnO, MeOH, 60 °C, then BzCl, 1,4-dioxane, RT, 33% (plus 21% β-190); (v) 162, NIS, AgOTf, CH₂Cl₂, 0 °C; (vi) CAN, MeCN, RT, 24% over two steps.

Overall, this route provided access to an atom-specific $^{13}$C labelled disaccharide, which has the potential to be glycosylated to a longer HS oligosaccharide. However, this disaccharide could only be installed at the reducing end due to the methoxy cap. An alternative approach to add the $^{13}$C label has also been tested in the Gardiner group in which an iduronate glycosyl donor was synthesised from $^{13}$C labelled cyanohydrin 178. Access to this building block in conjunction with disaccharide 192 allows for variation in the location of the $^{13}$C label in synthetic HS oligosaccharides. The issues observed in the glycosylation of 162 with $^{13}$C-labelled acceptor α-190 could be resolved by changing the protecting group at O-4 of the donor to trichloroacetyl, which has previously been used in glycosylation with the unlabelled analogue of α-190. Removal of the trichloroacetyl group with MeOH/pyridine would afford 192.

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8 This synthesis was performed by Agata Brzeźniak, MChem student, Gardiner group, University of Manchester, under supervision of the author.
9. ORTHOGONAL THIOGLYCOSIDE-BASED GLYCOSYLATION STRATEGY FOR HS OLIGOSACCHARIDE SYNTHESIS

9.1 Thioglycosides in Orthogonal Glycosylation

In an orthogonal glycosylation, the anomeric group of the glycosyl donor is activated using reagents or conditions which do not activate the anomeric group of the glycosyl acceptor. In addition, the two anomeric groups are different e.g. thioglycoside and glycosyl fluoride; if the two groups are the same this is instead a chemoselective glycosylation (Section 10). The use of orthogonal (or chemoselective) glycosylation removes the need for further manipulation of the anomeric centre of the reducing end residue after glycosylation, as the product will still bear a donor group which may be activated in a subsequent glycosylation (Scheme 42).

Scheme 42. Outline of orthogonal glycosylation strategy (protecting groups omitted for simplicity).

The first example of orthogonal donor groups for oligosaccharide synthesis was reported in 1994 by Kanie et al. Coupling of N-phthaloyl (Phth) protected glucosamine derivatives such as 193 and 194 (used as a model reaction due to the strong 1,2-trans directing nature of the N-phthaloyl group) was achieved by orthogonal activation of S-phenyl and glycosyl fluoride donor groups (Scheme 43).

Scheme 43. Reagents: (i) 194, NIS, AgOTf, CH₂Cl₂, –50 °C to RT, 85%; (ii) 196, Cp₂HfCl₂, AgClO₄, CH₂Cl₂, –78 °C to RT, 72%.
Subsequently, thioglycosides have been utilised in a number of orthogonal glycosylation approaches. In particular, the $S$-benzoxazolyl (SBox) and $S$-thiazolyl (STaz) thioimidates developed by Demchenko and co-workers have emerged as a new class of donors that can be used in orthogonal glycosylation strategies. Demchenko et al. first reported the use of SBox donors for 1,2-cis glycosylation as stable alternatives to previously tested thioimidate donors; the SBox group was also found to be suitable for 1,2-trans glycosylation. The STaz group was later introduced. STaz and SBox can be activated using stoichiometric silver triflate, which will not activate aryl or alkyl thioglycosides (e.g. SPh, SET), which themselves can be activated with NIS/Lewis acid. In addition, weak alkylating agents such as BnBr or MeI were found to activate STaz donors but not SBox donors. These thioimidate donors have been used in one-pot procedures and in automated oligosaccharide synthesis.

The orthogonal approach was exemplified by Demchenko and co-workers in the synthesis of hexasaccharide using five different donor groups (Scheme 44). The thiocyanate donor group of is activated with Cu(OTf)$_2$, which is a poor activator for STaz donors such as 199. Selective activation of STaz donor over SBox donor was achieved using BnBr, and MeOTf selectively activated the SBox group in the presence of glycosyl fluoride. Use of the original orthogonal F/SPh donor pair allowed access to thioglycoside 206. Finally, selective activation of SEt over an $O$-pentenyl donor using MeOTf afforded the desired hexasaccharide. Thioglycosides and $O$-pentenyl donors are termed ‘semi-orthogonal’ by Demchenko as the ability to selectively activate $O$-pentenyl over thioglycoside depends on the electronic effects of the protecting groups present on both the donor and acceptor.
Much of the development work on the thioimidate donors has been carried out on monosaccharides with simple protecting group motifs. However, there are limited examples of further application of the methodology. Sialic acids are found in many glycoconjugates implicated in immune response and cancers, therefore efficient methods for the production of sialic acid-containing oligosaccharides are desired. De
Meo et al. demonstrated the utility of SBox sialic acid donor 209 for di- and trisaccharide synthesis (Scheme 45).

Scheme 45. Reagents: (i) Bi(OTf)$_3$, CH$_2$Cl$_2$/THF, 3 Å mol. sieves, RT, 66%, 20:1 α/β.

Demchenko and co-workers used STaz and SBox donors to construct pseudo-tetrasaccharide repeat units of the bacterial cell wall of *Streptococcus pneumoniae*. Activation of STaz donor 212 in the presence of S-ethyl donor 213 was achieved using AgOTf, removal of the acetyl group at O-3 of the glucose residue afforded acceptor 214 (Scheme 46). Activation of SBox donor 215 was again achieved using AgOTf; as product 216 bears an anomeric S-ethyl moiety it could be immediately used in a subsequent glycosylation to afford desired pseudo-tetrasaccharide 218.

Scheme 46. Reagents: (i) AgOTf, DCE, 3 Å mol. sieves, RT, 81%; (ii) NaOMe, MeOH, RT, 90%; (iii) 215, AgOTf, DCE, 3 Å mol. sieves, RT, 76%; (iv) 217, NIS, TMSOTf, DCE, 4 Å mol. sieves, 0 °C, 70%.
9.2 Comparison to Current Glycosylation Strategies

The use of an orthogonal glycosylation strategy would offer advantages over other HS oligosaccharide assembly methods. The ‘reducing end cap’ strategy has been utilised by numerous groups (Section 3.3.2), but can be limiting in that the desired functionalisation for the reducing end (e.g. handle) must be installed early in the synthesis, prior to elongation steps. If the oligosaccharide is constructed from the non-reducing end down via a modular orthogonal approach, each oligosaccharide formed will bear an anomeric donor group which can be utilised for functionalisation. Previously in the Gardiner group, trichloroacetimidate donors, which can be selectively activated over thioglycosides, have been used to furnish di- and tetrasaccharides with a reducing end SPh moiety (Scheme 47). However, the trichloroacetimidate donors are prepared from the corresponding SPh donor, and are typically not stored for later use due to their susceptibility to hydrolysis. Adaptation of the synthesis to utilise shelf-stable SBox/STaz donors was therefore desired. An orthogonal approach would crucially remove the need for anomeric manipulation steps between glycosylations, and may be applicable to a one-pot strategy.

Scheme 47. (i) NBS, Me₂CO, RT; (ii) Cl₃CCN, DBU, CH₂Cl₂, RT, 85% for 219 (over two steps), 79% for 221 (over two steps); (iii) For 128: α-165 or β-165, TMSOTf, CH₂Cl₂, −40 °C to RT, 78%, for 222: α/β-220, TMSOTf, CH₂Cl₂, −40 °C to RT, 85%; (iv) CAN, MeCN/H₂O, 85%.
9.3 Synthesis of Glucoazide SBox and STaz Derivatives

Demchenko et al. have reported preparation of per-acetylated glucosamine SBox derivatives (with N-Phth, N-Ac, N-trifluoroacetyl or N-Troc protection) from glycosyl acetates or halides. Subsequent protecting group manipulations for these glucosamine derivatives were not reported. In order to access correctly protected glucoazide SBox and STaz derivatives for investigation of orthogonal glycosylations using these groups, O-trichloroacetimidate donors were utilised. Trichloroacetimidate donors 219 and 228–230 were prepared from the corresponding thioglycosides by hydrolysis using N-bromosuccinimide (NBS) in acetone followed by reaction with trichloroacetonitrile in the presence of DBU or K$_2$CO$_3$ (Scheme 48). The yield for hydrolysis of benzylidene derivative 173 was lower than the other substrates (39%), this is attributed to potential side-reaction of the benzylidene group with NBS.

Scheme 48. Reagents: (i) BnBr, NaH, THF, 60 °C, 77%; (ii) NBS, Me$_2$CO/H$_2$O, RT, 95% for 224, 78% for 225, 39% for 226, 89% for 227; (iii) Cl$_3$CCN, DBU, CH$_2$Cl$_2$, RT, 95% for 219, 92% for 228, 67% for 229; (iv) Cl$_3$CCN, K$_2$CO$_3$, CH$_2$Cl$_2$, RT, 77% for 230.

Trichloroacetimidate donors 219 and 228–230 were glycosylated with 2-mercaptobenzoxazole (HSBox) using TMSOTf as activator to afford the corresponding SBox glycosides (Scheme 49). A mixture of anomers was obtained for the more electron-rich (‘armed’) donors 219 and 230, whereas only the β anomer was obtained for 228 and 229.
Scheme 49. Reagents: (i) HSBox, TMSOTf, CH₂Cl₂, −50 °C to RT, 60% for 231, 58% for 232, 61% for 233, 47% for 234.

STaz donors 235–237 were prepared in an analogous manner using 2-mercaptop-2-thiazoline (HSTaz) as acceptor (Scheme 50). Only the β anomer of the product was obtained from these glycosylations. The presence of a C=N signal in the ¹³C NMR spectra confirmed that 235–237 were the desired S-linked products. However, in the glycosylation of 4-OPMB donor 219 N-linked product 238 was also isolated (34%), this has previously been observed for armed glycosyl halides reacting with HSTaz by Demchenko and co-workers.³³⁸

Scheme 50. (i) HSTaz, TMSOTf, CH₂Cl₂, −50 °C to RT, 36% for 235 (plus 34% of 238), 56% for 236, 71% for 237.

9.4 Synthesis of Iduronate SBox Glycoside

9.4.1 Attempted Synthesis from Methyl Glycosides

Conversion of methyl glycoside 240, accessible in four steps from cyanohydrin 239, to the corresponding SBox glycoside was attempted (Scheme 51). Direct conversion of methyl glycosides to S-phenyl thioglycosides using (trimethyl)phenylthiosilane and a Lewis acid such as ZnI₂ or TMSOTf has been reported.³⁵¹,³⁵² Therefore, silylation of HSBox was carried out using bis(trimethylsilyl)-acetamide (BSA) in anhydrous acetonitrile,³⁵³ and immediately reacted with 240 in anhydrous dichloromethane in the presence of ZnI₂. However, ¹H NMR showed no reaction of starting material 240.
Chapter Two: Results and Discussion

Formation of silylated reagent 241 could not be confirmed by $^1$H NMR, possibly due to instability to moisture, and this method was not further investigated.

A method for direct conversion of iduronate methyl glycoside 243 (in a mixture with the furanoside derivative) to the corresponding thioglycoside using PhSH and BF$_3$·OEt$_2$ has previously been developed in the Gardiner group. Following this procedure, diacetate 240 and diol 243 were reacted with HSBox in the presence of either BF$_3$·OEt$_2$ or TMSOTf. However, no reaction was observed by $^1$H NMR in any of these procedures, this is attributed to the reduced nucleophilicity of HSBox relative to PhSH. Due to the difficulty in accessing iduronate SBox derivatives directly from the methyl glycoside, an alternative synthesis was attempted from the glycosyl acetate, as these substrates are commonly used for thioimidate preparation by Demchenko and co-workers.

Scheme 51. Reagents: (i) KCN, MgCl$_2$·6H$_2$O, H$_2$O/EtOH, RT (32% over four steps); (ii) AcCl, MeOH, 60 °C; (iii) Ac$_2$O, DMAP, CH$_2$Cl$_2$, pyridine, RT, 56% (over 2 steps, plus 26% furanoside); (iv) HSBox, BSA, MeCN, 80 °C (to form 241), then 240, ZnI$_2$, CH$_2$Cl$_2$, RT; (v) HSBox, BF$_3$·OEt$_2$, CH$_2$Cl$_2$, 4 Å mol. sieves, RT; (vi) HSBox, TMSOTf, CH$_2$Cl$_2$, 4 Å mol. sieves, RT; (vii) NaOMe, MeOH, RT, 94%.

9.4.2 Synthesis from Glycosyl Acetate

Glycosyl acetate 249 was identified as a suitable substrate for conversion to the desired SBox glycoside. Iduronate 249 could be synthesised via a method previously developed in the Gardiner group (Scheme 52).

Firstly, cyanohydrin 239 was treated with HCl in THF to afford L-ido amide 245 (89%). Regioselective acetylation of 245 leaving a free hydroxyl at C-4 was achieved using 1 mol% DMAP. This procedure has been previously reported by the Gardiner group on 100 g scale, diacetylated product 246 was selectively crystallised out (57%) and a second batch isolated by column chromatography (9%). However, when the reaction was repeated on smaller scale (2.5 g) 246 was only isolated in 12% yield, due to difficult in separating the di- and
Chapter Two: Results and Discussion

triacetylated products by column chromatography. The triacetylated pyranoside and furanoside by-products from this reaction can be recycled to form amide 245 by stirring in THF/aqueous 4 M HCl.318

Reaction of 246 with isopentyl nitrite converted the amide group to the acid; subsequent methylation of the crude acid with iodomethane gave methyl ester 248 in 35% yield (over two steps). The mechanism of the reaction of 246 with isopentyl nitrite is believed to involve attack of water on an acylium ion intermediate to form carboxylic acid 247 as the major product.318 Finally, 248 was protected with the chloroacetyl group to furnish glycosyl acetate 249 (89%).

\[ \text{Mechanism of isoamyl nitrite-mediated amide to acid conversion (R = rest of monosaccharide):} \]

\[ R \]  

\[ \text{Scheme 52. Reagents: (i) 37\% HCl, THF, RT, 89\%; (ii) Ac}_2\text{O, DMAP, CH}_2\text{Cl}_2, \text{RT, 12\%; (iii) Isopentyl nitrite, AcOH, 90 °C; (iv) MeI, KHCO}_3, \text{DMF, RT, 35\% over two steps; (v) Chloroacetyl chloride, pyridine, CH}_2\text{Cl}_2, \text{RT, 89\%}.} \]

Reaction of glycosyl acetate 249 with HSBox and BF$_3$·OEt$_2$ afforded iduronate SBox derivative 250 (79%, Scheme 53). Multiple additions of HSBox and BF$_3$·OEt$_2$ were found to be required for product formation and the reaction took ~72 hours to reach completion. When no further additions of HSBox and BF$_3$·OEt$_2$ were made, the reaction reached only 20% conversion after 72 h (by $^1$H NMR). Heating the reaction to 40 °C did not reduce the reaction time required, however a final addition of TMSOTf was seen to drive the reaction to completion. These observations are attributed to the low reactivity of 249 as a glycosyl donor due to the presence of multiple electron withdrawing substituents, and to the relatively low nucleophilicity of the sulfur lone pair on HSBox due to delocalisation.
SBox glycoside 250 was obtained as a mixture of anomers (~9:1 α/β). A J₂₄ ‘W coupling’ was observed in the ¹H NMR spectrum suggesting 250 adopts the ¹C₄ conformation when dissolved in deuterated chloroform.³⁵⁷ The S-linked product was confirmed to have formed (as opposed to the less likely, but possible, N-linked product) firstly based on the absence of a C=S signal in the carbon NMR spectrum (expected to be seen at δ >200 ppm)³⁵⁸ and the presence of the C=N resonance at 162 ppm. Secondly, the UV-vis spectrum of 250 was recorded for comparison with literature spectra of N- and S-linked benzoxazolyl glycosides of peracetylated glucose.³⁵⁹ Bands were observed at λₘₐₓ 246 nm and 277 nm (C=N), indicating 250 is S-linked. No broad band was observed at ~300 nm, as would be expected for the N-linked product.³⁵⁵

Removal of the chloroacetyl group of 250 using thiourea afforded SBox glycoside 251 (72%).

![Scheme 53](image)

**Scheme 53.** Reagents: (i) HSBox, BF₃·OEt₂, TMSOTf, CH₂Cl₂, 0 °C to RT, 79%; (ii) Thiourea, EtOH, 70 °C, 71%.

9.5 Orthogonal Glycosylations to form HS Disaccharides

9.5.1 Glucoazide SBox Donors with Iduronate SPh Acceptor

Orthogonal glycosylations of glucoazide SBox donors 231–234 with iduronate SPh acceptor α-165 were evaluated using stoichiometric AgOTf as promoter following the work of Demchenko et al. (Scheme 54).³²⁹ Glycosylation of 4-OPMB glucosamine SBox donor 231 was unsuccessful (entry 1), the donor was consumed but no desired product α-128 was observed, however acceptor α-165 was recovered (47%). Stoichiometric AgOTf has the potential to cleave PMB groups,³⁶⁰ and therefore it is probable that this impaired the glycosylation, although formation of 4-OH product α-220 was not observed. In comparison, activation of tri-benzylated glucoazide SBox glycoside 234 afforded disaccharide α-254 in 57% yield (entry 4). Only the 1,2 cis product (α anomer) was observed (this was also the case in glycosylations of 232 and 233).

Glycosylation of less armed SBox donors 232 and 233 with α-165 afforded desired disaccharides 252 (21%, entry 2) and 253 (25%, entry 3). Reaction of benzylidene donor 233 did not proceed at room temperature but the donor was activated
at 40 °C. Both products 252 and 253 were converted to O-4 hydroxyl disaccharide α-220 by benzylamine-mediated chloroacetyl removal (54%) and regioselective reductive ring opening of 253 (62%).

Scheme 54. (i) α-165, AgOTf, CH₂Cl₂ (for 232–234) or DCE (for 231), 4 Å mol. sieves, –40 °C to RT; (ii) Benzylamine, Et₂O, 0 °C, 54% (from 252); (iii) Et₃SiH, BF₃·OEt₂, CH₂Cl₂, 0 °C, 62% (from 253).

9.5.2 Glucoazide STaz Donors with Iduronate SPh Acceptor

Evaluation of the corresponding STaz glycosides as glycosyl donors with iduronate S-phenyl acceptor α-165 was also carried out using stoichiometric AgOTf under the same conditions as for the SBox glycosides (Table 4). No activation of 4-O-chloroacetyl donor 236 or benzylidene protected donor 237 was observed (entries 2 and 4). This indicates that the STaz glucoazide donors display a lower reactivity towards AgOTf than the corresponding SBox glycosides. Activation of 236 and 237 with stoichiometric TMSOTf resulted in complete consumption of starting materials with no product formation observed (entries 3 and 5).

Glycosylation of 4-OPMB donor 235 resulted in formation of 4-hydroxyl disaccharide α-220 (21%) due to removal of the PMB group under the glycosylation conditions (entry 1). The outcome of these glycosylations indicates that electron donating (‘arming’) substituents such as PMB facilitate successful activation of STaz glucoazide donors.
Table 4. Glycosylations of glucoazide STaz donors with iduronate SPh acceptor.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>R¹</th>
<th>R²</th>
<th>Desired product</th>
<th>Conditions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>235</td>
<td>Bn</td>
<td>PMB</td>
<td>$\alpha$-127</td>
<td>AgOTf, DCE</td>
<td>21% yield of $\alpha$-220</td>
</tr>
<tr>
<td>2</td>
<td>236</td>
<td>Bn</td>
<td>ClAc</td>
<td>252</td>
<td>AgOTf, CH₂Cl₂</td>
<td>No reaction by $^1$H NMR</td>
</tr>
<tr>
<td>3</td>
<td>236</td>
<td></td>
<td></td>
<td></td>
<td>TMSOTf, CH₂Cl₂</td>
<td>Complete consumption of starting materials</td>
</tr>
<tr>
<td>4</td>
<td>237</td>
<td>PhCH</td>
<td></td>
<td>253</td>
<td>AgOTf, CH₂Cl₂</td>
<td>No reaction by $^1$H NMR</td>
</tr>
<tr>
<td>5</td>
<td>237</td>
<td></td>
<td></td>
<td></td>
<td>TMSOTf, CH₂Cl₂</td>
<td>Complete consumption of starting materials</td>
</tr>
</tbody>
</table>

N.B. All 4 Å molecular sieves, RT

9.5.3 Glucoazide STaz Donors with Iduronate SBox Acceptor

Selective activation of glucoazide STaz donors over iduronate SBox glycoside 251 was also evaluated using BnBr or MeI for activation as following the work of Demchenko et al. (Table 5). STaz donors 235 and 236 were not activated using BnBr (entries 1 and 3), and donor 235 could not be activated using MeI (entry 2). This indicates low reactivity of the glucoazide STaz donors compared to glucose STaz donors which can be activated under these conditions as shown by Demchenko et al.
Table 5. Glycosylations of glucoazide STaz donors with iduronate SBox acceptor.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>R^1</th>
<th>Desired product</th>
<th>Conditions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>235</td>
<td>PMB</td>
<td>255</td>
<td>BnBr, DCE, 55 °C</td>
<td>No reaction</td>
</tr>
<tr>
<td>2</td>
<td>235</td>
<td></td>
<td></td>
<td>MeI, DCE, RT</td>
<td>No reaction</td>
</tr>
<tr>
<td>3</td>
<td>236</td>
<td>ClAc</td>
<td>256</td>
<td>BnBr, DCE, 55 °C</td>
<td>No reaction</td>
</tr>
</tbody>
</table>

N.B. All with 4 Å molecular sieves

9.5.4  Glucoazide SPh Donors with Iduronate SBox Acceptor

Although selective activation of S-alkyl/S-aryl thioglycosides over SBox glycosides has not been widely reported, it was proposed that activation of glucoazide SPh donors over iduronate SBox glycoside 251 might be possible due to the low reactivity of iduronate monosaccharides. SBox glycosides have been shown to be stable in the presence of iodonium (di-γ-collidine) perchlorate (IDCP), a suitable activator for thioglycosides. Glucoazide donor 258 was prepared from diol 164 by regioselective benzoylation and chloroacetyl protection of O-4 (Scheme 55). Glycosylation of 258 over iduronate SBox glycoside 251 using IDCP resulted in consumption of 251 with no product formation observed (Scheme 55, entry 1). This suggests that IDCP activated the SBox moiety on 251 over the SPh group of 258.

NIS/catalytic triflate activation systems were also evaluated for selective activation of glucoazide SPh donors over iduronate SBox glycoside 251. Although NIS/catalytic triflate has been shown to activate the SBox moiety, stoichiometric amounts of triflate activators are usually required for effective activation. Therefore, it was hoped activation of the iduronate SBox group by NIS/catalytic triflate would occur more slowly than activation of glucosamine SPh. However, glycosylations of donors 162 (with NIS/TfOH) and 223 (with NIS/TMSOTf) with 251 resulted in complete consumption of donor with no observed formation of the desired products (entries 2 and 3). In both cases aglycone transfer i.e. migration of the SBox moiety to the donor was observed.
Scheme 55. Reagents: (i) 60% aq. AcOH, 120 °C, 75%; (ii) BzCl, Et₃N, CH₂Cl₂, 0 °C, 71%; (iii) Chloroacetyl chloride, pyridine, CH₂Cl₂, RT, 88%; (iv) Promoter, 4 Å mol. sieves, CH₂Cl₂, 0 °C.

9.5.5 Iduronate SBox Donor with Glucoazide SPh Acceptor

Use of iduronate SBox glycoside 250 as a donor for formation of IdoA-GlcN disaccharides was investigated. AgOTf was employed for the activation, as it is one of the most commonly used activators for the SBox group from the work of Demchenko et al. However, when 250 was activated using AgOTf in the presence of glucoazide SPh acceptors 174 and 257, hydrolysed donor 261 was obtained, along with recovered acceptor (Table 6). This suggests that although donor 250 was activated, it was not able to react with the free 4-OH of the acceptor. Taken in conjunction with the difficulty experienced in glycosylating a GlcN-IdoA-SPh disaccharide bearing an acetyl group at IdoA O-2 with a disaccharide acceptor (Section 10.4.3) it is possible that the acetyl group is having a detrimental influence on the glycosylation, rather than the low reactivity of the 4-hydroxyl of the acceptor.
Table 6. Attempted glycosylations of iduronate SBox donor with glucoazide SPh acceptors.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acceptor</th>
<th>R¹</th>
<th>Desired product</th>
<th>Conditions</th>
<th>Yield / %</th>
<th>Yield of 261 / %</th>
<th>Recovered acceptor / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>174</td>
<td>Bn</td>
<td>262</td>
<td>AgOTf, CH₂Cl₂, 4 Å mol. sieves, 0 °C</td>
<td>0</td>
<td>24</td>
<td>n.d.*</td>
</tr>
<tr>
<td>2</td>
<td>257</td>
<td>Bz</td>
<td>263</td>
<td>AgOTf, CH₂Cl₂, 4 Å mol. sieves, −20 °C</td>
<td>0</td>
<td>96</td>
<td>100</td>
</tr>
</tbody>
</table>

*Not determined: acceptor was not isolated, but was observed by TLC

9.6 Synthesis of Glucoazide SBox Derivatives from D-Glucosamine

Following investigations of orthogonal glycosylations with glucoazide SBox and STaz donors, development of a more practical and scalable synthesis from D-glucosamine was investigated. Glycosyl acetate 264, prepared in two steps from D-glucosamine, was converted to SBox glycoside 265 by reaction with HSBox and TMSOTf in 67% yield (Scheme 56).

Demchenko and co-workers have previously shown that peracetylated SBox derivatives with a non-participating O-2 group could be deprotected with NaOMe and subsequently benzylated with BnBr and NaH. SBox glycosides with an O-2 acetate group were found to undergo anomeric leaving group displacement on treatment with NaOMe/MeOH and were not stable to the basic conditions required for benzylation. When 265 was treated with NaOMe/methanol followed by BnBr and NaH, a complex mixture of products was obtained which could not be easily separated or identified, indicating the SBox moiety on 265 is not stable under these conditions. Removal of the acetate groups of 265 was achieved with K₂CO₃ in methanol to afford 266 (45%). A change in the anomeric ratio was observed by ¹H NMR, suggesting β-266 was less stable to the reaction conditions than α-266.

Benzylation of 266 with BnBr and NaH again resulted in a complex mixture of products, but the benzylidene group could be installed in 45% yield to give 267, again a reduction in the amount of β anomer present was observed. Attempted benzylation of the O-3 position of 267 resulted in a complex product mixture.

² Compound 264 was prepared by Dr Gavin J. Miller, Gardiner group, University of Manchester.
Scheme 56. Reagents: (i) HSBox, TMSOTf, CH$_2$Cl$_2$, 4 Å mol. sieves, RT, 67%; (ii) K$_2$CO$_3$, MeOH, RT, 45%; (iii) BnBr, NaH, THF, RT or 60 °C; (iv) Benzaldehyde dimethyl acetal, $\rho$TsOH, MeCN, 45%.

Overall, efforts to extend the current use of SBox/STaz in orthogonal glycosylations to synthesis of HS oligosaccharides met with some success, however further development is required to develop these test glycosylations into a synthetic strategy capable of delivering longer HS oligosaccharides. The glucoazide SBox donors were seen to be more reactive than the STaz derivatives, which (with the exception of ‘armed’ donor 255) could not be activated using AgOTf. However, the stability of the SBox moiety to the protecting group manipulations typically used for synthesis of the glucosamine building blocks was found to be poor, in particular benzylation under basic conditions was problematic. In order to utilise an orthogonal glycosylation strategy employing glucoazide SBox derivatives, a synthesis which avoids these conditions or installs the SBox moiety after protecting group manipulations is required. Iduronate 2-OAc SBox glycoside 250 was activated using stoichiometric AgOTf in the presence of glucoazide SPh acceptors, but no formation of disaccharide was observed and hydrolysis of the donor occurred. In light of the issues observed with use of an IdoA 2-OAc containing disaccharide as a glycosyl donor (Section 10.4.3), it is proposed that the corresponding 2-OBz SBox glycoside may be a more suitable glycosyl donor.
10. CHEMoselective Glycosylation Strategy for HS Oligosaccharide Synthesis

10.1 Synthetic Strategy

As previously described, chemoselective glycosylation occurs when a donor group is selectively activated in the presence of the same donor group on the glycosyl acceptor (Section 9.1). In an analogous manner to orthogonal glycosylation, chemoselective glycosylation removes the requirement for manipulation of the anomeric centre between glycosylations, thus improving the overall efficiency of oligosaccharide synthesis.

Previous work in the Gardiner group led to the discovery that S-phenyl thioglycoside l-ido [2.2.2] lactone β-270 could be used as acceptor in a chemoselective glycosylation with glucosazide donor 162 or 173 to afford lactone-containing disaccharides 270 and 271 (Scheme 57). Methanolysis of the lactone moiety and protection of IdoA O-2 with the benzoyl group afforded disaccharide building blocks β-128 and β-253, which have previously been prepared via other methods; β-128 has been used as a glycosyl donor but β-253 has not.

Scheme 57. Reagents: (i) PhSH, BF₃·OEt₂, 4 Å mol. sieves CH₂Cl₂, RT, 37%; (ii) KOH, THF/MeOH/H₂O, then TsCl, 1-methyl-imidazole, CH₂Cl₂, 85% over two steps, (iii) NIS, AgOTf, toluene, 0 °C, 81% for β-271 (plus 14% β-272), 57% for β-273; (iv) NaOMe, MeOH, RT; (v) BzCl, 1-methyl-imidazole, CH₂Cl₂, RT, 64% over two steps for β-128, 62% over two steps for β-253. Green colour indicates S-phenyl groups that activate under NIS/AgOTf conditions, red colour indicates those that do not.
The (2,6)-L-ido lactone structure has previously been employed as a protecting group motif in the work of Hung et al.. The lactone was formed during selective oxidation of C-6 on L-idose residues bearing a free hydroxyl at C-2 using TEMPO/BAIB (Scheme 58).\textsuperscript{157,159} This is thought to occur by intramolecular attack of the free hydroxyl on the aldehyde intermediate to form a lactol, which is then oxidised to form the lactone.\textsuperscript{364} Martin-Lomas et al. have previously utilised lactone 276 as a glycosyl acceptor.\textsuperscript{146}

\begin{center}
\includegraphics[width=\textwidth]{figure}
\end{center}

**Scheme 58.** Reagents: (i) TEMPO, BAIB, CH\textsubscript{2}Cl\textsubscript{2}, RT, 78%.

The lack of reactivity of 270 as a glycosyl donor (‘super-disarmed’) is interesting when compared to other thioglycoside donors locked into a similar conformation (B\textsubscript{2,5}), such as 277 and 278 which have been successfully used as donors by Toshima et al (Figure 19).\textsuperscript{365} However, 277 and 278 do not have an electron-withdrawing carboxylate group, which would be expected to reduce reactivity as a glycosyl donor. Other examples of conformationally locked uronic acid donors include galactosyl lactone 279, which has been shown to act as a donor by van Marel et al.\textsuperscript{366} This was used as the basis for development of 280 as a ‘conformationally armed’ glucuroyl donor by Furukawa and co-workers,\textsuperscript{367} in which the presence of all axial substituents increases reactivity.\textsuperscript{368} It should be noted that donors 279 and 280 are in a chair (\textsuperscript{1}C\textsubscript{4}) conformation and therefore their reactivity cannot be directly compared to that of lactone 270.
Chapter Two: Results and Discussion

Figure 19. (a) Compounds locked into the same conformation as lactone 270 (B$_{2,5}$) that function as glycosyl donors; (b) Conformationally-locked uronic acids that act as glycosyl donors.

Following the use of L-ido lactone 270 for synthesis of disaccharides, a strategy was devised to extend this chemoselective approach to the synthesis of longer HS oligosaccharides (Scheme 59). Removal of the 4-OPMB group of lactone-containing disaccharide $A$ (bearing either a benzyl or ester protecting group at O-6 of glucosamine depending on desired sulfation in the final product) would afford lactone-containing acceptor disaccharide $B$. Donor disaccharide $C$ could be obtained from opening of lactone-containing disaccharide $A$ followed by re-protection of O-2 as an ester. Chemoselective glycosylation of donor $C$ with acceptor $B$ would afford lactone-containing tetrasaccharide $D$ (where $n = 1$). Elongation of the chain could be achieved using an iterative cycle of glycosylation with acceptor disaccharide $B$, followed by lactone opening and re-protection of O-2 to give another donor oligosaccharide. Crucially, each different length of HS oligosaccharide made ($E$) bears a reactive thioglycoside group which can be used for conjugation e.g. to provide oligosaccharides bearing the amine tag for fluorescent labelling ($F$). This is a distinct advantage over previous methods employed in the Gardiner group in which the handle was installed at the start of the synthesis $^{246}$ This approach uses only thioglycoside donors, which are shelf-stable. The use of an $n+2$ approach allows access to an additional oligosaccharide lengths e.g. hexasaccharide, dodecasaccharide in comparison to the [4+4] approach also developed within the Gardiner group $^{246,369}$

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$^{1}$ This body of work was performed in conjunction with Robin Jeanneret, Gardiner group, University of Manchester.
Scheme 59. Reagents: (i) CAN, MeCN/H$_2$O, RT; (ii) NaOMe, MeOH, RT; (iii) BzCl or Ac$_2$O, 1-methyl-imidazole, CH$_2$Cl$_2$, RT; (iv) NIS, AgOTf, CH$_2$Cl$_2$, 0 °C; (v) N-Z-ethanolamine, NIS, AgOTf, CH$_2$Cl$_2$, 0 °C. Green colour indicates S-phenyl groups that activate under NIS/AgOTf conditions, red colour indicates those that do not (or are not expected to).
10.2 Synthesis of Monosaccharide Building Blocks

10.2.1 Synthesis of L-Ido Lactone

Cyanohydrin 239 was converted to diol 269 in 20% yield over two steps by reaction with acetyl chloride to form pyranoside/furanoside mixture of methyl glycosides 243/268, followed by reaction with PhSH and BF$_3$·OEt$_2$ (Scheme 60). Although this reaction is low yielding, it allows access to diol 269 in two steps from cyanohydrin starting material. Lactonisation of diol 269 was achieved by hydrolysis of the methyl ester with KOH, followed by reaction with TsCl and 1-methyl-imidazole (56% over two steps). This yield was reproducible, but is not consistent with previous work from the Gardiner group, in which 270 was formed in 85% yield over two steps following the same procedure. In addition, the anomers were found to only be partially separable by column chromatography, although it was previously reported that they were fully separable. However, lactone 270 could be used in glycosylations as a mixture of anomers (Section 10.4.1).

Scheme 60. Reagents: (i) AcCl, MeOH, 60 °C; (ii) PhSH, BF$_3$·OEt$_2$, 4 Å mol. sieves, CH$_2$Cl$_2$, 20% over two steps; (iii) KOH, THF/MeOH/H$_2$O, (iv) TsCl, 1-methyl-imidazole, CH$_2$Cl$_2$, 56%.

10.2.2 Synthesis of Glucoazide Donors

Suitable glucoazide donors were desired in order to be able to access HS oligosaccharides with differing sulfation patterns. In native HS, the possible positions for sulfation in glucosamine residues are O-6 and O-3 (O-3 being a rarer modification). Protection of O-3 and/or O-6 with an ester group will allow deprotection prior to O-sulfation, therefore a set of four donors 162, 281, 284 and 286 would allow access to the all possible O-3/O-6 sulfation patterns. 3,6-O-Dibenzylated donor 162 had already been synthesised (Section 6.2.2.1); 6-O-benzyolated analogue 281 was obtained by benzyolation of 161 in >99% yield (Scheme 61).

In order to prepare 3-OBz derivatives 284 and 286, the free O-3 hydroxyl of 159 was first protected with the benzoyl group in 85% yield. Regioselective reductive opening of the benzylidene group of 282 using dibutylboron triflate and BH$_3$·THF afforded 283, in contrast to the 3-OBn derivative (Section 6.2.2.1), however, the
reaction was not reliable (table, Scheme 61). The HMBC spectrum of 283 showed a cross peak between C-4 and the PMB group CH$_2$, thus confirming the benzylidene ring was successfully opened to the 6-OH product. Benzylation of the free 6-OH afforded 284 in 88% yield.

Scheme 61. Reagents: (i) BnBr, NaH, DMF, RT, 93%; (ii) Cyanuric chloride, NaBH$_4$, MeCN, RT, 84%; (iii) BzCl, Et$_3$N, DMAP, CH$_2$Cl$_2$, RT, >99%; (iv) BzCl, DMAP, pyridine, CH$_2$Cl$_2$, RT, 85%; (v) BH$_3$·THF, Bu$_2$BOTf, RT, 72%; (vi) BzCl, Et$_3$N, DMAP, CH$_2$Cl$_2$, RT, 88%.

For synthesis of 286, the free hydroxyl at C-6 had to be protected with a benzyl group, which was problematic in the presence of the O-3 ester due to the requirement for basic conditions. Reaction of 283 with BnBr and NaH did allow the product to be obtained in 60% yield, but variability was seen in the reaction (Table 7). Formation of 3,6-dibenzylated derivative 162 was observed in some instances, as a result of benzoyl group removal followed by benzylation of the free 3-OH (entries 1 and 2). Alternative reaction conditions employing benzyl trichloroacetimidate and TMSOTf$^{371}$ or camphorsulfonic acid$^{372}$ did not afford the desired product (entries 5 and 6).
Chapter Two: Results and Discussion

Table 7. Benzylation of O-6 of 283.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield of 286 / %</th>
<th>Yield of 3,6-OBn 162 / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BnBr, NaH, THF, 60 °C</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>BnBr, NaH, THF, 60 °C</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>BnBr, NaH, DMF, 60 °C</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>BnBr, NaH, DMF, 60 °C</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Benzyl 2,2,2-trichloroacetimidate, TMSOTf, CH₂Cl₂, RT</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Benzyl 2,2,2-trichloroacetimidate, camphorsulfonic acid, CH₂Cl₂, RT</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Decomposition of SM
<sup>b</sup> SM and impurities observed by NMR, no product observed

10.3 Synthesis of 4-para-Methoxybenzyl HS Disaccharides

10.3.1 Selective Activation of Glucoazide SPh Donors over Lactone

In order to begin development of the chemoselective glycosylation strategy, 4-OPMB lactone-containing disaccharides were required which could then be opened to form disaccharide donors, or the PMB group removed to give glycosyl acceptors. Synthesis of 6-OBz lactone-containing disaccharide 287 using NIS/AgOTf as promoter, as previously used within the Gardiner group for synthesis of 6-OBn analogue 271, gave a yield of 20% (Table 8, entry 1). In addition, 4-OPMB lactone 288 was observed as a by-product, showing that the PMB moiety is labile under these conditions. This is somewhat surprising given that the PMB group has been used previously in the Gardiner group in glycosylations employing NIS/catalytic triflate activation conditions. Stoichiometric triflic acid has been observed to remove PMB groups, and iodine, which may be present in impure NIS or be generated in the glycosylation, could potentially act as an oxidant to remove PMB.<sup>273</sup> IDCP was tested as an alternative promoter for the glycosylation, thus avoiding the use of NIS and triflate sources, but was not able to activate 6-OBz donor 281 or 6-OBn donor 162 (entries 3 and 5)
Attempts to reproduce the glycosylation of 6-OBn donor 281 with lactone 270 using NIS/AgOTf as previously reported within the Gardiner group were unsuccessful, with yields <40% obtained. It was proposed that this might be due to the quality of the reagents. Use of newly-purchased NIS with TMSOTf gave slightly increased yields for formation of 271 and 287 (entries 3 and 5). However, the yields obtained for synthesis of 6-OBn disaccharide 271 were still lower than previously reported.

Table 8. Glycosylation of 3-OBn, 4-OPMB glucoazide donors with lactone 270.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>R¹</th>
<th>Acceptor</th>
<th>Conditions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>281</td>
<td>Bz</td>
<td>α-270</td>
<td>NIS, AgOTf, CH₂Cl₂, 0 °C</td>
<td>20% isolated yield, formation of 4-PMB lactone 288 observed</td>
</tr>
<tr>
<td>2</td>
<td>281</td>
<td>β-270</td>
<td></td>
<td>IDCp, CH₂Cl₂, 0 °C</td>
<td>No activation of donor</td>
</tr>
<tr>
<td>3</td>
<td>281</td>
<td>α-270</td>
<td></td>
<td>NIS*, TMSOTf, CH₂Cl₂, 0 °C</td>
<td>28% isolated yield</td>
</tr>
<tr>
<td>4</td>
<td>162</td>
<td>Bn</td>
<td>β-270</td>
<td>IDCp, CH₂Cl₂, 0 °C</td>
<td>No activation of donor</td>
</tr>
<tr>
<td>5</td>
<td>162</td>
<td>β-270</td>
<td></td>
<td>NIS*, TMSOTf, CH₂Cl₂, 0 °C</td>
<td>37% isolated yield</td>
</tr>
</tbody>
</table>

*Newly purchased NIS was used

Glycosylation of corresponding 3-OBz donors 284 and 286 with lactone 270 was also evaluated (Table 9). The yields for these glycosylations were also low (<20%), even when new NIS was used (entry 4). In addition, 4-OPMB lactone 288 was observed to form in the glycosylation employing new NIS, suggesting that the PMB removal was not due to NIS quality. Following the issues observed during glycosylations with 4-OPMB thioglycoside donors, trichloroacetimidate donors were also evaluated.

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1 These reactions were carried out by Robin Jeanneret, Gardiner group, University of Manchester.
Table 9. Glycosylation of 3-OBz, 4-OPMB glucoazide donors with lactone 270.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>R^1</th>
<th>Acceptor</th>
<th>Conditions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>284</td>
<td>Bz</td>
<td>β-270</td>
<td>NIS, AgOTf, CH₂Cl₂, 0 °C</td>
<td>13% isolated yield, 28% recovered donor, 80% recovered acceptor</td>
</tr>
<tr>
<td>2</td>
<td>284</td>
<td></td>
<td>α-270</td>
<td>NIS, AgOTf, CH₂Cl₂, 0 °C</td>
<td>12% isolated yield</td>
</tr>
<tr>
<td>3</td>
<td>286</td>
<td>Bn</td>
<td>β-270</td>
<td>NIS, AgOTf, CH₂Cl₂, 0 °C</td>
<td>Product formation observed by NMR, multiple impurities formed</td>
</tr>
<tr>
<td>4</td>
<td>286</td>
<td></td>
<td>α-270</td>
<td>NIS*, TMSOTf, CH₂Cl₂, 0 °C</td>
<td>18% isolated yield, 15% isolated yield of 288</td>
</tr>
</tbody>
</table>

* Newly purchased NIS was used

10.3.2 Activation of Glucoazide Trichloroacetimidates over Lactone

Glycosylation of 4-OPMB trichloroacetimidate donors with lactone 270 was also investigated using 6-OBz trichloroacetimidate donor 292, prepared in two steps from 281, and 6-OBn donor 219 (Scheme 62). The outcome of these glycosylations was variable, with no product isolated in some cases and complex product mixtures observed. Although in one instance 6-OBz disaccharide 287 was obtained in 41% yield, 4-OH disaccharide 293 was also formed, showing PMB removal had occurred (entry 2). 4-OPMB lactone 288 was also observed as a by-product (entry 1), again this is surprising as the PMB group has previously been successfully used in glycosylations employing trichloroacetimidate donors. 155,318

Due to the labile nature of the PMB group in glycosylations employing both the thioglycoside and trichloroacetimidate donors, and the low yields of these glycosylations (including the inability to replicate the 81% yield for the formation of 271 previously reported within the Gardiner group 354), an alternative strategy was designed to develop the chemoselective glycosylation strategy which avoided the use of PMB.
Scheme 62. Reagents: (i) NBS, Me$_2$CO, RT, 92%; (ii) Cl$_3$CCN, K$_2$CO$_3$, CH$_2$Cl$_2$, RT, 95%, (iii) 270, TMSOTf, CH$_2$Cl$_2$, –50°C.

10.3.3 Methanolation of l-Iduronic Lactone with Et$_3$N/MeOH

To develop methanolysis conditions for the lactone which could be applied to oligosaccharides bearing esters e.g. 6-OBz disaccharide 287, investigation of lactone opening using Et$_3$N/MeOH was carried out. Lactone derivatives with 4-OTBDMS, which were available from previous work in the Gardiner group, were used as substrates. Lactone $\alpha$-294 could be opened in 12 h with Et$_3$N/MeOH but $\beta$-294 required an extended reaction time (72 h, Scheme 63). The rationale for this is that both faces of the carbonyl in $\beta$-294 are sterically hindered due to the TBDMS and SPh substituents, whereas the SPh moiety does not block the carbonyl face in $\alpha$-294.$^{354}$

\[\text{Scheme 62, Reagents: (i) NBS, Me}_2\text{CO, RT, 92%; (ii) Cl}_3\text{CCN, K}_2\text{CO}_3, \text{CH}_2\text{Cl}_2, \text{RT, 95%, (iii) 270, TMSOTf, CH}_2\text{Cl}_2, -50{}^\circ\text{C.}}\]

\[\text{10.3.3 Methanolation of l-Iduronic Lactone with Et}_3\text{N/MeOH}\]

To develop methanolysis conditions for the lactone which could be applied to oligosaccharides bearing esters e.g. 6-OBz disaccharide 287, investigation of lactone opening using Et$_3$N/MeOH was carried out. Lactone derivatives with 4-OTBDMS, which were available from previous work in the Gardiner group, were used as substrates. Lactone $\alpha$-294 could be opened in 12 h with Et$_3$N/MeOH but $\beta$-294 required an extended reaction time (72 h, Scheme 63). The rationale for this is that both faces of the carbonyl in $\beta$-294 are sterically hindered due to the TBDMS and SPh substituents, whereas the SPh moiety does not block the carbonyl face in $\alpha$-294.$^{354}$

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$k$ Compound 294 was prepared by Dr Steen U. Hansen, Gardiner group, University of Manchester.
The separate anomers of 4-OPMB disaccharide 287 were also subjected to Et₃N/MeOH mediated lactone opening, followed by benzylation of the IdoA O-2 hydroxyl. Heating in α-287 in Et₃N/MeOH (plus THF as co-solvent) afforded the desired 2-OH intermediate, as observed by TLC and ¹H NMR, however the subsequent benzylation was unsuccessful and product α-296 was not isolated. Treatment of β-287 for 3 days under the same conditions did not result in lactone opening.

Scheme 63. Reagents: (i) Et₃N, MeOH, RT, 90% for α-295 (12 h), 60% for β-295 (72 h); (ii) Et₃N, MeOH/THF, 60 °C, then BzCl, 1-methyl-imidazole, DMAP, CH₂Cl₂.

10.4 Chemoselective Glycosylation Strategy with Non-reducing End Cap

10.4.1 Synthesis of Tri-benzylated Donor Disaccharide

To avoid the issue of PMB removal in glycosylations, the strategy was adapted to replace the PMB moiety with a benzyl protecting group at O-4, which would still give the desired 4-OH product after deprotection. This could then be glycosylated with a 4-OH lactone-containing acceptor disaccharide to give a tetrasaccharide. Iterative additions of the lactone-containing acceptor disaccharide followed by lactone opening to give a glycosyl donor could then be carried out as in the previous 4-OPMB strategy. It should be noted that the donor and acceptor disaccharides are not derived from the same disaccharide in this strategy. In addition, replacement of the PMB protecting group with benzyl precludes the selective deprotection of the non-reducing end due to
the presence of other benzyl groups. Therefore, the resulting oligosaccharides could not be functionalised at the non-reducing end post-oligomerisation. In order to simplify development of the chemoselective glycosylation strategy, oligosaccharides with no GlcN O-6 or O-3 sulfation were chosen as initial targets; such that the method could later be applied to a more diverse range of sulfation patterns.

Glycosylation of tri-benzylated glucoazide thioglycoside 223 with lactone 270 afforded disaccharide 297 in 73% yield (using newly purchased NIS, Scheme 64). Due to the anomers of L-ido lactone 270 being only partially separable by column chromatography, the glycosylation to form 297 was tested using a mixture of acceptor anomers. This had no detrimental impact on purification of the product by column chromatography. Formation of β-linked disaccharide 298 was observed in the glycosylation (~6:1 α/β, determined by 1H NMR) as previously noted in the Gardiner group for the 4-OPMB analogue.354 Desired product 297 could be obtained without contamination by 298 after column chromatography, and β-298 was isolated and characterised.

The mixture of anomers of α/β-297 was then subjected to methanolysis to open the lactone. As opening of 4-OPMB disaccharide β-287 with Et3N/MeOH was unsuccessful, NaOMe was used instead for α/β-297. However, this resulted in formation of an impurity; monitoring of the reaction by TLC showed that the impurity began to form before the starting material was completely consumed. Desired product 300 was obtained as a mixture of anomers after acetylation of crude 2-OH intermediate 299 and column chromatography (90% yield over two steps).

Investigation of the behaviour of the individual anomers of 297 in the lactone opening reaction showed α-297 could be opened and acetylated without the need for column chromatography, as the impurity did not form for this anomer. It is postulated that the impurity formed in the opening of β-297 may be a product of elimination or epimerisation under basic conditions, and that the formation of this is enhanced due to the extended reaction time relative to α-297.
Scheme 64. **Reagents:** (i) NIS, AgOTf, toluene, 4 Å mol. sieves, 0 °C, 73%; (ii) NaOMe, CH₂Cl₂/MeOH, RT; (iii) Ac₂O, 1-methylimidazole, DMAP, CH₂Cl₂, RT, 90% over two steps.

10.4.2 Synthesis of Lactone-containing Acceptor Disaccharide

With donor disaccharide 300 in hand, synthesis of a lactone-containing acceptor disaccharide was then required. There have been multiple reports of the relatively low reactivity of GlcN 4-OH, for example Seeberger’s observation that 4-OH tetrasaccharide 67 could not be used as an acceptor (Scheme 14).

Zhu and Boons reported use of a 4-hydroxyl monosaccharide glucoazide donor, and glycosylation of a primary alcohol to a 4-OH tetrasaccharide has previously been performed in the Gardiner group. Based on this, glycosylation of 4-OH glucoazide donor 174 with lactone 270 was investigated (Scheme 65). Disaccharide 301 could be obtained in 59% yield. Formation of trisaccharide 302 was observed by mass spectrometry but this could not be isolated from the reaction. The use of two equivalents of acceptor was found to result in an improved impurity profile, it is proposed that the use of additional equivalents of acceptor decreases the likelihood of donor monosaccharide 174 reacting with the product to form 302. The additional lactone used could be recovered from the reaction (80% recovery of unreacted amount). Therefore, this 4-hydroxyl glycosylation strategy was used to furnish acceptor disaccharide 301, avoiding the need to protect O-4 of the monosaccharide and deprotect after the glycosylation.
Scheme 65. Reagents: (i) NIS, AgOTf, CH₂Cl₂, 4 Å mol. sieves, 0 °C, 59%.

10.4.3 Synthesis of Tetrasaccharide via Chemoselective Glycosylation

Synthesis of tetrasaccharide 303 via chemoselective activation of donor 300 over acceptor 301 was attempted (Table 10). Desired tetrasaccharide product 303 was formed in 35% yield using NIS/AgOTf as promoter in dichloromethane (entry 1). However, this result was not reproducible. Changing the reaction solvent to toluene resulted in no activation of the donor (entry 2). Use of newly purchased NIS and reduction of reaction temperature from 0 °C to –20 °C or –78 °C resulted in a maximum of 50% conversion of acceptor to product, with full consumption of donor (entries 5 and 6). This indicated decomposition of the donor was occurring under the glycosylation conditions. Use of the BSP/Tf₂O activating system resulted in decomposition of the starting materials, and iodine was not able to activate the donor (entries 3 and 4).
Table 10. Glycosylation of 2-OAc disaccharide donor 300 with lactone-containing disaccharide 301.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NIS/AgOTf, CH(_2)Cl(_2), 0 °C</td>
<td>35% isolated yield of 303, 56% recovered 301</td>
</tr>
<tr>
<td>2</td>
<td>NIS/AgOTf, toluene, 0 °C</td>
<td>No donor activation</td>
</tr>
<tr>
<td>3</td>
<td>BSP/Tf(_2)O, CH(_2)Cl(_2), –78 °C</td>
<td>Decomposition of starting materials</td>
</tr>
<tr>
<td>4</td>
<td>I(_2), CH(_2)Cl(_2), 0 °C</td>
<td>No donor activation</td>
</tr>
<tr>
<td>5</td>
<td>NIS/AgOTf, CH(_2)Cl(_2), –20 °C</td>
<td>1:1 ratio acceptor/product by (^1)H NMR</td>
</tr>
<tr>
<td>6</td>
<td>NIS/AgOTf, CH(_2)Cl(_2), –78 °C</td>
<td>1:1 ratio acceptor/product by (^1)H NMR</td>
</tr>
</tbody>
</table>

* NIS was newly purchased

Based on the poor outcomes of this glycosylation, 2-OBz disaccharide \(\alpha/\beta\)-254 was prepared as an alternative glycosyl donor. Methanolysis of disaccharide \(\alpha/\beta\)-297 followed by benzoylation of O-2 afforded \(\alpha/\beta\)-254 in 75% yield over two steps (Scheme 66). Glycosylation of this donor with lactone-containing disaccharide 301 proceeded smoothly using NIS/AgOTf activation in dichloromethane, affording tetrasaccharide 304 in 77% yield.

Methanolysis of the lactone moiety in tetrasaccharide 304 followed by benzoylation of IdoA O-2 under the same conditions as for disaccharide 297 (~0.2 eq. NaOMe in MeOH) was problematic. A complex mixture of products was formed in the methanolysis step indicating decomposition of the starting material, which is attributed to the extended reaction time (>24 h). Use of stoichiometric NaOMe in MeOH did not result in a reduced reaction time. Subsequently, it was found that treatment of \(\alpha\)-304...
with ~0.15 eq. NaOMe in MeOH resulted in opening of the lactone within 5 minutes, and the intermediate was successfully benzoylated to provide glycosyl donor \( \alpha-305 \).

Scheme 66. Reagents: (i) NaOMe, CH\(_2\)Cl\(_2\)/MeOH, RT; (ii) BzCl, 1-methyl-imidazole, DMAP, DCE, Δ, 75% over two steps for \( \alpha/\beta-254 \); (iii) 301, NIS, AgOTf, CH\(_2\)Cl\(_2\), 4 Å mol. sieves, 0 °C, 77%.

Synthesis of tetrasaccharide 304 via chemoselective activation of the SPh donor group of disaccharide 254 over the lactone SPh group in disaccharide 301 underpins the chemoselective strategy proposed for synthesis of HS oligosaccharides. The key features of this approach are that no anomic manipulations are required between glycosylation steps, and each oligosaccharide produced has a thioglycoside group which can be used for conjugation, for instance attachment of the amine tag for fluorescent labelling.

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\(^1\) This reaction was performed by Robin Jeanneret, Gardiner group, University of Manchester.
11. CONCLUSIONS

The aim of this project was to investigate the use of single molecule fluorescence techniques for the study of HS-protein binding. Firstly, synthesis of a HS disaccharide bearing a fluorescent label was undertaken. Fluorophore conjugation via a reducing end aldehyde tag was unsuccessful, both by direct reaction with Alexa Fluor 488 hydroxylamine and by reductive amination with mono-protected diamine linkers. It is proposed that the low reactivity of the aldehyde may be due to the additional equilibria between the ring closed and/or hydrate forms in solution. Following literature precedent for use of amines for conjugation of HS oligosaccharides, a primary amine tag was used for conjugation to Alexa Fluor 488 dye. Formation of the desired fluorescent conjugate was confirmed by proton NMR and mass spectrometry.

The fluorescence characteristics of Alexa Fluor 488-labelled disaccharide 178 were then analysed. Ensemble absorption and emission fluorescence spectra were in good agreement with the free dye and Alexa Fluor 488-labelled ssDNA, indicating a lack of dye perturbation by the disaccharide. Fluorescence correlation spectroscopy of 178 showed similar counts per molecule to that of the free dye, indicating a lack of perturbation in the dye when attached to the sugar. Single molecule measurements using an MFD set-up showed that Alexa Fluor 488 has the same fluorescence lifetime in 178 and labelled dsDNA (4.1 ns), and that the dye in 178 can be considered freely rotating as it has anisotropy of approximately zero.

Labelled disaccharide 178 was encapsulated in biotinylated vesicles and visualised using TIRF microscopy; the number of disaccharide molecules encapsulated in each vesicle could be determined from the number of photobleaching steps in the time trace. When vesicles were prepared using a 500 nM solution of disaccharide 178, single-step traces were predominantly seen, indicating one disaccharide molecule per vesicle. These experiments showed that disaccharide 178 can be encapsulated into vesicles and visualised, which may be useful for future experiments as vesicles can be used to mimic cellular/membrane conditions.

The interaction of labelled disaccharide 178 and FGF-1 was studied using FCS by measurement of diffusion time, binding of 178 to FGF-1 would be expected to result in a 2.4-fold increase in diffusion time, however no increase was observed. This is attributed to the relatively weak binding of disaccharides (K_d in micromolar range) to FGF-1 and the low concentration of disaccharide 178 (1 nM) required for FCS.
An additional aim of this research project was to further develop chemistry for the synthesis and functionalisation of HS oligosaccharides. A new method for atom-specific $^{13}$C enrichment in HS building blocks was developed by formation of $^{13}$C-labelled L-ido cyanohydrin using $^{13}$CN followed by transformation into a methoxy-capped HS disaccharide. It is proposed that this disaccharide could be incorporated at the reducing end of a HS oligosaccharide via glycosylation.

Orthogonal glycosylations for synthesis of HS disaccharides using the STaz and SBox thioimidate groups developed by Demchenko et al. were investigated. Glucoazide SBox donors were selectively activated over iduronate SPh acceptors. Glucoazide STaz derivatives were generally not activated using stoichiometric AgOTf (with the exception of the armed 4-OPMB derivative). Iduronate SBox glycoside 251 was prepared from the corresponding glycosyl acetate; however selective activation of both glucoazide STaz and SPh donors over the iduronate SBox acceptor was unsuccessful. Iduronate SBox glycoside 250 could be activated using stoichiometric AgOTf in the presence of a 4-OH glucoazide SPh acceptor, however the desired disaccharide did not form.

In order to provide further HS oligosaccharides for labelling and single molecule studies, a chemoselective glycosylation strategy employing [2.2.2] L-ido lactone 270 was developed. This was based on previous work in the Gardiner group which showed glucoazide SPh donors can be selectively activated in the presence of the lactone SPh group, and that the lactone SPh does not activate under normal NIS/AgOTf glycosylation conditions. However, attempts to perform this glycosylation with 4-OPMB donors with differing O-3 and O-6 protection gave low yields (including for direct repeats of previously reported glycosylations). In addition, PMB removal/migration was observed resulting in the formation of 4-OPMB lactone derivative 288.

Due to these issues, the strategy was re-designed with 4-OBn protection. Glycosylation of 3,4,6-tribenzylated donor 223 with lactone 270 proceeded in 73% yield, subsequent methanolysis and acetylation resulted in glycosyl donor 300. To afford the desired acceptor, 4-OH donor 174 was glycosylated with lactone 270 (58%). Formation of the tetrasaccharide was problematic with 2-OAc donor 300, but proceeded smoothly with 2-OBz donor 254. Although tetrasaccharide $\beta$-304 was not easily opened using NaOMe, it was subsequently shown that treatment of $\alpha$-304 with NaOMe resulted in lactone opening within 5 minutes, and resulting tetrasaccharide 305 should be
suitable as a glycosyl donor. This chemoselective lactone-based glycosylation strategy requires no anomeric manipulation steps between glycosylations, and each oligosaccharide formed has a thioglycoside group and can therefore be functionalised via glycosylation (e.g. attachment of the amine tag). The $n+2$ approach allows for all even-numbered lengths of oligosaccharide to be accessed, and inclusion of alternative disaccharide building blocks for programming sulfation into the sequence should also be possible.\textsuperscript{246}
12. FURTHER WORK
To continue development of single molecule fluorescence as a new method of investigating HS-protein binding, more fluorescently labelled HS oligosaccharides are required. These could be prepared using the chemoselective lactone-based glycosylation strategy outlined above, employing the amine tag method for fluorophore conjugation. A range of oligosaccharide lengths and sulfation patterns would be desired, which the lactone-based glycosylation strategy should facilitate. Investigation of the impact of different lengths of oligosaccharide-dye linker and of attaching the dye at the non-reducing end would allow a labelling method to be selected which has least impact on the binding capability/function of the HS oligosaccharides. The effect of the dye on binding of HS oligosaccharides to proteins would be investigated by comparing the labelled and unlabelled oligosaccharides using ITC. The addition of the amine tag also allows potential for other types of conjugation e.g. immobilisation.

The interaction of these fluorescently labelled oligosaccharides with proteins could then be studied using FCS, although it is expected that this would require implementation of the use of zero mode waveguides as binding would still be relatively weak and FCS requires very low concentrations. This technique could be developed into an effective method for screening which HS sequences bind to a given protein, as the protein does not need to be modified and crucially only a small amount (<10 µg) would be needed. This is particularly relevant for many chemokines which are relatively expensive (~£1000/mg).

Further investigation of binding at the single-molecule level could be undertaken using acceptor dye-labelled proteins, which would allow smFRET measurements between the oligosaccharide and protein. Molecular dynamics simulations would then be used to compute an overall structure of the HS-protein complexes using distances calculated from smFRET studies. Use of a single molecule method would remove averaging and could give new insights into the dynamics of HS-protein binding by showing rare or transient states in the binding, this is information which could not be accessed in ensemble methods such as NMR, or static methods such as X-ray crystallography. It is hoped that the knowledge gained about HS-protein binding, taken in conjunction with other binding studies and assays of biological activity, would have a practical application in the development of therapeutics for the multiple conditions in which HS-protein binding is relevant, including cancer, viral infection and Alzheimer’s disease.
Chapter Three: Experimental

13. EXPERIMENTAL

13.1 Synthesis

13.1.1 General Experimental Detail for Synthesis

Melting points were determined using a Stuart Scientific SMP10 apparatus and are uncorrected. IR spectra were recorded for solid samples using a Bruker Alpha-P ATR spectrometer. NMR spectra were recorded for solutions in CDCl$_3$, CD$_3$CO$_2$D, CD$_3$OD, D$_2$O or d$_6$-DMSO on Bruker Avance instruments (400 or 500 MHz) and were referenced to the residual solvent signal. Assignments were determined using COSY and HMQC experiments, and HMBC experiments where required. Where resonances in the $^{13}$C NMR spectrum are not assignable due to spectral overlap, the multiplicity of the carbon is given (from DEPT135). Coupling constants ($J$ values) are quoted to the nearest 0.1 Hz and are given as observed i.e. not made equal for coupling protons. For NMR assignment, protons are labelled H-1 to H-6 for the reducing end residue, then H-1’ to H-6’, H-1” to H-6” and so on towards the non-reducing end. If distinguishable, H-6 protons are denoted H-6ax (axial) and H-6eq (equatorial). Where $^1$H NMR data for a mixture of anomers in a ratio other than 1:1 is given, partial integrals are reported. Low resolution mass spectra were measured on a Micromass Platform II instrument with electrospray ionisation. Accurate mass measurements were obtained using a Micromass Q-TOF instrument with electrospray ionisation (University of Manchester) or a ThermoFisher LTQ Orbitrap XL instrument with nanospray ionisation (EPSRC National Mass Spectrometry Facility, Swansea). LCMS was performed using a Phenomenex Sphereclone ODS(2) column (4.6 mm x 250 mm, 5 µm), eluent water/acetonitrile (gradient 5% MeCN to 95% MeCN over 10 min, hold 20 min), UV detection (220 nm) and negative electrospray ionisation. Elemental analysis was performed by the Microanalytical Service, School of Chemistry, University of Manchester. Optical rotations were measured using a Bellingham and Stanley ADP410 or an Optical Activity AA-1000 polarimeter, and are given in units of deg cm$^3$ g$^{-1}$ dm$^{-1}$ with c quoted in g 100 mL$^{-1}$. UV-visible spectra were recorded using a Jasco V660 spectrophotometer. Analytical thin layer chromatography (TLC) was performed on Merck TLC Silica gel 60 plates (aluminium backed, UV$_{254}$ fluorescent indicator) with visualisation using UV light and/or cerium molybdate/phosphomolybdic acid stains. Preparative column chromatography was performed using Sigma-Aldrich silica gel (technical grade, 60 Å, 220–240 mesh, 35–75 µm) and the flash technique. $^{375}$ Dry column vacuum chromatography was performed as described by Pedersen and
Rosenbohm using Merck silica gel (60 Å, 15–40 µm). Preparative TLC was performed using Analtech Uniplate™ silica gel 60 plates (glass backed, UV_{254} fluorescent indicator, 1000 µm, 20 x 20 cm). All solvents used were of standard laboratory grade unless otherwise specified. ‘Petroleum ether’ refers to the fraction with boiling point 40–60 °C. Dichloromethane was dried using calcium hydride under nitrogen or activated 4 Å molecular sieves. Other anhydrous solvents were used as purchased or dried over activated 4 Å molecular sieves. pH 4.65 buffer (sodium acetate/acetic acid) was used as purchased (Sigma-Aldrich). IDCP was prepared according to the procedure reported by Lemieux and Morgan. Compositions of solvent mixtures are quoted as ratios of volume. Molecular sieves were activated by drying in an oven at 200 °C, or by heating under vacuum at 300 °C. Organic solutions were dried with anhydrous magnesium sulfate and concentrated by rotary evaporation under reduced pressure. Reaction mixtures were cooled in either ice/water/NaCl baths (0 to –20 °C), iPrOH/CO_{2}(s)/H_{2}O (–20 °C to –60 °C) or Me_{2}CO/CO_{2}(s) (–60 °C to –78 °C). Alexa Fluor 488 SDP ester was purchased from ThermoFisher Scientific. Cyanine3 dyes were purchased from Lumiprobe (Germany). Amberlite® and Dowex® were purchased from Sigma-Aldrich. Sephadex resins were purchased from GE.

13.1.2 Synthesis of Disaccharide with Aldehyde Tag 135

(S)-2,3-Bis(benzyloxy)propanol (2-azido-3,6-di-O-benzyl-4-O-p-methoxybenzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosiduronate 129

Disaccharide 128 (200 mg, 0.20 mmol) and (S)-(−)-2,3-dibenzylxyloxy-1-propanol (75 µL, 0.30 mmol) were dissolved in toluene (10 mL), concentrated in vacuo, and the process repeated twice. The mixture was dried under high vacuum for 2 h, then dissolved in anhydrous CH_{2}Cl_{2} (3 mL) under N_{2}, cooled to 0 °C, molecular sieves (~200 mg, 4 Å,
powdered, activated) were added and the reaction was stirred at 0 °C for 30 min. NIS (99 mg, 0.44 mmol) was added and the resulting pale red solution stirred at 0 °C for 30 min. AgOTf (7 mg, 30 µmol) was added and the reaction stirred at 0 °C for 30 min. TLC of the resulting deep red solution (3:1 hexane/EtOAc) showed no remaining disaccharide starting material. Na₂S₂O₃ (0.5 g) and NaHCO₃ (0.5 g) in water (10 mL) were added and the reaction stirred for 10 min at RT. The reaction mixture was filtered through Celite®, the phases separated and the aqueous phase washed with CH₂Cl₂ (3 x 10 mL). The combined organic phases were dried and concentrated in vacuo to an orange oil. Column chromatography (98:2 CH₂Cl₂/Et₂O) afforded the title compound 129 as a yellow glass (192 mg, 0.17 mmol, 82%); Rf 0.39 (3:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2866 (C–H), 2106 (N₃), 1764 (C=O), 1719 (C=O); MS (ES⁺) found m/z 1162 [M+NH₄⁺], HRMS found 1166.4622, C₆₆H₆₉N₃NaO₁₅ [M+Na⁺] requires 1166.4621; [α]D = –3.90 (c = 2.4, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.08 (2 H, dd, J = 8.4, 1.2 Hz, ArH), 7.40–7.37 (2 H, m, ArH), 7.39–7.19 (24 H, m, ArH), 7.11 (2 H, dd, J = 7.8, 2.0 Hz, ArH), 7.05 (2 H, dd, J = 6.6, 2.0 Hz, ArH), 6.84 (2 H, dd, J = 6.4, 2.0 Hz, ArH), 5.14–3.13 (2 H, m, H-1, H-2), 4.90 (1 H, d, J = 11.5 Hz, CH₂Ar), 4.86 (1 H, d, J = 1.9 Hz, H-5), 4.77 (1 H, d, J = 3.5 Hz, H-1’), 4.74 (1 H, d, J = 11.5 Hz, CH₂Ar), 4.64 (1 H, d, J = 11.9 Hz, CH₂Ar), 4.61–4.55 (3 H, m, 3 x CH₂Ar), 4.45 (2 H, app d, J = 12.7 Hz, 2 x CH₂Ar), 4.40 (2 H, app d, J = 10.5 Hz, 2 x CH₂Ar), 4.15–4.12 (3 H, m, H-3, 2 x CH₂Ar), 4.05 (1 H, t, J = 2.0 Hz, H-4), 3.93–3.87 (3 H, m, 3 x CH handle), 3.80–3.75 (5 H, m, 2 x H-6’, ArOCH₂), 3.69–3.62 (5 H, m, H-4’, H-5’, CO₂CH₂), 3.53 (2 H, dd, J = 5.3, 2.7 Hz, 2 x CH handle), 3.49 (1 H, t, J = 9.6 Hz, H-3’), 3.21 (1 H, dd, J = 10.4, 3.6 Hz, H-2’); δC (101 MHz, CDCl₃): 169.6 (C=O), 159.3 (C=O), 138.6 (Ar C), 138.2 (Ar C), 138.0 (Ar C), 137.9 (Ar C), 137.6 (Ar C), 133.3 (Ar CH), 130.6 (Ar CH), 130.1 (Ar CH), 129.7 (Ar CH), 129.5 (Ar CH), 128.9 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.3 (2 x Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 127.8 (2 x Ar CH), 127.7 (Ar CH), 127.6 (Ar CH), 113.7 (Ar CH), 100.1 (C-1’), 99.3 (C-1), 80.1 (C-3), 77.4 (CH), 76.7 (CH), 76.3 (C-4), 74.6 (CH₂Ar), 74.4 (CH₂Ar), 73.6 (CH₂Ar), 73.3 (CH₂Ar), 73.2 (CH), 72.4 (CH₂Ar), 71.7 (CH₂), 69.6 (CH₂), 68.4 (CH₂), 67.3 (CH), 67.1 (C-5), 63.7 (C-2’), 55.3 (ArOCH₃), 55.2 (CO₂CH₂).
To a stirred solution of disaccharide 129 (310 mg, 0.27 mmol) in MeCN (5 mL) and water (0.5 mL) was added CAN (295 mg, 0.54 mmol) and the resulting solution stirred at RT for 4 h. TLC (3:1 hexane/EtOAc) showed no remaining disaccharide starting material. CH$_2$Cl$_2$ (25 mL) and saturated aq. NaHCO$_3$ (10 mL) were added, the phases separated and the organic phase dried and concentrated in vacuo to a yellow oil. Column chromatography (4:1 to 2:1 hexane/EtOAc) afforded the title compound 130 as a yellow glass (229 mg, 0.20 mmol, 85%); R$_f$ 0.11 (3:1 hexane/EtOAc); IR (ν$_{max}$/cm$^{-1}$): 3030 (O–H), 2867 (C–H), 2106 (N$_3$), 1763 (C=O), 1718 (C=O); MS (ES$^+$) found m/z 1046 [M+Na]$^+$, HRMS found 1046.4049, C$_{58}$H$_{61}$N$_3$NaO$_{14}$ [M+Na]$^+$ requires 1046.4046; [α]$_D$ = −2.3 (c = 1.30, CH$_2$Cl$_2$); δ$_H$ (400 MHz, CDCl$_3$): 8.16 (2 H, dd, J = 8.3, 1.2 Hz, ArH), 7.49 (1 H, d, J = 7.3 Hz, ArH), 7.45–7.41 (2 H, m, ArH), 7.39–7.37 (3 H, m, ArH), 7.36–7.27 (20 H, m, ArH), 7.18 (2 H, dd, J = 8.0, 1.2 Hz, ArH), 5.15–5.17 (1 H, m, H-1), 5.15–5.14 (1 H, m, H-2), 4.88–4.84 (2 H, m, H-5, CH$_2$Ar), 4.75–4.72 (2 H, m, H-1′, CH$_2$Ar), 4.62 (1 H, d, J = 11.9 Hz, CH$_2$Ar), 4.58 (1 H, d, J = 12.3 Hz, CH$_2$Ar), 4.55 (1 H, d, J = 12.4 Hz, CH$_2$Ar), 4.51 (1 H, d, J = 12.0 Hz, CH$_2$Ar), 4.42 (1 H, d, J = 12.1 Hz, CH$_2$Ar), 4.39–4.34 (2 H, m, 2 x CH$_2$Ar), 4.27 (1 H, d, J = 11.0 Hz, CH$_2$Ar), 4.14–4.11 (2 H, m, H-3, CH$_2$Ar), 4.05 (1 H, t, J = 2.4 Hz, H-4), 3.92 (1 H, dd, J = 10.4, 5.6 Hz, CH handle), 3.86–3.82 (1 H, m, H-5′), 3.81–3.77 (2 H, m, 2 x CH handle), 3.73–3.66 (6 H, m, H-4′, 2 x H-6′, CO$_2$CH$_3$), 3.62 (1 H, dd, J = 10.1, 4.6 Hz, CH handle), 3.57 (1 H, dd, J = 5.3, 3.2 Hz, CH handle), 3.48 (1 H, dd, J = 10.1, 8.8 Hz, H-3′), 3.16 (1 H, dd, J = 10.0, 3.6 Hz, H-2′); δ$_C$ (101 MHz, CDCl$_3$): 171.2 (C=O), 169.5 (C=O), 138.5 (Ar C), 138.1 (Ar C), 138.0 (Ar C), 137.7 (Ar C), 137.5 (Ar C), 133.3 (Ar CH), 130.1 (Ar CH), 128.7 (Ar CH), 128.5 (Ar CH), 128.4 (2 x Ar CH), 128.3 (2 x Ar CH), 128.1 (Ar CH), 127.9 (3 x Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 127.6 (Ar CH),
127.5 (Ar CH), 99.3 (C-1’), 99.2 (C-1), 79.7 (C-3’), 76.7 (C-5’), 75.5 (C-4), 74.7
(CH2Ar), 73.7 (CH2Ar), 73.3 (CH2Ar), 72.9 (CH2Ar), 72.4 (C-3), 72.3 (CH2Ar), 70.5
(CH), 69.6 (CH2), 69.5 (CH2), 68.5 (CH2), 67.7 (C-2), 67.3 (C-5), 63.1 (C-2’), 52.2
(CO2CH3).

(S)-2,3-Bis(benzyloxy)propanl (2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-O-benzyl-α-L-idopyranosiduronic acid 131

To a stirred solution of disaccharide 130 (131 mg, 0.13 mmol) in THF (4 mL) was
added MeOH (1 mL) and the reaction cooled to 0 °C. LiOH monohydrate (21.6 mg,
0.51 mmol) was dissolved in water (1 mL) and added dropwise. The reaction mixture
was allowed to gradually warm to RT and stirred overnight. TLC (95:5 CH2Cl2/MeOH)
showed no remaining disaccharide starting material. HCl (0.3 mL, 1 M) was added and
the solvents removed in vacuo to give a white solid. Column chromatography (20:1
CH2Cl2/MeOH) gave the title compound 131 as a colourless glass (97 mg, 0.11 mmol,
85%); Rf 0.15 (95:5 CH2Cl2/MeOH); IR (νmax/cm−1): 3394 (O–H), 2868 (C–H), 2112
(N3), 1708 (C=O); MS (ES+) found m/z 928 [M+Na]+, HRMS found 928.3611,
C50H55N3NaO13 [M+Na]+ requires 928.3628; [α]D = +44.9 (c = 0.50, CH2Cl2), δH (400
MHz, CD3CO2D): 7.44–7.26 (25 H, m, Ar H), 5.14 (1 H, d, J = 4.0 Hz, H-1’), 5.04 (1 H,
s, H-1), 4.95–4.91 (2 H, m, CH2Ar, H-5), 4.80 (1 H, d, J = 11.0 Hz, CH2Ar), 4.74 (1 H,
d, J = 11.4 Hz, CH2Ar), 4.66 (1 H, d, J = 12.0 Hz, CH2Ar), 4.63–4.57 (3 H, m, 3 x
CH2Ar), 4.56 (1 H, d, J = 12.0 Hz, CH2Ar), 4.46 (1 H, d, J = 12.2 Hz, CH2Ar), 4.42 (1
H, d, J = 12.1 Hz, CH2Ar), 4.27–4.26 (1 H, m, H-4), 4.04–4.03 (1 H, m, H-3), 3.96–
3.95 (1 H, m, H-2), 3.91–3.78 (6 H, m, H-5’, 2 x H-6’, 3 x CH handle), 3.70–3.67 (5 H,
m, H-2’, H-3’, H-4’, 2 x CH handle); δC (101 MHz, CD3CO2D): 173.5 (C=O), 138.3
(Ar C), 138.0 (2 x Ar C), 137.9 (Ar C), 137.8 (Ar C), 133.6 (Ar CH), 129.9 (Ar CH),
128.3 (Ar CH), 128.2 (2 x Ar CH), 128.1 (2 x Ar CH), 127.8 (Ar CH), 127.7 (Ar CH),
129
127.6 (Ar CH), 127.5 (2 x Ar CH), 101.0 (C-1), 95.1 (C-1’), 80.7 (CH), 76.8 (CH), 75.2 (CH₂Ar), 73.3 (CH₂Ar), 73.0 (CH₂Ar), 71.9 (C-4), 71.8 (C-3), 71.7 (CH₂Ar), 70.3 (CH), 69.3 (CH₂), 68.3 (CH₂), 67.9 (CH₂), 66.8 (C-5), 65.6 (C-2), 63.4 (C-2’).

(S)-2,3-Bis(benzyloxy)propanyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-sulfo-α-D-glucopyranosyl)-(1→4)-3-O-benzyl-2-sulfo-α-L-idopyranosiduronic acid trisodium salt 132

To a stirred solution of disaccharide 131 (165 mg, 0.18 mmol) in anhydrous pyridine (3 mL) under N₂ at RT was added SO₃·pyridine (173 mg, 1.09 mmol) and the reaction stirred overnight. Saturated aq. NaHCO₃ (2 mL) was added and the mixture concentrated in vacuo to a white paste. Column chromatography (9:1, 4:1 CH₂Cl₂/MeOH) afforded the free acid form of disaccharide 132, which was then stirred with Amberlite IRC-86-Na⁺ resin in CH₂Cl₂/MeOH (5:1) for 12 h, filtered and the solvents removed to give the title compound 132 as a yellow glass (176 mg, 0.16 mmol, 86%); Rf 0.11 (95:5 CH₂Cl₂/MeOH); IR (νmax/cm⁻¹): 3438 (O–H), 2924 (C–H), 2171 (N₃), 1612 (C=O); MS (ES⁻) found 1137 [M−3Na+2H+DEA]⁻, HRMS found 1086.2615, C₅₀H₅₃N₃NaO₁₉S₂ [M−2Na+H]⁻ requires 1086.2618; [α]D = –44.9 (c = 0.10, CH₂Cl₂); δH (400 MHz, CD₃CO₂D): 7.47 (2 H, d, J = 7.2 Hz, Ar H), 7.39–7.35 (4 H, m, Ar H), 7.33–7.19 (19 H, m, Ar H), 5.30–5.29 (1 H, m, H-1’), 5.14–5.13 (1 H, m, H-1), 5.02 (1 H, d, J = 10.4 Hz, CH₂Ar), 4.92 (1 H, s, H-2), 4.79 (1 H, d, J = 11.6 Hz, CH₂Ar), 4.73 (1 H, d, J = 10.6 Hz, CH₂Ar), 4.66–4.57 (6 H, m, H-5, 5 x CH₂Ar), 4.49 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.45 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.30–4.29 (4 H, m, H-3, H-4, H-5’, CH handle), 3.96 (1 H, d, J = 6.8 Hz, CH handle), 3.87–3.83 (3 H, m, H-4’, 2 x H-6’), 3.66–3.65 (1 H, m, H-3’), 3.57–3.49 (4 H, m, H-2’, 3 x CH handle); δC (101 MHz, CD₃CO₂D): 165.6 (C=O), 138.2 (Ar C), 138.1 (Ar C), 138.0 (Ar C), 137.9 (Ar C), 137.6 (Ar C), 128.6 (Ar CH), 128.2 (2 x Ar CH), 128.1 (Ar CH), 128.0 (Ar
(S)-2,3-Dihydroxypropanyl (2-amino-2-deoxy-4-O-sulfo-α-D-glucopyranosyl)-(1→4)-2-sulfo-α-L-idopyranosiduronic acid trisodium salt 133

To a stirred solution of disaccharide 132 (50 mg, 44 µmol) in EtOH (2 mL) and water (1 mL) under N₂ was added Pd(OH)₂/C (50 mg) and the system purged with H₂. The reaction was stirred at 40 °C under H₂ overnight. TLC (6:5:3:1 EtOAc/pyridine/H₂O/AcOH) showed no remaining disaccharide starting material. The reaction mixture was filtered through Celite with EtOH and stripped to give the title compound 133 as a white powder (30 mg, 44 µmol, quant.); R_f 0.49 (6:5:3:1 EtOAc/pyridine/H₂O/AcOH); IR (ν_max/cm⁻¹): 3403 (O–H), 2926 (C–H), 1600 (C=O); MS (ES⁻) found 588 [M–3Na+2H]⁻, HRMS found 610.0367, C₁₅H₂₅NNaO₁₉S₂ [M–2Na+H]− requires 610.0365; [α]D = −5.0 (c = 2.50, H₂O); δH (400 MHz, D₂O): 5.19 (1 H, d, J = 2.8 Hz, H-1’), 5.09 (1 H, s, H-1), 4.52 (1 H, d, J = 1.6 Hz, H-5), 4.26–4.24 (2 H, m, H-2, H-3), 4.16 (1 H, t, J = 9.2 Hz, H-4’), 4.05 (1 H, s, H-4), 3.91–3.86 (1 H, m, CH handle), 3.83–3.79 (4 H, m, H-3’, H-5’, 2 x H-6’), 3.72–3.67 (1 H, m, CH handle), 3.63–3.54 (3 H, m, 3 x CH handle), 3.05 (1 H, d, J = 11.6 Hz, H-2’); δC (101 MHz, D₂O): 175.1 (C=O), 98.2 (C-1), 93.4 (C-1’), 76.2 (C-4’), 72.6 (CH), 71.3 (C-4), 70.3 (CH), 70.0 (CH), 69.9 (CH), 69.2 (CH₂), 66.5 (C-5), 63.6 (CH), 62.4 (CH₂), 59.7 (CH₂), 54.3 (C-2’).
(S)-2,3-Dihydroxypropanyl (2-deoxy-2-\(N\)-sulfo-4-\(O\)-sulfo-\(\alpha\)-d-glucopyranosyl)-(1\(\rightarrow\)4)-2-sulfo-\(\alpha\)-L-idopyranosiduronic acid tetrasodium salt 134

To a stirred solution of disaccharide 133 (30 mg, 46 \(\mu\)mol) in \(H_2O\) (2 mL) at RT was added NaHCO\(_3\) (39 mg, 0.46 mmol) and SO\(_3\cdot\)pyridine (36 mg, 0.30 mmol). The reaction was stirred at RT for 1 h, and a second addition of NaHCO\(_3\) (39 mg, 0.46 mmol) and SO\(_3\cdot\)pyridine (36 mg, 0.30 mmol) was made. The reaction was stirred for a further 1 h and a third addition of NaHCO\(_3\) (39 mg, 0.46 mmol) and SO\(_3\cdot\)pyridine (36 mg, 0.30 mmol) was made. The reaction was then stirred at RT overnight. TLC (6:5:3:1 EtOAc/pyridine/\(H_2O\)/AcOH) showed no remaining disaccharide starting material. The solvent was removed \(in\ vacuo\) to give a white paste, which was purified by size exclusion chromatography (Sephadex G-25, eluting with \(H_2O\)) to give the title compound 134 (25 mg, 33 \(\mu\)mol, 72%); \(R_f\) 0.08 (6:5:3:1 EtOAc/pyridine/\(H_2O\)/AcOH); IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)): 3392 (O–H), 2942 (C–H), 1611 (C=O); MS found 293 \([M-4\text{Na}+2\text{H}]^–\), HRMS found 333.5027, \(C_{15}H_{25}NO_{22}S_3\) \([M-4\text{Na}+2\text{H}]^–\) requires 333.5021; [\(\alpha\)]\(_D\) = −5.9 (c = 2.50, \(H_2O\)); \(\delta_H\) (400 MHz, \(D_2O\)): 5.33 (1 H, d, \(J = 3.6\) Hz, H-1’), 5.12 (1 H, d, \(J = 2.0\) Hz, H-1), 4.48 (1 H, d, \(J = 2.4\) Hz, H-5), 4.27–4.24 (1 H, m, H-2), 4.23–4.18 (2 H, m, H-3, H-4’), 4.03 (1 H, t, \(J = 2.8\) Hz, H-4), 3.91–3.85 (2 H, m, H-5’, \(CH\) handle), 3.81–3.79 (2 H, m, 2 x H-6’), 3.77–3.74 (1 H, m, H-3’), 3.69–3.68 (1 H, m, \(CH\) handle), 3.62 (2 H, dd, \(J = 6.6, 4.0\) Hz, 2 x \(CH\) handle), 3.55 (1 H, dd, \(J = 7.6, 6.4\) Hz, \(CH\) handle), 3.27 (1 H, dd, \(J = 10.6, 3.6\) Hz, H-2’); \(\delta_C\) (101 MHz, \(D_2O\)): 174.9 (C=O), 98.7 (CH), 96.9 (CH), 77.0 (CH), 75.9 (CH), 75.1 (CH), 70.2 (CH), 70.1 (CH), 69.4 (CH), 69.2 (CH\(_2\)), 68.1 (CH), 68.0 (CH), 62.5 (CH\(_2\)), 60.0 (CH\(_2\)), 57.8 (CH).
2-Oxoethyl (2-deoxy-2-N-sulfo-4-O-sulfo-α-D-glucopyranosyl)-2-sulfo-α-L-idopyranosiduronic acid tetrasodium salt 135

To a stirred solution of disaccharide 134 (20 mg, 26 µmol) in D₂O (1 mL) at RT was added NaIO₄ (6.2 mg, 28 µmol) and the reaction stirred for 1 h. ¹H NMR of the reaction mixture showed no remaining disaccharide starting material. The solvent was removed in vacuo and the residue purified by size exclusion chromatography (Sephadex G-25, using H₂O as eluent), to give the title compound 135 as a colourless glass (10 mg, 14 µmol, 54%); Rᵢ 0.08 (6:5:3:1 EtOAc/pyridine/H₂O/AcOH); IR (νₑₓₛ/cm⁻¹): 3349 (O–H), 1571 (C=O); MS (LCMS, ES⁻) found m/z 701 [M–Na]⁻, 599 [M–2Na-SO₃⁺H⁻], 498 [M–2Na-2(SO₃)+2H⁻], 339 [M–2Na]⁻²⁻, HRMS found 339.4703, C₁₄H₁₉NNa₂O₂S₃ [M–2Na]⁻ requires 339.4709; [α]D = +8.9 (c = 0.75, H₂O); δH (400 MHz, D₂O): 5.33 (1 H, d, J = 3.6 Hz, H-1’), 5.18–5.14 (2 H, m, H-1, CH(OR)₂ hydrate), 4.52 (1 H, d, J = 2.4 Hz, H-5), 4.29–4.27 (1 H, m, H-2), 4.25–4.20 (2 H, m, H-3, H-4’), 4.04 (1 H, t, J = 2.8 Hz, H-4), 3.87 (1 H, dt, J = 10.4, 3.2 Hz, H-5’), 3.82–3.80 (2 H, m, 2 x H-6’), 3.78–3.75 (1 H, m, H-3’), 3.68 (1 H, dd, J = 11.0, 4.5 Hz, CH₂ handle), 3.56 (1 H, dd, J = 11.0, 5.1 Hz, CH₂ handle), 3.29 (1 H, dd, J = 10.7, 3.5 Hz, H-2’); δC (101 MHz, D₂O): 174.8 (C=O), 99.3 (CH), 96.8 (CH), 88.3 (CH), 76.9 (CH), 75.9 (CH), 75.2 (CH), 71.0 (CH₂), 70.0 (CH), 69.4 (CH), 68.2 (CH), 68.1 (CH), 60.0 (CH₂), 57.8 (CH).
13.1.3 Attempted Conjugations to Disaccharide Aldehyde Tag

*Attempted Labelling of 135 with Alexa Fluor 488 Hydroxylamine Dye to give 140:*

Disaccharide 135 (0.81 mg, 1.12 µmol) was dissolved in pH 4.65 (NaOAc/AcOH) buffer (2.0 mL) and aniline (2 µL) was added. The reaction was stirred protected from light for 24 h, then neutralised with aq. NaOH (0.2 M) and purified by size exclusion chromatography (Sephadex G-25, eluting with H₂O).

*Attempted reductive amination of 135 with N-Z-ethylenediamine and NaCNBH₃ to give 141:*

Disaccharide 135 (10 mg, 13.8 µmol) was dissolved in pH 4.65 (NaOAc/AcOH) buffer (1.0 mL) and N-Z-ethylenediamine hydrochloride (3.4 mg, 14.5 µmol) was added. The reaction was stirred for 15 min, then NaCNBH₃ (1.8 mg, 0.029 mmol) was added and the reaction stirred at RT overnight. The solvent was removed and the residue co-evaporated with MeOH (10 mL) then purified by size exclusion chromatography (Sephadex G-25, eluting with H₂O).
3-[(2-Aminoethyl)amino]-1,2-propanediol 145

To D/L-glyceraldehyde (100 mg, 1.2 mmol) in pH 4.65 buffer (sodium acetate/acetic acid) was added N-Z-ethylenediamine hydrochloride (268 mg, 1.1 mmol) and the reaction stirred at RT for 15 min. NaCNBH$_3$ (146 mg, 2.3 mmol) was added and the reaction stirred at RT for 24 h. The solvent was removed to give the crude product as a white powder (147 mg) which was used in the hydrogenation without further purification; $\delta_H$ (400 MHz, D$_2$O): 7.39 (s, 5 H), 5.08 (3 H, s), 3.97 (2 H, t, $J = 8.1$ Hz), 3.53 (2 H, t, $J = 8.2$ Hz), 3.47–3.44 (1 H, m), 3.21–3.17 (2 H, m); MS (ES$^+$) found $m/z$ 269 [M+H]$^+$. This crude product was dissolved in H$_2$O under N$_2$, Pd(OH)$_2$/C (150 mg) added, the reaction placed under an atmosphere of H$_2$ and stirred under H$_2$ at RT overnight. Filtration of the mixture through Celite afforded the crude title compound 145 as a white powder (115 mg); $\delta_H$ (400 MHz, D$_2$O): 3.78 (2 H, br s), 3.48 (3 H, br d), 2.90 (2 H, s), 2.85 (2 H, d, $J = 6.2$ Hz); MS (ES$^+$) found $m/z$ 291 [2M+Na]$^+$. Data in agreement with reported syntheses.$^{378,379}$

Attempted reductive amination of 135 with N-Boc-ethylenediamine using aniline catalysis to give 146:

Disaccharide 135 (5 mg, 6.9 µmol) was added to a solution of N-Boc-ethylenediamine (5.5 mg, 34.5 µmol) and aniline (0.32 µL, 3.5 µmol) in pH 5.0 (NH$_4$OAc/AcOH) buffer (300 µL). The reaction was stirred for 7 days at 40 ºC, then purified by size exclusion chromatography (Sephadex G-25, eluting with H$_2$O). LCMS showed formation of aniline derivative 147 (found $m/z$ 779, [M–Na$]^-$).
13.1.4 Synthesis of Amine-tagged Disaccharide

2-(Carboxybenzyl-amino)-ethanyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-α-D-glucopyranosyl)-(1→4)-methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosiduronate 148

Disaccharide 128 (386 mg, 0.39 mmol) and N-Z-ethanolamine (92 mg, 0.47 mmol) were dissolved in toluene, concentrated in vacuo, and the process repeated twice. The mixture was dried under high vacuum for 1 h, then dissolved in anhydrous CH₂Cl₂ (5 mL) under N₂ and cooled to 0 °C. Molecular sieves (~300 mg, 4 Å, powdered, activated) were added and the reaction was stirred at 0 °C for 30 min. NIS (193 mg, 0.86 mmol) was added and the resulting pale red solution stirred at 0 °C for 30 min. AgOTf (10 mg, 40 µmol) was added and the reaction stirred at 0 °C for 30 min, then at RT for 1 h. TLC (2:1 hexane/EtOAc) showed no remaining disaccharide starting material. Na₂S₂O₃ (0.5 g) and NaHCO₃ (0.5 g) in water (10 mL) were added and the reaction stirred for 10 min at RT. The reaction mixture was filtered through Celite, the phases separated and the aqueous phase washed with CH₂Cl₂ (2 x 20 mL). The combined organic phases were dried and concentrated in vacuo to an orange oil. Column chromatography (2:1 hexane/EtOAc) afforded the title compound 148 as a yellow foam (340 mg, 0.32 mmol, 81%); Rₚ 0.24 (2:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2920 (C–H), 2106 (N₃), 1717 (C=O); MS (ES⁺) found m/z 1084 [M+NH₄⁺], HRMS found 1084.4558, C₅₉H₆₆N₅O₁₅ [M+NH₄⁺] requires 1084.4550; [α]D = -22.5 (c = 0.45, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.05–8.03 (2 H, m, ArH), 7.36–7.15 (21 H, m, ArH), 7.15–7.07 (2 H, m, ArH), 7.03–6.99 (2 H, m, ArH), 6.82–6.77 (2 H, m, ArH), 5.18 (1 H, t, J = 5.3 Hz, NH), 5.15–5.14 (1 H, m, H-1), 5.09 (1 H, t, J = 2.1 Hz, H-3), 5.02 (2 H, s, 2 x CH₂Ar), 4.84 (1 H, d, J = 11.6 Hz, CH₂Ar), 4.80–4.75 (2 H, m, H-1', H-5), 4.70 (1 H, d, J = 11.6 Hz, CH₂Ar), 4.58–4.54 (2 H, m, 2 x CH₂Ar), 4.43 (1 H, d, J = 11.3 Hz, CH₂Ar), 4.39 (1 H, d, J = 10.5 Hz, CH₂Ar) 4.18 (1 H, d, J = 10.8 Hz, CH₂Ar), 4.12 (1 H, t, J = 2.4 Hz, H-2), 4.07 (1 H, t, J = 3.2 Hz, H-4), 3.95 (1 H, d, J = 10.8 Hz, CH₂Ar),
3.88–3.85 (1 H, m, H-5'), 3.82–3.77 (2 H, m, H-6', CH₂ handle), 3.75 (3 H, s, ArOCH₃), 3.68–3.58 (6 H, m, H-6', H-4, CH₂ handle, CO₂CH₃), 3.49 (1 H, t, J = 9.6 Hz, H-3'), 3.39–3.35 (2 H, m, 2 x CH₂ handle), 3.18 (1 H, dd, J = 10.2, 3.4 Hz, H-2'); δC (101 MHz, CDCl₃): 169.3 (C=O), 165.6 (C=O), 159.3 (C=O), 156.4 (Ar C), 138.0 (Ar C), 137.9 (Ar C), 137.5 (Ar C), 136.6 (Ar C), 133.3 (Ar CH), 130.5 (Ar CH), 130.0 (Ar CH), 129.5 (Ar CH), 128.8 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.4 (2 x Ar CH), 128.1 (2 x Ar CH), 128.0 (2 x Ar CH), 127.8 (Ar CH), 127.7 (2 x Ar CH), 113.7 (Ar CH), 99.9 (C-1'), 99.2 (C-1), 80.0 (C-3'), 77.5 (CH), 75.6 (C-4), 74.7 (CH₂Ar), 74.5 (CH₂Ar), 73.6 (CH₂Ar), 73.5 (C-2), 72.6 (CH₂Ar), 71.8 (C-5'), 68.0 (C-3), 67.8 (CH₂), 67.7 (CH₂), 63.7 (C-2'), 73.5 (C-2'), 55.3 (ArOCH₃), 52.4 (CO₂CH₃), 40.8 (CH₂ handle).

2-(Carboxybenzyl-amino)-ethyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-α-D-glucopyranosyl)-(1→4)-3-O-benzyl-α-L-idopyranosiduronic acid 149

To a stirred solution of disaccharide 148 (334 mg, 0.31 mmol) in THF (4 mL) was added MeOH (1 mL) and the reaction was cooled to 0 °C. LiOH monohydrate (52 mg, 1.25 mmol) was dissolved in water (2 mL) and added dropwise. The reaction mixture was allowed to gradually warm to RT and stirred for 2 h. TLC (95:5 CH₂Cl₂/MeOH) showed no remaining disaccharide starting material. HCl (0.3 mL, 1 M) was added and the solvents removed in vacuo to give a white solid which was purified by size exclusion chromatography on Sephadex LH-20 (1:1 CH₂Cl₂/MeOH) to afford the title compound 149 as a yellow foam (276 mg, 0.29 mmol, 94%); Rf 0.33 (95:5 CH₂Cl₂/MeOH); IR (νmax/cm⁻¹): 3507 (O–H), 2924 (C–H), 2112 (N₃), 1719 (C=O); MS (ES⁺) found m/z 966 [M+NH₄]⁺, HRMS found 966.4138, C₅₁H₆₀N₉O₁₄ [M+NH₄]⁺ requires 966.4131; [α]D = −18.0 (c = 0.25, CH₂Cl₂); δH (400 MHz, CD₃CO₂D): 7.31–7.12 (20 H, m, ArH), 6.97–6.95 (2 H, m, ArH), 6.71–6.68 (2 H, m, ArH), 5.05–4.97 (4
H, m, H-1’, H-1, 2 x CH₂Ar), 4.84–4.77 (2 H, m, H-5, CH₂Ar), 4.71 (1 H, d, J = 11.1 Hz, CH₂Ar), 4.66 (1 H, d, J = 11.4 Hz, CH₂Ar), 4.58 (1 H, d, J = 10.4 Hz, CH₂Ar), 4.52 (2 H, app d, J = 12.3 Hz, 2 x CH₂Ar), 4.38–4.36 (2 H, m, 2 x CH₂Ar), 4.15 (1 H, m, H-4), 3.92 (1 H, t, J = 3.0 Hz, H-3), 3.88–3.84 (1 H, m, H-2), 3.75–3.46 (13 H, m, H-2’, H-3’, H-4’, H-5’, 2 x H-6’, 4 x CH₂ handle, ArOCH₃); δc (101 MHz, CDCl₂D): 173.5 (C=O), 171.1 (C=O), 159.4 (Ar C), 157.5 (Ar C), 138.0 (Ar C), 137.8 (Ar C), 137.7 (Ar C), 136.5 (Ar C), 133.6 (Ar CH), 130.0 (Ar CH), 129.9 (Ar CH), 129.7 (Ar CH), 129.4 (Ar CH), 129.3 (Ar CH), 128.8 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (2 x Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.8 (2 x Ar CH), 127.7 (2 x Ar CH), 127.6 (2 x Ar CH), 113.5 (Ar CH), 101.1 (C-1), 95.2 (C-1’), 80.9 (CH), 77.3 (CH), 75.3 (CH₂Ar), 74.3 (CH₂Ar), 73.1 (CH₂Ar), 71.9 (CH₂Ar), 71.8 (C-3), 70.6 (C-4), 67.7 (CH₂), 67.3 (CH), 67.2 (CH₂), 67.1 (C-5), 66.9 (CH₂Ar), 66.2 (C-2), 63.7 (C-2’), 54.5 (ArOCH₃), 53.6 (CH₂ handle).

2-(Carboxybenzyl-amino)-ethanlyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-α-D-glucopyranosyl)-(1→4)-2-O-sulfo-3-O-benzyl-α-L-idopyranosiduronic acid 150

Disaccharide 149 (276 mg, 0.29 mmol) was dissolved in anhydrous pyridine (6 mL), SO₃·pyridine was added (277 mg, 1.75 mmol) and the reaction stirred at RT overnight. TLC (92.5:7.5 CH₂Cl₂/MeOH) showed no remaining disaccharide starting material. The solvent was removed in vacuo to give a white solid which was purified by size exclusion chromatography on Sephadex LH-20 (1:1 CH₂Cl₂/MeOH) to give the title compound 150 as a yellow foam (242 mg, 0.24 mmol, 81%); Rf 0.18 (9:1 CH₂Cl₂/MeOH); IR (υmax/cm⁻¹): 3367 (O–H) 2919 (C–H), 2112 (N₃), 1715 (C=O); MS (ES⁻) found m/z 513 [M–2H]²⁻, HRMS found 513.1595, C₅₁H₅₄N₄O₁₇S [M–2H]²⁻ requires 513.1608; [α]D = +7.0 (c = 0.80, CH₂Cl₂); δH (400 MHz, CDCl₃CO₂D): 7.40–7.20 (20 H, m, ArH), 7.12–7.04 (2 H, m, ArH), 6.81–6.76 (2 H, m, ArH), 5.42 (1 H, br
Chapter Three: Experimental

139

5.19–5.09 (3 H, m, H-1’, 2 x CH2Ar), 4.96–4.91 (1 H, m, H-5), 4.90–4.77 (3 H, m, 3 x CH2Ar), 4.68–4.55 (4 H, m, H-1, H-3, 2 x CH2Ar), 4.51–4.45 (2 H, m, 2 x CH2Ar), 4.26–4.22 (2 H, m, 2 x CH2 handle), 4.06–4.02 (1 H, m, H-4), 3.59–3.77 (4 H, m, 2 x CH2Ar), 3.52–3.34 (3 H, m, 2 x CH2 handle, H-2’); δC (126 MHz, CD3CO2D): 159.3 (C=O), 157.7 (C=O), 138.2 (Ar C), 137.8 (Ar C), 137.7 (Ar C), 136.4 (Ar C), 136.2 (Ar C), 133.6 (Ar CH), 129.6 (Ar CH), 128.3 (Ar CH), 128.2 (2 x Ar CH), 128.1 (2 x Ar CH), 127.9 (Ar CH), 127.8 (2 x Ar CH), 127.6 (Ar CH), 113.5 (Ar CH), 99.3 (CH), 98.7 (CH), 80.3 (CH), 77.6 (CH), 75.0 (CH2Ar), 74.2 (CH2Ar), 73.0 (CH2Ar), 71.8 (CH2Ar), 71.4 (CH), 70.9 (CH), 70.8 (CH2), 69.3 (CH), 67.9 (CH2), 67.0 (CH2), 63.4 (CH), 60.7 (CH), 54.5 (ArOCH3), 40.3 (CH2 handle).

2-(Carboxybenzyl-amino)-ethanyl (2-amino-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-α-D-glucopyranosyl)-(1→4)-2-O-sulfo-3-O-benzyl-α-L-idopyranosiduronic acid 151

![Diagram]

To a stirred solution of disaccharide 150 (240 mg, 0.24 mmol) in THF (5 mL) was added aqueous NaOH (2 mL, 0.1 M). PMe3 in THF (1.0 mL, 1.0 M) was added dropwise. The reaction mixture was stirred for 2 h at RT. TLC (9:1 CH2Cl2/MeOH) showed no remaining disaccharide starting material. Aqueous HCl (0.1 M) was added until the solution reached pH ~7, and the solvents were removed in vacuo to give a white glass. Size exclusion chromatography on Sephadex LH-20 resin (1:1 CH2Cl2/MeOH) afforded the title compound 151 as a colourless glass (180 mg, 0.18 mmol, 75%); Rf 0.44 (9:1 CH2Cl2/MeOH); IR (νmax/cm–1): 3367 (O–H), 2920 (C–H), 1715 (C=O); MS (ES+) found m/z 1001 [M–H], HRMS found 1001.3387, C51H57N2O17S [M–H] requires 1003.3372; [α]D = +16.2 (c = 0.98, CH2Cl2); δH (400 MHz, CD3CO2D): 7.31–7.13 (20 H, m, ArH), 6.95–6.89 (2 H, m, ArH), 6.73–6.66 (2 H, m, ArH), 5.28 (1 H, d, J = 2.4 Hz, H-1’), 5.24 (1 H, s, H-1), 5.08–5.01 (2 H, m, 2 x
CH₂Ar), 4.94–4.89 (2 H, m, H-3, CH₂Ar), 4.77–4.70 (3 H, m, H-5, 2 x CH₂Ar), 4.62–4.42 (5 H, m, 5 x CH₂Ar), 4.26 (1 H, s, H-2), 4.12 (1 H, s, H-4), 3.93 (1 H, t, J = 9.6 Hz, H-3'), 3.82–3.70 (4 H, m, H-3, CH₂ handle), 3.65–3.54 (5 H, m, ArOCH₃, CH₂ handle, H-6'), 3.47 (1 H, dd, J = 10.0, 2.3 Hz, H-2'), 3.39–3.29 (2 H, m, 2 x CH₂ handle); δc (101 MHz, CD₂CO₂D): 159.4 (C=O), 157.7 (C=O), 138.1 (Ar C), 137.6 (Ar C), 137.6 (Ar C), 136.5 (Ar C), 129.8 (Ar CH), 129.7 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 127.5 (Ar CH), 127.3 (Ar CH), 113.6 (Ar CH), 98.6 (C-1), 91.5 (C-1’), 77.8 (CH), 77.2 (C-3’), 74.8 (CH₂Ar), 74.3 (CH₂Ar), 73.2 (CH₂Ar), 71.7 (CH), 71.4 (CH₂Ar), 69.7 (C-2), 69.5 (C-4), 67.4 (CH₂), 67.1 (CH₂), 66.9 (CH₂), 54.5 (ArOCH₃), 54.0 (C-2’), 40.5 (CH₂ handle).

2-(Carboxybenzyl-amino)-ethanly (3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-2-N-sulfo-α-D-glucopyranosyl)-(1→4)-2-O-sulfo-3-O-benzyl-α-l-idopyranosiduronic acid 152

To a stirred solution of disaccharide 151 (189 mg, 0.19 mmol) in dry pyridine (3 mL) was added SO₃·pyridine (300 mg, 1.89 mmol) and Et₃N (500 mL) and the reaction was stirred for 2 h at RT. TLC (9:1 CH₂Cl₂/MeOH) showed no remaining disaccharide starting material. Saturated aq. NaHCO₃ was added dropwise until effervescence ceased. The solvent was removed in vacuo to give an oil, size exclusion chromatography on Sephadex LH-20 resin (CH₂Cl₂) afforded the title compound 152 as a colourless glass (83 mg, 76.4 µmol, 40%); Rf 0.23 (9:1 CH₂Cl₂/MeOH); MS (ES⁻) found m/z 540 [M–2H]²⁻ requires 540.1439; [α]D = +17.8 (c = 2.50, CH₂Cl₂); δH (400 MHz, D₂O): 7.08–7.26 (20 H, m, ArH), 6.90–6.85 (2 H, m, ArH), 6.67–6.62 (2 H, m, ArH), 5.42–5.40 (1 H, m, H-1’), 5.15–5.12 (1 H, m, H-1), 5.07–5.02 (1 H, m, CH₂Ar), 4.95–4.91 (2 H, m, 2 x CH₂Ar), 4.68–4.60 (2 H, m, 2 x CH₂Ar), 4.59–4.50 (4 H, m, H-5, 3 x CH₂Ar), 4.48–4.43 (2 H, m, H-2, CH₂Ar), 4.38–
4.32 (2 H, m, H-3, CH$_2$Ar), 4.31–4.25 (2 H, m, 2 x CH$_2$Ar), 4.18–4.14 (1 H, m, H-4), 4.13–4.09 (2 H, m, 2 x H-6'), 4.06–3.99 (1 H, m, H-4'), 3.85–3.75 (1 H, m, H-5'), 3.69–3.60 (6 H, m, H-3', 2 x CH$_2$ handle, ArOC$_3$H), 3.47–3.44 (2 H, m, 2 x CH$_2$ handle), 3.40–3.36 (1 H, m, H-2'); δ$_C$ (101 MHz, CD$_3$CO$_2$D): 173.6 (C=O), 159.2 (Ar C), 157.6 (Ar C), 156.3 (Ar C), 139.1 (Ar C), 138.1 (Ar C), 137.8 (Ar C), 136.5 (Ar C), 130.4 (Ar CH), 129.7 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (2 x Ar CH), 127.9 (2 x Ar CH), 127.6 (Ar CH), 127.4 (Ar CH), 113.4 (Ar CH), 98.6 (CH), 98.0 (CH), 80.3 (CH), 76.9 (CH), 75.0 (CH$_2$Ar), 74.2 (CH$_2$Ar), 73.2 (CH), 73.1 (CH$_2$Ar), 73.0 (CH$_2$Ar), 71.9 (CH), 70.6 (CH), 68.2 (CH$_2$), 66.9 (CH$_2$), 66.8 (CH$_2$), 58.0 (CH), 54.5 (ArOC$_3$H), 46.5 (CH$_2$ handle).

2-Amino-ethanyl (2-deoxy-2-N-sulfo-α-D-glucopyranosyl)-(1→4)-2-O-sulfo-α-L-idopyranosiduronic acid trisodium salt 153

N-Sulfated disaccharide 152 (37 mg, 34 µmol) was dissolved in EtOH/H$_2$O (2 mL, 1:1), Pd/C (45 mg) was added under N$_2$, the reaction was purged with H$_2$/vacuum and the solution stirred under an atmosphere of H$_2$ for 2 d. The solution was filtered through Celite with H$_2$O/EtOH and the solvent removed in vacuo to give a white glass (32 mg) which was purified on Dowex® 50WX4 Na$^+$ resin (eluent H$_2$O) to give the title compound 153 as a white powder after lyophilisation (10 mg, 16 µmol, 47%); R$_f$ 0.09 (6:5:3:1 EtOAc/pyridine/H$_2$O/acetic acid); IR (ν$_{max}$/cm$^{-1}$): 3360 (O–H), 1763 (C=O); MS (ES$^-$) found m/z 557 [M–3Na+2H]$^-$, HRMS found 557.0595, C$_{14}$H$_{25}$N$_2$O$_{17}$S$_2$ [M–3Na+2H]$^-$ requires 557.0600; [α]$_D$ = +71.7 (c = 0.10, CH$_2$Cl$_2$); δ$_H$ (400 MHz, D$_2$O): 5.41 (1 H, t, J = 2.8 Hz, H-1'), 5.12 (1 H, t, J = 3.0 Hz, H-1), 4.54 (1 H, t, J = 3.1 Hz, H-5), 4.30–4.27 (1 H, m, H-2), 4.22–4.19 (1 H, m, H-3), 4.09 (1 H, t, J = 3.2 Hz, H-4), 4.01 (1 H, ddd, J = 11.5, 5.7, 3.6 Hz, H-6'), 3.83–3.75 (4 H, m, H-5', H-6', 2 x CH$_2$ handle), 3.61 (1 H, t, J = 9.8 Hz, H-3'), 3.44 (1 H, t, J = 9.3 Hz, H-4'), 3.30–3.23 (2 H, m, 2 x CH$_2$ handle), 3.20 (1 H, dd, J = 10.4, 3.4 Hz, H-2'); δ$_C$ (101 MHz, D$_2$O): 99.3
(C-1), 96.5 (C-1’), 76.5 (C-2), 75.7 (C-4), 71.7 (C-5’), 71.1 (C-3’), 69.9 (C-4’), 69.3 (C-5’ and C-3), 64.4 (C-6), 60.3 (CH₂ handle), 58.0 (C-2’), 39.2 (CH₂ handle).

13.1.5 Synthesis of Glucoazide Donor 162 from D-Glucosamine

Phenyl 3,4,6-tri-O-acetyl-2-deoxy-1-thio-2-N-trichloroacetamido-β-D-glucopyranoside 156

To a suspension of D-glucosamine hydrochloride (259 g, 1.20 mol) in MeOH (2.0 L) was added Et₃N (333 mL, 2.40 mol), followed by Cl₃COCl (134 mL, 1.20 mmol) dropwise. The reaction mixture was stirred at RT for 5 d, then filtered through Celite with MeOH (500 mL) and the solvent removed to give a yellow solid. To this crude intermediate was added anhydrous MeCN (390 mL), followed by Ac₂O (800 mL, 8.46 mmol) in portions. p-Toluenesulfonic acid (11 g, 0.058 mol) was added and the reaction mixture stirred at 60 °C overnight. The resulting dark brown solution was filtered through Celite, and the filtrate was concentrated to give a brown solid. This intermediate was dissolved in EtOAc (2.0 L) and washed with aq. HCl (3 x 200 mL, 1 M), saturated aq. NaHCO₃ (3 x 200 mL) and saturated aq. NaCl (2 x 200 mL). The organic phase was dried and the solvent was removed in vacuo to afford the glycosyl acetate intermediate (385 g) which was then dissolved in CH₂Cl₂ (1.0 L), and PhSH (103 mL, 1.01 mmol) and TMSOTf (140 mL, 0.78 mol) were added. The reaction mixture was stirred at RT overnight, then poured onto NaHCO₃ (120 g, 1.42 mol) in water (400 mL). Iodine (60 g, 0.23 mol) was added to oxidise remaining thiophenol and the solution stirred for 30 min. Na₂S₂O₃ (70 g, 0.44 mol) was added and the solution stirred for a further 30 min. The phases were separated and the aqueous phase extracted with EtOAc (2 x 200 mL). The combined organic phases were dried and the solvents removed in vacuo to give a brown solution which was recrystallised by heating in hexane/EtOAc (700 mL, 1:1) to give the title compound 156 as a light brown solid (325 g, 0.60 mmol, 50%); m.p. 165–166 °C (Lit. 168–169 °C), Rf 0.59 (1:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2947 (C–H), 1746 (C=O), 1718 (C=O); MS (ES⁺) found m/z 559 [M+NH₄]⁺, HRMS found 559.0463, C₂₀H₂₆Cl₃N₂O₈S [M+NH₄]⁺ requires
Chapter Three: Experimental

559.0470; \([\alpha]_D = +10.5\ (c = 0.20, \text{CH}_2\text{Cl}_2)\); \(\delta_H\ (400\ \text{MHz, CDCl}_3):\ 7.53-7.51\ (2\ \text{H, m, ArH}),\ 7.43\ (1\ \text{H, d, } J = 9.4\ \text{Hz, NH}),\ 7.34-7.28\ (3\ \text{H, m, ArH}),\ 5.42\ (1\ \text{H, dd, } J = 10.2, 9.6\ \text{Hz, H-3}),\ 5.06\ (1\ \text{H, t, } J = 9.8\ \text{Hz, H-4}),\ 4.83\ (1\ \text{H, d, } J = 10.4\ \text{Hz, H-1}),\ 4.25-4.09\ (3\ \text{H, m, H-2, 2 x H-6}),\ 3.79\ (1\ \text{H, ddd, } J = 10.0, 5.5, 2.6\ \text{Hz, H-5}),\ 2.07\ (3\ \text{H, s, C(O)CH}_3),\ 2.01\ (3\ \text{H, s, C(O)CH}_3),\ 1.83\ (3\ \text{H, s, C(O)CH}_3);\ \delta_C\ (101\ \text{MHz, CDCl}_3):\ 171.4\ (\text{C=O}),\ 170.6\ (\text{C=O}),\ 169.1\ (\text{C=O}),\ 161.8\ (\text{Ar C}),\ 133.5\ (\text{Ar CH}),\ 131.8\ (\text{Ar CH}),\ 129.0\ (\text{Ar CH}),\ 128.7\ (\text{Ar CH}),\ 86.7\ (\text{C-1}),\ 75.9\ (\text{C-5}),\ 73.3\ (\text{C-3}),\ 68.5\ (\text{C-4}),\ 62.4\ (\text{C-6}),\ 54.2\ (\text{C-2}),\ 20.8\ (\text{C(O)CH}_3),\ 20.6\ (\text{C(O)CH}_3),\ 20.3\ (\text{C(O)CH}_3).\ \text{Data in agreement with reported syntheses.}^{318,380}

Imidazole-1-sulfonyl azide 157

\[
\begin{align*}
\text{NaN}_3 & \rightarrow \text{N}_3^- & \text{S} & \overset{\text{O}}{\text{O}} & \overset{\text{N}}{\overset{\text{H}}{\text{N}}} & \overset{\text{HSO}_4^-}{\text{157}}
\end{align*}
\]

\(\text{NaN}_3\ (10.6\ \text{g, 0.16 mol})\) was suspended in dry acetonitrile \((80\ \text{mL})\) under \(\text{N}_2\). The mixture was cooled in a large ice bath for 30 min. Sulfuryl chloride \((13.2\ \text{mL, 0.16 mol})\) was added dropwise and the mixture was stirred overnight. The reaction mixture was cooled in a large ice bath for 30 min, then imidazole \((21.1\ \text{g, 0.31 mmol})\) was added portionwise over 10 min whilst keeping the reaction under a flow of \(\text{N}_2\). The reaction was stirred for 3 h in ice, then EtOAc \((163\ \text{mL})\) was added and the solution made basic \((\text{pH } \sim8)\) with saturated aq. NaHCO\(_3\). After effervescence had ceased, the phases were separated and the organic phase washed with H\(_2\)O, dried and cooled in ice for 30 min under \(\text{N}_2\). H\(_2\)SO\(_4\) \((8.67\ \text{mL, 0.16 mol})\) was added dropwise and the resulting mixture stirred for 1 h at RT, then ‘seeded’ with the desired product which resulted in precipitation of the \textit{title compound 157} as a white powder \((24.9\ \text{g, 0.092 mol, 57%})\), which was stored refrigerated until use. N.B. Due to the potential explosive characteristics of azides, \(\text{NaN}_3\) and 157 were handled only with plastic implements and a blast shield was employed for the procedure. Aqueous waste including washings from glassware was destroyed by addition of sodium nitrite \((2\ \text{equivalents relative to } \text{NaN}_3)\) followed by acidification to \(\text{pH } \sim1\) by addition of HCl and the solution stirred overnight.\(^{381}\)
Phenyl 2-azido-2-deoxy-1-thio-β-D-glucopyranoside 158

Prepared according to procedure reported by Hansen et al. Thioglycoside 156 (130 g, 0.24 mol) was suspended in MeOH (1.2 L) and K₂CO₃ (165 g, 1.20 mol) in H₂O (200 mL) was added. The reaction mixture was stirred at 60 °C overnight. The reaction mixture was then filtered through Celite with MeOH (3 x 100 mL). To the filtrate was added K₂CO₃ (70 g, 0.51 mol), and the solution was cooled to 0 °C. Imidazole-1-sulfonyl azide 157 (68.3 g, 0.25 mol) was added in four portions over 40 min. Cu승SO₄·5H₂O (600 mg, 2.40 mmol) was added and the suspension stirred for 3 d at RT. The reaction mixture was filtered and the methanol removed in vacuo. H₂O (200 mL) was added and the solution acidified to pH ~ 3 with aq. HCl (1 M), then extracted with EtOAc (3 x 300 mL). The organic phase was washed with saturated aq. NaHCO₃ (200 mL) and saturated aq. NaCl (200 mL), then dried and concentrated in vacuo to give the title compound 158 as a yellow foam (52.3 g, 0.17 mol, 73%); Rf 0.50 (9:1 CH₂Cl₂/MeOH); IR (νmax/cm⁻¹): 3278 (O–H), 2927 (C–H), 2110 (N₃); MS (ES⁺) found m/z 315 [M+NH₄]+, HRMS found 315.1124, C₁₂H₁₉N₄O₄S [M+NH₄]+ requires 315.1122; [α]D = −37.4 (c = 0.45, MeOH); δH (400 MHz, CD₃OD): 7.62–7.59 (2 H, m, ArH), 7.38–7.32 (3 H, m, ArH), 4.57 (1 H, d, J = 10.2 Hz, H-1), 3.91–3.87 (1 H, m, H-6), 3.73–3.68 (1 H, m, H-6), 3.45–3.41 (1 H, m, H-3), 3.34–3.31 (2 H, m, H-4, H-5), 3.17 (1 H, dd, J = 10.1, 9.3 Hz, H-2); δC (101 MHz, CDCl₃): 132.4 (Ar CH), 132.2 (Ar CH), 128.7 (Ar CH), 127.7 (Ar CH), 85.8 (C-1), 80.7 (CH), 77.0 (C-3), 69.7 (CH), 65.7 (C-2), 61.3 (C-6). Data in agreement with reported synthesis.

Phenyl 2-azido-2-deoxy-4,6-O-p-methoxybenzylidene-1-thio-β-D-glucopyranoside 159

Prepared according to procedure reported by Hansen et al. for α-159. To a stirred solution of triol 158 (52.3 g, 0.17 mol) in anhydrous MeCN (550 mL) under N₂ was added p-anisaldehyde dimethyl acetal (44.6 mL, 0.26 mol) and camphorsulfonic acid
(3.95 g, 0.017 mol). The solution was stirred at RT overnight, then quenched by addition of Et$_3$N (5 mL) and the solvents removed in vacuo. The residue was partitioned between CH$_2$Cl$_2$ (600 mL) and saturated aq. NaHCO$_3$ (300 mL). The organic phase was dried and the solvent removed to give an orange oil. Column chromatography (20:1, 10:1, 3:1 petroleum ether/ EtOAc + 10% CH$_2$Cl$_2$) afforded the title compound 159 as a yellow gum (43.0 g, 0.017 mol, 61%); R$_f$ 0.39 (3:1 hexane/ EtOAc); IR ($\nu_{\text{max}}$/cm$^{-1}$): 3359 (O–H), 2910 (C–H), 2112 (N$_3$); MS (ES$^+$) found m/z 416 [M+H]$^+$, HRMS found 416.1283, C$_{20}$H$_{22}$N$_3$O$_5$S [M+H]$^+$ requires 416.1280; $[\alpha]_D$ = –25.4 (c = 0.85, CH$_2$Cl$_2$); $\delta$H (400 MHz, CDCl$_3$): 7.57 (2 H, tdd, $J$ = 4.0, 1.6, 1.2 Hz, ArH), 7.40–7.35 (5 H, m, ArH), 6.91–6.88 (2 H, m, ArH), 5.48 (1 H, s, CHAr), 4.51 (1 H, d, $J$ = 10.2 Hz, H-1), 4.36–4.33 (1 H, m, H-6), 3.77 (3 H, s, ArOCH$_3$), 3.77–3.69 (2 H, m, H-3, H-6), 3.46–3.37 (2 H, m, H-4, H-5), 3.33 (1 H, dd, $J$ = 10.1, 9.1 Hz, H-2), 2.89 (1 H, d, $J$ = 2.8 Hz, OHH); $\delta$C (101 MHz, CDCl$_3$): 160.4 (Ar C), 133.7 (Ar CH), 130.9 (Ar CH), 129.2 (Ar CH), 128.7 (Ar CH), 127.6 (Ar CH), 113.8 (Ar CH), 101.89 (CHAr), 86.8 (C-1), 80.2 (CH), 74.1 (CH), 70.3 (CH), 68.4 (C-6), 65.1 (C-2), 55.3 (ArOCH$_3$). Data in agreement with reported synthesis.383

Phenyl 2-azido-3-O-benzyl-2-deoxy-4,6-O-p-methoxybenzylidene-1-thio-β-D-glucopyranoside 160

Prepared according to procedure reported by Hansen et al. for α-160.318 Glucosamine derivative 159 (25.98 g, 62.60 mmol) was dissolved in anhydrous DMF (150 mL) under N$_2$. BnBr (8.20 mL, 68.90 mmol) was added and the solution cooled to 0 °C. NaH (60% dispersion in mineral oil, 3.00 g, 75.12 mmol) was added in three portions over 30 min at 0 °C. The reaction mixture was stirred warming to RT overnight, then quenched by addition of EtOH (~ 1 mL). H$_2$O (150 mL) was added resulting in precipitation of the product, which was filtered off and washed with H$_2$O (3 x 100 mL) and hexane (3 x 50 mL) to give the title compound 160 as a yellow solid (29.40 g, 49.25 mmol, 77%); m.p. 138–140 °C; R$_f$ 0.52 (4:1 hexane/EtOAc); IR ($\nu_{\text{max}}$/cm$^{-1}$): 2931 (C–H), 2106 (N$_3$); MS (ES$^+$) found m/z 528 [M+Na]$^+$, HRMS found 528.1585, C$_{27}$H$_{27}$N$_3$NaO$_5$S [M+Na]$^+$ requires 528.1569; $[\alpha]_D$ = –114.0 (c = 0.60, CH$_2$Cl$_2$); $\delta$H (400 MHz, CDCl$_3$): 7.58 (2 H, dd, $J$ = 6.5, 3.1 Hz, ArH), 7.43–7.35 (10 H, m, ArH), 6.93 (2 H, d, $J$ = 8.9 Hz, ArH),
5.55 (1 H, s, CHAr), 4.92 (1 H, d, J = 11.0 Hz, CH₂Ar), 4.79 (1 H, d, J = 11.0 Hz, CH₂Ar), 4.51 (1 H, d, J = 10.2 Hz, H-1), 4.39 (1 H, dd, J = 10.5, 5.0 Hz, H-6), 3.84 (3 H, s, ArOCH₃), 3.82–3.77 (1 H, m, H-5), 3.70–3.62 (2 H, m, H-3, H-4), 3.50–3.45 (1 H, m, H-5), 3.40–3.36 (1 H, m, H-2); δC (101 MHz, CDCl₃): 160.2 (Ar C), 137.9 (Ar C), 133.9 (Ar CH), 129.2 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 127.3 (Ar CH), 1137 (Ar CH), 101.3 (CHAr), 86.6 (C-1), 81.3 (CH), 81.0 (CH), 77.4 (CH₂Ar), 75.2 (CH), 70.5 (C-6), 68.5 (C-2), 55.3 (ArOCH₃). Data in agreement with reported synthesis (N.B. melting point not reported).

Phenyl 2-azido-3-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-1-thio-β-D-glucopyranoside 161

Glucosamine derivative 160 (20.00 g, 39.60 mmol) was dissolved in anhydrous MeCN (300 mL) under N₂ and the solution was cooled to 0 °C. NaBH₄ (3.75 g, 99.00 mmol) was added and the solution stirred for 5 min. Cyanuric chloride (14.60 g, 79.10 mmol) was added and the reaction was stirred overnight at RT. The reaction was filtered through Celite with EtOAc (200 mL) and the solvent removed in vacuo. The residue was dissolved in CHCl₃ (200 mL) and washed with aq. NH₄OH (5 x 200 mL, 1%). The organic phase was dried and the solvent removed to reveal the title compound 161 as a white solid (16.80 g, 33.12 mmol, 84%); m.p. 120–124 °C; Rf 0.68 (1:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2906 (C–H), 2109 (N₃); MS (ES⁺) found m/z 530 [M+Na]^+, HRMS found 530.1741, C₂₇H₂₇N₃NaO₇S [M+Na]^+ requires 530.1725; [α]D = −6.0 (c = 0.20, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.56–7.54 (2 H, m, ArH), 7.38–7.34 (8 H, m, ArH), 7.22 (2 H, d, J = 8.7 Hz, ArH), 6.87 (2 H, d, J = 8.7 Hz, ArH), 4.88 (2 H, s, 2 x CH₂Ar), 4.76 (1 H, d, J = 10.6 Hz, CH₂Ar), 4.58 (1 H, d, J = 10.6 Hz, CH₂Ar), 4.46 (1 H, d, J = 10.2 Hz, H-1), 3.88 (1 H, dd, J = 12.1, 2.6 Hz, H-6), 3.81 (3 H, s, ArOCH₃), 3.69 (1 H, dd, J = 12.1, 4.7 Hz, H-6), 3.53–3.48 (2 H, m, H-3, H-4), 3.38–3.31 (2 H, m, H-2, H-5); δC (101 MHz, CDCl₃): 159.5 (Ar C), 137.6 (Ar C), 133.5 (Ar CH), 131.1 (Ar CH), 129.8 (Ar CH), 129.7 (Ar CH), 129.2 (Ar CH), 128.6 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 114.0 (Ar CH), 86.1 (C-1), 85.0 (CH), 79.7 (C-5), 77.0 (CH), 75.9 (CH₂Ar), 74.8 (CH₂Ar), 65.3 (C-2), 62.0 (C-6), 55.3 (ArOCH₃). Data in agreement with reported synthesis (N.B. only proton NMR data reported).
Phenyl 2-azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-1-thio-β-D-glucopyranoside 162

![Structure of 161 and 162](image)

To a stirred solution of 161 (15.00 g, 29.55 mmol) in anhydrous THF (150 mL) under N_2 at 0 °C was added NaH (1.54 g, 38.48 mmol) and the reaction was stirred for 20 min at 0 °C. BnBr (3.87 mL, 32.54 mmol) was added and the reaction stirred at 60 °C overnight. H_2O was added to quench the reaction and the solvents were removed in vacuo. The residue was dissolved in CH_2Cl_2 (200 mL) and washed with saturated aq. NaCl (3 x 50 mL), then the organic phase was dried and the solvent removed to give an orange solid. Column chromatography (99:1, 3:1 hexane/EtOAc) afforded the title compound 162 as a yellow gum (12.78 g, 21.38 mmol, 72%); R_f 0.44 (3:1 hexane/EtOAc); IR (ν_{max}/cm^{-1}): 2906 (C–H), 2108 (N_3); MS (ES^+) found m/z 615 [M+NH_4]^+, HRMS found 615.2630, C_{34}H_{39}N_4O_5S [M+NH_4]^+ requires 615.2636; [α]_D = −17.2 (c = 1.35, CH_2Cl_2); δ_H (400 MHz, CDCl_3): 7.64–7.62 (2 H, m, ArH), 7.39–7.27 (13 H, m, ArH), 7.15–7.12 (2 H, m, ArH), 6.86–6.83 (2 H, m, ArH), 4.88 (2 H, s, 2 x CH_2Ar), 4.73 (1 H, d, J = 10.5 Hz, CH_2Ar), 4.65 (1 H, d, J = 12.0 Hz, CH_2Ar), 4.58 (1 H, d, J = 12.0 Hz, CH_2Ar), 4.54 (1 H, d, J = 10.5, CH_2Ar), 4.43 (1 H, d, J = 10.1 Hz, H-1), 3.81 (3 H, s, ArOCH_3), 3.79–3.73 (2 H, m, 2 x H-6), 3.61 (1 H, t, J = 9.3 Hz, H-4), 3.54–3.46 (2 H, m, H-3, H-5), 3.36 (1 H, dd, J = 10.1, 9.3 Hz, H-2); δ_C (101 MHz, CDCl_3): 159.4 (Ar C), 138.2 (Ar C), 137.7 (Ar C), 133.6 (Ar CH), 131.2 (Ar CH), 130.0 (Ar CH), 129.6 (Ar CH), 129.0 (Ar CH), 128.6 (Ar CH), 128.4 (Ar CH), 128.2 (Ar CH), 128.0 (Ar CH), 127.6 (Ar CH), 127.6 (Ar CH), 113.9 (Ar CH), 85.9 (C-1), 85.1 (C-3), 79.4 (C-5), 77.2 (C-4), 75.9 (CH_2Ar), 74.8 (CH_2Ar), 73.5 (CH_2Ar), 68.7 (C-6), 65.1 (C-2), 55.3 (CO_2CH_3). Data in agreement with previously reported syntheses. 318,385
13.1.6 Attachment of Mono-protected Amine Tag to 166

Methyl (phenyl 2-O-benzoyl-3-O-benzyl-4-O-chloroacetyl-1-thio-L-idopyranoside) uronate 166

To a stirred solution of thioglycoside 165 (750 mg, 1.5 mmol) in anhydrous CH$_2$Cl$_2$ (15 mL) was added pyridine (400 µL), followed by chloroacetyl chloride (180 µL, 2.3 mmol) dropwise. The resulting orange solution was stirred for 3 h at RT under N$_2$. The reaction mixture was diluted with CH$_2$Cl$_2$ (100 mL), washed with aqueous HCl (50 mL, 1% v/v) and saturated aq. NaHCO$_3$ (50 mL), dried and the solvents removed in vacuo to give a brown oil. Column chromatography (2:1 hexane/EtOAc) afforded the title compound 166 as a white foam (858 mg, 1.50 mmol, >99%); $R_f$ 0.45 (2:1 hexane/EtOAc); IR ($\nu_{\text{max}}$/cm$^{-1}$): 2920 (C–H), 1762 (C=O), 1719 (C=O); MS (ES$^+$) found m/z 588 [M+NH$_4$]$^+$, HRMS found 593.1003, C$_{29}$H$_{27}$Cl$_3$NaO$_8$S [M+Na]$^+$ requires 593.1007; $[\alpha]_D$ = –64.1 (c = 0.57, CH$_2$Cl$_2$); $\delta$H (400 MHz, CDCl$_3$): 7.98–7.95 (2 H, m, ArH), 7.53–7.41 (3 H, m, ArH), 7.41–7.16 (10 H, m, ArH), 5.71 (1 H, s, H-1), 5.44 (1 H, d, $J = 1.9$ Hz, H-5), 5.38–5.37 (1 H, m, H-2), 5.29 (1 H, td, $J = 1.9$, 0.8 Hz, H-4), 4.86 (1 H, d, $J = 11.8$ Hz, CH$_2$Ar), 4.72 (1 H, d, $J = 11.8$ Hz, CH$_2$Ar), 3.94 (1 H, td, $J = 2.8$, 1.1 Hz, H-3), 3.83 (1 H, d, $J = 14.9$ Hz, CH$_2$Cl), 3.74–3.70 (4 H, m, CH$_2$Cl, CO$_2$CH$_3$); $\delta$C (101 MHz, CDCl$_3$): 168.4 (C=O), 166.4 (C=O), 165.1 (C=O), 136.7 (Ar C), 135.3 (Ar C), 133.8 (Ar CH), 131.3 (Ar CH), 129.9 (Ar CH), 129.1 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.2 (Ar CH), 127.8 (Ar CH), 86.5 (C-1), 73.0 (CH$_2$Ar), 71.4 (C-3), 69.4 (C-4), 68.6 (C-2), 66.7 (C-5), 52.7 (CO$_2$CH$_3$), 40.3 (CH$_2$Cl). Data in agreement with previous synthesis.\(^{318}\)

Methyl ((2-carboxybenzyl-amino)-ethyl) 2-O-benzoyl-3-O-benzyl-4-O-chloroacetyl-\(\alpha\)-L-idopyranoside) uronate 167
Thioglycoside 166 (462 mg, 0.81 mmol) and N-Z-ethanolamine (189 mg, 0.97 mmol) were dissolved in toluene, concentrated in vacuo, and the process repeated twice. The mixture was dried under high vacuum for 1 h, then dissolved in anhydrous CH₂Cl₂ (5 mL) under N₂ and cooled to 0 °C. Molecular sieves (~400 mg, 4 Å, powder, activated) were added and the reaction was stirred at 0 °C for 30 min. NIS (400 mg, 1.78 mmol) was added and the resulting pale red solution stirred at 0 °C for 30 min. AgOTf (20 mg, 80 µmol) was added and the reaction stirred warming to RT for 1 h. TLC (3:1 hexane/EtOAc) showed no remaining iduronate starting material. Na₂S₂O₃ (0.5 g) and NaHCO₃ (0.5 g) in water (10 mL) were added and the reaction stirred for 10 min at RT. The reaction mixture was filtered through Celite with CH₂Cl₂ (100 mL) and the phases separated. The organic phase was dried and concentrated in vacuo to a brown oil. Column chromatography (3:2 hexane/EtOAc) afforded the title compound 167 as a yellow foam (247 mg, 0.38 mmol, 46%); R₇ 0.60 (1:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2952 (C–H), 1761 (C=O), 1718 (C=O); MS (ES⁺) found m/z 678 [M+Na]⁺, HRMS found 656.1890, C₃₃H₃₅ClNO₁₁ [M+H]⁺ requires 656.1893; [α]D = −8.6 (c = 1.70, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.05–8.03 (2 H, m, ArH), 7.60 (1 H, tt, J = 7.5, 1.5 Hz, ArH), 7.47–7.43 (2 H, m, ArH), 7.40–7.29 (10 H, m, ArH); 5.35 (1 H, t, J = 1.8 Hz, H-4), 5.19–5.17 (2 H, m, H-1, H-2), 5.15–5.09 (3 H, m, H-5, 2 x CH₂Ar), 4.95 (1 H, d, J = 2.0 Hz, H-5), 4.84–4.74 (2 H, m, 2 x CH₂Ar), 3.97 (1 H, d, J = 14.8 Hz, CH₂Cl), 3.96 (1 H, dt, J = 5.8, 3.0 Hz, H-3), 3.89–3.82 (2 H, m, CH₂Cl, CH₂ handle), 3.80 (3 H, s, ArOCH₃), 3.64 (1 H, dt, J = 10.2, 5.0 Hz, CH₂ handle), 3.46–3.42 (2 H, m, 2 x CH₂ handle); δC (101 MHz, CDCl₃): 168.6 (C=O), 166.5 (C=O), 165.2 (C=O), 137.2 (Ar C), 136.6 (Ar C), 133.9 (Ar CH), 129.9 (Ar CH), 129.2 (Ar CH), 128.7 (Ar CH), 128.6 (Ar CH), 128.2 (Ar CH), 127.8 (Ar CH), 98.6 (C-1), 72.7 (CH₂Ar), 72.3 (C-3), 69.5 (C-4), 67.9 (CH₂ handle), 66.9 (C-2), 66.8 (CH₂Ar), 66.1 (C-5), 52.8 (CO₂CH₃), 40.8 (CH₂ handle), 40.4 (CH₂Cl).

Methyl ((2-carboxybenzyl-amino)-ethyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranoside) uronate 168
Monosaccharide 167 (240 mg, 0.37 mmol) was dissolved in EtOH (10 mL), thiourea (42 mg, 0.55 mmol) added and the reaction stirred at 70 °C for 3 h. The reaction mixture was extracted with CH₂Cl₂ (200 mL), washed with H₂O (100 mL) and the organic phase was dried and concentrated in vacuo to an orange oil. Column chromatography (1:1 hexane/EtOAc) afforded the title compound 168 as a colourless oil (174 mg, 0.30 mmol, 81%); Rₓ 0.43 (1:1 hexane/EtOAc); IR (vₓ/cm⁻¹): 3379 (O–H), 2950 (C–H), 1760 (C=O), 1716 (C=O); MS (ES⁺) found m/z 602 [M+Na]⁺, HRMS found 580.2176, C₃₁H₃₄N₁O₁₀ [M+H]⁺ requires 580.2177; [α]D = –22.5 (c = 0.50, CH₂Cl₂); δₓ (400 MHz, CDCl₃): 8.01 (2 H, dd, J = 8.4, 1.2 Hz, ArH), 7.61–7.56 (1 H, m, ArH), 7.42 (2 H, t, J = 7.7 Hz, ArH), 7.40–7.26 (10 H, m, ArH), 5.10 (1 H, dt, J = 2.7, 1.3 Hz, H-2), 5.02 (1 H, s, H-1), 4.97 (2 H, s, 2 x CH₂Ar), 4.77 (1 H, d, J = 1.7 Hz, H-5), 4.67 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.57 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.07–4.05 (1 H, m, H-4), 3.83 (1 H, t, J = 2.7 Hz, H-3), 3.80–3.75 (1 H, m, CH₂ handle), 3.72 (3 H, s, CO₂CH₃), 3.56–3.50 (1 H, m, CH₂ handle), 3.33–3.29 (2 H, m, 2 x CH₂ handle), 2.97 (1 H, br s, OHH); δₓ (101 MHz, CDCl₃): 169.9 (C=O), 165.2 (C=O), 156.5 (C=O), 137.6 (Ar C), 136.6 (Ar C), 133.9 (Ar CH), 129.9 (Ar CH), 129.0 (Ar CH), 128.8 (Ar CH), 128.6 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 127.8 (Ar CH), 98.8 (C-1), 74.7 (C-3), 72.3 (CH₂Ar), 68.2 (CH), 68.1 (CH), 67.8 (CH₂ handle), 67.7 (C-2), 66.8 (CH₂Ar), 52.6 (CO₂CH₃), 40.8 (CH₂ handle).

13.1.7 Synthesis of Disaccharides with Doubly-protected Amine Tag

N-Benzyl-N-(benzyloxy carbonyl)ethanolamine 170

Prepared according to procedure reported by Beshore and Dinsmore. To a stirred solution of N-benzylethanolamine (2.00 mL, 12.4 mmol) in EtOAc (30 mL) was added saturated aq. NaHCO₃ (15 mL) and the solution was cooled to 0 °C. Benzyl chloroformate (2.30 mL, 16.1 mL) was added dropwise with vigorous stirring and the reaction stirred at 0 °C for a further 20 minutes after the addition. The reaction mixture was diluted with water (30 mL) and extracted with EtOAc (2 x 30 mL). The combined organic phase was washed with aq. HCl (25 mL, 1 M), dried, and stripped to a yellow oil. Purification by column chromatography (100:0, 90:10, 80:20, 70:30 CH₂Cl₂/EtOAc) afforded the title compound 170 as a yellow oil (2.42 g, 8.5 mmol, 68%), Rₓ 0.43 (5:1 CH₂Cl₂/EtOAc); IR (vₓ/cm⁻¹): 3419 (O–H), 3030 (C–H), 1674
Chapter Three: Experimental

(C=O), 1716 (C=O); MS (ES\(^+\)) found \(m/z\) 286 [M+NH\(^+\)], HRMS found 286.1444, C\(_{17}\)H\(_{20}\)NO\(_3\) [M+H\(^+\)] requires 286.1438, \(\delta_\text{H}\) (400 MHz, CDCl\(_3\)): 7.36 (9 H, m, ArH), 7.21 (1 H, m, ArH), 5.19 (2 H, s, CH\(_2\)Ar), 4.59 (2 H, m, CH\(_2\)Ar), 3.73–3.67 (2 H, m, CH\(_2\) alkyl), 3.45–3.39 (2 H, m, CH\(_2\) alkyl), 2.86 (1 H, br s, OH); \(\delta_\text{C}\) (101 MHz, CDCl\(_3\)): 157.5 (C=O), 156.8 (C=O), 137.5 (Ar C), 136.3 (Ar C), 128.7 (Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.5 (Ar CH), 67.6 (CH\(_2\)), 67.5 (CH\(_2\)), 61.4 (CH\(_2\)). Data in agreement with previous synthesis.\(^{281}\)

Methyl ((2-(benzyl)carboxybenzyl-amino)-ethanyl 2-O-benzoyl-3-O-benzyl-4-O-chloroacetyl-\(\alpha\)-L-idopyranoside) uronate 171

Thioglycoside 166 (695 mg, 1.22 mmol) and \(N\)-benzyl-\(N\)-(benzyloxy carbonyl)ethanolamine 170 (416 mg, 1.46 mmol) were dissolved in toluene, concentrated \textit{in vacuo}, and the process repeated twice. The mixture was dried under high vacuum for 1 h, then dissolved in anhydrous CH\(_2\)Cl\(_2\) (7 mL) under N\(_2\) and cooled to 0 °C. Molecular sieves (~300 mg, 4 Å, powdered, activated) were added and the reaction was stirred at 0 °C for 30 min. NIS (603 mg, 2.68 mmol) was added and the resulting pale red solution stirred at 0 °C for 30 min. AgOTf (31 mg, 0.12 mmol) was added and the reaction stirred warming to RT for 1 h. TLC (3:1 hexane/EtOAc) showed no remaining iduronate starting material. Na\(_2\)S\(_2\)O\(_3\) (0.5 g) and NaHCO\(_3\) (0.5 g) in water (10 mL) were added and the reaction stirred for 10 min at RT. The reaction mixture was filtered through Celite with CH\(_2\)Cl\(_2\) (100 mL) and the phases separated. The organic phase was dried and concentrated \textit{in vacuo} to a yellow oil. Column chromatography (3:1, 2:1, 1:1 hexane/EtOAc) afforded the title compound 171 as an orange foam (697 mg, 0.93 mmol, 77%); \(R_f\) 0.38 (2:1 hexane/EtOAc); IR (\(\nu_\text{max}/\text{cm}^{-1}\)): 2951 (C–H), 1762 (C=O), 1720 (C=O), 1696 (C=O); MS (ES\(^+\)) found \(m/z\) 763 [M+Na\(^+\)], HRMS found 746.2346, C\(_{40}\)H\(_{41}\)ClNO\(_{11}\) [M+H\(^+\)] requires 746.2363; [\(\alpha\)]\(_D\) = –4.6 (c = 3.40, CH\(_2\)Cl\(_2\)); \(\delta_\text{H}\) (400 MHz, CDCl\(_3\)): 8.04 (2 H, d, \(J = 7.7\) Hz, ArH), 7.59 (1 H, t, \(J = 6.8\) Hz, ArH), 7.45 (2 H, t, \(J = 7.7\) Hz, ArH), 7.39–7.26 (11 H, m, ArH), 7.24–7.22 (2 H, m, ArH), 7.11 (1 H, d, \(J = 8.0\) Hz, ArH), 7.03 (1 H, d, \(J = 7.6\) Hz, ArH), 5.33 (1 H, s, H-4), 5.17–5.06 (4 H, m, 2
Chapter Three: Experimental

x CH₂Ar, H-1, H-2), 4.93–4.90 (1 H, m, H-5), 4.82 (1 H, d, J = 11.8 Hz, CH₂Ar), 4.75 (1 H, dd, J = 11.7, 6.0 Hz, CH₂Ar), 4.53 (2 H, m, CH₂Ar), 3.96–3.91 (3 H, m, 2 x CH₂Cl, H-3), 3.84–3.78 (5 H, m, 2 x CH₂ handle, CO₂CH₃), 3.41–3.39 (2 H, m, 2 x CH₂ handle); δC (101 MHz, CDCl₃): 168.5 (C=O), 166.4 (C=O), 165.1 (C=O), 156.5 (Ar C), 137.9 (Ar C), 137.1 (Ar C), 136.6 (Ar C), 133.8 (Ar CH), 129.8 (Ar CH), 129.2 (Ar CH), 128.7 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 127.3 (Ar CH), 127.2 (Ar CH), 98.7 (C-1), 72.8 (CH₂), 72.8 (C-3), 69.4 (C-4), 67.4 (CH₂), 66.8 (C-2), 65.8 (C-5), 52.7 (CO₂CH₃), 51.7 (CH₂) 46.7 (CH₂), 40.4 (CH₂). N.B. Rotamers were observed in ¹³C and ¹H NMR spectra.

Methyl (2-carboxybenzyl-amino)-ethanyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranoside uronate 172

Monosaccharide 171 (500 mg, 0.67 mmol) was dissolved in EtOH (20 mL), thiourea (76 mg, 1.01 mmol) added and the reaction stirred at 70 °C for 30 min. The reaction mixture was extracted with CH₂Cl₂ (200 mL), washed with H₂O (100 mL) and the organic phase was dried and concentrated in vacuo to an orange oil. Column chromatography (3:1, 2:1, 1:1 hexane/EtOAc) afforded the title compound 172 as a colourless oil (359 mg, 0.54 mmol, 80%); Rf 0.67 (1:1 hexane/EtOAc); IR (νmax/cm⁻¹): 3481 (O–H), 2951 (C–H), 1760 (C=O), 1696 (C=O); MS (ES⁺) found m/z 1361 [2M+Na]⁺, HRMS found 687.2910, C₃₈H₄₆N₂O₁₀ [M+NH₄]⁺ requires 687.2912; [α]₀ = −16.3 (c = 1.00, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.03 (2 H, d, J = 7.2 Hz, ArH), 7.62 (1 H, t, J = 7.4 Hz, ArH), 7.47 (2 H, t, J = 7.7, ArH), 7.39–7.26 (13 H, m, ArH), 7.16 (1 H, d, J = 6.7 Hz, ArH), 7.08 (1 H, d, J = 6.2 Hz, ArH), 5.20 (3 H, m, H-2, 2 x CH₂Ar), 5.09 (1 H, s, H-1), 4.90 (1 H, s, H-5), 4.83 (1 H, d, J = 11.9 Hz, CH₂Ar), 4.71 (1 H, dd, J = 11.9, 4.4 Hz, CH₂Ar), 4.58 (2 H, m, 2 x CH₂Ar), 4.17–4.14 (1 H, m, H-4), 4.03–3.95 (1 H, ddd, J = 16.8, 10.0, 6.4 Hz, CH₂ handle), 3.91 (1 H, t, J = 2.2 Hz, H-3), 3.85 (3 H, s, CO₂CH₃), 3.75–3.57 (2 H, m, 2 x CH₂ handle), 3.49–3.43 (1 H, m, CH₂ handle), 2.85 (1 H, app d, J = 11.6 Hz, OH); δC (101 MHz, CDCl₃): 169.8 (C=O), 169.8 (C=O), 156.5
Chapter Three: Experimental

(153 Ar C), 137.9 (Ar C), 137.3 (Ar C), 136.6 (Ar C), 133.8 (Ar CH), 129.8 (Ar CH), 128.7 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.1 (Ar CH), 128.0 (2 x Ar CH), 127.89 (Ar CH), 127.80 (Ar CH), 127.2 (Ar CH), 98.9 (C-1), 74.5 (C-3), 72.3 (CH_2Ar), 72.2 (C-4), 68.1 (C-5), 67.9 (CH_2Ar), 67.8 (C-2), 52.5 (CO_2CH_3), 51.6 (CH_2Ar), 46.7 (CH_2 handle), 45.7 (CH_2 handle). N.B. Rotamers were observed in $^{13}$C and $^1$H NMR spectra.

Phenyl 2-azido-2-deoxy-3,6-O-di-benzyl-1-thio-$\beta$-D-glucopyranoside 174

Prepared according to procedure reported by Hansen et al. To a stirred solution of benzylidene 173 (12.2 g, 25.7 mmol) in dry CH$_2$Cl$_2$ (100 mL) under N$_2$ at 0 °C was added triethylsilane (12.3 mL, 77.0 mmol) dropwise and BF$_3$·OEt$_2$ (9.7 mL, 77.0 mol) dropwise. The mixture was stirred at 0 °C for 3 h, quenched by addition of H$_2$O (25 mL) and neutralized with saturated aq. NaHCO$_3$. The phases were separated and the organic layer dried and concentrated to a yellow oil. Column chromatography (1:4, 1:3, 1:0 EtOAc/hexane) afforded the title compound 174 as a yellow liquid (10.4 g, 21.8 mmol, 84%); R$_f$ 0.36 (4:1 hexane/EtOAc); IR ($\nu_{\text{max}}$/cm$^{-1}$): 3456 (O–H), 2867 (C–H), 2107 (N$_3$); MS (ES$^+$) found 405 [M+NH$_4$]$^+$, HRMS found 405.1592; $[\alpha]_D = -50.0$ (c = 1.50, CH$_2$Cl$_2$); $\delta_H$ (400 MHz, CDCl$_3$): 7.64 (2 H, dd, $J = 7.4$, 2.1 Hz, ArH), 7.44–7.30 (13 H, m, ArH), 4.92 (1 H, d, $J = 11.1$ Hz, CH$_2$Ar), 4.90 (1 H, d, $J = 11.1$ Hz, CH$_2$Ar), 4.63 (1 H, d, $J = 11.9$ Hz, CH$_2$Ar), 4.59 (1 H, d, $J = 11.9$ Hz, CH$_2$Ar), 4.48 (1 H, d, $J = 9.8$ Hz, H-1), 3.83–3.77 (2 H, m, 2 x H-6), 3.69–3.63 (1 H, m, H-4), 3.48 (1 H, dt, $J = 9.6$, 4.4 Hz, H-5), 3.43–3.34 (3 H, m, H-2, H-3, OH); $\delta_C$ (101 MHz, CDCl$_3$): 137.8 (Ar C), 133.3 (Ar CH), 131.3 (Ar CH), 128.9 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 127.9 (Ar CH), 127.6 (Ar CH), 127.5 (Ar CH), 85.9 (C-1), 84.5 (C-3), 78.5 (C-5), 75.3 (CH$_2$Ar), 73.5 (CH$_2$Ar), 71.2 (C-4), 69.8 (C-6), 64.4 (C-2). Data in agreement with previously reported synthesis.
Phenyl 2-azido-4-O-chloroacetyl-2-deoxy-3,6-O-di-benzyl-1-thio-β-D-glucopyranoside 175

Glucosamine derivative 174 (1.04 g, 2.18 mmol) was dissolved in anhydrous CH₂Cl₂ under N₂. Pyridine (400 µL) was added and chloroacetyl chloride (260 µL, 3.27 mmol) was then added dropwise. The mixture was stirred at RT for 1 h. The solution was diluted with CH₂Cl₂ (100 mL), and the organic phase was washed with aq. HCl (100 mL, 0.1 M), and saturated aq. NaHCO₃ (100 mL). The organic phase was dried and the solvent removed in vacuo to give an orange solid. Column chromatography (5:1 hexane/EtOAc) afforded the title compound 175 as a yellow oil (1.17 g, 1.03 mmol, 73%); Rᵣ 0.48 (4:1 hexane/EtOAc); IR (ν_max/cm⁻¹): 2867 (C–H), 2108 (N₃), 1768 (C=O), 1747 (C=O); MS (ES⁺) found m/z 576 [M+Na]⁺, HRMS found 571.1766, C₂₈H₃₂ClN₄O₅S [M+NH₄]⁺ requires 571.1776; [α]D = −37.5 (c = 0.80, CH₂Cl₂); δ_H (400 MHz, CDCl₃): 7.50 (2 H, dd, J = 8.1, 1.5 Hz, Ar_H), 7.27–7.18 (13 H, m, Ar_H), 4.93 (1 H, tt, J = 9.4, 3.5 Hz, H-4), 4.76 (1 H, d, J = 11.4 Hz, CH₂Ar), 4.54 (1 H, d, J = 11.4, CH₂Ar), 4.45 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.42 (1 H, d, J = 11.8 Hz, CH₂Ar), 4.40 (1 H, d, J = 10.1, H-1), 3.68 (1 H, d, J = 14.8 Hz, CH₂Cl), 3.64–3.58 (5 H, m, H-5, 2 x H-6, CH₂Cl), 3.46 (1 H, d, J = 9.3 Hz, H-3), 3.32 (1 H, dd, J = 10.1, 9.3 Hz, H-2); δ_C (101 MHz, CDCl₃): 166.2 (C=O), 137.7 (Ar C), 137.5 (Ar C), 133.8 (Ar CH), 130.8 (Ar CH), 129.2 (Ar CH), 128.8 (Ar CH), 128.7 (Ar CH), 128.5 (Ar CH), 128.2 (2 x Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 86.2 (C-1), 82.4 (C-3), 76.7 (CH), 75.6 (CH₂Ar), 73.8 (CH₂Ar), 72.4 (C-4), 69.5 (C-6), 65.0 (C-2), 40.5 (CH₂Cl). Data in agreement with previous synthesis.³⁸⁷
Chapter Three: Experimental

2-((Benzyl)carboxybenzyl-amino)ethanyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-α-d-glucopyranosyl)-(1→4)-methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosiduronate 176

Donor 162 (381 mg, 0.64 mmol) and acceptor 172 (356 mg, 0.53 mmol) were dissolved in toluene, concentrated in vacuo, and the process repeated twice. The mixture was dried under high vacuum for 1 h, then dissolved in anhydrous CH₂Cl₂ (5 mL) under N₂ and cooled to 0 °C. Molecular sieves (~300 mg, 4 Å, powdered, activated) were added and the reaction was stirred at 0 °C for 30 min. NIS (298 mg, 1.33 mmol) was added and the resulting pale red solution stirred at 0 °C for 30 min. AgOTf (15 mg, 60 µmol) was added and the reaction stirred warming to RT for 1 h. TLC (2:1 hexane/EtOAc) showed no remaining donor. Na₂S₂O₅ (0.5 g) and NaHCO₃ (0.5 g) in water (10 mL) were added and the reaction stirred for 10 min at RT. The reaction mixture was filtered through Celite with CH₂Cl₂ (100 mL) and the phases separated. The organic phase was dried and concentrated in vacuo to a brown foam. Column chromatography (3:1, 2:1 hexane/EtOAc) afforded the title compound 176 as a white foam (165 mg, 0.14 mmol, 27%); Rf 0.34 (2:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2918 (C–H), 2106 (N₃), 1763 (C=O), 1696 (C=O); MS (ES⁺) found m/z 1174 [M+NH₄]⁺, HRMS found 1174.5023, C₄₀H₄₁ClNO₁₁ [M+H]⁺ requires 1174.5019; [α]D = -5.3 (c = 1.00, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.15 (2 H, d, J = 7.1 Hz, ArH), 7.60 (1 H, s, ArH), 7.46–7.28 (26 H, m, ArH), 7.18 (3 H, d, J = 8.0 Hz, ArH), 7.11 (2 H, t, J = 7.7 Hz, ArH), 5.23–5.18 (3 H, m, 3 x CH₂Ar), 5.14 (1 H, d, J = 5.4 Hz, H-1), 4.94 (1 H, d, J = 11.8 Hz, CH₂Ar), 4.86–4.80 (3 H, m, CH₂Ar, H-5, H'-1), 4.68 (1 H, d, J = 12.1 Hz, CH₂Ar), 4.63–4.58 (2 H, m, 2 x CH₂Ar), 4.55 (1 H, d, J = 10.8 Hz, CH₂Ar), 4.50 (1 H, d, J = 12.1 Hz, CH₂Ar), 4.37 (1 H, d, J = 11.2 Hz, CH₂Ar), 4.21–4.19 (1 H, m, H-2), 4.13–4.12 (2 H, m, H-4, CH₂Ar), 4.02–3.96 (2 H, m, H-3, H-4’), 3.91 (3 H, s, ArOCH₃), 3.88–3.83 (3 H, m, H-5’, 2 x H-6’), 3.79 (3 H, s, CO₂CH₃), 3.72 (2 H, d, J = 11.7 Hz, CH₂ handle), 3.67 (1 H, d, J = 9.6 Hz, CH₂ handle), 3.54 (2 H, t, J = 9.6 Hz, H-3’), 3.45 (2 H, m, 2 x CH₂
handle), 3.27 (1 H, dd, J = 10.2, 3.4 Hz, H-2’); δC (101 MHz, CDCl₃): 169.5 (C=O), 165.5 (C=O), 165.4 (C=O), 157.7 (Ar C), 156.5 (Ar C), 156.3 (Ar C), 156.2 (Ar C), 156.1 (Ar C), 139.0 (Ar CH), 137.9 (Ar CH), 137.8 (Ar CH), 136.7 (Ar CH), 132.6 (Ar CH), 130.0 (Ar CH), 129.6 (Ar CH), 129.4 (Ar CH), 129.2 (Ar CH), 128.8 (Ar CH), 128.6 (Ar CH), 128.5 (2 x Ar CH), 128.4 (Ar CH), 128.3 (2 x Ar CH), 128.2 (Ar CH), 128.1 (2 x Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.8 (2 x Ar CH), 127.7 (2 x Ar CH), 127.6 (2 x Ar CH), 127.3 (2 x Ar CH), 127.2 (Ar CH), 113.7 (Ar CH), 110.5 (C’-1), 99.3 (C-1), 80.1 (CH), 76.0 (C-4), 77.5 (CH), 74.6 (CH₂Ar), 73.6 (CH₂Ar), 72.6 (C-2), 72.5 (CH₂Ar), 71.7 (CH), 67.91, 67.8 (CH₂), 67.6 (CH₂), 63.8 (C’-2), 56.4 (ArOCH₃), 52.4 (CO₂CH₃), 51.5 (CH₂), 46.7 (CH₂), 45.7 (CH₂). N.B. Rotamers were observed in ¹³C and ¹H NMR spectra.

2-((Benzyl)carboxybenzyl-amino)-ethanyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-chloroacetyl-α-D-glucopyranosyl)-(1→4)-methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosiduronate 177

Acceptor 172 (132 mg, 0.20 mmol) and donor 175 (132 mg, 0.24 mmol) were dissolved in anhydrous toluene and the solvent removed in vacuo. This process was repeated twice and the resulting solid was dried under high vacuum for 1 h. The solid was dissolved in anhydrous CH₂Cl₂ (3 mL) under N₂ and cooled to 0 °C, molecular sieves (~200 mg, 4 Å, powdered, activated) were added and the reaction was stirred for 30 min. NIS (99 mg, 0.44 mmol) was added and the mixture was stirred for 30 min. AgOTf (5 mg, 0.02 mmol) was added and the solution was stirred warming to RT for 1 h. Saturated aq. Na₂S₂O₃ (2 mL) and saturated aq. NaHCO₃ (2 mL) were added and the mixture was stirred for 10 min. The resulting solution was filtered through Celite with CH₂Cl₂ (100 mL). The phases were separated and the aqueous phase was washed with CH₂Cl₂ (2 x 50 mL). The combined organic phases were dried and concentrated in vacuo to give an orange oil. Column chromatography (2:1 hexane/EtOAc) afforded the
**Chapter Three: Experimental**

*title compound* 177 as a colourless oil (66 mg, 59 \(\mu\)mol, 30\%). Data for 177: \(R_f\) 0.30 (2:1 hexane/EtOAc); IR \((\nu_{\text{max}}/\text{cm}^{-1}): 2924 (\text{C–H}), 2108 (\text{N}_3), 1765 (\text{C} = \text{O}), 1697 (\text{C} = \text{O}); \) MS (ES\(^+\)) found \(m/z\) 1130 \([\text{M+NH}_4]^+\), HRMS found 1130.4156, \(C_{60}H_{65}ClIN_{15}O_{15}\) \([\text{M+NH}_4]^+\) requires 1130.4160; \([\alpha]_D = -8.8\) (c = 0.25, CH\(_2\)Cl\(_2\)); \(\delta_H\) (400 MHz, CDCl\(_3\)): 8.10–7.99 (2 H, m, Ar\(\text{H}\)), 7.27–7.11 (4 H, m, Ar\(\text{H}\)), 7.07–6.97 (4 H, m, Ar\(\text{H}\)), 5.10–5.04 (4 H, m, H-1, H-5, H-4', CH\(_2\)Ar), 4.99 (1 H, d, \(J = 10.3\) Hz, CH\(_2\)Ar), 4.81 (1 H, d, \(J = 11.7\) Hz, CH\(_2\)Ar), 4.78–4.66 (2 H, m, 2 x CH\(_2\)Ar), 4.62 (1 H, app t, \(J = 3.2\) Hz, H-1'), 4.51–4.32 (3 H, m, 3 x CH\(_2\)Ar), 4.39 (1 H, d, \(J = 10.4\) Hz, CH\(_2\)Ar), 4.33 (1 H, d, \(J = 11.6\) Hz, CH\(_2\)Ar), 4.09–4.07 (1 H, m, H-6'), 4.01 (1 H, dd, \(J = 10.5, 6.4\) Hz, H-5), 3.96–3.94 (1 H, m, H-6'), 3.92–3.71 (5 H, m, H-2, H-4, 2 x CH\(_2\)handle), 3.65 (3 H, s, CO\(_2\)CH\(_3\)), 3.54 (1 H, d, \(J = 14.5\) Hz, CH\(_2\)Cl), 3.50–3.43 (3 H, m, H-3, H-3', CH\(_2\)Cl), 3.36–3.32 (2 H, m, 2 x CH\(_2\) handle), 3.22 (1 H, dd, \(J = 10.2, 3.5\) Hz, H-2'); \(\delta_C\) (101 MHz, CDCl\(_3\)): 169.5 (C=O), 166.1 (C=O), 165.6 (C=O), 156.5 (Ar C), 156.3 (Ar C), 153.7 (Ar C), 133.7 (Ar CH), 130.0 (Ar CH), 129.9 (Ar CH), 128.6 (Ar CH), 128.5 (2 x Ar CH), 128.4 (2 x Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 127.9 (2 x Ar CH), 127.8 (2 x Ar CH), 127.2 (Ar CH), 99.3 (C'-1), 98.9 (C-1), 77.3 (CH), 75.0 (CH), 73.6 (CH\(_2\)), 72.6 (CH\(_2\)), 72.3 (C-4), 68.2 (CH), 67.5 (CH\(_2\)), 67.4 (CH\(_2\)), 67.3 (CH), 63.3 (C'-2), 52.4 (CO\(_2\)CH\(_3\)), 45.0 (CH\(_2\)), 40.5 (CH\(_2\)Cl).

### 13.1.8 Labelling of Disaccharide 153 with Alexa Fluor 488 SDP Ester

To disaccharide 153 (1.38 mg, 2.2 \(\mu\)mol) in 0.1 M NaHCO\(_3\) (100 \(\mu\)L, pH 8) was added Alexa Fluor 488 5-SDP ester (1.0 mg, 1.32 \(\mu\)mol, 0.6 equivalents) in DMSO* (50 \(\mu\)L). The resulting mixture was shaken for 96 h protected from light (aluminium foil). The resulting solution was lyophilised to give labelled disaccharide 178 (and residual unlabelled 153) as an orange solid; HRMS found 267.5085, \(C_{35}H_{34}N_4O_{27}\) \([\text{M–5Na}+\text{H}]^{+}\)
requires 267.5079, and 357.0135, C_{35}H_{34}N_{4}O_{27} [M–5Na+2H]^{3–} requires 357.0129; δ_{H} (400 MHz, CDCl\textsubscript{3}) for labelled/unlabelled mixture: 8.24 (1 H, d, J = 1.5 Hz), 7.97 (1 H, dd, J = 8.0, 1.7 Hz), 7.56 (1 H, s), 7.41 (1 H, d, J = 7.9 Hz), 7.23 (2 H, dd, J = 9.3, 0.7 Hz), 6.93 (2 H, d, J = 9.3 Hz), 5.38 (1 H, d, J = 3.3 Hz), 5.34 (1 H, d, J = 3.4 Hz), 5.16 (1 H, d, J = 2.4 Hz), 5.09 (1 H, d, J = 3.4 Hz), 4.50 (1 H, d, J = 2.5 Hz), 4.48 (1 H, d, J = 2.5 Hz), 4.27–4.25 (2 H, m), 4.19–4.16 (2 H, m), 4.07–4.03 (2 H, m), 3.99–3.94 (2 H, m), 3.81–3.74 (5 H, m), 3.69–3.56 (4 H, m), 3.44–3.38 (2 H, m), 3.19–3.14 (3 H, m).

* N.B. 0.6 equivalents of dye were used to avoid potential problematic separation of free dye and disaccharide, which have similar molecular weights. Single use (ampule) deuterated DMSO (Cambridge Isotopes) was used to ensure purity of the solvent.

13.1.9 Synthesis of \textsuperscript{13}C Labelled Disaccharide

3-\textit{O}-Benzy1-1,2-isopropylidene-\textalpha-L-idofuran-(\textsuperscript{13}C)-nitrile 187

Prepared according to the procedure for the unlabelled analogue as reported by Hansen \textit{et al.}\textsuperscript{317} To diacetone-\textit{D}-glucose (377 g, 1.45 mol) in THF (800 mL) was added BnCl (180 mL, 1.56 mol), Bu\textsubscript{4}NHSO\textsubscript{4} (17 g), and NaOH (200 g) in H\textsubscript{2}O (200 mL). The mixture was heated to 60 °C overnight. The reaction mixture was cooled and washed with saturated aq. NaCl (500 mL). The organic phase was separated and the aqueous phase washed with EtOAc (3 x 200 mL). The organic phases were combined and solvents removed to yield the crude benzylated product, which was filtered through silica with EtOAc to give a yellow oil. The resulting product was dissolved in AcOH/H\textsubscript{2}O (1.0 L, 4:1) and heated to 60 °C for 3 h. The solvents were removed and then the mixture co-evaporated with toluene (2 x 300 mL) to yield the crude diol as a yellow oil. This intermediate was dissolved in a mixture of EtOH/H\textsubscript{2}O (1.8 L, 5:4), NaIO\textsubscript{4} (324 g, 1.52 mol) was added portion-wise and the mixture stirred using an overhead stirrer (heavy precipitate present) for 1 h. The reaction mixture was filtered through Celite and the EtOH removed \textit{in vacuo}. The solid was washed with CH\textsubscript{2}Cl\textsubscript{2} (500 mL) to remove residual product, and the aqueous filtrate washed with CH\textsubscript{2}Cl\textsubscript{2}
(1.0 L). The resulting organic phases were combined and the solvent removed \textit{in vacuo} to give the crude aldehyde as a yellow oil (368 g).

A portion of the crude aldehyde (7.6 g, 27.2 mmol) was dissolved in EtOH/H$_2$O (100 mL, 1:1), MgCl$_2$·6H$_2$O (5.52 g, 27.2 mmol) and K$_{13}$CN (1.80 g, 27.2 mmol) were added and the solution was stirred for 5 d at RT. $^1$H NMR showed a ~1:1 ratio of ido:gluco products. H$_2$O (75 mL) was added and the solution was stirred overnight, $^1$H NMR showed a ~90:10 ratio of ido/gluco products. The solution was stirred overnight at RT, then filtered through Celite with CH$_2$Cl$_2$ (500 mL). The phases were separated and the organic phase was dried and the solvent removed \textit{in vacuo} to give a yellow solid. Crystallisation of the ido product was achieved by dissolving the crude product in EtOAc (25 mL) and adding hexane (100 mL) in portions, yielding the title compound $^{187}$ as two crops of beige solid (total 3.49 g, 11.4 mmol, 38% over four steps); m.p. 146–149 °C (Lit. $^{317}$ for unlabelled analogue 150–151 °C); $R_f$ 0.40 (20:1 CH$_2$Cl$_2$/EtOAc); IR ($\nu_{\text{max}}$/cm$^{-1}$): 3383 (O–H), 2877 (C–H); MS (ES$^+$) found m/z 324 [M+NH$_4$]$^+$, HRMS found 329.1201, [M+Na]$^+$ requires 329.1194; $[\alpha]_D$ = –57.9 (c = 0.62, CH$_2$Cl$_2$); $\delta$H (400 MHz, CDCl$_3$): 7.41–7.32 (5 H, m, ArH), 6.00 (1 H, d, J = 3.7 Hz, H-1), 4.77 (1 H, t, J = 7.3 Hz, H-4), 4.70 (1 H, d, J = 10.8 Hz, CH$_2$Ar), 4.66 (1 H, d, J = 3.7 Hz, H-2), 4.58 (1 H, d, J = 11.2 Hz, CH$_2$Ar), 4.47 (1 H, ddd, J = 4.47 (1 H, ddd, J = 7.4, 3.8, 2.5 Hz, H-3), 4.21 (1 H, d, J = 3.8 Hz, H-5), 2.72 (1 H, br s, OH), 1.51 (3 H, s, C(CH$_3$)$_2$), 1.34 (3 H, s, C(CH$_3$)$_2$); $\delta$C (101 MHz, CDCl$_3$): 136.3 (Ar C), 128.8 (Ar CH), 128.5 (Ar CH), 128.2 (Ar CH), 117.2 ($^{13}$C=N), 112.9 (O$_2$C(CH$_3$)$_2$), 105.8 (C-1), 82.3 (C-2), 82.1 (C-5), 81.1 (C-3), 72.9 (CH$_2$Ar), 61.0 (C-4), 27.0 (CH$_3$), 26.4 (CH$_3$). Data in agreement with the unlabelled analogue.$^{317}$

Methyl (methyl 2,4-di-O-acetyl-3-O-benzyl-$\alpha$/-$\beta$-L-idopyranoside)-($^{13}$C)-uronate $^{188}$

Prepared according to the unlabelled analogue as reported by Miller \textit{et al.} $^{320}$ $^{13}$C labelled cyanohydrin $^{187}$ (5.71 g, 18.64 mmol) was dissolved in anhydrous MeOH (25 mL) under N$_2$ and cooled to 0 °C. AcCl (2.65 mL, 37.27 mmol) was added dropwise and the mixture was stirred for 3 h at 60 °C. AcCl (0.66 mL, 9.32 mmol) was added and
the mixture stirred for a further 5 h at 60 °C. AcCl (0.66 mL, 9.32 mmol) was added and the mixture stirred for a further 14 h at 60 °C. TLC (3:2 hexane/EtOAc) showed no remaining starting material. The solvent was removed, the residue taken up in EtOAc (100 mL), H₂O (100 mL) was added and the mixture shook vigorously for 5 min. The organic phase was washed with saturated aq. NaHCO₃ (100 mL) and saturated aq. NaCl (100 mL), dried and the solvent removed in vacuo to give a brown oil. Column chromatography (1:1, 2:1 hexane/EtOAc) afforded the intermediate pyranoside/furanoside mixture as an orange oil. This crude intermediate was dissolved in anhydrous CH₂Cl₂ (40 mL) under N₂, acetic anhydride (2.74 mL, 29.07 mmol), pyridine (2.81 mL, 34.85 mmol) and DMAP (177 mg, 1.45 mmol) were added and the solution stirred at RT overnight. TLC (2:3 hexane/EtOAc) showed no remaining starting material. The solvent was removed and the residue was co-evaporated with toluene (2 x 50 mL) to give an orange oil. Column chromatography (2:1 hexane/EtOAc) afforded the title compound 188 as a yellow oil (1.93 g, 4.85 mmol, 26%, ~1:1 α/β) along with the furanoside by-product as a yellow oil (1.82 g, 4.58 mmol, 32%, ~1:1 α/β).

Data for α/β-188: Rf 0.50 (3:2 hexane/EtOAc); IR (νmax/cm⁻¹): 2961 (C–H), 1729 (C=O); MS (ES⁺) found m/z 415 [M+NH₄]⁺, HRMS found 420.1344, C₁₈¹³CH₂₄NaO₉ [M+Na]⁺ requires 420.1352; δH (400 MHz, CDCl₃): 7.39–7.29 (10 H, m, Ar H), 5.17 (1 H, ddd, J = 2.9, 2.0, 0.8 Hz, H-2α), 5.14 (1 H, ddt, J = 3.2, 2.1, 1.0 Hz, H-4β), 4.99 (1 H, m, H-2β), 4.89–4.86 (3 H, m, H-1α, H-4α, H-5α), 4.78 (1 H, d, J = 1.8 Hz, H-1β), 4.76–4.69 (4 H, m, 4 x CH₂Ar), 4.64 (1 H, dd, J = 6.6, 2.2 Hz, H-5β), 3.95 (1 H, td, J = 3.3, 1.2 Hz, H-3β), 3.77 (6 H, d, J = 3.8 Hz, 2 x 1³CO₂CH₃), 3.73 (1 H, td, J = 2.7, 1.2 Hz, H-3α), 3.56 (3 H, s, OCH₃), 3.46 (3 H, s, OCH₃), 2.08 (3 H, s, C(O)CH₃), 2.04–2.02 (9 H, m, 3 x C(O)CH₃); δC (101 MHz, CDCl₃): 170.0 (C=O), 169.7 (C=O), 169.6 (C=O), 169.3 (C=O), 168.8 (1³C=O), 167.9 (1³C=O), 137.1 (Ar C), 136.8 (Ar C), 128.4 (Ar CH), 128.2 (Ar CH), 128.0 (Ar CH), 127.6 (2 x Ar CH), 99.3 (C-1α), 98.7 (C-1β), 73.0 (C-3β), 72.9 (CH₂Ar), 72.1 (d, J = 68.3 Hz, C-5β), 72.0 (CH₂Ar), 71.8 (C-3α), 67.5 (C-2α), 67.3 (C-4β), 66.9 (CH), 66.8 (CH), 65.6 (d, J = 68.8 Hz, C-5α), 57.1 (OCH₃), 56.1 (OCH₃), 52.3 (d, J = 2.7 Hz, 1³CO₂CH₃), 52.2 (d, J = 2.7 Hz, 1³CO₂CH₃), 20.8 (C(O)CH₃), 20.7 (C(O)CH₃), 20.6 (C(O)CH₃), 20.5 (C(O)CH₃). Data in agreement with unlabelled analogue.³²⁰
Data for corresponding α/β-furanoside: Rf 0.60 (2:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2935 (C–H), 1745 (C=O), 1718 (C=O); MS (ES⁺) found m/z 415 [M+NH₄]⁺, HRMS found 420.1370, C₁₈H₂₄NaO₉ [M+Na]⁺ requires 420.1352; δH (400 MHz, CDCl₃): 7.33–7.23 (10 H, m, ArH), 5.57 (1 H, dd, J = 7.1, 5.4 Hz, H-5β), 5.33 (1 H, dd, J = 5.5, 2.1 Hz, H-5α), 5.19 (1 H, s, H-2α), 5.14 (1 H, d, J = 4.6, H-1β), 4.98 (1 H, dd, J = 7.9, 4.6 Hz, H-2β), 4.90 (1 H, s, H-1α), 4.71–4.67 (3 H, m, H-4β, H-4α, CH₂Ar), 4.59 (1 H, t, J = 8.9 Hz, CH₂Ar), 4.57 (1 H, t, J = 8.9, CH₂Ar), 4.50 (1 H, t, J = 7.9 Hz, H-3β), 4.43 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.12 (1 H, dd, J = 6.3, 1.4 Hz, H-3α) 3.72 (3 H, d, J = 3.8 Hz, 1₃CO₂CH₂), 3.56 (3 H, d, J = 3.8 Hz, 1₃CO₂CH₂), 3.35 (3 H, s, OCH₃), 3.31 (3 H, s, OCH₃), 2.12 (3 H, s, (O)(CH₃), 2.10 (3 H, s, (O)(CH₃)), 2.05 (6 H, s, 2 x (O)(CH₃)); δC (101 MHz, CDCl₃): 168.6 (1₃C=O), 168.5 (1₃C=O), 168.4 (C=O), 167.9 (C=O), 167.7 (C=O), 167.6 (C=O), 137.3 (Ar C), 137.1 (Ar C), 128.5 (Ar CH), 128.3 (Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 127.6 (Ar CH), 115.3 (Ar CH), 107.2 (C-1α), 99.8 (C-1β), 81.3 (C-3α), 80.0 (CH), 79.4 (C-2α), 78.9 (C-3β), 77.6 (C-2β), 75.1 (CH), 73.4 (CH₂Ar), 72.5 (CH₂Ar), 71.9 (d, J = 58.0 Hz, C-5β), 70.4 (d, J = 58.0 Hz, C-5α), 55.7 (OCH₃), 55.2 (OCH₃), 52.6 (d, J = 2.4 Hz, 1₃CO₂CH₃), 52.2 (d, J = 2.7 Hz, 1₃CO₂CH₃), 20.8 (2 x C(O)CH₃), 20.7 (2 x C(O)CH₃).

Methyl (methyl 3-O-benzyl-α/β-L-idopyranoside)-(1³C)-uronate 189

Prepared according to the unlabelled analogue as reported by Miller et al.³²⁰ Pyranoside 188 (1.73 g, 4.36 mmol, ~1:1 α/β) was dissolved in anhydrous MeOH (20 mL) under N₂. NaOMe in MeOH (500 µL, 20–30 wt%) was added dropwise and the solution was stirred at RT for 1 h. Amberlite 120 H⁺ resin was added and the solution was stirred for 15 min until neutralised. The solution was filtered through Celite with MeOH (100 mL). The solvent was removed in vacuo to yield a yellow solid. Column chromatography (1:1 hexane/EtOAc) afforded the title compound 189 as an orange oil (778 mg, 2.48 mmol, 57%, ~1:1 α/β); Rf 0.23 (1:1 hexane/EtOAc); IR (νmax/cm⁻¹): 3458 (O–H), 2951 (C–H), 1692 (C=O); MS (ES⁺) found m/z 650 [2M+Na]⁺, HRMS found 336.1139, C₁₄H₂₆NaO₇ [M+Na]⁺ requires 336.1140; δH (400 MHz, CDCl₃): 7.38–7.28 (10 H, m, ArH), 4.87 (1 H, s, H-1α), 4.74 (1 H, dd, J = 5.9, 1.6 Hz, H-5α), 4.70 (1 H, d, J = 8.7 Hz, H-1β), 4.68 (2 H, app d, J = 8.8 Hz, 2 x CH₂Ar), 4.60 (2 H, app d, J = 11.9 Hz, 2 x
$CH_2Ar$, 4.51 (1 H, dd, $J = 6.0$, 1.4 Hz, H-5β), 4.10–4.09 (1 H, m, H-3α), 4.04–4.02 (1 H, m, H-4β), 3.94–3.92 (1 H, m, H-3β), 3.85–3.82 (2 H, m, H-2β, H-2α), 3.80 (6 H, app t, $J = 3.5$ Hz, 2 x $^{13}$CO$_2$CH$_3$), 3.74 (1 H, m, H-4 α), 3.60 (3 H, s, OCH$_3$), 3.46 (3 H, s, OCH$_3$); δ$_C$ (101 MHz, CDCl$_3$): 170.9 ($^{13}$C=O), 169.7 ($^{13}$C=O), 137.9 (Ar C), 137.3 (Ar C), 128.7 (Ar CH), 128.6 (Ar CH), 128.3 (Ar CH), 128.0 (Ar CH), 127.8 (Ar CH), 102.6 (C-1α), 99.5 (C-1β), 75.5 (C-3β), 75.1 (C-4α), 74.5 (d, $J = 68.1$ Hz, C-5β), 72.7 (CH$_2$Ar), 71.9 (CH$_2$Ar), 68.5 (CH), 68.3 (C-3α), 67.9 (C-4β), 67.8 (d, $J = 67.6$ Hz, H-5α), 66.8 (CH), 57.1 (OCH$_3$), 56.3 (OCH$_3$), 52.5 (2 x $^{13}$CO$_2$CH$_3$). Data in agreement with unlabelled analogue.$^{320}$

Methyl (methyl 2-O-benzoyl-3-O-benzyl-$\alpha$-L-idopyranoside)-(13C)-uronate α-190

![Diagram](image)

Prepared according to the unlabelled analogues as reported by Miller et al.$^{320}$ Diol 189 (734 mg, 2.34 mmol, 1:1 α/β) was dissolved in anhydrous MeOH (20 mL) under N$_2$, Bu$_2$SnO (612 mg, 2.46 mmol) was added and the white suspension was stirred at 60 °C for 3 h resulting in a colourless solution. The solvent was removed in vacuo, the intermediate was dried under high vacuum for 3 h, then dissolved in anhydrous 1,4-dioxane under N$_2$. BzCl (300 µL, 2.57 mmol) was added dropwise and the solution was stirred overnight at RT. A further amount of BzCl (50 µL, 0.43 mmol) was added and the mixture was stirred for a further 2 h. The solvent was removed in vacuo to yield a yellow solid, column chromatography (20:1, 15:1 toluene/acetone) yielded the title compound α-190 as a yellow oil (323 mg, 0.77 mmol, 33%), and β-190 as a yellow oil (207 mg, 0.49 mmol, 21%).

Data for α-190: R$_f$ 0.18 (2:1 hexane/EtOAc); IR ($\nu_{\text{max}}$/cm$^{-1}$): 3332 (O–H), 2914 (C–H), 1715 (C=O); MS (ES$^+$) found m/z 857 [2M+Na]$^+$, HRMS found 440.1396, C$_{21}$H$_{24}$NaO$_8$ [M+Na]$^+$ requires 440.1402; [α]$_D$ = +9.1 (c = 1.50, CH$_2$Cl$_2$); δ$_H$ (400 MHz, CDCl$_3$): 7.24 (2 H, d , $J = 7.2$ Hz, ArH), 7.43 (1 H, m, ArH), 7.31–7.16 (7 H, m, ArH), 5.10 (1 H, app s, H-4), 4.90 (1 H, app s, H-1), 4.80 (1 H, dd, $J = 5.7$, 1.3 Hz, H-5), 4.71 (1 H, d, $J = 12.0$ Hz, CH$_2$Ar), 4.57 (1 H, d, $J = 12.0$ Hz, CH$_2$Ar), 4.01–3.99 (1 H, m, H-3), 3.80–3.79 (1 H, m, H-2), 3.73 (3 H, d, $J = 3.8$ Hz, $^{13}$CO$_2$CH$_3$), 3.42 (3 H, s, OCH$_3$); δ$_C$ (101 MHz, CDCl$_3$): 170.0 ($^{13}$C=O), 165.0 (C=O), 137.5 (Ar C), 133.7 (Ar...
CH), 129.8 (Ar CH), 129.0 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 100.0 (C-1), 74.4 (C-2), 72.0 (CH₂Ar), 67.9 (C-3), 67.7 (d, J = 67.1 Hz, C-5), 67.7 (C-4), 56.3 (OCH₃), 52.4 (d, J = 2.6 Hz, ¹³CO₂CH₃). Data in agreement with unlabelled analogue.¹³⁸

Data for β-190: Rf 0.26 (2:1 hexane/EtOAc); IR (ν<sub>max</sub>/cm⁻¹): 3505 (O–H), 2952 (C–H), 1715 (C=O); MS (ES⁺) found m/z 435 [M+NH₄]⁺, HRMS found 440.1405, C₂₁H₂₆NaO₈ [M+Na]⁺ requires 440.1402; [α]D = +87.7 (c = 0.48, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.07 (2 H, dd, J = 8.3, 1.2 Hz, Ar H), 7.60–7.56 (1 H, m, Ar H), 7.48–7.43 (2 H, m, Ar H), 7.39–7.32 (5 H, m, Ar H), 5.33 (1 H, dd, J = 5.9, 2.2 Hz, H-2), 4.96 (1 H, d, J = 2.1 Hz, H-1), 4.83–4.77 (2 H, m, 2 x CH₂Ar), 4.65 (1 H, dd, J = 5.9, 2.2 Hz, H-5), 4.12–4.08 (2 H, m, H-3, H-4), 3.85 (3 H, d, J = 3.8 Hz, ¹³CO₂CH₃), 3.57 (3 H, s, OCH₃); δC (101 MHz, CDCl₃): 169.8 (¹³C=O), 165.6 (C=O), 137.3 (Ar C), 133.5 (Ar CH), 130.2 (Ar CH), 129.9 (Ar CH), 129.3 (Ar CH), 129.1 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 127.9 (Ar CH), 125.3 (Ar CH), 99.5 (C-1), 75.7 (CH), 73.4 (d, J = 66.8 Hz, C-5), 73.0 (CH₂Ar), 69.3 (C-2), 68.8 (CH), 57.5 (OCH₃), 52.4 (d, J = 2.6 Hz, ¹³CO₂CH₃). Data in agreement with unlabelled analogue.¹⁴¹

Methyl (2-azido-3,6-O-di-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 2-O-benzoyl-3-O-benzyl-α-l-ido-pyranoside-(¹³C)-uronate 192

Glycosyl donor 162 (522 mg, 0.88 mmol) and acceptor α-190 (304 mg, 0.73 mmol) were combined in anhydrous toluene (~3 mL) and the solvent removed in vacuo. This process was repeated twice and the donor/acceptor mixture dried under high vacuum overnight. The mixture was dissolved in anhydrous CH₂Cl₂ (5 mL) under N₂, molecular sieves (~500 mg, 4 Å, powdered, activated) were added and the mixture was stirred for 1 h at RT. NIS (205 mg, 0.91 mmol) was added, the mixture was cooled to 0 °C and stirred for 30 min. AgOTf (18 mg, 70 µmol) was added and the mixture was stirred warming to RT for 1 h. TLC (2:1 hexane/EtOAc) showed no remaining glycosyl donor.
Saturated aq. NaHCO₃ (2 mL) and saturated aq. Na₂S₂O₃ (2 mL) were added and the mixture was stirred for 10 min. The reaction mixture was filtered through Celite and the phases were separated. The organic phase was dried and concentrated in vacuo to give an orange oil. Column chromatography (1:1 hexane/EtOAc, two columns) yielded 191 as a yellow foam (291 mg). An analytical sample of 191 was obtained by preparative TLC (20:1 toluene/acetone). Disaccharide 191 (221 mg, 0.24 mmol) was dissolved in MeCN (5 mL), H₂O (0.5 mL) and CAN (200 mg, 0.37 mmol) were added, and the solution was stirred at RT for 3 h. CAN (132 mg, 0.24 mmol) was added and the mixture stirred for 1 h. The solution was partitioned between CH₂Cl₂ (100 mL) and saturated aq. NaHCO₃ (100 mL), the phases were separated, and the aqueous phase was extracted with CH₂Cl₂ (2 x 50 mL). The combined organic phases were dried and the solvent removed in vacuo to give a yellow oil. Column chromatography (3:1 hexane/EtOAc) yielded the title compound 192 as a colourless oil (127 mg, 0.16 mmol, 24% over two steps).

Data for 191: Rf 0.44 (2:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2926 (C–H), 2106 (N₃), 1718 (C=O); MS (ES⁺) found m/z 922 [M+NH₄]⁺, HRMS found 922.3959, C₄₉H₅₇N₄O₁₃ [M+NH₄]⁺ requires 922.3950; [α]D = -29.7 (c = 0.95, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.01 (2 H, d, J = 7.1 Hz, ArH), 7.35–7.12 (20 H, m, ArH), 7.04 (2 H, dd, J = 5.1, 2.7 Hz, ArH), 6.98 (2 H, d, J = 8.6 Hz, ArH), 6.77 (2 H, d, J = 8.6 Hz, ArH), 5.04 (1 H, s, H-2), 4.98 (1 H, s, H-1), 4.85 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.77–4.72 (1 H, m, H-5), 4.68 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.61 (1 H, d, J = 3.3 Hz, H-1’), 4.51 (2 H, m, 2 x CH₂Ar), 4.39 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.33 (1 H, d, J = 10.5 Hz, CH₂Ar), 4.08 (1 H, d, J = 10.7 Hz, CH₂Ar), 4.04–4.02 (1 H, m, H-3), 3.93 (1 H, s, H-4), 3.83–3.80 (2 H, m, H-5’, CH₂Ar), 3.74 (3 H, s, ArOCH₂), 3.72–3.70 (1 H, m, H-6’), 3.66 (3 H, d, J = 3.7 Hz, ¹³CO₂CH₃), 3.61–3.55 (2 H, m, H-4’, H-6’), 3.44–3.39 (4 H, m, H-3’, OCH₂), 3.12 (1 H, dd, J = 10.2, 3.4 Hz, H-2’); δC (101 MHz, CDCl₃): 169.6 (¹³C=O), 165.4 (Ar C), 159.2 (Ar C), 159.2 (Ar C), 137.4 (Ar C), 133.2 (Ar CH), 130.6 (Ar CH), 129.9 (Ar CH), 129.4 (Ar CH), 128.8 (Ar CH), 128.4 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.0 (2 x Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 127.65 (Ar CH), 113.7 (Ar CH), 100.4 (C-1), 100.0 (C-1’), 80.0 (C-3’), 77.4 (C-4’), 75.9 (C-4), 74.6 (CH₂Ar), 74.4 (CH₂Ar), 73.6 (CH₂Ar), 72.9 (C-3), 72.4 (CH₂Ar), 71.7 (C-5’), 67.8 (C-2 and C-6’), 67.1 (d, J = 65.5 Hz, C-5), 63.7 (C-2’), 56.2 (OCH₃), 55.3 (ArOCH₂), 52.3 (d, J = 2.7 Hz, ¹³CO₂CH₃). Data in agreement with unlabelled analogue.²⁸⁸
Data for 192: Rf 0.25 (3:1 hexane/EtOAc); IR (νmax/cm⁻¹): 3499 (O–H), 2926 (C–H), 2106 (N3), 1717 (C=O); MS (ES⁺) found m/z 802 [M+NH4]+, HRMS found 802.3364, C₄H₄N₄O₁₂ [M+NH4]+ requires 802.3375; [α]D = −9.4 (c = 1.24, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.18–8.15 (2 H, m, ArH), 7.52–7.47 (1 H, m, ArH), 7.45–7.40 (4 H, m, ArH), 7.38–7.27 (11 H, m, ArH), 7.21–7.17 (2 H, m, ArH), 5.13–5.12 (1 H, m, H-2), 5.10 (1 H, s, H-1), 4.91 (1 H, d, J = 11.9 Hz, CH₂Ar), 4.86–4.83 (1 H, m, H-5), 4.77 (1 H, d, J = 11.9 Hz, CH₂Ar), 4.65 (1 H, d, J = 3.5 Hz, H-1'), 4.58 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.54 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.31 (1 H, d, J = 11.0 Hz, CH₂Ar), 4.14 (1 H, d, J = 10.9 Hz, CH₂Ar), 4.12–4.11 (1 H, m, H-3), 4.03–4.02 (1 H, m, H-4), 3.88–3.82 (1 H, m, H-5'), 3.77 (1 H, dd, J = 10.2, 3.6 Hz, H-6'), 3.73 (3 H, d, J = 3.8 Hz, ¹³CO₂CH₃), 3.67 (1 H, t, J = 9.2 Hz, H-4''), 3.60 (1 H, dd, J = 10.2, 4.6 Hz, H-6''), 3.50 (3 H, s, OCH₃), 3.47–3.43 (1 H, m, H-3'), 3.14 (1 H, dd, J = 10.2, 3.5 Hz, H-2'); δC (101 MHz, CDCl₃): 169.6 (C=O), 168.8 (C=O), 165.6 (Ar C), 138.0 (Ar C), 137.7 (Ar C), 137.4 (Ar C), 133.4 (Ar CH), 130.0 (Ar CH), 128.7 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.2 (Ar CH), 127.9 (2 x Ar CH), 100.3 (C-1), 99.2 (C-1'), 79.7 (C-3'), 75.2 (C-4), 74.7 (CH₂Ar), 73.7 (CH₂Ar), 72.5 (CH₂Ar), 72.6 (C-3), 72.4 (C-4'), 70.6 (C-5'), 69.5 (C-6'), 68.2 (C-2), 67.2 (d, J = 68.4 Hz, C-5), 63.0 (C-2'), 56.2 (OCH₃), 52.30 (d, J = 2.7 Hz, ¹³CO₂CH₃). Data in agreement with unlabelled analogue. ³²⁵

13.1.10 Synthesis of Glucosamine Trichloroacetimidates 219, 228–330

2-Azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-1-O-trichloroacetimidate-α/β-D-glucopyranoside α/β-219

To a stirred solution of glucosamine derivative 224 (749 mg, 1.48 mmol) in anhydrous CH₂Cl₂ (5 mL) under N₂ was added Cl₃CCN (742 μL, 7.40 mmol) and DBU (11 μL, 74 μmol). The resulting solution was stirred at RT for 90 min. The solvents were removed to give a brown oil which was purified by column chromatography (3:1 hexane/EtOAc + 1% Et₃N) to give the title compound 219 as a yellow gum which was used immediately for glycosylation (916 mg, 1.41 mmol, 95%, 5:1 α/β; Rf 0.35, 0.42 (3:1 hexane/EtOAc); IR (νmax/cm⁻¹) 2917 (C–H), 2109 (N3); MS (ES⁺) found m/z 671
Phenyl 2-azido-3,4,6-tri-O-benzyl-2-deoxy-1-thio-β-D-glucuronate 223

To a stirred solution of triol 158 (1.63 g, 5.45 mmol) in anhydrous THF (30 mL) under N₂ at 0 °C was added NaH (785 mg, 19.60 mmol). The solution was stirred for 10 minutes at 0 °C. BnBr (2.07 mL, 17.40 mmol) was added and the solution stirred at reflux for 2 d. The reaction was quenched with H₂O and the solvents were removed in vacuo to give a yellow solid which was washed thoroughly with cold petroleum ether to afford the title compound 223 as a yellow solid (2.38 g, 4.19 mmol, 77%); m.p. 95–97 °C; Rf 0.73 (3:1 cyclohexane/EtOAc); IR (υmax/cm⁻¹): 2911 (C–H), 2110 (N₃); MS (ES⁺) found m/z 585 [M+NH₄]^⁺, HRMS found 585.2513, C₃₃H₃₇N₄O₄S [M+NH₄]^⁺ requires 585.2530; [α]D = -51.2 (c = 4.30, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.66 (2 H, dd, J = 8.1, 1.5 Hz, ArH), 7.40–7.24 (18 H, m, ArH), 4.91 (1 H, d, J = 10.5 Hz, CH₂Ar), 4.88 (1 H, d, J = 10.5 Hz, CH₂Ar), 4.84 (1 H, d, J = 10.9 Hz, CH₂Ar), 4.67 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.63 (1 H, d, J = 10.9 Hz, CH₂Ar), 4.59 (1 H, d, J = 11.9 Hz, CH₂Ar), 4.46 (1 H, d, J = 10.1 Hz, H-1), 3.85–3.77 (2 H, m, 2 x H-6), 3.66 (1 H, t, J = 9.4 Hz, H-4), 3.58–3.50 (2 H, m, H-3, H-5), 3.40 (1 H, dd, J = 10.1, 9.3 Hz, H-2); δC (101 MHz, CDCl₃): 138.2 (Ar C), 137.9 (Ar C), 137.6 (Ar C), 133.7 (Ar CH), 131.2 (Ar CH), 130.8 (2 x Ar CH), 129.9 (2 x Ar CH), 129.7 (Ar C), 125.7 (2 x Ar CH), 123.4 (2 x Ar CH), 123.0 (Ar C), 113.3 (2 x Ar CH), 109.5 (2 x Ar CH), 107.9 (Ar CH), 105.3 (2 x Ar CH), 102.4 (Ar CH), 91.1 (C-1α), 89.0 (C-2α), 87.7 (C-3α), 86.6 (C-4α), 84.3 (C-5α), 82.2 (C-6α), 81.1 (2 x C-6), 77.7 (2 x C-6), 63.1 (C-2α), 55.3 (ArOCH₃). Data in agreement with previous synthesis³¹⁸ (N.B. synthesis first reported by Cirillo et al.³⁸⁹ but data not reported).
129.0 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.7 (Ar CH), 127.6 (Ar CH), 86.0 (C1), 85.1 (C-3), 79.4 (C-5), 77.6 (C-4), 76.0 (CH2Ar), 75.1 (CH2Ar), 73.5 (CH2Ar), 68.8 (C-6), 65.1 (C-2). Data in agreement with previous syntheses (N.B. synthesis first reported by Du et al. but data not reported, data reported (except melting point) by Greenberg et al.)

2-Azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-α/β-D-glucopyranose α/β-224

Thioglycoside 162 (5.00 g, 8.36 mmol) was dissolved in acetone (150 mL) and cooled to 0 °C. NBS (1.49 g, 8.36 mmol) was added and the reaction was stirred at 0 °C for 30 min. Saturated aqueous NaHCO3 solution (5 mL) was added, and the solvent was removed in vacuo. The residue was partitioned between CH2Cl2 (100 mL) and H2O (100 mL), the phases separated, and the organic phase dried and concentrated in vacuo to a brown oil. Column chromatography (3:1 hexane/EtOAc) afforded the title compound 224 as a brown solid (4.05 g, 8.0 mmol, 95%, ~1:1 α/β); Rf 0.35 (2:1 hexane/EtOAc); IR (νmax/cm⁻¹): 3397 (O–H), 2915 (C–H), 2107 (N=O); MS (ES⁺) found m/z 528 [M+Na]+. HRMS found 528.2100, C28H31N3NaO6 [M+Na]+ requires 528.2105; δH (400 MHz, CDCl3): 7.41–7.29 (20 H, m, ArH), 7.05 (4 H, app t, J = 9.2 Hz, ArH), 6.82–6.79 (3 H, m, ArH), 5.31–5.30 (1 H, m, H-1α), 4.90–4.77 (2 H, m, 2 x CH2Ar), 4.82 (1 H, d, J = 10.8 Hz, CH2Ar), 4.75–4.70 (2 H, m, 2 x CH2Ar), 4.61–4.56 (2 H, m, 2 x CH2Ar), 4.54–4.49 (2 H, m, CH2Ar, H-1β), 4.46–4.40 (2 H, m, 2 x CH2Ar), 4.08–4.04 (1 H, m, H-5α), 4.00 (1 H, dd, J = 10.1, 9.0 Hz, H-3α), 3.79 (6 H, s, 2 x ArOCH3), 3.68–3.34 (10 H, m, H-2α, H-2β, H-3β, H-4α, H-4β, H-5β, 2 x H-6α, 2 x H-6β); δC (101 MHz, CDCl3) 159.4 (2 x Ar C), 137.9 (2 x Ar C), 137.7 (2 x Ar C), 129.7 (Ar CH), 129.6 (Ar CH), 128.7 (Ar CH), 128.5 (2 x Ar CH), 128.2 (Ar CH), 128.1 (2 x Ar CH), 128.0 (Ar CH), 127.9 (4 x Ar CH), 113.9 (2 x Ar CH), 96.2 (C-1β), 92.0 (C-1α), 83.1 (CH), 80.2 (C-3α), 78.2 (CH), 77.4 (CH), 75.6 (2 x CH2Ar), 74.8 (CH), 74.7 (CH2Ar), 73.5 (2 x CH2Ar), 70.6 (C-5α), 68.6 (2 x C-6), 67.4 (CH), 64.0 (CH), 63.5 (CH), 55.3 (2 x ArOCH3). Data in agreement with previous synthesis (N.B. 1H and 13C NMR data reported only).
2-Azido-3,6-di-O-benzyl-4-O-chloroacetyl-2-deoxy-α/β-D-glucopyranose α/β-225

Glucosamine derivative 175 (350 mg, 0.63 mmol) was dissolved in acetone (10 mL) and cooled to 0 °C. NBS (112 mg, 0.65 mmol) was added and the mixture stirred for 30 min. Saturated aq. NaHCO₃ (2 mL) was added and the solvent was removed *in vacuo*. The solid was partitioned between CH₂Cl₂ (100 mL) and H₂O (100 mL). The organic phase was dried and the solvent removed *in vacuo* to give a yellow oil. Column chromatography (3:1, 2:1 hexane/EtOAc) afforded the title compound 225 as a white solid (229 mg, 0.49 mmol, 78%, ~1:1 α/β); Rₓ 0.45 (4:1 hexane/EtOAc); IR (ν_{max}/cm⁻¹): 3394 (O–H), 2867 (C–H), 2100 (N₃), 1755 (C=O); MS (ES⁺) found m/z 484 [M+Na]⁺, HRMS found 479.1692, C₂₂H₂₈ClN₄O₆ [M+NH₄]⁺ requires 479.1688; δH (400 MHz, CDCl₃): 7.36–7.27 (20 H, m, Ar H), 5.35 (1 H, d, J = 3.4 Hz, H-1α), 5.12 (1 H, dd, J = 10.1, 9.3 Hz, H-4α), 5.04 (1 H, ddd, J = 10.4, 6.5, 3.5 Hz, H-4β), 4.86 (1 H, d, J = 11.4 Hz, CH₂Ar), 4.85 (1 H, d, J = 11.6 Hz, CH₂Ar), 4.64–4.58 (3 H, m, H-1β, 2 x CH₂Ar), 4.53–4.45 (4 H, m, 4 x CH₂Ar), 4.16–4.12 (1 H, m, H-6α), 4.00 (1 H, t, J = 9.7 Hz, H-3α), 3.65–3.43 (12 H, m, H-2α, H-2β, H-3β, H-5α, H-5β, 3 x H-6, 4 x CH₂Cl); δC (101 MHz, CDCl₃): 166.4 (C=O), 166.3 (C=O), 137.2 (Ar C), 137.2 (Ar C), 128.6 (2 x Ar CH), 128.5 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 128.1 (2 x Ar CH), 96.2 (C-1β), 91.8 (C-1α), 80.2 (CH), 77.6 (C-3α), 75.0 (CH₂Ar), 73.7 (CH₂Ar), 73.6 (CH₂Ar), 72.7 (CH), 72.2 (CH), 68.9 (2 x C-6), 68.4 (CH), 67.0 (CH), 63.7 (CH), 40.5 (2 x CH₂Cl).

2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-α/β-D-glucopyranose α/β-226

Glucosamine derivative 173 (872 mg, 1.83 mmol) was dissolved in acetone (20 mL) and the solution was cooled to 0 °C. NBS (652 mg, 3.66 mmol) was added and the solution was stirred for 1 h warming to RT. The solvent was removed *in vacuo* to give a brown oil. Column chromatography (4:1 hexane/EtOAc) yielded the title compound 226 as a yellow solid (271 mg, 0.71 mmol, 39%, ~1:1 α/β); Rₓ 0.51 (3:1 hexane/EtOAc); IR (ν_{max}/cm⁻¹): 3431 (O–H), 2921 (C–H), 2110 (N₃); MS (ES⁺) found m/z 384 [M+H]⁺.
HRMS found 384.1555, C_{20}H_{22}N_{3}O_{5} [M+H]^+ requires 384.1554; δ_H (400 MHz, CDCl_3): 7.55–7.49 (4 H, m, ArH), 7.46–7.30 (16 H, m, ArH), 5.62 (1 H, s, CHPh), 5.59 (1 H, s, CHPh), 5.22 (1 H, t, J = 3.4 Hz, H-1α), 4.99 (1 H, d, J = 11.0 Hz, CH_2Ar), 4.96 (1 H, d, J = 11.2 Hz, CH_2Ar), 4.83 (2 H, app dd, J = 11.1, 3.8 Hz, 2 x CH_2Ar), 4.55 (1 H, dd, J = 9.0, 5.4 Hz, H-1β), 4.36–4.28 (3 H, m, 2 x H-6α, β-OH), 4.13 (2 H, app tt, J = 9.7, 2.4 Hz, H-3α, H-4β), 3.82–3.71 (4 H, m, H-3β, H-5β, 2 x H-6β), 3.65–3.60 (2 H, m, H-4α, α-OH), 3.48 (1 H, dd, J = 9.9, 3.5 Hz, H-2α), 3.44–3.37 (2 H, m, H-2β, H-5α); δ C (101 MHz, CDCl_3): 137.7 (Ar C), 137.6 (Ar C), 137.5 (Ar C), 128.5 (Ar CH), 128.4 (Ar CH), 128.3 (2 x Ar CH), 128.0 (Ar CH), 126.0 (2 x Ar CH), 101.5 (PhCH), 101.4 (PhCH), 96.5 (C-1β), 92.7 (C-1α), 82.8 (CH), 81.5 (CH), 79.0 (CH), 76.3 (CH), 75.2 (CH_2Ar), 75.0 (CH_2Ar), 68.9 (C-6β), 68.5 (C-6α), 67.2 (CH), 66.4 (CH), 63.6 (C-2α), 62.7 (CH). Data in agreement with previous syntheses (N.B. 13C NMR data not reported).^{392–394}

2-Azido-3,4,6-tri-O-benzyl-2-deoxy-α/β-D-glucuronate α/β-227

Thioglycoside 223 (210 mg, 0.37 mmol) was dissolved in acetone (3 mL) and cooled to 0 °C. NBS (66 mg, 0.37 mmol) was added and the reaction was stirred at 0 °C for 30 min. Saturated aq. NaHCO_3 solution (2 mL) was added, and the solvent was removed in vacuo. The residue was partitioned between CH_2Cl_2 (30 mL) and H_2O (30 mL), the phases separated, and the organic phase dried and concentrated in vacuo. Column chromatography (10:1, 2:1 petroleum ether/EtOAc) afforded the title compound 227 as a white solid (157 mg, 0.33 mmol, 89%, ~1:1 α/β); R_f 0.43 (2:1 hexane/EtOAc); IR (ν_{max}/cm^{-1}): 3394 (O–H), 2294 (C–H), 2105 (N=O); MS (ES^+) found m/z 493 [M+NH_4]^+, HRMS found 493.2433, C_{27}H_{33}O_{5}N_4 [M+NH_4]^+ requires 493.2445; δ_H (400 MHz, CDCl_3): 7.32–7.20 (26 H, m, ArH), 7.11–7.05 (4 H, m, ArH), 5.25 (1 H, t, J = 3.5 Hz, H-1α), 4.83–4.79 (3 H, m, 3 x CH_2Ar), 4.76–4.71 (3 H, m, 3 x CH_2Ar), 4.53–4.41 (7 H, m, H-1β), 6 x CH_2Ar), 4.02 (1 H, ddd, J = 10.1, 4.0, 2.7 Hz, H-5α), 3.82–3.92 (2 H, m, H-3α, H-3β), 3.64–3.28 (9 H, m, H-2α, H-2β, H-4α, H-4β, H-5β, 2 x H-6α, 2 x H-6β); δ_C (101 MHz, CDCl_3): 137.8 (Ar C), 137.7 (Ar C), 137.6 (Ar C), 137.5 (Ar C), 128.5 (Ar CH), 128.5 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.8 (2 x Ar CH), 96.2 (C-1β), 92.1 (C-1α), 83.1 (CH), 80.1
(CH), 78.5 (CH), 77.7 (CH), 75.6 (CH₂Ar), 75.6 (CH₂Ar), 75.1 (CH), 73.5 (CH₂Ar), 70.7 (CH), 68.6 (2 x C-6), 67.5 (CH), 64.0 (CH). Data in agreement with reported syntheses (N.B. first reported by Kinzy and Schmidt, further data reported by Inoue et al.)

2-Azido-3,6-di-O-benzyl-4-O-chloroacetyl-2-deoxy-1-O-trichloroacetimidate-α/β-D-glucopyranoside α/β-228

Glucosamine derivative 225 (186 mg, 0.40 mmol) was dissolved in anhydrous CH₂Cl₂ (3 mL) under N₂. Cl₃CCN (200 µL, 2.0 mmol) and DBU (3 µL, 0.02 mmol) were added and the solution stirred at RT for 1.5 h. DBU (10 µL) was added and the mixture was stirred for an additional 15 min. The solvent was removed in vacuo to give a brown oil. Column chromatography (3:1 hexane/EtOAc) afforded the title compound 228 as a yellow oil (224 mg, 0.37 mmol, 92%, α anomer with <5% β anomer). Data for α/β-228:

Rf 0.45 (4:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2916 (C–H), 2019 (N₃), 1770 (C=O), 1673 (C=N); MS (ES⁺) found m/z 629 [M+Na]⁺, HRMS found 627.0344, C₂₄H₂₄Cl₄N₄NaO₆ [M+Na]⁺ requires 627.0348. NMR data for α-228 δH (400 MHz, CDCl₃): 7.28–7.19 (10 H, m, ArH), 6.37 (1 H, d, J = 3.5 Hz, H-1), 5.24 (1 H, t, J = 9.8, H-4), 4.80 (1 H, d, J = 11.3 Hz, CH₂Ar), 4.57 (1 H, d, J = 11.3 Hz, CH₂Ar), 4.41 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.34 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.08–4.00 (2 H, m, H-3, H-5), 3.69 (1 H, dd, J = 10.1, 3.5 Hz, H-2), 3.56 (1 H, d, J = 14.7 Hz, CH₂Cl), 3.51 (1 H, d, J = 14.8 Hz, CH₂Cl), 3.43 (2 H, td, J = 11.1, 3.8 Hz, 2 x H-6); δC (101 MHz, CDCl₃): 166.0 (C=O), 160.5 (C=N), 137.4 (Ar C), 137.3 (Ar C), 128.6 (Ar C), 128.4 (Ar C), 128.2 (Ar C), 128.1 (2 x Ar C), 127.9 (Ar C), 94.4 (C-1), 90.8 (CCl₃), 77.6 (CH), 75.0 (CH₂Ar), 73.7 (CH₂Ar), 72.0 (C-4), 71.3 (CH), 68.4 (C-6), 62.8 (C-2), 40.5 (CH₂Cl).
2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-1-O-trichloroacetimidate-α/β-D-glucopyranoside α/β-229

Glucosamine derivative 226 (418 mg, 1.09 mmol) was dissolved in anhydrous CH$_2$Cl$_2$ (5 mL) under N$_2$. Cl$_3$CCN (786 µL, 5.45 mmol) and DBU (15 µL, 0.11 mmol) were added and the solution was stirred at RT for 1 h. The solvent was removed in vacuo to give a brown foam. Column chromatography (5:1 hexane/EtOAc) yielded the title compound 229 as a white foam (387 mg, 0.73 mmol, 67%, α anomer with <5% β anomer). Data α/β-229: R$_f$ 0.43 (4:1 hexane/EtOAc); IR (ν$_{\text{max}}$/cm$^{-1}$): 2926 (C–H), 2110 (N$_3$), 1671 (C=N); MS (ES$^+$) found m/z 568 [M+K]$^+$, HRMS found 549.0458, C$_{22}$H$_{21}$Cl$_3$N$_4$NaO$_5$ [M+Na]$^+$ requires 549.0475. NMR data for α-229: δ$_H$ (400 MHz, CDCl$_3$): 7.54–7.55 (2 H, m, Ar$_H$), 7.46–7.31 (8 H, m, Ar$_H$), 6.40 (1 H, d, $J$ = 3.7 Hz, H-1), 5.65 (1 H, s, PhC$_H$), 5.06 (1 H, d, $J$ = 10.8 Hz, C$_H$$_2$Ar), 4.87 (1 H, d, $J$ = 11.0 Hz, C$_H$$_2$Ar), 4.37 (1 H, dd, $J$ = 10.4, 4.9 Hz, H-6), 4.21 (1 H, t, $J$ = 9.6 Hz, H-3), 4.08 (1 H, dt, $J$ = 9.8, 4.9 Hz, H-5), 3.89–3.78 (2 H, m, H-4, H-6), 3.75 (1 H, dd, $J$ = 9.9, 3.7 Hz, H-2); δ$_C$ (101 MHz, CDCl$_3$): 160.9 (C=N) 137.6 (Ar C), 136.9 (Ar C), 129.2 (Ar CH), 128.5 (2 x Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.0 (Ar CH), 125.9 (Ar CH), 101.4 (PhCH), 94.9 (C-1), 82.2 (C-4), 76.3 (C-3), 75.2 (CH$_2$Ar), 68.6 (C-6), 65.2 (C-5), 62.5 (C-2). Data in agreement with previous syntheses.$^{386,397}$

2-Azido-3,4,6-tri-O-benzyl-2-deoxy-1-O-trichloroacetimidate-α/β-D-glucopyranoside α/β-230

Glucosamine derivative 227 (157 mg, 0.33 mmol) was dissolved in anhydrous CH$_2$Cl$_2$ (3 mL) under argon. Cl$_3$CCN (74 µL, 0.74 mmol) and K$_2$CO$_3$ (154 mg, 1.11 mmol) were added and the suspension was stirred at RT overnight. The solid was filtered off and the solvent was removed in vacuo to afford the title compound 230 as a yellow oil
(153 mg, 0.25 mmol, 77%, β anomer with ~15% α anomer). Data for α/β-230: Rf 0.69
(2:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2915 (C–H), 2107 (N₃), 1672 (C=N); MS (ES⁺)
found m/z 322 [M+K+H]²⁺, HRMS found 641.1091, C₂₉H₂₉Cl₁₃N₄NaO₅ [M+Na]⁺
requires 641.1096. NMR data for β-230: δH (400 MHz, CDCl₃): 7.38–7.27 (12 H, m,
ArH), 7.20–7.16 (3 H, m, ArH), 5.63 (1 H, d, J = 8.4 Hz, H-1), 4.83–4.81 (3 H, m, 3 x
CH₂Ar), 4.64–4.52 (3 H, m, 3 x CH₂Ar), 3.80–3.67 (5 H, m, H-2, H-4, 2 x H-6), 3.61–
3.53 (2 H, m, H-3, H-5); δC (101 MHz, CDCl₃): 161.1 (C=N), 137.9 (Ar C), 137.7 (Ar
C), 128.5 (Ar CH), 128.4 (Ar CH), 128.0 (Ar CH), 127.9 (2 x Ar CH), 96.9 (C-1), 83.0 (CH), 77.2 (CH), 76.1 (CH), 75.7 (CH₂Ar), 75.1 (CH₂Ar), 73.5
(CH₂Ar), 68.0 (C-6), 65.8 (C-2). Data in agreement with reported synthesis.³⁹⁵

13.1.11 Glucoazide SBox Derivatives from Trichloroacetimidates

Benoxazolyl 2-azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-1-thio-α/β-d-
gluconurate α/β-231

Trichloroacetimide donor 219 (392 mg, 0.60 mmol) and HSBox (76 mg, 0.50 mmol)
were combined in anhydrous toluene and the solvent removed in vacuo. This process
was repeated twice. The resulting mixture was dried under high vacuum overnight, then
dissolved in anhydrous CH₂Cl₂ (2 mL) under N₂. The solution was cooled to ~50 °C,
molecular sieves (~300 mg, 4 Å, powdered, activated) were added and the mixture was
stirred for 30 min. TMSOTf (18 µL, 0.10 mmol) was added and the mixture stirred
warming to RT for 1.5 h. TLC (3:1 hexane/EtOAc) showed remaining donor. The
mixture was filtered through Celite with CH₂Cl₂ (100 mL) and the solvent was removed
in vacuo to give a brown solid. Column chromatography (4:1 hexane/EtOAc) afforded
the title compound 231 as a yellow oil (194 mg, 0.30 mmol, 60%, ~1:1 α/β); Rf 0.57
(3:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2806 (C–H), 2107 (N₃); MS (ES⁺) found m/z 639.5
[M+H]⁺, HRMS found 661.2078, C₃₅H₃₄N₆NaO₆S [M+Na]⁺ requires 661.2097; δH (400
MHz, CDCl₃): 7.68–7.66 (1 H, m, ArH), 7.34–7.12 (31 H, m, ArH), 6.85–6.79 (4 H, m,
ArH), 6.55 (1 H, d, J = 3.0 Hz, H-1α), 6.01 (1 H, d, J = 9.4 Hz, H-1β), 4.91 (1 H, d, J =
10.5 Hz, CH₂Ar), 4.88 (1 H, d, J = 10.5 Hz, CH₂Ar), 4.78 (1 H, d, J = 10.5 Hz, CH₂Ar),
4.71 (1 H, d, \( J = 11.5 \) Hz, \( CH_2Ar \)), 4.61–4.54 (3 H, m, 3 x \( CH_2Ar \)), 4.52–4.41 (7 H, m, H-2\( \alpha \), H-5\( \beta \), 5 x \( CH_2Ar \)), 4.19–4.12 (1 H, m, H-2\( \beta \)), 3.96–3.94 (2 H, m, H-3\( \alpha \), H-5\( \alpha \)), 3.83–3.78 (2 H, m, H-3\( \beta \), H-6), 3.76 (3 H, s, ArOCH\( 3 \)), 3.75 (3 H, s, ArOCH\( 3 \)), 3.71–3.66 (5 H, m, H-4\( \alpha \), H-4\( \beta \), 3 x H-6); \( \delta_c \) (101 MHz, CDCl\( 3 \)): 159.5 (C=N), 147.2 (Ar C), 147.1 (Ar C), 138.0 (Ar C), 137.8 (Ar C), 136.8 (Ar C), 129.8 (Ar CH), 129.7 (Ar CH), 129.5 (Ar CH), 128.7 (Ar CH), 128.6 (Ar CH), 128.5 (2 x Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 127.5 (Ar CH), 125.0 (Ar CH), 124.9 (Ar CH), 124.8 (Ar CH), 124.1 (Ar CH), 114.3 (Ar CH), 114.0 (Ar CH), 113.9 (Ar CH), 110.7 (Ar CH), 109.9 (Ar CH), 85.1 (C-1\( \beta \)), 83.8 (CH), 82.4 (C-1\( \alpha \)), 78.0 (CH), 77.6 (CH), 76.8 (CH), 76.2 (CH), 73.7 (CH\( 2Ar \)), 73.5 (CH\( 2Ar \)), 73.7 (CH), 73.5 (CH\( 2Ar \)), 73.4 (CH\( 2Ar \)), 72.7 (CH\( 2Ar \)), 72.4 (CH\( 2Ar \)), 68.9 (C-6), 67.9 (C-6), 55.4 (2 x ArOCH\( 3 \)).

Benzoxazolyl 2-Azido-3,6-di-O-benzyl-4-O-chloroacetyl-2-deoxy-1-thio-\( \beta \)-d-glucuronate 232

Trichloroacetimidate donor 228 (534 mg, 0.88 mmol) and HSBox (111 mg, 0.73 mmol) were combined in anhydrous toluene (~3 mL) and the solvent removed in vacuo. The process was repeated twice. The solid was dried under high vacuum for 3 h, then dissolved in anhydrous CH\( _2Cl_2 \) (5 mL) under N\(_2\) and cooled to –50 °C. TMSOTf (26 µL, 0.15 mmol) was added and the solution was stirred for 1 h warming to RT. TLC (3:1 hexane/EtOAc) showed no remaining donor. The reaction was quenched with Et\(_3\)N and the solvent removed in vacuo to give a brown oil. Column chromatography (5:1, 4:1 hexane/EtOAc) yielded the title compound 232 as a colourless oil (253 mg, 0.43 mmol, 58%); \( R_f \) 0.73 (3:1 hexane/EtOAc); IR (v\(_{\text{max}}\)/cm\(^{-1}\)): 2871 (C–H), 2109 (N\(_3\)), 1770 (C=O); MS (ES\(^+\)) found m/z 595 [M+H]\(^+\), HRMS found 617.1239, C\(_{29}\)H\(_{27}\)ClN\(_4\)NaO\(_6\)S [M+Na]\(^+\) requires 617.1238; [\( \alpha \)]\(_D\) = –2.0 (c = 0.80, CH\(_2Cl_2\)); \( \delta_H \) (400 MHz, CDCl\( 3 \)): 7.44–7.42 (1 H, m, ArH), 7.26–7.24 (1 H, m, ArH), 7.19–7.15 (3 H, m, ArH), 7.12–7.00 (9 H, m, ArH), 5.18 (1 H, d, \( J = 10.5 \) Hz, H-1), 5.00 (1 H, t, \( J = 9.6 \) Hz, H-4), 4.69 (1 H, d, \( J = 11.4 \) Hz, \( CH_2Ar \)), 4.47 (1 H, d, \( J = 11.4 \) Hz, \( CH_2Ar \)), 4.24 (1 H, d, \( J = 11.6 \) Hz,
CH₂Ar), 4.19 (1 H, d, J = 11.7 Hz, CH₂Ar), 3.63 (1 H, dd, J = 10.5, 9.3 Hz, H-2), 3.55 (1 H, dt, J = 9.9, 4.2 Hz, H-5), 3.46 (1 H, t, J = 9.8 Hz, H-3), 3.39–3.30 (4 H, m, 2 x H-6, 2 x CH₂Cl); δ C (101 MHz, CDCl₃): 166.0 (C=O), 160.6 (C=N), 152.0 (Ar C), 141.6 (Ar C), 137.4 (Ar C), 137.3 (Ar C), 128.7 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 124.7 (Ar CH), 119.1 (Ar CH), 110.3 (Ar CH), 84.4 (C-1), 82.4 (C-3), 77.6 (C-5), 75.5 (CH₂Ar), 73.7 (CH₂Ar), 72.0 (C-4), 68.9 (C-6), 65.3 (C-2), 40.5 (CH₂Cl).

Benzoxazolyl 2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-1-thio-β-D-glucuronate 233

Trichloroacetimidate donor 229 (188 mg, 0.36 mmol) and HSBox (45 mg, 0.30 mmol) were combined in anhydrous toluene (~3 mL) and the solvent removed in vacuo. The process was repeated twice. The solid was dried under high vacuum for 3 h, then dissolved in anhydrous CH₂Cl₂ (5 mL) under N₂ and cooled to –50 °C. TMSOTf (11 µL, 0.06 mmol) was added and the solution was stirred for 1 h warming to RT. TLC (3:1 hexane/EtOAc) showed no remaining donor. The reaction was quenched with Et₃N and the solvent removed in vacuo to give a brown oil. Column chromatography (4:1 hexane/EtOAc) yielded the title compound 233 as a white foam (95 mg, 0.18 mmol, 61%); Rf 0.57 (3:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2869 (C–H), 2109 (N₃), 1600 (C=N); MS (ES⁺) found m/z 539 [M+Na]⁺, HRMS found 539.1379, C₂₇H₂₄N₄O₅NaS [M+Na]⁺ requires 539.1365; [α]D = –16.7 (c = 0.55, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.68–7.65 (1 H, m, ArH), 7.52–7.48 (3 H, m, ArH), 7.44–7.30 (10 H, m, ArH), 5.61 (1 H, s, PhCH), 5.40 (1 H, d, J = 10.2, H-1), 5.00 (1 H, d, J = 11.0 Hz, CH₂Ar), 4.86 (1 H, d, J = 11.0 Hz, CH₂Ar), 4.39 (1 H, dd, J = 10.4, 4.9 Hz, H-6), 3.85–3.79 (3 H, m, H-2, H-3, H-4), 3.76 (1 H, d, J = 10.2 Hz, H-6), 3.70–3.63 (1 H, m, H-5); δC (101 MHz, CDCl₃): 160.4 (C=N), 152.0 (Ar C), 141.6 (Ar C), 137.4 (Ar C), 137.0 (Ar C), 129.2 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.4 (Ar CH), 128.1 (Ar CH), 126.0 (Ar CH), 124.7 (Ar CH), 124.7 (Ar CH), 119.2 (Ar CH), 110.2 (Ar CH), 101.4 (CHPh), 84.6 (H-1), 81.3 (CH), 81.0 (CH), 75.3 (CH₂Ar), 71.0 (C-5), 68.4 (C-6), 65.0 (C-2).
Benzoxazolyl 2-Azido-3,4,6-tri-O-benzyl-2-deoxy-1-thio-\(\alpha/\beta\)-D-glucuronate \(\alpha/\beta\)-234

Trichloroacetimidate donor 230 (75 mg, 0.12 mmol) and HSBox (36 mg, 0.24 mmol) were combined and the flask was purged with vacuum/argon. The solid was dissolved in anhydrous CH\(_2\)Cl\(_2\) (2 mL) under argon and cooled to –60 °C. TMSOTf (4 \(\mu\)L, 24 \(\mu\)mol) was added and the solution was stirred for 1 h warming to RT. TLC (3:1 hexane/EtOAc) showed no remaining donor. The reaction was quenched with Et\(_3\)N and the solvent removed in vacuo. Column chromatography (9:1, 2:1, 1:2 hexane/EtOAc) yielded the title compound 234 as a colourless oil (34 mg, 56 \(\mu\)mol, 47%, 2:5 \(\alpha/\beta\)). 

\[ \text{IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)): 2867 (C–H), 2108 (N=O)} \]

\[ \text{MS (ES\textsuperscript{+}) found m/z 609 [M+H\textsuperscript{+}], HRMS found 609.2159, C\textsubscript{34}H\textsubscript{33}N\textsubscript{4}O\textsubscript{5}S [M+H\textsuperscript{+}] requires 609.2166; \}} \]

\[ \delta\text{H (400 MHz, CDCl\textsubscript{3})}: 7.59–7.54 (1.39 H, m, ArH), 7.40–7.36 (1.49 H, m, ArH), 7.30–7.16 (21.60 H, m, ArH), 7.14–7.08 (2.79 H, m, ArH), 6.50 (0.38 H, d, \(J = 5.3\) Hz, H-1\(\alpha\)), 5.24 (1 H, d, \(J = 10.5\) Hz, H-1\(\beta\)), 4.83–4.80 (2.81 H, m, 4 x CH\(_2\)Ar), 4.74 (1.44 H, dd, \(J = 10.8, 4.0\) Hz, CH\(_2\)Ar), 4.54–4.46 (2.89 H, m, 4 x CH\(_2\)Ar), 4.38 (1 H, d, \(J = 12.0\) Hz, CH\(_2\)Ar), 4.32 (0.40 H, d, \(J = 12.0\), CH\(_2\)Ar), 4.06–4.02 (0.36 H, m, H-5\(\alpha\)), 3.99 (0.42 H, dd, \(J = 10.2, 5.3\) Hz, H-2\(\alpha\)), 3.80–3.51 (7.81 H, m, H-2\(\beta\), H-3\(\alpha\), H-3\(\beta\), H-4\(\alpha\), H-4\(\beta\), H-5\(\beta\), 2 x H-6\(\alpha\), 2 x H-6\(\beta\)); \delta\text{C (101 MHz, CDCl\textsubscript{3})}: 160.9 (2 x C=), 151.9 (Ar C), 141.7 (Ar C), 137.9 (Ar C), 137.8 (Ar C), 137.7 (Ar C), 137.5 (Ar C), 128.6 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 127.6 (Ar CH), 124.5 (Ar CH), 119.1 (Ar CH), 110.2 (Ar CH), 86.2 (C-1\(\alpha\)), 85.1 (CH), 84.4 (C-1\(\beta\)), 82.0 (CH), 80.1 (CH), 77.6 (CH), 77.4 (CH), 75.9 (CH\(_2\)Ar), 75.9 (CH\(_2\)Ar), 75.2 (CH\(_2\)Ar), 75.1 (CH\(_2\)Ar), 74.0 (CH), 73.5 (CH\(_2\)Ar), 73.5 (CH\(_2\)Ar), 68.3 (C-6), 67.9 (C-6), 65.6 (CH), 63.5 (CH).
13.1.12 Glucoazide STaz Derivatives from Trichloroacetimidates

Thiazolyl 2-Azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-1-thio-α/β-D-glucuronate 235

Trichloroacetimidate donor 219 (387 mg, 0.60 mmol) and HSTaz (60 mg, 0.50 mmol) were combined in anhydrous toluene and the solvent removed in vacuo. This process was repeated twice. The solid was dried under high vacuum overnight, then dissolved in anhydrous CH$_2$Cl$_2$ (2 mL) under N$_2$. The solution was cooled to −50 °C, molecular sieves (~300 mg, 4 Å, powdered, activated) were added and the reaction was stirred for 30 min. TMSOTf (18 µL, 0.10 mmol) was added and the mixture was stirred for 1.5 h warming to RT. TLC (2:1 hexane/EtOAc) showed no remaining donor. The solution was filtered through Celite with CH$_2$Cl$_2$ (100 mL) and the solvent removed in vacuo to give an orange oil. Column chromatography (3:1 hexane/EtOAc) to give the title compound 235 as a colourless oil (109 mg, 0.18 mmol, 36%) along with N-linked product 238 (104 mg, 0.17 mmol, 34%).

Data for 235: R$_f$ 0.22 (3:1 hexane/EtOAc); IR ($\nu_{\text{max}}$/cm$^{-1}$): 2868 (C–H), 2108 (N$_3$); MS (ES$^+$) found m/z 607 [M+H]$^+$, HRMS found 607.2041, C$_{31}$H$_{35}$N$_4$O$_5$S$_2$ [M+H]$^+$ requires 607.2049; [α]$_D$ = −9.5 (c = 0.55, CH$_2$Cl$_2$); δ$_H$(400 MHz, CDCl$_3$): 7.30–7.17 (10 H, m, ArH), 7.01–6.97 (2 H, m, ArH), 6.74–6.71 (2 H, m, ArH), 5.09 (1 H, d, J = 10.1 Hz, H-1), 4.80 (2 H, s, 2 x CH$_2$Ar), 4.63 (1 H, d, J = 10.5 Hz, CH$_2$Ar), 4.55 (1 H, d, J = 12.1 Hz, CH$_2$Ar), 4.45 (1 H, d, J = 12.0 Hz, CH$_2$Ar), 4.36 (1 H, d, J = 10.5 Hz, CH$_2$Ar), 4.12 (2 H, t, J = 8.5, 0.8 Hz, 2 x CH$_2$), 3.70 (3 H, m, ArOCH$_3$), 3.56–3.60 (3 H, m, H-2, 2 x H-6), 3.54–3.51 (3 H, m, H-2, H-3, H-5), 3.30–3.26 (2 H, t, J = 8.8 Hz, 2 x CH$_2$); δ$_C$(101 MHz, CDCl$_3$): 159.4 (C=N), 138.0 (Ar C), 137.7 (Ar C), 130.0 (Ar CH), 129.6 (Ar CH), 128.6 (Ar CH), 128.4 (Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.7 (Ar CH), 113.9 (Ar CH), 85.2 (C-3), 83.9 (C-1), 79.8 (C-5), 77.2 (C-4), 75.8 (CH$_2$Ar), 74.7 (CH$_2$Ar), 73.5 (CH$_2$Ar), 74.5 (CH$_2$Ar), 68.4 (C-6), 65.6 (C-2), 64.2 (CH$_2$), 55.3 (ArOCH$_3$), 35.3 (CH$_2$).
Data for 238: R_f 0.37 (3:1 hexane/EtOAc); IR (ν_max/cm⁻¹): 2909 (C–H), 2105 (N=O); MS (ES⁺) found m/z 607 [M+H]⁺, HRMS found 629.1888, C₃₁H₃₄N₄NaO₅S₂ [M+Na]⁺ requires 629.1868; [α]_D = +19.9 (c = 1.70, CH₂Cl₂); δ_H (400 MHz, CDCl₃): 7.30–7.18 (10 H, m, ArH), 7.03–7.00 (2 H, m, ArH), 6.75–6.72 (2 H, m, ArH), 5.78 (1 H, d, J = 9.7 Hz, H-1), 4.83 (1 H, d, J = 10.7 Hz, CH₂Ar), 4.80 (1 H, d, J = 10.7 Hz, CH₂Ar), 4.65 (1 H, d, J = 10.5 Hz, CH₂Ar), 4.49 (1 H, d, J = 12.1 Hz, CH₂Ar), 4.46 (1 H, d, J = 10.5 Hz, CH₂Ar), 4.41 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.09 (1 H, dt, J = 10.7, 7.7 Hz, CH₂Ar), 3.89 (1 H, d, J = 10.7, 7.8 Hz, 2 x CH₂), 3.70 (3 H, s, ArOC₃H₃), 3.69–3.63 (3 H, m, H-3, H-4, H-6), 3.60 (1 H, dd, J = 11.1, 1.7 Hz, H-6), 3.52–3.48 (2 H, m, H-2, H-5), 3.24 (2 H, td, J = 7.7, 1.9 Hz, CH₂), δ_C (101 MHz, CDCl₃): 198.54 (C=S), 157.0 (Ar C), 135.5 (Ar C), 135.2 (Ar C), 127.5 (Ar CH), 127.1 (Ar CH), 126.1 (Ar CH), 126.0 (Ar CH), 125.7 (Ar CH), 125.6 (Ar CH), 125.4 (Ar CH), 125.3 (Ar CH), 111.5 (Ar CH), 81.5 (CH), 80.5 (C-1), 75.2 (CH), 74.8 (CH), 73.4 (CH₂Ar), 72.3 (CH₂Ar), 71.1 (CH₂Ar), 65.8 (C-6), 60.7 (C-2), 52.9 (ArOCH₃), 48.4 (CH₂), 25.9 (CH₂).

Thiazolyl 2-Azido-3,6-di-O-benzyl-4-O-chloroacetyl-2-deoxy-1-thio-β-D-glucuronate 236

Trichloroacetimidate donor 228 (486 mg, 0.80 mmol) and HSTaz (80 mg, 0.67 mmol) were combined in anhydrous toluene (~3 mL) and the solvent removed in vacuo. The process was repeated twice. The solid was dried under high vacuum for 3 h, then dissolved in anhydrous CH₂Cl₂ (5 mL) under N₂ and cooled to −50 °C. TMSOTf (24 µL, 0.13 mmol) was added and the solution was stirred for 1 h warming to RT. TLC (3:1 hexane/EtOAc) showed no remaining donor. The reaction was quenched with Et₃N and the solvent removed in vacuo to give a brown oil. Column chromatography (3:1 hexane/EtOAc) yielded the title compound 236 as a colourless oil (210 mg, 0.37 mmol, 55%); R_f 0.37 (3:1 hexane/EtOAc); IR (ν_max/cm⁻¹): 2870 (C–H), 2108 (N=O); MS (ES⁺) found m/z 563 [M+H]⁺, HRMS found 585.1005, C₂₅H₂₇Cl₅N₄NaO₅S₂ [M+Na]⁺ requires 585.1009; [α]_D = +4.8 (c = 0.83, CH₂Cl₂); δ_H (400 MHz, CDCl₃): 7.32–7.19 (10 H, m, ArH), 5.19 (1 H, d, J = 10.5 Hz, H-1), 5.09 (1 H, t, J = 9.6 Hz, H-
4), 4.81 (1 H, d, J = 11.5 Hz, CH$_2$Ar), 4.58 (1 H, d, J = 11.5 Hz, CH$_2$Ar), 4.42 (1 H, d, J = 11.7 Hz, CH$_2$Ar), 4.37 (1 H, d, J = 11.7 Hz, CH$_2$Ar), 4.15 (2 H, t, J = 8.1, 2 x CH$_2$), 3.64–3.44 (7 H, m, H$_2$-2, H$_3$-3, H$_5$-5, 2 x H$_6$-6, 2 x CH$_2$Cl), 3.32 (2 H, t, J = 8.1 Hz, 2 x CH$_2$); δ$_C$ (101 MHz, CDCl$_3$): 166.0 (C=O), 162.7 (C=N), 137.5 (Ar C), 137.4 (Ar C), 128.6 (Ar CH), 128.4 (Ar CH), 128.2 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 127.9 (Ar CH), 83.9 (C-1), 82.3 (CH), 77.4 (CH), 75.4 (CH$_2$Ar), 73.6 (CH$_2$Ar), 72.1 (C-4), 69.0 (C-6), 65.3 (CH), 64.1 (CH$_2$), 40.5 (CH$_2$Cl), 35.4 (CH$_2$). Thiazolyl 2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-1-thio-β-D-glucuronate

![Thiazolyl 2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-1-thio-β-D-glucuronate](image)

Trichloroacetimidate donor 229 (169 mg, 0.32 mmol) and HSTaz (32 mg, 0.27 mmol) were combined in anhydrous toluene (∼3 mL) and the solvent removed in vacuo. The process was repeated twice. The solid was dried under high vacuum for 3 h, then dissolved in anhydrous CH$_2$Cl$_2$ (5 mL) under N$_2$ and cooled to −50 °C. TMSOTf (10 µL, 0.05 mmol) was added and the solution was stirred for 1 h warming to RT. TLC (2:1 hexane/EtOAc) showed no remaining donor. The reaction was quenched with Et$_3$N and the solvent removed in vacuo to give a yellow oil. Column chromatography (3:1 hexane/EtOAc) yielded the title compound 237 as a colourless glass (93 mg, 0.19 mmol, 71%); R$_f$ 0.64 (2:1 hexane/EtOAc); IR (ν$_{max}$/cm$^{-1}$): 2925 (C–H), 2109 (N$_3$); MS (ES$^+$) found m/z 485 [M+Na]$^+$, HRMS found 485.1322, C$_{23}$H$_{24}$N$_4$NaO$_4$S$_2$ [M+Na]$^+$ requires 485.1317; [α]$_D$ = −6.9 (c = 0.55, CH$_2$Cl$_2$); δ$_H$ (400 MHz, CDCl$_3$): 7.49–7.47 (2 H, m, ArH), 7.42–7.30 (8 H, m, ArH), 5.58 (1 H, s, CHPh), 5.26 (1 H, d, J = 10.5 Hz, H-1), 4.95 (1 H, d, J = 11.1 Hz, CH$_2$Ar), 4.82 (1 H, d, J = 11.1 Hz, CH$_2$Ar), 4.38 (1 H, dd, J = 10.5, 5.0 Hz, H-6), 4.24 (2 H, td, J = 8.1, 3.1 Hz, 2 x CH$_2$), 3.79–3.70 (3 H, m, H-3, H-4 H-6), 3.67–3.62 (1 H, m, H-2), 3.58–3.53 (1 H, m, H-5), 3.40 (2 H, t, J = 8.1 Hz, 2 x CH$_2$); δ$_C$ (101 MHz, CDCl$_3$): 162.6 (C=N), 149.3 (Ar C), 137.5 (Ar C), 137.0 (Ar C), 129.1 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.1 (Ar CH), 126.0 (Ar CH), 101.4 (CHPh), 84.2 (H-1), 81.3 (CH), 81.0 (CH), 70.7 (CH$_2$Ar), 68.4 (C-5), 65.0 (C-6), 64.2 (C-2), 63.5 (CH$_2$), 35.4 (CH$_2$).
13.1.13 Synthesis of Iduronate Methyl Glycosides

3-O-Benzyl-1,2-O-isopropyldiene-\(\alpha\)-L-idofuranonitrile 239

Prepared according to the procedure reported by Hansen et al.\(^{317}\) Crude aldehyde 41 (368 g) was dissolved in EtOH/H\(_2\)O (1:1, 4.0 L), MgCl\(_2\)·6H\(_2\)O (323 g, 1.59 mol) and KCN (103 g, 1.59 mmol) were added and the solution was stirred for 5 d at RT, then filtered through Celite with EtOAc (500 mL). The phases were separated, the aqueous washed with EtOAc (3 x 200 mL). The combined organic phase was dried and the solvent removed \textit{in vacuo} to give a yellow solid. Crystallisation of the ido product was achieved by dissolving the crude product in EtOAc (100 mL) and adding hexane (400 mL) in portions, yielding the \textit{title compound} 239 as three crops of off-white solid (total 143 g, 0.47 mol, 32% over four steps); m.p. 145–146 °C (Lit.\(^{317}\) 150–151 °C); \(R_f\) 0.40 (20:1 CH\(_2\)Cl\(_2\)/EtOAc); IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)): 3383 (O–H), 2877 (C–H); MS (ES\(^+\)) found m/z 306 [M+H]\(^+\), HRMS found 306.1325, \(C_{16}H_{20}NO_5\) [M+H]\(^+\) requires 306.1336; \([\alpha]_D = -57.9\) (c = 0.62, CH\(_2\)Cl\(_2\)); \(\delta_H\) (400 MHz, CDCl\(_3\)): 7.41–7.32 (5 H, m, ArH), 6.00 (1 H, d, \(J = 3.7\) Hz, H-1), 4.77 (1 H, t, \(J = 7.3\) Hz, H-4), 4.70 (1 H, d, \(J = 10.8\) Hz, CH\(_2\)Ar), 4.66 (1 H, d, \(J = 3.7\) Hz, H-2), 4.58 (1 H, d, \(J = 11.2\) Hz, CH\(_2\)Ar), 4.47 (1 H, ddd, \(J = 7.4, 3.8, 2.5\) Hz, H-3), 4.21 (1 H, d, \(J = 3.8\) Hz, H-5), 2.72 (1 H, br s, OH), 1.51 (3 H, s, C(CH\(_3\))\(_2\)), 1.34 (3 H, s, C(CH\(_3\))\(_2\)); \(\delta_C\) (101 MHz, CDCl\(_3\)): 136.3 (Ar C), 128.8 (Ar CH), 128.5 (Ar CH), 128.2 (Ar CH), 117.3 (C=N), 112.7 (O\(_2\)C(CH\(_3\))\(_2\)), 105.6 (C-1), 82.3 (C-2), 82.0 (C-5), 81.1 (C-3), 72.8 (CH\(_2\)Ar), 61.0 (C-4), 27.0 (CH\(_3\)), 26.4 (CH\(_3\)). Data in agreement with previous synthesis.\(^{317}\)

Methyl (methyl 2,4-di-O-acetyl-3-O-benzyl-\(\alpha/\beta\)-L-idopyranoside) uronate \(\alpha/\beta\)-240

Prepared according to the procedure reported by Miller et al.\(^{320}\) To a stirred solution of cyanohydrin 239 (5.00 g, 16.3 mmol) in anhydrous MeOH (30 mL) under N\(_2\) at 0 °C
was added AcCl (2.32 mL, 32.6 mmol) dropwise. The reaction was stirred at 60 °C for 3 h, AcCl (0.60 mL, 8.4 mmol) was added and the reaction stirred for a further 19 h. AcCl (0.60 mL, 8.4 mmol) was added and the reaction stirred for 21 h. The solvents were removed in vacuo and the residue partitioned between EtOAc (75 mL) and water (75 mL). The phases were separated and the aqueous phase washed with EtOAc (2 x 30 mL). The combined organic phases were washed with saturated aq. NaHCO₃ (75 mL) and saturated aq. NaCl (75 mL), dried and concentrated to a yellow syrup. Column chromatography (1:1, 2:1 EtOAc/hexane) afforded a mixture of the furanoside and pyranoside products, which was used in the next step without further purification. To this mixture of methyl glycosides (3.90 g) was added DMAP (15 mg, 0.12 mmol), anhydrous CH₂Cl₂ (40 mL) and anhydrous pyridine (2.4 mL, 30 mmol). Acetic anhydride (2.94 ml, 31.2 mmol) was added dropwise and the reaction stirred at RT for 18 h. The solvents were removed in vacuo and the residue co-evaporated with toluene (2 x 100 mL). Column chromatography (3:2 hexane/EtOAc) afforded the title compound 240 as a yellow oil (2.80 g, 7.0 mmol, 56%, ~1:2 α/β) along with furanoside 268 (1.7 g, 4.28 mmol, 26%, ~1:1 α/β).

Data for α/β-240: Rf 0.50 (3:2 hexane/EtOAc); IR (νmax/cm⁻¹): 2954 (C–H), 1733 (C=O); MS (ES⁺) found m/z 414 [M+NH₄]⁺, HRMS found 397.1493, C₁₉H₂₅O₉ [M+H]⁺ requires 397.1494; δH (400 MHz, CDCl₃): 7.39–7.29 (7.97 H, m, ArH), 5.19 (0.47 H, ddd, J = 2.9, 2.0, 0.8 Hz, H-2α), 5.16 (1 H, ddt, J = 3.2, 2.1, 1.0 Hz, H-4β), 5.01 (1 H, m, H-2β), 4.91–4.89 (1.30 H, m, H-1α, H-4α, H-5α), 4.80 (1 H, d, J = 1.8 Hz, H-1β), 4.79–4.74 (2.6 H, m, 3 x CH₂Ar), 4.64 (1 H, dd, J = 6.6, 2.2 Hz, H-5β), 3.97 (1 H, td, J = 3.3, 1.2 Hz, H-3β), 3.79 (4.30 H, s, CO₂CH₃), 3.73 (0.45 H, td, J = 2.7, 1.2 Hz, H-3α), 3.57 (3 H, s, β OCH₃), 3.47 (1.26 H, s, α OCH₃), 2.08 (3 H, s, C(O)CH₃), 2.05–2.04 (6 H, m, C(O)CH₃); δC (101 MHz, CDCl₃): 171.2 (C=O), 170.1 (C=O), 169.9 (C=O), 169.6 (C=O), 169.0 (C=O), 168.1 (C=O), 137.3 (Ar C), 136.9 (Ar C), 128.6 (Ar CH), 128.4 (Ar CH), 128.2 (Ar CH), 127.9 (Ar CH), 127.8 (2 x Ar CH), 99.6 (C-1α), 98.9 (C-1β), 73.2 (CH₂Ar), 73.1 (C-3β), 72.3 (C-5β), 72.1 (CH₂Ar), 71.9 (C-3α), 67.8 (CH), 67.5 (CH), 67.0 (2 x CH), 65.8 (CH), 57.4 (OCH₃), 56.3 (OCH₃), 53.5 (CO₂CH₃), 52.4 (CO₂CH₃), 21.0 (C(O)CH₃), 20.9 (C(O)CH₃), 20.8 (C(O)CH₃), 20.7 (C(O)CH₃). Data in agreement with previous synthesis. ³²₀
Methyl (methyl 3-O-benzyl-α/β-methoxy-L-idopyranoside) uronate α/β-243

To methyl glycoside 240 (250 mg, 0.63 mmol, ~1:2 α/β) in anhydrous MeOH (10 mL) under N₂ was added sodium methoxide powder (3.4 mg, 0.06 mmol) and the reaction stirred for 1 h. Amberlite H⁺ resin was added and the reaction was stirred for 15 min. The solvent was removed in vacuo to give a yellow syrup. Column chromatography (1:1 hexane/EtOAc) afforded the title compound 243 as a colourless oil (185 mg, 0.59 mmol, 94%, ~1:2 α/β); Rᶠ 0.23 (1:1 hexane/EtOAc); IR (νₒₚ/ cm⁻¹): 3453 (O–H), 2917 (C–H), 1737 (C=O); MS (ES⁺) found m/z 335 [M+Na]⁺, HRMS found 335.1100, C₁₅H₂₀NaO₇ [M+Na]⁺ requires 335.1102; δH (400 MHz, CDCl₃): 7.41–7.29 (7.59 H, m, ArH), 4.87 (0.49 H, s, H-1α), 4.74 (0.47 H, dd, J = 5.9, 1.6 Hz, H-5α), 4.72–4.68 (2.55 H, m, H-1β, 2 x CH₂Ar), 4.64–4.61 (1.49 H, m, 2 x CH₂Ar), 4.51 (1 H, dd, J = 6.0, 1.4 Hz, H-5β), 4.10–4.09 (0.48 H, m, H-3α), 4.04–4.02 (1 H, m, H-4β), 3.94–3.92 (1 H, m, H-3β), 3.85–3.82 (1.44 H, m, H-2β, H-2α), 3.83 (4.21 H, s, 2 x CO₂H₂), 3.74 (0.55 H, m, H-4 α), 3.60 (3 H, s, β OCH₃), 3.46 (1.3 H, s, α OCH₃); δC (101 MHz, CDCl₃): 171.2 (C=O), 170.7 (C=O), 169.6 (C=O), 137.9 (Ar C), 137.3 (Ar C), 128.6 (Ar CH), 128.5 (Ar CH), 128.2 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 102.5 (C-1α), 99.4 (CH), 75.5 (CH), 75.2 (CH), 74.4 (C-5α), 72.6 (CH₂Ar), 71.8 (CH₂Ar), 68.4 (CH), 68.3 (CH), 67.8 (C-4α), 67.7 (C-3α), 66.7 (CH), 56.9 (CO₂CH₃), 56.2 (CO₂CH₃), 52.5 (OCH₃), 52.4 (OCH₃). Data in agreement with previous synthesis.³²⁰

13.1.14 Iduronate SBox Glycoside from Glycosyl Acetate 249

3-O-Benzyl-L-idopyranuronamide 245

Prepared according to the procedure reported by Hansen et al.³¹⁸ To a stirred solution of cyanohydrin 239 (10.50 g, 34.4 mmol) in THF (20 mL) at 0 °C was added HCl (80 mL, 36%) and the reaction stirred for 4 h at RT. The reaction mixture was poured slowly onto saturated aq. NaHCO₃, the phases separated and the aqueous phase washed with
THF (50 mL, 30 mL, 3 x 15 mL). The combined organic phase was dried and the solvent removed \textit{in vacuo} to give a brown oil, which was stirred in CHCl$_3$ (10 mL) for 12 h. The resultant precipitate was filtered to afford the \textit{title compound} 245 as a brown powder (8.74 g, 27.4 mmol, 89%, mixture of anomers, ratio not determined); R$_f$ 0.31 (10:1 CHCl$_3$/MeOH); IR ($\nu_{\max}$/cm$^{-1}$): 3410 (O–H), 2886 (C–H), 1702 (C=O); MS (ES$^+$) found m/z 306 [M+Na]$^+$, HRMS found 306.0948, C$_{13}$H$_{17}$NNaO$_6$ [M+Na]$^+$ requires 306.0948; $\delta$H (400 MHz, d$_6$-DMSO, major anomer): 7.38–7.33 (5 H, m, Ar H), 6.87 (1 H, br s, NH), 6.76 (1 H, d, $J$ = 8.0 Hz, OH), 5.10 (1 H, d, $J$ = 6.8 Hz, OH), 4.86–4.82 (2 H, m, H-1, OH), 4.63 (2 H, s, 2 x CH$_2$Ar), 4.07 (1 H, d, $J$ = 1.6 Hz, H-5), 3.86–3.58 (1 H, m, H-4); $\delta$C (101 MHz, d$_6$-DMSO): 172.0 (C=O), 138.1 (Ar C), 128.3 (Ar CH), 128.2 (Ar CH), 127.5 (Ar CH), 93.0 (C-1), 76.5 (C-3), 75.0 (C-5), 70.9 (CH$_2$Ar), 68.0 (C-4), 66.3 (C-2). Data in agreement with previous synthesis.\textsuperscript{318}

1,2-Di-O-acetyl-3-O-benzyl-\beta-L-idopyranuronamide 246

Prepared according to the procedure reported by Hansen \textit{et al.}\textsuperscript{318} To a stirred solution of amide 245 (2.50 g, 8.7 mmol) in CH$_2$Cl$_2$ (25 mL) at 0 °C was added Ac$_2$O (1.96 mL, 20.7 mmol) and DMAP (11 mg, 90 µmol). The reaction was stirred for 5 h at RT. Ethanol (10 mL) was added and the solvents removed \textit{in vacuo}. The residue was partitioned between EtOAc (50 mL) and water (40 mL), and the phases were separated. The organic phase washed with saturated aq. NaHCO$_3$ (40 mL), dried and the solvents were removed \textit{in vacuo}. Column chromatography (EtOAc, two columns) followed by crystallisation from hexane/EtOAc afforded the \textit{title compound} 246 as a white powder (390 mg, 1.06 mmol, 12%); m.p. 148–153 °C (Lit.\textsuperscript{318} 148–152 °C); R$_f$ 0.20 (EtOAc); IR ($\nu_{\max}$/cm$^{-1}$): 2885 (C–H), 1753 (C=O), 1730 (C=O), 1699 (C=O); MS (ES$^+$) found m/z 390 [M+Na]$^+$, HRMS found 367.1493, C$_{17}$H$_{22}$NO$_8$ [M+H]$^+$ requires 367.1494; [$\alpha$]$_D$ = +69.2 (c = 0.83, CH$_2$Cl$_2$); $\delta$H (400 MHz, CDCl$_3$): 7.38–7.32 (5 H, m, ArH), 6.47 (1 H, br s, NH), 6.07 (1 H, d, $J$ = 1.2 Hz, H-1), 5.88 (1 H, s, H-5), 5.13–5.12 (1 H, m, H-2), 4.69 (2 H, s, CH$_2$Ar), 4.58 (1 H, d, $J$ = 1.6 Hz, H-5), 4.19 (1 H, br s, H-4), 3.90 (1 H, t, $J$ = 3.2 Hz, H-3), 2.58 (1 H, br s, OH), 2.17 (3 H, s, C(O)CH$_3$), 2.14 (3 H, s, C(O)CH$_3$);
δC (101 MHz, CDCl₃): 170.4 (C=O), 169.2 (C=O), 168.5 (C=O), 136.7 (Ar C), 128.6 (Ar CH), 128.3 (Ar CH), 127.9 (Ar CH), 90.4 (C-1), 76.1 (C-5), 74.7 (C-3), 72.7 (CH₂Ar), 67.2 (C-2), 66.5 (C-4), 21.0 (C(O)CH₃), 20.8 (C(O)CH₃). Data in agreement with previous synthesis.³¹⁸

Methyl 1,2-Di-O-acetyl-3-O-benzyl-β-L-idopyranuronate 248

Prepared according to the procedure reported by Hansen et al.³¹⁸ To a stirred solution of amide 246 (500 mg, 1.40 mmol) in AcOH (10 mL) under N₂ was added isopentyl nitrite (0.72 mL, 5.4 mmol). The reaction was heated to 90 °C for 5 h. The cooled reaction mixture was co-evaporated with toluene (3 x 20 mL) and the crude material dissolved in DMF (5 mL) under N₂. KHCO₃ (137 mg, 1.37 mmol) was added, then MeI (85 µL, 1.37 mmol) was added dropwise and the reaction stirred for 18 h. KOAc (100 mg, 1.37 mmol) was added to quench excess MeI and the reaction was stirred for 18 h. The reaction mixture was diluted with CH₂Cl₂ (20 mL) and washed with water (4 x 10 mL). The organic phase was dried and solvent removed in vacuo to give an orange oil. Column chromatography (2:1, 1:1 hexane/EtOAc) gave the title compound 248 as a colourless syrup (190 mg, 0.50 mmol, 35%); Rf 0.19 (1:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2939 (C–H), 1756 (C=O), 1754 (C=O), 1724 (C=O); MS (ES⁺) found m/z 400 [M+NH₄]⁺, HRMS found 400.1611, C₁₈H₂₆NO₉ [M+NH₄]⁺ requires 400.1608; [α]D = +39.2 (c = 0.93, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.34–7.26 (5 H, m, ArH), 6.03 (1 H, d, J = 1.6 Hz, H-1), 5.12–5.10 (1 H, m, H-2), 4.68–4.60 (3 H, m, H-5, 2 x CH₂Ar), 3.95–3.94 (1 H, m, H-4), 3.82 (1 H, t, J = 3.2 Hz, H-3), 3.76 (3 H, s, CO₂CH₃), 2.09 (3 H, s, C(O)CH₃), 2.05 (3 H, s, C(O)CH₃); δC (101 MHz, CDCl₃): 169.1 (C=O), 168.4 (C=O), 168.3 (C=O), 136.7 (Ar C), 128.6 (Ar CH), 128.3 (Ar CH), 127.9 (Ar CH), 90.2 (C-1), 75.4 (C-5), 74.9 (C-3), 72.7 (CH₂Ar), 67.6 (C-4), 66.8 (C-2), 52.4 (CO₂CH₃), 21.0 (C(O)CH₃), 20.7 (C(O)CH₃). Data in agreement with previous synthesis.³¹⁸
Methyl 1,2-Di-O-acetyl-3-O-benzyl-4-O-chloroacetyl-β-L-idopyranuronate 249

Prepared according to the procedure reported by Hansen et al. To a stirred solution of methyl ester 248 (283 mg, 0.74 mmol) in anhydrous CH₂Cl₂ (5 mL) under N₂ was added pyridine (90 µL) and chloroacetyl chloride (83 µL, 1.04 mmol). The reaction was stirred under N₂ for 90 min at RT, diluted with CH₂Cl₂ (20 mL), washed with HCl (15 mL, 1% v/v) and saturated aq. NaHCO₃ (15 mL), dried and solvents removed in vacuo to give a yellow foam. Column chromatography (2:1 hexane/EtOAc) gave the title compound 249 as a white powder (302 mg, 0.66 mmol, 89%); m.p. 141–142 °C (Lit. 143–145 °C), Rf 0.83 (2:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2931 (C–H), 1775 (C=O), 1753 (C=O); MS (ES⁺) found m/z 481 [M+Na]⁺, HRMS found 481.0882, C₂₀H₂₃ClNaO₁₀ [M+Na]⁺, requires 481.0887; [α]D = +16.3 (c = 0.60, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.37–7.36 (5 H, m, ArH), 6.10 (1 H, d, J = 1.6 Hz, H-1), 5.22–5.21 (1 H, m, H-4), 5.07–5.06 (1 H, m, H-2), 4.83 (1 H, d, J = 2.4 Hz, H-5), 4.76 (2 H, s, 2 x CH₂Ar), 3.89 (1 H, d, J = 14.7 Hz, CH₂Cl), 3.84 (1 H, d, J = 14.4 Hz, CH₂Cl), 3.83 (1 H, t, J = 3.0 Hz, H-3), 3.78 (3 H, s, CO₂CH₃), 2.11 (6 H, s, 2 x C(O)CH₃); δC (101 MHz, CDCl₃): 169.9 (C=O), 168.5 (C=O), 167.1 (C=O), 166.4 (C=O), 136.5 (Ar C), 128.7 (Ar CH), 128.5 (Ar CH), 128.0 (Ar CH), 90.0 (C-1), 73.2 (CH₂Ar), 73.0 (C-5), 72.8 (C-3), 68.6 (C-4), 65.8 (C-2), 52.8 (CO₂CH₃), 40.3 (CH₂Cl), 20.9 (C(O)CH₃), 20.8 (C(O)CH₃). Data in agreement with previous synthesis.

Methyl (benzoxazolyl 2-O-acetyl-3-O-benzyl-4-O-chloroacetyl-1-thio-α-L-idopyranoside) uronate 250

To a stirred solution of 249 (515 mg, 1.12 mmol) and HSBox (338 mg, 2.24 mmol) in anhydrous CH₂Cl₂ (8 mL) under N₂ was added molecular sieves (~300 mg, 4 Å, powdered, activated) and the reaction stirred for 20 min at RT. The reaction was cooled to 0 °C, BF₃·OEt₂ (281 µL, 2.24 mmol) added dropwise and the reaction was stirred warming to RT for a total of 120 h. Additions of HSBox (338 mg, 2.24 mmol) and
Chapter Three: Experimental

BF₃·OEt₂ (568 µL, 4.52 mmol) were made at 20, 26, 48, 54 and 120 h, followed by a final addition of HSBox (338 mg, 2.24 mmol) and TMSOTf (404 µL, 2.24 mmol). The reaction was stirred for 20 h at RT. The reaction mixture was filtered through Celite with CH₂Cl₂ (100 mL) and washed with aqueous NaOH (100 mL, 1% w/v) and water (100 mL). The organic phase was dried and the solvent removed to give a brown solid. Column chromatography (hexane/EtOAc gradient) afforded the title compound 250 as a white foam (484 mg, 0.88 mmol, 79%, α anomer with <10% β anomer). The α anomer was formed exclusively in a lower-yielding repeat of the procedure, was purified in an analogous manner to α/β-250 by column chromatography. After this purification, α-250 was crystallised by dissolution in EtOAc followed by addition of hexane (1:5 ratio) to obtain a white powder. Data for α-250: m.p. 144–145 °C; Rᵣ 0.60 (3:2 hexane/EtOAc); IR (νmax/cm⁻¹): 2957 (C–H), 1774 (C=O), 1747 (C=O); MS (ES⁺) found m/z 550 [M+H]⁺, HRMS found 550.0919, C₂₅H₂₅ClNO₉S [M+H]⁺ requires 550.0933; elemental analysis calcd (%) for C₂₅H₂₄ClNO₉S, C 54.60, H 4.40, N 2.55, Cl 6.45, S 5.83, found C 54.63, H 4.66, N 2.61, Cl 6.32; [α]D = –149.1 (c = 1.1, CH₂Cl₂); λmax (EtOH)/nm 246 (ε/dm mol cm⁻¹, 11,150) and 277 (9,249); δH (400 MHz, CDCl₃): 7.63–7.61 (1 H, m, ArH), 7.47–7.35 (6 H, m, ArH), 7.29 (2 H, m, ArH), 6.69 (1 H, s, H-1), 5.28 (1 H, td, J = 1.9, 0.8 Hz, H-4), 5.21 (1 H, d, J = 1.8 Hz, H-5), 5.16 (1 H, dt, J = 2.5, 1.2 Hz, H-3), 4.85 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.79 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.06 (1 H, d, J = 14.7 Hz, CH₂Cl), 4.03–3.99 (2 H, m, H-2, CH₂Cl), 3.74 (3 H, s, CO₂CH₃), 2.12 (3 H, s, C(O)CH₃); δC (101 MHz, CDCl₃): 169.5 (C=O), 167.6 (C=O), 166.4 (C=O), 161.6 (C=N), 152.0 (Ar C), 141.7 (Ar C), 136.2 (Ar C), 128.7 (Ar CH), 128.5 (Ar CH), 127.9 (Ar CH), 124.6 (Ar CH), 124.5 (Ar CH), 119.1 (Ar CH), 110.2 (Ar CH), 82.4 (C-1), 73.3 (CH₂Ar), 70.4 (C-2), 68.8 (CH), 67.9 (CH), 67.8 (C-3), 52.8 (CO₂CH₃), 40.3 (CH₂Cl), 20.9 (C(O)CH₃).

Methyl (benzoxazolyl 2-O-acetyl-3-O-benzyl-1-thio-α-L-idopyranoside) uronate 251

S-Benzoxazolyl thioglycoside 250 (333 mg, 0.61 mmol, α anomer) was dissolved in EtOH (50 mL), thiourea (69 mg, 0.91 mmol) was added and the reaction was stirred at 70 °C for 3 h. A second addition of thiourea (69 mg, 0.91 mmol) was made and the
reaction was stirred at 70 °C for 90 min. The reaction mixture was extracted with CH$_2$Cl$_2$ (200 mL), washed with H$_2$O (100 mL) and the organic phase was dried and concentrated in vacuo to an orange oil. Column chromatography (3:2 hexane/EtOAc) afforded the title compound 251 as a white foam (216 mg, 0.46 mmol, 75%); $R_f$ 0.20 (3:2 hexane/EtOAc); IR ($
$max/cm$^{-1}$): 3476 (O–H), 2951 (C–H), 1736 (C=O); MS (ES$^+$) found m/z 474 [M+H$^+$], HRMS found 474.1210, C$_{23}$H$_{24}$NO$_8$S [M+H$^+$] requires 474.1217; $[\alpha]_D$ = –13.8 (c = 0.20, CH$_2$Cl$_2$); $\lambda_{\text{max}}$(EtOH)/nm 201 (ε/dm mol cm 16,147), 247 (18,291) and 278 (35,328); $\delta$H (400 MHz, CDCl$_3$): 7.49–7.46 (1 H, m, ArH), 7.32–7.30 (1 H, m, ArH), 7.30–7.21 (5 H, m, ArH), 7.19–7.12 (2 H, m, ArH), 6.52 (1 H, s, H-1), 5.15–5.13 (1 H, m, H-3), 4.98 (1 H, d, $J$ = 1.7 Hz, H-5), 4.68 (1 H, d, $J$ = 11.7 Hz, CH$_2$Ar), 4.54 (1 H, d, $J$ = 11.8 Hz, CH$_2$Ar), 4.03–3.99 (1 H, m, H-4), 3.71–3.68 (1 H, m, H-2), 3.64 (3 H, s, CO$_2$CH$_3$), 2.02 (3 H, s, C(O)CH$_3$); $\delta$C (101 MHz, CDCl$_3$): 168.9 (C=O), 168.8 (C=O), 161.9 (C=N), 152.0 (Ar C), 141.7 (Ar C), 136.4 (Ar C), 128.7 (Ar CH), 128.4 (Ar CH), 128.0 (Ar CH), 124.6 (Ar CH), 124.5 (Ar CH), 119.1 (Ar CH), 110.2 (Ar CH), 82.7 (C-1), 72.8 (CH$_2$Ar), 72.5 (C-2), 70.4 (C-5), 68.7 (C-3), 67.9 (C-4), 52.5 (CO$_2$CH$_3$), 21.0 (C(O)CH$_3$).

13.1.15 Synthesis of Disaccharides using Glucoazide SBox/STaz Donors

Phenyl (2-azido-3,6-di-O-benzyl-4-O-chloroacetyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 2-O-benzoyl-3-0-benzyl-1-thio-α-L-idopyranosiduronate 252

S-Benzoazolyl donor 232 (154 mg, 0.26 mmol) and acceptor 165 (109 mg, 0.22 mmol) were combined in anhydrous toluene (~3 mL) and the solvent removed in vacuo. The process was repeated twice. The solid was dried under high vacuum for 3 h, then dissolved in anhydrous CH$_2$Cl$_2$ (5 mL) under N$_2$, and molecular sieves (~200 mg, 4 Å, powdered, activated) were added. The solution was stirred for 1 h at RT. The solution was cooled to ~40 °C. AgOTf (113 mg, 0.44 mmol) was added and the solution was stirred for 2 h warming to RT. TLC (4:1 hexane/EtOAc) showed no remaining donor. The solution was filtered through Celite with CH$_2$Cl$_2$ (100 mL) and the solvent was
removed in vacuo to give a yellow oil. Column chromatography (two columns, first column 3:1 hexane/EtOAc, second column 4:1 hexane/EtOAc) yielded the title compound 252 as a colourless oil (44 mg, 46.7 µmol, 21%) plus recovered 165 from the first column (43 mg, 0.087 mmol, 39%). Data for product 252: Rf 0.28 (4:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2952 (C–H), 2107 (N3), 1764 (C=O); MS (ES⁺) found m/z 961 [M+Na]⁺, HRMS found 955.2991, C49H52ClN4O12S [M+NH4]⁺ requires 955.2985; [α]D = −8.2 (c = 0.60, CH2Cl2); δH (400 MHz, CDCl3): 8.20–8.17 (2 H, m, ArH), 7.55–7.53 (2 H, m, ArH), 7.51–7.47 (2 H, m, ArH), 7.45–7.39 (3 H, m, ArH), 7.39–7.24 (15 H, m, ArH), 7.06–7.04 (1 H, m, ArH), 5.83 (1 H, dd, J = 10.1, 9.4 Hz, H-4′), 5.00 (1 H, d, J = 11.7 Hz, CH2Ar), 4.79 (1 H, d, J = 11.7 Hz, CH2Ar), 4.69 (1 H, d, J = 3.8 Hz, H-1′), 4.47 (1 H, d, J = 11.7 Hz, CH2Ar), 4.40 (1 H, d, J = 11.7 Hz, CH2Ar), 4.25 (1 H, td, J = 2.9, 0.9 Hz, H-3), 4.05 (1 H, t, J = 2.4 Hz, H-4), 3.98 (1 H, d, J = 11.1 Hz, CH2Ar), 3.89–3.79 (2 H, m, H-5′, CH2Ar), 3.73 (3 H, s, CO2CH3), 3.58 (1 H, d, J = 14.5 Hz, CH2Cl), 3.55–3.46 (3 H, m, H-3′, H-6′, CH2Cl), 3.41 (1 H, dd, J = 10.6, 3.0 Hz, H-6′), 3.33 (1 H, dd, J = 10.1, 3.6 Hz, H-2′); δC (101 MHz, CDCl3): 169.2 (C=O), 165.6 (C=O), 137.4 (Ar C), 137.4 (Ar C), 137.0 (Ar C), 135.3 (Ar C), 133.4 (Ar CH), 131.3 (Ar CH), 130.0 (Ar CH), 129.1 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.2 (Ar CH), 127.8 (2 x Ar CH), 127.7 (Ar CH), 99.4 (H-1′), 86.9 (H-1), 78.3 (CH), 76.4 (CH), 74.6 (CH2Ar), 73.7 (CH2Ar), 72.8 (CH2Ar), 72.4 (C-4′), 71.6 (C-3), 69.6 (CH), 69.2 (CH), 68.4 (C-6′), 68.3 (CH), 63.5 (C-2′), 52.4 (CO2CH3), 40.4 (CH2Cl).

Phenyl (2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-α-d-glucopyranosyl)-(1→4)-methyl 2-O-benzoyl-3-O-benzyl-1-thio-α-L-idopyranosiduronate 253

S-Benzoxazolyl donor 233 (78 mg, 0.15 mmol) and acceptor 165 (62 mg, 0.13 mmol) were combined in anhydrous toluene (~3 mL) and the solvent removed in vacuo. The process was repeated twice. The solid was dried under high vacuum for 2 h, then dissolved in anhydrous CH2Cl2 (3 mL) under N2, and molecular sieves (~200 mg, 4 Å, powder, activated) were added. The solution was stirred for 1 h at RT. The solution
was cooled to –60 °C. AgOTf (67 mg, 0.26 mmol) was added and the solution was stirred warming to RT overnight, then at 40 °C for 3 h. TLC (3:1 hexane/EtOAc) showed no remaining donor. The solution was filtered through Celite with CH₂Cl₂ (100 mL) and the solvent was removed in vacuo to give a yellow oil. Column chromatography (4:1 hexane/EtOAc) yielded the title compound 253 as a yellow gum (28 mg, 32.6 µmol, 25%); Rₓ 0.48 (3:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2922 (C–H), 2107 (C=O); MS (ES⁺) found m/z 877 [M+NH₄]⁺, HRMS found 877.3114, C₄⁷H₄₉N₄O₁₁S [M+NH₄]⁺ requires 877.3113; [α]D = –3.9 (c = 0.40, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.16–8.13 (2 H, m, Ar H), 7.60–7.53 (2 H, m, Ar H), 7.50–7.46 (4 H, m, Ar H), 7.43–7.29 (15 H, m, Ar H), 7.14–7.08 (2 H, m, Ar H), 5.82 (1 H, s, H-1), 5.50 (1 H, s, PhCH), 5.44–5.42 (1 H, m, H-2), 5.40 (1 H, d, J = 1.9 Hz, H-5), 5.01 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.79 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.72 (1 H, d, J = 3.6 Hz, H-1’), 4.36 (1 H, dd, J = 10.4, 5.3 Hz, H-6eq’), 4.32 (1 H, d, J = 10.9 Hz, CH₂Ar), 4.24 (1 H, td, J = 2.9, 1.1 Hz, H-3), 4.13–4.10 (1 H, m, H-4), 4.06–3.96 (1 H, m, H-5’), 3.83 (1 H, d, J = 10.7 Hz, CH₂Ar) 3.80 (3 H, s, CO₂CH₃), 3.63 (1 H, t, J = 10.2 Hz, H-6ax’), 3.55–3.52 (2 H, m, H-3’, H-4’), 3.24 (1 H, dd, J = 9.7, 3.7 Hz, H-2’); δC (101 MHz, CDCl₃): 169.2 (C=O), 165.5 (C=O), 137.8 (Ar C), 137.5 (Ar C), 137.1 (Ar C), 135.5 (Ar C), 133.3 (Ar CH), 131.4 (Ar CH), 130.0 (Ar CH), 129.1 (Ar CH), 128.8 (Ar CH), 128.5 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 127.9 (Ar CH), 126.1 (Ar CH), 101.4 (CHPh), 100.4 (C-1’), 87.1 (C-1), 82.3 (CH), 76.8 (CH), 76.7 (CH), 74.7 (CH₂Ar), 72.8 (CH₂Ar), 72.3 (C-3), 69.2 (C-2), 68.5 (C-6), 68.3 (C-5), 63.5 (CH), 63.3 (CH), 52.3 (CO₂CH₃). Data in agreement with previous synthesis¹⁴⁶ (N.B. literature ¹H NMR assigns triplet at 3.63 as H-5’; HMQC and DEPT135 spectra show cross peak to C-6’).

Phenyl (2-azido-3,4,6-tri-O-benzyl-2-deoxy-α-d-glucopyranosyl)-(1→4)-methyl 3-O-benzyl-2-O-benzoyl-1-thio-α/β-L-idopyranosiduronate 254

*From glycosylation of glucosamine SBox donor 234:*
Glycosyl donor α/β-234 (27 mg, 0.044 mmol), glycosyl acceptor α-165 (18 mg, 0.037 mmol) and molecular sieves (~20 mg, 4 Å, powdered, activated) were combined and the flask was purged with argon/vacuum. Anhydrous CH₂Cl₂ (1 mL) was added, the solution was cooled to −50 °C, and the reaction was stirred for 10 min. AgOTf (14 mg, 0.053 mmol) was added and the reaction was stirred for 2 h warming to RT. The reaction mixture was filtered through Celite with CH₂Cl₂ (50 mL) and the solvent removed in vacuo. Column chromatography (80:1 toluene/acetone) afforded the title compound α-254 as a colourless oil (20 mg, 0.021 mmol, 57%). For β-254 data see Section 13.1.27.

Data for α-254: Rf 0.53 (3:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2922 (C–H), 2105 (N=O), 1764 (C=O), 1718 (C=O); MS (ES⁺) found m/z 970 [M+NH₄]⁺, HRMS found m/z 974.3284, C₅₄H₅₃N₃NaO₁₁S [M+Na]⁺ requires 974.3293; [α]D = −34.2 (c = 0.85, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.18–8.16 (2 H, m, ArH), 7.61–7.59 (2 H, m, ArH), 7.54 (2 H, d, J = 7.4 Hz, ArH), 7.46–7.43 (2 H, m, ArH), 7.41–7.25 (18 H, m, ArH), 7.23–7.19 (2 H, m, ArH), 7.20 (2 H, dd, J = 7.5, 1.9 Hz, ArH), 7.16 (2 H, dd, J = 7.5, 1.9 Hz, ArH), 5.83 (1 H, app s, H-1), 5.49 (1 H, m, H-2), 5.43 (1 H, d, J = 2.0 Hz, H-5), 5.04 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.85–4.83 (2 H, m, H-1’, CH₂Ar), 4.70 (1 H, d, J = 10.9 Hz, CH₂Ar), 4.64 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.56–4.51 (2 H, m, 2 x CH₂Ar), 4.29 (1 H, td, J = 2.8, 0.8 Hz, H-3), 4.16–4.15 (2 H, m, H-4, CH₂Ar), 3.98–3.96 (2 H, m, H-5’, CH₂Ar), 3.87 (1 H, dd, J = 10.9, 2.4 Hz, H-6’), 3.81 (3 H, s, CO₂CH₃), 3.76–3.71 (2 H, m, H-4’, H-6’), 3.55 (1 H, app t, J = 9.6 Hz, H-3’), 3.32 (1 H, dd, J = 10.2, 3.5 Hz, H-2’); δC (101 MHz, CDCl₃): 169.2 (C=O), 165.5 (C=O), 138.4 (Ar C), 137.9 (2 x Ar C), 137.2 (Ar C), 135.5 (Ar C), 133.3 (Ar CH), 132.5 (Ar CH), 131.5 (Ar CH), 130.0 (Ar CH0, 2 x Ar C), 132.8 (Ar CH), 128.8 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.3 (2 x Ar CH), 128.1 (2 x Ar CH), 127.8 (2 x Ar CH), 127.7 (2 x Ar CH), 127.6 (Ar CH), 100.6 (C-1’), 87.0 (C-1), 80.2 (C-3’), 77.7 (C-4’), 76.9 (C-4), 74.8 (CH₂Ar), 74.6 (CH₂Ar), 73.6 (CH₂Ar), 72.8 (CH₂Ar), 72.5 (C-3), 71.8 (C-5’), 69.2 (C-2), 68.4 (C-5), 67.8 (C-6’), 63.9 (C-2’), 52.3 (CO₂CH₃);
13.1.16 Preparation of 4-Hydroxyl Disaccharide 220

Phenyl (2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl -2-O-benzoyl-3-O-benzyl-1-thio-α-L-idopyranosiduronate 220

*From attempted glycosylation of 4-OPMB donor 235 with acceptor 165:*

![Chemical structure](image)

STaz donor 235 (126 mg, 0.21 mmol) and acceptor 165 (86 mg, 0.17 mmol) were combined in anhydrous toluene (~3 mL) and the solvent removed *in vacuo*. The process was repeated twice. The solid was dried under high vacuum overnight, then dissolved in anhydrous DCE (3 mL) under N₂, and molecular sieves (~200 mg, 4 Å, powdered, activated) were added. The solution was stirred for 1.5 h at RT, then cooled to –40 °C. AgOTf (87 mg, 0.34 mmol) was added and the solution was stirred warming to RT overnight. TLC (2:1 hexane/EtOAc) showed no remaining donor. The solution was filtered through Celite with CH₂Cl₂ (100 mL) and the solvent was removed *in vacuo* to give a yellow foam. Column chromatography (4:1 hexane/EtOAc) yielded the *title compound* 220 as a colourless gum (32 mg, 37.2 µmol, 21%).

*From 253 by regioselective reductive benzylidene opening:*

![Chemical structure](image)

Disaccharide 253 (37 mg, 43 µmol) was dissolved in anhydrous CH₂Cl₂ (2 mL) under N₂ and the solution was cooled to 0 °C. Et₃SiH (20 µL, 0.13 mmol) was added, followed by BF₃·OEt₂ (16 µL, 0.13 mmol) dropwise. The solution was stirred at 0 °C for 3 h, then partitioned between CH₂Cl₂ (50 mL) and saturated aq. NaHCO₃ (50 mL). The phases were separated and the organic phase was dried and the solvent removed *in vacuo* to give a yellow gum. Column chromatography (3:1 hexane/EtOAc) yielded the *title compound* 220 as a yellow gum (23 mg, 26.7 µmol, 62%).
From 252 by chloroacetyl group removal:

Disaccharide 252 (30 mg, 32 µmol) was dissolved in Et₂O (2 mL). The solution was cooled to 0 °C and benzylamine (10 µL, 0.96 mmol) was added. The solution was stirred at 0 °C for 3 h, benzylamine (100 µL, 0.96 mmol) was added and the solution was stirred at RT for 2 h. The solution was partitioned between EtOAc (50 mL) and HCl (50 mL, 1 M) and the organic phase was separated, dried and the solvent removed in vacuo to give a colourless oil. Column chromatography (3:1 hexane/EtOAc) yielded the title compound 220 as a yellow gum (15 mg, 17.4 µmol, 54%); \( R_f \) 0.40 (2:1 hexane/EtOAc); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)): 3411 (O–H), 2924 (C–H), 2108 (N₃), 1763 (C=O); MS (ES⁺) found m/z 879 [M+NH₄]+, HRMS found 884.2829, \([M+Na]^{+}\) requires 884.2838; [\( \alpha \)]D = -11.1 (c = 0.55, CH₂Cl₂); \( \delta_H \) (400 MHz, CDCl₃): 8.16 (2 H, dd, \( J = 8.4, 1.4 \) Hz, ArH), 7.55–7.53 (2 H, m, ArH), 7.49–7.27 (19 H, m, ArH), 5.80 (1 H, d, \( J = 0.3 \) Hz, H-1), 5.41 (1 H, td, \( J = 2.2, 0.8 \) Hz, H-2), 5.36 (1 H, d, \( J = 2.2 \) Hz, H-5), 4.98 (1 H, d, \( J = 11.6 \) Hz, CH₂Ar), 4.77 (1 H, d, \( J = 11.7 \) Hz, CH₂Ar), 4.71 (1 H, d, \( J = 3.5 \) Hz, H-1'), 4.58 (1 H, d, \( J = 12.0 \) Hz, CH₂Ar), 4.53 (1 H, d, \( J = 12.0 \) Hz, CH₂Ar), 4.24–4.20 (2 H, m, H-3, CH₂Ar), 4.09–4.05 (2 H, m, H-4, CH₂Ar), 3.86–3.81 (1 H, m, H-5), 3.76 (1 H, dd, \( J = 10.1, 3.5 \) Hz, H-6'), 3.74 (3 H, s, CO₂CH₃), 3.65 (1 H, t, \( J = 9.2 \) Hz, H-4'), 3.59 (1 H, dd, \( J = 10.1, 4.7 \) Hz, H-6'), 3.41 (1 H, dd, \( J = 10.1, 8.8 \) Hz, H-3'), 3.20 (1 H, dd, \( J = 10.2, 3.5 \) Hz, H-2'); \( \delta_C \) (101 MHz, CDCl₃): 169.2 (C=O), 165.6 (C=O), 138.0 (Ar C), 137.6 (Ar C), 137.1 (Ar C), 135.3 (Ar C), 133.4 (Ar CH), 131.5 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.1 (Ar CH), 127.9 (Ar CH), 99.7 (C-1'), 86.8 (C-1), 79.9 (C-3'), 76.0 (C-4), 74.72 (CH₂Ar), 73.8 (CH₂Ar), 72.8 (CH₂Ar), 72.5 (C-4'), 72.2 (C-3), 70.6 (C-5'), 69.6 (C-6'), 69.4 (C-2), 68.6 (C-5), 63.2 (C-2'), 53.8 (CO₂CH₃). Data in agreement with reported synthesis.²⁴⁶
13.1.17 Synthesis of Glucoazide Donor 258

Phenyl 2-Azido-3-β-O-benzoyl-2-deoxy-1-thio-β-D-glucopyranoside 164

Prepared according to procedure reported by Hansen et al. Glucosamine derivative 173 (5.46 g, 11.4 mmol) was dissolved in 60% aq. acetic acid (24 mL) and heated to 120 °C to dissolve. The solution was stirred at 120 °C for 3 h. The mixture was cooled to RT and poured onto aq. NaHCO₃ (100 mL, 40% w/v), then extracted with EtOAc (3 x 100 mL). The organic phase was dried and the solvent removed in vacuo to give a yellow oil. The solid was purified by silica plug (3:1, 2:1, 0:1 hexane/EtOAc) to afford the title compound 164 as a yellow oil (3.30 g, 8.52 mmol, 75%); R_f 0.41 (2:1 hexane/EtOAc); [α]D = –18.1 (c = 0.45, CH2Cl2); IR (ν_max/cm⁻¹): 3444 (O–H), 2879 (C–H), 2107 (N₃); MS (ES⁺) found m/z 405 [M+NH₄⁺], HRMS found 410.1156, C₁₉H₂₁N₃NaO₄S [M+Na]⁺ requires 410.1151; δH (400 MHz, CDCl₃): 7.55–7.52 (2 H, m, ArH), 7.37–7.28 (8 H, m, ArH), 4.88 (1 H, d, J = 11.1 Hz, CH₂Ar), 4.82 (1 H, d, J = 11.1 Hz, CH₂Ar), 4.46 (1 H, d, J = 9.9 Hz, H-1), 3.86 (1 H, dd, J = 12.0, 3.2 Hz, H-6), 3.76 (1 H, dd, J = 11.9, 4.8 Hz, H-6), 3.57 (1 H, t, J = 9.1 Hz, H-5), 3.38–3.27 (3 H, m, H-2, H-3, H-4), 2.81 (1 H, br s, O-H); δC (400 MHz, CDCl₃): 137.9 (Ar C), 133.2 (Ar CH), 131.5 (Ar CH), 129.2 (Ar CH), 128.6 (Ar CH), 128.4 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 86.3 (C-1), 84.7 (CH), 79.6 (CH), 75.5 (CH₂Ar), 70.4 (C-5), 64.9 (C-2), 62.3 (C-6). Data in agreement with previous synthesis (N.B. data reported by Tatai and Fügedi, synthesis reported by Mizuno et al. but data not reported).

Phenyl 2-azido-2-deoxy-6-O-benzoyl-3-O-benzyl-1-thio-β-D-gluco-pyranoside 257

Prepared according to procedure reported by Hansen et al. Glucosamine derivative 164 (1.44 g, 3.72 mmol) was dissolved in anhydrous CH₂Cl₂ (15 mL) under N₂. Triethylamine (570 µL, 4.09 mmol) was added and the solution was cooled to 0 °C. BzCl (430 µL, 3.72 mmol) was added and the mixture was stirred warming to RT over 30 min. The mixture was poured onto aq. HCl (25 mL, 1% v/v), the phases were
separated and the organic phase was washed with sat aq. NaHCO₃ (25 mL) and brine (25 mL). The organic phase was dried and the solvent removed in vacuo to yield a yellow solid. Column chromatography (3:1 hexane/EtOAc) afforded the title compound 257 as a white powder (1.30 g, 2.65 mmol, 71%); m.p. 149–151 °C (Lit. 318 152–155 °C); Rf 0.73 (2:1 hexane/EtOAc); IR (νmax/cm⁻¹): 3450 (O–H), 2905 (C–H), 2111 (N₃), 1697 (C=O); MS (ES⁺) found m/z 509 [M+NH₄⁺]; HRMS found 509.1847, C₂₆H₂₉N₄O₅S [M+NH₄⁺] requires 509.1853; [α]D = –50.0 (c = 0.07, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.09–8.07 (2 H, m, ArH), 7.64–7.61 (3 H, m, ArH), 7.50 (2 H, dd, J = 8.0, 7.5 Hz, ArH), 7.41–7.28 (6 H, m, ArH), 7.20 (2 H, dd, J = 8.0, 6.9 Hz, ArH), 4.95 (1 H, d, J = 10.9 Hz, CH₂Ar), 4.86 (1 H, d, J = 11.0 Hz, CH₂Ar), 4.71 (1 H, dd, J = 12.6, 3.4 Hz, H-6), 4.63 (1 H, dd, J = 12.1, 2.0 Hz, H-6), 4.50 (1 H, d, J = 10.0 Hz, H-1), 3.58 (1 H, dd, J = 3.8, 2.0 Hz, H-5), 3.54 (1 H, t, J = 9.0 Hz, H-4), 3.43 (1 H, t, J = 8.8 Hz, H-3), 3.33 (1 H, dd, J = 9.9, 9.3 Hz, H-2); δC (101 MHz, CDCl₃): 167.0 (C=O), 137.7 (Ar C), 133.9 (Ar C), 133.5 (Ar CH), 129.9 (Ar CH), 129.0 (Ar CH), 128.8 (Ar CH), 128.5 (Ar CH), 128.3 (2 x Ar CH), 85.9 (C-1), 84.3 (C-3), 78.0 (C-5), 75.8 (CH₂Ar), 69.8 (C-4), 64.5 (C-2), 63.3 (C-6). Data in agreement with previous synthesis. 318

Phenyl 2-azido-4-O-chloroacetyl-3-O-benzyl-2-deoxy-1-thio-β-D-glucopyranoside 258

Glucosamine derivative 257 (986 mg, 2.00 mmol) was dissolved in anhydrous CH₂Cl₂ (15 mL) under N₂. Pyridine (400 µL) and chloroacetyl chloride (238 µL, 3.00 mmol) were added and the solution was stirred at RT for 1.5 h. The solution was diluted with CH₂Cl₂ (100 mL) and washed with aq. HCl (50 mL, 1% v/v) and saturated aq. NaHCO₃ (50 mL). The organic phase was dried and the solvent was removed in vacuo to give a brown oil. Column chromatography (3:1 hexane/EtOAc) afforded the title compound 258 as a yellow foam (1.00 g, 0.17 mmol, 88%); Rf 0.48 (4:1 hexane/EtOAc); IR (νmax/cm⁻¹): 2880 (C–H), 2221 (N₃), 1771 (C=O), 1718 (C=O); MS (ES⁺) found m/z 585 [M+NH₄⁺], HRMS found 590.1117, C₂₈H₂₆ClN₃NaO₅S [M+Na⁺] requires 590.1129; [α]D = –39.5 (c = 0.85, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.05–8.02 (2 H, m, ArH), 7.63–7.58 (1 H, m, ArH), 7.56–7.53 (2 H, m, ArH), 7.49–7.49 (2 H, m, ArH), 7.49–7.49 (2 H, m, ArH), 7.49–7.49 (2 H, m, ArH).
To a stirred solution of glycosyl acetate α/β-264 (3.50 g, 9.40 mmol, ~2:1 α/β) and HSBox (2.84 g, 18.80 mmol) in anhydrous CH₂Cl₂ (50 mL) under argon was added molecular sieves (~4 g, 4 Å, beads, activated) and the reaction stirred for 10 min at 0 °C. TMSOTf (3.40 mL, 18.80 mmol) was added dropwise and the reaction stirred warming to RT overnight. The reaction mixture was filtered with CH₂Cl₂ (50 mL), washed with aq. NaOH (3 x 100 mL, 1% w/v) and H₂O (3 x 100 mL). The organic phase was dried and solvent removed in vacuo to give a brown foam. Dry column vacuum chromatography (1:0, 9:1, 8:1 petroleum ether/EtOAc gradient) afforded the title compound 265 as a yellow foam (2.93 g, 6.31 mmol, 67%, ~1:1 α/β); Rf 0.47 (5:1 toluene/acetone); IR (νmax/cm⁻¹): 2942 (C–H), 2111 (N=O); MS (ES⁺) found m/z 465 [M+H]+, HRMS found 465.1079, C₁₉H₂₁NaO₈S [M+H]+ requires 465.1075; δH (400 MHz, CDCl₃): 7.84–7.64 (2 H, m, ArH), 7.52–7.46 (2 H, m, ArH), 7.36–7.27 (4 H, m, ArH), 6.58 (1 H, d, J = 5.5 Hz, H-1α), 5.42 (1 H, d, J = 10.5 Hz, H-1β), 5.30–5.21 (2 H, m, H-3α, H-3β), 5.14–5.05 (2 H, m, H-4α, H-4β), 4.40–4.35 (1 H, m, H-5α), 4.31–4.21 (3 H, m, H-2α, H-6α, H-6β), 4.14–4.03 (2 H, m, H-6β), 4.00–3.88 (3 H, m, H-2β, H-5β, H-6u), 2.20–1.99 (18 H, m, 6 x C(O)CH₃); δC (101 MHz, CDCl₃): 170.6 (2 x C=O), 169.9 (2 x C=O), 169.8 (2 x C=O), 160.1 (C=N), 152.1 (C=N), 141.6 (Ar C), 125.0 (Ar CH), 124.8 (Ar CH), 119.4 (Ar CH), 119.2 (Ar CH), 110.4 (Ar CH), 85.0 (C-
1α), 83.7 (C-1β), 76.6 (CH), 74.7 (CH), 72.3 (CH), 70.5 (C-5α), 68.1 (CH), 61.6 (C-6), 61.2 (C-6), 61.0 (CH).

Benzoxazolyl 2-Azido-2-deoxy-1-thio-α/β-D-glucuronate α/β-266

Acetylated glucoazide derivative 265 (1.58 g, 3.49 mmol, ~1:1 α/β) was dissolved in methanol (15 mL) and K₂CO₃ (2.35 g, 17.00 mmol) was added in water (3 mL). The reaction was stirred at RT for 5 min, then filtered and the methanol removed in vacuo. The solution was acidified to pH ~4 with 1 M HCl, then extracted with EtOAc (2 x 50 mL). The organic phase was washed with saturated aq. NaHCO₃ (100 mL) and saturated aq. NaCl (100 mL), dried and the solvents removed to give a foam. Purification by silica plug (1:0, 99:1, 95:5 CH₂Cl₂/MeOH) afforded the title compound 266 as a yellow foam (533 mg, 1.58 mmol, 45%, ~3:1 α/β); R_f 0.60 (9:1 CH₂Cl₂/MeOH); IR (ν_max/cm⁻¹): 3336 (O–H), 2917 (C–H), 2110 (N₃); MS (ES⁺) found m/z 361 [M+Na]⁺, HRMS found 361.0578, C₁₃H₁₄N₄O₅S [M+Na]⁺ requires 361.0577; δ_H (400 MHz, CD₃OD): 7.63–7.59 (1.35 H, m, ArH), 7.58–7.53 (1.34 H, m, ArH), 7.38–7.32 (2.74 H, m, ArH), 6.55 (1 H, d, J = 5.2 Hz, H-1α), 5.38 (0.35 H, d, J = 10.1 Hz, H-1β), 3.99 (1 H, dd, J = 10.2, 5.3 Hz, H-2α), 3.90–3.84 (1.36 H, m, H-5α, H-6β), 3.78–3.72 (2.25 H, m, 2 x H-6α, H-6β), 3.67–3.47 (3.55 H, m, H-2β, H-3α, H-3β, H-4α, H-4β, H-5β); δ_C (101 MHz, CD₃OD): 163.6 (C=N), 163.1 (C=N), 153.2 (Ar C), 153.1 (Ar C), 142.6 (Ar C), 126.0 (Ar CH), 126.0 (Ar CH), 125.9 (Ar CH), 119.7 (Ar CH), 119.6 (Ar CH), 111.3 (Ar CH), 111.3 (Ar CH), 87.7 (C-1α), 85.3 (C-1β), 82.9 (CH), 78.7 (CH), 77.2 (CH), 75.4 (CH), 71.1 (CH), 70.9 (CH), 66.9 (CH), 64.8 (C-2α), 62.4 (C-6β), 61.9 (C-6α).

Benzoxazolyl 2-Azido-4,6-O-benzylidene-2-deoxy-1-thio-α/β-D-glucuronate α/β-267

To a stirred solution of triol 266 (300 mg, 0.89 mmol, ~3:1 α/β) in anhydrous acetonitrile (10 mL) was added molecular sieves (~300 mg, 4 Å, powdered, activated), benzaldehyde dimethyl acetal (160 µL, 1.06 mmol) and p-toluenesulfonic acid (15 mg,
The reaction mixture was stirred at RT for 2 d, then filtered through Celite with CH₂Cl₂ (100 mL) and the solvents removed in vacuo to give a brown oil. Column chromatography (1:0, 99:1, 98:2 CH₂Cl₂/MeOH) yielded the title compound [267] as an orange foam (171 mg, 0.40 mmol, 45%), ~2:1 α/β; Rf 0.39 (2:1 hexane/EtOAc); IR (νmax/cm⁻¹): 3326 (O–H), 2872 (C–H), 2110 (N₃); MS (ES⁺) found m/z 427 [M+H]⁺, HRMS found 427.1068, C₂₀H₁₉N₄O₅S [M+H]⁺ requires 427.1071; δH (400 MHz, CDCl₃): 7.72–7.68 (1.49 H, m, ArH), 7.43–7.47 (5.36 H, m, ArH), 7.45–7.40 (4.77 H, m, ArH), 7.37–7.28 (3.04 H, m, ArH), 6.57 (1 H, d, J = 5.4 Hz, H-1α), 5.57–5.55 (1.47 H, m, 2 x CHPh), 5.42 (0.48 H, d, J = 10.5 Hz, H-1β), 4.37 (0.46 H, dd, J = 10.4, 4.2 Hz, H-6β), 4.25 (1 H, dd, J = 10.3, 5.0 Hz, H-6α), 4.16 (1 H, td, J = 9.7, 4.9 Hz, H-5α), 4.04 (1 H, dd, J = 9.9, 5.5 Hz, H-2α), 3.98–3.89 (1.41 H, m, H-3α, H-3β), 3.81–3.72 (2.26 H, m, H-2β, H-6α, H-6β), 3.67–3.61 (2.49 H, m, H-4α, H-4β, H-5β); δC (101 MHz, CDCl₃): 161.3 (C=N), 160.5 (C=N), 152.0 (Ar C), 151.9 (Ar C), 141.5 (Ar C), 141.4 (Ar C), 136.7 (Ar C), 136.6 (Ar C), 129.6 (Ar CH), 129.2 (Ar CH), 129.1 (Ar CH), 128.5 (Ar CH), 128.3 (Ar CH), 126.9 (Ar CH), 126.4 (Ar CH), 124.8 (Ar CH), 124.7 (Ar CH), 124.6 (2 x Ar CH), 119.1 (Ar CH), 110.2 (Ar CH), 102.2 (CHPh), 102.1 (H₂C), 86.1 (C-1α), 84.5 (C-1β), 81.0 (CH), 80.2 (CH), 74.3 (CH), 73.5 (CH), 71.2 (CH), 70.8 (CH), 68.3 (2 x C-6), 65.6 (C-5α), 65.4 (CH), 63.3 (C-2α).

13.1.19 Synthesis of L-Ido Lactone
Phenyl (methyl 3-O-benzyl-1-thio-α,β-L-idopyranoside) uronate α/β-[269]

To a stirred solution of cyanohydrin [239] (60.0 g, 0.20 mol) in MeOH (250 mL) at 0 °C was added AcCl (30.0 mL, 0.62 mmol) dropwise. The reaction was stirred at 60 °C for 12 h, then cooled to at 0 °C. AcCl (10.0 mL, 0.14 mol) was added and the reaction stirred at 60 °C for 24 h. The solvents were removed in vacuo and the residue partitioned between EtOAc (350 mL) and water (300 mL) and the phases mixed vigorously for 5 min. The phases were separated and the organic phase was washed with saturated aq. NaHCO₃ (400 mL) and saturated aq. NaCl (400 mL), dried and concentrated to give pyranoside/furanoside mixture [243/268] as a brown syrup, which was used without further purification. The mixture was dissolved in anhydrous CH₂Cl₂.
(1.0 L) under \(N_2\). Molecular sieves (~65 g, 4 Å, powdered, activated), PhSH (23 mL, 0.23 mol) and \(BF_3\cdot\text{OEt}_2\) (74 mL, 0.60 mol) were added and the reaction was stirred at RT for 2 h. The reaction mixture was filtered into a solution of \(\text{NaHCO}_3\) (200 g, 2.38 mol) in \(\text{H}_2\text{O}\) (1.2 L) to quench (heavy foaming observed). Iodine was added until the dark red colour persisted and the solution was stirred for 10 min. Saturated aq. \(\text{Na}_2\text{S}_2\text{O}_3\) was added and the solution stirred for 30 min until the dark red colour was removed. The mixture was filtered through Celite, the phases were separated, and the aqueous phase extracted with \(\text{EtOAc}\) (3 x 500 mL). The combined organic phase was dried and the solvent removed. Dry column vacuum chromatography (1:0, 4:1 petroleum ether/EtOAc) afforded the \textit{title compound 269} as a yellow foam (18.40 g, 0.047 mol, 24% over two steps, \(\sim 1:1 \alpha/\beta\)).

Data for the individual anomers was obtained by removal of the TBDMS group of \(\alpha\)-295 and \(\beta\)-295. Example procedure: TBDMS protected iduronate \(\beta\)-295 (114 mg, 0.23 mmol) was dissolved in dry THF (2 mL) and the solution cooled to 0 °C. TBAF (250 µL, 1.0 M in THF) was added dropwise and the reaction mixture stirred at 0 °C for 30 min. The solvents were removed \textit{in vacuo} and the crude product purified by column chromatography (1:1 hexane/EtOAc) to afford \(\beta\)-269 as a yellow oil (61 mg, 0.16 mmol, 70%).

Data for \(\beta\)-269: \(R_f\) 0.38 (1:1 hexane/EtOAc); IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)) 3436 (O–H), 2950 (C–H), 1740 (C=O); MS (ES\(^+\)) found \(m/z\) 408 [M+NH\(_4\)]\(^+\), HRMS found 408.1472, \(C_{20}H_{26}NO_6S\) [M+NH\(_4\)]\(^+\) requires 408.1475; \([\alpha]_D = +37.9\) (c = 0.10, \(\text{CH}_2\text{Cl}_2\)); \(\delta_H\) (400 MHz, CDCl\(_3\)):

- 7.56–7.54 (2 H, m, Ar H),
- 7.34–7.26 (8 H, m, Ar H),
- 5.20 (1 H, d, \(J = 1.1\) Hz, H-1),
- 4.63 (1 H, d, \(J = 11.2\) Hz, \(\text{CH}_2\text{Ar}\)),
- 4.59 (1 H, d, \(J = 12.1\) Hz, \(\text{CH}_2\text{Ar}\)),
- 4.53 (1 H, d, \(J = 1.3\) Hz, H-5),
- 4.15 (1 H, dt, \(J = 3.0, 1.4\) Hz, H-4),
- 4.01–4.00 (1 H, m, H-2),
- 3.95 (1 H, t, \(J = 3.2\) Hz, H-3),
- 3.82 (3 H, s, \(\text{CO}_2\text{CH}_3\));
- \(\delta_C\) (101 MHz, CDCl\(_3\)):
  - 169.8 (C=O),
  - 137.4 (Ar C),
  - 134.3 (Ar C),
  - 131.6 (Ar CH),
  - 129.2 (Ar CH),
  - 128.8 (Ar CH),
  - 128.3 (Ar CH),
  - 127.9 (Ar CH),
  - 127.7 (Ar CH),
  - 87.2 (C-1),
  - 76.2 (C-5),
  - 75.5 (C-3),
  - 72.7 (\(\text{CH}_2\text{Ar}\)),
  - 69.7 (C-2),
  - 67.6 (C-4),
  - 52.8 (\(\text{CO}_2\text{CH}_3\)).

Collected data reported in ref. 354.

Data for \(\alpha\)-269: \(R_f\) 0.44 (1:1 hexane/EtOAc); IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)) 3423 (O–H), 2950 (C–H), 1757 (C=O); MS (ES\(^+\)) found \(m/z\) 408 [M+NH\(_4\)]\(^+\), HRMS found 408.1472, \(C_{20}H_{26}NO_6S\) [M+NH\(_4\)]\(^+\) requires 408.1475; \([\alpha]_D = -226.8\) (c = 0.55, \(\text{CH}_2\text{Cl}_2\)); \(\delta_H\) (400 MHz, CDCl\(_3\)):
Chapter Three: Experimental

7.54–7.52 (2 H, m, ArH), 7.45–7.25 (8 H, m, ArH), 5.59 (1 H, s, H-1), 5.25 (1 H, d, \(J = 1.5\) Hz, H-5), 4.79 (1 H, d, \(J = 11.9\) Hz, \(CH_2Ar\)), 4.57 (1 H, d, \(J = 11.9\) Hz, \(CH_2Ar\)), 4.20–4.17 (2 H, m, H-2, H-4), 3.84 (1 H, td, \(J = 3.3, 0.9\) Hz, H-3), 3.82 (3 H, s, \(CO_2CH_3\)); \(\delta C\) (101 MHz, CDCl\(_3\)): 170.7 (C=O), 137.4 (Ar C), 136.3 (Ar C), 130.9 (Ar CH), 129.0 (Ar CH), 128.6 (Ar CH), 128.0 (Ar CH), 127.7 (Ar CH), 127.3 (Ar CH), 89.4 (C-1), 74.4 (C-3), 72.4 (\(CH_2Ar\)), 68.9 (CH), 68.8 (CH), 68.5 (CH), 52.6 (\(CO_2CH_3\)).

Data collected matched those previously reported (N.B. titled as \(\beta\)).

Phenyl (3-\(O\)-benzyl-1-thio-\(\alpha\-)L-idopyranoside) urono-2,6-lactone \(\mathbf{a-270}\) and phenyl (3-\(O\)-benzyl-1-thio-\(\beta\-)L-idopyranoside) urono-2,6-lactone \(\mathbf{\beta-270}\)

Prepared according to the procedure reported by Hansen \textit{et al}.\(^{354}\) To a stirred solution of diol \(\alpha/\beta-269\) (30.0 g, 76.92 mmol, \(\sim 2:3\) \(\alpha/\beta\)) in THF (360 mL) at 0 °C was added LiOH monohydrate (3.60 g, 0.086 mol) in H\(_2\)O (90 mL). The reaction was stirred overnight warming to RT. Aqueous HCl (200 mL, 0.5 M) was added and the product was extracted with EtOAc (2 x 200 mL). The organic phase was dried and the solvents were removed \textit{in vacuo} to give the desired intermediate as a yellow foam (28.90 g, 76.78 mmol). A portion of this intermediate (3.90 g, 10.00 mmol) was dissolved in dry CH\(_2\)Cl\(_2\) (100 mL) under N\(_2\) at 0 °C. 1-Methyl-imidazole (1.59 mL, 20.00 mmol) and TsCl (2.05 g, 10.75 mmol) were added. The reaction was stirred at 0 °C for 2.5 h, then extracted with CH\(_2\)Cl\(_2\) (200 mL) and H\(_2\)O (2 x 200 mL). The organic phase was dried and the solvents were removed \textit{in vacuo}. Column chromatography (40:1 toluene/acetone) gave the \textit{title compounds} \(\mathbf{a-270}\) (1.39 g, 3.86 mmol, 37% over two steps) and \(\mathbf{\beta-270}\) (0.71 g, 1.98 mmol, 19%) as yellow solids (anomers partially separable).

Data for \(\mathbf{a-270}\): R\(_f\) 0.25 (3:1 hexane/EtOAc); IR (\(\nu_{max}/\text{cm}^{-1}\)): 3345 (O–H), 2859 (C–H), 1775 (C=O); MS (ES\(^+\)) found \(m/z\) 381 [M+Na]\(^+\), HRMS found 381.0768, C\(_{19}H_{18}NaO_5S\) [M+Na]\(^+\) requires 381.0769; [\(\alpha\)]\(_D\) = \(-236.7\) (c = 0.55, CH\(_2\)Cl\(_2\)); \(\delta H\) (400 MHz, CDCl\(_3\)): 7.51–7.29 (10 H, m, ArH), 5.51 (1 H, dd, \(J = 2.0, 1.6\) Hz, H-1), 4.97 (1 H, ddd, \(J = 3.6, 2.0, 0.4\) Hz, H-2), 4.86 (1 H, d, \(J = 12.0\) Hz, \(CH_2Ar\)), 4.72 (1 H, d, \(J = 12.0\) Hz, \(CH_2Ar\)), 4.20–4.17 (2 H, m, H-2, H-4), 3.84 (1 H, td, \(J = 3.3, 0.9\) Hz, H-3), 3.82 (3 H, s, \(CO_2CH_3\)).
4.49 (1 H, t, J = 3.6 Hz, H-4), 4.30 (1 H, d, J = 3.6 Hz, H-5), 3.88 (1 H, td, J = 3.6, 1.6 Hz, H-3); δC (101 MHz, CDCl₃): 168.4 (C=O), 136.8 (Ar C), 135.6 (Ar C), 131.2 (Ar CH), 129.4 (Ar CH), 128.7 (Ar CH), 128.4 (Ar CH), 128.1 (Ar CH), 127.9 (Ar CH), 83.1 (C-1), 80.5 (C-3), 74.7 (C-2), 72.5 (CH₂Ar), 71.9 (C-5), 71.2 (C-4). Data in agreement with previous synthesis.354

Data for β-270: Rf 0.19 (3:1 hexane/EtOAc); IR (νmax/cm⁻¹): 3326 (O–H), 2923 (C–H), 1785 (C=O); MS (ES⁺) found m/z 381 [M+Na]+, HRMS found 381.0768, C₁₉H₁₈NaO₅S [M+Na]+ requires 381.0769; [α]D = +149.8 (c = 0.56, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.52–7.50 (2 H, m, ArH), 7.38–7.30 (8 H, m, ArH), 5.70 (1 H, d, J = 0.8 Hz, H-1), 4.86 (1 H, dt, J = 4.4, 0.8 Hz, H-2), 4.70 (1 H, J = 12.0 Hz, CH₂Ar), 4.63 (1 H, J = 12.0 Hz, CH₂Ar), 4.35 (1 H, d, J = 4.4 Hz, H-5), 4.20–4.18 (1 H, m, H-4), 3.87 (1 H, ddd, J = 4.4, 2.0, 0.8 Hz, H-3); δC (101 MHz, CDCl₃): 168.7 (C=O), 136.7 (Ar C), 132.7 (Ar C), 132.5 (Ar CH), 129.3 (Ar CH), 128.8 (Ar CH), 128.5 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 80.9 (C-1), 79.1 (C-3), 77.0 (C-2), 72.5 (CH₂Ar), 71.8 (C-5), 71.5 (C-4). Data in agreement with previous synthesis.354

13.1.20 Synthesis of 4-OPMB Lactone-containing Disaccharide 271
Phenyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-α-D-glucopyranosyl)-(1→4)-3-O-benzyl-1-thio-α-L-idopyranosidurono-2,6-lactone β-271

Acceptor β-270 (314 mg, 0.87 mmol) and donor 162 (569 mg, 0.90 mmol) were dissolved in dry CH₂Cl₂ (15 mL) under argon. Molecular sieves (~600 mg, 4 Å, beads, activated) were added and the mixture was cooled to −10 °C and stirred 10 min. NIS (243 mg, 1.08 mmol) was added and the reaction was stirred for 10 min at −10 °C. TMSOTf (33 µL, 0.18 mmol) was added and the reaction was stirred for 10 min. TLC (2:1 cyclohexane/EtOAc) showed no remaining glycosyl donor. The solvents were removed in vacuo to give a brown oil. Column chromatography (10:1 hexane/EtOAc + 20% CH₂Cl₂) afforded the title compound β-271 as a white foam (283 mg, 0.33 mmol, 37%) along with the corresponding β-linked disaccharide β-272 as a colourless oil (35 mg, 0.041 mmol, 5%).
Data for **β-271**: Rf 0.15 (4:1 hexane/EtOAc); IR (ν_{max}/cm⁻¹): 2912 (C-H), 2105 (N=), 1785 (C=O); MS (ES⁺) found m/z 868 [M+Na]⁺, HRMS found 868.2836, C₄ᵣH₅N₃NaO₁₀S [M+Na]⁺ requires 868.2875; [α]D = +132.5 (c = 0.28, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.55–7.52 (2 H, m, ArH), 7.43–7.30 (18 H, m, ArH), 7.09 (2 H, d, J = 8.6 Hz, ArH), 6.84 (2 H, m, ArH), 5.73 (1 H, d, J = 0.8 Hz, H-1), 5.02 (1 H, d, J = 3.8 Hz, H-1'), 4.94 (1 H, J = 10.7 Hz, CH₂Ar), 4.88–4.85 (2 H, m, H-2, CH₂Ar), 4.76 (1 H, d, J = 11.8 Hz, CH₂Ar), 4.74–4.66 (3 H, m, 3 x CH₂Ar), 4.64 (1 H, J = 3.9 Hz, H-5), 4.52–4.47 (2 H, m, 2 x CH₂Ar), 4.08 (1 H, dd, J = 3.7, 2.5 Hz, H-4), 4.04 (1 H, dd, J = 4.0, 2.4 Hz, H-3), 3.94–3.74 (5 H, m, H-3', H-4', H-5', 2 x H-6'), 3.82, (3 H, s, ArOC(H)₃), 3.34 (1 H, J = 10.3, 3.7 Hz, H-2'); δC (101 MHz, CDCl₃): 166.9 (C=O), 159.3 (Ar C), 137.8 (Ar C), 137.8 (Ar C), 136.4 (Ar C), 132.6 (Ar C), 132.5 (Ar CH), 130.0 (Ar C), 129.4 (Ar CH), 129.2 (Ar CH), 128.7 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.1 (Ar CH), 128.0 (2 x Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 113.8 (Ar CH)), 100.3 (C-1'), 81.0 (C-1, C-4), 79.7 (CH), 77.8 (C-3), 77.6 (CH), 76.5 (C-2), 75.5 (CH₂Ar), 74.6 (CH₂Ar), 73.6 (CH₂Ar), 72.4 (CH₂Ar), 71.9 (CH), 70.3 (C-5), 68.0 (C-6'), 63.2 (C-2'), 55.3 (ArOCH₃).

Data for **β-272**: Rf 0.31 (3:1 cyclohexane/EtOAc); IR (ν_{max}/cm⁻¹): 2904 (C-H), 2105 (N=), 1787 (C=O); MS (ES⁺) found m/z 863 [M+Na]⁺, HRMS found 863.3322, C₄ᵣH₅N₄O₁₀S [M+NH₄]⁺ requires 863.3320; [α]D = -0.5 (c = 0.38, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.51–7.49 (2 H, m, ArH), 7.38–7.24 (23 H, m, ArH), 7.08 (2 H, d, J = 8.7 Hz, ArH), 6.82 (2 H, d, J = 8.7 Hz, ArH), 5.68 (1 H, d, J = 0.8 Hz, H-1), 4.88 (1 H, d, J = 10.8 Hz, CH₂Ar), 4.83–4.79 (2 H, m, H-2, CH₂Ar), 4.75–4.72 (2 H, m, 2 x CH₂Ar), 4.56 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.49–4.41 (4 H, m, H-5, 3 x CH₂Ar), 4.40–4.36 (2 H, m, H-4, H-1'), 4.06 (1 H, dd, J = 4.1 Hz, 2.0 Hz, H-3), 3.79 (3 H, s, ArOC(H)₃), 3.65–3.60 (3 H, m, H-4', 2 x H-6'), 3.43–3.37 (3 H, m, H-2', H-3', H-5'); δC (101 MHz, CDCl₃): 166.2 (C=O), 159.4 (Ar C), 137.8 (Ar C), 137.7 (Ar C), 132.5 (Ar CH), 129.7 (Ar CH), 129.2 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.1 (Ar CH), 128.0 (2 x Ar CH), 127.8 (Ar CH), 113.9 (AR CH), 101.1 (C-1'), 82.9 (CH), 81.2 (C-1), 77.4 (C-3), 77.2 (C-4), 76.9 (CH), 76.6 (C-2), 75.7 (CH₂Ar), 75.3 (C-5), 74.8 (CH₂Ar), 73.5 (CH₂Ar), 72.1 (CH₂Ar), 69.3 (CH), 68.5 (C-6'), 66.2 (C-2'), 55.3 (ArOCH₃). N.B. Formation previously reported, data not previously reported.³⁵⁴
13.1.21 Synthesis of Glucoazide Donors 281, 284 and 286

Phenyl 2-Azido-6-O-benzoyl-3-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-1-thio-β-D-glucopyranoside 281

Glucosamine derivative 161 (12.34 g, 24.31 mmol) was dissolved in anhydrous CH₂Cl₂ (70 mL). BzCl (3.10 mL, 35.90 mmol), Et₃N (3.72 mL, 26.69 mmol) and DMAP (297 mg, 2.43 mmol) were added and the reaction was stirred at RT for 2 h. H₂O (20 mL) was added and the solution stirred for 30 min. The phases were separated and the organic phase washed with aq. HCl (50 mL, 0.1 M) and saturated aq. NaHCO₃ (50 mL), then dried and concentrated in vacuo to reveal the title compound 281 as a yellow oil (14.50 g, 24.26 mmol, >99%); Rf 0.43 (4:1 cyclohexane/EtOAc); IR (ν max/cm⁻¹): 2958 (C–H), 2107 (N₃), 1717 (C=O); MS (ES⁺) found m/z 634 [M+Na]⁺, HRMS found 650.1705, C₃₄H₃₃K₃O₆S [M+K]⁺ requires 650.1727; [α]D = –1.1 (c = 1.60, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.05 (2 H, dt, J = 8.1, 1.5 Hz, ArH), 7.68–7.62 (1 H, m, ArH), 7.62–7.59 (2 H, m, ArH), 7.53–7.49 (2 H, m, ArH), 7.46–7.36 (5 H, m, ArH), 7.32–7.28 (1 H, m, ArH), 7.26–7.22 (2 H, m, ArH), 7.20–7.16 (2 H, m, ArH), 6.85–6.83 (2 H, m, ArH), 4.96 (1 H, d, J = 10.4 Hz, CH₂Ar), 4.93 (1 H, d, J = 10.4 Hz, CH₂Ar), 4.83 (1 H, d, J = 10.6 Hz, CH₂Ar), 4.72 (1 H, dd, J = 12.0, 1.8, H-6), 4.59 (1 H, d, J = 10.6 Hz, CH₂Ar), 4.49–4.45 (2 H, m, H-1, H-6), 3.76 (3 H, s, ArOCH₃), 3.67–3.60 (3 H, m, H-3, H-4, H-5), 3.39 (1 H, t, J = 9.6 Hz, H-2); δC (101 MHz, CDCl₃): 166.0 (C=O), 159.6 (C=O), 137.5 (Ar C), 134.2 (Ar CH), 133.3 (Ar CH), 130.5 (Ar C), 129.9 (Ar CH), 129.8 (Ar CH), 129.4 (Ar C), 129.0 (Ar CH), 128.7 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 114.0 (Ar CH), 85.6 (C-1), 85.2 (CH), 77.3 (CH), 76.6 (CH), 76.1 (CH₂Ar), 74.8 (CH₂Ar), 64.9 (C-2), 63.0 (C-6), 55.2 (ArOCH₃).

Phenyl 2-azido-3-O-benzoyl-2-deoxy-4,6-O-p-methoxybenzylidene-1-thio-β-D-glucopyranoside 282
To a stirred solution of 159 (5.32 g, 12.82 mmol) in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (30 mL) was added BzCl (1.63 mL, 14.08 mmol), anhydrous pyridine (5.18 mL, 0.064 mol) and DMAP (312 mg, 2.56 mmol). The solution was stirred at RT for 1 h, then diluted with CH\textsubscript{2}Cl\textsubscript{2} (70 mL) and washed with aq. HCl (50 mL, 0.1 M), and saturated aq. NaHCO\textsubscript{3} (50 mL). The organic phase was dried and the solvent removed to give an orange solid, which was purified by dissolving in the minimum amount of CH\textsubscript{2}Cl\textsubscript{2} and adding petroleum ether to give the \textit{title compound} 282 as an off-white solid (5.65 g, 10.70 mmol, 85%); m.p. 79–83 °C; R\textsubscript{f} 0.34 (4:1 petroleum ether/EtOAc); IR (\nu_{\text{max}}/\text{cm}^{-1}): 2982 (C–H), 2106 (N\textsubscript{3}), 1784 (C=O); MS (ES\textsuperscript{+}) found m/z 520 [M+H]\textsuperscript{+}, HRMS found 520.1566, C\textsubscript{27}H\textsubscript{26}N\textsubscript{3}O\textsubscript{6}S [M+H]\textsuperscript{+} requires 520.1542; [\alpha]_D = –36.5 (c = 0.30, CH\textsubscript{2}Cl\textsubscript{2}); \delta H (400 MHz, CDCl\textsubscript{3}): 7.97–7.95 (2 H, m, ArH), 7.53–7.47 (3 H, m, ArH), 7.38–7.30 (5 H, m, ArH), 7.20 (2 H, t, J = 9.2 Hz, ArH), 6.73 (2 H, d, J = 8.8 Hz, ArH), 5.41 (1 H, t, J = 9.5 Hz, H-3), 5.37 (1 H, s, ArCH), 4.62 (1 H, d, J = 10.1 Hz, H-1), 4.31 (1 H, dd, J = 10.5, 4.8 Hz, H-6\textsubscript{eq}), 3.72 (1 H, t, J = 10.2 Hz, H-6\textsubscript{ax}), 3.67 (3 H, s, ArOC\textsubscript{H}\textsubscript{3}), 3.63 (1 H, t, J = 9.5 Hz, H-4), 3.54 (1 H, dd, J = 9.7, 4.9 Hz, H-5), 3.49 (1 H, t, J = 9.8 Hz, H-2); \delta C (101 MHz, CDCl\textsubscript{3}): 165.4 (C=O), 160.2 (Ar C), 133.9 (Ar CH), 133.5 (Ar CH), 130.9 (Ar CH), 130.0 (Ar CH), 129.4 (Ar CH), 129.2 (Ar CH), 129.0 (Ar CH), 128.6 (Ar CH) 127.5 (Ar CH), 113.7 (Ar CH), 101.6 (ArCH), 87.4 (C-1), 78.5 (C-4), 73.6 (C-3), 71.0 (C-5), 68.5 (C-6), 64.1 (C-2), 55.3 (ArOCH\textsubscript{3}).

Phenyl 2-azido-3-O-benzoyl-2-deoxy-4-O-p-methoxybenzyl-1-thio-β-D-glucopyranoside 283

To a stirred solution of 282 (544 mg, 1.05 mmol) in BH\textsubscript{3}.THF (3.14 mL, 1.0 M) at 0 °C was added a solution of Bu\textsubscript{2}BOTf in CH\textsubscript{2}Cl\textsubscript{2} (1.16 mL, 1.0 M) dropwise. The solution was stirred for 1 h at 0 °C, then quenched by addition of Et\textsubscript{3}N and methanol, and the solvent removed \textit{in vacuo}. Column chromatography (5:1, 3:1 petroleum ether/EtOAc) afforded the \textit{title compound} 283 (395 mg, 0.76 mmol, 72%); R\textsubscript{f} 0.35 (3:1 petroleum ether/EtOAc); IR (\nu_{\text{max}}/\text{cm}^{-1}): 3217 (O–H), 2912 (C–H), 2114 (N\textsubscript{3}), 1720 (C=O); MS (ES\textsuperscript{+}) found m/z 539 [M+NH\textsubscript{4}]\textsuperscript{+}, HRMS found 539.1950, C\textsubscript{27}H\textsubscript{31}N\textsubscript{4}O\textsubscript{6}S [M+ NH\textsubscript{4}]\textsuperscript{+} requires 539.1959; [\alpha]_D = –10.4 (c = 0.60, CH\textsubscript{2}Cl\textsubscript{2}); \delta H (400 MHz, CDCl\textsubscript{3}): 8.05–8.02
(2 H, m, ArH), 7.63–7.57 (3 H, m, ArH), 7.48–7.44 (2 H, m, ArH), 7.38–7.34 (3 H, m, ArH), 7.05–7.00 (2 H, m, ArH), 6.68–6.65 (2 H, m, ArH), 5.42 (1 H, t, J = 9.6 Hz, H-3), 4.65 (1 H, d, J = 10.2 Hz, H-1), 4.51 (1 H, d, J = 10.8 Hz, CH2Ar), 4.46 (1 H, d, J = 10.8 Hz, CH2Ar) 3.96–3.93 (1 H, m, H-6), 3.80–3.72 (2 H, m, H-4, H-6), 3.69 (3 H, s, ArOC3H3), 3.52–3.45 (2 H, m, H-2, H-5); δ C (101 MHz, CDCl3): 165.4 (C=O), 159.4 (Ar C), 133.5 (Ar CH), 133.3 (Ar CH), 131.3 (Ar C), 129.9 (Ar CH), 129.8 (Ar CH), 129.3 (Ar CH), 129.3 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 113.8 (Ar CH), 86.5 (C-1), 79.7 (C-5), 76.5 (C-4), 74.7 (C-3), 74.4 (CH2Ar), 63.8 (C-2), 61.6 (C-6), 55.1 (ArOCH3).

Phenyl 2-azido-3,6-di-O-benzoyl-2-deoxy-4-O-p-methoxybenzyl-1-thio-β-D-glucopyranoside 284

To a stirred solution of glucosamine derivative 283 (1.75 g, 3.36 mmol) in anhydrous CH2Cl2 (20 mL) was added BzCl (428 µL, 3.69 mmol), Et3N (514 µL, 3.69 mmol) and DMAP (42 mg, 0.34 mmol). The solution was stirred for 2 h at RT. H2O (5 mL) was added and the solution was stirred for 30 min at RT. The solution was washed with H2O (50 mL), aq. HCl (50 mL, 0.1 M) and saturated aq. Na2CO3 (50 mL). The organic phase was dried and the solvent removed in vacuo to give a white solid, which was purified by dissolving in the minimum amount of CH2Cl2 and adding petroleum ether to afford the title compound 284 as a white solid (1.86 g, 2.97 mmol, 88%); m.p. 130–132 °C; Rf 0.40 (3:1 cyclohexane/EtOAc); IR (νmax/cm–1): 2956 (C–H), 2109 (N3), 1724 (C=O); MS (ES+) found m/z 643 [M+NH4]+, HRMS found 626.1931, C34H32N3O7S [M+NH4]+ requires 626.1961; elemental analysis calcd (%) for C34H28N3O7S, C 65.27, H 4.99, N 6.72, S 5.12, found C 65.03, H 5.01, N 6.70, S 4.95; [α]D = −7.0 (c = 0.20, CH2Cl2); δH (400 MHz, CDCl3): 8.10–8.04 (4 H, m, ArH), 7.67–7.59 (4 H, m, ArH), 7.53–7.47 (4 H, m, ArH), 7.33–7.29 (1 H, m, ArH), 7.20 (2 H, td, J = 7.3, 1.3 Hz, ArH), 7.01 (2 H, d, J = 8.7 Hz, ArH), 6.63 (2 H, d, J = 8.7 Hz, ArH), 5.47 (1 H, dd, J = 9.8, 9.1 Hz, H-3), 4.69 (1 H, dd, J = 12.0, 2.2, H-6), 4.63 (1 H, d, J = 10.0 Hz, H-1), 4.51 (1 H, dd, J = 12.0, 4.4 Hz, H-6), 4.47 (1 H, d, J = 10.8 Hz, CH2Ar), 4.44 (1 H, d, J = 10.8 Hz, CH2Ar), 3.80 (1 H, ddd, J = 9.8, 4.4, 2.2 Hz, H-5), 3.71 (1 H, dd, J = 10.9, 7.9 Hz, H-4).
3.65 (3 H, s, ArOCH₃), 3.49 (1 H, t, J = 10.0 Hz, H-2); δC (101 MHz, CDCl₃): 166.0 (C=O), 159.4 (C=O), 134.0 (Ar CH), 133.6 (Ar CH), 133.3 (Ar CH), 130.6 (Ar C), 130.0 (Ar CH), 129.9 (Ar CH), 129.8 (Ar CH), 129.3 (Ar C), 129.0 (Ar CH), 128.7 (Ar C), 128.6 (Ar CH), 128.5 (Ar CH), 113.8 (Ar CH), 85.9 (C-1), 77.5 (C-5), 76.6 (C-3), 74.8 (C-4), 74.4 (CH₂Ar), 63.4 (C-2), 63.0 (C-6), 55.1 (ArOCH₃).

Phenyl 2-azido-3-O-benzoyl-6-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-1-thio-β-D-glucopyranoside 286

To a stirred solution of glucosamine derivative 283 (1.91 g, 3.66 mmol) in anhydrous THF (10 mL) at 0 °C was added NaH (205 mg, 5.12 mmol) and the solution was stirred for 20 min. BnBr (0.53 µL, 4.39 mmol) was added and the solution was stirred overnight at 60 °C. The solvent was removed in vacuo, and the residue partitioned between CH₂Cl₂ (100 mL) and saturated aq. NaCl (50 mL). The organic phase was dried and concentrated in vacuo to a yellow oil. Column chromatography (1:0, 9:1 hexane/EtOAc) afforded the title compound 286 as a colourless foam (1.35 g, 2.21 mmol, 60%), along with 3,6-O-dibenzylated compound 162 (216 mg, 0.36 mmol, 10%).

Data for product 286: Rf 0.35 (4:1 cyclohexane/EtOAc); IR (νmax/cm⁻¹): 2959 (C–H), 2119 (N₃), 1717 (C=O); MS (ES⁺) found m/z 629 [M+NH₄]⁺, HRMS found 650.1724, C₃₄H₃₃N₃O₆S [M+NH₄]⁺ requires 650.1727; [α]D = -2.9 (c = 0.35, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.06–8.04 (2 H, m, ArH), 7.68–7.63 (1 H, m, ArH), 7.61–7.59 (2 H, m, ArH), 7.53–7.49 (2 H, m, ArH), 7.46–7.36 (6 H, m, ArH), 7.25–7.22 (2 H, m, ArH), 7.17 (2 H, td, J = 7.3, 1.3 Hz, ArH), 6.86–6.82 (2 H, m, ArH), 4.96 (1 H, d, J = 10.4 Hz, CH₂Ar), 4.92 (1 H, d, J = 10.4 Hz, CH₂Ar), 4.83 (1 H, d, J = 10.6 Hz, CH₂Ar), 4.73–4.70 (2 H, m, H-4, H-6), 4.59 (1 H, d, J = 10.7 Hz, CH₂Ar), 4.49–4.44 (2 H, m, H-1, H-6), 3.76 (3 H, s, ArOCH₃), 3.66–3.59 (2 H, m, H-3, H-5), 3.39 (1 H, dd, J = 10.0, 9.3 Hz, H-2); δC (101 MHz, CDCl₃): 166.0 (C=O), 159.5 (C=O), 137.4 (Ar C), 134.2 (Ar CH), 133.7 (Ar CH), 133.2 (Ar CH), 130.4 (Ar C), 129.8 (3 x Ar CH), 129.4 (Ar C), 129.1 (Ar CH), 128.9 (Ar CH), 128.7 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.4 (2 x Ar CH), 128.2 (Ar CH), 127.8 (Ar CH), 127.6 (Ar CH), 127.0 (Ar CH), 114.0 (Ar
CH), 85.6 (C-1), 85.2 (CH), 77.2 (CH), 76.6 (CH), 76.1 (CH₂Ar), 74.8 (CH₂Ar), 64.9 (CH₂Ar), 63.0 (C-6), 55.2 (ArOCH₃).

13.1.22 Synthesis of Lactone-containing Disaccharides 287, 289 and 290
Phenyl (2-azido-6-O-benzoyl-3-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-α-D-glucopyranosyl)-(1→4)-3-O-benzyl-1-thio-α/β-L-idopyranosidurono-2,6-lactone 287

From glycosylation of SPh donor 281 with lactone α-270:

Acceptor α-270 (348 mg, 0.97 mmol) and donor 281 (600 mg, 1.00 mmol) were dissolved in dry CH₂Cl₂ (10 mL) under argon. Molecular sieves (~600 mg, 4 Å, powdered, activated) were added and the mixture was cooled to 0 °C. NIS (270 mg, 1.20 mmol) was added and the reaction was stirred for 10 min at 0 °C. AgOTf (25 mg, 0.097 mmol) was added and the reaction was stirred for 1 h warming to RT. TLC (2:1 cyclohexane/EtOAc) showed no reaction. A catalytic amount of AgOTf was added and the reaction was stirred for a further 1 h. TLC (2:1 cyclohexane/EtOAc) showed no reaction. NIS (270 mg, 1.20 mmol) and AgOTf (25 mg, 0.097 mmol) were added and the reaction was stirred at RT overnight. The reaction was quenched by the addition of saturated aq. Na₂S₂O₃ (5 mL), stirred for 10 minutes, then filtered through Celite with CH₂Cl₂ (3 x 15 mL). The phases were separated and the organic phase was dried and the solvents removed in vacuo to give a yellow solid. Column chromatography (15:1, 10:1, 5:1, 3:1 cyclohexane/EtOAc) afforded the title compound α-287 as a white foam (169 mg, 0.20 mmol, 20%); Rf 0.36 (5:1 cyclohexane/EtOAc); IR (νmax/cm⁻¹): 2922 (C–H), 2105 (N₃), 1786 (C=O); MS (ES) found m/z 877 [M+NH₄⁺], HRMS found 877.3113, [α]D = −41.3 (c = 0.30, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.10–7.99 (2 H, m, ArH), 7.56 (1 H, tt, J = 8.8, 1.6 Hz, ArH), 7.50–7.30 (17 H, m, ArH), 7.20 (2 H, dd, J = 8.6, 2.8 Hz, ArH), 6.80 (2 H, dd, J = 8.6, 2.8 Hz, ArH), 5.50 (1 H, t, J = 1.8 Hz, H-1), 5.06 (1 H, d, J = 3.6 Hz, H-1'), 4.94 (1 H, t, J = 3.0 Hz, H-2), 4.93–4.87 (2 H, m, 2 x CH₂Ar), 4.85 (1 H, d, J = 11.6 Hz, CH₂Ar), 4.80 (1 H, d, J = 10.8 Hz, CH₂Ar), 4.77 (1 H, d, J = 11.6 Hz, CH₂Ar), 4.64 (1 H, dd, J = 12.4, 2.0 Hz, H-6'), 4.63 (1 H, J = 3.6 Hz, H-5), 4.57 (1 H, d, J = 10.8 Hz, CH₂Ar), 4.49 (1 H, dd, J = 12.4, 4.0 Hz, H-6'), 4.41 (1 H, t, J = 3.6 Hz, H-4), 4.06–4.00
(1 H, m, H-5’), 4.02 (1 H, dt, J = 3.6, 1.2 Hz, H-3), 3.93 (1 H, dd, J = 10.4, 8.8 Hz, H-3’), 3.76 (3 H, s, ArOCH3), 3.67 (1 H, t, J = 8.8 Hz, H-3’), 3.40 (1 H, dd, J = 10.4, 3.6 Hz, H-2’); δC (101 MHz, CDCl3): 167.1 (C=O), 166.1 (C=O), 159.5 (Ar C), 137.6 (Ar C), 136.6 (Ar C), 136.1 (Ar C), 136.0 (Ar C), 135.6 (Ar C), 133.1 (Ar CH), 131.2 (Ar CH), 129.7 (Ar CH), 129.5 (Ar C), 129.3 (Ar CH), 128.8 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 127.8 (Ar CH), 114.0 (Ar CH), 99.9 (C-1’), 83.4 (C-1), 80.4 (C-2), 79.9 (C-3’), 78.6 (C-3), 77.5 (C-4’), 75.7 (CH2Ar), 74.8 (CH2Ar), 74.4 (C-2), 72.5 (CH2Ar), 70.4 (C-5), 70.3 (C-5’), 63.3 (C-2’), 62.9 (C-6), 55.2 (ArOCH3).

From glycosylation of trichloroacetimidate donor 292 with lactone β-270:

Glycosyl donor α/β-292 (578 mg, 0.87 mmol) and glycosyl acceptor β-270 (253 mg, 0.71 mmol) were combined in anhydrous CH2Cl2 (6 mL) under argon, and the mixture was cooled to –50 °C. TMSOTf (1.3 µL, 0.73 µmol) in anhydrous CH2Cl2 (1 mL) was added. The solution was stirred for 30 min at –50 °C, TLC (3:1 cyclohexane/EtOAc) showed no consumption of donor. TMSOTf (5 µL, 0.027 mmol) was added and the reaction was stirred for 3 h warming to RT. TLC (3:1 cyclohexane/EtOAc) showed no remaining donor. Et3N was added to quench the reaction, and the solvents were removed in vacuo to give a yellow foam. Column chromatography (9:1 cyclohexane/EtOAc) afforded the title compound β-287 as a white foam (248 mg, 0.29 mmol, 41%); Rf 0.33 (5:1 cyclohexane/EtOAc); IR (νmax/cm–1): 2945 (C–H), 2105 (N3), 1786 (C=O), 1719 (C=O); δH (400 MHz, CDCl3): 8.10–8.06 (2 H, m, ArH), 7.54–7.32 (18 H, m, ArH), 7.24–7.20, (2 H, m, ArH), 6.84–6.80, (2 H, m, ArH), 5.70 (1 H, d, J = 0.8 Hz, H-1), 4.98–4.91 (3 H, m, H-1’, 2 x CH2Ar), 4.87 (1 H, d, J = 4.4 Hz, H-2), 4.82 (1 H, d, J = 10.4 Hz, CH2Ar), 4.73 (1 H, d, J = 11.6 Hz, CH2Ar), 4.67–4.50 (5 H, m, H-5, 2 x H-6’, 2 x CH2Ar), 4.10–4.04 (2 H, m, H-4, H-5’), 4.04 (1 H, dd, J = 4.2, 2.4 Hz, H-3), 4.64 (1 H, dd, J = 10.4, 8.8 Hz, H-3’), 3.75 (3 H, s, ArOCH3), 3.66 (1 H, dd, J = 9.6, 8.8 Hz, H-4’), 3.42 (1 H, J = 10.4, 4.0 Hz, H-2’); δC (101 MHz, CDCl3): 166.9 (C=O), 166.1 (C=O), 159.5 (Ar C), 137.6 (Ar C), 137.0 (Ar C), 136.4 (Ar C), 136.2 (Ar C, Ar CH), 130 (Ar CH), 129.7 (2 x Ar CH), 129.5 (Ar C), 129.2 (Ar CH), 128.8 (Ar
CH), 128.7 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 114.0 (Ar CH), 100.1 (C-1’), 80.9 (C-1, C-4), 79.9 (C-3), 77.8 (C-3’), 77.4 (C-4’), 76.5 (C-2), 75.7 (CH₂Ar), 74.8 (CH₂Ar), 72.5 (CH₂Ar), 70.6 (CH), 70.3 (CH), 63.29 (C-2’), 63.0 (C-6’), 55.3 (ArO)

Phenyl (2-azido-3,6-di-O-benzoyl-2-deoxy-4-O-p-methoxybenzyl-α-D-glucopyranosyl)-(1→4)-(3-O-benzyl-1-thio-α-L-idopyranosidurono-2,6-lactone α-289

Glycosyl donor 284 (2.37 g, 3.79 mmol) and glycosyl acceptor α-270 (1.32 g, 3.79 mmol) were combined in anhydrous CH₂Cl₂ (20 mL) under argon and the solution cooled to 0 °C. Molecular sieves (~2 g, 4 Å, powdered, activated) were added, followed by NIS (1.02 g, 4.54 mmol), and the reaction was stirred for 10 min at 0 °C. A catalytic amount of AgOTf was added and the reaction was stirred for 1 h warming to RT. TLC (2:1 cyclohexane/EtOAc) showed low conversion to product. A catalytic amount of AgOTf was added and the reaction was stirred at RT overnight. TLC (2:1 cyclohexane/EtOAc) showed unreacted donor. A catalytic amount of AgOTf was added and the reaction was stirred at RT overnight. TLC (2:1 cyclohexane/EtOAc) showed unreacted donor. NIS (1.70 g, 7.56 mmol) was added, followed by catalytic AgOTf, and the reaction was stirred at RT overnight. TLC (2:1 cyclohexane/EtOAc) showed no remaining donor. Saturated aq. Na₂S₂O₃ (10 mL) was added and the reaction was stirred for 10 min, then filtered through Celite with CH₂Cl₂ (100 mL). The phases were separated and the organic phase dried and the solvent removed to give a yellow foam. Column chromatography (9:1 hexane/EtOAc) afforded the title compound α-289 as a white foam (384 mg, 0.44 mmol, 12%); Rr 0.48 (2:1 cyclohexane/EtOAc); IR (vmax/cm⁻¹): 2929 (C–H), 2108 (N=O), 1785 (C=O), 1722 (C=O); MS (ES⁺) found m/z 891 [M+NH₄]⁺, HRMS found 891.2897, C₄₇H₄₇N₄O₁₂S [M+NH₄]⁺ requires 891.2906; [α]D = +4.0 (c = 0.15, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.14–8.10 (2 H, m, ArH), 7.65–7.58 (3 H, m, ArH), 7.53–7.28 (13 H, m, ArH), 7.08–7.04 (2 H, m, ArH), 6.67–6.64 (2 H, m, ArH), 5.82 (1 H, dd, J = 10.8, 9.0 Hz, H-3’), 5.52 (1 H, t, J = 1.8 Hz, H-1’), 5.22 (1 H, d, J = 3.7 Hz, H-1’), 4.97 (1 H, dd, J = 3.1, 2.3, H-2), 4.86 (1
H, d, $J = 11.2$ Hz, $CH_2Ar$), 4.78–4.72 (3 H, m, H-5, H-6’, $CH_2Ar$), 4.63–4.58 (1 H, m, H-6’), 4.57–4.50 (2 H, m, 2 x $CH_2Ar$), 4.48 (1 H, t, $J = 3.7$ Hz, H-4), 4.24 (1 H, ddd, $J = 10.0, 3.5, 2.2$, H-5’), 4.11 (1 H, ddd, $J = 5.3, 3.6, 1.7$ Hz, H-3), 3.85 (1 H, dd, $J = 9.8, 9.2$ Hz, H-4’), 3.64 (3 H, s, ArOCH$_3$), 3.39 (1 H, dd, $J = 10.8, 3.7$, H-2’); $\delta_c$ (101 MHz, CDCl$_3$): 167.1 (C=O), 166.2 (C=O), 165.4 (C=O), 159.4 (Ar C), 136.5 (Ar C), 135.5 (Ar C), 133.6 (Ar CH), 133.2 (Ar CH), 132.4 (Ar CH), 131.2 (Ar CH), 131.1 (Ar CH), 130.1 (Ar CH), 130.0 (Ar C), 129.9 (Ar CH), 129.8 (Ar CH), 129.4 (Ar CH), 129.3 (Ar CH), 129.0 (Ar CH), 128.7 (2 x Ar CH), 128.6 (2 x Ar CH), 128.5 (Ar CH), 128.4 (2 x Ar CH), 128.1 (Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 113.8 (Ar CH), 100.5 (C-1’), 83.2 (C-1), 81.2 (C-4), 79.0 (C-3), 75.1 (C-4’), 74.3 (CH$_2$Ar), 74.2 (C-2), 72.9 (CH$_2$Ar), 72.4 (C-3’), 70.4 (C-5’), 70.1 (C-5), 62.9 (C-6’), 61.3 (C-2’), 55.1 (ArOCH$_3$).

Phenyl (2-azido-3-O-benzoyl-6-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-$\alpha$-D-glucopyranosyl)-(1→4)-3-O-benzyl-1-thio-$\alpha$-L-idopyranosidurono-2,6-lactone $\alpha$-290

Glycosyl donor 286 (577 mg, 0.94 mmol) and glycosyl acceptor $\alpha$-270 (329 mg, 0.94 mmol) were combined in anhydrous CH$_2$Cl$_2$ (15 mL) under argon and the solution cooled to 0 °C. Molecular sieves (~2 g, 4 Å, powdered, activated) were added, and the reaction was stirred for 10 min. NIS (254 mg, 1.13 mmol) was added, and the reaction was stirred for 10 min at 0 °C. TMSOTf (34 µL, 0.19 mmol) was added and the reaction was stirred for 15 min warming to RT. TLC (3:1 cyclohexane/EtOAc) showed no remaining glycosyl donor. The reaction was quenched with Et$_3$N, filtered through Celite with CH$_2$Cl$_2$ (85 mL), and the solvent removed $\textit{in vacuo}$ to give a brown oil. Column chromatography (1:1, 2:1, 0:1 toluene/CH$_2$Cl$_2$) afforded the title compound $\alpha$-290 as a white foam (148 mg, 0.17 mmol, 18%), along with 4-OPMB lactone $\alpha$-288 (69 mg, 0.14 mmol, 15%).

Data for product $\alpha$-290: $R_f$ 0.46 (2:1 cyclohexane/EtOAc); IR ($\nu_{\text{max}}$ cm$^{-1}$): 2919 (C–H), 2105 (N$_3$) 1785 (C=O), 1718 (C=O); MS (ES$^+$) found $m/z$ 877 [M+NH$_4]^+$, HRMS found 877.3111, C$_{47}$H$_{46}$N$_4$O$_{11}$S [M+NH$_4]^+$ requires 877.3119; $[\alpha]_D = -2.9$ (c = 0.35, CH$_2$Cl$_2$); $\delta_H$ (400 MHz, CDCl$_3$): 7.93–7.90 (2 H, m, ArH), 7.47–7.43 (1 H, m, ArH), 7.41–7.37 (4
H, m, ArH), 7.34–7.20 (11 H, m, ArH), 7.16–7.06 (4 H, m, ArH), 6.72–6.69 (2 H, m, ArH), 5.40 (1 H, dd, J = 2.0, 1.5 Hz, H-1), 4.96 (1 H, d, J = 3.7 Hz, H-1'), 4.85–4.81 (3 H, m, ArH), 4.78–4.66 (3 H, m, 3 x CH2Ar), 4.55 (2 H, dd, J = 10.4, 2.8 Hz, H-5, H-6'), 4.49 (1 H, d, J = 10.6 Hz, CH2Ar), 4.40 (1 H, dd, J = 12.3, 3.9 Hz, H-6'), 4.31 (1 H, t, J = 3.7 Hz, H-4), 3.97–3.91 (2 H, m, H-3, H-5'), 3.88 (1 H, dd, J = 10.4, 3.7 Hz, H-2'), δH (400 MHz, CDCl3): 7.48 (2 H, m, ArH), 7.32–7.24 (8 H, m, ArH), 7.22 (2 H, d, J = 8.8 Hz ArH), 6.88 (2 H, d, J = 8.8 Hz, ArH), 5.50 (1 H, dd, J = 2.0, 1.6 Hz, H-1), 4.95 (1 H, ddd, J = 3.2, 2.0, 0.4 Hz, H-2), 4.76 (1 H, d, J = 12.0 Hz, CH2Ar), 4.59 (1 H, d, J = 11.8 Hz, CH2Ar), 4.56 (1 H, d, J = 11.6 Hz, CH2Ar), 4.47 (1 H, d, J = 11.6 Hz, CH2Ar), 4.41 (1 H, d, J = 3.6 Hz, H-5), 4.49 (1 H, td, J = 3.6, 0.4 Hz, H-4), 3.88 (1 H, td, J = 3.6, 1.2 Hz, H-3); δC (101 MHz, CDCl3): 167.2 (C=O), 159.7 (Ar C), 136.6 (Ar C), 136.7 (Ar C), 135.7 (Ar C), 131.0 (Ar CH), 129.7 (Ar CH), 129.3 (Ar C), 128.6 (Ar CH), 128.0 (Ar CH), 127.8 (Ar CH), 114.0 (Ar CH), 83.3 (C-1), 79.0 (C-3), 76.9 (C-4), 75.1 (C-2'), 71.9 (CH2Ar), 71.2 (CH2Ar), 69.3 (C-5), 55.1 (ArOCH3).

13.1.23 Preparation of Trichloroacetimidate Donor 292

2-Azido-6-O-benzoyl-3-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-α/β-D-glucopyranose α/β-291

Thioglycoside 281 (2.08 g, 3.40 mmol) was dissolved in acetone (30 mL), the solution was cooled to 0 °C, and NBS (605 mg, 3.40 mmol) was added. The reaction mixture
was stirred at 0 °C for 30 min, then quenched by addition of saturated aq. NaHCO₃. The solvent was removed in vacuo and the residue partitioned between CH₂Cl₂ (50 mL) and H₂O (50 mL). The organic phase was concentrated to a yellow oil, purification by column chromatography (1:0, 10:1, 3:1 petroleum ether/EtOAc) afforded the title compound 291 as a white solid (1.63 g, 3.14 mmol, 92%, α anomer with ~20% β anomer). Data for α/β-291: δH (400 MHz, CDCl₃): 8.02–8.97 (2 H, m, ArH), 7.60–7.54 (1 H, m, ArH), 7.46–7.31 (7 H, m, ArH), 7.24–7.17 (2 H, m, ArH), 6.83–6.78 (2 H, m, ArH), 5.31 (1 H, d, J = 3.0 Hz, H-1), 4.95 (2 H, s, 2 x CH₂Ar), 4.89–4.83 (1 H, m, CH₂Ar), 4.62–4.54 (2 H, m, H-6, CH₂Ar), 4.49–4.41 (1 H, m, CH₂Ar), 4.21 (1 H, dt, J = 9.7, 2.8 Hz, H-5), 4.08 (1 H, dd, J = 10.1, 9.0 Hz, H-3), 3.73 (3 H, s, CH₃), 3.71–3.63 (1 H, m, H-4), 3.47–3.42 (1 H, m, H-2); δC (101 MHz, CDCl₃): 166.3 (C=O), 159.4 (Ar C), 137.6 (Ar CH), 129.7 (3 x Ar CH), 129.6 (Ar C), 128.6 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 114.0 (Ar CH), 92.0 (C-1), 80.3 (C-3), 77.4 (C-4), 75.8 (CH₂Ar), 74.8 (CH₂Ar), 69.4 (C-5), 64.1 (C-2), 63.0 (C-6), 55.2 (ArCH₃). Data in agreement with previous synthesis (N.B. only ¹H and ¹³C NMR data reported).³¹⁸

2-Azido-6-O-benzoyl-3-O-benzyl-2-deoxy-4-O-p-methoxybenzyl-1-O-trichloroacetimidate-α/β-D-glucopyranoside α/β-292

To a stirred solution of glucosamine derivative 291 (872 mg, 1.68 mmol) in anhydrous CH₂Cl₂ (20 mL) was added Cl₃CCN (337 µL, 3.36 mmol) and K₂CO₃ (696 mg, 5.04 mmol). The reaction was stirred at RT overnight, then Cl₃CCN (300 µL, 2.99 mmol) was added and the reaction stirred for a further 30 min. The reaction mixture was filtered and the solvent removed in vacuo to reveal the title compound 292 as a yellow foam (1.06 g, 1.60 mmol, 95%, ~ 5:2 α/β); Rf 0.48, 0.60 (3:1 cyclohexane/EtOAc); IR (νmax/cm⁻¹): 3340 (N–H), 2897 (C–H), 2111 (N₃), 1717 (C=O); MS (ES⁺) found m/z 537 [M+NH₄]⁺, HRMS found 542.1900, C₂₈H₂₉N₃NaO₇ [M+Na]⁺ requires 542.1898; δH
(400 MHz, CDCl₃): 8.04–7.96 (3 H, m, ArH), 7.59–7.54 (2 H, m, ArH), 7.46–7.31 (12 H, m, ArH), 7.23–7.18 (3 H, m, ArH), 6.82–6.78 (3 H, m, ArH), 6.43 (1 H, d, J = 3.6 Hz, H-1α), 5.32 (0.41 H, dd, J = 6.8, 3.6 Hz, H-1β), 5.00–4.94 (3 H, m, 3 x CH₂Ar), 4.85–4.81 (1.60 H, m, 2 x CH₂Ar), 4.60–4.44 (5 H, m, 2 x H-6α, 2 x H-6β, 2 x CH₂Ar), 4.24–4.20 (0.50 H, m, H-5β), 4.17 (1 H, ddd, J = 10.1, 3.4, 2.3 Hz, H-5α), 4.12–4.05 (1.51 H, m, H-3α, H-3β), 3.80–3.65 (8 H, m, H-2α, H-4α, H-4β, 2 x ArOCH₃), 3.49–3.46 (0.55 H, m, H-2β); δC (101 MHz, CDCl₃): 166.2 (C=O), 166.0 (C=N), 161.0 (Ar C), 159.6 (Ar C), 137.4 (Ar C), 133.2 (Ar CH), 129.8 (Ar CH), 129.7 (3 x Ar CH), 129.6 (Ar CH), 129.2 (Ar C), 128.6 (2 x Ar CH), 128.5 (Ar CH), 128.4 (2 x Ar CH), 128.2 (3 x Ar CH), 128.1 (Ar CH), 114.0 (2 x Ar CH), 94.6 (C-1α), 92.1 (C-1β), 90.8 (CCl₃), 80.3 (C-3α), 77.6 (CH), 77.2 (CH), 77.0 (CH), 75.8 (CH₂Ar), 75.1 (CH₂Ar), 74.9 (CH₂Ar), 72.0 (C-5α), 69.5 (C-5β), 64.1 (C-2β), 63.2 (C-2α), 62.9 (C-6β), 62.6 (C-6α), 55.2 (ArOCH₃). Data in agreement with previous synthesis.³¹⁸

13.1.24 Hydrolysis of 4-OTBDMS Lactone 294 with Et₃N/MeOH
Phenyl (methyl 3-O-benzyl-4-O-tert-butyldimethylsilyl-1-thiophenyl-α-L-idopyranoside) Uronate α-295

Lactone α-294 (15 mg, 40 µmol) was dissolved in MeOH (2 mL), Et₃N (5 µL, 40 µmol) was added, and the reaction mixture was stirred for 18 h. The solvent was removed and the crude product purified by column chromatography (7:1 hexane/EtOAc) to afford the title compound α-295 as a colourless glass (17 mg, 36 µmol, 90%); Rf 0.38 (7:1 cyclohexane/EtOAc); IR (ʋmax/cm⁻¹): 3475 (O–H), 2950 (C–H), 1754 (C=O); MS (ES⁺) found m/z 522 [M+NH₄]⁺, HRMS found 522.2329, C₂₆H₄₀NO₆SSi [M+NH₄]⁺ requires 522.2340; [α]₀ = −131.9 (c = 0.85, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.53–7.50 (2 H, m, ArH), 7.42–7.23 (8 H, m, ArH), 5.66 (1 H, s, H-1), 5.28 (1 H, d, J = 1.5 Hz, H-5), 4.84 (1 H, d, J = 12.2 Hz, CH₂Ar), 4.56 (1 H, d, J = 12.2 Hz, CH₂Ar), 4.18–4.17 (1 H, m, H-4), 4.06–4.05 (1 H, m, H-2), 3.79 (3 H, s, CO₂CH₃), 3.66 (1 H, t, J = 2.8 Hz, H-3), 0.83 (9 H, s, OSi(CH₃)₃), 0.02 (3 H, s, OSi(CH₃)₂), 0.03 (3 H, s, OSi(CH₃)₂); δC (101 MHz, CDCl₃): 169.8 (C=O), 137.3 (Ar C), 136.6 (Ar CH), 130.7 (Ar CH), 129.0 (Ar CH), 128.6 (Ar CH), 128.2 (Ar CH), 127.9 (Ar CH), 127.1 (Ar CH), 89.7 (C-1), 74.0
(C-3), 72.2 (CH₂Ar), 69.9 (C-4), 69.1 (C-5), 68.9 (C-2), 52.3 (CO₂CH₃), 25.5 (OSi(CH₃)₃), 17.8 (OSi(CH₃)₃), −4.8 (OSi(CH₃)₂), −5.6 (OSi(CH₃)₂). Collected data reported in ref. 354.

Phenyl (methyl 3-O-Benzy1-4-O-tert-butyldimethylsilyl-1-thiophenyl-β-L-idopyranoside) Uronate β-295

Lactone β-294 (2.70 g, 5.70 mmol) was dissolved in MeOH (30 mL), Et₃N (790 µL, 5.70 mmol) was added, and the reaction mixture stirred at RT for 72 h. Et₃N (790 µL, 5.70 mmol) was added and the reaction mixture stirred for another 3 h. The solvent was removed and the crude product purified by column chromatography (7:1 hexane/EtOAc) to afford the title compound β-295 as a colourless glass (1.73 g, 3.41 mmol, 60%) along with recovered starting material (500 mg, 1.06 mmol, 19%). Data for product β-295: Rf 0.30 (7:1 cyclohexane/EtOAc); IR (ν max/cm⁻¹): 3354 (O–H), 2951 (C–H), 1770 (C=O); MS (ES⁺) found m/z 522 [M+NH₄⁺], HRMS found 522.2328, C₂₆H₄₀NO₆SSi[M+NH₄⁺] requires 522.2340; [α]D = +52.9 (c = 0.9, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.57–7.55 (2 H, m, ArH), 7.37–7.29 (8 H, m, ArH), 5.19 (1 H, d, J = 1.1 Hz, H-1), 4.68 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.58–4.55 (2 H, m, CH₂Ar, H-5), 4.12 (1 H, t, J = 1.6 Hz, H-4), 3.94–3.93 (1 H, m, H-2), 3.81 (3 H, s, CO₂CH₃), 3.75 (1 H, t, J = 3.2 Hz, H-3), 0.86 (9 H, s, OSi(CH₃)₃), 0.00 (3 H, s, OSi(CH₃)₂), −0.01 (3 H, s, OSi(CH₃)₂); δC (101 MHz, CDCl₃): 169.2 (C=O), 137.1 (Ar C), 135.7 (Ar CH), 130.7 (Ar CH), 128.9 (Ar CH), 128.7 (Ar CH), 128.4 (Ar CH), 128.0 (Ar CH), 127.0 (Ar CH), 87.5 (C-1), 76.8 (C-5), 75.4 (C-3), 72.4 (CH₂Ar), 69.8 (C-2), 69.0 (C-4), 52.3 (CO₂CH₃), 25.6 (OSi(CH₃)₃), 17.9 (OSi(CH₃)₃), −4.9 (OSi(CH₃)₂), −5.4 (OSi(CH₃)₂). Collected data reported in ref. 354.
13.1.25 Synthesis of Donor Disaccharide 300

Phenyl (2-azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-3-O-benzyl-1-thio-α/β-L-idopyranosidurono-2,6-lactone α/β-297

Glycosyl donor 223 (602 mg, 1.06 mmol) and glycosyl acceptor α/β-270 (306 mg, 0.85 mmol, ~5:8 α/β anomers) were combined in anhydrous toluene (7 mL) under argon and the solution was cooled to 0 °C. Molecular sieves (~900 mg, 4 Å, powdered, activated) were added, and the reaction was stirred for 10 min. NIS (287 mg, 1.28 mmol) was added, and the reaction was stirred for 10 min at 0 °C. AgOTf (11 mg, 0.043 mmol) was added and the reaction was stirred for 10 min warming to RT. TLC (3:1 cyclohexane/EtOAc) showed no remaining glycosyl donor. The solvent was removed in vacuo to give a red oil. Column chromatography (3:2, 2:1, CH2Cl2/hexane + 1% EtOAc) afforded the title compound α/β-297 as a yellow foam (503 mg, 0.62 mmol, 73%). The separate anomers α-297 and β-297 were isolated in separate, lower-yielding procedures using single anomers of 270. β-linked disaccharide β-298 was also isolated from glycosylation using β-270 as acceptor.

Data for α-297: Rf 0.56 (3:1 cyclohexane/EtOAc); IR (νmax/cm–1): 2901 (C–H), 2105 (N3) 1785 (C=O), 1718 (C=O); MS (ES+) found m/z 833 [M+NH4]+, HRMS found m/z 833.3219, C46H49N4O9S [M+NH4]+ requires 833.3215; [α]D = –23.9 (c = 3.35, CH2Cl2); δH (400 MHz, CDCl3): 7.53–7.49 (4 H, m, ArH), 7.44–7.27 (19 H, m, ArH), 7.18–7.15 (2 H, m, ArH), 5.51 (1 H, dd, J = 2.0, 1.2 Hz, H-1), 5.10 (1 H, d, J = 3.6 Hz, H-1’), 4.94 (1 H, dd, J = 3.6, 2.0 Hz, H-2), 4.89 (1 H, d, J = 10.4 Hz, CH2Ar), 4.85 (1 H, d, J = 12.0 Hz, CH2Ar), 4.84 (1 H, d, J = 10.4 Hz, CH2Ar), 4.80 (1 H, d, J = 11.6 Hz, CH2Ar), 4.79 (1 H, d, J = 10.8 Hz, CH2Ar), 4.66 (1 H, d, J = 12.0 Hz, CH2Ar), 4.66 (1 H, d, J = 3.6 Hz, H-5), 4.53 (1 H, d, J = 11.2 Hz, CH2Ar), 4.49 (1 H, d, J = 12.4 Hz, CH2Ar), 4.42 (1 H, t, J = 3.6 Hz, H-4), 4.02 (1 H, td, J = 3.6, 1.2 Hz, H-3), 3.93–3.85 (2 H, m, H-3’, H-5’), 3.82 (1 H, dd, J = 11.0, 3.0 Hz, H-6’), 3.79–3.72 (2 H, m, H-4, H-6’), 3.41 (1 H, dd, J = 10.2, 3.6 Hz, H-2’); δC (101 MHz, CDCl3): 167.2 (C=O), 137.9 (Ar C), 137.8 (2 x Ar C), 136.6 (Ar C), 135.4 (Ar C), 131.2 (Ar CH), 129.3 (Ar CH), 128.6 (Ar CH), 213
128.5 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 100.2 (C-1’), 83.3 (C-1), 80.3 (C-4), 79.7 (C-3’), 78.7 (C-3), 77.9 (C-4’), 75.5 (CH2Ar), 74.9 (CH2Ar), 74.4 (C-2), 73.6 (CH2Ar), 72.4 (CH2Ar), 71.8 (C-5’), 70.4 (C-5), 68.0 (C-6’), 63.2 (C-2’).

Data for β-297: Rf 0.53 (3:1 cyclohexane/EtOAc); IR (νmax/cm−1): 2916 (C–H), 2104 (N3) 1784 (C=O), 1718 (C=O); MS (ES+) found m/z 833 [M+NH4]+, HRMS found m/z 838.2760, C46H43N3NaO9S [M+Na]+ requires 838.2769; [α]D = +120.0 (c = 3.50, CH2Cl2); δH (400 MHz, CDCl3): 7.59–7.55 (2 H, m, ArH), 7.50–7.29 (21 H, m, ArH), 7.24–7.21 (2 H, m, ArH), 5.77 (1 H, d, J = 0.8 Hz, H-1), 5.05 (1 H, d, J = 3.6 Hz, H-1’), 4.95 (1 H, d, J = 10.4 Hz, CH2Ar), 4.91 (1 H, d, J = 4.0 Hz, H-2), 4.89 (1 H, d, J = 10.4 Hz, CH2Ar), 4.84 (1 H, d, J = 11.2 Hz, CH2Ar), 4.79 (1 H, d, J = 12.0 Hz, CH2Ar), 4.72 (1 H, d, J = 12.0 Hz, CH2Ar), 4.70 (1 H, d, J = 12.0 Hz, CH2Ar), 4.68 (1 H, d, J = 4.0 Hz, H-5), 4.59 (1 H, d, J = 10.8 Hz, CH2Ar), 4.53 (1 H, d, J = 12.0 Hz, CH2Ar), 4.13 (1 H, dd, J = 4.0, 2.4 Hz, H-4), 4.05 (1 H, dd, J = 4.0, 2.4 Hz, H-3), 3.97–3.87 (3 H, m, H-3’, H-5’, H-6’), 3.84–3.79 (2 H, m, H-4’, H-6’), 3.47 (1 H, dd, J = 10.4, 4.0 Hz, H-2’); δC (101 MHz, CDCl3): 167.0 (C=O), 138.0 (Ar C), 137.8 (Ar C), 136.5 (Ar C), 132.7 (Ar C), 132.5 (Ar CH), 129.3 (Ar CH), 128.8 (Ar CH), 128.6 (Ar CH), 128.5 (2 x Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 100.4 (C-1’), 81.0 (C-1, C-4), 79.9 (C-3’), 77.9 (C-4’), 77.8 (C-3), 76.6 (C-2), 75.5 (CH2Ar), 75.0 (CH2Ar), 73.6 (CH2Ar), 72.5 (CH2Ar), 71.9 (C-5’), 70.4 (C-5), 68.0 (C-6’), 63.2 (C-2’).

Data for β-linked β-298: Rf 0.40 (3:1 hexane/EtOAc); IR (νmax/cm−1): 29867 (C–H), 2110 (N3), 1788 (C=O); MS (ES+) found m/z 838 [M+Na]+, HRMS found m/z 838.2759, C46H43N3NaO9S [M+Na]+ requires 838.2769; [α]D = +58.9 (c = 0.50, CH2Cl2); δH (400 MHz, CDCl3): 7.45 (2 H, dd, J = 7.5, 2.0 Hz, ArH), 7.32–7.18 (21 H, m, ArH), 7.14–7.11 (2 H, m, ArH), 5.64 (1 H, d, J = 0.8 Hz, H-1), 4.84 (1 H, d, J = 10.7 Hz, CH2Ar), 4.79–4.74 (3 H, m, H-2, 2 x CH2Ar), 4.69 (1 H, d, J = 11.8 Hz, CH2Ar), 4.52 (1 H, d, J = 11.6 Hz, CH2Ar), 4.51 (1 H, d, J = 10.8 Hz, CH2Ar), 4.44–4.39 (3 H, m, H-5, 2 x CH2Ar), 4.35 (1 H, d, J = 7.7 Hz, H-1’), 4.32 (1 H, dd, J = 4.0, 1.9 Hz, H-4), 4.02 (1 H, dd, J = 4.0, 2.0 Hz, H-3), 3.66–3.58 (3 H, m, H-4’, 2 x H-6’), 3.41–3.31 (3 H, m, H-2’, H-3’, H-5’); δC (101 MHz, CDCl3): 166.2 (C=O), 138.0 (2 x Ar C), 137.8 (Ar C), 137.7 (Ar C), 136.7 (Ar C), 132.7 (Ar C), 132.5 (Ar CH), 129.2 (Ar CH), 214
128.7 (Ar CH), 128.6 (Ar CH), 128.5 (2 x Ar CH), 128.4 (Ar CH), 128.2 (Ar CH), 128.1 (2 x Ar CH), 128.0 (3 x Ar CH), 127.8 (2 x Ar CH), 101.1 (C-1’), 82.9 (CH), 81.3 (C-1), 77.4 (CH), 77.3 (CH), 77.2 (CH), 76.6 (CH), 75.7 (CH2Ar), 75.3 (CH), 75.2 (CH2Ar), 75.1 (CH2Ar), 72.1 (CH2Ar), 69.3 (CH), 68.4 (C-6’), 66.3 (CH).

Phenyl (2-azido-3,4,6-tri-O-benzyl-2-deoxy-α-d-glucopyranosyl)-(1→4)-methyl 2-O-acetyl-3-O-benzyl-1-thio-α/β-L-idopyranosiduronate α/β-300

Disaccharide α/β-297 (697 mg, 0.85 mmol, ~1:2 α/β) was dissolved in anhydrous CH2Cl2/MeOH (8 mL, 1:1) and NaOMe (15 µL, 25–30 wt% in MeOH) was added. The reaction was closely monitored by TLC to reduce impurity formation and was quenched at 3 h by addition of AcOH. The solvent was removed to give 2-OH intermediate 298 as a white foam, which was used in the acetylation step without further purification. This intermediate was dissolved in anhydrous CH2Cl2 (8 mL), Ac2O (159 µL, 1.70 mmol), 1-methyl-imidazole (135 µL, 1.69 mmol) and DMAP (10 mg, 0.085 mmol) were added and the reaction was stirred for 2 h. The solvent was removed in vacuo and the residue purified by column chromatography (100:1 toluene/acetone) to afford the title compound α/β-300 as a white foam (688 mg, 0.77 mmol, 91%, ~1:1.75 α/β). The separate anomers α-300 and β-300 were isolated in separate, lower-yielding procedures using single anomers of disaccharide starting material. A sample of 2-OH intermediate α-299 was obtained by silica plug purification (1:1 hexane/ EtOAc) of methanalysis using α-297 as starting material.

Data for 2-OH intermediate α-299: Rf 0.50 (3:1 cyclohexane/ EtOAc); IR (νmax/cm⁻¹): 3488 (O–H), 2917 (C–H), 2109 (N₁) 1737 (C=O); MS ES⁺ found m/z 865 [M+NH₄]⁺, HRMS found m/z 865.3477, C₄₇H₅₃N₄O₁₀S [M+NH₄]⁺ requires 865.3467; [α]D = −27.6 (c = 1.45, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.46–7.43 (2 H, m, ArH), 7.37–7.30 (4 H, m, ArH), 7.28–7.17 (17 H, m, ArH), 7.07–7.05 (2 H, m, ArH), 5.62 (1 H, s, H-1), 5.32 (1 H, d, J = 1.6 Hz, H-5), 4.96 (1 H, d, J = 3.8 Hz, H-1’), 4.74 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.72–4.69 (3 H, m, 3 x CH₂Ar), 4.54 (2 H, m, 2 x CH₂Ar), 4.43 (1 H, d, J =
11.4 Hz, CH₂Ar), 4.38 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.19 (1 H, t, J = 1.5 Hz, H-4), 4.03 (1 H, dt, J = 11.7, 1.3 Hz, H-2), 3.95 (1 H, d, J = 11.7 Hz, OH), 3.84 (1 H, t, J = 2.4 Hz, H-3), 3.73 (1 H, app q, J = 8.5 Hz, H-3′), 3.77 (3 H, s, CO₂CH₃), 3.64–3.67 (2 H, m, H-4′, H-6′), 3.54–3.58 (2 H, m, H-2′, H-6′), 3.50 (1 H, dt, J = 9.8, 2.1 Hz, H-5′); δc (101 MHz, CDCl₃): 169.3 (C=O), 138.2 (Ar C), 137.7 (Ar C), 137.5 (Ar C), 137.0 (Ar C), 136.2 (Ar C), 130.8 (Ar C), 129.0 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.4 (2 x Ar CH), 128.2 (Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 127.2 (Ar CH), 94.9 (C-1′), 89.8 (C-1), 81.2 (C-3′), 77.5 (C-4) 75.9 (CH₂Ar), 74.7 (CH₂Ar), 73.6 (CH₂Ar), 72.6 (CH₂Ar), 71.7 (CH), 71.5 (CH), 70.9 (C-3), 68.6 (C-2), 68.0 (C-5), 67.7 (C-6′), 63.6 (C-2′), 52.6 (CO₂CH₃).

Data for α-300: Rf 0.55 (3:1 cyclohexane/EtOAc); IR (νmax/cm⁻¹): 2923 (C–H), 2105 (N₃), 1764 (C=O), 1736 (C=O); MS (ES⁺) found m/z 907 [M+NH₄]+, HRMS found m/z 907.3583, C₄₉H₅₃N₄O₁₁S [M+NH₄]+ requires 907.3583; [α]D = −6.1 (c = 13.15, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.52–7.49 (2 H, m, ArH), 7.43–7.27 (21 H, m, ArH), 7.16–7.13 (2 H, m, ArH), 5.63 (1 H, d, J = 1.2 Hz, H-1), 5.27 (1 H, d, J = 2.4 Hz, H-5), 5.20–5.18 (1 H, m, H-2), 4.90 (1 H, d, J = 3.2 Hz, H-1′), 4.86 (1 H, d, J = 11.6 Hz, CH₂Ar), 4.80 (1 H, d, J = 10.4 Hz, CH₂Ar), 4.77 (1 H, d, J = 11.2 Hz, CH₂Ar), 4.76 (1 H, d, J = 10.4 Hz, CH₂Ar), 4.68 (1 H, d, J = 11.6 Hz, CH₂Ar), 4.59 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.51 (1 H, d, J = 11.6 Hz, CH₂Ar), 4.45 (1 H, d, J = 12.0 Hz, CH₂Ar), 4.12 (1 H, t, J = 2.8 Hz, H-4), 3.99 (1 H, td, J = 3.2, 0.7 Hz, H-3), 3.86–3.78 (2 H, m, H-3′, H-5′), 3.77–3.69 (2 H, m, H-4′, H-6′), 3.74 (3 H, s, CO₂CH₃), 3.64 (1 H, dd, J = 11.0, 1.8 Hz, H-6′), 3.37 (1 H, dd, J = 10.2, 3.2 Hz, H-2′), 2.13 (3 H, s, C(O)CH₃); δc (101 MHz, CDCl₃): 170.2 (C=O), 169.3 (C=O), 138.3 (Ar C), 137.8 (Ar C), 137.7 (Ar C), 137.1 (Ar C), 135.4 (Ar C), 131.2 (Ar CH), 129.0 (Ar CH), 128.6 (Ar CH), 128.5 (Ar CH), 128.4 (Ar CH), 128.3 (Ar CH), 128.1 (Ar CH), 128.0 (2 x Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 127.6 (Ar CH), 127.5 (Ar CH), 127.3 (Ar CH), 97.8 (C-1′), 86.2 (C-1), 80.1 (CH), 77.9 (C-4′), 75.4 (CH₂Ar), 74.6 (CH₂Ar), 73.6 (CH₂Ar), 73.3 (C-4), 72.8 (CH₂Ar), 71.8 (C-3), 71.6 (CH), 68.9 (C-5), 68.7 (C-2), 67.8 (C-6′), 63.5 (C-2′), 52.3 (CO₂CH₃), 21.0 (C(O)CH₃).

Chapter Three: Experimental

Data for β-300: Rf 0.39 (3:1 cyclohexane/EtOAc); IR (νmax/cm⁻¹): 2924 (C–H), 2106 (N₃), 1768 (C=O), 1736 (C=O); MS (ES⁺) found m/z 907 [M+NH₄]+, HRMS found m/z 907.3585, C₄₉H₅₅N₄O₁₁S [M+NH₄]+ requires 907.3583; [α]D = +61.5 (c = 0.65,
CH$_2$Cl$_2$); $\delta_{1}H$ (400 MHz, CDCl$_3$): 7.60–7.58 (2 H, m, ArH), 7.19 (2 H, dd, $J$ = 7.8, 1.7 Hz, ArH), 5.18 (1 H, d, $J$ = 1.8 Hz, H-1), 5.14 (1 H, t, $J$ = 1.6 Hz, H-2), 4.85 (1 H, d, $J$ = 10.5 Hz, CH$_2$Ar), 4.82–4.75 (4 H, m, H-1’, 3 x CH$_2$Ar), 4.70 (1 H, d, $J$ = 11.8 Hz, CH$_2$Ar), 4.62 (1 H, d, $J$ = 12.0 Hz, CH$_2$Ar), 4.57–4.54 (2 H, m, H-5, CH$_2$Ar), 4.48 (1 H, d, $J$ = 10.1, 8.7 Hz, H-3’), 3.91 (1 H, dd, $J$ = 10.8, 1.9 Hz, H-6’), 3.43 (1 H, dd, $J$ = 10.2, 3.6 Hz, H-2’), 2.21 (3 H, s, C(O)C$_2$H$_5$)

13.1.26 Synthesis of Acceptor 301 and 2-OAc Tetrasaccharide 303

Phenyl (2-azido-3,6-di-O-benzyl-2-deoxy-a-D-glucopyranosyl)-(1→4)-3-O-benzyl-1-thio-β-L-idopyranosidurono-2,6-lactone 301

Glycosyl donor 174 (624 mg, 1.31 mmol) and glycosyl acceptor β-270 (940 mg, 2.62 mmol) were combined in anhydrous CH$_2$Cl$_2$ (18 mL) under argon and the solution cooled to 0 °C. Molecular sieves (~600 mg, 4 Å, powdered, activated) were added, and the reaction was stirred for 10 min. NIS (398 mg, 1.77 mmol) was added, and the reaction was stirred for 10 min at 0 °C. AgOTf (16 mg, 0.062 mmol) was added and the reaction was stirred for 20 min warming to RT. $^1$H NMR showed no remaining glycosyl donor. The reaction was filtered through Celite with CH$_2$Cl$_2$ (100 mL), saturated aq. Na$_2$S$_2$O$_3$ (20 mL) was added and the phases were mixed vigorously and then separated. The organic phase was dried and the solvent removed in vacuo to give a yellow oil. Column chromatography (100:1, 30:1 toluene/acetone) afforded the title compound 301.
as a yellow foam (548 mg, 0.76 mmol, 58%) plus recovered acceptor $\beta$-270 (538 mg, 1.50 mmol, 80% of unreacted amount). Data for product 301: $R_f$ 0.53 (2:1 cyclohexane/EtOAc); IR ($\nu_{\text{max}}$/cm$^{-1}$): 3512 (O–H), 2923 (C–H), 2104 (N$_3$), 1783 (C=O); MS (ES$^+$) found $m/z$ 743 [M+NH$_4$]$^+$, HRMS found $m/z$ 743.2747, C$_{39}$H$_{43}$N$_4$O$_9$S [M+NH$_4$]$^+$ requires 743.2745; $[\alpha]_D = -2.3$ (c = 0.35, CH$_2$Cl$_2$); $\delta$ H (400 MHz, CDCl$_3$): 7.51–7.48 (2 H, m, Ar H), 7.42–7.28 (18 H, m, Ar H), 5.69 (1 H, d, $J = 0.8$ Hz, H-1), 4.94 (1 H, d, $J = 4.0$ Hz, H-1'), 4.88 (1 H, d, $J = 11.2$ Hz, CH$_2$Ar), 4.86–4.82 (2 H, m, H-2, CH$_2$Ar), 4.73 (1 H, d, $J = 12.0$ Hz, CH$_2$Ar), 4.66–4.63 (2 H, m, H-2, CH$_2$Ar), 4.64 (1 H, d, $J = 12.0$ Hz, CH$_2$Ar), 4.57 (1 H, d, $J = 4.0$ Hz, H-5), 4.55 (1 H, d, $J = 12.0$ Hz, CH$_2$Ar), 4.05 (1 H, dd, $J = 4.0$, 2.2 Hz, H-4), 4.01 (1 H, dd, $J = 4.0$, 2.2 Hz, H-3), 3.87–3.72 (5 H, m, H-3', H-4', H-5', 2 x H-6'), 3.34 (1 H, $J = 10.0$, 3.6 Hz, H-2'), 2.41 (1 H, d, $J = 2.8$ Hz, OH); $\delta$ C (101 MHz, CDCl$_3$): 167.0 (C=O), 137.9 (Ar C), 137.7 (Ar C), 136.4 (Ar C), 132.6 (Ar C) 132.5 (Ar CH), 129.2 (Ar CH), 128.7 (Ar CH), 128.6 (Ar CH), 128.5 (2 x Ar CH), 128.1 (Ar CH), 128.0 (Ar CH), 127.8 (2 x Ar CH), 100.3 (C-1'), 81.0 (C-1), 80.9 (C-4), 79.3 (CH) , 77.9 (C-3), 76.5 (C-2), 75.2 (CH$_2$Ar), 73.7 (CH$_2$Ar), 72.5 (CH$_2$Ar), 72.0 (CH), 71.0 (CH), 70.4 (C-5), 69.3 (C-6'), 62.6 (C-2'). N.B. Formation of trisaccharide 302 was observed by mass spectrometry (found $m/z$ 1110.4299, C$_{59}$H$_{64}$N$_7$O$_{13}$S [M+NH$_4$]$^+$ requires 1110.4277).

Phenyl (2-azido-3,4,6-tri-O-benzyl-2-deoxy-4-$\alpha$-$\beta$-glucopyranosyl)-(1$\rightarrow$4)-(methyl-2-O-acetyl-3-$\alpha$-$\beta$-benzyl-1-thio-$\beta$-$\beta$-idopyranosidurono-2,6-lactone 303

![Chemical Structure](image_url)

Glycosyl donor $\alpha$/$\beta$-300 (235 mg, 0.26 mmol) and glycosyl acceptor 301 (153 mg, 0.21 mmol) were combined in anhydrous CH$_2$Cl$_2$ (5 mL) under argon and the solution cooled to 0 °C. Molecular sieves (~400 mg, 4 Å, powdered, activated) were added, and
the reaction was stirred for 10 min. NIS (74 mg, 0.33 mmol) was added, and the reaction was stirred for 10 min at 0 °C. AgOTf (2.7 mg, 0.011 mmol) was added and the reaction was stirred for 3 h warming to RT. The reaction mixture was filtered through Celite with CH₂Cl₂ (50 mL) and the solvent removed in vacuo to give a red oil. Column chromatography (60:1 toluene/acetone) afforded the title compound 303 as a white foam (111 mg, 0.074 mmol, 35%); Rf 0.18 (3:1 cyclohexane/EtOAc); IR (νmax/cm⁻¹): 2926 (C–H), 2106 (N₃), 1767 (C=O), 1736 (C=O); MS (ES⁺) found m/z 1522 [M+NH₄]⁺, HRMS found m/z 1522.5787, C₈₂H₈₈N₇O₂₀S [M+NH₄]⁺ requires 1522.5799; [α]D = +50.6 (c = 1.85, CH₂Cl₂); δH (400 MHz, CDCl₃): 7.51–7.49 (4 H, m, ArH), 7.38–7.27 (32 H, m, ArH), 7.18–7.14 (4 H, m, ArH), 7.18 (1 H, d, J = 0.8 Hz, H-1), 5.69 (1 H, d, J = 4.2 Hz, H-2), 4.57–4.81 (11 H, m, H-5, H-1’’’), 4.45 (1 H, d, J = 12.1 Hz, CH₂Ar), 4.30 (1 H, d, J = 1.3 Hz, H-5’’), 4.15–4.23 (3 H, m, H-2’’, 2 x CH₂Ar), 4.06–4.00 (2 H, m, H-3, H-4), 3.93–3.92 (1 H, m, H-4’’’, 2 x H-6’’, CO₂CH₃), 3.86–3.60 (14 H, m, H-3’, H-4’, H-5’, 2 x H-6’, H-3’’, H-3’’’, H-4’’’, 2 x H-6’’’, CO₂CH₃), 3.48–3.44 (1 H, m, H-2’’), 3.33 (1 H, dd, J = 9.8, 3.8 Hz, H-2’’’), 1.83 (3 H, s, C(O)CH₃); δC (101 MHz, CDCl₃): 168.6 (C=O), 167.0 (C=O), 138.5 (Ar C), 138.3 (Ar C), 137.8 (Ar C), 137.6 (Ar C), 137.2 (Ar C), 136.5 (Ar C), 132.8 (Ar C), 132.4 (Ar CH), 129.1 (Ar CH), 129.0 (Ar CH), 128.7 (Ar CH), 128.5 (Ar CH), 128.4 (3 x Ar CH), 128.3 (Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 128.1 (Ar CH), 128.0 (3 x Ar CH), 127.9 (Ar CH), 127.8 (Ar CH), 127.7 (Ar CH), 127.5 (2 x Ar CH), 127.4 (Ar CH), 127.1 (Ar CH), 125.3 (Ar CH), 124.0 (Ar CH), 100.0 (C-1’’’), 97.9 (C-1’’’), 97.1 (C-1’’), 80.9 (CH), 80.7 (C-1), 78.2 (CH), 78.1 (CH), 77.8 (CH), 76.6 (CH), 76.5 (C-2), 75.5 (CH₂Ar), 75.4 (CH₂Ar), 74.8 (CH₂Ar), 73.4 (CH₂Ar), 73.3 (CH₂Ar), 72.8 (C-4’’’), 72.6 (CH₂Ar), 72.5 (CH₂Ar), 72.3 (CH), 71.9 (CH), 71.7 (CH), 71.5 (CH), 70.8 (C-5’’’), 70.2 (C-5), 68.6 (C-6), 67.8 (C-6), 63.9 (C-2’’’), 63.2 (C-2’’’’), 52.4 (CO₂CH₃), 27.0 (C(O)CH₃).
13.1.27 Synthesis of Tetrasaccharide 304 from Donor Disaccharide 254

Phenyl (2-azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-methyl 3-O-benzyl-2-O-benzoyl-1-thio-α/β-L-idopyranosiduronate 254

*From methanolysis and O-2 benzoylation of lactone-containing disaccharide α/β-297:*

![Chemical Structure](image)

To a stirred solution of α/β-297 (460 mg, 0.564 mmol, ~1:1.7 α/β) in dry MeOH/CH₂Cl₂ (4 mL, 1:1) was added NaOMe in MeOH (0.6 mL, 0.18 M). After stirring for 2 h, AcOH (0.6 mL of a 0.175 M solution in CH₂Cl₂) was added and the solvents removed in vacuo to give the intermediate 2-OH disaccharide as a white foam which was used without further purification. The intermediate was dissolved in dry DCE (8 mL), DMAP (15 mg, 0.12 mmol), 1-methyl-imidazole (0.10 mL, 1.25 mmol) and BzCl (0.15 mL, 1.29 mmol) were added and the reaction was stirred for 3.5 h at RT. The solvents were removed in vacuo and the crude product purified by column chromatography to give α/β-254 as a white foam as a mixture of anomers (404 mg, 0.424 mmol, 75%, ~1:1.4 α/β). The anomers were partially separable by column chromatography allowing pure samples of each anomer to be obtained (for α-254 see Section 13.1.15).

Data for β-254: R₇ 0.56 (10:1 toluene/acetone); IR (ν_max/cm⁻¹): 2911 (C–H), 2106 (N₃), 1768 (C=O), 1720 (C=O); MS (ES⁺) found m/z 970 [M+NH₄]⁺, HRMS found m/z 974.3283, C₅₅H₅₃N₃NaO₁₁S [M+Na]⁺ requires 974.3293; [α]D = +45.3 (c = 0.33, CH₂Cl₂); δH (400 MHz, CDCl₃): 8.28–8.25 (2 H, m, ArH), 7.65–7.61 (2 H, m, ArH), 7.47–7.26 (22 H, m, ArH), 7.23–7.19 (2 H, m, ArH), 7.13–7.10 (2 H, m, ArH), 5.35 (1 H, d, J = 1.9 Hz, H-1), 5.33–5.32 (1 H, m, H-2), 4.93 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.83 (1 H, d, J = 11.7 Hz, CH₂Ar), 4.73 (1 H, d, J = 3.5 Hz, H-1’), 4.69–4.66 (2 H, m, 2 x CH₂Ar), 4.61 (1 H, d, J = 1.6 Hz, H-5), 4.55–4.50 (2 H, m, 2 x CH₂Ar), 4.38 (1 H, t, J = 2.6 Hz, H-3), 4.07–4.06 (1 H, m, H-4), 4.04 (1 H, d, J = 10.6, CH₂Ar), 4.00–3.97 (1 H, m, H-5’), 3.88 (1 H, dd, J = 10.9, 2.2 Hz, H-6’), 3.80 (3 H, s, CO₂CH₃), 3.78–3.75 (2 H, m, H-6’, CH₂Ar), 3.70 (1 H, t, J = 9.5 Hz, H-4’), 3.49 (1 H, dd, J = 10.2, 9.0 Hz, H-3’),
3.28 (1 H, dd, \(J = 10.3, 3.5\) Hz, H-2'); \(\delta_c\) (101 MHz, CDCl\(_3\)): 168.5 (C=O), 166.1 (C=O), 138.4 (Ar C), 137.9 (Ar C), 137.0 (Ar C), 134.9 (Ar C), 133.3 (Ar CH), 131.2 (2 x Ar CH), 129.6 (Ar CH), 129.1 (Ar CH), 128.9 (Ar CH), 128.6 (Ar CH), 128.4 (2 x Ar CH), 128.3 (2 x Ar CH), 128.2 (Ar CH), 128.1 (Ar CH), 127.8 (2 x Ar CH), 127.7 (2 x Ar CH), 127.6 (Ar CH), 100.1 (C-1'), 85.9 (C-1), 80.2 (C-3'), 77.7 (C-4'), 75.8 (C-5), 75.7 (C-4), 74.8 (CH\(_2\)Ar), 74.6 (CH\(_2\)Ar), 73.6 (CH\(_2\)Ar), 73.2 (C-3), 73.0 (CH\(_2\)Ar), 71.7 (C-5'), 70.0 (C-2), 67.8 (C-6'), 63.8 (C-2'); 52.3 (CO\(_2\)C\(_3\)H\(_3\)).

Phenyl (2-azido-3,4,6-tri-O-benzyl-2-deoxy-4-\(\alpha\)-\(\delta\)-glucopyranosyl)-(1→4)-(methyl 2-O-benzoyl-3-O-benzyl-\(\alpha\)-\(\delta\)-idopyranosyluronate)-(1→4)-(2-azido-3,6-di-O-benzyl-2-deoxy-\(\alpha\)-\(\delta\)-glucopyranosyl)-(1→4)-3-O-benzyl-1-thio-\(\beta\)-\(\delta\)-idopyranosidurono-2,6-lactone 304

Glycosyl donor \(\alpha/\beta\)-254 (235 mg, 0.26 mmol), glycosyl acceptor 301 (153 mg, 0.21 mmol) and molecular sieves (~400 mg, 4 Å, powdered, activated) were combined in a flask and purged with argon. Anhydrous CH\(_2\)Cl\(_2\) (2.1 mL) was added and the solution was cooled to \(-25\) °C and stirred for 10 min. NIS (28 mg, 0.12 mmol) was added, and the reaction was stirred for 10 min at \(-25\) °C. AgOTf (1 mg, 4 \(\mu\)mol) was added and the reaction was stirred for 3 h warming to RT. The reaction mixture was filtered through Celite with CH\(_2\)Cl\(_2\) (50 mL) and saturated aq. Na\(_2\)S\(_2\)O\(_3\) (50 mL) was added. The phases were mixed vigorously, then separated and the aqueous phase washed with CH\(_2\)Cl\(_2\) (5 x 20 mL). The combined organic phase was dried and the solvent was removed \textit{in vacuo}. Column chromatography (50:1 toluene/acetone) afforded the title compound \(\beta\)-304 as a white foam (95 mg, 0.061 mmol, 77%); \(R_f\) 0.48 (10:1 toluene/acetone); IR (\(\nu_{\text{max}}/\text{cm}^{-1}\))): 2867 (C–H), 2106 (N\(_3\)) 1787 (C=O), 1764 (C=O); MS (ES\(^+\)) found \(m/z\) 1584 [M+NH\(_4\)]\(^+\), HRMS found \(m/z\) 1589.5501, \(C_{87}H_{86}N_{16}NaO_{20}S\) [M+Na]\(^+\) requires 1589.5510; \([\alpha]_D = +54.1\) (c = 1.40, CH\(_2\)Cl\(_2\)); \(\delta_h\) (400 MHz, CDCl\(_3\)): 8.07 (2 H, dd, \(J =

221
8.2, 1.1 Hz, ArH), 7.58–7.55 (2 H, m, ArH), 7.50–7.48 (2 H, m, ArH), 7.19 (39 H, m, ArH), 7.57 (1 H, d, J = 0.8 Hz, H-1), 5.50 (1 H, d, J = 1.2 Hz, H-1’’), 5.22 (1 H, t, J = 2.5 Hz, H-2’’), 5.00 (1 H, d, J = 3.9 Hz, H-1’), 4.97–4.88 (5 H, m, H-2, H-1’’), 3 x CH2Ar), 4.85 (1 H, d, J = 11.2 Hz, CH2Ar), 4.78 (1 H, d, J = 11.8 Hz, CH2Ar), 4.74 (1 H, d, J = 10.8 Hz, CH2Ar), 4.72–4.65 (3 H, m, 3 x CH2Ar), 4.64 (1 H, d, J = 4.0 Hz, H-5’’), 4.59–4.48 (4 H, m, 4 x CH2Ar), 4.29–4.25 (2 H, m, H-3’’, H-5), 4.14–4.08 (5 H, m, H-3, H-4, H-4’, H-4’’, CH2Ar), 3.96 (1 H, d, J = 10.0 Hz, H-5’’), 3.90 (1 H, dt, J = 9.9, 2.4 Hz, H-5’’’’), 3.85 (1 H, dd, J = 10.8, 2.2 Hz, H-6’’’’), 3.81–3.73 (4 H, m, H-3’, H-4’’, 2 x H-6’), 3.71 (1 H, dd, J = 10.8, 1.7 Hz, H-6’’’’), 3.61 (1 H, t, J = 9.6 Hz, H-3’’’’), 3.48 (3 H, s, CO2CH3), 3.45 (1 H, dd, J = 9.2, 5.4 Hz, H-2’), 3.28 (1 H, dd, J = 10.3, 3.5 Hz, H-2’’’’); δC (101 MHz, CDCl3): 169.0 (C=O), 166.9 (C=O), 165.4 (C=O), 138.3 (Ar C), 137.9 (2 x Ar C), 137.8 (Ar C), 137.6 (Ar C), 137.4 (Ar C), 136.4 (Ar C), 133.3 (Ar CH), 132.7 (Ar CH), 132.4 (Ar CH), 130.0 (Ar CH), 129.5 (Ar CH), 129.3 (Ar CH), 129.1 (Ar CH), 128.8 (Ar CH), 128.6 (Ar CH), 128.5 (2 x Ar CH), 128.4 (2 x Ar CH), 128.3 (Ar CH), 128.2 (2 x Ar CH), 128.1 (Ar CH), 128.1 (Ar CH), 127.9 (Ar CH), 127.8 (2 x Ar CH), 127.7 (2 x Ar CH), 127.6 (Ar CH), 127.4 (Ar CH), 125.4 (Ar CH), 100.2 (C-1’), 100.0 (C-1’’’’), 98.4 (C-1’’’’), 81.1 (C-1), 80.9 (CH), 80.0 (C-3’’’’), 78.3 (CH), 77.8 (CH), 77.6 (CH), 76.5 (CH), 75.9 (CH), 74.9 (CH2Ar), 74.7 (CH2Ar), 74.5 (CH), 73.7 (CH), 73.6 (CH2Ar), 72.9 (CH2Ar), 72.5 (CH2Ar), 71.9 (C-5’), 71.7 (C-5’’’’), 70.3 (C-5), 68.4 (C-2), 68.2 (C-6’), 68.2 (CH), 67.7 (C-6’’’’), 63.7 (C-2’’’’), 63.4 (C-2’), 51.9 (CO2CH3).

13.2 Fluorescence Measurements
13.2.1 Steady-state Ensemble Spectroscopy
Absorption and emission spectra were acquired on Cary 60 (Agilent Technologies) and Fluoromax (HORIBA Scientific) spectrometers respectively. Emission spectra were recorded under magic angle conditions and background fluorescence from the solvent was negligible. Samples were prepared in 20 mM Tris, 10 mM MgCl2, pH 7.5 buffer.

The labelled ssDNA sequence used for comparison of absorption and emission spectra was 5’ TGG CGA CGG CAG CGA GGC TTA GCG GCA AAA AAA AAA AAA AAA AAA AAA AAA AGC CGC X, where X = T-(Alexa Fluor 488) at position 64 (Purimex GmbH, Germany).
13.2.2 Fluorescence Correlation Spectroscopy

FCS measurements were performed on the same confocal microscope used for MFD measurements. Samples were prepared in Nunc Lab-Tek II chambered cover glasses (4 wells, VWR) and excited via the linearly polarised light of a pulsed 488 nm laser (Becker & Hickl). The laser light was focused into the sample solution by a water immersion objective lens (UPLAPO 60x, numerical aperture = 1.2, Olympus, UK) mounted in an inverted microscope (IX-71, Olympus, UK). The average laser power at the sample was ca. 170 µW. As each molecule traverses the detection volume, a burst of fluorescence photons is generated. The emission photons were subsequently divided into parallel and perpendicular components via a polarising beamsplitter (Linos, Germany) and then into wavelength ranges above and below 595 nm using a dichroic beamsplitter (z595 DCXR, Chroma, USA) before being detected using four avalanche photodiodes (PDM50C, MPD, Italy, for emission wavelengths <595 nm (green) and SPCM-AQRH-14, Perkin Elmer, USA, for emission wavelengths >595 nm (red)). For the FCS measurements reported, the use of two APDs along the <595 nm emission path allowed simultaneous independent measurement of parallel and perpendicular components of the fluorescence (relative to the excitation beam). The signals from both detectors were routed to a digital autocorrelator (ALV-7002, ALV GmbH, Germany) connected to a computer. Five million photons were typically collected for each correlation measurement with count rates of ca. 100 kHz. All measurements are reported for a temperature of 22±1 °C. Autocorrelation functions, G(t), were fitted to Equation 7. Samples were prepared in 20 mM Tris, 10 mM MgCl$_2$, pH 7.5 buffer. FGF-1 was used as supplied by PeproTech (UK).

$$G(t) = C + \left(\frac{1}{N}\right) \cdot \left(\frac{1}{1 + \frac{t}{|D|}}\right) \cdot \left(\frac{1}{\sqrt{1 + \frac{t}{V^2}} \cdot |D|}\right) \cdot \left(1 - |t_f| + t_f \cdot e^{-\frac{t}{t_s}}\right)$$

**Equation 7.** Fitting equation for autocorrelation functions in FCS measurements, where $C$ is a constant, $N$ is the number of molecules in the confocal volume, $D$ is the diffusion time, $V$ is a measure of the detection volume defined as $z_0/w_0$, where $z_0$ is the Rayleigh range and $w_0$ is the beam waist, $t_f$ is the triplet fraction and $t_s$ is the triplet lifetime.

13.2.3 Multi-parameter Confocal Fluorescence Spectroscopy

For single-molecule measurements in solution, a home-built multiparameter fluorescence detection (MFD) setup was used, which is based around a confocal
microscope with photon-counting detection (Becker and Hickl) and pulsed laser excitation, allowing the simultaneous measurement of fluorescence intensity, colour, lifetime and polarisation. The Alexa Fluor 488 fluorophore was excited by a linearly polarised laser (480 nm, 40 MHz, ~ 60 ps FWHM; Picoquant, Germany). The laser light was focused into the dilute solution of labelled molecules by a water immersion objective (UPLAPO 60x, S4 numerical aperture = 1.2, Olympus, UK). The diffusion correlation time for Rhodamine 110 was ca. 200 µs. The average laser power at the sample was ca. 180 µW. Each molecule generates a burst of fluorescence photons as it traverses the detection volume. This photon train is divided into parallel and perpendicular components via a polarising beamsplitter (Linos, Germany) and then into wavelength ranges above and below 595 nm by using a dichroic beamsplitter (z595 DCXR, Chroma, USA). Additionally, red (HQ 710/130 nm, AHF Analysentechnik, Germany) and green (HQ 525/50 nm, Chroma, USA) bandpass filters in front of the detectors ensure that only fluorescence photons coming from the acceptor and donor molecules are registered. Detection is performed using four avalanche photodiodes (SPCM-AQRH-14, Perkin Elmer, USA for the red and PDM50C, MPD, Italy for the green). The signals from all detectors are routed, via delay lines, to a time-correlated single photon-counting board (SPC 132, Becker and Hickl, Germany) connected to a computer. Bursts of fluorescence photons are distinguished from the background of 1–2 kHz by applying threshold intensity criteria (0.1 ms interphoton time and 100 photons minimum per burst). All measurements were recorded in 20 mM Tris, 10 mM MgCl₂, 1 mM vitamin C, pH 7.5 buffer at 21±1°C. Measurement buffers were cleaned using activated charcoal prior to use. Data analysis for MFD used software written by the group of Prof. Claus Seidel (Heinrich Heine Universität, Düsseldorf). Sample dilutions were prepared in Nunc Lab-Tek II chambered cover glasses (ThermoFisher, UK).

13.2.4 Encapsulation of Disaccharide in Lipid Vesicles

Small unilamellar vesicles were prepared by the extrusion method. Briefly, a solution of 98 mol% L-α-phosphatidylcholine (Egg, Chicken) (Egg-PC) and 2 mol% 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotinyl) (sodium salt) (Biotinyl-PE) in chloroform was dried using a flow of nitrogen. All lipids were purchased from Avanti Polar Lipids, USA. The dried lipid film was stored under vacuum for 3–4 hours after which it was rehydrated with 20 mM Tris, 6% w/v D-glucose, 0.04 mg mL⁻¹ catalase, 1 mg mL⁻¹ glucose oxidase, 2 mM 6-hydroxy-
2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), pH 8 buffer. For single-molecule encapsulation, 100 nM disaccharide 178 was included. For multi-molecule encapsulation >500 nM disaccharide 178 was added. After mixing of the hydrated lipids (10 mg mL\(^{-1}\)) unilamellar vesicles were prepared with a miniextruder (Avanti Polar Lipids, USA) using a polycarbonate membrane of 100 nm pore size. After extrusion, vesicle solutions were stored at 4 °C in hydration buffer prior to use. The vesicle size distribution was measured by dynamic light scattering (Zetasizer, Malvern Instruments, UK).

### 13.2.5 TIRF Microscopy

Single-molecule TIRF imaging was performed using objective-type total-internal reflection fluorescence microscopy, where the labelled disaccharide constructs were encapsulated within Egg-PC vesicles. After cleaning, slides and coverslips were treated with aminosilane (Flurochem) and coated in a monolayer of a 100:1 mixture of MeO-poly(ethylene glycol)-NHS and biotin-poly(ethylene glycol)-NHS (MW = 5000; Iris Biotech, Germany). The biotinylated surfaces were then incubated with 0.2 mg mL\(^{-1}\) neutravidin (Sigma Aldrich) for 10 minutes and subsequently washed with buffer. Biotynlated Egg-PC vesicles were then flushed onto the neutravidin-coated surfaces at a concentration of 0.01 mg mL\(^{-1}\) and allowed to incubate for 10 minutes at room temperature. Prior to use the samples were diluted in 20 mM Tris, 6 % (w/v) D-glucose (Sigma Aldrich), 0.04 mg mL\(^{-1}\) catalase (Sigma Aldrich), 1 mg mL\(^{-1}\) glucose oxidase (Sigma Aldrich) 2 mM 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox, Sigma Aldrich), pH 8 buffer.

All measurements were performed in microfluidic flow cells. Flow cells containing immobilised vesicles were mounted on an inverted microscope (IX71, Olympus). Excitation light (488 nm Stradus (Laser 2000, UK) diode laser) was provided via the evanescent wave of a totally internally reflected beam. For proper angles of incidence, TIR takes place at the interface between the lower coverslip and the sample. The intensity of the evanescent wave decays exponentially upon penetrating the sample, with a decay length of approximately 100–200 nm. Fluorescence emission was collected using a 100 x 1.49 numerical aperture oil-immersion objective lens (Olympus) and separated from scattered excitation via a 500 nm dichroic mirror (Chroma Technology Corp. USA). Fluorescence was subsequently collimated (DV2 Multichannel Imaging System, Photometrics) and imaged onto an electron multiplying
charged coupled device (EMCCD) camera (Evolve, Photometrics). All measurements were performed at room temperature (21±1 °C). Approximately 200 visible spots within the field of view were observed. The fluorescence intensities of the spots were detected using a cooled EMCCD camera (Evolve, Photometrics, UK) and movies were recorded using ImagePro-Plus 7.0 software with an exposure time of 50 ms. All movies were analysed by in-house lab routines written in ImagePro-Plus 7.0. The fluorescence vs. time trajectories were viewed and analysed using MATLAB procedures written in-house. To extract the true number of stepwise photobleaching events, a 1D edge detection algorithm was employed. Briefly, the algorithm constructs a series of scaled derivatives from each raw intensity trajectory before identifying local maxima and minima in the scaled derivatives to identify the presence of sharp edges (bleaching events). By tracking the number of sharp edges identified per trace prior to the fluorescence signal reaching the background intensity, the number of bleaching events occurring in the data was evaluated.
14. REFERENCES


247. G. J. Miller, personal communication.


