Design, synthesis and evaluation of tool compounds for studying endosulfatases



Alice Kennett

St Peter's College

University of Oxford

A thesis submitted for the degree of

Doctor of Philosophy

Hilary 2022

Declaration of authorship

The work presented in this thesis is my own, except where otherwise stated. No part of this thesis has been submitted for any other degree at this or any other university.

Alice Kennett

April 2022

Abstract

The extracellular endo-6-*O*-sulfatases (Sulfs) catalyse the hydrolysis of the glucosamaine 6-*O*-sulfate groups within heparan sulfate (HS).[1] By controlling the sulfation status of HS, the endosulfatases modulate the bioavailability of various signalling ligands that use HS chains to interact with their cell-surface receptors and thus play an important role in the regulation of these pathways. GlcNS-6-sulfamate derivatives have been reported as weak inhibitors of the Sulfs; however, more potent inhibitors of these enzymes are required to probe the biological relevance of endosulfatases in disease models.[2] This work describes the synthesis of tool compounds for targeting endosulfatases for enzymatic and in-cell experiments, which can be categorised into inhibitors, target engagement probes and targeted protein degradation.

Firstly, a small library of putative Sulf inhibitors, based on GlcNS-6-sulfamate monosaccharide were prepared, however when these compounds were evaluated as inhibitors of Sulf-2 only very weak inhibition was observed even at high concentration (<30% at 500 μM). In an attempt to increase the potency of these analogues, the size of the HS-fragment was increased to the disaccharide IdoA(2S)-GlcNS(6Sulfamate). This was synthesised in 15-steps in 3% overall yield. When this disaccharide was evaluated for Sulf-2 inhibition at 1 mM, nearly complete inhibition of the enzyme was observed. The IC_{50} value of this compound was determined to be 42.6 μM ± 18.3.

A multivalent display approach was utilised to improve potency further via altering the presentation of the disaccharide inhibitor to Sulf-2. A glycopolymer was designed with a polynorbornene backbone to allow for maximal control over chain length and low polydispersity. Polymerisation of an NHS-activated norbornene carboxylate monomer through ring-opening metathesis polymerization chemistry followed by an amide coupling

reaction to install the disaccharide inhibitor was found to be superior to polymerisation of a sulphated-disaccharide functionalised norbornene monomer. The glycopolymer with the highest disaccharide loading (20%) was evaluated as a Sulf-2 inhibitor, using the effective molarity of the disaccharide to investigate the multivalent effect and it was found to be approximately 5-fold more potent than the parent disaccharide, (IC_{50} = 9 μM ± 4.75).

Small molecule target engagement probes were designed and synthesised using two photo-affinity techniques for proximity labelling. First, the disaccharide IdoA(2S)-GlcNS(6Sulfamate) was functionalised with a diazirine and a terminal alkyne, using traditional PAL probe design. However, it was anticipated that traditional photoaffinity labelling of Sulf-2 in the extracellular matrix would be challenging, a second approach by the MacMillan group was utilised, which takes advantage of catalytic diazirine activation to achieve greater than single stoichiometric labelling. To this end, the IdoA(2S)-GlcNS(6Sulfamate) was tethered to a cell-impermeable Ir-photocatalyst via a CuAAC reaction. The target engagement probes were investigated in MCF-7 cells, that are reported to express high levels of HSulf-2, and pleasingly the disaccharide-based probes labelled various extracellular proteins. These proteins were successfully off-competed by a high concentration of the 'parent' inhibitor. Identification of these proteins by LC-MS/MS is on-going.

Finally, a probe was designed for targeted protein degradation using LYTAC technology. It was envisioned that the HSulf-2 inhibiting glycopolymer could be end-functionalised with an azide group. It was planned that this would be joined to the poly(M6P-ala) glycopeptide reported by the Bertozzi group via a CuAAC reaction. Synthesis of both end-labelled polymers is on-going and is high priority for future work.

Acknowledgements

I would like to thank Prof. Angie Russell for giving me the opportunity to study for a DPhil in her group, for allowing me to make this project my own and for being a supportive mentor. I also want to thank the EPSRC and OxStem, without their funding this project wouldn't have been possible. Thank you to the CRL staff for all that you do, to the mass spec and NMR staff for your hard work and help. And thank you Romain for the supply of Sulf-2.

Thank you to the AJR group members. I am very grateful to some of the excellent postdocs that have supported me through the past four years: Kate, Liam, Seb and Carole. Thank you all so much. I don't think I could have made it without all of your support. And to the DPhil girls: Isabel, Rachel, Purnima and Ria– I miss you all, the group hasn't been the same this last year. And to Hannah and Jess – thank you for being such wonderful friends, I am truly lucky to have had you in the hood next to me.

I am grateful for all the scientific support and advice I have received. Special shout outs to:

- Carole for all the early morning chats and constant support in G8
- George for always having ideas and suggestions
- Andia for teaching me everything I know about cell culture
- Maarten for enduring the painful Sulf isolation attempts
- Nader for all the NMR advice and for running my VT NMRs
- Sophie and Maryam for guidance for polymer chatacterisation
- Victor for the MS training, especially my MALDI adventure

Finally, to my friends and family – Thank you for encouraging me not to quit every time I suggested it. It has been worth it in the end. Thank you for your love and support, and thank you Mum for trying to fix everything for me (even when you don't understand chemistry).

Abbreviations

4-MU	4-Methylumbelliferone
4-MUS	4-Methylumbelliferone sulfate
Ac	Acetyl
AFM	Atomic force microscopy
Aka	Acceptor nucleophilic constant
aq.	Aqueous
ARSA	Arylsulfatase A
ARSB	Arylsulfatase B
ASGPR	Asialoglycoprotein receptor
ATP	Adenosine 5'-triphosphate
ATS	Adenosine 5′-phosphosulfate
BCA	Bicinchoninic acid
Bn	Benzyl
b.r.s.m.	Based on recovered starting material
BSA	Bovine serum albumin
cat.	Catalytic
Cbz	Carboxybenzyl
CI-M6PR	Cation-independent mannose 6-phosphate receptor
COSY	Correlation spectroscopy
CSA	(1S)-(+)-10-Camphorsulfonic acid
CuAAC	Copper(I)-catalyzed azide-alkyne cycloaddition
dec.	Decomposed
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMEM	Dulbecco's modified eagle's medium
DMF	Dimethylformamide
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DTT	Dithiothreitol
EC_{50}	Half maximal effective concentration
EDC.HCl	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetraacetic acid
EMATE	Estrone sulfamate
eq.	Equivalents
ESI	Electrospray ionisation
Et	Ethyl
FGE	Formylglycine generating enzyme
FGF	Fibroblast growth factor
Fgly	Formylglycine
Fgly-OH	Formylglycine hydrate
Fmoc	9-fluorenylmethoxycarbonyl

FT-IR	Fourier transform infrared spectroscopy
GCMS	Gas Chromatography Mass Spectrometry
GlcA	Glucuronic acid
GlcNAc	*N*-Acetylglucosamine
GPC	Gel permeation chromatography
h	Hour(s)
HEK-293	Human embryonic kidney 293 cells
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMBC	Heteronuclear multiple bond correlation spectroscopy
HPLC	High performance liquid chromatogrpahy
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
HS	Heparan sulfate
HSPG	Heparan sulfate proteoglycnas
HSQC	Heteronuclear single quantum coherence spectroscopy
IC_{50}	Half maximal inhibitory concentration
IdoA	Iduronic acid
$ImSO_2N_3$	Imidazole-1-sulfonyl azide
ITC	Isothermal titration calorimetry
LB	Luria-Bertani broth
LC -MS/MS	Liquid Chromatography with tandem mass spectrometry
LCMS	Liquid chromatography–mass spectrometry
LTR	Lysosome targeting receptors
LYTACs	Lysosome Targeting Chimeras
m	Meta
M	Molar
MAPK	Mitogen-activated protein kinase
Me	Methyl
min	Minute(s)
mp	Melting point
MS	Mass spectrometry
Ms	Mesyl
MS	MS Mass spectrometry
NCA	Amino acid *N*-carboxyanhydrides
NCPA	*N*-phenoxycarbonyl α-amino acids
NHS	*N*-Hydroxysuccinimide
Ni_NTA	Nickel-nitriloacetic acid
NIS	*N*-Iodosuccinimide
NMR	NMR Nuclear magnetic resonance
$ONOO^-$	peroxy nitrite
p	Para
PAL	PAL Photoaffinity label
PAL	Photoaffinity labelling

PAPS	3'-Phosphoadenosine-5'-phosphosulfate
PAS	Sulfatase from *Pseudomonas aeruginosa*
PBS	Phosphate-buffered saline
Ph	Phenyl
PhMe	Toluene
PIDA	(Diacetoxyiodo)benzene
PP_i	Inorganic pyrophosphate
PROTACs	Proteolysis targeting chimeras
p-TsOH	p-Toluenesulfonic acid
R	Unspecified substituent
RAW	RAW 264.7 Cell Line murine - Macrophage
rhGAA	Recombinant human acid alpha-glucosidase
ROMP	Ring-opening metathesis polymerization
RRV	Relative reactivity value
rt	Room temperature
SAR	Structure-Activity Relationship
satd	Saturated
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SPR	Surface plasmon resonance
Sulf-1	Endosulfatase 1
Sulf-2	Endosulfatase 2
TAMRA	5-Carboxytetramethylrhodamine
TASF	Tris(dimethylamino)sulfonium difluorotrimethylsilicate
TBAF	Tetra-n-butylammonium fluoride
TBDPS	*tert*-Butyldiphenylsilyl
TCE	Trichloroethyl
TCEP	Tris(2-carboxyethyl)phosphine
TEAB	Triethylammonium bicarbonate
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy, free radical
Tf	Trifyl
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
TGF-β	Transforming growth factor β
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TPD	Targeted protein degredation
Ts	Tosyl
UV	Ultraviolet
VEGF	Vascular endothelial growth factor
μw	Microwave

CONTENTS

Declaration of authorship ..... ***iii***

Abstract ..... ***v***

Acknowledgements ..... ***vii***

Abbreviations ..... ***viii***

1. Introduction ***1***

1.1 Sulfation 1

1.2 Sulfatases 2

1.3 Mechanism of reaction of the sulfatases 6

1.4 Endosulfatases 8

1.5 Endosulfatase substrate specificity 10

1.6 Heparan sulfate proteoglycans 12

1.7 HS, Sulfs and signalling 15

1.8 Disease relevance of Sulfs 17

1.9 Developments in the Chemical Synthesis of Heparin and Heparan Sulfate 17

1.10 Modular chemical synthesis 18

1.11 Solid phase synthesis 18

1.12 Chemoenzymatic approaches 20

1.13 Degredation/digestion of Heparin 21

1.14 Chemical synthesis of *O*-glycoslyations 22

1.15 Mechanism of *O*-glycosylation reactions and the consequence on stereochemistry 25

1.16 Small molecule inhibitors of endosulfatases 27

1.17 Sulfamate-containing Sulf inhibitors 28

1.18 Mechanism of inactivation of sulfatases by sulfamate inhibitors 31

1.19 Summary 32

2. Glucosamine-6-sulfamate analogues as inhibitors of endosulfatases ***35***

2.1 Introduction 35

2.2 Synthesis of inhibitor 27 36

2.3 Modifications at C1 37

2.4 Modifications at C2 39

2.5 Modifications at C6 40

2.6 Synthesis of non-hydrolysable glucosamine-6-sulfamate analogue 44

2.7 Sulf activity assay 47

2.8 MSulf-2 activity assay using conditioned medium 47

2.9 Protein isolation 49

2.10 4-Methylumbelliferone sulfate (4-MUS) assay 55
2.11 Evaluation of glucosamine-6*O*-sulfamate inhibitors of HSulf-2 57
2.12 Summary 60
3. Design and synthesis of small molecule endosulfatase inhibitors 62
3.1 Introduction 62
3.2 Rational for GlcN-Ido relating to FGF 63
3.3 Rational for IdoA-GlcN relating to Sulfs 64
3.4 Inhibitor design 66
3.5 Synthesis of IdoA-GlcNS(6Sulfamate) 66
3.6 Synthesis of IdoA(2S)-GlcNS(6Sulfamate) 75
3.7 Mannose model system 77
3.8 Redesign of idose building block 78
3.9 Biological Evaluation of compounds 88 and 89 82
3.10 Summary 83
4. Design and synthesis of an amino-functionalised disaccharide intermediate 85
4.1 Introduction 85
4.2 Retrosynthetic analysis of intermediate 163 86
4.3 Synthesis of intermediate 153 86
4.4 Orthogonally protected thioglycoide donors 89
4.5 Glycosyl acetate donor 130 93
4.6 Fischer glycosylation 95
4.7 Triacetate glycosyl donor investigation 98
4.8 Remaining synthesis towards amino-functionalised 163 102
4.9 Summary 105
5. Target engagement probes via proximity labelling 106
5.1 Introduction 106
5.2 Affinity-based chemical proteomics 106
5.3 Design of photoaffinity labelling (PAL) probes to target endosulfatases 108
5.4 Synthesis of minimalist linkers 171 and 174 109
5.5 Synthesis of biphenyl trichloroethylsulfamate based PAL, 176 110
5.6 Synthesis of IdoA2S-GlcNS6S based PAL, 187 115
5.7 Catalytic activation of diazirines 116
5.8 Synthesis of cell non-permeable Ir photocatalyst 119
5.9 Synthesis of IdoA(2S)-GlcNS(6Sulfamate)-Ir probe 124
5.10 Characterisation of 208 127
5.11 Biochemical evaluation of 187 as a HSulf-2 inhibitor 131
5.12 Target engagement study 132

5.13 Summary 134
6. Synthesis of an IdoA(2S)-GlcNS(6Sulfamate) containing glycolpolymer 136
6.1 Introduction 136
6.2 Polymerisation 138
6.3 Redesign of monomer 140
6.4 Activated ester ROMP-polymer 143
6.5 Biochemical evaluation of glycopolymers, 226, 227 and 228 147
6.6 End functionalisation of living polymer 148
6.7 Lysosome targeting chimeras (LYTAC) 150
6.8 Synthesis of poly(M6P) as a ligand CI-M6PR 153
6.9 Future work: Construction of LYTAC 156
6.10 Summary 157
7. Thesis summary and future work 159
7.1 Future work 160
8. Chemistry experimental 162
8.1 General Experimental 162
8.2 General procedures 163
8.3 ^{1}H NMR assignment of the anomeric configuration 164
8.4 Compound preparations 166
9. Biology experimental methods 316
9.1 Materials 316
9.2 Cell culture 316
9.3 HSulf-2 activity assay (4-MUS assay) 316
9.4 MSulf-2 isolation 317
9.5 Target engagement study 319
10. Appendix 322
10.1 NMR data for Compound 89 322
10.2 NMR and HPLC data for Compound 208 323
10.3 NMR data for compound 187 325
10.4 NMR data for 175 326
10.5 Diazirine activation via Dexter energy transfer NMR experiment data 327
10.6 Polymer chacterisation 328
10.7 ^{1}H NMR data for glycopolymers 226, 227 and 228 329
11. Bibliography 331

1. Introduction

This chapter introduces endosulfatases (Sulfs) as an important class of enzymes that have been linked to a variety of diseases. The physiological substrate of the Sulfs is heparan sulfate (HS), which is an important macromolecule involved in many extracellular signalling pathways. This molecule is challenging to synthesise, and so mimics and fragments are often used as an alternative in biochemical studies, and current chemical methodologies to access these will be discussed. In the absence of commercially available antibodies, the Sulfs are difficult to study, therefore this thesis aims to produce a series of Sulf probes to inhibit and detect the enzymes. A summary of known sulfatase inhibitors will be provided, introducing the sulfamate 'warhead' that has been successful for irreversible inhibition of steroid sulfatase.

1.1 Sulfation

Sulfatases are enzymes that catalyse the hydrolysis of sulfate esters and sulfamates on a diverse range of substrates. To appreciate the importance of sulfatases, it is necessary to understand the process of sulfation and its relevance in biological systems. Sulfotransferases are enzymes that catalyse the transfer of a sulfate moiety from 3'-phosphoadenosine 5'-phosphosulfate (PAPS, **1**) onto a hydroxyl group of a substrate, as shown in figure 1.[3] PAPS is a very high energy molecule, requiring two equivalents of adenosine triphosphate (ATP) for its biosynthesis, and this means that sulfation is an energy intensive process for a cell. Furthermore, sulfotransferases and sulfatases have been identified in most species, which implies that sulfation is an important biological reaction.[3] The main roles of sulfation include: hormone regulation, detoxification/metabolism, molecular recognition, cell signaling, and viral entry into cells.[4]

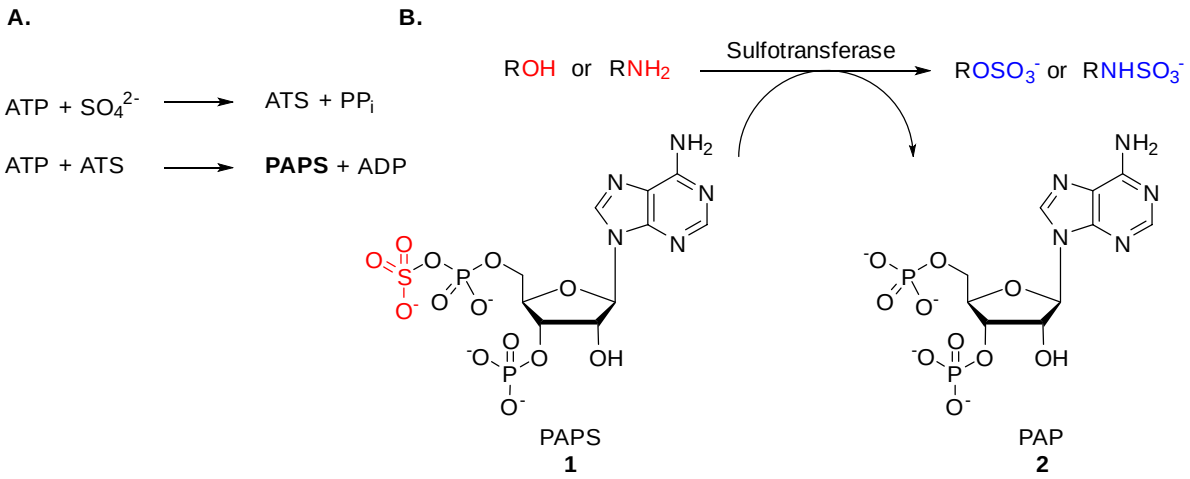


Figure 1: A. Biosynthesis of PAPS; ATP adenosine triphosphate, PPi pyrophosphate, ATS adenosine 5'-phosphosulfate, PAPS 3'-phosphoadenosine 5'-phosphosulfate, ADP adenosine diphosphate. **B.** Sulfotransferase reaction converting an alcohol or amine into a sulfate or sulfamate using PAPS as the source of 'sulfate'.

1.2 Sulfatases

Barbeyron et al. have recently collated all the genomic data of the sulfatases and proposed a classification system based on sequence homology.[5] There are four distinctive families of sulfatases based on their catalytic mechanism, as shown in figure 2. Class I requires a formylglycine residue for catalytic activity;[6] class II are alkyl sulfatases that contain an Fe-centered active site that oxidises the alkyl chain and subsequent hydrolysis releases an equivalent of inorganic sulfate to produce the corresponding alkyl aldehyde;[7] class III are also alkyl sulfatases but have a Zn^{2+} centred active site and the mechanism of desulfation involves cleaving the C-O bond by nucleophilic attack of hydroxide at the carbon atom, thus leading to inversion of stereochemistry;[8] and finally, class IV sulfatases have a Zn^{2+} -centred active site that is conserved with two other hydrolase families (glyoxalases II and metallo-beta-lactamases) but has a yet uncharacterised mechanism of hydrolysis.[9]

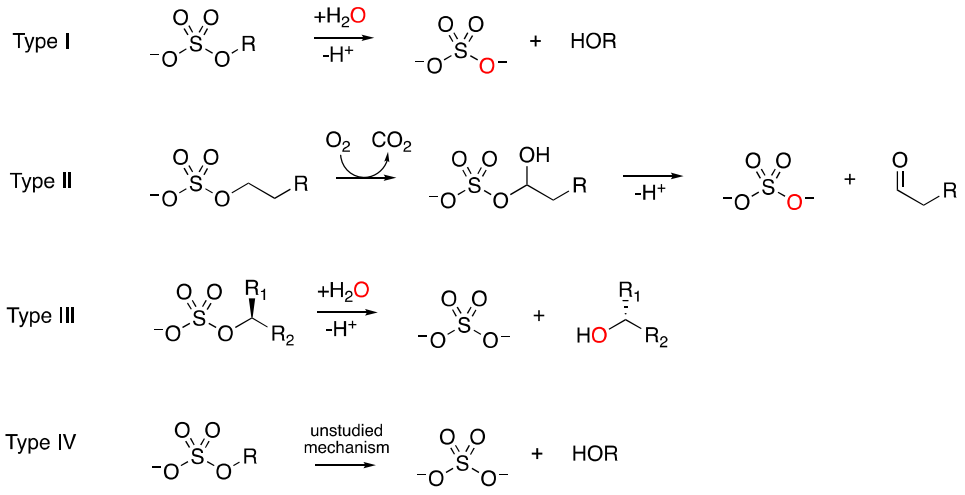
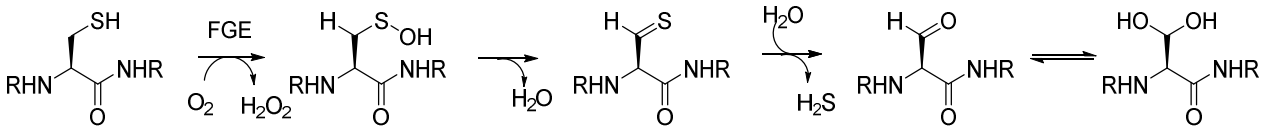


Figure 2: Classes of sulfatases based on the active site sequence, structure, and mechanism

Class 1 sulfatases all have a high degree of similarity in the active site sequences and structures, and catalytic mechanisms. The type 1 sulfatases are the largest family and are divided into 73 subfamilies corresponding to either a known substrate specificity or to an uncharacterised substrate.[10] Class 1 sulfatases hydrolyse the sulfate esters in many biologically relevant compounds including hydrophobic substrates (glucosinolate, steroid, and thyronine sulfates), amphiphilic substrates (sulfated glycans) and hydrophilic substrates (simple mono- and disaccharide sulfates). This family of enzymes share a high sequence similarity (20-60%), particularly in the *N*-terminal region that contains a conserved active site, which comprises of a formylglycine (FGly) residue that is installed post-translationally.[1] Class I sulfatases contain a pentapeptide sequence (C/S-X-P-X-R) that directs a post-translational modification (PTM) of the cysteine (or serine, as in some prokaryotic sulfatases) into a formylglycine (FGly) residue. This PTM is installed by the formylglycine generating enzyme (FGE). FGly is present in the active site of all known class I sulfatases and is essential for catalytic activity.[11] In eukaryotic sulfatases, the formylglycine-generating enzyme (FGE) uses molecular oxygen to oxidize the cysteine to an aldehyde and this occurs in the endoplasmic reticulum during late stage protein translocation, as shown in scheme 1.[11] The sole function of FGE is to activate sulfatases,

and a deficiency of this enzyme in humans leads to inactivation of all class I sulfatases and causes a rare and fatal autosomal disorder called multiple sulfatase deficiency.[12,13]



Scheme 1: Post-translational modification of the amino acid cysteine by a formylglycine-generating enzyme using molecular oxygen to give formylglycine.

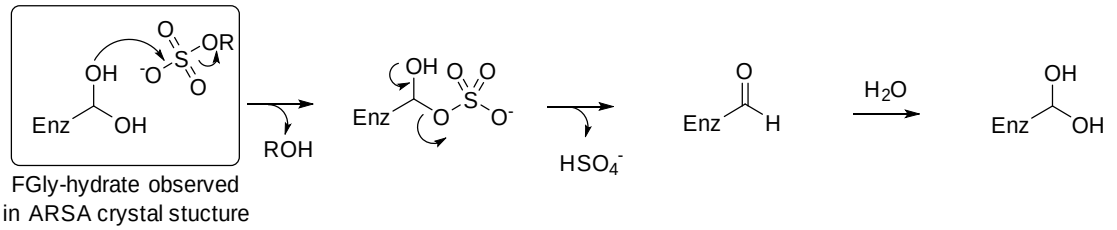
Mammalian sulfatases are located in various subcellular locations and in the extracellular matrix (ECM), as summarised in table 1. The sulfatases in the endoplasmic reticulum and Golgi network are membrane bound, and the lysosomal and extracellular sulfatases are soluble. Steroid sulfatase (STS, also known as arylsulfatase C (ARSC)) is one of the most widely studied eukaryotic sulfatase, which has gained attention due to its involvement in hormone dependant cancers. For this reason, it has been the targeted with small molecule inhibitors, perhaps most successfully with arylsulfamates and the mechanism of inhibition has been investigated (as discussed in section 1.14). However, due to their conserved active sites, other eukaryotic sulfatases (such as arylsulfatase from *P. aeruginosa* (PARS) or Sulfatase from *Aerobacter aerogenes*) have also been used in the mechanism studies due to the ease of accessing large quantities of enzyme via standard recombinant expression and affinity purification methods.

Table 1: Summary of mammalian Class I sulfatases; cellular location, pH optimum and physiological substrate (if known). Abbreviations: ER endoplasmic reticulum, ECM extracellular matrix, HS heparan sulfate.

Enzyme	Abbreviation	Subcellular localization	pH optimum	Physiological substrate
Arylsulfatase A	ARSA	Lysosome	4.5	Sulfatide
Arylsulfatase B	ARSB	Lysosome	5.6	Dermatan sulfate, chondroitin sulfate
Arylsulfatase C (steroid sulfatase)	ARSC/STS	ER	7.0	Steroid sulfatases
Arylsulfatase D	ARSD	ER	7.0	Unknown
Arylsulfatase E	ARSE	Golgi apparatus	7.0	Unknown
Arylsulfatase F	ARSF	ER	7.0	Unknown
Arylsulfatase G	ARSG	ER	7.0	Unknown
Arylsulfatase I	ARSI	ER	7.0	Unknown
Glucuronate 2-sulfatase	GlcA2S	Lyosome	4.6	HS, dermatan sulfate
Iduronate-2-sulfate sulfatase	IdoA2S	Lysosome	4.5	HS, dermatan sulfate
Galactose-6-sulfatase	GalN6S	Lysosome	4.0	Chondroitin sulfate, keratan sulfate
Glucosamine 6-sulfatase	GlcN6S	Lysosome	5.2	HS, keratan sulfate
Glucosamine 3-sulfatase	GlcN3S	Lysosome		HS
Sulfamidase	SGSH/ARSK	Lysosome	5.6	HS
Endosulfatase 1	Sulf-1	ECM	7.5	HS
Endosulfatase 2	Sulf-2	ECM	7.5	HS

1.3 Mechanism of reaction of the sulfatases

For many years, the mechanism of sulfatases was unknown; however, the discovery that the activity of sulfatases is dependent on this unique post-translational modification to generate a FGly residue, in combination with the X-ray crystal structure of ARSA has led to the suggested mechanism of desulfation, as shown in scheme 2.[14,i] The active site of ARSA showed a two-fold electron density around the FGly residue, which was cautiously interpreted as an aldehyde hydrate. It is thought that this hydrate is a key intermediate in the catalytic cycle, as sulfatases lacking the formylglycine residue are inactive, and furthermore, if mutated to a serine the enzyme is capable of performing stoichiometric desulfation, highlighting that the higher oxidation level of this residue is required for catalysis.[15]



Scheme 2: The proposed mechanism of hydrolysis of sulfate esters based on the interpretation of the hydrated FGly in ARSA

The proposed mechanism (scheme 2), proceeds via an S_N2 reaction at the sulfur centre, which involves a pentaco-ordinate transition state and inversion of stereochemistry. Other proposed mechanisms would proceed with retention of stereochemistry. Chai et al. prepared phenyl [(R)-^{16}O,^{17}O,^{18}O]sulfate in order to study the stereochemical outcome of sulfate transfer to using an arylsulfatase from *aspergillus oryzae.*[16] The reaction occurred with inversion of configuration at the sulfur atom, providing evidence of involvement of a

[i] DOI: 10.2210/pdb1auk/pdb

pentaco-ordinate transition state consistent with S_N2 at the sulfur centre. To date, this finding has not been confirmed with a better characterised sulfatase.

A crystal structure of a bacterial sulfatase from *pseudomonas aeruginosa* (PAS)[ii] was determined at 1.3 Å resolution; the structure of the active site strongly resembles that of ARSA and ARSB.[6] This crystal structure shows the formylglycine hydrate ligated with inorganic sulfate at high resolution. The orientation of this sulfate is optimal for nucleophilic attack, with one face of the tetrahedral sulfate situated 2.96 Å from one hydroxyl of the forymlglycine hydrate. The crystal structure shows the presence of a calcium ion, which stabilises the charge and binds the substrate during catalysis. To date, this is the highest resolution crystal structure of any sulfatase active site and it is likely that all class I sulfatases have a similar active site geometry, based on the known sequence homology. Following publication of the PAS crystal structure, Marino et al. took a quantum mechanical approach to elucidate the reaction mechanism of the sulfate ester hydrolysis.[17] The largest energy barrier to overcome was found to be the nucleophilic attack of the forymlglycine hydrate (FGly-OH) on the sulfate ester. The calculated bond distances showed the shortening of the FGlyO-S bond and the lengthening of the S-OR bond during the transition state. This indicates that the nucleophilic attack of FGly-OH onto S occurs in a synchronous transition state, with bond cleavage of S-OR, further supporting the hypothesis that the mechanism of sulfate hydrolysis occurs via S_N2 reaction at the sulfur center.

[ii] DOI: 10.2210/pdb3HX6/pdb

1.4 Endosulfatases

There are 15 known mammalian sulfatases, and of these only two are located in the extracellular matrix: the HS 6-*O*-endosulfateses, sulfatase 1 (Sulf-1) and sulfatase 2 (Sulf-2).[18] These two enzymes are closely related in structure (having 63-65% sequence homology), and appear to have overlapping roles, which is highlighted by the low perinatal survival when both enzymes are genetically removed, but no major abnormalities are caused by the single knockout of either Sulf1 or Sulf2. The Sulfs remove the 6-*O*-sulfate group from glucosamine residues within heparan sulfate (HS) (figure 3), and have a strong preference for [Glc/IdoA(2S)-GlcNS(6S)] trisulfated disaccharide. Once desulfated, the [Glc/IdoA(2S)-GlcNS] disaccharide has a much lower affinity for the Sulfs, thus allowing release of the substrate.[19] The trisulfated disaccharide unit has a low abundance within HS, and therefore subtle Sulf-induced modifications induce major functional consequences, highlighting the importance of both sulfation and the role of the Sulfs.[20] Regulation of the 6-*O*-sulfation status of HS by action of the Sulfs has been linked to the regulation of various signalling pathways, some of which are discussed in section 1.7.

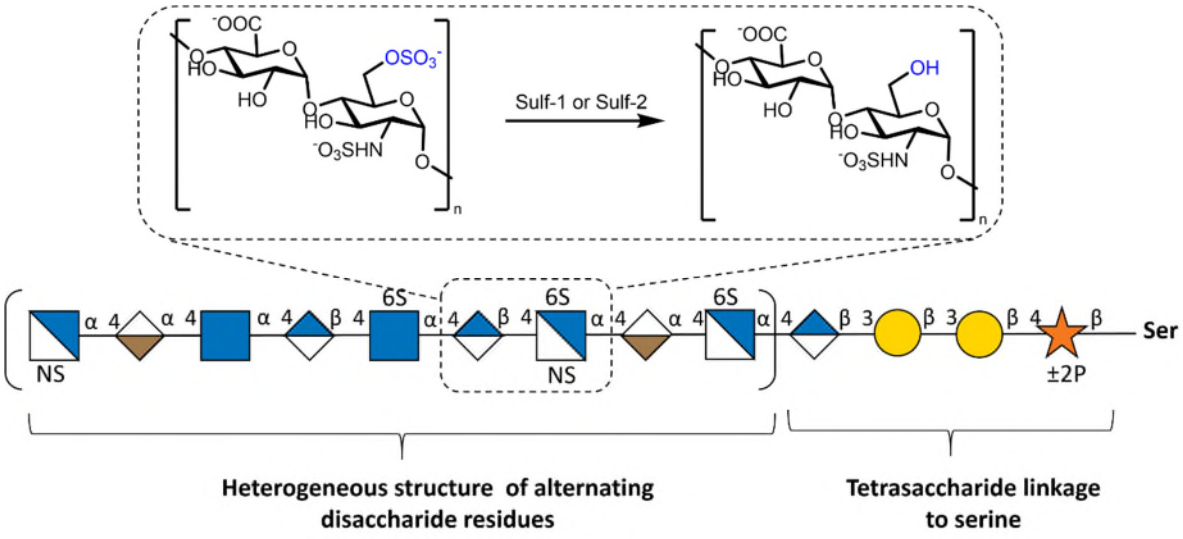


Figure 3: Structure of HS, highlighting the disaccharide [Glc/IdoA(2S)-GlcNS(6S)] for which Sulfs have a strong preference.

As with the other class I sulfatases, Sulf-1 and Sulf-2 contain a cysteine residue that is post-translationally modified to the catalytically active formylglycine residue. However, the Sulfs have several characteristics that distinguish them from the other sulfatases: the length of the amino acid sequence (~800 residues long), compared to 500-600 residues of most other sulfatases; they are located in the extracellular matrix and have an optimal activity in neutral pH; and finally, they are endo-acting, preferentially removing sulfate groups internal to the polysaccharide chain.[18]

To date, the mammalian endosulfatases have not been solved by X-ray crystal structure. However, due to the highly conserved primary sequence of the active site, and the conservation of tertiary structure of the active site across four class I sulfatases, it is reasonable to hypothesise that the endosulfatases catalytic activity is consistent with the other enzymes in the sulfatase family.

The primary structure of the Sulfs is divided into two domains: the *N*-terminus containing the catalytic site, and the hydrophilic *C*-terminus responsible for binding HS. The Sulfs are translated as a pre-proprotein that contains a short signal peptide, which is post-translationally cleaved to form a proprotein. Maturation of the Sulfs occurs by cleavage within the hydrophilic domain by a furin-type protease to form two-subunits that are linked by a disulfide bridge, as shown in figure 4.[21] Shorter splice variants lacking the catalytic sites have been identified in many species, which while catalytically inactive may still play a crucial role in the regulation of local HS concentrations. For example, the splice variants exert opposing effects on Wnt signalling: the full-length enzyme activates the pathway, while the shorter splice variant inhibits it.[22]

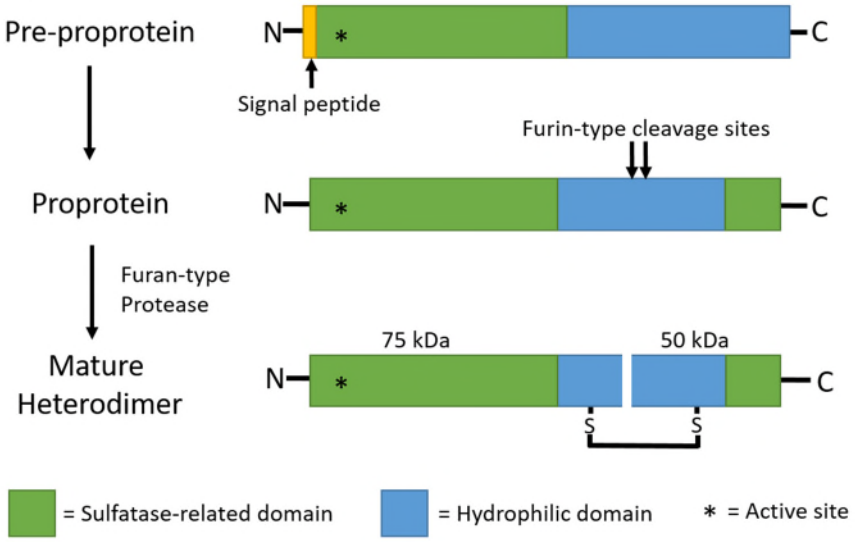


Figure 4: Post-translational modifications involved in production of mature Sulf enzymes

A study by Milz et al. has investigated the role of the hydrophilic C-terminal domain, which contains a high number of basic residues.[23] They identified two HS binding sites, and postulated cooperativity between the primary binding site in the catalytic domain and the secondary binding site in the hydrophilic domain. Mutated enzyme lacking the hydrophilic domain has significantly reduced activity, supporting the hypothesis that the primary function of this domain is HS binding.

1.5 Endosulfatase substrate specificity

Many studies into the substrate specificity of Sulfs involve the enzymatic remodelling of HS, followed by digestion by heparin lyases, then analysis of the resulting disaccharides by mass spectrometry (MS).[24] This methodology has also been coupled with scintillation counting-MS, where ^{35}S-radiolabeled HS polysaccharides are used as the substrate. Using these methods, quantitative analysis of the disaccharide composition has been accomplished, and it is reported that Sulfs have a strong preference for [Glc/IdoA(2S)-GlcNS(6S)] trisulfated disaccharide.[19] A similar approach has been adapted for evaluation

of sulfation patterns of HS *in vivo*, which allows the comparison of HS composition across different organs.[25]

Saad et al. investigated the endolytic activity of the Sulfs on smaller substrates, including low molecular weight heparin, a fully-sulfated decasaccharide from heparin, tetrasaccharide (ΔUA2S-GlcNS6S-Ido2S-GlcNS6S) and disaccharide.[26] The decasaccharide substrate showed the greatest decrease in glucosamine-6-*O*-sulfation by Sulf action, but no desulfation product was observed for the disaccharide. The smallest fragment that was found to be a substrate of HSulf-2 was the tetrasaccharide, although this was desulfated to a much lesser extent than the decasaccharide. Furthermore, during the time course of the work described in this thesis, a publication by Chiu et al. took the approach of synthesising HS oligiosaccharide fragments, with defined sulfation patterns, and evaluated the substrate specificity of Sulf-1 (figure 5).[27] This identified the trisaccharide [IdoA(2S)-GlcNS(6S)-IdoA(2S)] **3** as the minimal substrate for Sulf-1, evidenced by approximately 8-fold increase in released sulfate compared to tetrasaccahride [GlcNS(6S)-IdoA(2S)-GlcNS(6S)-IdoA(2S)] **4**. Finally, disaccharide [GlcNS(6S)-IdoA(2S)] **5** was found to be too short a fragment to be recognised as a substrate, and therefore experienced minimal desulfation under the assay conditions.

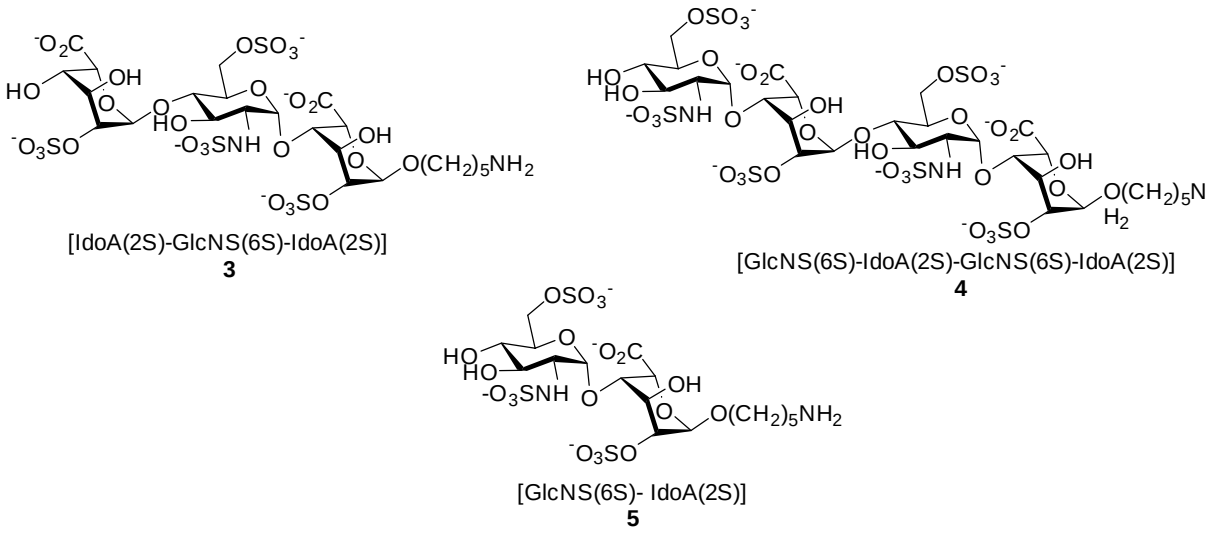


Figure 5: Structures of HS fragments used to investigate the substrate specificity of Sulfs

1.6 Heparan sulfate proteoglycans

Heparan sulfate proteoglycans (HSPGs) are a family of cell-surface and secreted glycoproteins that are covalently attached to HS chains.[28] HSPGs are involved in a wide range of cellular processes by direct interaction and complex formation with numerous signalling proteins, growth factors, and extracellular matrix components. Many of these interactions are mediated by specific structural motifs within the HS chains.[29] The biochemical roles of HS include mediating protein-protein interactions, acting as a co-receptor and sequestering signalling molecules, thereby inhibiting signalling cascades.

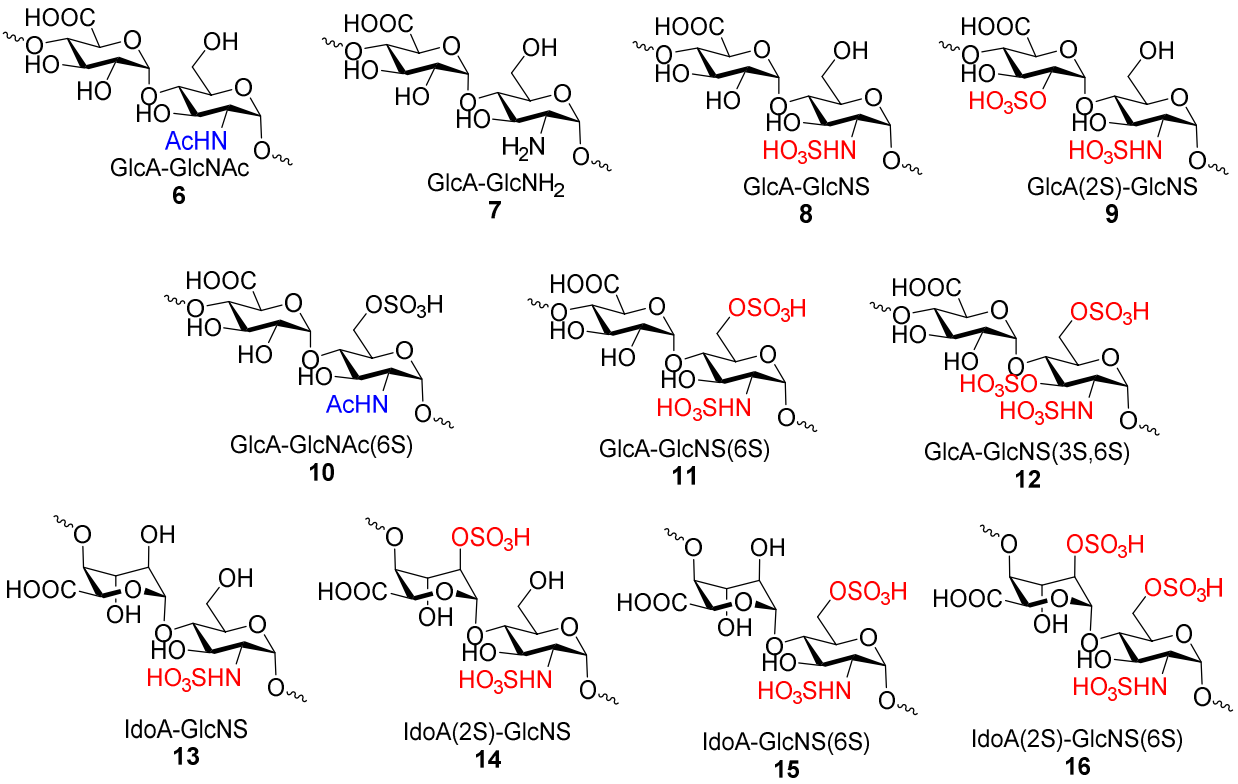


Figure 6: Disaccharide subunits found in HS. GlcA = β-*D*-glucuronic acid; GlcNAc = N-acetyl-*D*-glucosamine; $GlcNH_2$ = glucosamine; IdoA = α-*L*-iduronic acid, 2S = 2-O-sulfate, 3S = 3-O-sulfate, 6S = 6-O-sulfate, NS = N-sulfate.

HS glycans are highly sulfated, linear polysaccharides that are synthesised in the Golgi apparatus.[30] The HS polysaccharide chains are composed of alternating 1→4-linked α-D-glucosamine (GlcN) and β-D-glucuronic acid (GlcA) or α-L-iduronic acid (IdoA) residues, as shown in figure 7.[31] On average HS chains consist of approximately 150 disaccharide subunits. Figure 6 shows the typical disaccharide units found in HS. GlcA-GlcNAc **6** is the major disaccharide unit and contains no sulfate groups, and the predominant uronic acid epimer is D-glucuronic acid.[32] HS is made up of NS domains (highly sulfated) and NA domains (which contain unsulfated, N-acetylated disaccharide units). Biosynthesis is initiated at the attachment site on the protein core, a tetrasaccahdride linkage to serine, shown in figure 7, and is followed by chain polymerisation by glycosyltransferases which extend the chain from glucuronic acid (GlcA) and *N*-acetylglucosamine (GlcNAc)

precursors.[33] The chains then undergo sequential modification by various enzymatic transformations involving *N*-deacetylation/*N*-sulfation, sulfation of the 2-*O*-, 3-*O*- and 6-*O*-hydroxyl of glucosamine and C5-epimerization of GluA to L-iduronic acid (IdoA) catalyse deacetylation, sulfation and epimerization reactions, respectively. In addition, HS is degraded by endosulfatases and heparanases, which cleave polymeric HS into shorter chain oligosaccharides. These transformations may result in approximately 20 different disaccharide subunits that can be arranged in different ways to create specific HS epitopes (structures given in figure 6), that consequently have large structural heterogeneity. This makes characterisation of the HS chain, and the identification of specific bind epitopes of proteins, very challenging.

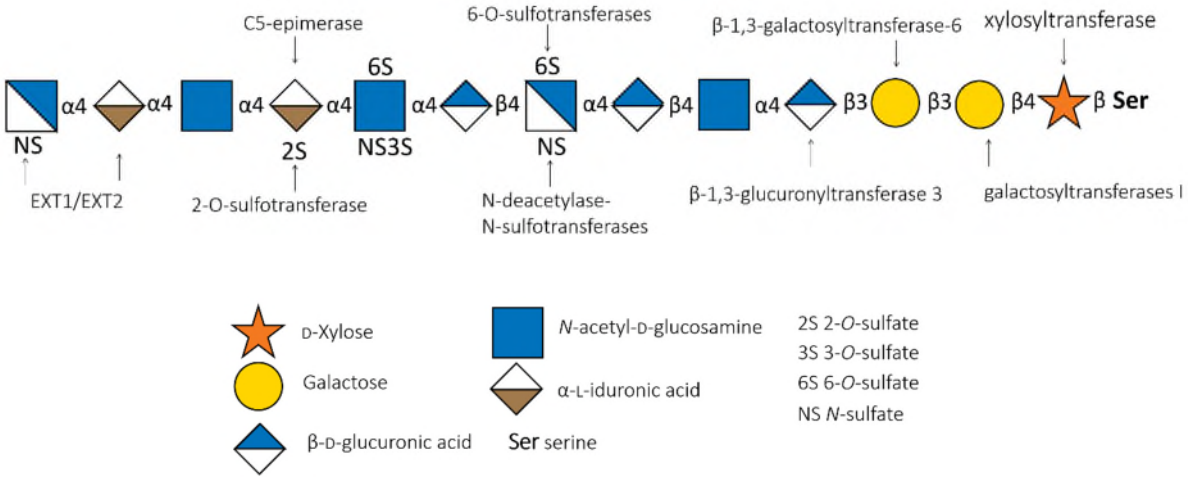


Figure 7: HS structure highlighting enzymes involved in HS biosynthesis

Binding to HSPGs may enrich binding proteins in the extracellular matrix or sequester ligands away from their receptors, thereby modulating the spatial distribution and activity of biologically active proteins such as growth factors and cytokines.[34] In this way, the alteration of the sulfation status of HS through modulation of the Sulfs has been identified as a potential therapeutic opportunity to regulate these signalling pathways and the bioavailability of different HS-binding proteins and thereby inhibit disease progression.

1.7 HS, Sulfs and signalling

Electrostatic interactions between the hydroxyl and sulfate groups of HS and basic amino acids of proteins allows HS to bind various proteins. Examples of growth factors that bind HS include fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β).[35] In these examples, HS acts as the co-receptor and affects the relationship between the growth factor and its receptor, and this in turn affects the signal sent to the target cell.

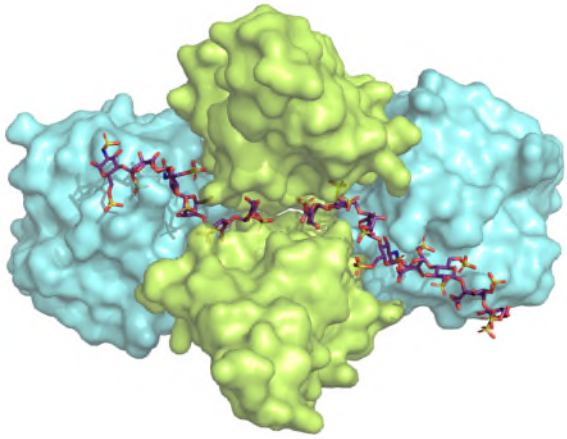


Figure 8: Crystal structure of a ternary FGF2-FGFR1-HS complex (two heparin decasaccharides) FGFs in blue, binding domains of FGFR in green. Recreated on Pymol. PDB ID: 1fq9.[36]

The highly sulfated domains of HS facilitate the formation of a ternary complex between FGF2 and its receptor. The crystal structure of the dimeric 2:2:2 FGF:FGFR:HS complex has been resolved at 3.0 Å resolution (figure 8), and shows that HS interacts with both FGFR and FGF simultaneously, thereby increasing FGF-FGFR binding.[36] Furthermore, HS interacts with the FGFR in the adjoining complex, showing that HS also promotes FGFR dimerization (figure 9). Dimerization of FGFR activates the receptor tyrosine kinase, which in turn activates a phosphorylation cascade through the mitogen-activated protein kinase (MAPK) pathway, leading to increased cell proliferation, angiogenesis and survival. The crystal structure of the ternary complex has revealed that the glucosamine-6-

O-sulfate enabled critical hydrogen-bonding and salt-bridge interactions between HS and FGF/FGFR.[36] Enzymatic action of Sulf-1 in reducing the glucosamine-6-*O*-sulfate groups on HS lowers the affinity of HS with FGF and FGFR, effectively down-regulating the MAPK signalling pathway. This in turn reduces cell proliferation, angiogenesis and cell survival. Interestingly, the effect of Sulf-2 activity has been reported to be opposing to that of Sulf-1, in one study Sulf-2 was found to enhance FGF2 signalling.[37]

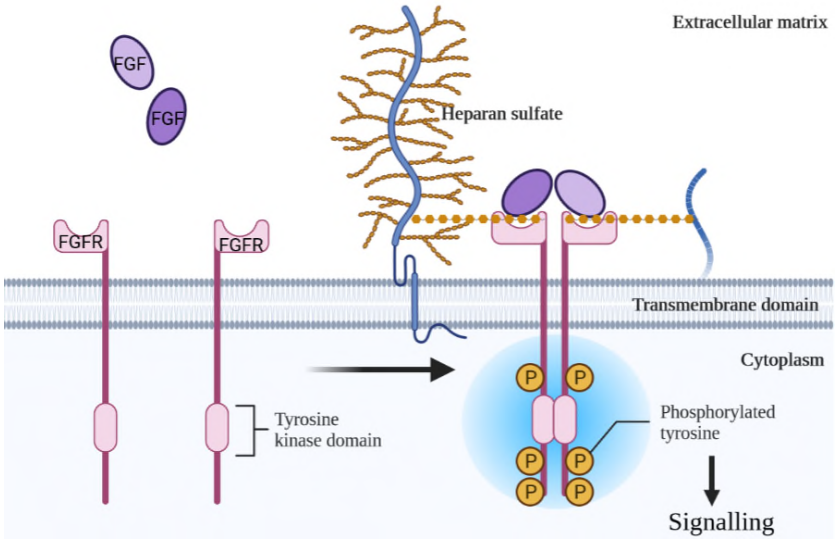


Figure 9: Formation of a ternary FGF:FGFR:HS complex leads to FGFR dimerization and transphosphorylation to activate signalling. Figure made using BioRender.com.

Another signalling pathway that is regulated by Sulfs is the canonical Wnt/β-catenin pathway.[38] HS interactions with Wingless/Wnt has been shown to regulate the Wnt concentration gradient and protein stabilisation in the extracellular matrix (figure 10).[39] Through interactions mediated by sulfate groups, HSPGs sequester Wnt ligands on the cell surface to prevent binding of the Wnt ligand with the trans-membrane Frizzled receptor, and this induces β-catenin degredation.[38] When HS is desulfated by the Sulfs, the Wnt ligands are released and this increases binding to the Frizzled receptor, leading to

stabilisation of β-catenin, which is able to regulate gene expression in the nucleus. Ai et al. proposed this 'catch or present' mechanism of Sulf1 regulation of Wnt signalling.[38]

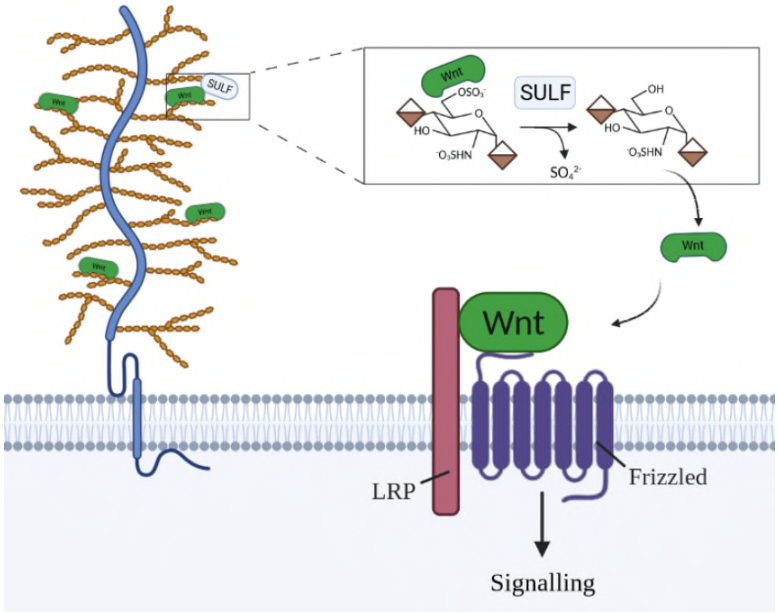


Figure 10: Desulfation of HS causes release of Wnt ligand that is able to bind to Frizzled receptor and activates Wnt signalling. Figure made using BioRender.com.

1.8 Disease relevance of Sulfs

As a consequence of the dynamic remodelling of sulfation within HS chains, and their role in regulating important signalling pathways, the Sulfs have been linked to a range of diseases including arthritis, atherogenic dyslipidemia[40], chronic renal fibrosis[41], inflammation[42], wound healing[43] and cirrhosis.[44] In addition, an increasing number of publications are identifying the altered expression of Sulf1 or Sulf2 in cancers, including brain[45], breast[46], ovarian[47], hepatocellular[48], pancreatic[49,50], gastric and colorectal cancer[51], melanoma and urothelial carcinomas.[52]

1.9 Developments in the Chemical Synthesis of Heparin and Heparan Sulfate

Accessing structurally defined units of heparin sulfate is a challenge due to its structural complexity, both in terms of the saccharide backbone composition and the variation of

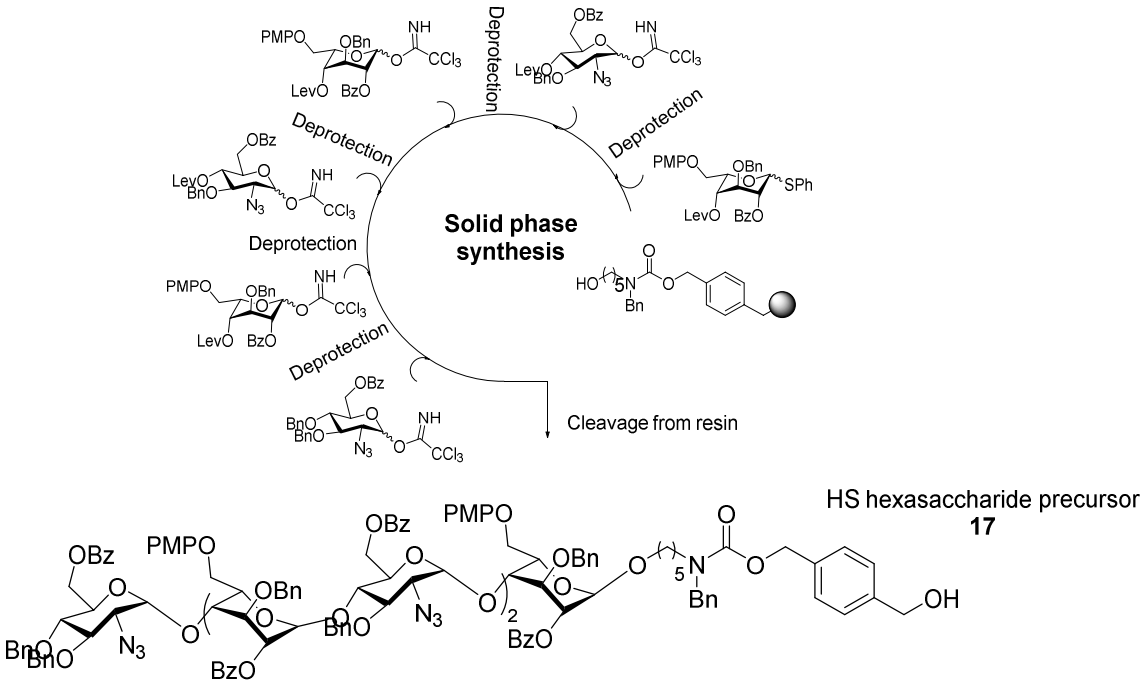
sulfated positions. Several chemical approaches and technologies have been used to access these glycan fragments, and these will be summarized below.

1.10 Modular chemical synthesis

The modular synthesis of oligosaccharides requires coupling of mono- or disaccharide fragments via *O*-glycosylation reactions, followed by regio- or chemo-selective installation of sulfate groups. This method often requires careful choice of protecting groups and usually demands a high number of orthogonal groups. The overall yields are often very low over a large number of synthetic steps, many of which are protection/deprotection manipulations. One method to overcome the low yield of multiple steps is to programme the glycoside reactivity to allow for one-pot sequential *O*-glycosylation reactions. This strategy still requires synthesis of a large number of key intermediates, with protecting groups chosen not only to allow for the desired orthogonality, but also to influence the donor relative reactivity value (RRV) to allow for controllable reactivity.

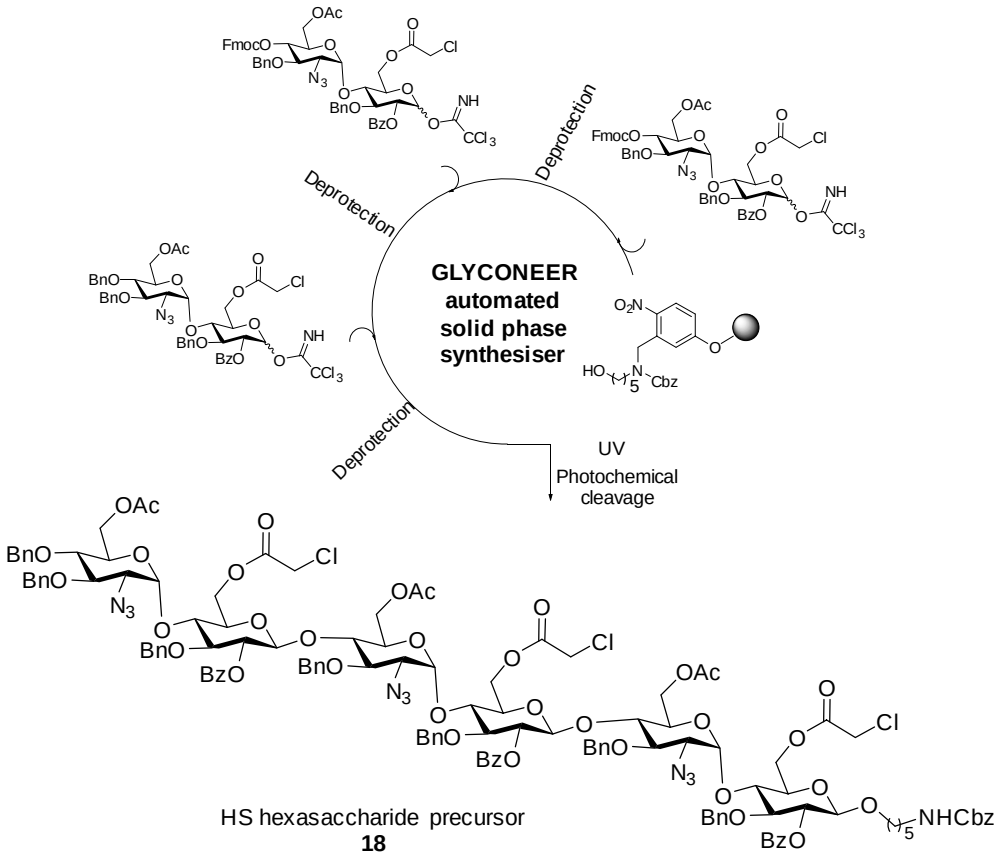
1.11 Solid phase synthesis

Development of solid phase synthesis of glycans has been more challenging than for peptides due to the requirement of high yielding, stereoselective *O*-glycosylation reactions. Guedes et al. reported the use of solid phase synthesis to assemble monosaccharide building blocks into a HS hexasaccharide precursor **17**, scheme 3.[53] The first *O*-glycosylation reaction to attach the monomer to the carboxystyrene resin, followed by successive cycles of delevulination and glycosylation (with alternating Ido- and GlcN-donors), gave hexasaccahride precursor in 11% yield over 14 steps. The major advantage of this synthetic method is the time saved by resin 'purification' and such sequence of reactions can be completed in 2-3 weeks, rather than 2-3 months of solution phase reactions and chromatography purification.



Scheme 3: Synthesis of HS hexasaccharide precursor **17** using solid-phase synthesis, published by Guedes et al.

Furthermore, the use of solid supported glycan synthesis has been adapted for application on an automated synthesiser called Glyconeer™.[54] In 2019, Budhadev et al. reported the use of Glyconeer to synthesise HS hexasaccharide precursor **18** using three repeated cycles of glycosylation with a disaccharide building block and *O*-Fmoc deprotection, followed by photochemical resin cleavage through a UV flow reactor, (scheme 4).[55] In addition, the quenched disaccharide glycosyl donor was recovered and recycled by converting it into the glycosyl trichloroacetimidate building block. Purification *via* column chromatography gave the product in 30% overall yield on a 15 mg scale in 6 h. If compared with solution phase synthesis of a similar substrate, this method is superior with respect to overall yield and time efficiency.



Scheme 4: Synthesis of HS hexasaccharide precursor **18** using Glyconeer™ (automated glycan solid-phase synthesiser), published by Budhadev et al.

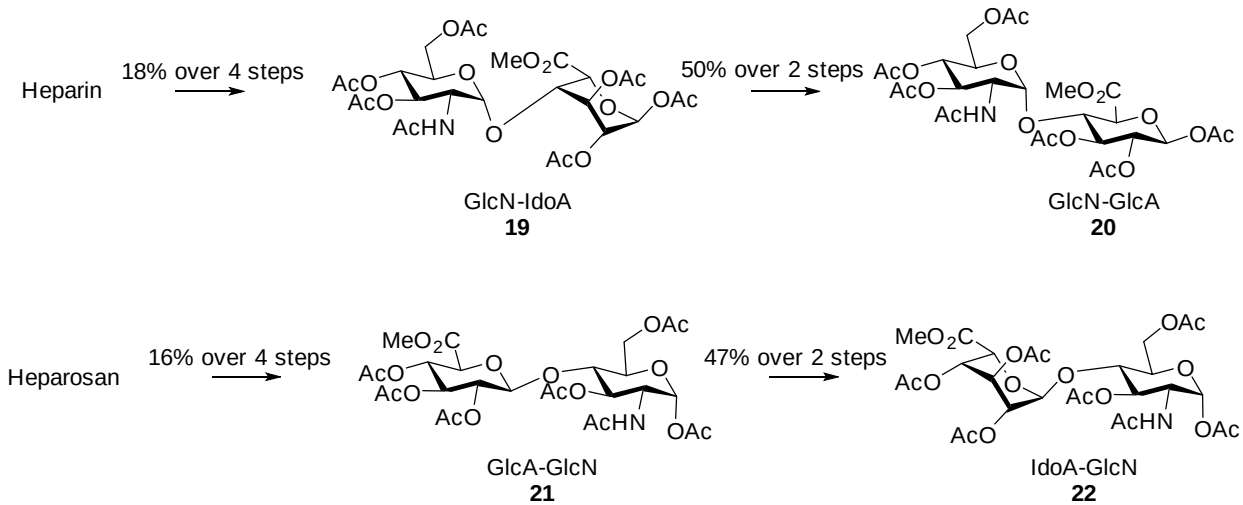
1.12 Chemoenzymatic approaches

Chemoenzymatic synthesis of heparan sulfate fragments using HS biosynthetic enzymes is an attractive alternative to chemical synthesis methods. As early as 2003, Kuberan et al. reported the chemoenzymatic synthesis of an antithrombin III–binding pentasaccharide in just 6 steps using a polysaccharide from *Escherichia coli* as a starting material.[56] The authors claim that this method is 100 times faster than the chemical synthesis of the same substrate, however the product was only isolated in microgram scale. This study established the capability of chemoenzymatic synthesis towards HS fragments. A breakthrough allowing for larger scale preparation was the successful expression of the HS biosynthesis enzymes in *Escherichia coli*. For example, Xu et al. reported the 10- and 12-step

chemoenzymatic syntheses of two heparin fragments in moderate yield (37-45%) to isolate up to 49 mg.[57]

1.13 Degredation/digestion of Heparin

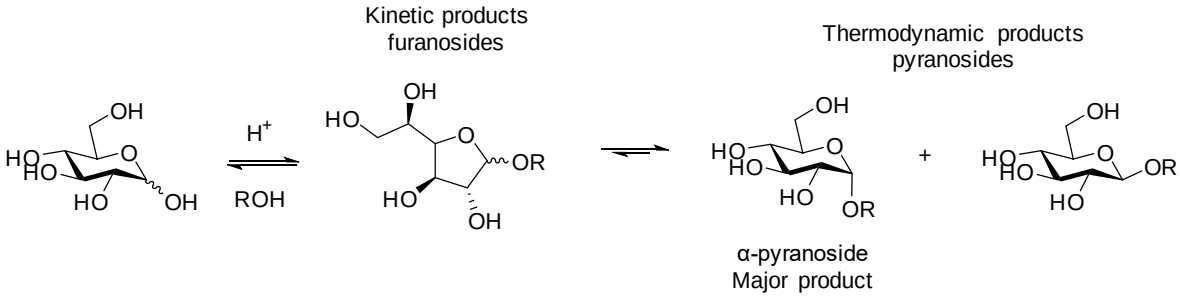
Pawar et al. have reported an efficient and cost-effective method to generate the four core disaccharide units of HS from natural polysaccharides, (scheme 6).[58] Firstly, the D-GlcN-L-IdoA disaccharide unit **19** was accessed in a one-pot three step procedure from heparin in 18% overall yield. Secondly, in a similar manner, heparosan (the non-sulfated bioprecurosor of heparin, isolated from *Escherichia coli* K5) was hydrolysed under slightly milder acidic conditions, esterified and acetylated to obtain GlcA–GlcN disaccharide unit **21** in 16% overall yield. Finally, two additional HS disaccharides **20** and **22** were obtained via C5 epimerisation, which proceeded in moderate to good yield, along with recovery of the starting material. This allowed the rapid access to multigram quantities of important HS building blocks, which can be easily converted into glycosyl donors for either modular synthesis or solid phase supported synthesis of libraries of HS analogues.



Scheme 6: Preparation of four key disaccharide building blocks by degredation of naturally occurring polysaccharides heparin and heparosan, reported by Pawar et al.

1.14 Chemical synthesis of *O*-glycosylations

Fischer glycosylation was the earliest synthetic glycosylation method and was developed by Emil Fischer in the 1890s, and remains one of the most commonly used protocols for the synthesis of simple (i.e. methyl) glycosides. Typically, the reaction involves a solution of an unprotected sugar in an alcoholic solvent, in the presence of a strong acid catalyst, at reflux for prolonged reaction times to produce the corresponding glycoside (scheme 7). The furanoside is initially formed as the kinetic product, however this is in equilibrium with the thermodynamic pyranoside product(s), of which the α-anomer is more stable due to the anomeric effect.[59]



Scheme 7: Fischer glycosylation reaction of glucose and methanol, showing reaction composition and equilibrium between species. The reaction is under thermodynamic control.

In the 1950's, Edward and Lemieux defined the *anomeric effect* as the tendency of an electronegative substituent in the C1 position to be in the axial position.[60,61] There are two theories behind this empirically overserved phenomenon. The first is based on the stabilizing orbital interactions between the non-bonding electron pair on the ring oxygen with the empty σ* orbital of the C−X bond, (figure 11, A.); and the second is based on dipole moment theory, which predicts that the equatorial position is destabilised due to the partial alignment of the dipoles of the heteroatoms, as shown in figure 11, B. In comparison, when in the axial position, the dipoles of the heteroatoms are in opposite directions.

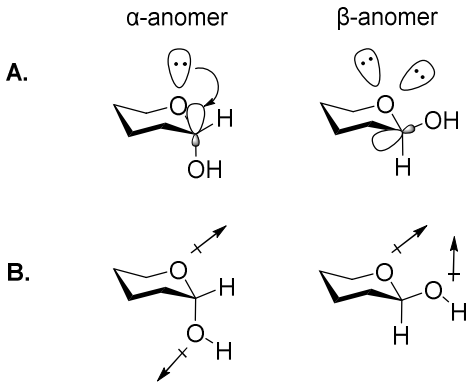


Figure 11: A. molecular orbital theory explanation of the anomeric effect and B. dipole moment theory explanation.

Recent developments in the Fischer glycosylation include the use of solid supported acid catalysts to simplify reaction work-up[62], microwave irradiation[63] or ultrasonic irradiation[64] to reduce reaction times and continuous flow reaction set up[65], which offers the benefit of scale-up without reaction re-optimisation.

Fischer glycosylation is most valuable when the desired glycoside corresponds to a cheap, commercially available and low boiling alcohol, such that it can be used in excess, but easily removed before isolation of the corresponding glucoside.[65] When the alcohol does not satisfy these criteria, alternative chemical *O*-glycosylation reactions are utilised. Whilst various *O*-glycosylation reactions have been developed, most methods involve the condensation of an alcohol (glycosyl acceptor) with a glycosyl donor (a sugar that contains a leaving group installed at the anomeric carbon), in the presence of a promotor to 'activate' the anomeric position leaving group. Amongst the most popular donors are glycosyl trichloroacetimidates[66 ,67], fluorides[68], sulfoxides[69], *n*-pentenyl[70] and thioglycosides[71,72] (figure 12). These reactions are highly moisture sensitive, as water will compete with the acceptor to form an undesired anomeric-hydroxyl containing side-product. They are therefore are often performed in the presence of molecular sieves as a drying agent.

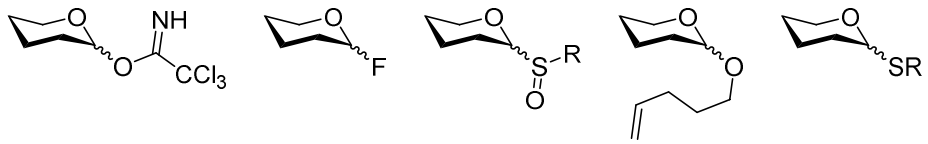


Figure 12: Structures of commonly used glycosyl donors, left to right: trichloroacetimidates, fluorides, sulfoxides, *n*-pentenyl and thioglycosides.

Thioglycosides are stable in many conditions that are employed for protecting group manipulations. Typically these reactions are performed at low tempertature (−78 °C - −40 °C), and one of the most common 'promotor' reagent system used to activate the anomeric-thio group is NIS/TfOH.[73] Many other promotor systems have also been developed (and these have been reviewed by Das et al).[74] Thioglycosides have been used in 'one-pot' sequential glycosylation reactions to synthesise oligosaccharides. The selectivity is facilitated by the relative reactivity of the individual thioglycosides (modulated by the choice of protecting groups).[75] For example, a more reactive thioglycoside donor is reacted with a moderately reactive thioglycoside donor, then on completion, a less reactive thioglycoside is added with more promotor reagent to give a trisaccahride. The primary advantage of this method is improved overall synthetic efficiency by avoiding the need to isolate intermediates.

Glycosyl trichloroacetimidates are another very popular choice of glycosyl donor, due to their ease of formation and high reactivity, leading to good yields with favourable stereocontrol. Glycosyl trichloroacetimidates are readily prepared by reaction of an anomeric hydroxyl group with trichloroacetonitrile in the presence of a base. The glycosylation reactions can be then activated using a range of Lewis acid catalysts e.g. TMSOTf or $BF_3{\cdot}OEt_2$, at relatively high temperature (0 °C). As in the case of thioglycosides, many additional 'promotor' reagents have have been investigated and have been reviewed by Zhu and Schmidt.[76]

1.15 Mechanism of *O*-glycosylation reactions and the consequence on stereochemistry

In the presence of a participating group in the C2 position, for example O-Ac or N-Ac, the oxacarbenium ion formed upon activation of the leaving group is stabilised on the α-face. This leads to attack on the β-face by the acceptor, resulting in high β-selectivity.

In the absence of a C2 participating group, the glycosylation mechanism goes through a continuum, with unimolecular S_N1-like and bimolecular S_N2-like at either end of the spectrum. The position along this spectrum for a given donor and acceptor pair is poorly understood, explaining why the stereochemical outcome is difficult to predict. The leaving group on the anomeric carbon is first activated by addition of a promotor (e.g. TMSOTf), and this leads to the formation of several possible intermediates on a spectrum between a covalently-bonded intermediate to a formal oxocarbenium ion, shown in figure 13. The reaction of the former with an acceptor tends to favour nucleophilic substitution in a S_N2-like manner to give predominantly the β-glycoside, while the latter reacts via an S_N1-like mechanism to give predominantly the α-glycoside, as shown in figure 13. The reactivity of a given acceptor and donor will affect how they react along this mechanistic spectrum. In general, more reactive donors (higher RRV values) will favour α-glycosylation, and more reactive acceptors (higher acceptor nucleophilic constant, AKa, values) will favour β-glycosylation.

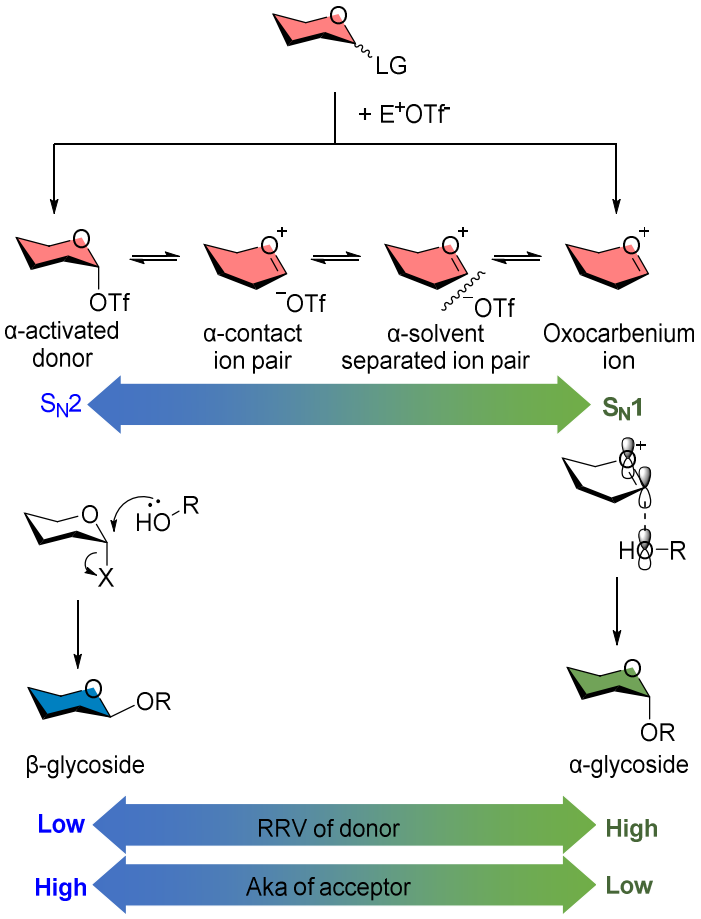


Figure 13: Mechanism of glycosylation reaction showing the S_N1-to-S_N1 continuum and the donor and acceptor parameters that inform where on the spectrum a given donor/acceptor pair will be.

These *O*-glycoylation reactions are highly sensitive to the donor and acceptor substrates, in addition to the reaction conditions (solvent, and temperature) making the stereoselective outcome difficult to predict. In 1988, Fraser-Reid introduced the concept of armed/disarmed effects to describe the relative reactivities of glycosyl donors based on their (non-participating) protecting groups.[77] Ether-protected glycosyl donors were more reactive (termed 'armed'), than the corresponding ester-protected glycosyl donor (termed 'disarmed'). Additionally, when two glycosyl donors were reacted together, it was found that if the 'disarmed' glycoside contained free-hydroxyl, a selective coupling could be achieved with the disarmed counterpart taking on the role of the glycosyl accepter.[78] More recently, this concept has been extended to predict the stereochemical outcome of an *O*-

glycosylation reaction.[79] Guidelines for the prediction software, GlycoComputer[80], was released in 2021 and uses Wong's relative reactivity value (RRV)[81] or donors, and Wang's acceptor nucleophilic constant (AKa) of acceptors[80] to estimate the stereochemical outcome of a reaction.

1.16 Small molecule inhibitors of endosulfatases

Over the past decade, there has been increasing interest in designing Sulf inhibitors since their identification as a potential therapeutic target (figure 14). In 2010, Hossain et al. reported the potent inhibition of Sulf1 and Sulf2 by PI-88 **23**, a heparin mimetic originally developed as a heparanase inhibitor.[82] Then in 2013, Zheng et al. reported OKN-007 **24**, a novel phenyl-sulfonyl inhibitor of HSulf-2, (although the inhibitory data were unpublished), that had antitumor effects in multiple cancer cell lines.[83] This molecule had previously been in clinical trials for stroke patients, and thus had passed human safety trials. However both of these inhibitors had been developed for alternative applications, and thus raises the question of selectivity.

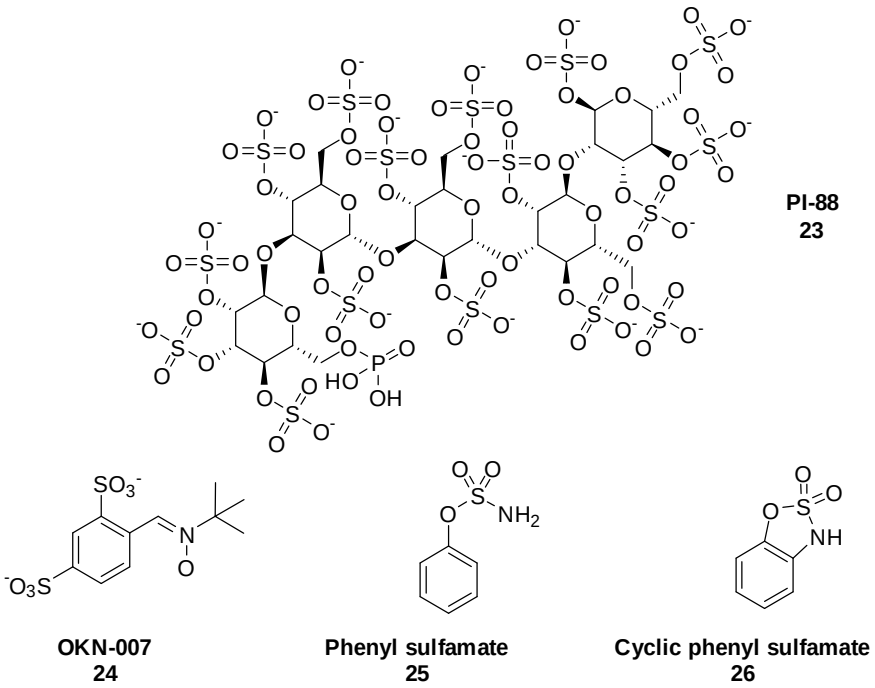


Figure 14: Structures of early non-selective HSulf inhibitors

1.17 Sulfamate-containing Sulf inhibitors

Taking inspiration from the wealth of SAR knowledge generated for steroid sulfatase inhibition, the sulfamate pharmacophore was investigated for endosulfatase inhibition.[2] The sulfamate motif was first verified as an inhibitor of the endosulfases using the phenyl sulfamate **25** and cyclic phenyl sulfamate **26** by Schelwies et al., however these inhibitors were more potent against arylsulfatase from *Pseudomonas aeruginosa* (PARS).[2] This is not surprising considering these analogues were developed by Hanson et al. to be pan-sulfatase mechanism-based inhibitors.[84] Scheilwies et al. validated phenyl sulfamate as an inhibitor of HSulf-1/2, before synthesising glucosamine-based small molecule inhibitors. The design involved the introduction of the sulfamate motif as a bioisostere for the sulfate group in the context of the HS substrate in an attempt to improve the potency and specificity of the inhibitors. As a proof of concept, the preliminary work began with the use of the smallest, most relevant unit of HS, α-GlcNS6S, a disulfated monosaccharide. Inhibitors were designed, one with the primary sulfamate group **27** (predicted to be irreversible

inhibitors) and one with a *N*,*N*-dimethyl sulfamate group **28** (predicted to be competitive, reversible inhibitors). The biochemical characterisation of these compounds revealed that the sulfamate inhibitors had an IC_{50} values of 95 and 180 μM against HSulf-1, and 130 and 240 μM against HSulf-2, but perhaps more importantly, were more selective for the Sulfs than other sulfatases investigated. The *N*,*N*-dimethyl sulfamate analogues were only 2-fold less potent than the primary sulfamate analogues, a much smaller difference in potency than has been reported for the SAR of steroid sulfatase inhibitors, indicating that the endosulfatases are tolerant to increased steric bulk of the *N*,*N*-dimethyl sulfamate or act via a different mechanism. Finally, under the reported assay conditions, no irreversible inhibition was observed for either sulfamate analogues in comparison to the steroid sulfamate inhibitors, which may be attributed to the higher pKa of the parent alkoxide compared to lower pKa of the phenoxide in the case of aryl sulfamate inhibitors.

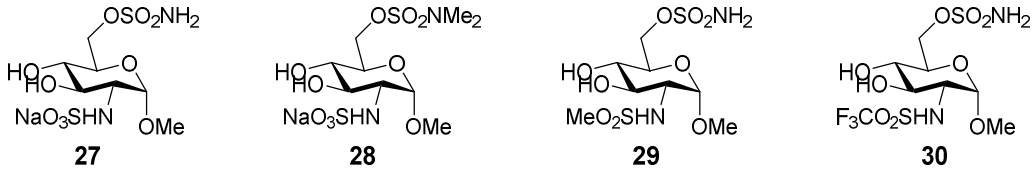
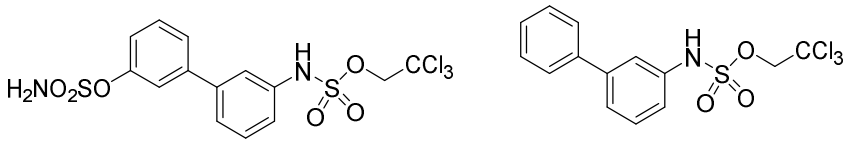


Figure 15: Structures of glucosamine-6-*O*-sulfamate small molecule inhibitors of the Sulfs

In 2015, Miller et al. aimed to replicate the inhibitory activity of the glucosamine-6-sulfamate inhibitors, and improve the potency by exploring the structure activity relationship of the series.[85] This SAR included an investigation into the effect of hydrogen bond donor ability of the 2*N*-hydrogen atom by synthesising the 2*N*-trifluoromethylsulfonamide **30** and the 2*N*-methylsulfonamide **29** to alter the acidity of this proton. However, all compounds synthesised were found to have minimal inhibition of Sulf-2 at 1 mM. In 2016, the same authors reported the design of saccharide mimics that contained the aryl sulfamate motif that has been previously reported as inhibiting type-I

sulfatases.[86] The design was aided by overlaying biphenyl and biphenyl ether analogues with a heparin-derived trisaccharide, so that the substitution patterns of the polar groups on the biphenyl analogues could be superimposed with the energy minimised 3D-structure of the saccharide. In total, 32 analogues were synthesised and evaluated as inhibitors of Sulf-2, and it emerged that a trichloroethylsulfamate group, initially used as a protected-sulfate intermediate, was a superior pharmacophore for sulf-2 inhibition compared to the previously reported sulfamate group, compounds **31** and **32**. Unfortunately, the compounds reported did not display good selectivity for Sulf-2 compared to other type I sulfatases, despite having an improved potency. These compounds were not tested for time-dependent inhibition and so it remains unknown whether these compounds exhibit irreversible or competitive binding. Without a more detailed structure-activity relationship, it is unclear whether the trichloroethylsulfamate is mimicking the 6*O*-sulfate or the 2*N*-sulfate in HS.



31
167 μM

32
254 μM

Figure 16: Structures of biphenyl trichloroethylsulfamate inhibitors

Three saturated analogues of the biphenyl series were prepared in an attempt to mimic the 3D character of the saccharide ring and improve potency, shown in figure 17. Unfortunately, when these analogues were evaluated as inhibitors of Sulf-2, none caused more than 40% inhibition at 1 mM and therefore were not progressed for IC_{50} determination. (reference: Annalisa Bertoli, PhD thesis).

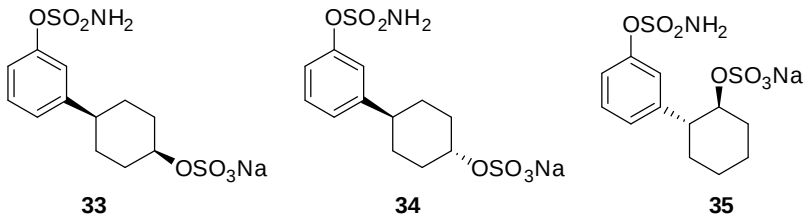


Figure 17: Structures of saturated 'B' ring analogues of the biphenyl sulfamate inhibitors

1.18 Mechanism of inactivation of sulfatases by sulfamate inhibitors

Aryl-*O*-sulfamates are irreversible, active-site directed inhibitors of estrone sulfatase. Inhibitors for other sulfatases have not been extensively developed, but a study by Bojarová and Williams demonstrated that simple aryl sulfamates inactivate sulfatases from multiple sources, providing evidence that the sulfamate pharmacophore will effectively inhibit any class 1 sulfatase.[87] Although, the mechanism of action has yet to be confirmed, the current hypothesis is sulfamoyl transfer to the active site FGly residue.

Sulfamate is a good isostere of sulfate, therefore the mechanism of sulfamoyl transfer may be similar to sulfuryl transfer. The requirement of a cleavable S-OAr bond was shown by Liu et al., who synthesised the α,α-difluorosulfonamide derivative of EMATE as a non-hydrolysable analogue. This analogue was found to be a reversible inhibitor of estrone sulfatase, suggesting that the irreversible inhibition caused by sulfamate analogues requires cleavage of the S-OAr bond.[88] A physical-organic chemistry study by Bojarova et al. reported evidence suggesting that the catalyzed and spontaneous hydrolysis of aryl sulfamates share a similar transition state, implying they react via the same mechanism.[89] Quantum mechanical calculations suggested that this mechanism is an E1cB-like pathway that produces sulfonylamine.[90]

A break-through study by Potter reporting the crystal structure of PARS[iii] inactivated with 667-coumate has given insight into the dead-end adduct.[91] The crystal structure determined to 2.0 Å resolution shows the only covalent modification in the active site is sulfamoyl transfer to one hydroxyl of the FGly residue, however at this resolution it is not possible to distinguish between *N*- or *O*-linked adducts. The current hypothesis proposes that the mechanism of inactivation involves the enzyme catalysed hydrolysis of sulfamates via an E1cB-pathway followed by nucleophilic attack of the nitrogen atom of $HNSO_2$ to produce the *N*-linked sulfamoylated FGly residue as the dead-end product (figure 18).

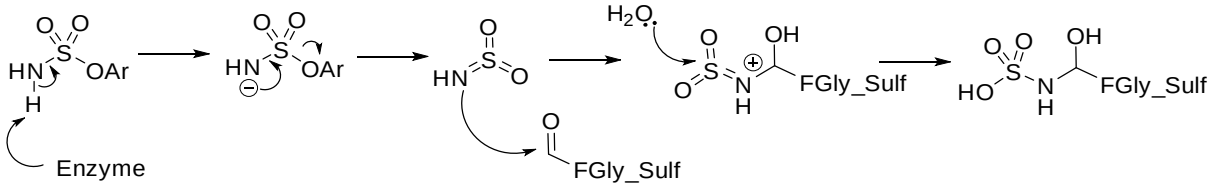


Figure 18: The proposed mechanism leading to the N- sulfamoylated enzyme adduct

To date, the majority of inactivation studies of sulfatases have used aryl sulfamates as inhibitors. It is not currently known whether the minimum pharmacophore is the sulfamate group or the entire phenylsulfamate unit, and so it is still not clear whether this mechanism of inactivation applies in the case of alkyl sulfamates. A series of long chain sulfamates has been tested against esterone sulfatase, however no inhibition was observed up to concentrations of 10 mmol/L.[92] It should be noted that the analogous sulfate was not tested as a substrate so the lack of inhibition may reflect the binding affinity.

1.19 Summary

It is clear from an increasingly large body of literature that heparan sulfate is implicated in many areas of disease. Two endosulfatases have been identified that modify HS sulfation,

[iii] PDB not available

specifically in the glucosamine-6*O* position. While structurally subtle, this modification has great biological consequences relating to aforementioned disease areas. The Sulfs are difficult to study due to a lack of potent and selective inhibitors or ligands. Therefore, the aim of this thesis is to develop chemical probes to target Sulfs in cells. Thesis content is summarised in figure 19.

Chapter 2 describes the re-synthesis of the putative inhibitor glucosamine-6-sulfamate, and its biochemical evaluation as a Sulf inhibitor in an attempt to settle the uncertainty arising from the different findings of Schelweis et al and Miller et al. Following on from this, a small library of glucosamine-6-sulfamates are prepared to establish a structure activity relationship, focussing on the sulfamate warhead (modification at C6), the anomeric substituent (modification at C1) and the acidity of the *N*H via modification at C2.

Chapter 3 describes the synthesis of putative disaccharide inhibitors, which are designed to increase the inhibitory potency of the small molecules developed in chapter 1.

Chapter 4 describes the modification of the trisulfated disaccharide inhibitor developed in chapter 3 so that it can be used as a key intermediate in the synthesis of target engagement probes (chapter 5) and a multivalent display (chapter 6).

Chapter 5 describes the synthesis of target engagement probes, firstly using a photoaffinity labelling approach with a diazirine and alkyne handle, and secondly using an iridium photocatalyst-conjugate for the catalytic activation of an intermolecular diazirine.

Chapter 6 describes the synthesis of a multivalent display of the disaccharide inhibitor using a norbornenyl polymer that could be modified by amide couplings, and the work towards using this glycopolymer as a ligand for incorporation into a molecule for targeted degradation using LYTAC technology.

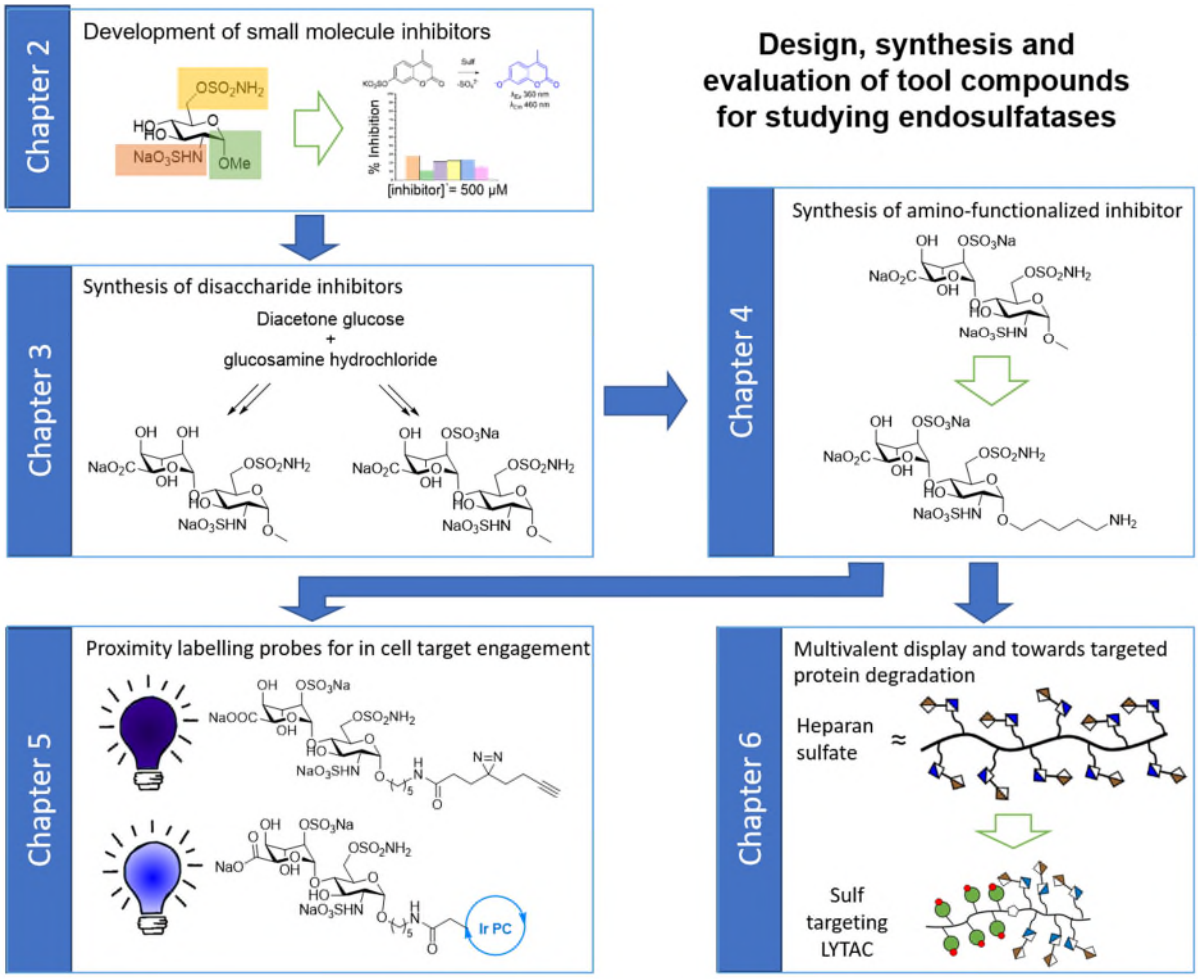


Figure 19: Summary of the content described in this thesis

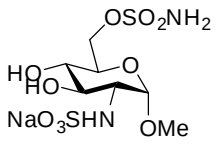
2. Glucosamine-6-sulfamate analogues as inhibitors of endosulfatases

This chapter describes the design and synthesis of a library of putative endosulfatase inhibitors based on the monosaccharide GlcNS(6S), and efforts towards isolation of Sulf-2 for development of an endosulfatase activity assay.

2.1 Introduction

Two publications that have investigated glucosamine-6-sulfamate as an inhibitor of Sulf-2 report dramatically different inhibitory data. Schelwies et al. report an IC_{50} value of 130 μM, using purified recombinant enzyme in their assay protocol.[2] In contrast, Miller et al. report no inhibition at 1 mM against polymer bound Sulf-2 (enzyme concentration not quantified).[85] The disagreement in the reported activity of **27** may be due to the practical differences in the assay protocols. Other than the enrichment method of Sulf-2 mentioned above, another difference in the assay protocols is the enzyme/inhibitor preincubation prior to addition of the assay reagent. Schelwies et al. preincubated the enzyme with the inhibitor before performing a 10-fold dilution into the final assay solution for activity determination, but reported the IC_{50} values relating to the original concentration. On the other hand, Miller et al. reported the concentration of the inhibitor in the final assay concentration. It is clear that these assay formats are not directly comparable, nonetheless the question of whether **27** is an inhibitor of Sulfs remains.

The work described in this thesis began with the synthesis of **27**, following the procedure described by Miller et al.[85]

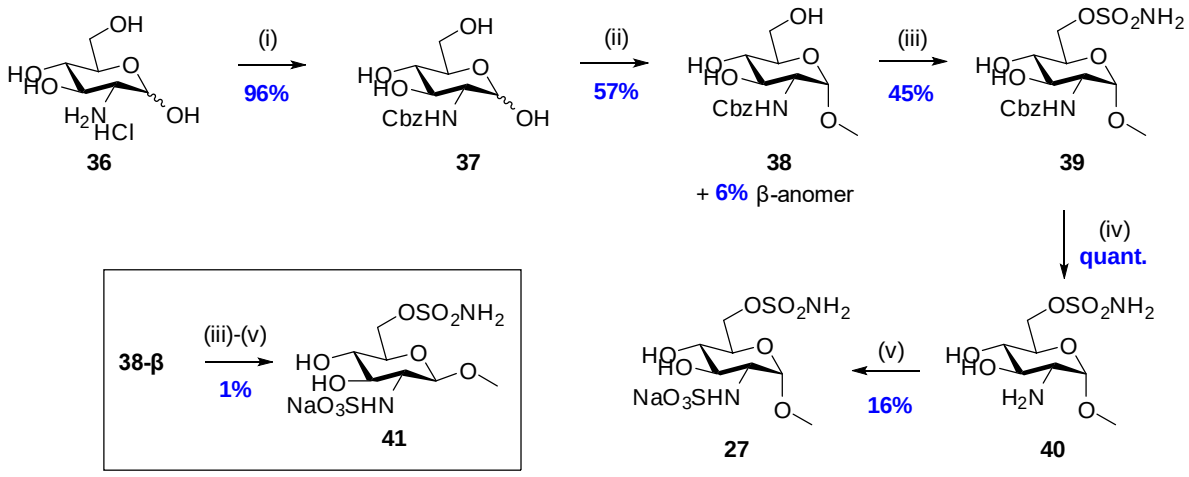


27

Figure 20: Structure of glucosamine-6*O*-sulfamate **27**

2.2 Synthesis of inhibitor 27

Glucosamine hydrochloride (**36**) was selectively *N*-protected with benzyl chloroformate in basic aqueous medium in 96% yield. The anomeric methoxy group was installed under Fischer glycosylation conditions. A temperature of 85 °C for 16 h gave a mixture of anomers (ratio α/β 9:1), which were separated by column chromatography (isolated yields: α-glycoside 57%, β-glycoside 6%, scheme 8). The two anomers were assigned by ^{1}H NMR, based on the C(1)*H* signal chemical shift and coupling constant, as discussed in Chapter 7 (subsection ^{1}H NMR assignment of the anomeric configuration). The α-methyl glycoside C(1)*H* doublet at 4.68 ppm had a coupling of 3.1 Hz, and the β-methyl glycoside C(1)*H* doublet at 4.27 ppm had a coupling of 8.1 Hz. The α–anomer (**38**) was then subjected to the regioselective sulfamoylation conditions at –40 °C to give **39** in a 45% yield. Finally, deprotection of the Cbz-protecting group by Pd/C catalysed hydrogenation (quantitative yield), followed by *N*-sulfation with sulfur trioxide-pyridine complex at pH 9 (16% yield), gave **27** as a sodium salt in 4% yield over five steps.



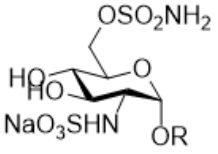
Scheme 8: Synthesis of putative inhibitor **27**. Reagents and conditions: (i) CbzCl, $NaHCO_3$, H_2O, rt, 16 h, 96%; (ii) HCl, CH_3OH, 85 °C, 16 h, α-glycoside 73%, β-glycoside 6%; (iii) $ClSO_2NH_2$, DMF/PhMe, −40 °C, 16 h, 45%; (iv) H_2 (balloon), 10% Pd/C, CH_3OH, rt, 16 h, quant.; (v) SO_3·Py, H_2O (pH 9-10), rt, 16 h; 16%.

The corresponding β-anomer of **27** (**41**) was prepared following the synthetic sequence outlined in scheme 8, from the methyl β-glycoside (**38-β**) isolated from the Fischer glycosylation (scheme 8, step ii), in 1% yield over 5 steps.

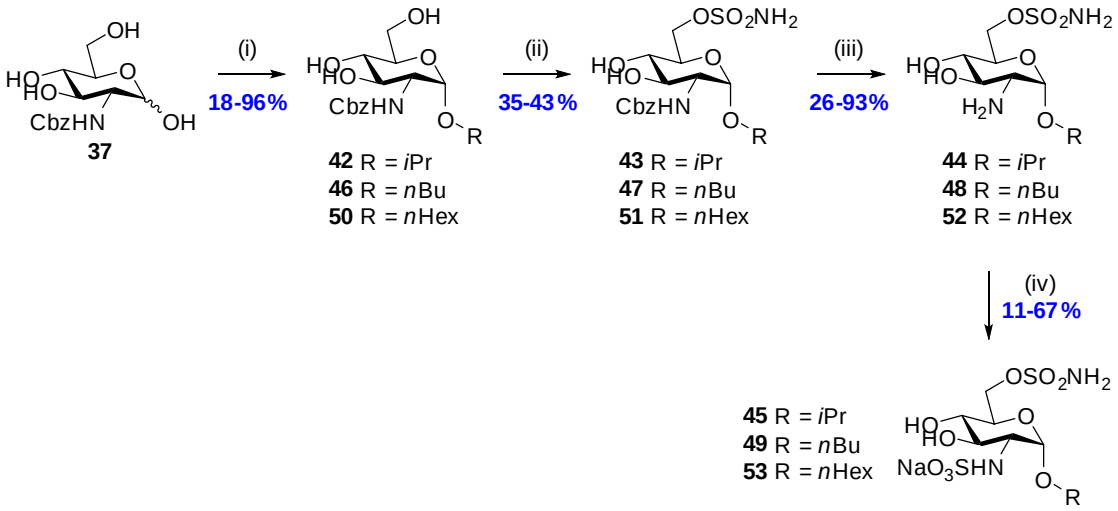
2.3 Modifications at C1

To assess what modifications are tolerated at the anomeric carbon, a series of increasingly lipophilic alcohols were inserted into this position via an acid catalysed Fischer glycosylation reaction (table 2).

Table 2: Summary of alkyl glycoside analogues synthesised

	Compound	*R*
	**45**	*i*Pr
	49	*n*Bu
	53	*n*Hex

A series of alternative alkyl glucosides were synthesised according to scheme 9. The synthesis began from *N*-Cbz glucosamine, which was reacted with *iso*-propanol, *n*-butanol and *n*-hexanol under Fischer glycosylation conditions using HCl (4M in dioxane, final reaction concentration 1.25M). Compared to the reaction conditions described previously, the reaction temperature was lowered and reaction time shortened due to decomposition. For this reason, whether the reaction had reached equilibrium was not determined. The *iso*-propyl analogue resulted in a low yield (18%), which is hypothesised to be due to increased sterics compared to the other alcohols investigated. Nonetheless, the desired α–*O*-glycosides were isolated in moderate to good yields (18-69%), scheme 9 and table 3. The anomeric configuration was assigned by ^{1}H NMR; *i*-Pr **42** C(1)*H* doublet at 4.81 ppm with a coupling constant of 2.7 Hz; *n*-Bu **46** C(1)*H* doublet at 4.49 ppm with a coupling constant of 3.5 Hz; *n*-Hex **50** C(1)*H* doublet at 4.79 ppm with a coupling constant of 3.5 Hz. The α-glycosides were obtained then subjected to regioselective sulfamoylation with sulfamoyl chloride at –40 °C to afford the sulfamate intermediates in 35-43% yield. The hydrogenation labile *N*-Cbz protecting group was removed using standard conditions of H_2 (balloon), 10% loading Pd/C in methanol (26-93%), and finally *N*-sulfation with sulphur trioxide-pyridine complex at pH 9-10, gave alkyl glycosides **45**, **49** and **53** as sodium salts in 11-67% yield, scheme 9 and table 3.



Scheme 9: Synthetic route to alkyl glycoside analogues. Reagents and conditions: (i) Requisite alcohol, HCl (4M in dioxane), 60 °C, 3 h, 18-63%; (ii) $ClSO_2NH_2$, DMF/$PhCH_3$, −40 °C, 35-43%; (iii) H_2 (balloon), 10% w/w Pd/C, CH_3OH, rt, 16 h, 26-93%; (iv) $Py.SO_3$, H_2O, pH 9-10, rt, 16 h, 11-67%.

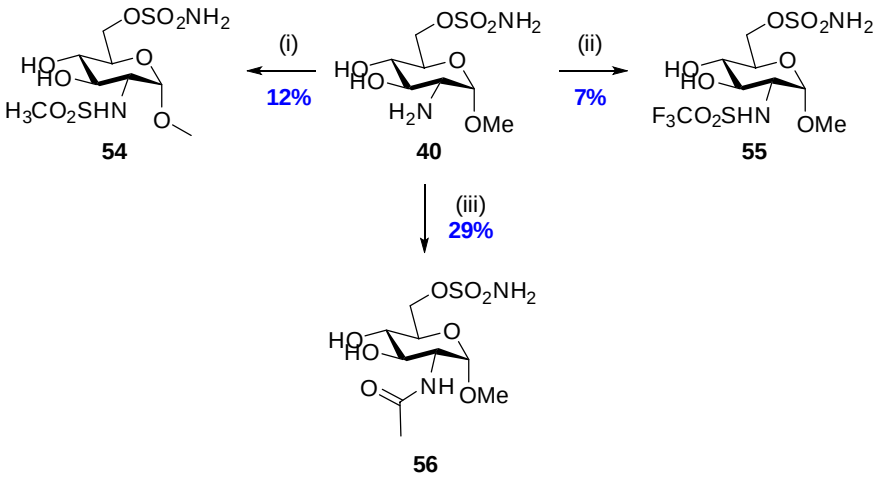
Table 3: Yields of reactions (i)-(iv) described in scheme 9 for sulfamate analogues 45, 49 and 53

Compound	*R*	*Yield (i)*	*Yield (ii)*	*Yield (iii)*	*Yield (iv)*
45	*i*Pr	18%	36%	88%	11%
49	*n*Bu	69%	35%	26%	36%
53	*n*Hex	63%	43%	93%	67%

2.4 Modifications at C2

Myette et al. published a homology model of the 6-*O*-sulfatase from *Flavobacterium heparinum* with the monosaccharide GlcNS6S docked into the active site.[93] Using this model, the authors proposed that the *N*-sulfate residue acts to increase the acidity of the *N*-H atom, therefore increasing its hydrogen bond donor ability, which is hypothesised to affect binding by interacting with an aspartic acid residue within the active site. To investigate the importance of the acidity of this *N*-H, the sulfate moiety was replaced by both a mesylate and a triflate. Analogues **54** and **55** were prepared from intermediate amine **41** using methanesulfonyl chloride and trifluoromethanesulfonic anhydride respectively,

and were isolated in low yield (12% and 7%, respectively). Finally, amine **40** was reacted with acetyl chloride in pyridine at 0 °C to afford *N*-acetyl glucosamine **56** in 29% yield. These low yields were primarily due to difficulty in compound isolation, perhaps instability on silica column chromatography, however the progress of the reaction was determined complete by TLC.



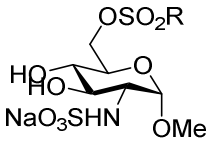
Scheme 10: Synthesis of putative inhibitors **54**, **55** and **56**. Reagents and conditions: (i) H_3CSO_2Cl, DIPEA, CH_2Cl_2, 0 °C - rt, 1 h, 12%; $(CF_3SO_2)_2O$, Et_3N, CH_2Cl_2/1,4-dioxane, 0 °C, 1 h, 7%; (iii) AcCl, pyridine, 0 °C - rt, 1 h, 29%.

2.5 Modifications at C6

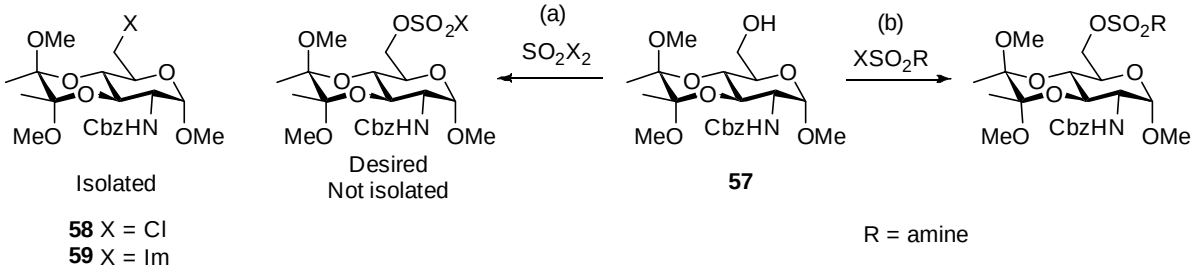
The structure activity relationship explored by Miller et al. focused primarily on modifications at the C1 and C2 positions. The modification at C1 involved additional hydrophilic functional groups, for example a primary alcohol and a carboxylate group. The modifications at C2 involved substituting the *N*-sulfate with *N*-mesylate and *N*-triflate. Arguably, the most important functional group, the 6-*O*-sulfamate was not investigated, despite the fact that this group is hypothesised to be the sulfatase inhibiting pharmacophore. As discussed in Chapter 1, the aryl sulfamate group leads to mechanism-based irreversible inhibition of the steroid sulfatases. Schelwies et al. compared the *N*-unsubstituted and the (*N*,*N*-dimethyl)sulfamate warheads, and reported that both analogues fully inhibited Sulf-

1 and sulf-2 at 5 mM.[2] However, in the case of the steroid sulfatase inhibitors, the *N*-methyl and *N*,*N*-dimethylsulfamates do not exhibit irreversible inhibition profiles, in comparison to the primary sulfamate analogue.[94] This indicates that the structure-activity relationship (SAR) allows for modification at the *N*-sulfamate and that it may lead to a change in inhibitory action. In order to establish a SAR of the endosulfatase-inhibiting glucosamine-6-sulfamate analogues, a series of compounds were designed focussing on modification at the C6 position, shown in table 4.

Table 4: Summary of sulfamate analogues synthesised

	Compound	*R*
	**27**	NH_2
	61	$N(CH_3)_2$
	65	$NHCH_3$
	69	$N(CH_2CH_3)_2$
	73	$N(CH_2)_5$

In order to have complete flexibility regarding the choice of amine, it was desirable to develop optimised conditions for the synthesis of the *N*-substituted sulfamates that could be easily run in parallel to synthesise a library of compounds. With this in mind, it was proposed that an activated sulfate group, which could undergo nucleophilic substitution at S (rather than C), would be a suitable reactive intermediate for library formation, scheme 11 (a).

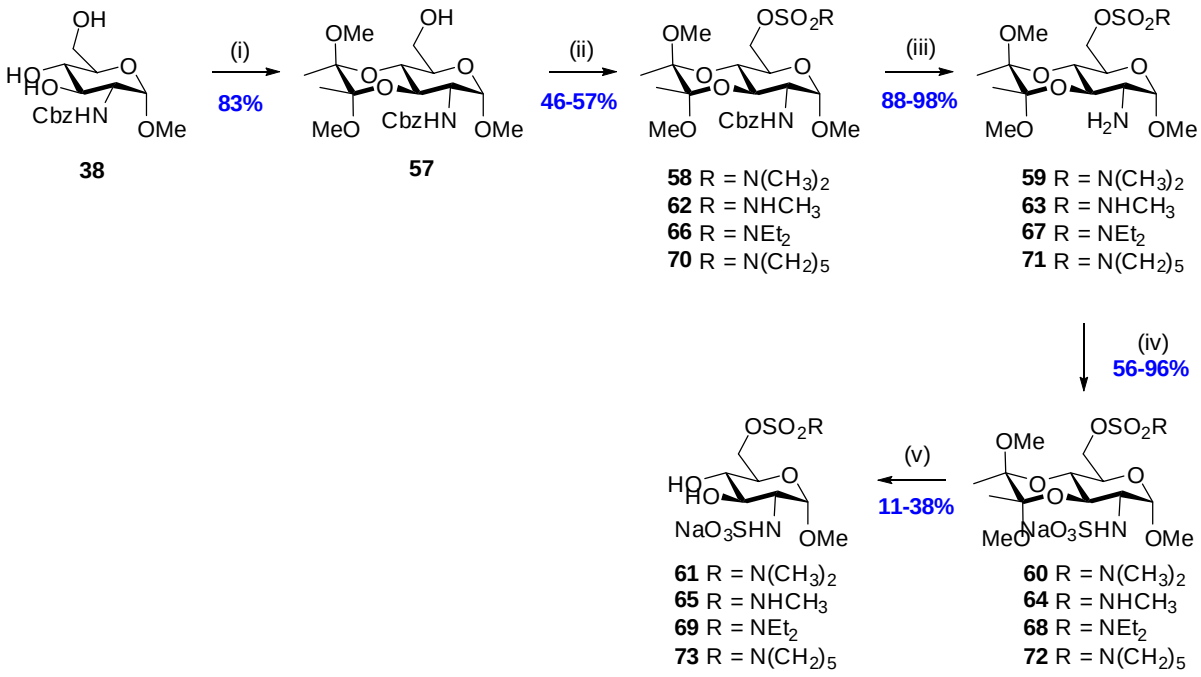


Scheme 11: Routes to 6-*O*-sulfamates investigated. Reagents and conditions*:* (a) **57**, NaH, THF, 0 °C, 15 min; then SO_2Cl_2 or 1,1′-sulfonyldiimidazole, 0 °C – rt, 1 h (also attempted at –78 °C); (b) (i) Chlorosulfonic acid, RNH_2, CH_2Cl_2, 0 °C, 30 min; (ii) sulfamic acid, $SOCl_2$ or triphenylphosphine ditriflate (generated *in situ* from $Ph_3P{=}O$, Tf_2O); then, **57**, CH_2Cl_2, –78 °C – rt, 18 h.

First, it was hypothesised that glucosamine-6-chlorosulfate could be reacted with any primary or secondary amine to produce the desired sulfamate. Following a procedure described by Simpson and Widlanski[95] the chlorosulfate synthesis was attempted by adding alcohol **57** to a solution of sulfuryl chloride. Unfortunately, this only yielded the 6-chloroglucosamine directly **58** (X = Cl). In an attempt to control the reactivity of the activated intermediate, an alternative sulfating reagent was used (1,1′-sulfonyldiimidazole), because the intermediate would be less electrophilic with respect to S_N2 reaction at C. However, this also yielded the undesired product, tentatively assigned as the 6-substituted glucosamine **59** (X = Im) by mass spectrometry, scheme 11 (a).

Having been unsuccessful in initial attempts to sulfate the glucosamine intermediate **57**, efforts were turned towards synthesising the desired sulfamoyl chlorides or synthetic equivalents, scheme 11 (b). Addition of sulfuryl chloride to benzyl amine was difficult to control and the undesired sulfamide product was obtained, as observed by mass spectrometry. Another route involved the formation of the more stable sulfamic acid followed by activation with triphenylphosphine ditriflate[96] or thionyl chloride[97]. Unfortunately, these alternative approaches did not yield any of the desired products.

Consequently, it was decided to use a series of commercially available *N*,*N*-dialkylated sulfamoyl chlorides. These were reacted with the orthogonally protected alcohol **57**, as shown in scheme 12. The synthesis of this key intermediate began from α-methylglucoside **38**, with the selective protection of 3- and 4-hydroxyls. This was achieved by reaction with 2,3-butanedione, trimethyl orthoformate and catalytic sulfonic acid in refluxing methanol, to give **57** in a high yield as a single diastereomer (83%). Next, the 6-OH was reacted with the commercially available *N*,*N*-dialkylated sulfamoyl chlorides and NaH in THF at 0 °C to give the desired sulfamate in moderate yield (46-57%). Subsequent cleavage of the amine-Cbz protecting group was achieved under standard hydrogenation conditions, with 10% Pd on activated carbon under atmospheric H_2, which proceeded very effectively with all analogues to give the desired primary amine in excellent yield (88-89%). The *N*-sulfation was then achieved by reaction of the primary amine with sulphur trioxide-pyridine complex at pH 9-10, monitored by ^{1}H NMR spectroscopy, and further equivalents of sulphur trioxide-pyridine complex were added until no starting material was observed (>95% conversion by ^{1}H NMR). All analogues were isolated in moderate to excellent yields (56-96%). Finally, bis-acetal deprotection under acidic conditions gave the desired 6-*O*-sulfamates **61**, **65**, **69** and **73** in 11-38% yield. The isolated yields of these analogues was low due to a challenging and inefficient purification, however the conversion to product was high (monitored by TLC and MS). Enough material was isolated for full characterisation and biological evaluation, and therefore the purification was not optimised further.



Scheme 12: Synthetic route to glucosamine-6-*O*-sulfamate analogues. Reagents and conditions: (i) 2,3-butanedione, $CH(OMe)_3$, cat. CSA, CH_3OH, 80 °C, 83%; (ii) $ClSO_2R$, THF, 0 °C – rt, 46-57%; (iii) H_2 (balloon), 10% Pd/C, CH_3OH, rt, 16 h, 88-98%; (iv) $Py.SO_3$, H_2O, pH 9-10, rt, 16 h, 56-96%; (v) TFA, H_2O, rt, 2 h, 11-38%.

Table 5: Yields of reactions (ii)-(v) described in scheme 12 for sulfamate analogues **61**, **65**, **69** and **73**

Compound	*R*	*Yield (ii)*	*Yield (iii)*	*Yield (iv)*	*Yield (v)*
61	$N(CH_3)_2$	57%	88%	96%	11%
65	$NHCH_3$	46%	91%	96%	33%
69	$N(CH_2CH_3)_2$	50%	98%	77%	13%
73	$N(CH_2)_5$	49%	90%	54%	38%

A limitation of this route was the reliance on the commercial availability of the sulfamoyl chlorides, many of which were moisture sensitive.

2.6 Synthesis of non-hydrolysable glucosamine-6-sulfamate analogue

In order to probe the mechanism of inhibition, it was desirable to have a structurally related, but inactive analogue of glucosamine-6-sulfamate **27**. Based on the proposed mechanism

of inhibition of the sulfamate-based inhibitors involving the transfer of sulfate to the enzyme,[94] it was hypothesised that the replacement of the sulfamate moiety with a methylene-sulfonamide would be non-hydrolysable and therefore would act via a different mechanism of inhibition. The carbanion ($Glc6CH_2^-$) is an extremely poor leaving group in comparison to the oxyanion ($Glc6O^-$), therefore preventing sulfamoyl transfer to the endosulfatase and thus any observed inhibition of Sulfs affected by the methylene-sulfonamide analogue would be due to competitive binding.

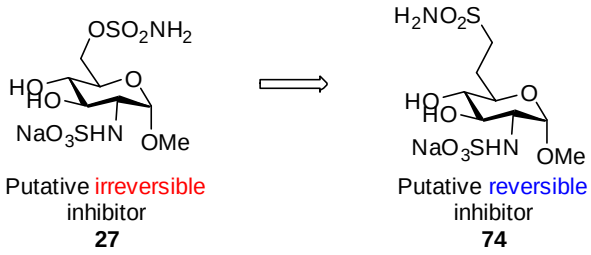
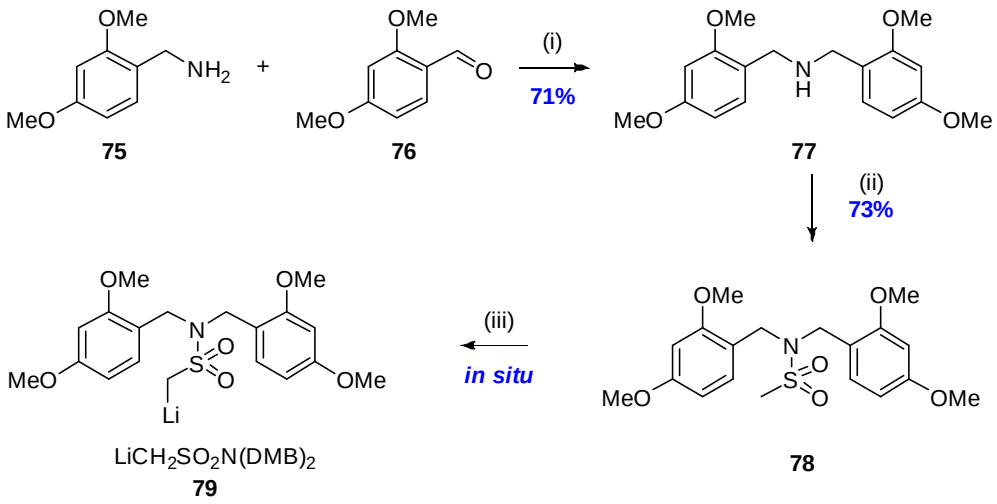


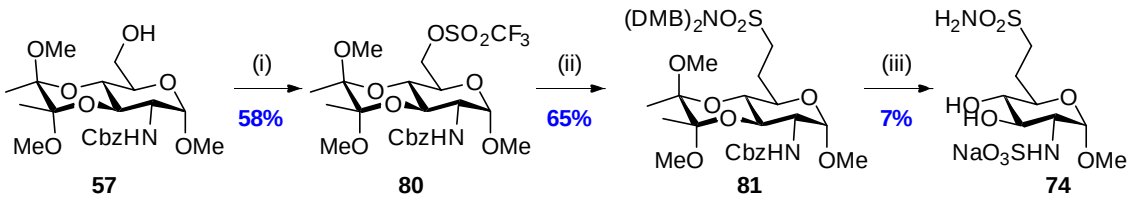
Figure 21: Design of glucosamine-6-CH_2-sulfonamide 74 as non-hydrolysable analogue of inhibitor **27**

The synthesis of non-hydrolysable analogue **74** began with the triflation of alcohol **57** by reaction with triflic anhydride in the presence of Et_3N at 0 °C to give **80** in 58% yield. The methylene sulphonamide unit was then introduced by nucleophilic substitution of the triflate with $LiCH_2SO_2N(DMB)_2$ (**79**).[98] This nucleophile was generated *in situ* by deprotonation of methylsulfonamide **78** with *n*-BuLi at −78 °C. Methylsulfonamide **78** was prepared following the synthetic route outlined in scheme 13; a reductive amination of 2,4-dimethoxybenzylamine (**75**) and 2,4-dimethoxybenzaldehyde (**76**) using $NaBH_4$ as the reducing agent (achieved in 71% yield), was followed by mesylation of the secondary amine (**77**) by reaction with mesyl chloride in the presence of Et_3N to give the desired methylsulfonamide **78** in 73% yield.



Scheme 13: Synthesis of $LiCH_2SO_2N(DMB)_2$ (**79**). Reagents and conditions: (i) Na_2SO_4, CH_3OH, 65 °C, 4 h; then, 0 °C, $NaBH_4$, 16 h, 71%; (ii) MsCl, Et_3N, $PhCH_3$, 0 °C, 2 h, 73%; (iii) n-BuLi, THF, −78 °C, 15 min, assumed quantitative, generated in situ.

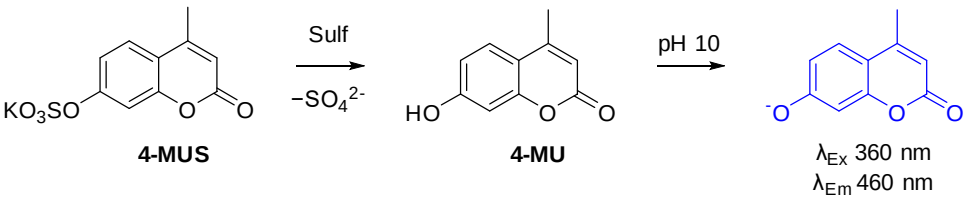
Nucleophilic substitution of triflate **80** with the carbanion nucleophile was achieved in moderate yield (65%). Finally, a three reaction sequence of hydrogenation-*N*-sulfation-hydrolysis gave the putative non-hydrolysable inhibitor **74** in 7% yield over three steps; (1) the carboxybenzyl group was deprotected in 46% yield by palladium catalyzed hydrogenation; (2) addition of sulfur trioxide pyridine complex to the amine intermediate in water at pH 9-10 resulted in the sulfation of the amino group in 37% yield; (3) deprotection of the two *N*-2,4-dimethoxybenzyl and 3,4-bisacetal units was achieved using TFA in water.



Scheme 14: Synthesis of putative inactive control. Reagents and conditions: (i) Tf_2O, Et_3N, CH_2Cl_2, 0 °C, 1 h, 58%; (ii) $LiCH_2SO_2N(DMB)_2$, THF, -78 °C, 5 h, 65%; (iii) 1. H_2 (balloon), 10% w/w Pd/C, CH_3OH, rt, 16 h, 46%; 2. $Py.SO_3$, H_2O, pH 9-10, rt, 16 h, 37%; 3. TFA, H_2O, rt, 2 h, 52%.

2.7 Sulf activity assay

Two main methods are utilised for the measurement of Sulf activity. These differ in the nature of the substrate; (1) colourimetric/fluorogenic pseudosubstrates, whose spectral properties change upon modification by the enzyme and can be measured using a UV-vis spectrometer; (2) heparin, which is closer to the physiological substrate and requires digestion into disaccharides prior to analysis by HPLC. Although heparin is closer to HS, fluorogenic substrates are more suitable for high-throughput screening due to ease of assay set up. 4-Methylumbelliferone sulfate potassium salt (4-MUS) is a pseudo-substrate that is commonly used to detect sulfatase activity by monitoring the hydrolysis to the fluorescent parent phenol methylumbelliferone (4-MU, pKa 7.8, λ_{Ex} 360 nm, λ_{Em} 460 nm), scheme 15. One limitation of this probe is the requirement for basification of the assay solution before fluorescent measurement, which has two major consequences; (i) this additional step in the assay format limits its use as a high-throughput screening format and (ii) the fluorescence intensity is subtly dependent on pH, meaning the measurement is prone to errors between assays.



Scheme 15: Desulfation of 4-methylumbelliferone sulfate (4-MUS) to 4-methylumbelliferone (4-MU), which is subsequently deprotonated to give the fluorescent anion that is excited at 360 nm and emits at 460 nm.

2.8 MSulf-2 activity assay using conditioned medium

Using conditioned medium from a genetically modified MSulf-2 overexpressing cell line was an attractive source of enzyme due to avoiding the need for transient transfection. With

this in mind, MSulf2 and MSulf2ΔCC[iv] overexpressing RAW cell lines were generously gifted by Dr Maarten Swart and were used to obtain conditioned medium containing the active and inactive MSulf-2, respectively. The conditioned medium was removed after 24 h, and the sulfatase activity was measured using 4-MUS (10 mM) in assay buffer (50 mM HEPES, 2.5 mM $CaCl_2$, 250 mM NaCl; pH 7.5) (v/v 1:1, conditioned medium/4-MUS in assay buffer). The reaction was incubated at 37 °C, then quenched by addition of 1M Tris buffer (pH 10.4) before measuring fluorescence at 460 nm following excitation at 355 nm. The results are shown in figure 22. Only approximately two-fold difference in fluorescence was measured comparing the active and inactive MSulf-2 conditioned medium, which was significantly lower than the typical fluorescence obtained when using the sulfatase from *Aerobacter aerogenes* as a positive control (although this was under different assay conditions, and so not directly comparable). The high background activity in the 'negative control' conditioned medium may be due to the endogenous expression of Sulf1, or other secreted enzymes that may have promiscuous sulfatase activity. Many derivatives of 4-methylumbelliferone are used for a variety of simple hydrolyase activity assays, for example 4-methylumbelliferyl glucuronide used for β-glucuronidase activity and 4-methylumbelliferyl phosphate used for phosphatase activity. Additionally, there are numerous reports of promiscuity in catalytic activity of some phosphatases which are able to hydrolyse sulfate esters. [99], [100] The consequence of this high background activity in the neagitve control was that the window of activity of the assay was narrow, and therefore limits the usefulness of the assay because of a potential overlap between the positive and negative controls.

[iv] MSulf2ΔCC the essential catalytic cysteine 88 has been mutated to an alanine residue

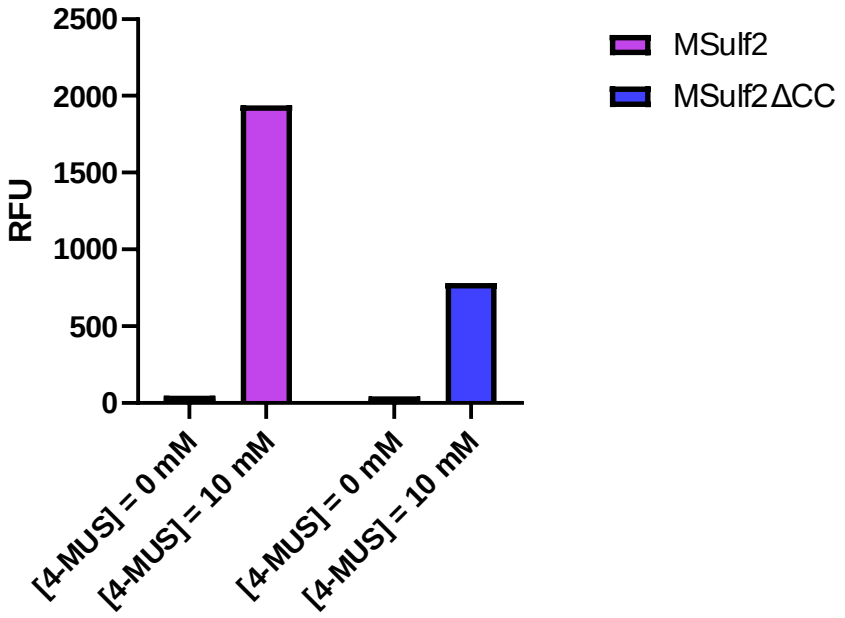


Figure 22: Bar graph of Sulf activity from conditioned medium of genetically modified RAW cells, supplemented with either 0 or 10 mM 4-MUS in assay buffer 50 mM HEPES, 2.5 mM $CaCl_2$, 250 mM NaCl, (pH 7.5). The reaction was incubated at 37 °C for 1 h and quenched with 1M Tris buffer (pH 10.4) and read at 460 nm following excitation at 355 nm in FLUOstar Omega plate reader (BMG Labtech) using Omega data analysis software. RFU = relative fluorescence units.

Following on from this result, it was decided to attempt the isolation of the active MSulf2 enzyme to generate a more concentrated sample to use in the assay protocol, comparable to that used with the sulfatase from *Aerobacter aerogenes.*

2.9 Protein isolation

The mouse sulfatase-2 gene was purchased as plasmid bacteria as agar stab from Addgene (pcDNA3.1/Myc-His(-)-MSulf-2 was a gift from Professor Steven Rosen, Addgene plasmid # 13008). The plasmid was aplified in *Escherichia coli* DH5α cells on LB agar plates contianing ampicillin for antibiotic selection. The DNA was purified using QIAprep® Spin Miniprep Kit, and analysed using restriction digest followed by gel electrophoresis (Figure 23, B.) and sequencing from the T7 promotor.

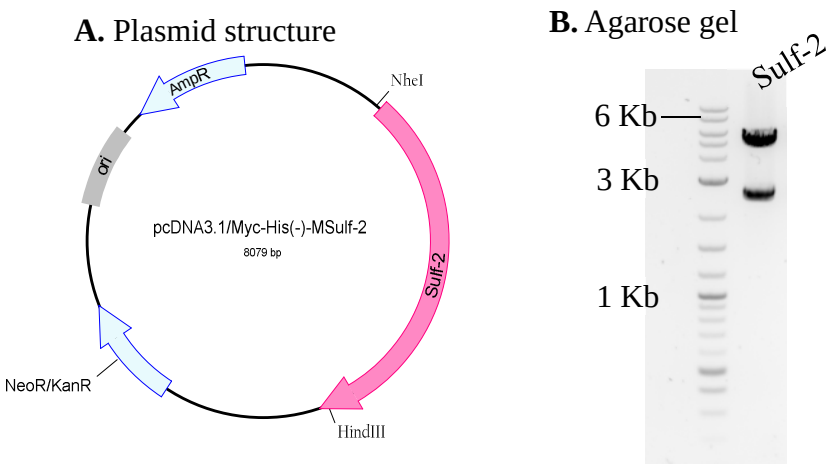


Figure 23: A. Schematic of plasmid used as the DNA cloning vector. Growth strain DH5α *Escherichia coli*. Backbone size without insert = 5500 bp, insert size = 2600 bp. Shown on the plasmid diagram are the genes encoded (AmpR and NeoR/KanR for ampicillin, neomycin/kanamycin resistance respectively), its origin of replication (ori), restriction sites (5′ cloning site: XhoI and 3′ cloning site: HindIII) and protein purification tags: Myc epitope tag and 6×His affinity tag on the C terminal of the Sulf-2 gene insert. B. 1% Agarose gel of restriction digest, using TAE buffer with Sybr Safe, Quick-Load® 2-Log DNA Ladder

The Sulf-2 plasmid was transfected into HEK-293 cells using jetPRIME® transfection reagent according to the manufactures protocol. The cells were seeded for 24 h until approximately 80% confluent prior to transfection, then were incubated at 37 °C for 18 h, after which the medium was changed for 0.5% FBS containing medium, and incubated at 37 °C for a further 18 h. The cell supernatant was collected and the cells were lysed for analysis by western blot. Heparin supplementation (200 μg/mL) and $NaClO_3$ (300 mM) treatment were investigated as additives to maximise the efficiency of protein production and secretion, as shown in figure 24. The hypothesis was that by having an excess of heparin (a substrate of Sulf-2) in the cellular environment, the cell would produce and secrete more enzyme as the demand from the substrate was higher. On the other hand, $NaClO_3$ is an inhibitor of heparan sulfate biosynthesis[101], and so it was hypothesised that if there was no substrate for the Sulf-2 to act on, then the cell may secrete it more readily after production. After the 24 h incubation, the supernatant was collected from the cells

and the proteins were precipitated by treatment with TCA. The cells were lysed, and both the cell lysate and the protein precipitate were denatured in preparation for analysis by western blot. As it can be seen in figure 24, the heparin supplementation gave the greatest 'yield' of sulf-2 protein in both the supernatant and the cell lysate and so this protocol was chosen to take forward for isolation attempts.

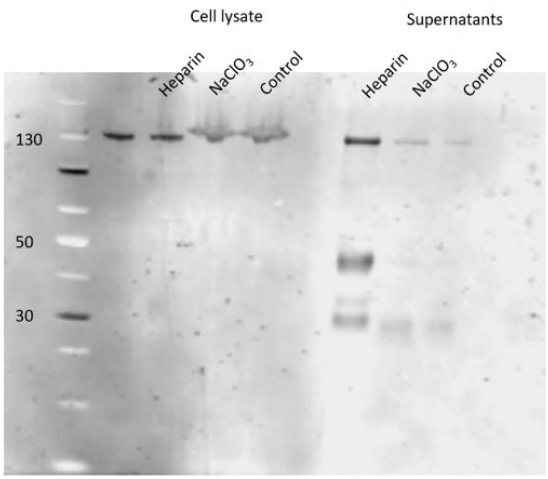


Figure 24: Western blot of Sulf-2 expression in HEK293 cells using heparin, sodium chlorate or no additive to optimise Sulf-2 'yield'. Anti-6×His.

Cells were seeded into a tissue-culture treated 10 cm dish and grown until 80% confluency was achieved. They were then transfected with the Sulf-2 plasmid using jetPRIME® reagent, lysed after 24 h, and the supernatant was collected. It was decided to purify only the supernatant because, according to the western blot analysis, a significant amount of protein was excreted into the extracellular matrix and it was hypothesised that this would be mature enzyme i.e. undergone all post translational modifications required for activity.

A Ni-NTA (nickel-nitrilotriacetic acid) resin column was used to purify the supernatant, utilising the high affinity of the Ni with the His-tag. The nitrilotriacetic acid binds to nickel tightly as it has four chelating sites, compared to other nickel-resins, which reduced the nickel leaching into the sample. This can especially be an issue for enzymes that require a

metal ion cofactor, such as endosulfatases, which have a Ca^{2+} ion in the active site that is essential for binding of substrates and catalytic turnover.

The column was equilibrated with a buffer that contains a low concentration of imidazole to minimise background interaction with proteins that contain many histidine residues, and to enhance the selectivity of the affinity purification. Due to the addition of the heparin in the expression protocol, it was necessary to use a 1M NaCl 'wash' to displace the heparin from the protein. The target enzyme was then eluted from the Ni-resin using buffers of increasing imidazole concentration, as shown in figure 25.

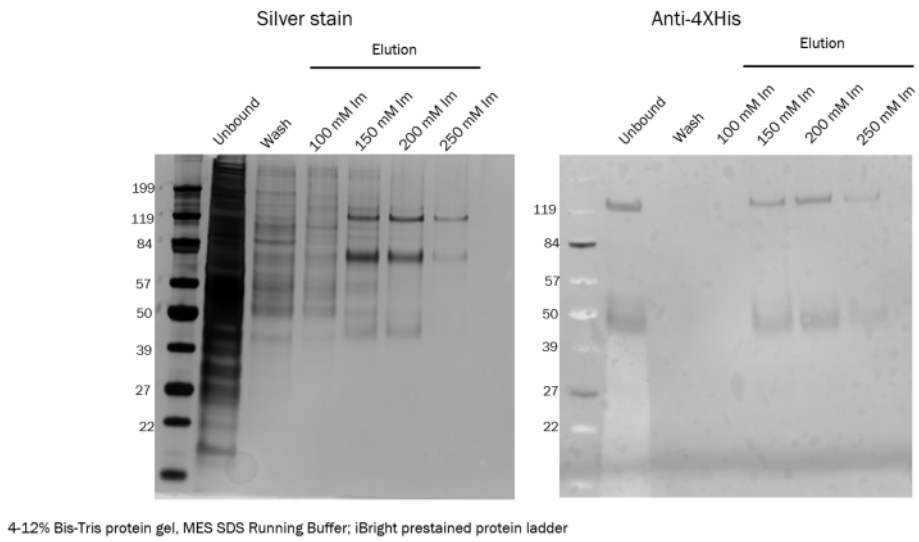


Figure 25: Gel electrophoresis of Sulf-2 purification via His-Tag Ni-TCA affinity chromatography, using an imidazole containing buffer. SDS-PAGE gels with silver staining (left), western blot with monoclonal Anti-4×His antibody produced in mouse (right). 4-12% Bis-Tris protein gel, MES SDS Running Buffer; iBright prestained protein ladder in lane 1.

As can be seen from the comparison of the silver stained SDS-PAGE gel and the western blot of the same samples (visualised with anti-4×His antibody conjugated to a fluorescent secondary antibody), a proportion of the His-tagged protein did not bind to the column ('unbound' well). Furthermore, there was significant background mixtures of protein still eluting after significant washing.

The two bands that were observed in the western blot (at approximately 120 kDa and 50 kDa), are likely to be due to the reduction of the disulfide bonds linking two units (75 kDa and 50 kDa fragments). The C-terminal fragment (50 kDa unit) contains the His-tag, and therefore is observed on the western blot, however the larger *N*-terminal fragment (75 kDa unit) can be seen on the silver stained SDS-PAGE gel. To confirm this analysis of the subunits, the protein sample was analysed by gel electrophoresis before and after reduction, as shown in figure 26. The 'non-reduced' sample does not contain the band at 50 kDa, confirming that the lower molecular weight band is likely to be the result of disulfide bond reduction.

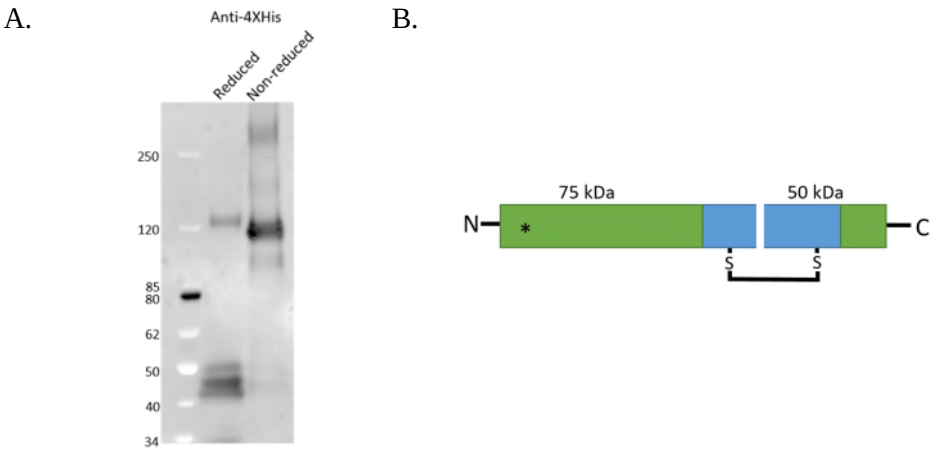


Figure 26: A. Gel electrophoresis of Sulf-2 comparing 'Reduced' and 'Non-reduced' sample preparation. Western blot analysis with monoclonal Anti-4×His antibody produced in mouse. 4-12% Bis-Tris protein gel, MES SDS Running Buffer; iBright prestained protein ladder in lane 1. **B.** Schematic representation of the two subunits, molecular weights indicated, of Sulf enzymes linked by a disulfide bond; * represents active site. His tag on C-terminal.

To optimise the purification further, the overexpression protocol was repeated, but on loading the Ni-resin column, the supernatant was double eluted prior to imidazole exchange. Additionally, it was decided to increase the number of fractions collected at a lower imidazole concentration in an effort to increase the purity of the Sulf-2 containing fractions. The results are shown in figure 27.

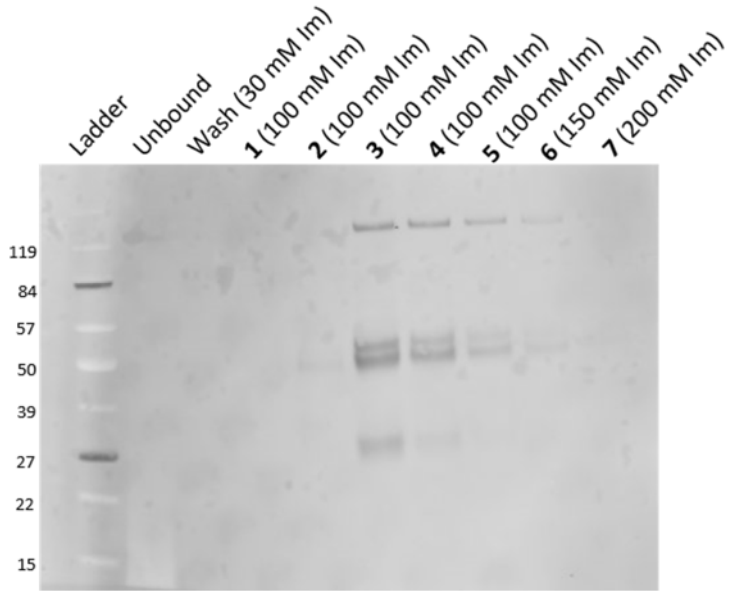


Figure 27: Gel electrophoresis of Sulf-2 purification via His-Tag Ni-TCA affinity chromatography, using an imidazole containing buffer. Western blot analysis with monoclonal Anti-4×His antibody produced in mouse. 4-12% Bis-Tris protein gel, MES SDS Running Buffer; iBright prestained protein ladder in lane 1.

Sulf-2 containing fractions 3-6 were combined and concentrated using a centrifugal Amicon® Ultra filter with a 30 kDa molecular weight cut-off. The quantity of protein was measured using a Pierce™ BCA Protein Assay Kit. However, despite concentrating the pooled Sulf-2 containing fractions, the protein concentration was too low to be accurately measured using the standard BCA assay. Furthermore, when the protein sample was assayed for arylsulfatase activity against the pseudo-substrate 4-MUS, no fluorescence was detected even with prolonged reaction times (sulfatase from *Aerobacter aerogenes* was used as a positive control).

Following on from this result, it was difficult to determine whether the lack of observed activity was a result of the low protein concentration or immature protein production. During the time course of this thesis, a publication by Seffouh et al.[20] described an optimised isolation protocol for HSulf-2, which required large scale transfection of 3D culture FreeStyle HEK 293-F cells, followed by two-step purification of cation-exchange,

then size-exclusion chromatography. On discussion with the corresponding author, it was apparent that this scale of protein isolation was too challenging and expensive to be undertaken in this work, however Professor Romain Vivès generously gifted purified recombinant HSulf-2 for activity testing.

2.10 4-Methylumbelliferone sulfate (4-MUS) assay

Before testing the inhibitory data of the small molecule inhibitors, the fluorescent assay was optimised with respect to the enzyme concentration and the assay time scale. The assay buffer (50 mM Tris base, 10 mM $MgCl_2$, pH 7.5) and 4-MUS concentration (10 mM) was used as previously reported.[20] Different concentrations of HSulf-2 were measured for arylsulfatase activity at 37 °C, quenched by addition of 1 mM Tris base (pH 11.5) and analysed on a fluorescence plate reader (4-MU, λ_{Ex} 350 nm, λ_{Em} 460 nm). The results are summarised in figure 28, A (n = 1), which indicated that the largest window of activity was observed using HSulf-2 concentration at high concentration (160 ng/μL) after four hours. Furthermore, the activity of HSulf-2 was found to be linear over this time period indicating that enzyme denaturation at this temperature was not significant during the assay time course (figure 28, B).

A **B**

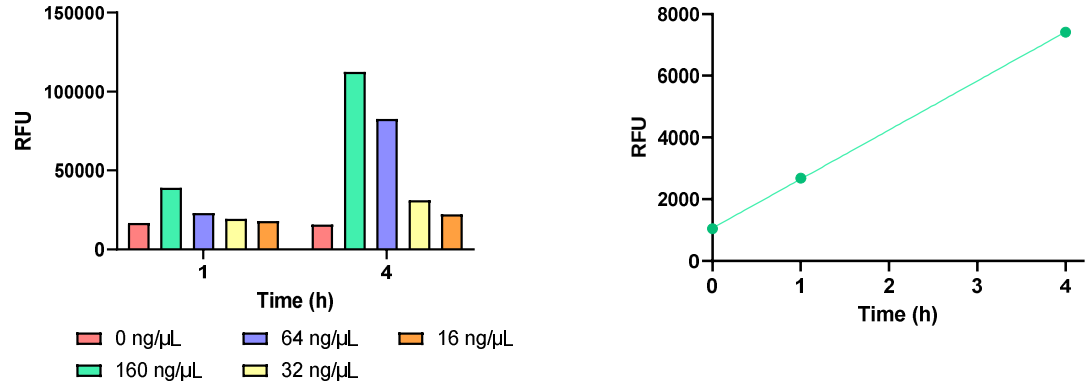


Figure 28: A. HSulf-2 arylsulfatase activity at different concentrations, measured as by hydrolysis of 4-MUS. Concentrations of HSulf-2 investigated: 0, 160, 64, 32 and 16 ng/µL in assay buffer (10 mM 4-MUS, 50 mM Tris base, 10 mM $MgCl_2$, (pH 7.5), 37 °C for either 1 h or 4 h. **B.** Linear activity of HSulf-2 for duration of assay. 160 ng/µL HSulf-2, 10 mM 4-MUS, 50 mM Tris base, 10 mM $MgCl_2$, (pH 7.5), 37 °C, 4 h, then quenched by addition of 1 mM Tris base (pH 11.5) and analysed on a fluorescence plate reader (4-MU, λ_{Ex} 350 nm, λ_{Em} 460 nm). Inhibition values are reported as percentages of the uninhibited control values. RFU = relative fluorescence units.

In order to validate these assay conditions for use as an inhibition assay, two literature inhibitors of HSulf-2; (OKN-007 (**24**) and biphenyl trichloroethylsulfamate (**31**), structures shown in figure 29), were used to test the inhibition of the hydrolysis of 4-MUS to 4-MU by HSulf-2. Inhibition values are reported as percentages of the uninhibited control values, calculated using the following equations:

$$\%\ Activity = \frac{RFU\ with\ inhibitor}{RFU\ control} \times 100$$

$$\%\ Inhibition = 100\ - \%\ Activity$$

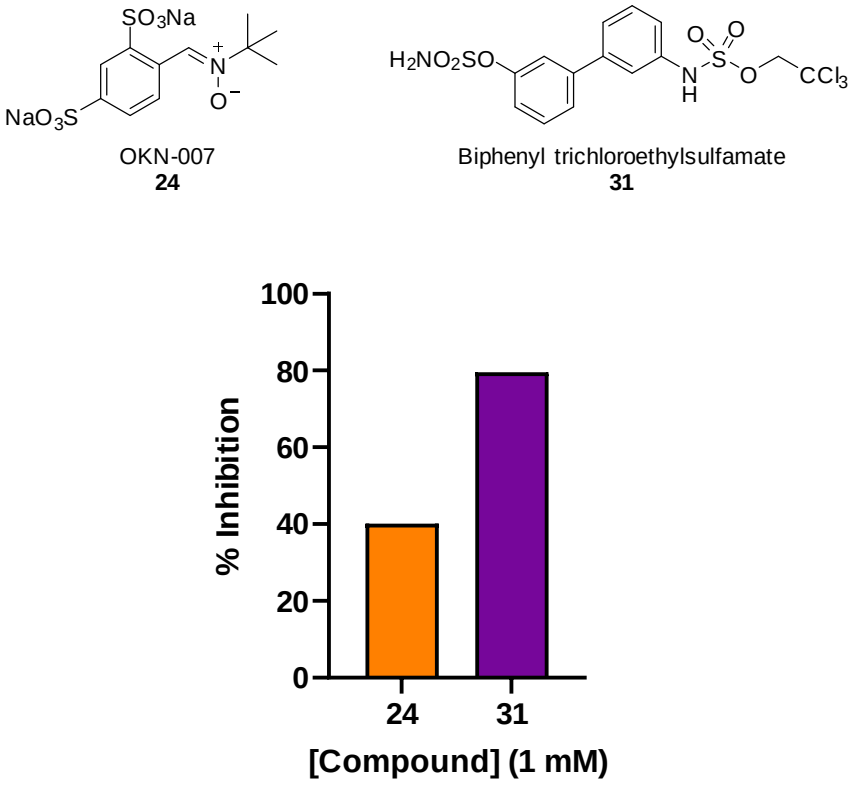


Figure 29: HSulf-2 inhibition data for OKN-007 and biphenyl trichloroethylsulfamate. Data represented as the mean ± SD, (n = 2). 160 ng/μL HSulf-2, 10 mM 4-MUS, 50 mM Tris base, 10 mM $MgCl_2$, (pH 7.5), 37 °C, 4 h, then quenched by addition of 1 mM Tris base (pH 11.5) and analysed on a fluorescence plate reader (4-MU, λ_{Ex} 350 nm, λ_{Em} 460 nm). Inhibition values are reported as percentages of the uninhibited control values. RFU = relative fluorescence units.

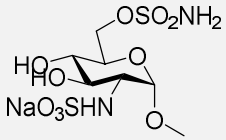
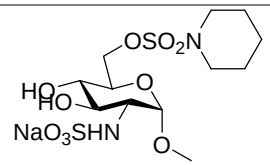
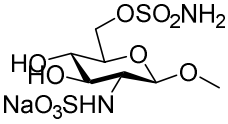
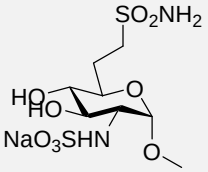
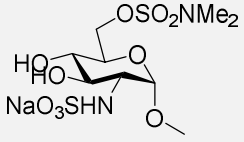
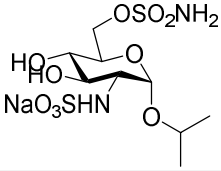
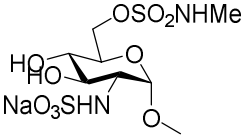
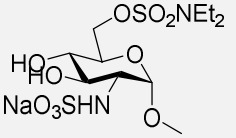
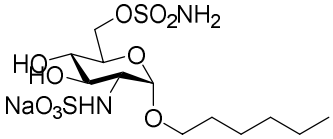
Reuillon et al. report 99% inhibition of HSulf-2 by biphenyl trichloroethylsulfamate **31** at 1 mM.[86] In the work of this thesis, **31** was found to inhibit HSulf-2 by 83% at 1 mM, which was considered to be comparable considering slight differences in assay protocols. Following on from this result, it was decided that the enzyme assay was sensitive enough to be able to test inhibitor potency.

2.11 Evaluation of glucosamine-6*O*-sulfamate inhibitors of HSulf-2

The compounds were screened at a single concentration (500 μM) to obtain a value of percentage inhibition of the desulfation of 4-MUS.

The inhibition data for the small molecule inhibitors synthesised are summarised in table 6. The parent glucosamine-6-*O*-sulfamate **27** was found to be superior to other GlcNS analogues investigated, displaying weak inhibition of 28% at 500 μM (table 6, entry 1). All analogues caused less than 25% inhibition of HSulf-2 at 500 μM under these assay conditions, and due to the low inhibition observed, it is difficult to draw strong conclusions on the structure activity relationship of these analogues.

Table 6: HSulf-2 inhibition data for glucosamine-6-*O*-sulfamate analogues. Assay conditions: Inhibitor (500 μM), HSulf-2 (160 ng/μL) in assay buffer (10 mM 4-MUS, 50 mM Tris base, 10 mM $MgCl_2$, (pH 7.5), 37 °C for 4 h. Quenched by addition of one assay volume of 1 mM Trisbase (pH 11.5), doubling the final assay volume before analysis on a fluroesence plate reader (4-MU, λ_{Ex} 350 nm, λ_{Em} 460 nm). Data represented as the mean ± SD, (n = 2).[v]

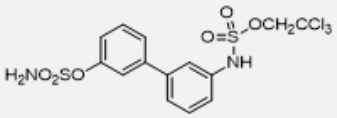
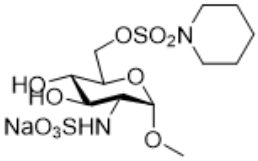
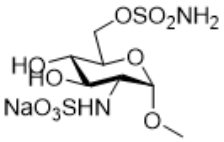
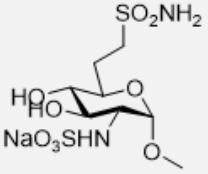
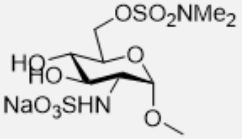
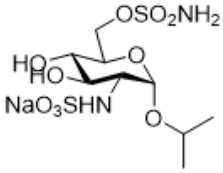
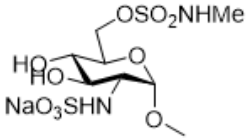
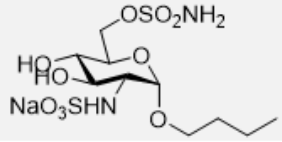
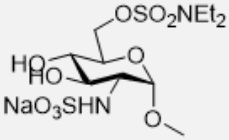
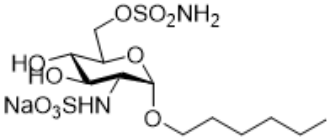
Entry	*Compound*	*% Inhibition*	*Entry*	*Compound*	*% Inhibition*
1		28 ±2	6		24 ±2
2		19 ±5	7		15 ±1
3		11 ±1	8		22 ±2
4		21 ±1	9		22 ±2
5		23 ±1	10		1 ±1

[v] *N*-mesylate and *N*-triflate analogues were not included in the biological evaluation due to decomposition of the samples. Re-synthesis was not prioritised due to the general trend observed with the monosaccharides (<30% inhibition at 500 μM), predicting that these analogues would also have low potency.

The *C*-linked sulfonamide **74** (table 6, entry 7) is approximately 2-fold less potent than the parent sulfamate (table 6, entry 1), however without further studies to investigate the mode of inhibition it is unclear whether this analogue is inhibiting via a different mechanism. Other modifications to the sulfamate headgroup were also reasonably well tolerated (i.e. comparable activity to the parent primary sulfamate), with the exception of the dimethylsulfamate, which only inhibited HSulf-2 by 11% (table 6, entry 3), which is 3-fold less potent than **27**. Due to the low potency, none of these analogues were of interest for further studies.

Selectivity of the compounds against sulfatases was assessed by comparison of inhibitory activity against sulfatase from *Aerobacter aerogenes* (purchased from Sigma Aldrich, CAS 9016-17-5), under the same assay conditions. All glucosamine-based analogues were found to be inactive against sulfatase from *Aerobacter aerogenes* at 500 μM, data shown in table 7. In this study, the biphenyl trichloroethylsulfamate **31** was used as a positive control, which caused 100% inhibition of the sulfatase from *Aerobacter aerogenes* at 500 μM, which agrees with the findings of Reuillon et al., that this compound is potentially a pan-sulfatase inhibitor. This data shows that the glucosamine analogues show some potential selectivity for endosulfatases over a generic arylsulfatase, however further investigation is required to know whether they are selective over the other polysaccharide sulfatases.

Table 7: Sulfatase from Aerobacter aerogenes inhibition data for glucosamine-6-*O*-sulfamate analogues. Data represented as the mean ± SD, (n = 2). Assay conditions: Inhibitor (500 μM), Sulfatase from Aerobacter aerogenes (172 ng/μL) in assay buffer (10 mM 4-MUS, 50 mM Tris base, 10 mM $MgCl_2$, (pH 7.5), 37 °C for 1 h. Quenched by addition of one assay volume of 1 mM Trisbase (pH 11.5), doubling the final assay volume before analysis on a fluroesence plate reader (4-MU, λ_{Ex} 350 nm, λ_{Em} 460 nm). Data represented as the mean ± SD, (n = 2).

Entry	*Compound*	*% Inhibition*	*Entry*	*Compound*	*% Inhibition*
1		100 ± 0.03	6		2.0 ±0.2
2		1.6 ±2.3	7		0.0 ± 3.3
3		0.2 ± 1.7	8		1.8 ± 0.6
4		2.0 ± 0.7	9		0.0 ± 1.4
5		2.1 ± 0.3	10		4.1 ± 0.5

2.12 Summary

A small library of thirteen glucosamine-6*O*-sulfamate analogues was prepared as putative inhibitors of Sulf-2. The Sulf-2 activity assay was developed, initially using conditioned medium as the source of enzyme. However due to limited assay sensitivity, recombinant enzyme was isolated, albeit in very low quantities. It was apparent that a large-scale enzyme preparation protocol was required, which was not feasible for the scope of this thesis. Recombinant HSulf-2 was generously donated and the glucosamine-6*O*-sulfamate analogues were evaluated as inhibitors of endosulfatases. All glucosamine-6-*O*-sulfamates

investigated showed modest inhibitory action against HSulf-2 at 500 μM (<30%). This finding agrees with the original report by Schelwies et al.,[2] suggesting that, albeit at low potency, the glucosamine analogues are indeed inhibitors of the Sulfs. Furthermore, these compounds were found to have minimal inhibition against the sulfatase from *Aerobacter aerogenes* (<4% at 500 μM), indicating potential selectivity over arylsulfatases.

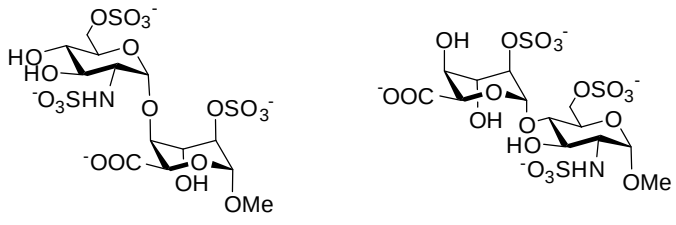
3. Design and synthesis of small molecule endosulfatase inhibitors

This chapter describes the design and synthesis of sulfatated analogues of IdoA-GlcN as putative inhibitors of Sulf1/2.

3.1 Introduction

Mimicking an enzyme's natural substrate, or a fragment of the active substrate, is an approach to inhibitor design that is commonly used to identify an active molecule without requiring large compound libraries and high-throughput screening. These substrate-mimic inhibitors are recognised by the enzyme active site and prevent the natural substrate from binding, but are not themselves turned over, thereby affecting competitive inhibition. If the substrate-mimic is chemically altered to incorporate a reactive group that can form a covalent bond upon binding, then irreversible inhibition can be achieved. When a fragment of the substrate is mimicked in inhibitor design, it usually follows that increasing the natural substrate character, e.g. increasing the fragment size, increases the potency obtained.[102]

With this in mind, it was decided to extend the HS fragment length from the monosaccharide (as described in Chapter 1), to the disaccharide. It opened up the opportunity for 2 potential disaccharides: the GlcNS(6S)-IdoA(2S) (**82**) and IdoA(2S)-GlcNS(6S) (**83**), shown in figure 30.



Trisulfated GlcN-IdoA **82**

Trisulfated IdoA-GlcN **83**

Figure 30: Structure of two HS disaccharides

3.2 Rational for GlcN-Ido relating to FGF

Hu et al. investigated the binding affinities of various sulphated disaccharides with FGF in order to understand better the substrate specificity.[103] The GlcN-IdoA **82** fragment binds to FGF with high affinity – which is the signalling pathway that the work in this thesis is aiming to affect downstream of Sulf inhibition. A second benefit of using a disaccharide with high affinity for FGF is that the fragment itself may induce FGF-FGFR complex formation and thus increase FGF signalling. However, this 'off-target' may also prove to be a limitation, as binding to FGF may decrease the local 'free' concentration of the inhibitor at its desired target.

Hu et al. synthesised a number of sulfated GlcN-IdoA disaccharide analogues and measured their association constants with FGF-1 via isothermal titration calorimetry (ITC).[103] From the 48 disaccharides investigated, only four analogues showed any detectable affinity towards FGF-1, and of these, the trisulfated GlcNS6S- IdoA2S (**84**, Figure 31) had the weakest dissociation affinity (K_D = 21.2 μM), which was approximately 30-fold weaker than 3 kDa heparin (K_D = 0.746 μM). Of the disaccharides investigated, all the analogues that associated with FGF-1 contained GlcN *N*- and the IdoA 2*O*- sulfonate moieties, demonstrating the importance of these residues during binding. It is noteworthy that the disaccharide without GlcN 6*O*-sulfate displayed the greatest affinity for FGF-1, only 5-fold weaker than 3 kDa heparin (~9-mer).[103] The experimental binding dissociation constants reported in this paper do not provide any insight into how these sulfated disaccharides influence FGF-signalling, in particular the authors do not address the binding affinities between the disaccharides and recombinant fibroblast growth factor receptors (FGFR). A limitation of not considering either FGF-HS-FGFR complex formation or

downstream signalling events is that the specific sulfation pattern requirements for FGF signalling in a biological system may not be identified.

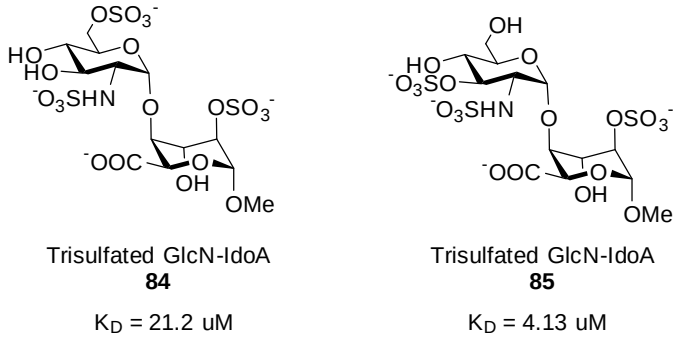


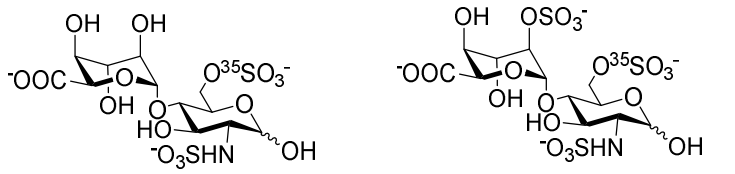
Figure 31: Dissociation Constants of two trisulfated disaccharides that bind to FGF-1 (determined by ITC)[103]

Schlessinger et al. reported the crystal structure of the FGF1-HS-FGFR complex and identified that the GlcN 6*O*-sulfate group hydrogen bonds to FGF and FGFR simultaneously, and is therefore critical for complex formation.[36] The FGF-FGFR complex has been shown to be facilitated by a disaccharide (sucrose octasulfate), which then also interacts with an adjoining FGFR in order to induce receptor dimerization.[104] This demonstrates that highly sulfated small molecules are able to interact sufficiently with the heparin binding sites on FGF and FGFR to induce FGFR dimerization. This indicates that a high level of sulfation may shorten the polysaccharide length requirement compared to physiological heparan sulfate.

3.3 Rational for IdoA-GlcN relating to Sulfs

The synthesis of orthogonally protected GlcN-Ido disaccharide intermediates were reported by Hu et al., and while this could be easily adapted to synthesise the 6-*O*-sulfamate analogue, the data suggest that the IdoA-GlcN analogue is more likely to be a potent and selective inhibitor of the endosulfatases.[103]

The substrate specificity of Sulf1/2 was investigated by Pempe et al. who identified that Sulf-2 is particularly effective in removing the 6-*O*-sulfate group from the trisulfated disaccharide unit consisting of 2-*O*-sulfated uronic acid and *N*-sulfated 6-*O*-sulfated glucosamine residues.[19] This result is in agreement with the substrate specificity identified by Ai et al., who reported that the trisulfated disaccharide IdoA2S-GlcNS6S is extensively 6-*O*-desulfated by Sulf-1/2, but could not detect activity on IdoA-GlcNS6S disaccharide units even with extended reaction times.[38] A limitation of this study was that it used either heparinase I or II to digest the [^{35}S]GAGs of HS prior to structural analysis of the disaccharide fragments via HPLC anion exchange chromatography, with product quantification by scintillation counting. As it used heparinase as a digestion enzyme, it is unclear whether the true fragment specificity is the disaccarhide or whether a larger epitope is required for efficient 6-*O*-desulfation.



Disulfated IdoA-GlcN
86

Trisulfated IdoA2S-GlcNS6S
87

Figure 32: Structures of two disaccharide fragments from digested HS after treatment with Sulf-2, identified as substrates of Sulf-2 (determined by scintillation counting LC-MS)[19]

Saad et al. reported the quantification of the 6-*O*-desulfation by HSulf-2 of UA2S-GlcNS6S subunit within different length substrates with a mass spectrometry assay.[26] Desulfation of the full length heparin (20-100 disaccharide subunits), and low molecular weight heparin (10-20 disaccharide subunits) was found to be comparable at 37% and 36% decrease of 6-O-sulfation when compared to the enzyme free control. When the disaccharide substrate was investigated, no change in 6-*O*-sulfation was observed, indicating that this fragment is

not a substrate of HSulf-2. This high affinity of Sulf-1/2 with heparan sulfate is attributed to the presence of a hydrophilic domain, which is unique to Sulf-1/2 compared to the other sulfatases, and has been found to bind selectively to heparan sulfate and does not interact with chondroitin sulfate, dermatan sulfate or desulfated heparin.[23]

3.4 Inhibitor design

In this current work, using disaccharide **83** as a template for inhibitor design, it was hypothesised that the sulfamate moiety could be used as a bioisostere to replace the 6-*O*-sulfate in the D-glucosamine unit (as a continuation of the work investigated in chapter 2), and compounds **88** and **89** were designed, shown in figure 33. Taking into consideration the fragment specificity within full length HS, compound **88** was expected to be less potent than **89**, however there was limited information available about its binding with Sulf-1/2 and so it was still included as a putative inhibitor and for comparison. It should also be noted that it was considered that these analogues did not necessarily need to bind to the active site to affect an inhibitory activity, and binding to the hydrophilic domain at any position might be enough to disrupt binding of HS to Sulf-1/2.

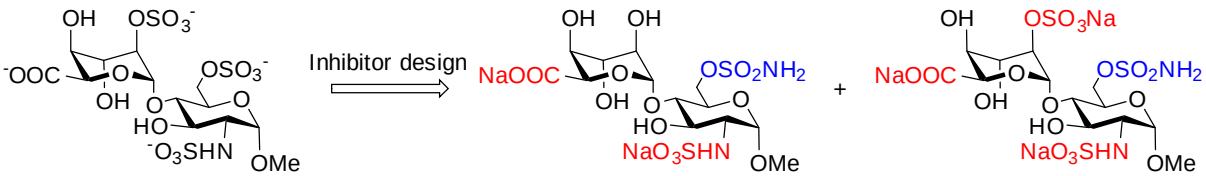
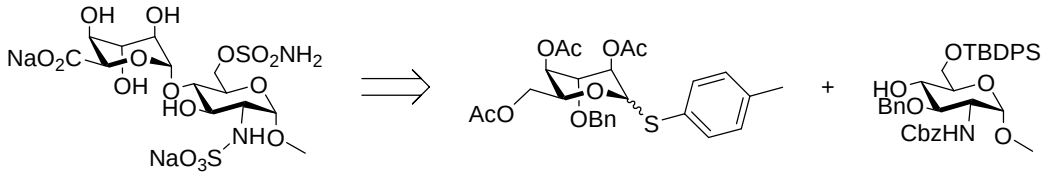


Figure 33: Endosulfatase inhibitors using disaccharide IdoA2S-GlcNS6S as a template

3.5 Synthesis of IdoA-GlcNS(6Sulfamate)

Retrosynthetic analysis of **88** identified idose glycosyl donor **90** and glucosamine glycosyl acceptor **93**, which could be coupled in a chemical glycosylation reaction initiated by treatment with NIS and TfOH at −78 °C. It was proposed that the presence of the idose C-

2 acetate protecting group would participate in stabilising the intermediate oxocarbenium ion and promote the formation of the desired α-anomer in a stereoselective manner.

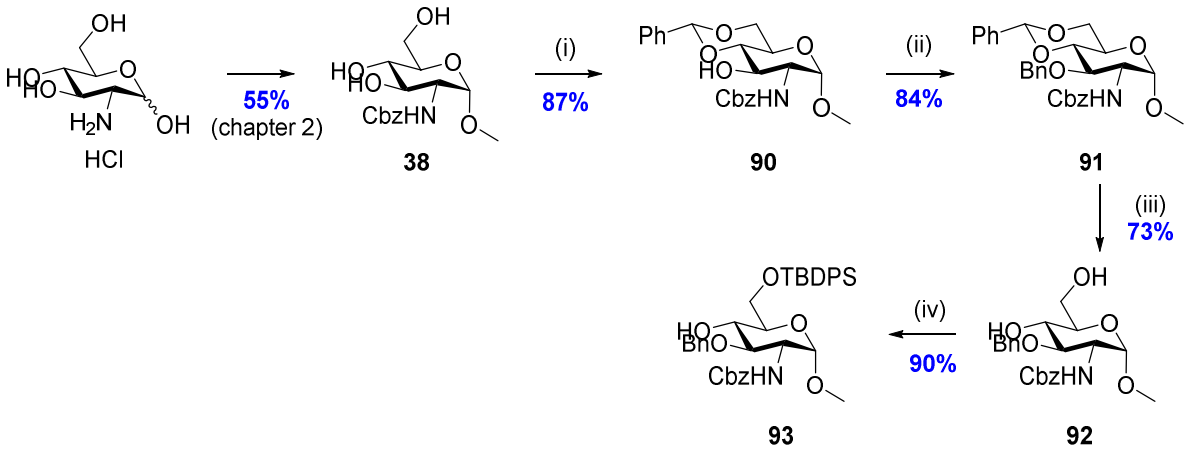


88 **100** **93**

Figure 34: Retrosynthetic analysis of compound **88**

The synthesis of the glucosamine glycosyl acceptor began with protection of the primary amine of glucosamine by reaction with benzyl chloroformate, followed by Fischer glycosylation in methanol to prepare methyl glycoside **38** (55% over two steps), as optimised in chapter 2 (page 37, scheme 8). Next, to temporarily protect the 4-OH and 6-OH a double protection strategy of a benzylidine acetal was employed by reacting the diol with benzaldehyde dimethyl acetal and catalytic camphorsulfonic acid, which gave the desired acetal in 87% yield. This is a versatile protecting group that may be introduced in either acidic or basic conditions and it can be completely removed or regioselectively opened under reducing conditions by choice of Lewis acid catalyst to give either 4-OBn or 6-OBn. When the benzylidene acetal is introduced under acidic conditions, due to the reversible nature of the acetal formation, only a single stereoisomer is obtained because the bulky phenyl substituent goes in the more thermodynamically stable, equatorial position where the 1,3-diaxial interactions and gauche interactions are minimized. The 3-OH was then protected using benzyl bromide and sodium hydride, which resulted in the fully protected glucosamine **91** in 58% yield. This yield was lower than expected, which was attributed to the relatively acidic proton on the carbamate nitrogen atom. Finally, selective acetal hydrolysis using 70% acetic acid in water at 65 °C removed the benzylidene acetal,

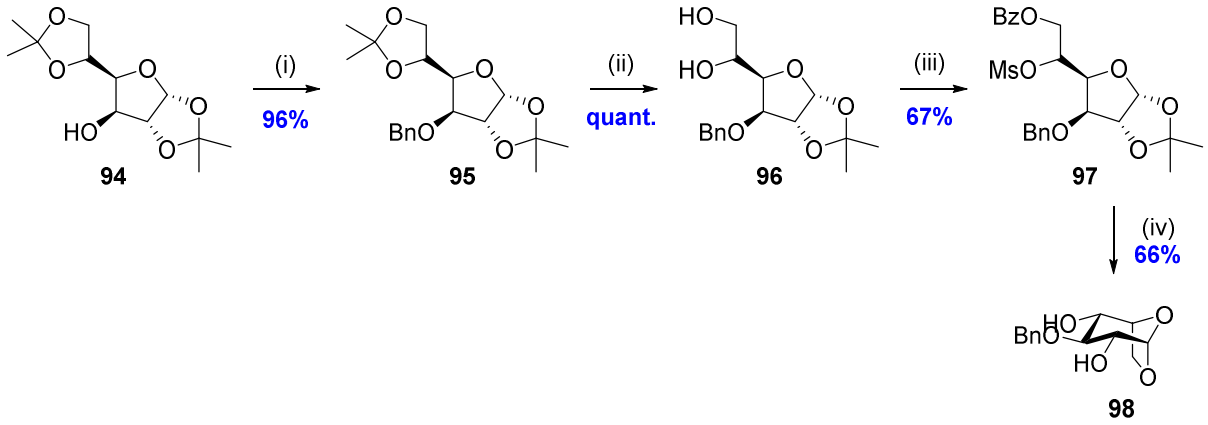
and selective protection of the primary alcohol was achieved by reaction with bulky *tert*-butyldiphenylchlorosilane, which gave glycosyl acceptor **93** in 57% over two steps.



Scheme 16: Synthesis of Glc-glycosyl accpetor **93** from glucosamine hydrochloride. Reagents and conditions: (i) $PhCH(OMe)_2$, cat. CSA, DMF, 80 °C, 16 h, 87%; (ii) NaH, BnBr, DMF, rt, 16 h, 84%; (iii) $AcOH/H_2O$ (v/v 7:3), 65 °C, 16 h, 73%; (iv) TBDPSCl, cat. DMAP, Et_3N, CH_2Cl_2, rt, 16 h, 90%.

The synthetic route to idose glycosyl donor began with the synthesis of 1,6-anhydro-3-*O*-benzyl-β-L-idopyranose (**98**) from diacetone-D-glucose, following a procedure described by Lee et al.[105] The 3-OH benzyl protection of diacetone-D-glucose was achieved using a four equivalent excess of sodium hydride and benzyl bromide, which gave fully protected glucose **91** in 84% yield. Subsequent selective acetal hydrolysis using 50% acetic acid in water at 45 °C produced the corresponding 4,6-diol in quantitative yield. This diol underwent a one-pot benzoylation-mesylation, by first adding the benzoyl chloride to a solution of **96** in pyridine and CH_2Cl_2 for 4 h at 0 °C, followed by the addition of an excess of mesyl chloride to yield the corresponding furanose as a single isomer in 67% yield. The outcome of this reaction was scale dependent, as on a larger scale the minor side-product, the bisbenzoylated furanose, became more significant, and this was attributed to the reaction being more sensitive to the rate of benzoyl chloride addition. The critical reaction in this synthetic procedure was the inversion of stereochemistry at C-5 to form the L-*ido*

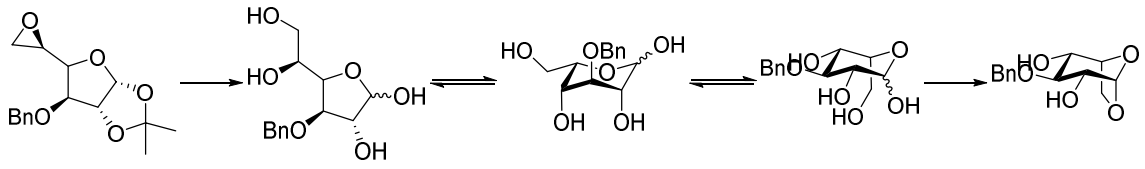
building block and the inversion was achieved by forming an epoxide intermediate through intramolecular S_N2 reaction of a 6-*O* alkoxide (by deprotection of the benzoyl ester). A solvent screen by Hung et al. determined that the dehydration reaction was best performed in a mixture of dilute sulphuric acid and 1,4-dioxane, as in their hands the reaction required high temperatures (160 °C), and so they initially investigated high boiling solvents such as ethylene glycol and diglyme.[106] 1,4-Dioxane was found to be the optimal solvent because it could be removed by evaporation prior to work-up, and therefore isolation of compound **98** was easier (62% reported when using 1,4-dioxane compared to 52% with diglyme).



Scheme 17: Synthesis of anhydro-idose **98** from diacetone glucose. Reagents and conditions: (i) NaH, BnBr, DMF, 0 °C to rt, 16 h, 96%; (ii) AcOH/H_2O (v/v 1:1), 45 °C, 16 h, quant.; (iii) BzCl, pyridine, CH_2Cl_2, 0 °C, 4 h; then, MsCl, rt, 16 h, 67%; (iv) 1. KO^{t}Bu, ^{t}BuOH/CH_2Cl_2, 0 °C, 1h; 2. H_2SO_4, 1,4-dioxane, 120 °C, 16 h, 66%.

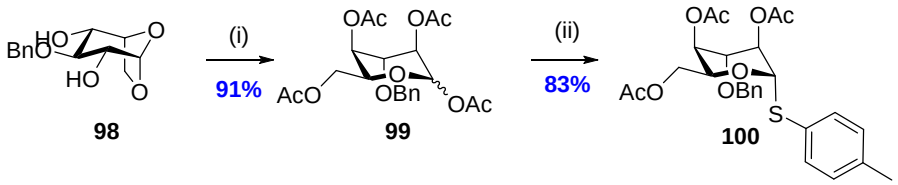
Treatment of compound **97** with *t*-BuOK in *tert*-butyl alcohol and CH_2Cl_2 at 0 °C led to the rapid formation of the intermediate epoxide (scheme 18). This was treated with H_2SO_4 (0.6 N) without workup, which affected the epoxide ring opening and isopropylidene ring cleavage, and it was then dehydrated to give **98** in 66% yield. The difficulty of this reaction lay in monitoring the conversion to anhydrosugar from the tetrol– and extended reaction times did not have a significant effect on the isolated yield, however for the purposes of

this thesis the 52% isolated yield of the desired L-*ido* compound **98** was sufficient and thus was not optimised further.



Scheme 18: Proposed intermediates of L-ido anhydrosugar formation **98**.

Treatment of compound **98** with acetic anhydride and catalytic trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave tetracetate in 91% yield. Compound **99** was then converted into thioglycoside **100** by reaction with 4-methylbenzenethiol and $BF_3{\cdot}OEt_2$ at rt in 83% yield.



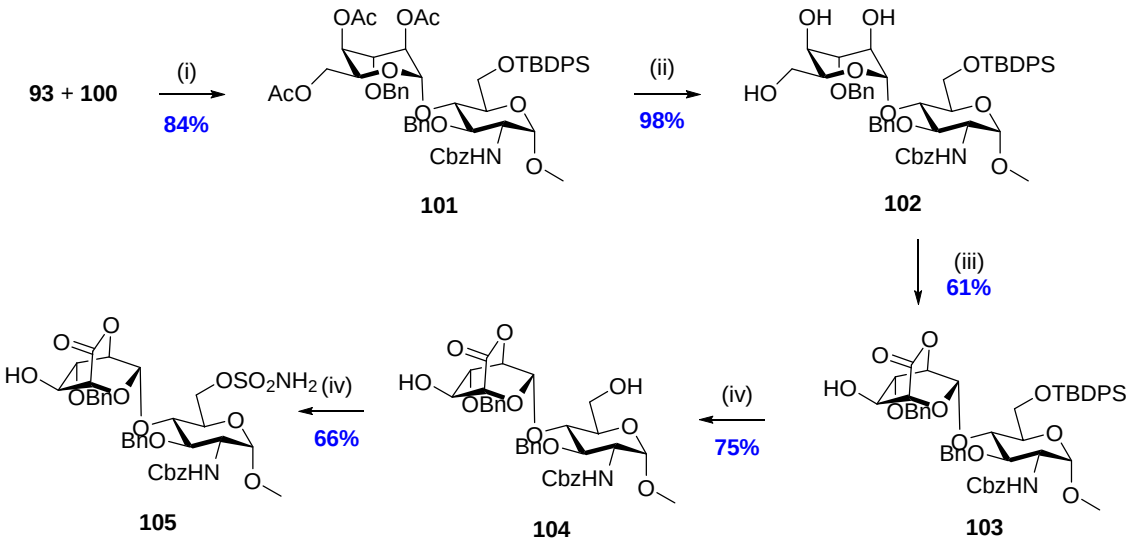
Scheme 19: Synthesis of Ido-glycosyl donor **100**. Reagents and conditions: (i) TMSOTf, Ac_2O, CH_2Cl_2, rt, 91%; (ii) TolSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 16 h, 83%.

With the donor and acceptor in hand, the thioglycoside donor **100** was activated using the NIS/TfOH reagent system, which proceeded effectively in the glycosylation reaction with glucose building block **93** to afford the desired α-linked disaccharide **101** in 84% yield (anomeric configuration assigned by ^{1}H NMR, 5.18 (s, 1H, C(1')H)[vi]. Moreover, as neighbouring group participation of the 2-*O*Ac on the glycosyl acceptor in addition to the axial C−O bonds perpendicular to the plane of the molecule causes favourable charge-dipole interaction when the positively charged oxycarbenium ion forms, this ensured high stereoselectivity and enabled the exclusive formation of the α–glycosidic bond, which was

[vi] See chapter 7 for explanation about magnitude of *J* couplings for idose anomers

confirmed by ^{1}H NMR spectroscopy. Subsequently, the acetate esters of compound **101** were removed using standard conditions with catalytic $NaOCH_3$ to produce triol **102** in 98% yield. Then, following a protocol described by van den Bos et al., the primary alcohol was subjected to oxidation with catalytic 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) in the presence of (diacetoxyiodo)benzene (PIDA) as co-oxidant.[107] A lactol was formed through intramolecular acetal formation by nucleophilic attach of the 2-OH onto the newly formed aldehyde, and it was subsequently oxidized by the TEMPO/PIDA reagent system to yield compound **103** in 61% yield. The lactone intermediate was incorporated into the design of the synthesis for two reasons: (i) the idose C-6 was required to be at the carboxylic acid oxidation level, and (ii) the lactone functions as an intrinsic protecting group for the 2-OH of the L-*ido* subunit.

It was envisioned that the desilyation of compound **103** could be accomplished using acetic acid buffered TBAF, however when this reagent system was trialled the desired alcohol **104** was only isolated in 39% yield. The major side-reaction was the lactone hydrolysis, presumably by adventitious water in the TBAF solution. In order to improve this yield and prevent premature 'deprotection' of the 2-OH, an anhydrous source of fluoride was investigated. Tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) is a mild source of anhydrous fluoride and, unlike many other sources of fluoride where the anion is 'naked' the fluoride exists in a Lewis acid-base adduct with trimethylsilylfluoride, and therefore deactivates the basicity of 'naked fluoride'. In addition to desilylation of base-sensitive substrates, TASF has proven useful for selective desilylation of primary TBDPS in the presence of a secondary TBS ether.[108] Satisfyingly, desilyation of compound **103** was accomplished using TASF gave compound **104** in an improved yield of 75%.



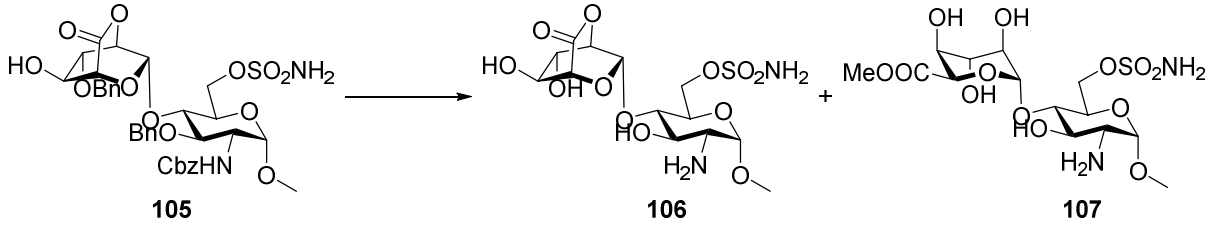
Scheme 20: Synthesis of disaccharide **105** from glycosyl donor **100** and glycosyl acceptor **93**. Reagents and conditions: (i) NIS, TfOH, 4Å molecular sieves, CH_2Cl_2, −60 °C, 2 h, 84%; (ii) $NaOCH_3$, CH_3OH, rt, 24 h, 98%; (iii) TEMPO, $PhI(OAc)_2$, CH_2Cl_2/H_2O, rt, 16 h, 61%; (iv) TASF, DMF, rt, 16 h, 75%; (v) NH_2SO_2Cl, $DMF/PhCH_3$, −20 °C, 16 h, 66%.

Regioselective sulfamoylation of diol **104** was initially attempted using commercially available sulfamoyl chloride (H_2NSO_2Cl) in DMF/PhMe at –20 °C for 24 h. The reaction was treated with an additional 0.15 equivalents of H_2NSO_2Cl, however the reaction was quenched because the formation of an additional new compound was observed by TLC and compound **105** was isolated in 66% yield.

Next, the Pd/C-mediated hydrogenolysis to cleave all benzyl and Cbz groups of compound **105** was attempted, however under standard conditions the benzyl groups remained (as monitored by ^1H NMR spectroscopy, table 8, entry 1). In an attempt to push the reaction to completion, the pressure of hydrogen was increased from atmospheric pressure to 4.5 bar, however the same result occurred (table 8, entry 2). The reactivity difference between Pd/C and $Pd(OH)_2/C$ varies widely, with many reports claiming that certain chemoselectivity may be achieved using one rather than the other,[109] however the variability experienced is likely to be Pd or PdO particle size and homogeneity of the

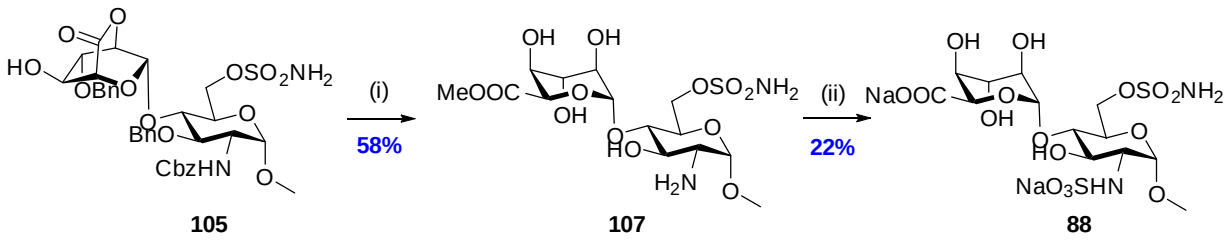
distribution on the carbon support, which can vary between chemical vendors. Crawford et al. investigated the reaction outcome of the hydrogenolysis of a challenging substrate that required 25 groups to be reduced under hydrogen atmosphere, and found that pH and hydrogen pressure had minimal effect on reaction outcome, but the catalyst quality was most important.[110] It was hypothesised that the limiting factor in these reactions was not the hydrogen quantity, but instead was likely to be either the reaction temperature or the steric hindrance of the substrate. To overcome this, a catalytic transfer hydrogenation protocol described by Hanessian et al. was attempted in this thesis, in which cyclohexene was used as the hydrogen donor using 20% palladium hydroxide on carbon in refluxing methanol and under these conditions it was found that the lactone underwent a solvolysis reaction to form the methyl ester and 'free' 2-OH.[111] At this point in the synthesis, the deprotection of the 2-OH was acceptable, and more importantly, full deprotection of the benzyl ethers was achieved to give compound **107** in 58% yield (table 8, entry 3). The reaction was not monitored to identify any of the intermediates, however it is plausible that the lactone solvolysis occured before the idose 3-*O* debenzylation, and that the cleavage of the [2,2,2]-bicylic ring system was sufficient to decrease the steric hindrance of the substrate coordinating with the heterogeneous catalyst. Further investigation would be required to understand whether it was the altered substrate or the increased temperature that caused the success of this reaction.

Table 8: Summary of conditions investigated for the debenzylation of compound **105**. Reagents and conditions: (1) H2 (balloon), Pd/C, CH_3OH, rt 16 h; (2) H_2 (4.5 bar in parr hydrogenator) $Pd(OH)_2/C$, CH_3OH, rt, 16 h; (3) cyclohexene, $Pd(OH)_2/C$, CH_3OH, 80 °C, 16 h.



Entry	*Conditions*	*Reaction outcome*
1	H_2 (balloon), Pd/C	Cbz deprotection, 2 × Bn remain
2	H_2 (4.5 bar), $Pd(OH)_2/C$	No debenzylation observed by NMR and *m/z*
3	Cyclohexene, $Pd(OH)_2/C$	58% isolated yield of **107**

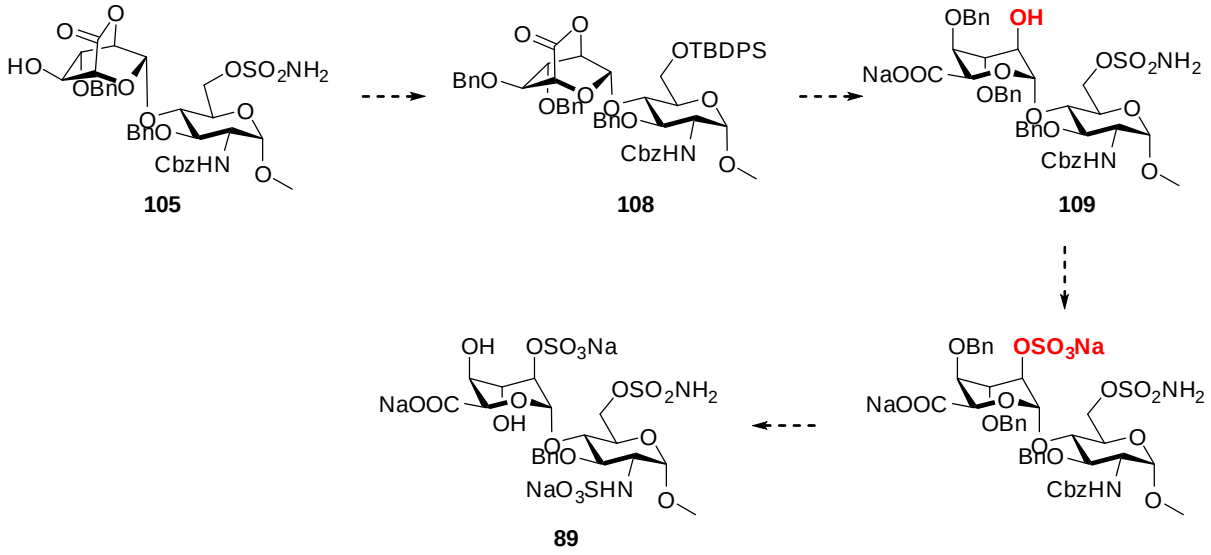
The final step of the synthesis to inhibitor **88** was the chemoselective sulfation of the primary amine **107**. Compound **107** was subjected to sulphur-trioxide pyridine complex in basic aqueous medium. The optimised reaction protocol, as described in Chapter 2, was utilised and the conversion was monitored by ^{1}H NMR spectroscopy. The purification of this analogue proved challenging owing to the high inorganic salt concentration of the reaction mixture, and compound **88** was found to be too polar for purification via reversed phase chromatography. An ion-pair reversed-phase HPLC method was evaluated, using aqueous 2M triethylammonium bicarbonate (a volatile buffer), as the mobile phase. Following purification, the product containing fractions were concentrated and eluted through a Dowex® 50WX8 Na^+-form column using water as the eluent to give the sodium salt of compound **88** in 22% yield. The low yield was due to difficulties in purification rather than poor conversion – the crude reaction mixture was monitored by ^{1}H NMR, and was determined complete (95% by NMR).



Scheme 21: Synthesis of compound **88** from intermediate **105**. Reagents and conditions: (i) cyclohexene, $Pd(OH)_2/C$, CH_3OH, 80 °C, 16 h, 58%; (ii) SO_3·Py, H_2O (pH 9-10), rt, 16 h, 22%.

3.6 Synthesis of IdoA(2S)-GlcNS(6Sulfamate)

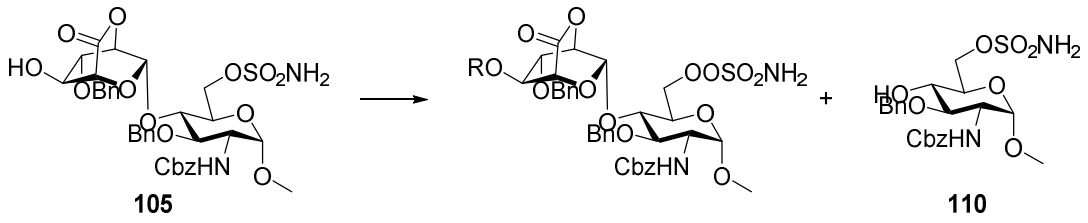
It was hypothesised that the iduronic acid 2-*O*SO_3Na group would be very important for recognition with Sulf1/2 and therefore it was a high priority to synthesise the small molecule inhibitor that contained this moiety. It was initially envisioned that the synthetic route for the synthesis of IdoA-GlcNS(6Sulfamate) could be easily adapted to allow for the desired modification at the *Ido*-2-*O*H position. To achieve this regioselectivity, the 4'-*O*H of compound **105** would require protection so that once the lactone was hydrolysed the disaccharide would be orthogonally protected (except for the C-2 hydroxyl).



Scheme 22: Proposed synthesis of compound **89** from intermediate **105**

Initial efforts involved formal deprotonation of the 4-*O*H by sodium hydride and treatment of the alkoxide with benzyl bromide (DMF and THF were investigated as solvents, table 9, entry 1). Only glucosamine monomer **110** was isolated from these reactions, indicating fragmentation of the glycosidic bond. This cleavage of the glycosidic bond was attributed with the formation of the alkoxide, and as previously discussed this lactone was found to be very reactive towards nucleophiles. To avoid fragmentation an inorganic base was investigated. Unfortunately, the reaction of **105** with benzyl bromide and K_2CO_3 did not produce any fully protected **108** as monitored by TLC and mass spectrometry (table 9, entry 2). More reactive electrophiles (methyl iodide and allyl bromide) were investigated using these reaction conditions, but all attempts led to the same outcome. To avoid the strongly basic conditions that are required for the Williamson ether synthesis, an alternative acid catalysed benzylation was attempted, table 9, entry 3. Compound **105** was subjected to benzyl 2,2,2-trichloroacetimidate in the presence of triflic acid as the promotor, but this reaction also did not afford any desired product.

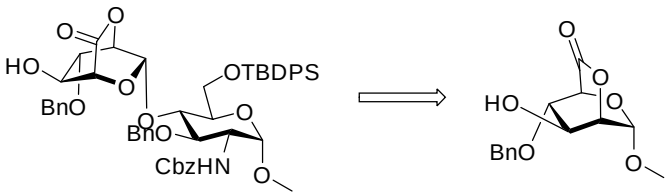
Table 9: Summary of conditions investigated for the 4'-O-benzylation of compound **105**. Reagent and conditions: (1) NaH, BnBr, DMF or THF, 0 °C to rt, 16 h; (2) BnBr, K_2CO_3, DMF, rt, 16 h; (3) BnTCA, TfOH, CH_2Cl_2, rt, 16 h. Reaction outcome assessed by TLC and LRMS analysis of crude mixture.



Entry	*Conditions*	*Reaction outcome*
1	NaH, BnBr, DMF or THF	Only **110** isolated after aqueous work-up
2	BnBr, K_2CO_3, DMF	No reaction
3	BnTCA, TfOH, CH_2Cl_2	No reaction

3.7 Mannose model system

To investigate the optimal benzylation conditions further it was decided to use a model system, so that the analysis was simple and precious material was not wasted on trial reactions. Hashimoto et al. have reported conditions for the synthesis of a 2,6-lactone mannoside, and as an intermediate in their building block synthesis the 4-*O*H was successfully benzylated under acidic conditions, and so it was proposed that a similar system could be used as the model. In comparison with the substrate reported by Hashimoto, in which a pseudo-axial hydroxyl was benzylated, to mimic compound **105** more closely, a pseudo-equatorial hydroxyl was required for the model substrate, and therefore the synthetic route was altered to allow for benzylation of the 3-*O*H under the test conditions.

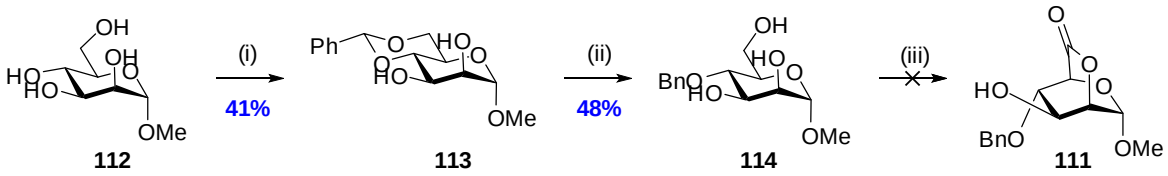


105 **111**

Figure 35: Comparison of structure of compound **105** and model substrate **111** to be used to investigate 4'-O-benzylation.

Commercially available methyl-α-D-mannopyranoside was subjected to benzylidine acetal formation using the previously optimised conditions for the glucosamine compound **90** (benzaldehyde dimethyl acetal in DMF and catalysed by addition of CSA). Owing to the *cis*-orientation of the 2- and 3-hydroxyls in mannose, acetal formation of this diol occurred concurrently with the desired acetal and so the 4,6-*O*-benzylidene acetal was only isolated in 41% yield. Compound **113** was then subjected to regioselective reducing conditions to cleave open the ring to give the 6-hydroxyl and 4-*O*-benzyl ether.[112] The reductive ring opening was attempted using borane as the reducing agent and TMSOTf as the acid at 0

°C, which proceeded with excellent regioselectivity, however the isolated yield was only 48%. The resulting triol was subjected to the TEMPO/BAIB reagent system in an attempt to synthesise the 2,6-lactone **111**. Surprisingly, under these conditions the reaction stalled at the aldehyde intermediate as the 2-hydroxyl did not cyclise to form the hemiacetal. This disappointing result meant that the model system was unable to be easily synthesised, and thus it was not possible to trial benzylation conditions on a substrate that mimicked compound **105**.

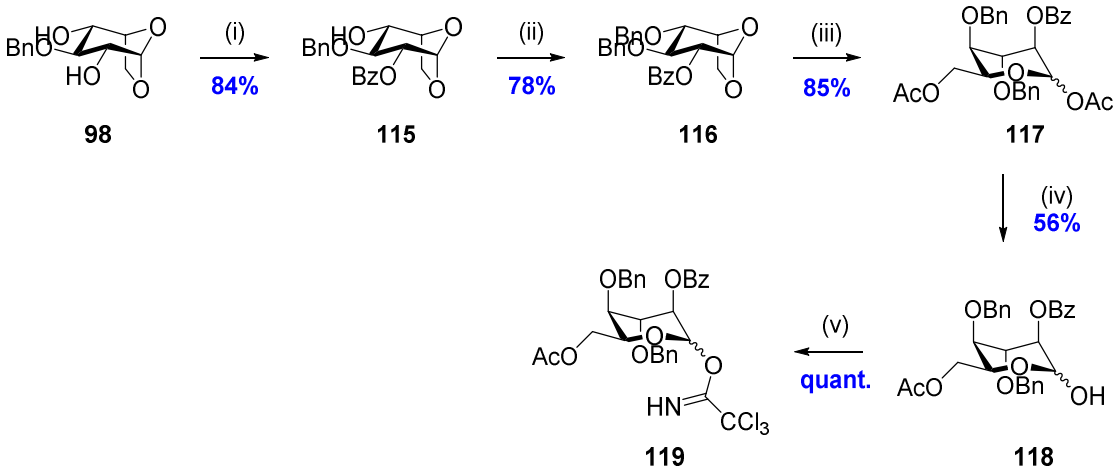


Scheme 23: Synthesis of model substrate **111** from methyl α-D-mannopyranoside (**112**). Reagents and conditions: (i) $PhCH(OMe)_2$, cat. CSA, DMF, rt, 16 h, 41%;' (ii) BH_3·THF, TMSOTf, CH_2Cl_2, 0 °C, 16 h, 48%; (iii) $PhI(OAc)_2$, TEMPO, CH_2Cl_2, rt, 16 h, only aldehyde was observed by crude ^1H NMR.

These unsatisfactory results using the model system led to investigation of an alternative *Ido*-glycosyl acceptor such that no protecting group chemistry was required to be performed on lactone intermediate **105**.

3.8 Redesign of the idose building block

Following on from the unsuccessful attempts to benzylate the 4-*O*H of the idose unit, it was decided to redesign the idose glycosyl donor so that the protecting group was in place prior to the glycosylation reaction. The synthetic route for the redesigned isdose glucosyl donor **119** is shown in scheme 24.



Scheme 24: Synthesis of Ido-glycosyl donor **119** from intermediate **98**. Reagents and conditions: (i) BzCl, pyridine, CH_2Cl_2, 0 °C to rt, 16 h, 84%; (ii) BnBr, Ag_2O, CH_2Cl_2, rt, 3 d, 78%; (iii) Ac_2O, $Cu(OTf)_2$, rt, 16 h, 85%; (iv) NH_3, CH_3OH, 0 °C, 16 h, 56% (75% b.r.s.m.); (v) Trichloroacetonitrile, K_2CO_3, CH_2Cl_2, rt, 16 h, quantitative.

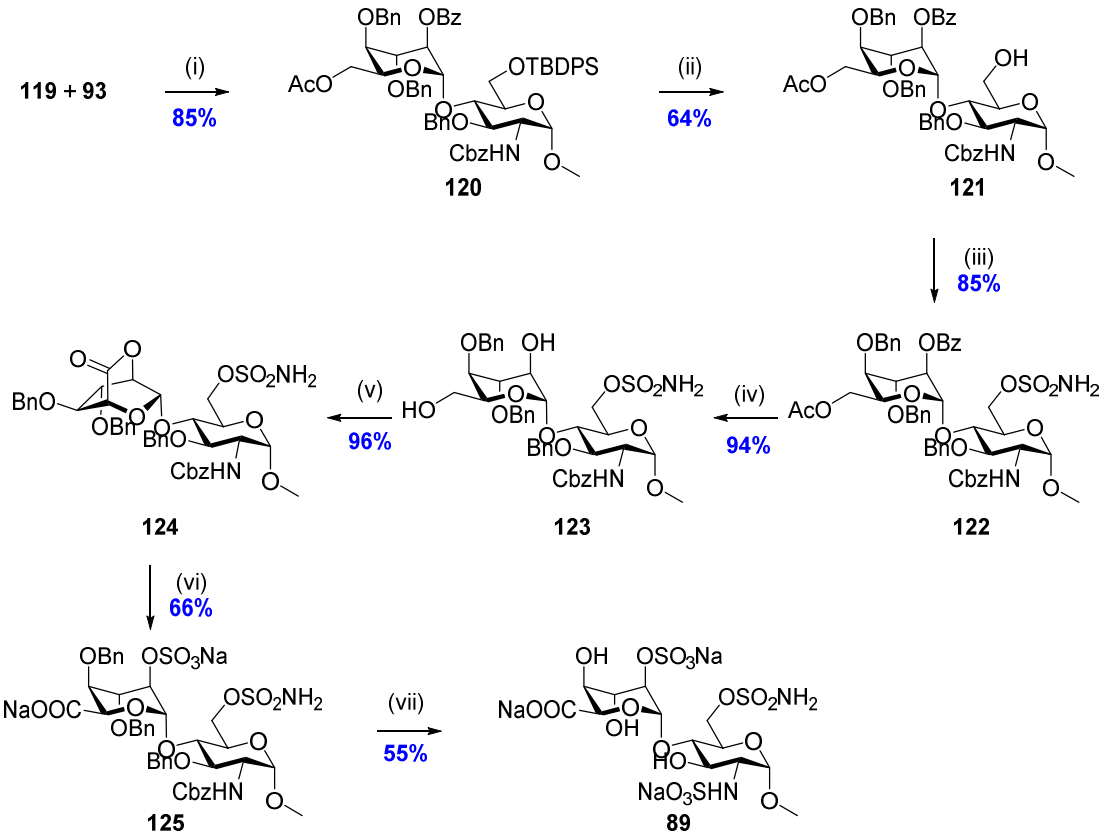
Starting from intermediate 1,6-anhydrosugar **98** it was possible to take advantage of the inherent difference in reactivity of the 2- and 4-hydroxyls. The inductive effect of the two oxygen atoms at C-1 leads to increased nucleophilicity of the 2-*O*H with electrophiles, thus in basic solution the 2-*O*H exclusively reacts with benzoyl chloride and compound **115** was isolated in 84% yield. Subsequent 4-*O*-benzylation of alcohol **115** with Ag_2O and benzyl bromide gave fully protected 1,6-anhydrosugar **116** in 78% yield. 1,6-Anhydrosugar ring opening of compound **116** with acetic anhydride, using copper(II) trifluoromethanesulfonate ($Cu(OTf)_2$) as the catalyst, gave compound **117** in good yield, 85%. Next, the regioselective 1-*O*-deacetylation of compound **117** was achieved using ammonia in methanol solution, however the conversion was slow at 0 °C and when the temperature was raised and the reaction time extended, the regioselectivity of the deacetylation was compromised. Finally, alcohol **118** was reacted with trichloroacetonitrile and K_2CO_3 to obtain glycosyl donor **119** in quantitative yield.

With the orthogonally protected *ido*-glycosyl donor in hand, the glycosylation reaction between **119** and glycosyl acceptor **93** was activated using TMSOTf, which proceeded

effectively to afford the desired disaccharide **120** in 85% yield, (anomeric configuration assigned by ^{1}H NMR, 5.28 (s, 1H, C(1')*H*))[vii]. Moreover, as with the 2-*O*Ac neighbouring group participation in section 3.5, the 2-*O*-benzoyl ester also ensured high stereoselectivity and enabled the exclusive formation of the α–glycosidic bond, which was once again confirmed by ^{1}H NMR spectroscopy. A modification of the order of synthetic steps was then attempted in order to improve the yield of the desilyation reaction. Instead of the deacetylation-lactone formation-desilyation sequence employed in section 3.5, it was decided to desilyate the 6-*O*-TBDPS group using acetic acid buffered TBAF. The buffered reaction conditions were utilised to prevent hydrolysis of the ester protecting groups, which are inherently less reactive towards nucleophiles than the [2,2,2]-bicyclic lactone, and this reaction which proceeded to afford **121** in 64% yield.

Subsequent sulfamoylation of the primary alcohol was achieved in 85% yield by altering the previously optimised conditions to increase the equivalents of sulfamoyl chloride (2 equivalents), and raising the reaction temperature to 0 °C. For substrate **121** there was no possibility of undesired side-products as a result of poor regioselectivity because of the protecting groups on the other hydroxyl moieties. Subsequently, the base-labile protecting groups of compound **122** (one acetate ester and one benzoyl ester), were removed by subjecting **122** to catalytic $NaOCH_3$ in CH_3OH to produce diol **123** in 94% yield.

[vii] See chapter 7 for explanation about magnitude of *J* couplings for idose anomers



Scheme 25: Synthesis of inhibitor **89** from glucosyl donor **119** and acceptor **93**. Reagents and conditions: (i) cat. TMSOTf, 4Å molecular sieves, CH_2Cl_2, 0 °C, 1 h, 85%; (ii) TBAF (1M in THF), AcOH, THF, rt, 16 h, 64%; (iii) $ClSO_2NH_2$, DMF, rt, 16 h, 85%; (iv) $NaOCH_3$, CH_3OH, rt, 16 h, 94%; (v) TEMPO, $PhI(OAc)_2$, CH_2Cl_2/H_2O, rt, 16 h, 96%; (vi) 1. LiOH, THF/H_2O; 2. SO_3·Py, DMF, 100 °C under microwave irradiation, 15 min, 66%; (vii) 1. $Pd(OH)_2/C$, H_2 (balloon), CH_3OH/PBS (v/v 9:1), rt, 16 h; 2. SO_3·Py, H_2O (pH 9-10), rt, 16 h, 55%.

The resulting diol **123** was then subjected to oxidation with the TEMPO/PIDA reagent system to afford lactone **124** in 96% yield. Following on from the lactone formation, it was immediately hydrolysed in basic aqueous medium, and the resulting 2-OH moiety was treated with sulphur trioxide-pyridine complex under microwave irradiation. After elution through a Dowex® 50WX8 Na^+-form column, using water as the eluent, **125** was isolated in 66% yield over two steps. Finally, the hydrogenolysis-labile protecting groups were cleaved by subjecting **125** to $Pd(OH)_2/C$ catalysed hydrogenation in methanol and aqueous phosphate buffered saline (20 mM, pH 7.4), to give a primary amine intermediate, which

was successively subjected to sulphur trioxide pyridine complex in basic aqueous medium to afford final compound **89** in 55% over two steps as the tri-sodium salt.

3.9 Biological Evaluation of compounds 88 and 89

The inhibitory activity of compounds **88** and **89** toward HSulf-2 were evaluated using the fluorescent assay monitoring the hydrolysis of 4-MUS to 4-MU, as previously discussed in chapter 2. The compounds were initially tested at a single concentration (500 μM), data shown in figure 36. At this single concentration, inhibitor 89 was approximately 2-fold more efficient than inhibitor **88**.

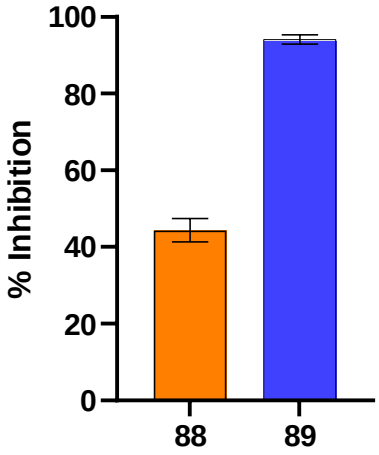


Figure 36: HSulf-2 inhibition data for **88** and **89** at 500 μM. Data represented as the mean ± SD, (n = 2). 120 ng/μL HSulf-2, 10 mM 4-MUS, 50 mM Tris base, 10 mM $MgCl_2$, (pH 7.5), 37 °C, 4 h, then quenched by addition of 1 mM Tris base (pH 11.5) and analysed on a fluorescence plate reader (4-MU, λ_{Ex} 350 nm, λ_{Em} 460 nm). Inhibition values are reported as percentages of the uninhibited control values.

A dose response curve was generated for inhibitor **89**, by testing at 6 concentrations across the range of 100 nM – 1 mM (figure 37). The IC_{50} value was determined as 42.6 μM ± 18.3.

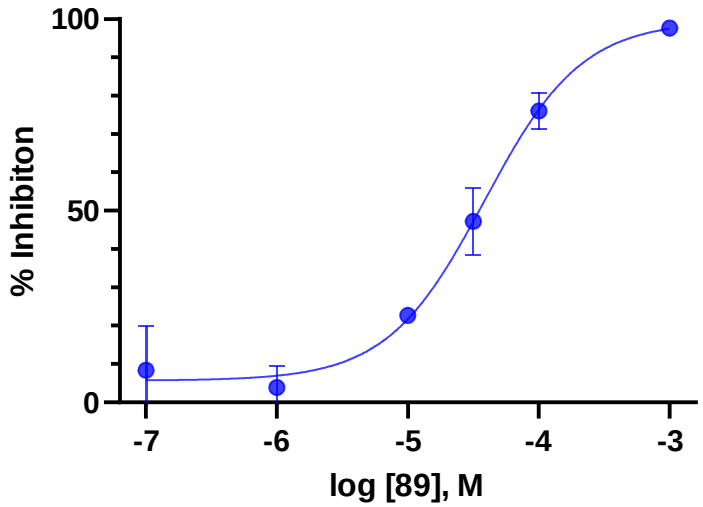
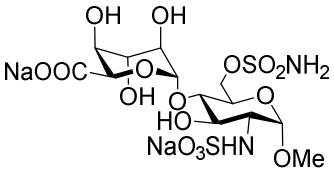


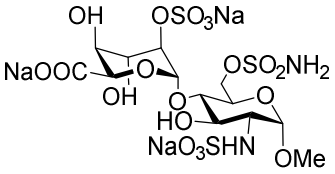
Figure 37: IC_{50} curve for inhibitor **89** against HSulf-2. IC_{50} = 42.6 µM ± 18.3. Data presented as mean ±SD (n=2). 160 ng/µL HSulf-2, 10 mM 4-MUS, 50 mM Tris base, 10 mM $MgCl_2$, (pH 7.5), 37 °C, 4 h, then quenched by addition of 1 mM Tris base (pH 11.5) and analysed on a fluorescence plate reader (4-MU, λEx 350 nm, λEm 460 nm). Inhibition values are reported as percentages of the uninhibited control values.

3.10 Summary

This chapter describes the design and synthesis of two sulfatated analogues of IdoA-GlcN as putative inhibitors of Sulf-1/2. In order to increase the potency of the small molecule inhibitors of endosulfatases described in chapter 2, the HS fragment size was increased to a disaccharide scaffold. The inhibitor design was guided by the substrate specificity of Sulf1/2 within heparan sulfate. Using this as a starting point, two inhibitors **88** and **89** were designed incorporating the sulfamate inhibitor pharmacophore in the GlcN 6*O* position. The synthetic routes towards these inhibitors were successfully executed and the analogues were evaluated using the fluorescent 4-MUS assay. The preliminary sulfatase inhibition data indicate that the disaccharide sulfamates have superior potency against Sulf-2 compared with the series of monosaccharides. The most potent disaccharide **89**, determined by comparison of percentage inhibition at a single concentration (500 µM), was progressed for dose-response analysis, and the IC_{50} value was determined to be 42.6 µM ± 18.3.



88



89

Figure 38: Summary of regioselectively sulfated HS disaccharides synthesised in Chapter 3

4. Design and synthesis of an amino-functionalised disaccharide intermediate

This chapter describes the design and synthesis of an amino-functionalised disulfated IdoA-GlcN intermediate for use in the target engagement probe of inhibitor **89** (as will be discussed in chapter 5) and the multivalent display of inhibitor **89** (as will be discussed in chapter 6).

4.1 Introduction

At this point in the project, two new avenues of further research were of interest: (i) target engagement studies to identify the selectivity of inhibitor binding within a cellular environment, and (ii) multivalent display of the small molecule ligand to increase the potency of inhibition. It was envisioned that both of these ideas may involve the synthesis of a common building block, which is a modified analogue of **89** that includes a functional group 'handle' that could subsequently be reacted to tether it to a desired scaffold.

A primary amine was chosen as the functional group 'handle' as it can be reacted at a late stage in the synthesis to form an amide, which is stable in biological environments and allows for a versatile range of potential coupling partners (figure 38).

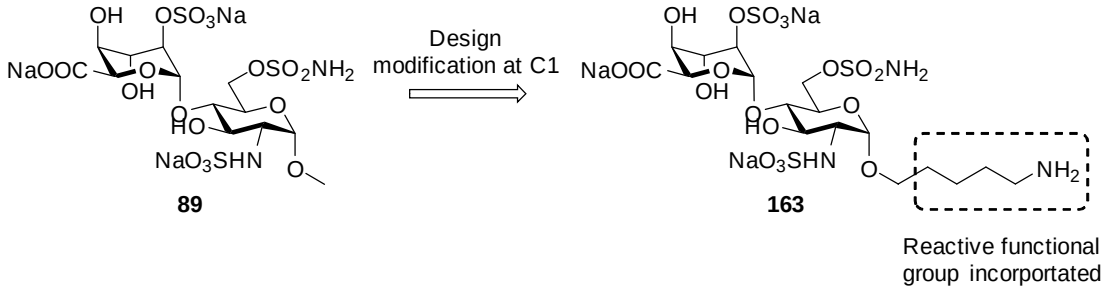
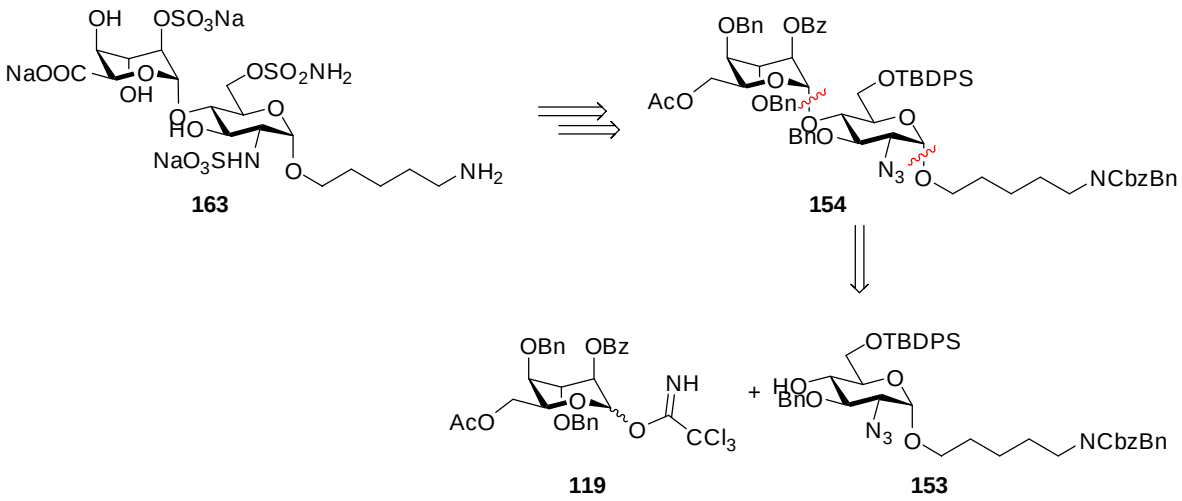


Figure 38: Modification of inhibitor **89** to incorporate a reactive functional group and structure of intermediate **163**.

4.2 Retrosynthetic analysis of intermediate 163

Synthesis of amine functionalised dimer **163** was planned to follow a similar synthetic route as compound **89** with some modifications. The addition of a second amine into the molecule required orthogonal protecting groups, and it was proposed that the linker amine could be protected with benzyl and carboxybenzyloxy groups, and therefore deprotected by a hydrogenation simultaneously with the other *O*-benzyl groups. The glucosamine C(2)-*N* residue could be masked using an azide, which is stable against a variety of reactions and could be easily unveiled prior to the H_2/Pd reduction step using mild conditions in the Staudinger reduction.

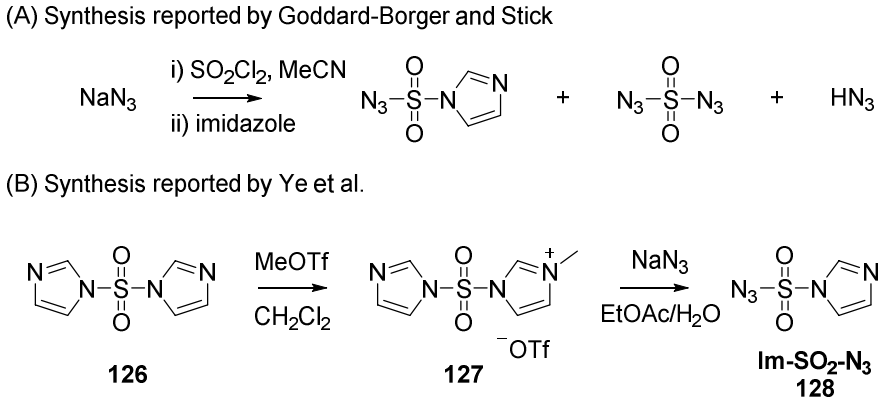


Scheme 26: Retrosynthetic analysis of intermediate **163** identifying functional group interconversions (protecting group strategies and regioselecitve sulfation reactions) and a chemical glycosylation reaction using Ido-glycosyl donor **119**.

4.3 Synthesis of intermediate 153

In order to install the azide functionality, a diazotransfer reaction of glucosamine hydrochloride was employed. Despite being well documented in the literature, the azide transfer reaction on glucosamine hydrochloride proved more challenging than expected. Initial attempts utilised the 'safe' azide transfer reagent imidazole-1-sulfonyl azide (Im-

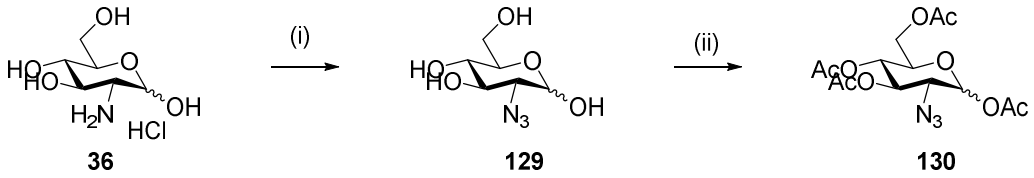
SO_2-N_3),[113] which was synthesised according to a procedure described by Ye et al., shown in scheme 27.[114]



Scheme 27: Summary of procedures to synthesise imidazole-1-sulfonyl azide

Following this procedure, *N,N'*-sulfuryldiimidazole (**126**) was methylated using methyl triflate to produce **127** as a triflate salt, which precipitated out of the reaction mixture and required no further purification. Triflate salt **127** was reacted with sodium azide under biphasic conditions for 1 h, after which time the organic layer was separated to give the crude Im-SO_2-N_3 (**128**) solution, which was used immediately in the reaction with glucosamine hydrochloride. Following subsequent peracetylation of the intermediate tetrol under standard conditions, only 24% yield was obtained over two steps (table 10, entry 1).

Table 10: Summary of diazotransfer reagent and conditions investigated for the synthesis of 1,3,4,6-Tetra-O-acetyl-2-azido-2-deoxyglucose **130**. Reagents and conditions: (i) diazotransfer reagent, $CuSO_4$, K_2CO_3, CH_3OH, rt, 16 h; (ii) Ac_2O, cat. DMAP, pyridine, rt, 16 h.



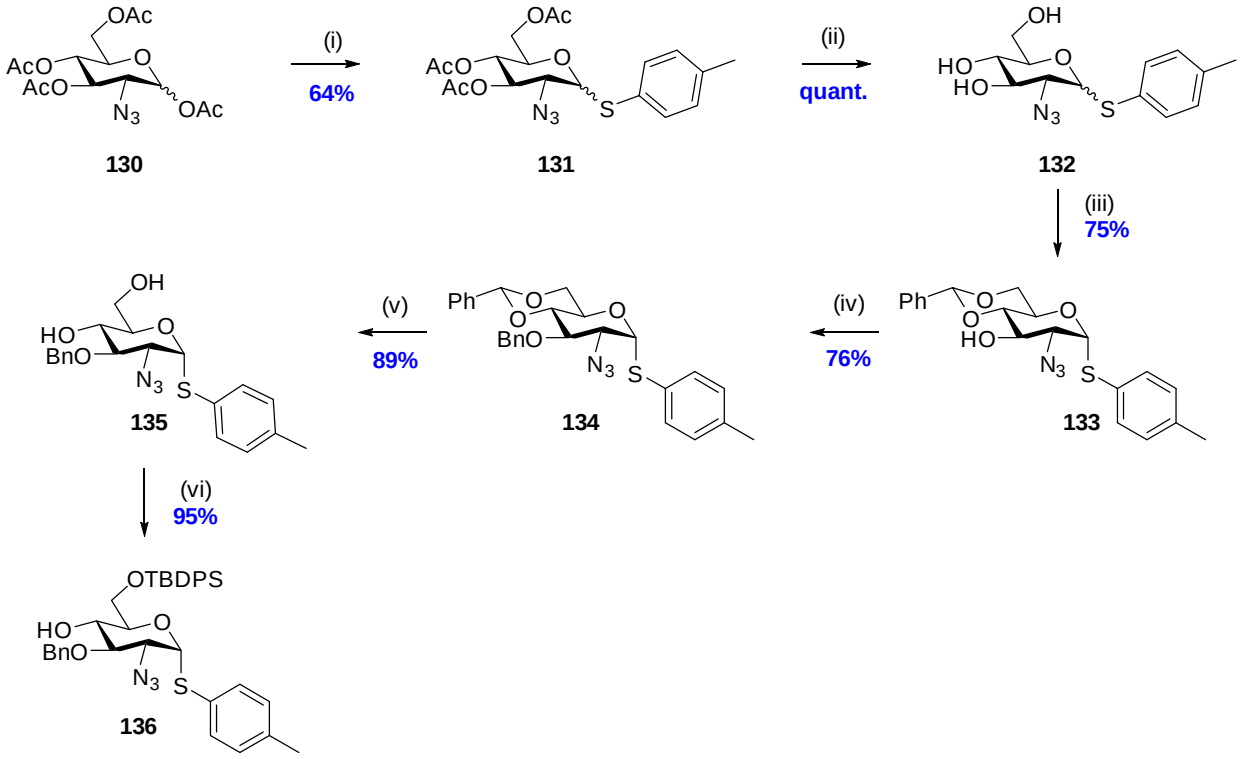
36 **129** **130**

Entry	*Transfer reagent*	*Transfer reagent formation conditions*	*Isolated yield*
1	Im-SO_2-N_3	1.2 eq. NaN_3, 0.9 eq. EtOAc	24%
2	TfN_3	2.4 eq. NaN_3, 1.2 eq. Tf_2O, H_2O/PhMe	25%
3	TfN_3	1.6 eq. NaN_3, 1.2 eq. Tf_2O, MeCN	64%
4	TfN_3	10 eq. NaN_3, 2 eq. Tf_2O, CH_2Cl_2/H_2O	79%

In an attempt to increase this yield, it was decided that the more reactive diazotransfer reagent TfN_3 should be investigated. Initially, to minimize the explosion risk, TfN_3 was prepared as a solution in toluene, an inert high boiling solvent, to avoid any accidental concentration of the explosive intermediate, following a procedure described by Titz et al.[115] This protocol afforded a low isolated yield of 25% after peracetylation (table 10, entry 2). Secondly, anhydrous acetonitrile was investigated as a solvent in an attempt to minimise Tf_2O hydrolysis. The formation of TfN_3 in acetonitrile was monitored and quantified by ^{19}F NMR using 1,4-difluorobenzene as an internal standard, and the equivalents of glucosamine.HCl were calculated based on the measured concentration of TfN_3. The TfN_3 was then used in a 1.2 equivalent excess in the diazotransfer reaction, which gave a pleasing increase in yield after peracetylation of 64% (table 10, entry 3). Finally, the equivalents of Tf_2O and NaN_3 were increased to 2 and 10 respectively, using a CH_2Cl_2/H_2O biphasic reaction mixture. The diazotransfer reaction afforded the azido-sugar in 79% yield after peracetylation (table 10, entry 4). These conditions for formation of TfN_3 and subsequent

diazotransfer gave consistently high yields over a range of scales, from 200 mg to 20 g of glucosamine hydrochloride, providing that high quality Tf_2O was used.

4.4 Orthogonally protected thioglycoide donors



Scheme 28: Synthesis of thioglycoside **136** from peracetylated **130**. Reagents and conditions: (i) p-thiocresol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 16 h, 64%; (ii) $NaOCH_3$, CH_3OH, rt, 16 h, quant.; (iii) $PhCH(OMe)_2$, p-TsOH, DMF, rt, 16 h, 75%; (iv) NaH, BnBr, TBAI, DMF, rt, 16 h, 76%; (v) $AcOH/H_2O$ (v/v 7:3), 65 °C, 16 h, 89%; (vi) TBDPSCl, cat. DMAP, Et_3N, CH_2Cl_2, rt, 16 h, 95%. [viii]

Following on from the azide transfer protocol, the anomeric acetate was exchanged for a thiotoluene by treatment of **130** with *p*-thiocresol and $BF_3.OEt_3$ in CH_2Cl_2. Subsequent global acetate deprotection using sodium methoxide in methanol gave **132** in 64% yield over two steps. The 4,6-diol was then protected using a benzylidene acetal using benzaldehyde dimethyl acetal and catalytic *p*-TsOH, which was achieved in 75% yield.

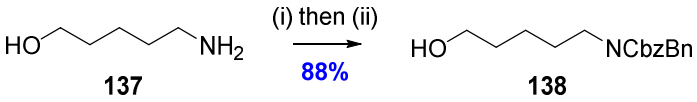
[viii] **130**-**132** isolated as a mixture of anomers. The batch used for characterisation in the experimental has a defined and stated anomeric ratio.

Following on from the diol protection, the 3-*O* position was then protected using a benzyl ester by reacting alcohol **133** with benzyl bromide and NaH in DMF, which produced fully protected intermediate **134** in 76% yield. Finally, acid-catalysed hydrolysis of the benzylidine acetal and selective protection of the primary 6-*O* alcohol using *tert*-butyl(chloro)diphenylsilane gave glycosyl donor **136** in 85% yield over two steps.

The final step in this proposed synthesis was the glycosylation reaction of a thioglycoside and an alcohol. Glycosylation reactions typically involve an activator-mediated nucleophilic displacement of a leaving group on a glycosyl donor by the hydroxyl group of the glycosyl acceptor. Any other potentially reactive hydroxyl groups on either the acceptor or donor need to be masked by protecting groups. These protecting groups also have a profound influence on the mechanism, rate and stereoselectivity of the glycosylation. This reaction involves many potential cationic intermediates and can proceed via mechanistic pathways ranging from S_N1 to S_N2. In the absence of a participating group at the C-2 position, the glycosylation proceeds via an oxycarbenium ion and thus often leads to an anomeric mixture. This mixture can be influenced by a number of factors including temperature, pressure, solvent and promoter choice and the structure of the glycosyl donor (steric and electronic effects of protecting groups, conformation and torsional effects, and the nature of the leaving group). This means that predicting the reaction outcome remains a challenge to synthetic chemists, although progress has been made in recent years.

With this glycosyl donor in hand, the *N*-protected 5-aminopentan-1-ol glycosyl acceptor (**138**) was prepared by reductive amination of 5-aminopentan-1-ol (**137**) with benzaldehyde using sodium borohydride as the reducing agent, followed by chemoselective

benzyloxycarbonyl formation by reacting the aminoalcohol with benzyl chloroformate in aqueous $NaHCO_3$ affording a 88% yield over two steps.



Scheme 29: Synthesis of *N*,*N*-Cbz,Bn-5-aminopentan-1-ol. Reagents and conditions: (i) PhCHO, $NaBH_4$, CH_3OH, 0°C, 2 h; (ii) CbzCl, $NaHCO_3$, H_2O/1,4-dioxane, rt, 16 h, 88%.

The glycosylation reaction using **138** as the glycosyl acceptor was attempted with various thioglycoside intermediates. Initially, the glycosylation between **136** and **138** was attempted at −78 °C in CH_2Cl_2 using TMSOTf and NIS to activate the thioester glycosyl donor, under these conditions only the β-anomer was isolated in 46% yield (table 11, entry 1). This glycosyl donor is considered 'armed' due to the 3-*O*-benzyl ether and the 6-*O*-silyl group stabilising the oxacarbenium ion intermediate, and therefore increasing the reactivity. The C2-N_3 did not influence the stereochemical outcome of the glycosylation reaction, but lessened the reactivity of the donor. To investigate whether using an ethereal co-solvent would influence the stereochemistry, this reaction was repeated using CH_2Cl_2/THF 1:1. Using this alternative solvent system, the crude product was formed α/β ratio 1:3 observed by ^{1}H NMR spectroscopy (table 11, entry 2). The solvent-coordination hypothesis for increased α-selectivity predicts that the ethereal solvent coordinates with the anomeric carbon of the oxocarbenium cation preferentially on the β-face of the ring, and thus the nucleophilic attacks on the α-face (figure 39). However, CH_2Cl_2 remains the primary solvent as this increases the rate of glycosylation reactions.

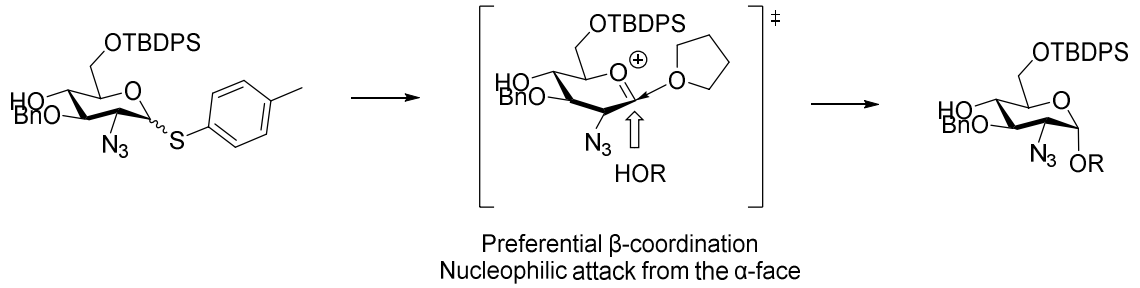
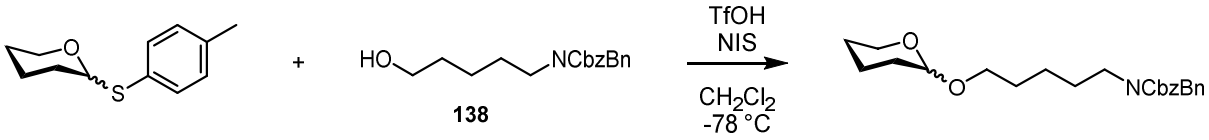
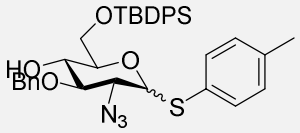
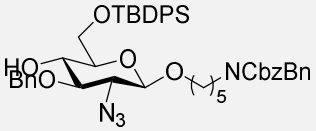
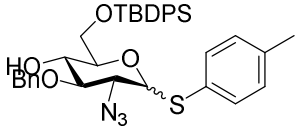
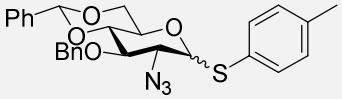
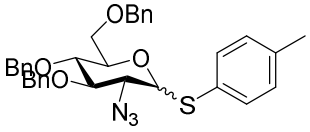
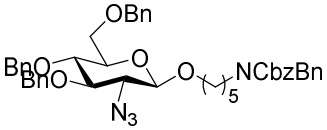


Figure 39: Explanation of ethereal solvent participation effecting greater α-selectivity

Table 11: Summary of GlcN-glycosyl donors investigated in the glycosylation reaction with alcohol **138**. Reagents and conditions: NIS, cat. TfOH, 4Å molecular sieves, CH_2Cl_2, −78 °C.



Entry	*Glycosyl donor*	*Reaction temperature*	*Reaction outcome*
1	 **136**	–78 °C→ –40 °C	Isolated 46% yield  **β-140**
2	 **136**	–78 °C→ –40 °C [(a)]	Crude α/ β 1:3 [(b)] isolated **α-140** 10%, **β-140** 46%
3	 **134**	–78 °C→ rt	No reaction
4	 **139**	–78 °C→ –40 °C	63% isolated yield  **β-141**

(a) Deviations from standard conditions, solvent CH_2Cl_2/THF 1:1; (b) α/β ratio determined by crude NMR integration

Thioglycoside **134**, which was an intermediate on route to **136** (scheme 28), was trialled as it was in hand (table 11, entry 3). Surprisingly, this reaction did not give any conversion to either stereoisomer of the product nor the hydrolysed glycoside, implying that the donor was not activated by TfOH/NIS at −78 °C. The nature of the inactivating effect of the 4,6-benzylidine acetal has been experimentally determined to be due to the electronic and torsional effects of locking the ring in the $^4C_1$ conformation.[116] After 16 h, having warmed to room temperature gradually, this glycosylation reactions had not turned the characteristic brown colour to indicate the formation of iodine, as observational evidence that the oxocarbenium ion had failed to form.[117]

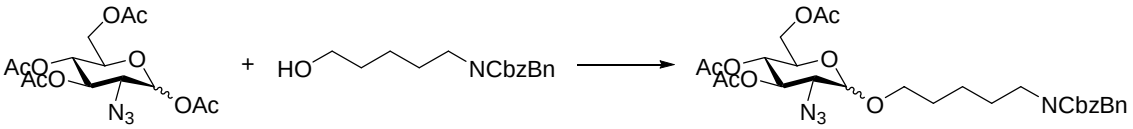
Finally, in an attempt to make the glycosyl donor more reactive to increase α-selectivity, the 3,4,6-*O*-tribenzyl thiocresol glycoside **139** was prepared by treating triol **132** with NaH and benzyl bromide in DMF in 77% yield. This analogue was synthesised to investigate whether the glycosyl donor could be activated despite having the 'disarming' C2-N_3 functional group, despite not having the required orthogonal protecting groups for this particular synthetic route. When this 'armed' glycosyl donor was reacted with alcohol **138** and triflic acid and NIS at −78 °C, only the β-glycoside **141** was isolated in 63% yield (table 11, entry 4).

4.5 Glycosyl acetate donor 130

A literature search for examples of α-selective glycosylation reactions suggested Lewis acid catalysed reaction conditions may allow access to the desired glycoside via the glycosyl acetate donor **130**. Starting from the glycosyl acetate was an attractive route because the α-selectivity arises from in situ anomerisation of the initially formed β-glycoside, and therefore the stereochemical outcome would not depend on the nature of protecting groups on the glycosyl donor.

Firstly, activation using $BF_3.OEt_2$ and 1.2 equivalents of alcohol **138** at 40 °C for 16 h gave no conversion to the desired glycoside (table 12, entry 1).[118]

Table 12: Summary of glycosylation reactions using acetate donor **130**. Reagents and conditions: (i) **130** (1 eq.), **138** (1.2 eq.), $BF_3 \cdot OEt_3$, 4Å molecular sieves, CH_2Cl_2, 40 °C, 16 h; (ii) **130** (1 eq.), **138** (4 eq.), $Cu(OTf)_2$, DCE, 130 °C under microwave irradiation, 25 min; (iii) **130** (1 eq.), **138** (1 eq.), $FeCl_3$, $CaSO_4$, DCE, 85 °C, 16 h.



130 **138** **142**

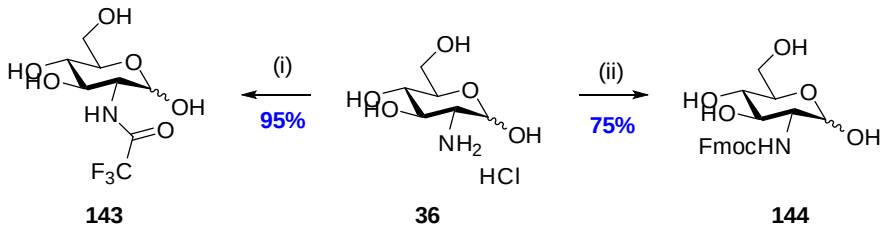
Entry	*Alcohol eq.*	*Activation conditions*	*Reaction outcome*
1	1.2	$BF_3.OEt_2$, CH_2Cl_2, 40 °C	No reaction
2	4	$Cu(OTf)_2$, DCE, 130 °C	No reaction
3	1	$FeCl_3$, $CaSO_4$, DCE, 85 °C	Decomposition

Secondly, microwave assisted $Cu(OTf)_2$ catalysed glycosylation conditions were investigated (as reported by Frem et al.[119]), however no conversion to product was observed (table 12, entry 2). In their report, Frem et al. claim that the excellent yield and good α-diastereoselectivity can be achieved due to thermodynamic control.

Thirdly, a procedure by Wei et al. reported highly selective α-glycosidation of glycosamine penta-acetates using $FeCl_3$ and demonstrated that the scope of the acceptor alcohol tolerated both phenols and primary alkyl alcohols.[120] This was an attractive approach for the installation of the 5-aminopentanol (**138**) into the anomeric position of glycosyl acetate **130**, and it was hypothesised that the 2-azido moiety would not drastically effect stereoselectivity. However, when glycosyl acetate **130** was reacted with two equivalents of alcohol **138** and $FeCl_3$ (150 mol%) in the presence of anhydrous $CaSO_4$, the reaction was unsuccessful, giving a complex mixture of decomposition products, (table 12, entry 3).

4.6 Fischer glycosylation

Fischer glycosylation is commonly used for the synthesis of simple alkyl glycosides from free sugars.[121] The reaction is acid-catalysed and uses the alcohol as the solvent at reflux. Under acidic conditions, hydroxyls do not require protection, however the primary amine would need an orthogonal protecting group (with respect to the *N*-CbzBn of the alcohol). The protecting groups chosen were azido, trifluoroacetate and Fmoc, all of which were expected to be non-directing and orthogonal to the hydrogenation-labile Cbz and Bn on the alcohol **138**.

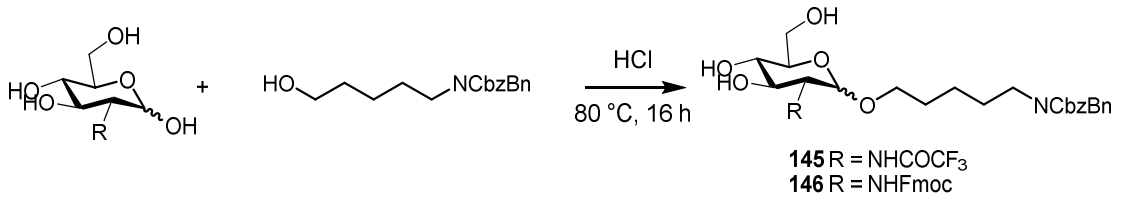


143 **36** **144**

Scheme 30: Synthesis of N-protected glucosamine intermediates as substrates for Fischer glycosylation reactions. Reagents and conditions: (i) ethyl trifluoroacetate, Et_3N, CH_3OH, rt, 16 h, 95%; (ii) FmocOSu, $NaHCO_3$, H_2O/acetone, rt, 16 h, 76%.

Initially, the reaction of 2-azido-glucosamine and alcohol **138** (five equivalent excess) was investigated, however after a short reaction time of 1 h, the reaction mixture had decomposed, (table 13, entry 1).

Table 13: Summary of Fischer glycosylation conditions investigated. Reagents and conditions: HCl (final reaction concentration 1.25M), 80 °C, reaction time as stated.

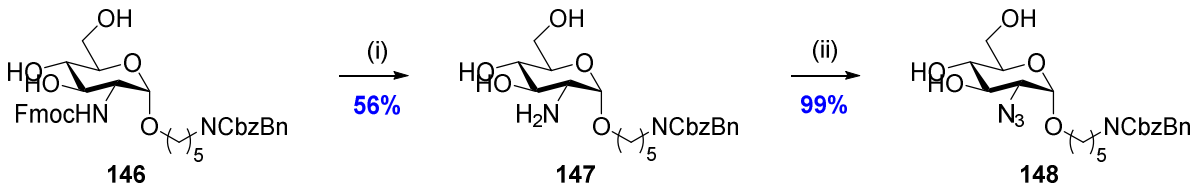


R =	*Reaction observation*	*Reaction time*	*Isolated α*
N_3	Full decomposition	1 h	–
NHTFA	Deprotection of *N*-TFA	1 h	6%
NHFmoc	Some decomposition	16 h	30%
NHFmoc	Crude ratio α/β 80:20	6 h, 65 °C	63%

The second attempt used *N*-trifluoroacetate analogue **143**, which was easily prepared from glucosamine hydrochloride and ethyl trifluoroacetate in 95% yield (scheme 30). This protecting group was expected to be considerably less β-directing than the analogous acetyl analogue due to the electron-withdrawing fluorine atoms, however this electron poor carbonyl is labile under acidic conditions, especially at raised temperatures, and the isolated yield from this reaction was lowered due to considerable detrifluoroacetylation (table 13, entry 2).

Finally, the 9-fluorenylmethoxycarbonyl (Fmoc) protected glucosamine **144** was investigated, which was prepared using Fmoc-OSu in basic aqueous conditions in excellent yield 88%. This group can be deprotected with secondary amines such as piperidine or morpholine. When *N*-Fmoc glucosamine was reacted with 5 equivalents of alcohol **138** and catalytic anhydrous HCl (4 M in 1,4-dioxane) at 85 °C for 16 h, the isolated yield of the desired α-glycoside **146** was only 30% (table 13, entry 3). This low yield was assumed to be due to some decomposition of the Fmoc group at higher temperatures. Indeed, when

trying to obtain NMR characterisation of **146** in DMSO at 90 °C, significant decomposition was observed after a short time. Hock et al. have reported the thermal decomposition of *N*-Fmoc in DMSO as an alternative deprotection strategy that avoids the use of base for sensitive substrates.[122] To limit the decomposition, the temperature was lowered to 65 °C for a shorter reaction time (6 h) and after this time the anomeric ratio of products was α/β, 4:1, measured by LCMS. The desired α-anomer was isolated in 63% yield. The Fischer glycosylation conditions are usually only used when the desired alcohol has a low boiling point, so that it can be used in a very large excess and then removed under vacuum. Alcohol **138** does not have a low enough boiling point to remove it under reduced pressure, and so the number of equivalents was kept low. The excess of alcohol **138**, being less poplar than the desired products, caused the column chromatography purification to be more difficult as the product eluted more quickly with less separation of the two anomers. This meant that the Fischer glycosylation approach using *N*-Fmoc glucosamine was not practically feasible to scale this reaction beyond 5 g.



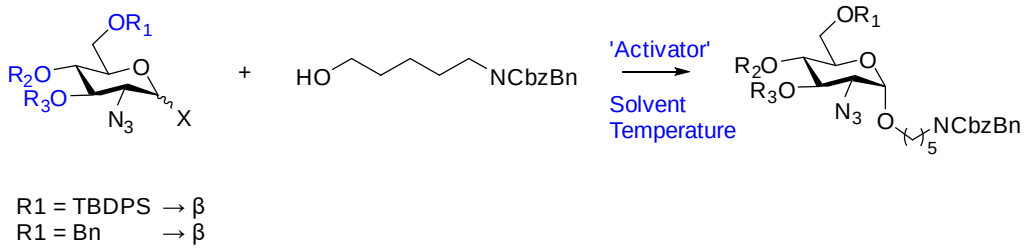
Scheme 31: Synthesis of intermediate **146** from O-glycoside **144**. Reagents and conditions: (i) piperidine, CH_2Cl_2, rt, 30 min, 56%; (ii) TfN_3, $CUSO_4$, K_2CO_3, CH_3OH/H_2O, rt, 16 h, 99%.

The Fmoc-deprotection was achieved by reacting **146** with an excess of piperidine to give primary amine **147**. Only a moderate 56% yield was isolated from this reaction because, despite the relatively low boiling point of piperidine (106 °C), the large excess affected the column chromatography. Finally, the primary amine **147** was then converted into the desired azido-functionality using the diazotransfer protocol described in section 4.3 in 99%

yield. Following this synthetic route, triol intermediate **148** was isolated in 26% yield over 4 steps.

4.7 Triacetate glycosyl donor investigation

In a continued effort to find a procedure that could be used to access the α-*O*-glycoside **148** on large scale, attention was turned back to 2-azido-2-deoxy-glucosamine glycoside donors. The fixed functionalities for the reaction are the 2-azido donor – a deactivating group and the non-reactive alcohol acceptor. The parameters that could be changed were the temperature, solvent, donor leaving group and the donor protecting groups on the 3,4 and 6-hydroxyls, summarized in scheme 32.



Scheme 32: *O*-chemical glycosylation conditions with 2-azido-2-deoxy-glucosamine glycoside donors. Variable parameters in blue.

Ngoje and Li have investigated the stereoselectivity of 2-azido-2-deoxy thioglycoside donors and found that the triacetate glycosyl donor **130** gave a modest α-selectivity (α/β, 3.5:1), and proposed that the C-4 acetate is the most important remote directing group for (β) stereoselectivity.[123] The 4-acetyl-3,6-dibenzyl glycosyl donor gave a modest β-selectivity (α/β, 1:3) and to investigate the participation of the C-4 acetyl to the stereochemical outcome, the benzoyl ester and the trichloroacetate ester were prepared as extreme 'participating' groups. As hypothesised, the C4 benzoyl ester glycoylation resulted in higher β-selectivity (α/β, 1:6) and the trichloroacetyl ester a lower β-selectivity (α/β, 1:1.2). Indeed, the best result for α-selectvity was obtained with the 4-benzyl-3,6-diacetyl

glycosyl donor (α/β, >20:1).[123] Yu et al. provided evidence for the 4-*O*-acyl participation of peractylated glycosides via the isolation and characterisation of 1,2,4-*O*-orthoacetyl products.[124] When the 2-*O*-acetyl group was deuterium labelled there was no deuterium incorporated into the orthoester product suggesting that the 4-*O*-acetyl interacts with the anomeric carbon to form a B_{14} intermediate, effectively blocking the α-face and favouring formation of the β-product. Alternatively, acetyl groups in the 3 and 6 positions participate to favour the α-product by blocking reaction from the β-face in $^1C_4$ intermediates. The study by Ngoje and Li implies that the 6-*O*-acetyl group is most important for the α-selectivity of 2-azido-2-deoxy thioglycoside donors.[123]

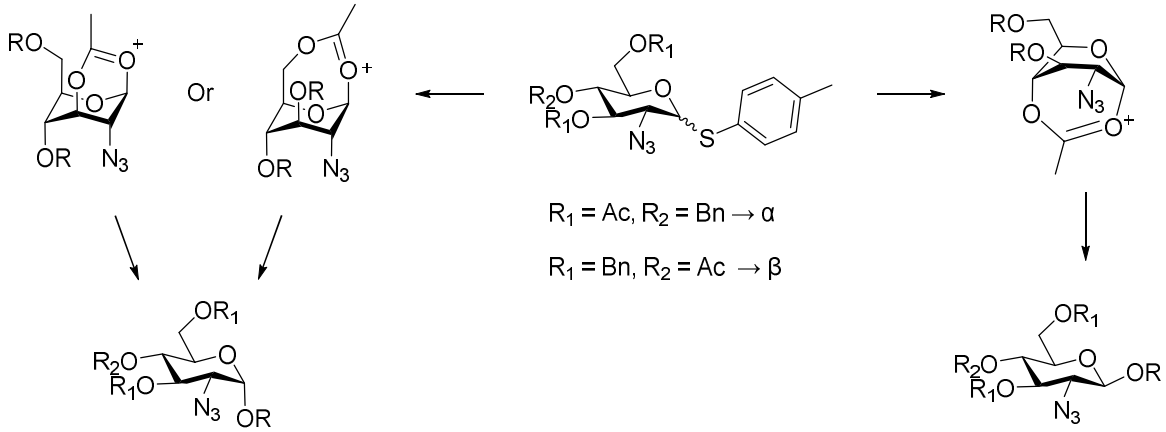


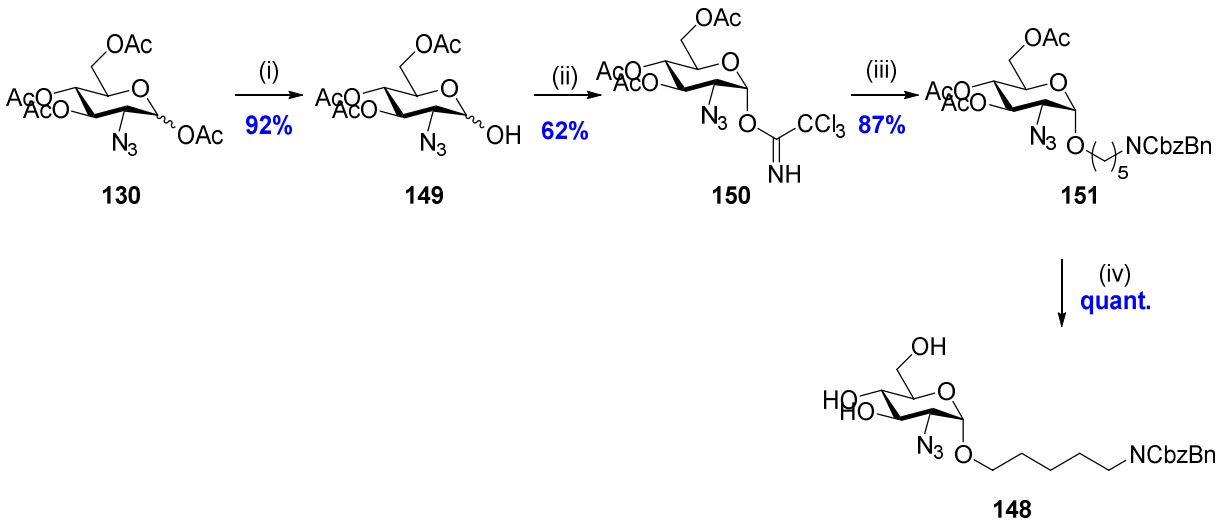
Figure 40: Summary of remote α/β-directing effects of 3-, 4- and 6-OAc reported by Ngoje and Li[123]

According to this study, the optimal glycoside donor, using only acetyl and benzyl protecting groups, is 3,6-*O*-diacetyl-4-*O*-benzyl analogue, however this was not investigated in this study because (i) it would require a 6 step synthesis from peracetylated **130**; (ii) the 4-*O*-benzyl is not orthogonal and therefore would require different protecting groups during the synthesis and (iii) if an orthogonal 'benzyl' was used instead (e.g. dimethoxybenzyl) the route would still require several protecting group manipulations before getting to the desired donor **148**. For these reasons, despite the excellent α-selectivity reported (α/β, >20:1) this protecting group choice was not attempted, instead the

3,4,6-*O*-triacetyl donor **130** was investigated, which was reported to induce some α-selectivity (α/β, 7:2).

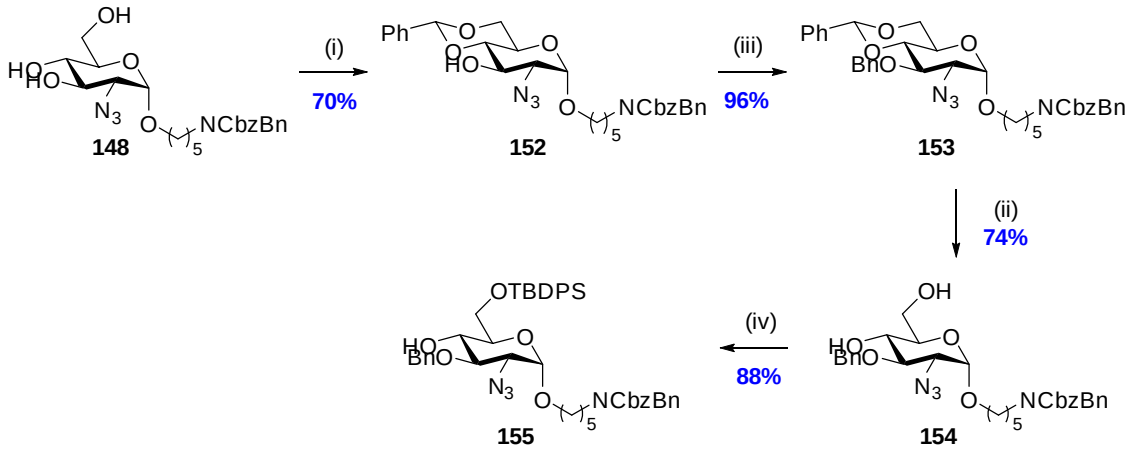
Considering the general trends for promoting α-selectivity (higher reaction temperatures and using an ethereal co-solvent), the temperature was raised to 0 °C, which required the donor leaving group to be changed from the thiotoluene to trichloroacetimidate, and Et_2O was used as a co-solvent to promote attack on the α-face.

Trichloroacetimidate glycosyl donor **130** was synthesised according to scheme 3. The synthesis began with the chemoselective anomeric acetate deprotection of **130**, which was achieved by reaction with dimethylamine in THF at 0 °C to afford hemiacetal **149** in 92% yield. Compound **149** was then reacted with trichloroacetonitrile and catalytic DBU in CH_2Cl_2 to give the α-trichloroacetimidate in 62% yield. This product was reacted with *N*,*N*-benzyl,carboxybenzyl-5-amino-pentan-1-ol **138** under strictly anhydrous conditions at 0 °C to give the desired α-glycoside **151** in 87% yield (scheme 33). In comparison to the previous route to intermediate **148** (described in section 4.6, 26% over 4 steps), this route is superior and gave triol **148** in 39% yield over 5 steps.



Scheme 33: Alternative synthesis to triol **148** from tetra-acetate **130**. Reagents and conditions: (i) dimethylamine, THF, 0 °C, 2 h, 92%; (ii) trichloroacetonitrile, DBU, CH_2Cl_2, rt, 4 h, 62%; (iii) **138**, TMSOTf, 4Å molecular sieves, CH_2Cl_2/Et_2O, 0 °C, 10 min, 54%; (iv) $NaOCH_3$, CH_3OH, rt, 16 h, quantitative.

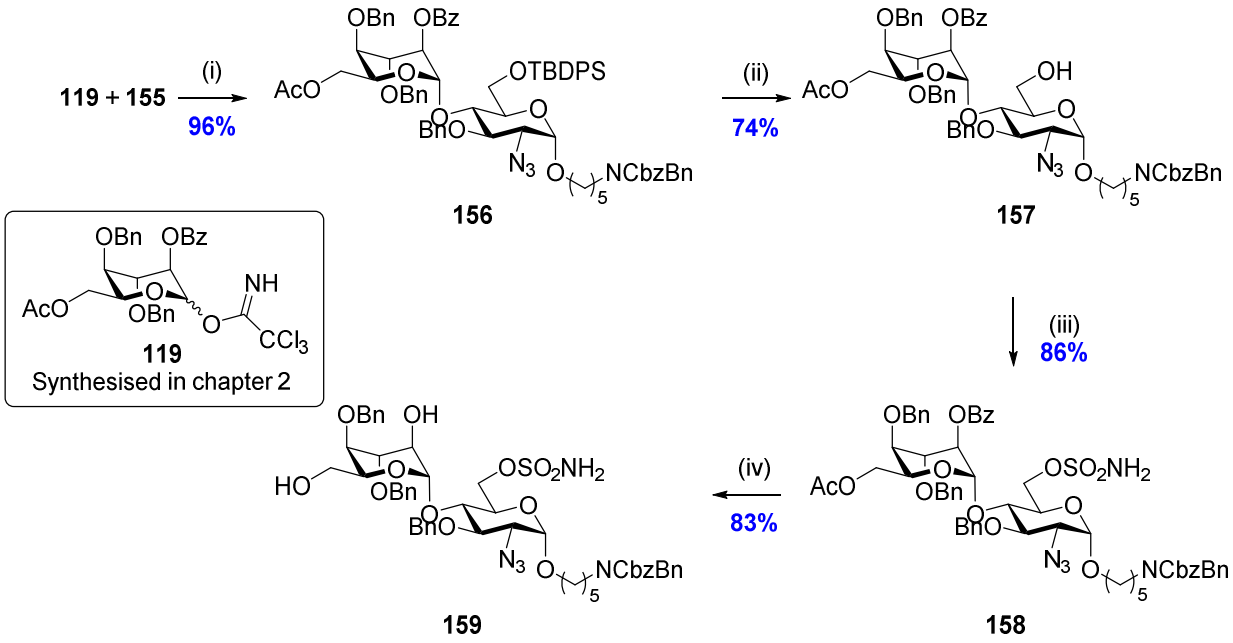
The remaining synthesis towards acceptor **155** required selective 3-*O*-benzylation. This was achieved by first protecting the 4,6-diol by reaction with benzaldehyde dimethyl acetal and catalytic CSA, then benzylation with benzyl bromide and sodium hydride and then acetal hydrolysis using 70% aq. acetic acid, to give diol **154** in 50% over three steps. Finally, chemoselective protection of the primary 6-*O*-hydroxyl using the sterically bulky *tert*-butyldiphenylchlorosilane under basic conditions afforded the desired acceptor **155** in 88% yield (scheme 34).



Scheme 34: Synthesis of GlcN-glycosyl acceptor **155**. Reagents and conditions: (i) $PhCH(OMe)_2$, cat. CAS, MeCN, 60 °C, 6 h, 70%; (ii) NaH, BnBr, DMF, 0 °C to rt, 16 h, 96%; (iii) $AcOH/H_2O$ (v/v 7:3), 65 °C, 6 h, 86%; (iv) TBDPSCl, cat. DMAP, Et_3N, CH_2Cl_2, rt, 16 h, 88%.

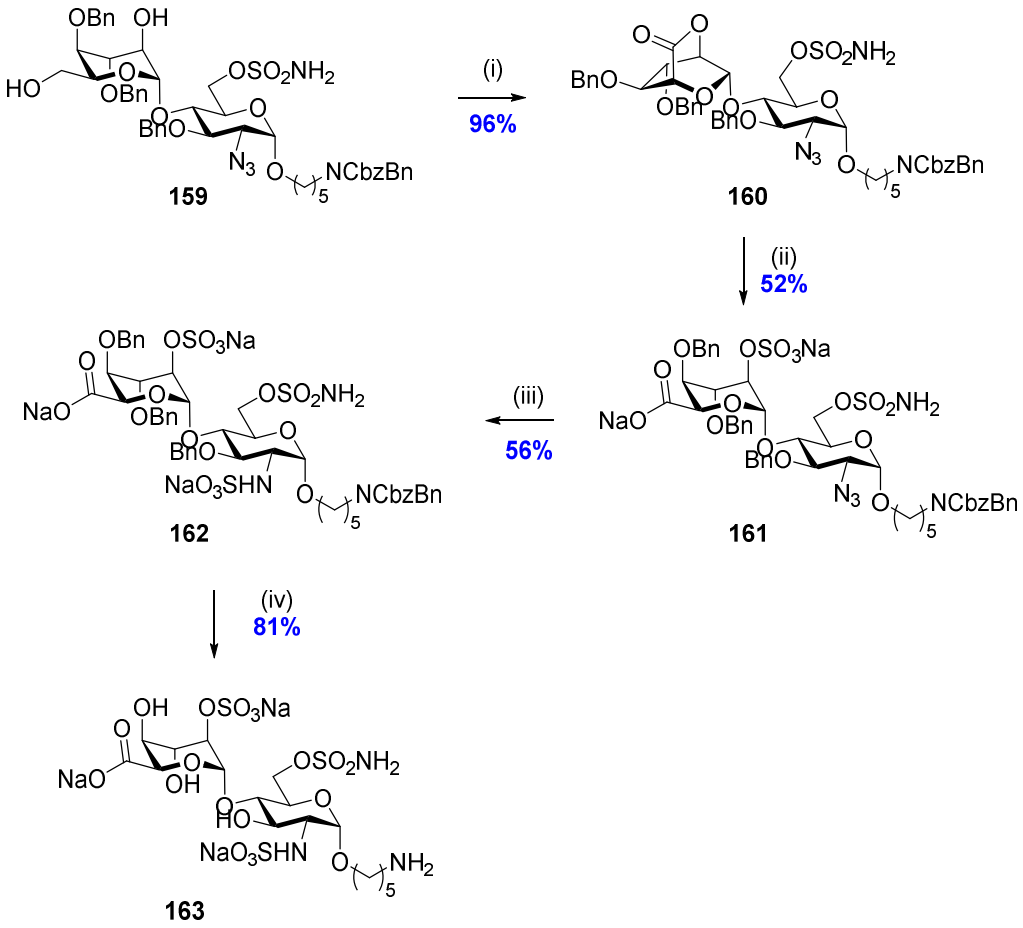
4.8 Remaining synthesis towards amino-functionalised 163

With the donor and acceptor in hand, the trichloroacetimidate donor **119** (synthesised in chapter 2, page 78) was activated using TMSOTf which proceeded effectively in the glycosylation reaction with GlcN-glycosyl acceptor **155** to give the desired disaccharide **156** in 96% yield. The procedure to ensure maximum yield involved pre-drying the donor and acceptor by azeotroping with $PhCH_3$ (× 3), followed by 1 h under high vacuum, then stirring under an atmosphere of N_2 in anhydrous solvent with 4Å molecular sieves for 30 min. Using this procedure, this reaction was robust to scale up and this excellent yield was reproduced on 1 mmol and 3 mmol scale.



Scheme 35: Synthesis of disaccharide **159** from glysosyl donor **119** and acceptor **153**. Reagents and conditions: (i) TMSOTf, 4Å molecular sieves, CH_2Cl_2/Et_2O, 0 °C, 10 min, 96%; (ii) TBAF, AcOH, THF, rt, 16 h, 74%; (iii) $ClSO_2NH_2$, DMF, 0 °C to rt, 16 h, 86%; (iv) $NaOCH_3$, CH_3OH, rt, 16 h, 83%.

Subsequently, using the optimised order of reactions outlined in Chapter 2, the 6-*O*-desilyation was affected by subjecting compound **156** to acetic acid buffered TBAF to give compound **157** in 74% yield. The resulting primary alcohol was subjected to sulfamoylation conditions with $ClSO_2NH_2$ to give compound **158** in 86% yield. Next, the 6'-*O*-acetate and 2-*O*-benzoyl esters of compound **158** were removed using standard conditions with catalytic $NaOCH_3$ to produce diol **159** in 83% yield.



Scheme 36: Synthesis amino-functionalised intermediate **163**. Reagents and conditions: (i) TEMPO, $PhI(OAc)_2$, H_2O/CH_2Cl_2, rt, 16 h, 96%; (ii) 1. LiOH, H_2/THF, 2 h, rt; 2. SO_3·Py, DMF, 60 °C, 16 h, 52%; (iii) 1. PMe_3, NaOH, THF, rt, 16 h; 2. SO_3·Py, H_2O (pH 9-10), 56%; (iv) H_2 (balloon), $Pd(OH)_2/C$, CH_3OH/PBS (v/v 9:1), rt, 16 h, 81%.

Then, the resulting diol **159** was subjected to oxidation with the TEMPO/PIDA reagent system to give lactone **160** in excellent yield (96%). The resulting lactone was subjected to a hydrolysis-sulfation two step protocol, in which the lactone was hydrolysed using previously utilised conditions of LiOH, and the resulting 2-OH was sulphated using SO_3·Py under forcing conditions (60 °C under microwave irradiation), to give **161** in 52% yield over two steps. This low yield was not due to poor conversion, but isolation difficulties. The resulting azide intermediate was subjected to a Staudinger reduction with trimethylphosphine and aq 1M NaOH to afford the amine intermediate by removing the excess PMe_3 under vacuum and the crude product was immediately subjected to sulfation

with SO_3·Py in basic aqueous medium to afford the **162** in 56% yield over two steps. Finally, global deprotection of the hydrogenolysis-labile protecting groups was achieved by subjecting **162** to $Pd(OH)_2/C$ catalysed hydrogenation in methanol and aqueous phosphate buffered saline (20 mM, pH 7.4), to give primary amine **163** in 81% yield after purification and ion-exchange chromatography.

4.9 Summary

This chapter describes the design and synthesis of an amino-functionalised IdoA(2S)-GlcNS(6Sulfamate) building block, **163**. It was synthesised in 2% yield over 17 steps. The most challenging step proved to be the installation of the aminopenan-1-ol (**138**) at the anomeric carbon of the glucosamine residue with the desired α-connectivity. Several methods were explored, including Fischer glycosylation and various chemical *O*-glycoylation reaction conditions. The Fischer glycosylation reactions produced the α-product through thermodynamic control but this method was not scalable. The glycosyl acceptor for the chemical glycosylation reactions was thoroughly investigated. Initially, thioglycoside donors were trialled using various 3-,4- and 6-*O*-protecting groups, however these produced the undesired β-anomer as the major product. A breakthrough was found with changing by the donor's leaving group to the trichloroacetimidate, which allowed for a higher reaction temperature, which typically favours the α-product. Et_2O was added as a co-solvent and using this protocol the desired stereochemistry was installed with excellent yield (87%). The remaining synthetic route was performed using optimised reaction protocols described in chapter 3 with minimal difficulties.

5. Target engagement probes via proximity labelling

This chapter describes the design and synthesis of target engagement probes based on the IdoA(2S)-GlcNS(6Sulfamate) motif and a potent, but not endosulfatase selective, biphenylsulfamate reported by Reuillon et al.[86]

5.1 Introduction

Proving small molecule target engagement in cells is essential for attributing any induced pharmacological effects with protein-small molecule interaction as opposed to other 'off-target' mechanisms. Small molecule-protein binding can be measured easily using purified protein, and while these are essential data to obtain, it does not always translate to binding within a living system. Target engagement aims to measure if, and to what extent, a compound interacts with the target protein in cells. There are many emerging methodologies to probe target engagement, however many of these methods involve the modification of the target protein, ligand or both.[125] Proximity based methods requiring a chemical modification only of the ligand of interest can be used on non-engineered disease systems. These methods include affinity-based chemical proteomics and ligand-directed protein labelling.[126]

5.2 Affinity-based chemical proteomics

The design of affinity-based chemical probes typically begins with attaching (i) a reactive group to the ligand and (ii) a bio-orthogonal ligation coupling group (figure 41). The former is required for covalently bonding the tool compound to the target protein. Photocrosslinkers such as a carbene, nitrene or radical precursors are commonly used as the reactive group that can be activated under UV-irradiation to form the highly reactive species. The positioning of the reactive group is important, as it must be near a suitable

amino acid for bonding when the ligand is bound to the protein. Once cross-linked, this protein-probe complex can be analysed using the reporter group, usually via click chemistry when the reporter is an alkyne, e.g. for pull-down/LC-MS/MS analysis or fluorescence conjugation (figure 42).

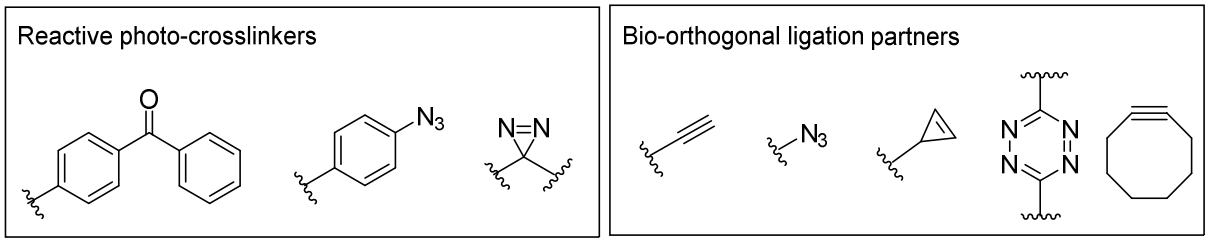


Figure 41: Example functional groups used as photo-crosslinkers and bio-orthogonal ligation coupling partners

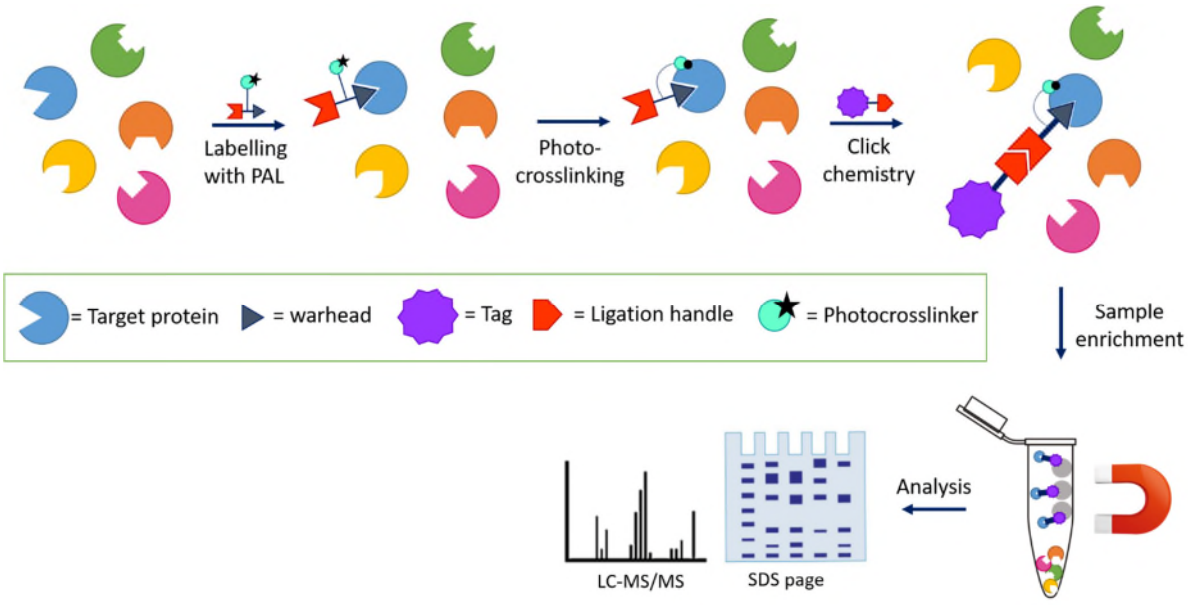


Figure 42: Workflow of small molecule target identification using affinity-based chemical proteomics

Diazirines are three membered rings, comprised of two nitrogen atoms and one carbon atom. Upon UV-irradiation (350 nm), the diazirine is promoted from its singlet ground state to its singlet excited state, and subsequent homolytic cleavage of the σ C-N bond produces molecular nitrogen and a singlet carbene, which is able to insert into neighbouring C-H or X-H bonds. Undesired isomerism of the diazirine to the diazo-compound

contributes to background reactivity as the diazo-isomer has a longer half-life, and is able to react in the dark (figure 43).

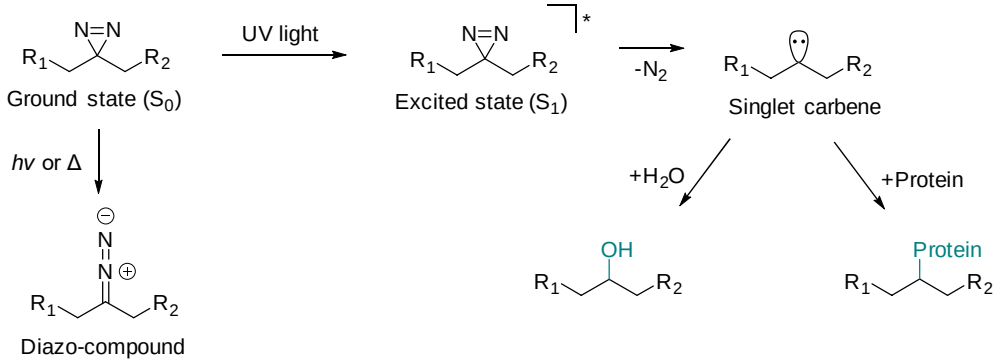
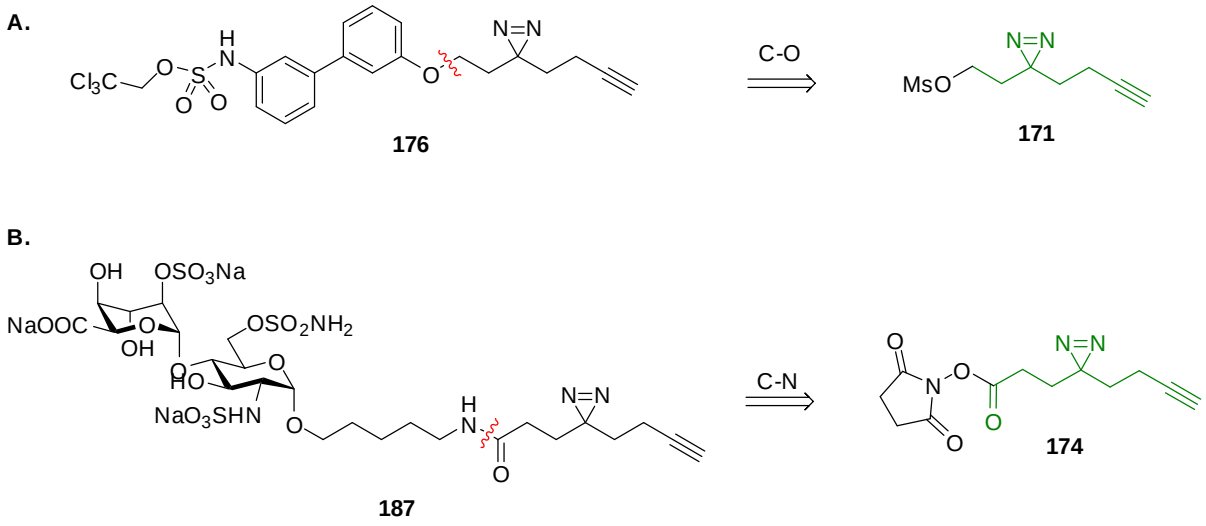


Figure 43: Diazirine activation upon UV irradiation

5.3 Design of photoaffinity labelling (PAL) probes to target endosulfatases

With this in mind, it was hypothesised that proximity labelling probes based on the disaccharide IdoA(2S)-GlcNS(6Sulfamate) could allow for direct determination of endosulfatase engagement in cells, and give an indication of selectivity of the small molecule inhibitors. The design of the two PAL probes to target endosulfatases is shown in scheme 37. The first probe, based on the biphenyl trichloroethylsulfamate Sulf-2 inhibitior **31** (reported by Reuillon et al.), is shown in scheme 37, A, and the second probe was based on the IdoA(2S)-GlcNS(6sulfamate) ligand developed in Chapter 3 of this thesis (scheme 37, B). In order to simplify the synthesis of the PAL probes it was decided to utilise the so-called *minimalist* linker described by Li et al.,[127] which can be easily tethered to the target molecule via amide coupling or an alkylation reaction. The structure of this linker is highlighted in green in scheme 37.

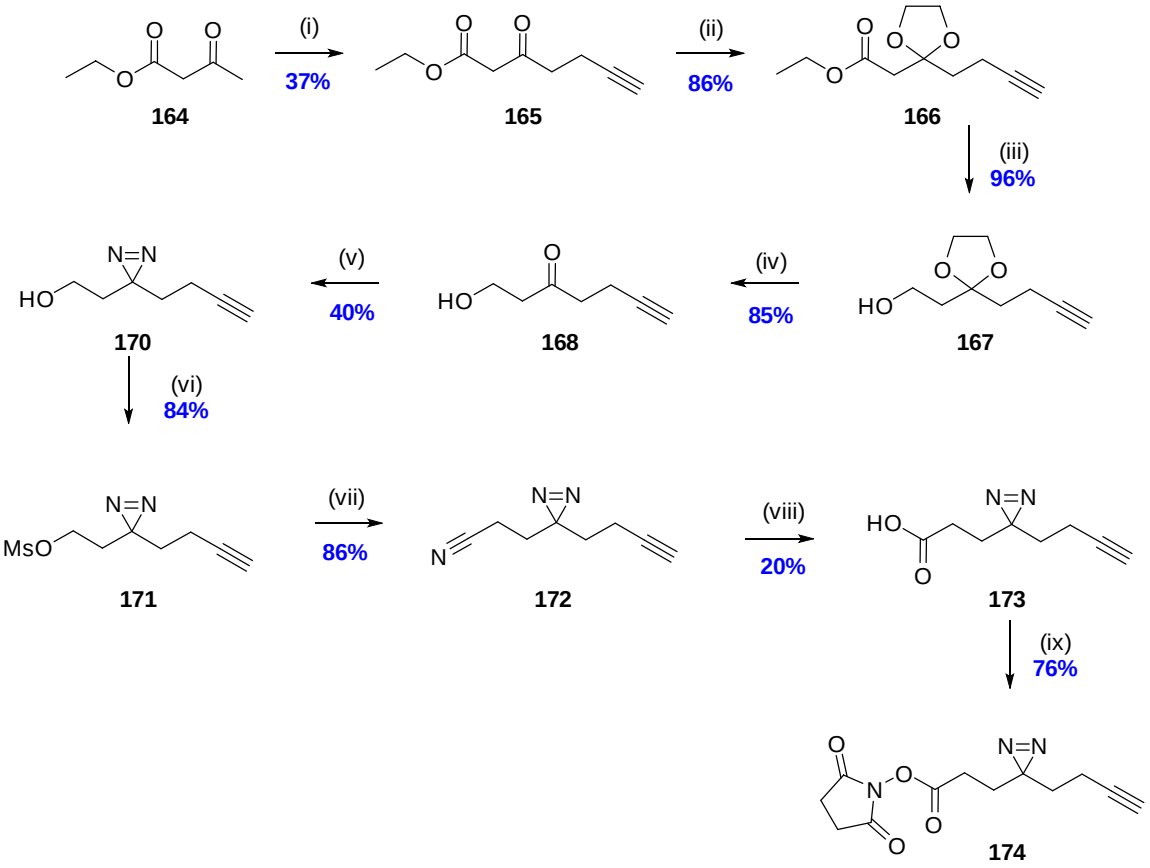


Scheme 37: Retrosynthetic analysis of photoaffinity labelling probes **176** and **187**. Minimalist linker reported by Li et al. coloured in green.[127]

5.4 Synthesis of minimalist linkers 171 and 174

Ethyl acetoacetate **164** was first subjected to alkylation with propargyl bromide via the dianion to give exclusive α-alkylation **165** in 37% yield after purification via distillation using a Kugelrohr apparatus. The β-ketone was protected by forming a cyclic acetal **166** from ethylene diol catalysed by $BF_3 \cdot OEt_2$ in 86% yield. Next, the ester was reduced by treatment with $LiAlH_4$ in excellent yield, and subsequent acid hydrolysis of the acetal gave compound **168** in 82% yield over two steps. To synthesise the 'reactive group', compound **168** was subjected to liquid ammonia then hydroxylamine-*O*-sulfonic acid to obtain the diaziridine intermediate, which was oxidised with iodine to afford diazirine **170** in 40% yield. Subsequently, the primary alcohol was converted into a leaving group by reaction with mesyl choride and this alkylating reagent reacted with potassium cyanide to give nitrile **172** in 72% yield over two steps. Basic hydrolysis of the nitrile to the corresponding carboxylic acid was not as easy as expected. Only 20% yield of the desired acid was obtained after 8 h at reflux in 10% aq. NaOH, however the nitrile starting material could

be recovered and reacted again. Finally, reaction of compound **173** with *N*-hydroxysuccinimide and EDC.HCl gave activated ester **174** in 76% yield.



Scheme 38: Synthesis of minimalist linker **171** and activated ester **174**. Reagents and conditions: (i) LDA, THF, −78 °C, 15 min; then propargyl bromide, −78 °C to rt, 16 h, 37%; (ii) ethylene glycol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C to rt, 16 h, 86%; (iii) $LiAlH_4$, Et_2O, 0 °C, 1 h, 96%; (iv) 6 M HCl, THF, rt, 16 h, 85%; (v) 1. NH_3 (l), −78 °C, 4 h; then H_2NOSO_3H, CH_3OH, −78 °C, 16 h; 2. I_2, Et_3N, CH_2Cl_2, 0 °C, 4 h, 40%; (vi) MsCl, Et_3N, THF, 0 °C, 2 h, 84%; (vii) KCN, DMF, 70 °C, 16 h, 86%; (viii) 10% NaOH, 100 °C, 8 h, 20%;(ix) HOSu, EDC.HCl, MeCN, rt, 16 h, 76%.

5.5 Synthesis of biphenyl trichloroethylsulfamate based PAL, 176

A PAL based on the biphenyl trichloroethylsulfamate Sulf-2 inhibitior **31** reported by Reuillon et al. was designed, and it was proposed that this analogue could act as a comparison for the target selectivity of saccharide and non-saccharide based Sulf1/2 inhibtiors.[86] The authors report the trichloroethylsulfamate group as a novel

pharmacophore for sulfatase inhibition. The most potent Sulf-2 inhibitor reported was **31** (IC_{50} = 167 μM). The phenyl sulfamate group was not required for Sulf-2 selectivity, however, it did improve the potency. When this phenyl sulfamate is removed, as in compound **175** (IC_{50} = 254 μM), the selectivity of inhibition across three sulfatases (Sulf-2, ARSA and ARSB) improves.

Table 14: Sulfatase IC_{50} data for biphenyl trichloroethylsulamate inhibitors reported by Reuillon et al.[86]

Compound	*Sulf-2 IC_{50} (μM)*	*ARSA IC_{50} (μM)*	*ARSB IC_{50} (μM)*
31	167 ± 5	55 ± 11	130 ± 6
175	254 ± 3	No inhibition	30 ± 11

It was therefore decided that for the work in this thesis the minimalist photoaffinity linker could be attached through the phenyl group in substitution of the sulfamate in compound **31**.

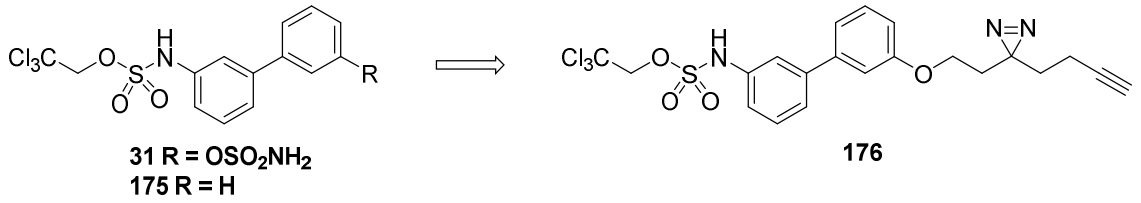
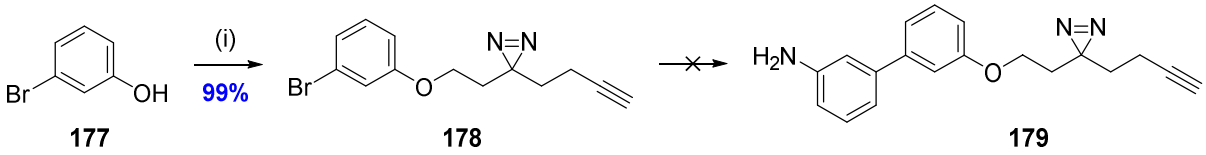


Figure 44: Design of biphenyl trichloroethylsulfamate-based PAL **176** from two biphenyl Sulf-2 inhibitors **31** and **175**

Following the order of steps described by Reuillon et al. to synthesise **176**, linker **171** was reacted with 3-bromophenol **177** and Na_2CO_3 to give compound **178** in 99% yield. Subsequently, compound **178** was subjected to Suzuki-Miyaura reaction conditions with 3-aminophenylboronic acid. However, this did not produce any desired product and in addition, the starting material bromide **178** was not recovered. This was unexpected, as the diazirine functional group is reported to be stable under these reaction conditions.[128] Furthermore, a model reaction of 3-bromoanisole with 3-aminophenylboronic acid under

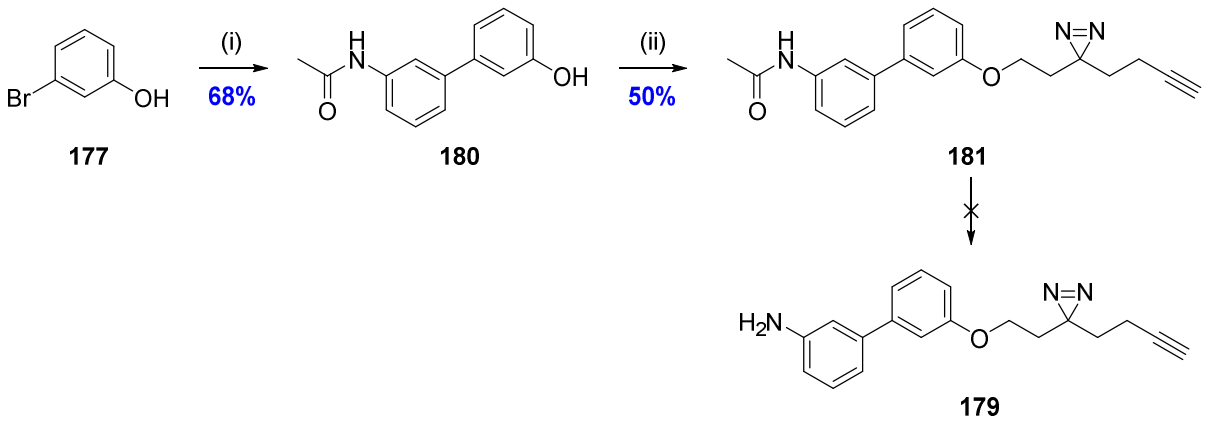
identical conditions but with a stoichiometric additive of 1-octyne, gave full conversion to the corresponding biphenyl indicating that the reaction conditions are tolerant of a terminal alkyne.



177 178 179

Scheme 39: Synthesis of aniline **179**. Reagents and conditions: (i) **171**, Cs_2CO_3, MeCN, 85 °C, 4 h, 99%.

Following the unsuccessful attempt of synthesising intermediate **179** in two steps from 3-bromophenol **177**, it was apparent that the Suzuki-Miyaura reaction needed to be performed prior to the addition of the photoaffinity linker. The revised synthesis of compound **179** started with the Suzuki-Miyaura coupling reaction of 3-bromophenol **177** and a protected-aminophenylboronic acid, as shown in scheme 40. Initially, an acetate group was chosen, as the acetamide functionality was thought to be stable under Suzuki-Miyaura and alkylation reaction conditions.



177 180 181 179

Scheme 40: Attempted synthesis of PAL-biphenyl aniline **179**. Reagents and conditions: (i) 3-acetamidophenylboronic acid, $Pd(dppf)Cl_2$, Na_2CO_3, DME, H_2O, 80 °C, 30 min, 68%; (ii) **171**, Cs_2CO_3, MeCN, 80 °C, 4 h, 50%.

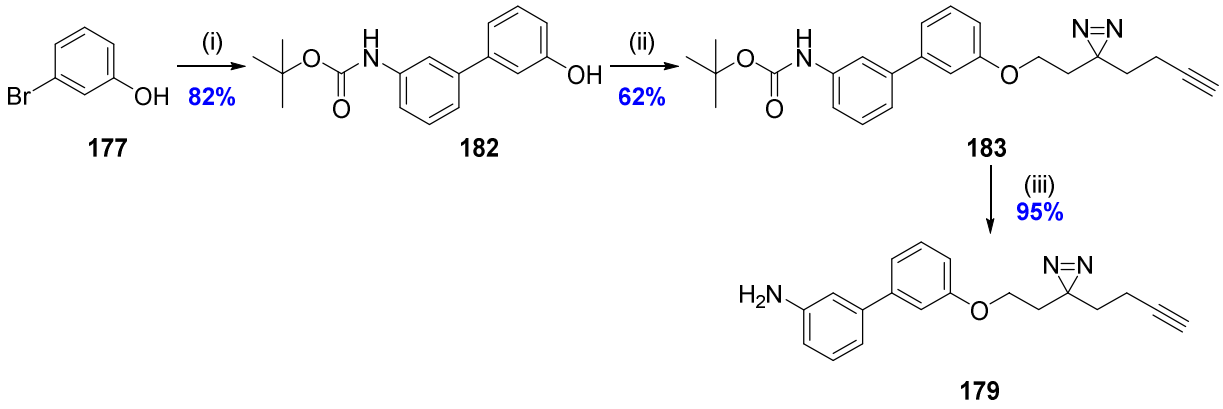
The Suzuki-Miyaura cross-coupling of 3-bromophenol and 3-acetamidophenylboronic acid in the presence of $Pd(dppf)Cl_2$ and sodium carbonate gave **180** in 86% yield. Reacting

the subsequent phenol with minimalist linker **171** under microwave irradiation in acetonitrile at 80 °C produced compound **181** in 50% yield. Cleavage of the *N*-acetate was attempted using sodium hydroxide in H_2O/THF at ambient temperature for 16 h, however no product was observed by ^{1}H NMR spectroscopy. Alternative conditions were investigated, including LiOH/H_2O_2 and ONOO- (peroxynitrite), however no conditions yielded the desired aniline.

Table 15: Summary of conditions trialled for the deprotection of the N-acetate on compound **181**.

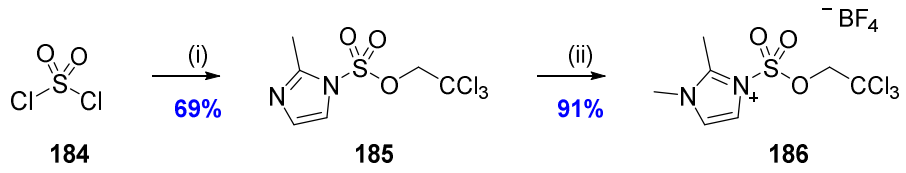
Entry	*Conditions*	*Reaction outcome*
1	NaOH in H_2O/THF, reflux	No deprotection monitored by ^{1}H NMR
2	LiOH, H_2O_2, THF, rt	No deprotection monitored by ^{1}H NMR
3	$NaNO_2$, H_2O_2, NaOH, HCl to generate $ONOO^-$, THF, rt	No deprotection monitored by ^{1}H NMR

Following the difficulting in cleaving the *N*-acetate protecting group, it was decided to change to a *N*-Boc protecting group from the beginning of the synthetic route (scheme 41). The revised synthesis of **179** began with a Suzuki-Miyaura cross-coupling of 3-bromophenol and 3-(*N*-Boc-amino)phenylboronic acid in the presence of Pd(dppf)Cl_2 and Na_2CO_3, which gave intermediate **182** in 82% yield. The subsequent alkylation of phenol **182** with minimalist linker **171** gave *N*-Boc **183** in 62% yield. The *N*-Boc deprotection proceeded effectively in the presence of TFA to afford corresponding aniline **179** in 95% yield, after basification.



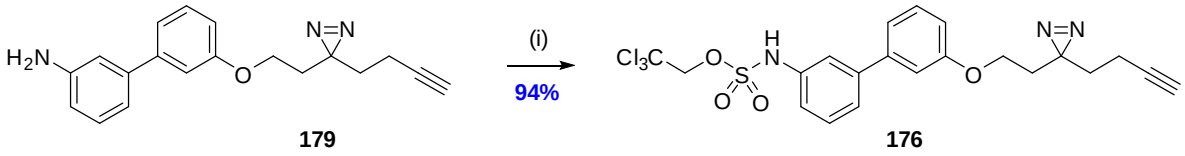
Scheme 41: Synthesis of PAL-biphenyl aniline **179**. Reagents and conditions: (i) 3-(N-Boc-amino)phenylboronic acid, Pd(dppf)Cl_2, Na_2CO_3, DME, H_2O, 80 °C, 30 min, 82%; (ii) **171**, Cs_2CO_3, MeCN, 80 °C, 4 h, 62%; (iii) TFA, CH_2Cl_2, rt, 6 h, 95%, (82% pure by HPLC).

Next, 2,2,2-trichloroethyl (TCE) chlorosulfate was prepared, and used without isolation, by reaction of sulfuryl chloride with one equivalent of 2,2,2-trichloroethanol and pyridine (scheme 42). The pyridinium chloride was removed via vacuum filtration. The crude TCE chlorosulfate was reacted directly with 2-methylimidazole to obtain 2-methylimidazole-1-sulfonate **185** in 69% yield. Finally, 2-methylimidazole-1-sulfonate **185** was subjected to methylation with triethyloxonium tetrafluoroborate (also known as Meerwein's salt), to afford **186** in 91% yield.



Scheme 42: Synthesis of sulfuryl imidazolium salt **186**. Reagents and conditions: (i) 2,2,2-trichloroethanol, pyridine, CH_2Cl_2, 0 °C; then, 2-methylimidazole, rt, 16 h, 69%; (ii) triethyloxonium tetrafluoroborate, CH_2Cl_2, 0 °C to rt, 16 h, 91%.

Finally, intermediate **179** was subjected to trichloroethylsulfamate formation using **186**, to give biphenyl trichloroethylsulfamate-based PAL **176** in 94% yield.

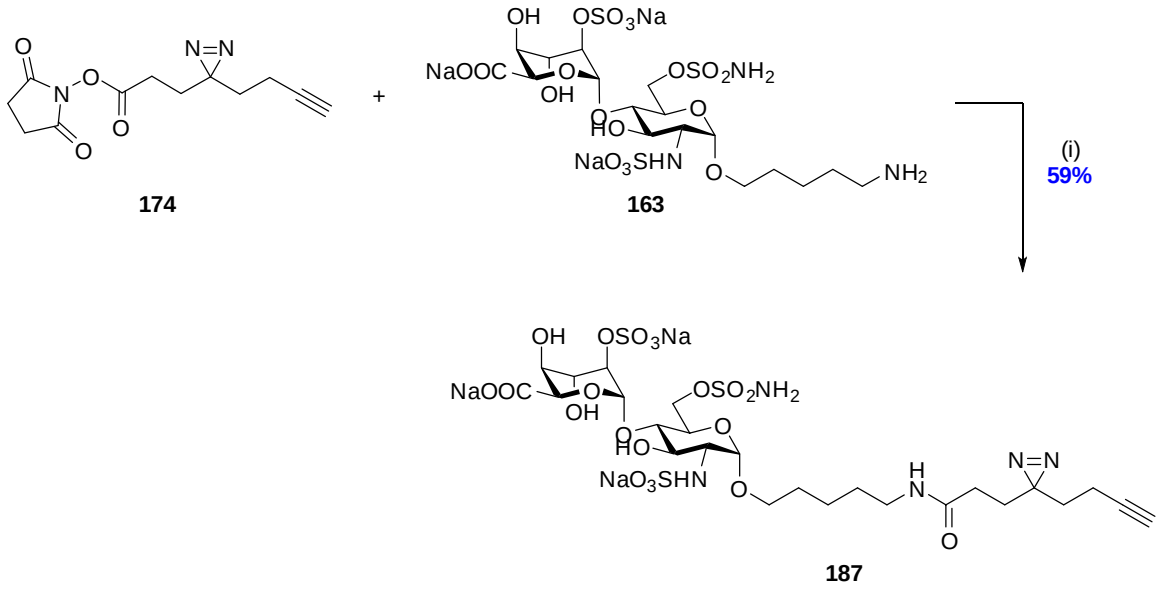


179 **176**

Scheme 43: Synthesis of biphenyl trichloroethylsulfamate-based PAL. Reagents and conditions: (i) **184**, THF, 67 °C, 30 h, 94%.

5.6 Synthesis of IdoA2S-GlcNS6S based PAL, 187

Photoaffinity labelling probe **187** was prepared by reacting minimalist linker activated NHS-ester with IdoA(2S)-GlcNS(6Sulfamate)-functionalised amine **163** in the presence of *N*-methylmorpholine in DMF at ambient temperature for 16 h, as shown in scheme 44. Purification via reversed phase chromatography using water as the eluent, followed by elution from a Dowex® 50WX8 Na^+-form column using water as the eluent to give the tri-sodium salt **187** in 59% yield.



174 **163** **187**

Scheme 44: Synthesis of IdoA2S-GlcNS6S-based PAL **187**. Reagent and conditions: (i) N-methylmorpholine, DMF, rt, 16 h, 59%.

In summary, two 'classical' photoaffinity labelling probes have been synthesised using a bifunctional linker containing a diazirine and alkyne.

5.7 Catalytic activation of diazirines

One of the main practical limitations of diazirine-containing probes is that the carbene generated upon UV irradiation rapidly reacts with water, which leads to an estimated 99% formation of the hydrated product rather than reacting with a protein. In addition, these 'quenched' probes compete for binding with unreacted diazirine-probes, reducing labelling efficiency further. These probes often suffer from low cross-linking yields and high background noise due to low receptor and protein abundance in combination with short carbene half-lives.

Geri et al. have reported a novel proximity labelling technique that utilises a bioactive small molecule conjugated to an iridium photocatalyst, for activation of diazirine molecules via Dexter energy transfer.[129] During this event, an iridium photocatalyst absorbs blue light to become excited into its triplet-excited state, and then transfers the energy from the incident light to a nearby ground state diazirine molecule. This results in the formation of an excited triplet state of the diazirine, and the photocatalyst returns to its ground state. The diazirine triplet excited state then releases nitrogen to give a triplet carbene and spin equilibration of this carbene to the singlet, which then then either cross-links with a nearby protein or is quenched by the aqueous environment (as shown in figure 45). Crucial to the success of this method is the fact that aryl diazirines have a triplet energy ($\approx$ 60 kcal mol^{-1})[130] that corresponds to the upper limit of Ir photoredox catalysts.

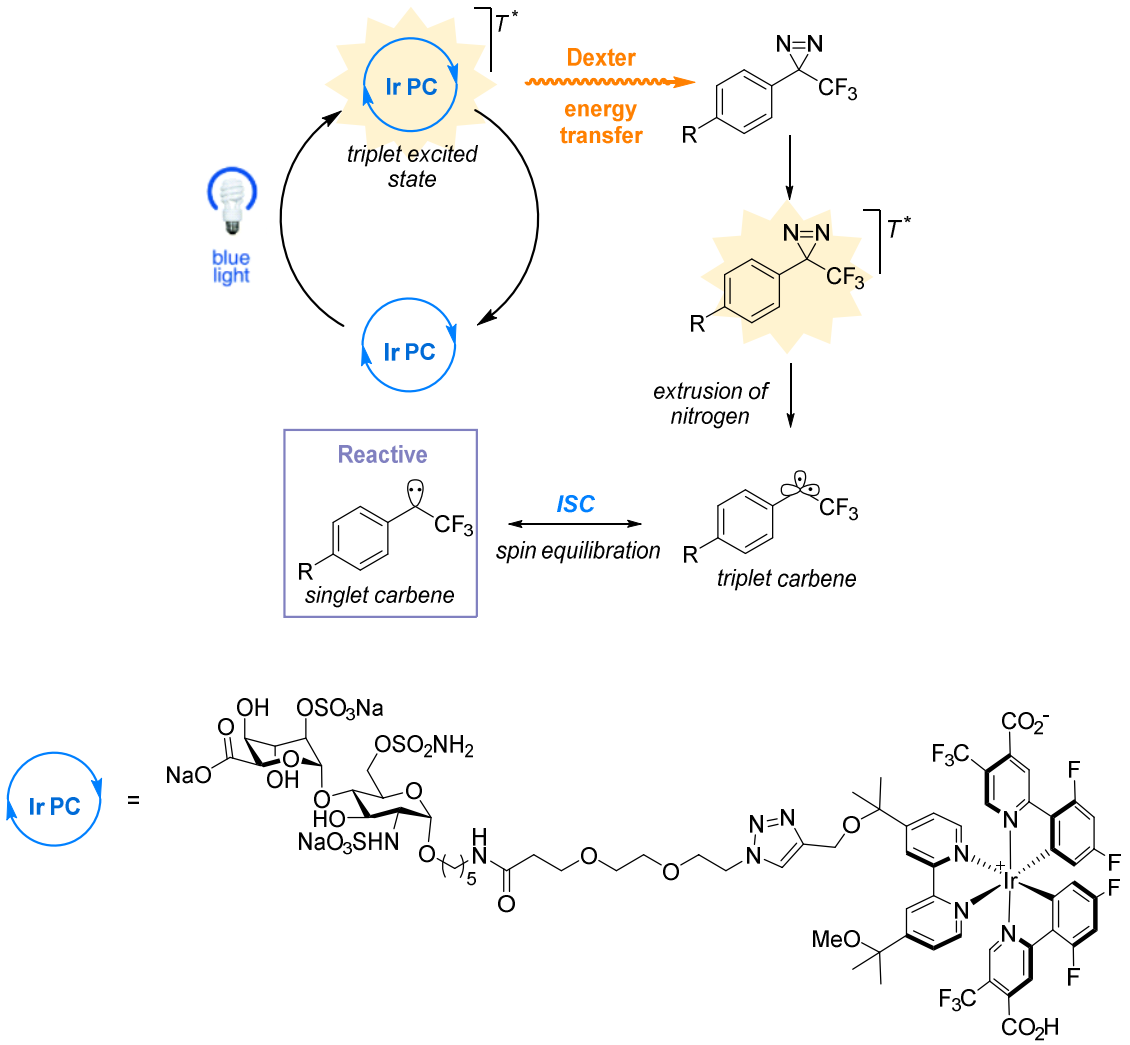


Figure 45: Events leading to generation of a reactive singlet carbene initiated by irradiation of an Ir-photocatalyst (Ir PC) with blue light.

This technique results in a 4-5 nm labelling radius from the Ir photocatalyst due to the distance over which energy is transferred (limiting the diazirine activation radius to 1 nm). Then the carbene can only react with a protein in a further 3 nm radius (limited by the rate of diffusion) due to its the half-life in aqueous solution ($t_{1/2} < 2$ ns). A benefit of this method is the possibility of multiple labelling events occuring from one Ir-conjugated biomolecule if an excess of diazirine molecules is used. Furthermore, the blue light does not directly activate the diazirine, which limits the hydrolysis quenching of carbenes on unbound molecules, thus improving the sensitivity of the analysis (figure 46). However, due to the

'labelling radius', some proteins in the microenvironment of the target protein may also be labelled (i.e. not the protein that the small molecule ligand is directly interacting with), resulting in a more complex data analysis and the requirement of additional controls.

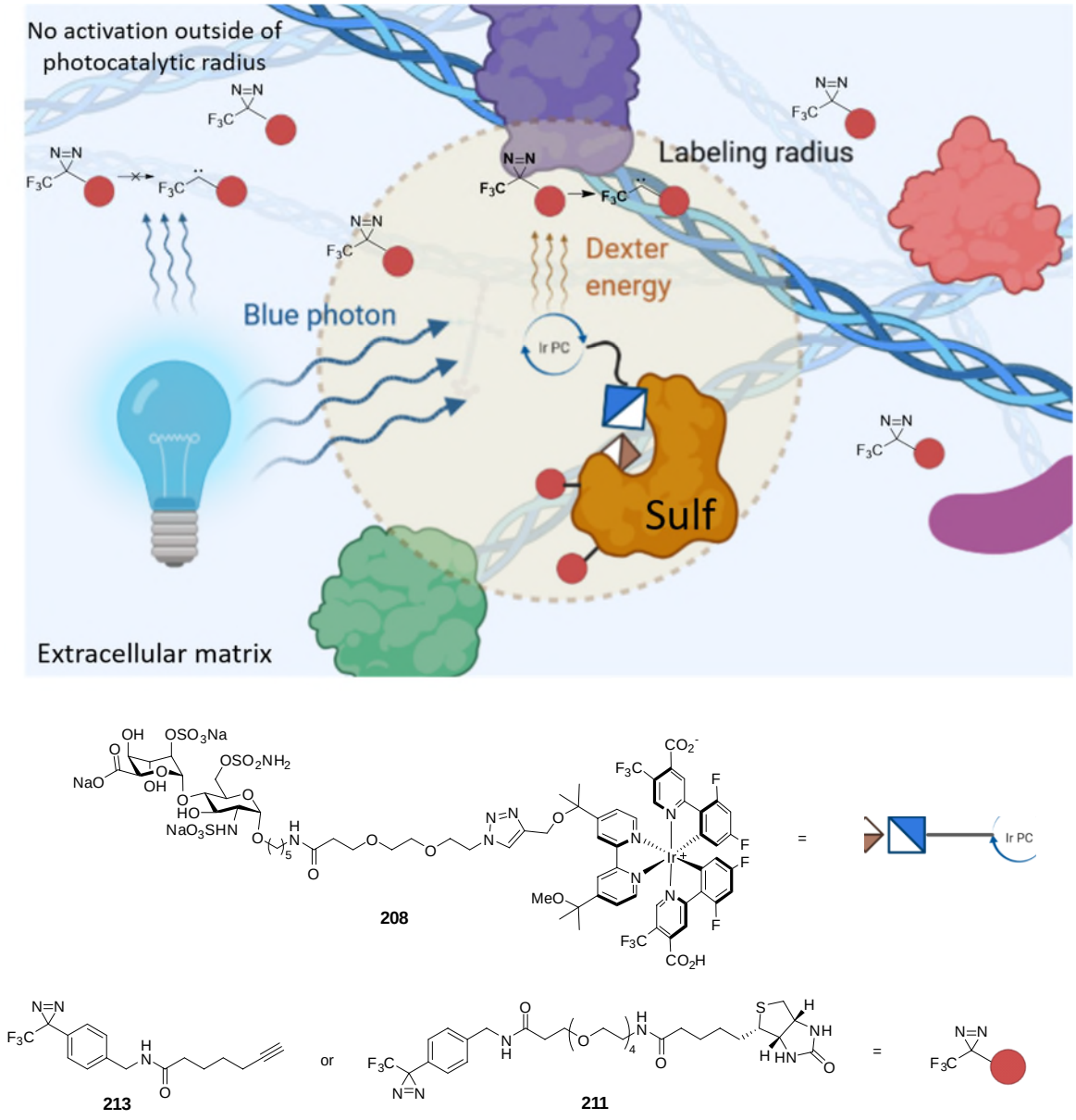


Figure 46: A. When blue light is used to excite the Ir photocatalyst-conjugated disaccharide, it transfers energy to the surrounding microenvironment (labelling radius) and any diazirine-containing molecules in that microenvironment are activated into a carbene that are able to react with a protein (or H_2O). Importantly, the blue light does not activate any diazirine-contianing molecules outside the labelling radius. Figure prepared using Biorender.com

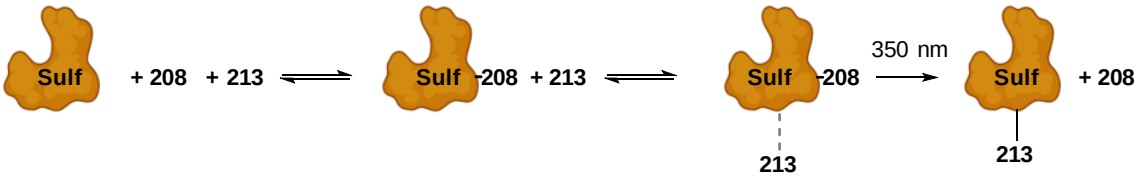


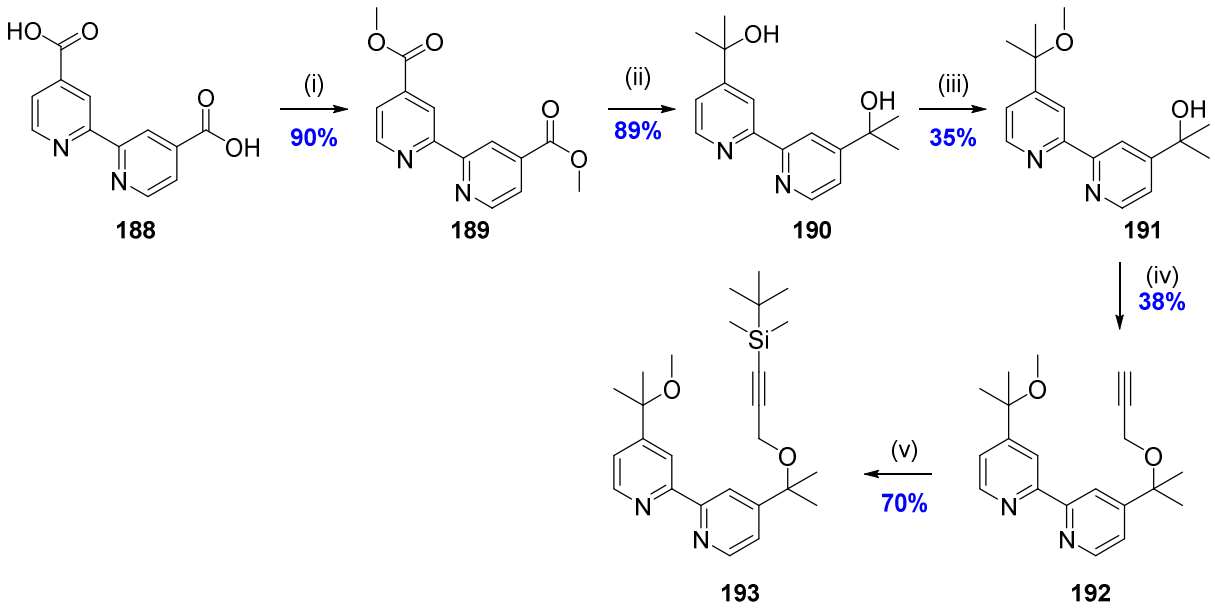
Figure 47: Equilibrium showing Ir-conjugated disaccharide ligand **208,** endosulfatase and diazirine-contianing molecule **213**. Note, **213** does not need to complex with Sulf however once activated the carbene must diffuse (maximum 3 nm) and react with Sulf for labelling to occur.

5.8 Synthesis of cell non-permeable Ir photocatalyst

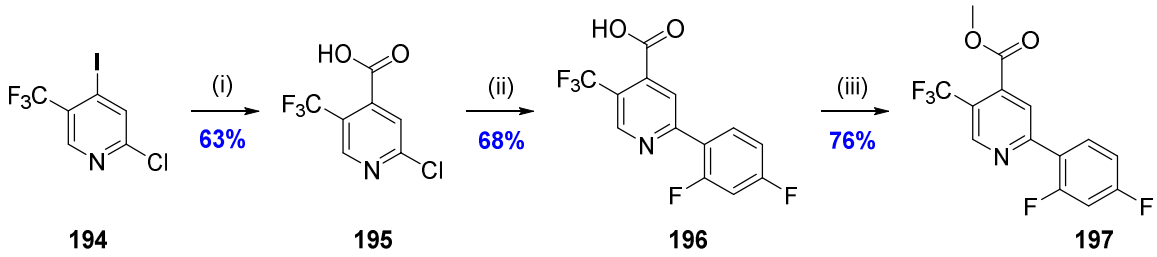
The bipyridine ligand **193** was synthesised in five steps from 2,2′-bipyridine-4,4′-dicarboxylic acid following the synthetic route outlined in scheme 45. The synthesis began with an acid catalysed esterification of **188** in methanol, which proceeded very effectively to give dimethyl [2,2'-bipyridine]-4,4'-dicarboxylate **189** in 90% yield. The resulting diester was reacted with an excess of methyl magnesium bromide (3M in THF, 6 equivalents) at −78 °C to produce dialcohol **190** in 89% yield. Subsequent monoalkylation of compound **190** was achieved using NaH in THF to form the monoalkoxide anion, followed by addition of methyl iodide at 80 °C for 16 h. Using the procedure described by Geri et al., the conversion for this reaction was low, resulting in only 35% isolated yield of the mono-methyl ether **191**, however the unreacted bisalcohol **190** was easily recovered. The second alkylation to install the terminal alkyne by reaction with propargyl bromide, proceeded in similar fashion to give bis-ether **192** in 38% yield. Finally, *tert*-butyl dimethyl silyl protection of the terminal alkyne was achieved by reacting compound **192** with *n*-BuLi (1.6 M in hexanes) in THF at −78 °C to form the carbanion, then reaction with *tert*-butyldimethylsilyl trifluoromethanesulfonate to give ligand **193** in 70% yield.

Synthesis of the substituted 2-phenylpyridine ligand **197** began with a lithium-halogen exchange reaction of 2-chloro-4-iodo-5-(trifluoromethyl)pyridine **194** with *n*-BuLi at −78 °C in THF, followed by quenching of the lithium salt by pouring the reaction mixture over

freshly crushed solid CO_2. This reaction proceeded efficiently to give carboxylic acid **195** in 86% yield. Subsequent Suzuki-Miyaura cross-coupling of compound **195** with (2,4-difluorophenyl)boronic acid in the presence of Pd(dppf)Cl_2 and Cs_2CO_3 gave **196** in 68% yield. Due to the zwitterionic nature of compound **196** the work-up procedure was optimised to exclude an aqueous work-up because it was found to be water soluble at all pH values, which lead to dramatically reduced yields. Simply concentrating the crude reaction mixture directly onto Celite® for dry-loading for column chromatography was optimal for maximising yield. Finally, compound **196** was subjected to oxalyl chloride in the presence of catalytic DMF, and the resulting acid chloride immediately quenched with an excess of methanol to give methyl ester **197** in 76% yield.



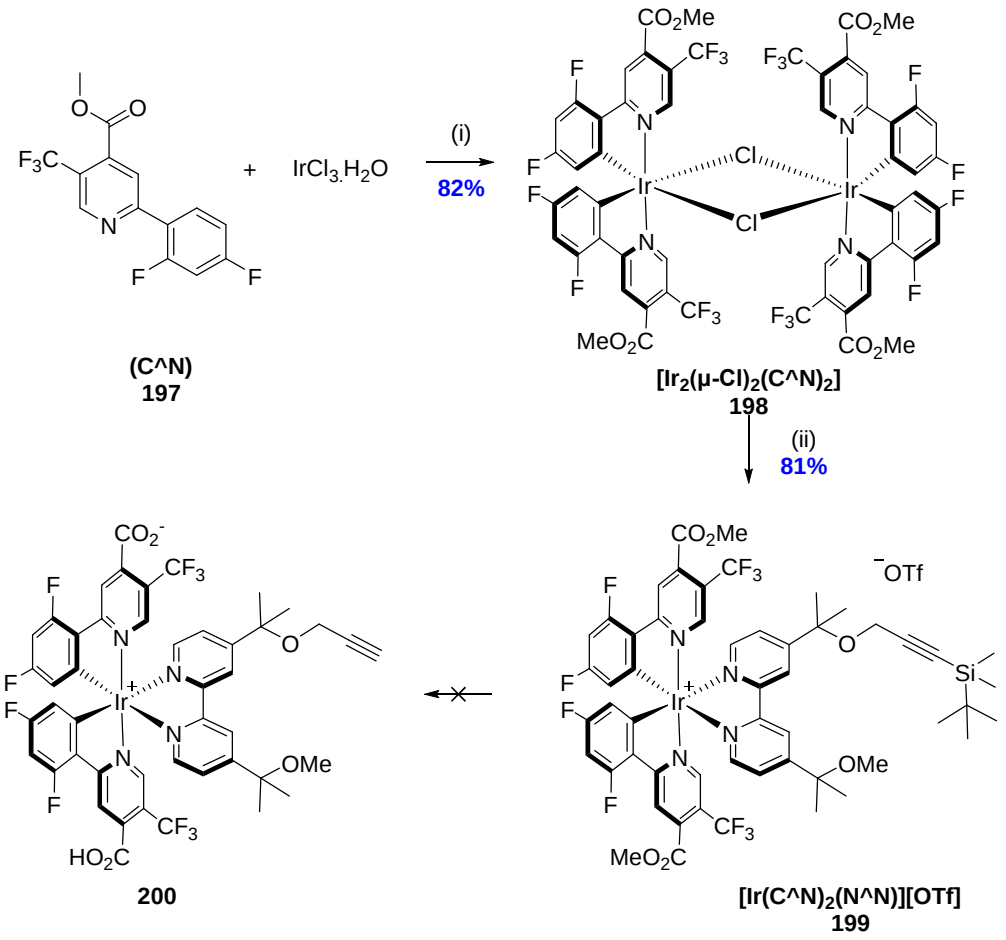
Scheme 45: Synthesis of bipyridine ligand **193**. Reagents and conditions: (i) H_2SO_4, CH_3OH, 80 °C, 16 h, 90%; (ii) MeMgBr, THF, −78 °C to rt, 2.5 h, 89%; (iii) NaH, THF, 0 °C, 1 h, then, MeI, 80 °C, 16 h, 35%; (iv) NaH, DMF, 0 °C, 1 h, then, propargyl bromide, 80 °C, 16 h, 38%; (v) n-BuLi, THF, −78 °C, 15 min; then, TBSOTf, rt, 1 h, 70%.



194 195 196 197

Scheme 46: Synthesis of 2-phenylpyridine derived ligand **197**. Reagents and conditions: (i) n-BuLi, THF, −78 °C; then, CO_2(s), rt, 30 min, 63%; (ii) (2,4-difluorophenyl)boronic acid, $Pd(dppf)Cl_2.CH_2Cl_2$, Cs_2CO_3, 1,4-dioxane, H_2O, 100 °C, 4 h, 68%; (iii) oxalyl chloride, cat. DMF, CH_2Cl_2, rt, 1 h; then, CH_3OH, rt, 1 h; 76%.

With both ligands in hand the Ir photocatalyst synthesis was attempted. Dimer **198** was synthesised by heating $IrCl_3 \cdot H_2O$ and ligand **197** in a thoroughly degassed mixture of H_2O and 2-ethoxyethanol at 135 °C for 16 h. The methyl-ester **197** was used rather than acid **196**, because it was predicted that a tetracarboxylate would exhibit high aqueous solubility, therefore rendering the usual protocol of precipitation into H_2O impractical. Indeed, when $IrCl_3 \cdot H_2O$ and ligand **197** were reacted under these conditions, the desired dimer **198** was isolated in 82% yield, as shown in scheme 47. Using a modified procedure described by Sauvageot et al., ligand **193** was reacted with $[Ir_2(\mu\text{-}Cl)_2(C\text{^}N)_2]$ dimer **198** in CH_3OH/CH_2Cl_2 at 75 °C for 16 h, followed by ion exchange by reaction with an excess of AgOTf gave $[Ir(C\text{^}N)_2(N\text{^}N)][OTf]$ complex **199** in 81% yield.[131] $[Ir_2(\mu\text{-}Cl)_2(C\text{^}N)_2]$ **198** and $[Ir(C\text{^}N)_2(N\text{^}N)][OTf]$ **199** are novel racemic iridium complexes and their structures were assessed by comparison to NMR spectroscopic data of published iridium complexes, which have been further analysed by X-ray crystallography.

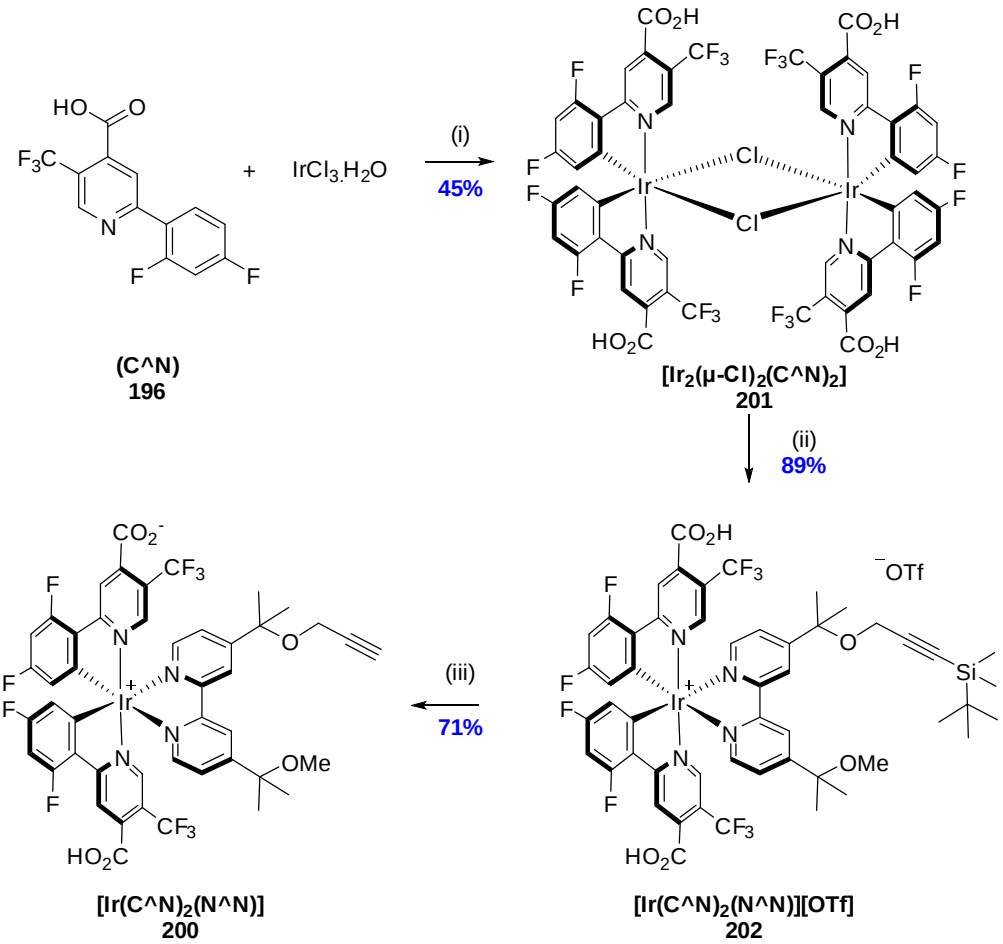


Scheme 47: Attempted synthesis of **200**. Reagents and conditions: (i) H_2O, 2-ethoxyethanol, 135 °C, 16 h, 82%; (ii) **193**, CH_3OH, CH_2Cl_2, 75 °C, 16 h; then, AgOTf, rt, 30 min, 81%.

Finally, silyl deprotection and methyl ester hydrolysis was attempted by subjecting iridium complex **199** to TBAF in THF and then, after aqueous work-up, the crude alkyne containing complex was immediately subjected to ester hydrolysis conditions using 1M LiOH at ambient temperature. This reaction immediately led to decomposition on addition of the base, as observed by rapid discolouration of the mixture and confirmed by ^{1}H spectroscopy. This was unexpected. Fan et al. have reported the successful ligand ester hydrolysis of [Ir(ppy-COOMe)$_3$] complexes (ppy-COOMe = methyl 3-(pyridin-2-yl)benzoate)[132] and also Huang et al. have also reported the hydrolysis of [Ir(C^N)$_2$(N^N)][Cl] complexes[133], indicating that complex **199** should be stable with respect to basic hydrolysis conditions. It was hypothesised that LiOH was too reactive due

to the Lewis acidity of the Li^+ cation in water, and therefore two alternative bases were investigated (NaOH and $Ba(OH)_2$) however both caused decomposition.

This result necessitated an alternative approach to the synthesis of [Ir(C^N)2(N^N)] complex **200**. It was decided to re-evaluate the isolation protocol for the $[Ir_2(\mu\text{-}Cl)_2(C\text{^}N)_2]$ dimer when four carboxylate groups were present on the molecule. Ligand **196** was reacted with $IrCl_3 \cdot H_2O$ in H_2O and 2-ethoxyethanol at 135 °C for 16 h, and when the isolation procedure reported by Geri was attempted, as expected, no desired product precipitated from the reaction mixture.[129] Instead the reaction mixture was acidified by addition of aq. 1M HCl to pH ≈ 3 and extracted with EtOAc. The presence of a large quantity of 2-ethoxyethanol made the work-up slightly more challenging, but it could be fully removed by recrystalling the $[Ir_2(\mu\text{-}Cl)_2(C\text{^}N)_2]$ dimer from boiling EtOAc, which gave **201** in 45% yield (considerably lower than without the water-solubilising tetracarboxylate functionality).



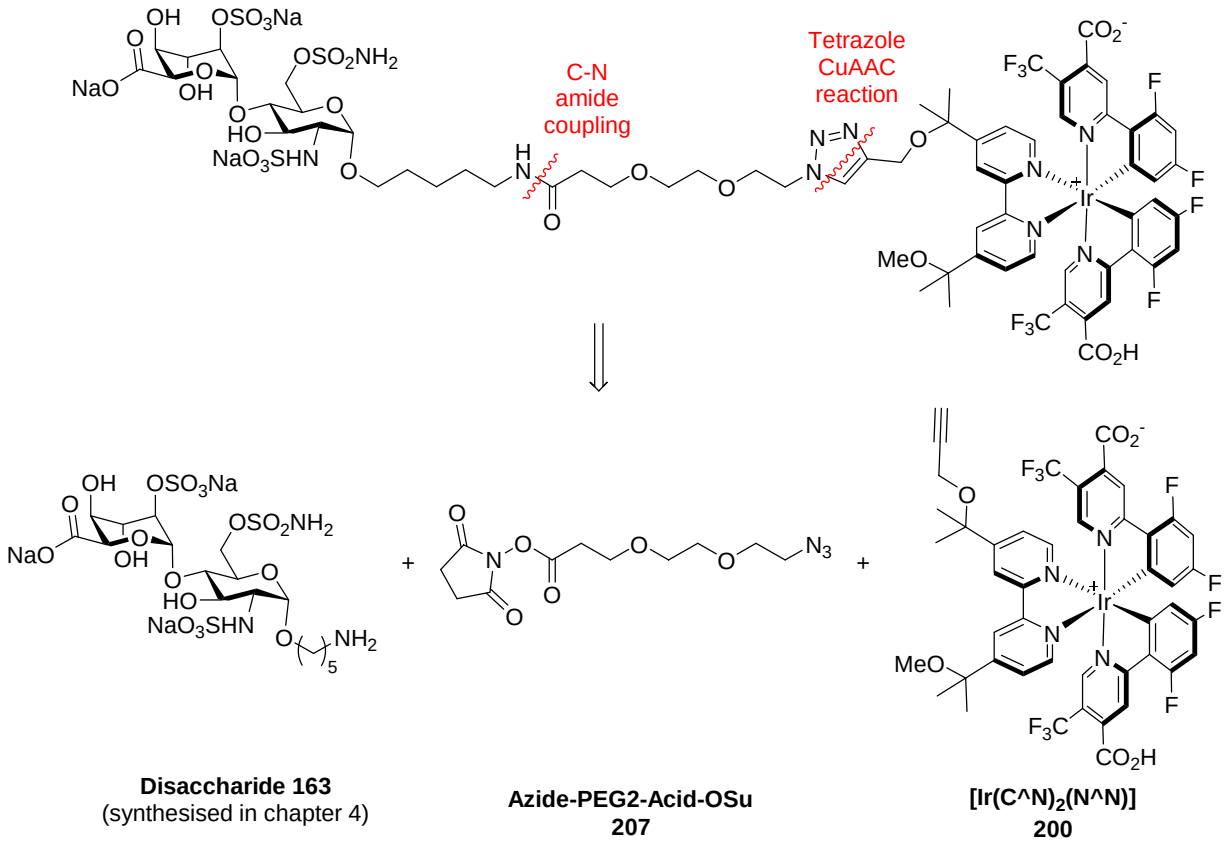
Scheme 48: Synthesis of [Ir(C^N)2(N^N)] complex **200**. Reagents and conditions: (i) H_2O, 2-ethoxyethanol, 135 °C, 16 h, 45%; (ii) **193**, CH_3OH, CH_2Cl_2, 75 °C, 16 h; then, AgOTf, rt, 30 min, 89%; (iii) TBAF (1M in THF), MeCN, rt, 16 h, 71%.

Subsequent reaction of ligand **193** with $[Ir_2(\mu\text{-}Cl)_2(C^N)_2]$ dimer **201**, under the conditions previously optimised, proceeded very effectively to give $[Ir(C^N)_2(N^N)][OTf]$ complex **202** in 89% yield. Finally, silyl deprotection using TBAF in THF at ambient temperature gave **200** in 71% yield. This concluded the synthesis of the alkyne-functionalised Ir photocatalyst, ready for CuAAC reaction with an azide-functionalised biomolecule.

5.9 Synthesis of IdoA(2S)-GlcNS(6Sulfamate)-Ir probe

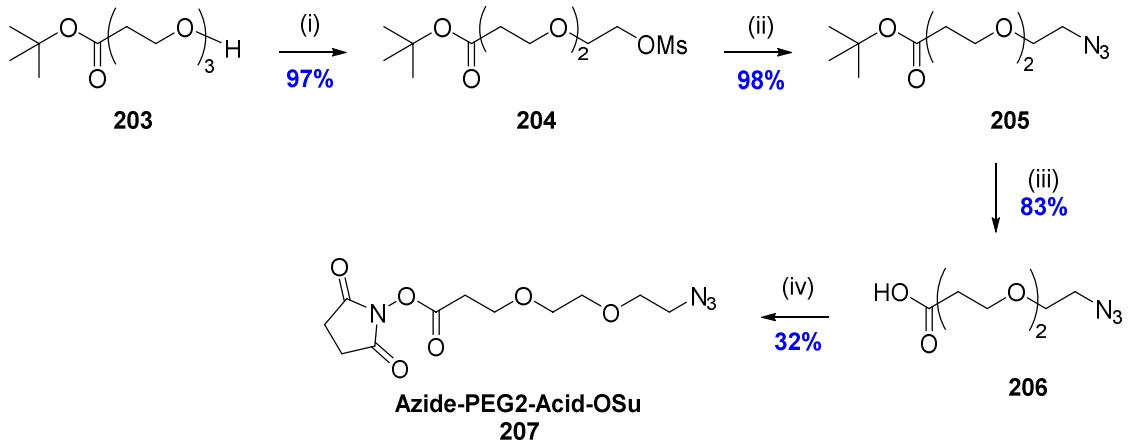
The disaccharide IdoA(2S)-GlcNS(6Sulfamate) unit needed to be functionalised with an azide to allow for its attachment to $[Ir(C^N)_2(N^N)]$ complex **200** via an azide-alkyne Huisgen cycloaddition reaction (CuAAC = Copper(I)-catalyzed Azide-Alkyne

Cycloaddition). It was therefore decided to append a PEG-3-azide onto the disaccharide through an amide coupling reaction, as outlined in scheme 50.



Scheme 49: Retrosynthetic analysis of IdoA(2S)-GlcNS(6Sulfamate)-Ir probe

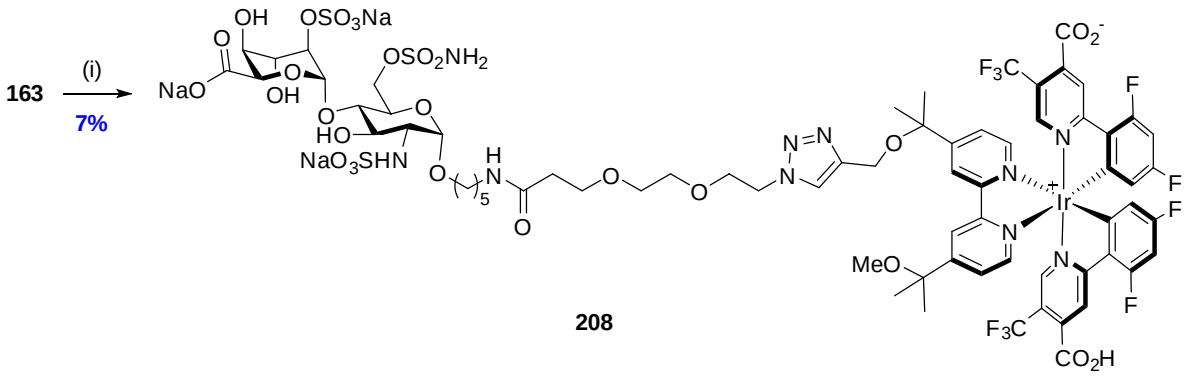
Azide-PEG2-Acid-OSu **207** was synthesised in four steps from hydroxy-PEG2-*t*-butyl ester as outlined in scheme 50. *tert*-Butyl 3-(2-(2-hydroxyethoxy)ethoxy)propanoate **203** was first subjected to mesylation conditions using mesyl chloride in the presence of Et_3N to convert the primary alcohol into a leaving group, which was then reacted with sodium azide in an S_N2 reaction to afford **205** in 95% yield over two steps. The *tert*-butyl ester was hydrolysed using TFA and finally the carboxylic acid was converted into an activated ester by reaction with *N*-hydroxysuccinimide in the presence of EDC.HCl, to give Azide-PEG2-Acid-OSu **207** in 32% yield.



Scheme 50: Synthesis of Azide-PEG2-Acid-OSu **207**. Reagents and conditions: (i) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 97%; (ii) NaN_3, DMA, 80 °C, 4 h, 98%; (iii) TFA, CH_2Cl_2, rt, 2 h, 83%; (iv) N-hydroxysuccinimide, EDC.HCl, CH_2Cl_2, rt, 16 h, 32%.

The final steps in the synthesis of IdoA(2S)-GlcNS(6Sulfamate)-Ir probe **208** were to combine the three building blocks, which required one amide coupling reaction and one CuAAC reaction. The amide coupling reaction of the disaccharide primary amine **163** and Azide-PEG2-Acid-OSu **207** was achieved in the presence of *N*-methylmorpholine in DMF at ambient temperature. Finally, the azide group on the resulting intermediate was reacted with the alkyne group on Ir-photocatalyst **200** in a copper(I) catalysed azide-alkyne cycloaddition reaction, using $CuSO_4$ as the catalyst and ascorbic acid as a reducing agent, which proceeded to afford **208** in 7% yield over two steps. (**208** was isolated as a 1:1 mixture of diastereomers)[ix]

[ix] 1:1 ratio of diastereomers analysed by analytical HPLC



Scheme 51: Synthesis of IdoA(2S)-GlcNS(6Sulfamate)-Ir probe. Reagents and conditions: (i) 1. **207**, N-methylmorpholine, DMF, rt, 16 h; 2. **200**, DIPEA, $CuSO_4$, sodium ascorbate, H_2O, MeCN, rt, 16 h; 7%.

5.10 Characterisation of 208

The in-cell target engagement experiments also required the an intermolecular diazirine group for protein labelling. It was necessary to investigate the activation of a diazirine via Dexter energy transfer from the excited triplet state of **208**. To confirm that the spectral properties of the Ir-photocatalyst had not been altered dramatically by conjugation of the IdoA(2S)-GlcNS(6sulfamate) inhibitor, the UV-vis absorbance spectrum of compound **208** (0.1 μM in H_2O) was obtained (figure 48). Crucially, compound **208** absorbs at 440 nm, which was required for the experimental set-up using a Kessil 440 nm lamp (PR160L-440).

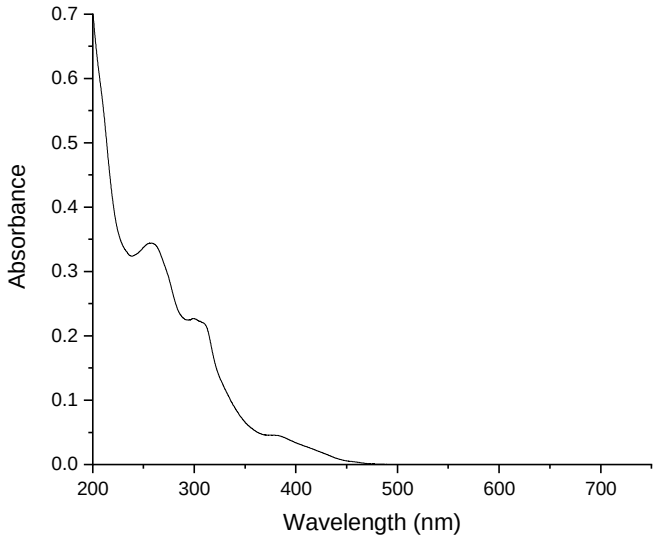
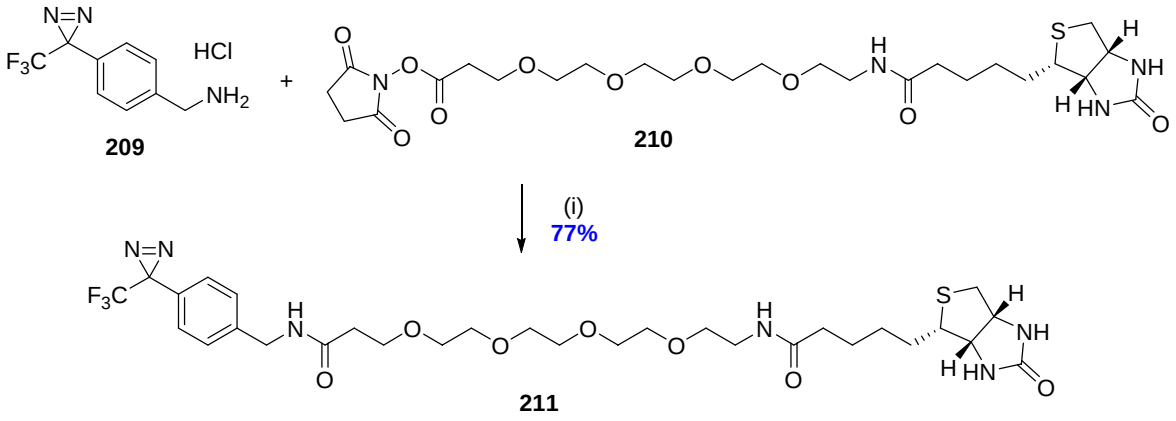


Figure 48: UV-vis absorbance spectrum of compound **208** (0.1 μM in H_2O)

Next, attention turned towards preparation of the diazirine containing labelling molecule to use in the target engagement study. In the original publication by Geri et al. a biotin conjugated diazirine **211** was used (scheme 52). Compound **211** was synthesised in 77% yield via an amide coupling reaction of the commercially available 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzenemethanamine hydrochloride **209** and Biotin-PEG4-NHS ester **210**.



Scheme 52: Synthesis of biotin conjugated diazirine-molecule **211**. Reagents and conditions: (i) DIPEA, CH_2Cl_2, rt, 16 h, 77%.

In order to confirm that upon photoirradiation with 440 nm light, the Ir-photocatalyst is able to transfer energy and activate the diazirine, catalytic amount of IdoA(2S)-GlcNS(6Sulfamate)-Ir PC catalyst **208** (0.1 mg) was added to diazirine-PEG4-biotin **211** in a quartz NMR tube and the reaction was irradiated with 440 nm light for 20 min.[x] The results are shown in figure 49. An unexpected fragmentation of the biotin unit was observed to occur before the full activation of the diazirine, which was observed which was most likely due to the high reaction concentration.

[x] No change to the ^{1}H NMR spectrum of **209** was observed with photoirradiation (440 nm) without photocatalyst **206**. Spectra given in the appendix

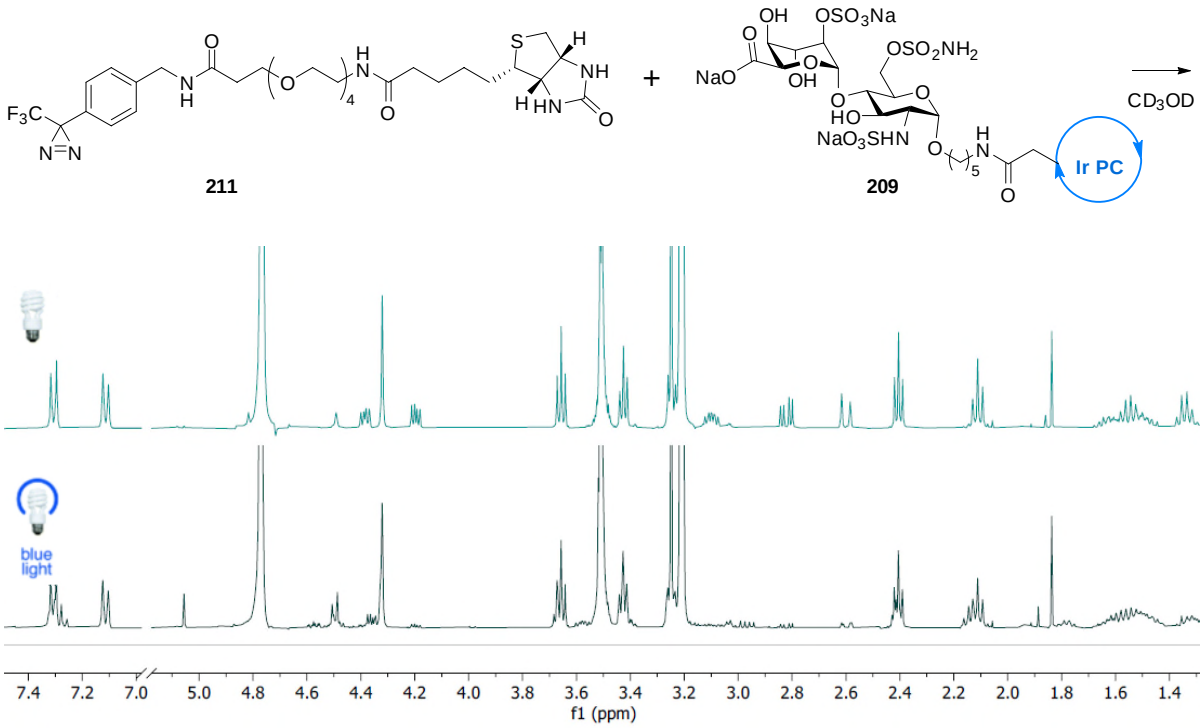
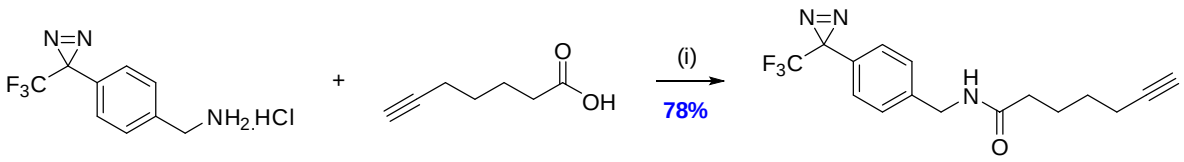


Figure 49: ^{1}H NMR spectra of a mixture of **208** (0.1 mg) and **211** (5 mg) in 500 µL CD_3OD. Top: before blue light irradiation. Bottom: with high intensity 440 nm irradiation for 20 min. Experiments performed in a Quartz NMR tube using Kessil 440 nm lamp (PR160L-440) positioned 20 cm from NMR tube.

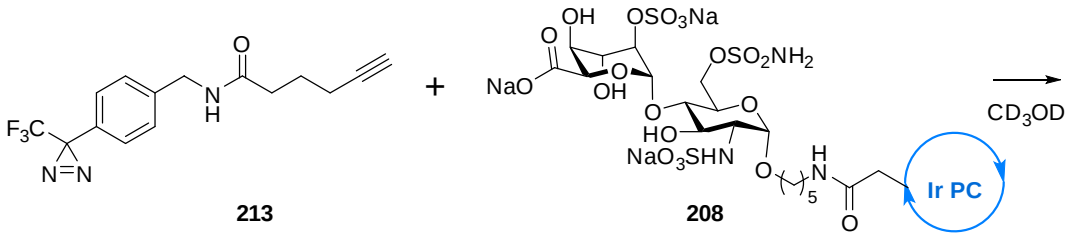
Comparison of the ^{19}F NMR spectra indicated the formation of a new fluorine containing species (δ_F (471 MHz, MeOH-*d*$_4$) -78.3 ppm), however the signal from the trifluoromethyl diazirine remained (δ_F (471 MHz, MeOH-*d*$_4$) -67.1 ppm). This finding indicated that the decomposition of the biotin was not entirely due to the carbene formation. For this reason, it was decided to utilise an alternative diazirine-containing molecule for the target engagement studies, which could be subsequently reacted to attach the biotin group. As an alternative *N*-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)hept-6-ynamide **213** was prepared according to scheme 53.



209 **212** **213**

Scheme 53: Synthesis of N-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)hept-6-ynamide **213**. Reagents and conditions: (i) HATA, DIPEA, CH_2Cl_2, rt, 16 h, 78%.

When the diazirine activation experiment was repeated using **213** (5 mg) and **208** (0.1 mg) with photoirradiation at 440 nm for 20 min, complete consumption of **213** was observed by ^{1}H NMR (figure 50).



213 **208**

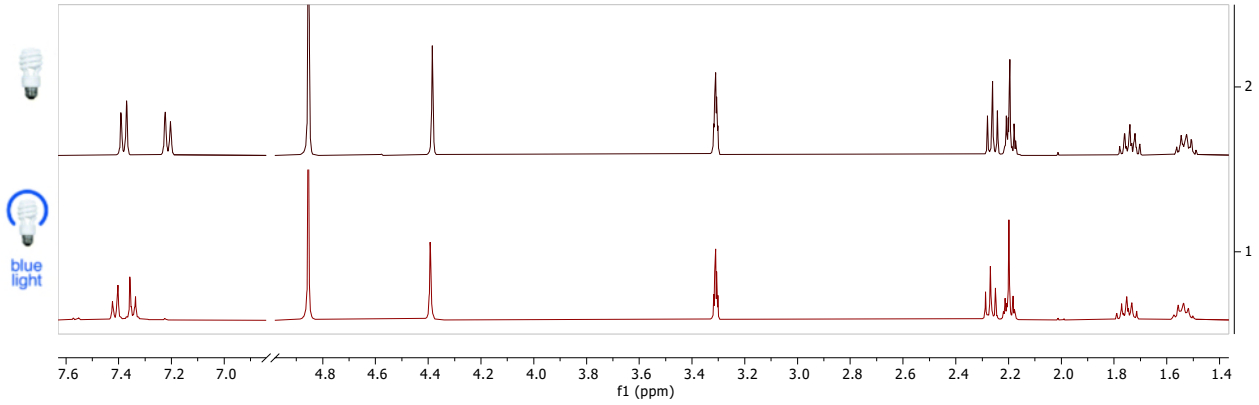
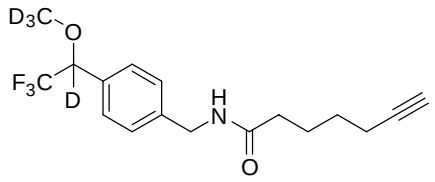


Figure 50: ^{1}H NMR spectra of a mixture of **208** (0.1 mg) and **213** (5 mg) in 500 µL CD_3OD. Top: before blue light irradiation. Bottom: with high intensity 440 nm irradiation for 20 min. Experiments performed in a Quartz NMR tube using Kessil 440 nm lamp (PR160L-440) positioned 20 cm from NMR tube.

The reaction was further characterised by ^{19}F NMR, which indicated near complete consumption of **213** and the appearance of two new fluorine containing species (δ_F (377 MHz, MeOH-d_4) -84.7 (minor new species), -78.3 (major new species), -67.2 (**213**))[xi].

[xi] 19F NMR spectra given in the appendix

LCMS analysis of the reaction confirmed that the carbene had inserted into CD_3OD to give **214** (figure 51) (*m/z* (ESI^+) 332 ($[M+H]^+$), 354 ($[M+Na]^+$)).



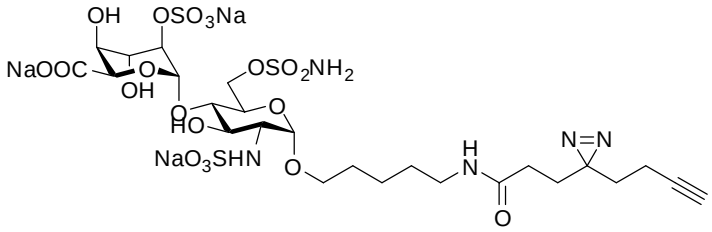
214

Figure 51: Structure of **214**, formed by carbene insertion into methanol-d_4

This simple NMR experiment gave confidence that the IdoA(2S)-GlcNS(6Sulfamate)-Ir photocatalyst **208** is able to be excited at 440 nm (as predicted by the UV-vis absorbance spectrum, figure 48) and is able to transfer energy to an intermolecular diazirine functionality to initiate carbene formation. This allowed progression of compounds **208** and **213** into a target engagement assay.

5.11 Biochemical evaluation of 187 as a HSulf-2 inhibitor

Before evaluating the target engagement probes in cells, it was necessary to evaluate these probes as mimics of the parent inhibitor **89**. Probe **187** as an inhibitor of HSulf-2 using the biochemical assay described in Chapter 2. It is important to have similar activity to the parent probe **89** in order to be a good target engagement probe. Target engagement probe **208** was not evaluated because of the possibility of the conjugated iridium-photocatalyst interfering with the fluorescent readout.



187

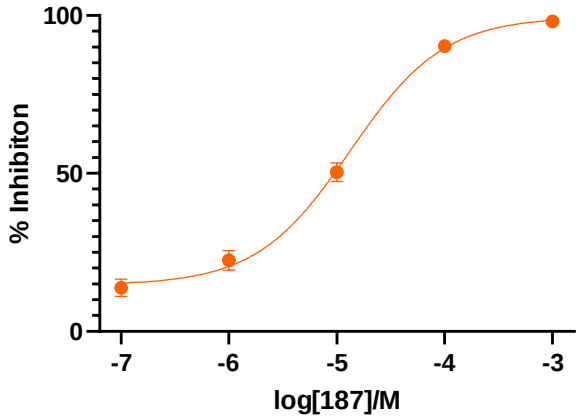


Figure 52: Dose-response curve for target engagement probe **187**. Data represented as the mean ± SD (n = 2).

Pleasingly, the IC_{50} value of 13.7 μM was comparable to the parent inhibitor **89**, and therefore **187** was progressed into an in-cell target engagement study. MCF-7 cell line was chosen, because it has been reported that Sulf-2 is upregulated in these breast cancer cells and the arylsulfatase activity of HSulf-2 has been detected in MCF-7 conditioned medium.[134]

5.12 Target engagement study

MCF-7 cells were grown to 70% confluency, then incubated with serum-free media for 16 h to accumulate secreted proteins, then treated with target engagement probe (50 μM) with/without disaccharide **89** (500 μM) as a competitor. The probes were covalently bonded to their target proteins by activation of the diazirine by UV or blue light. The supernatant was isolated, and the proteins were concentrated using TCA-precipitation protocol described by Nie et al.[135] The precipitate was dissolved in lysis buffer and the

protein concentration was determined by a bicinchoninic acid (BCA) assay, then all samples were adjusted to 1 mg/mL concentration. 100 μg of sample was subjected to copper catalysed azide-alkyne Huisgen cycloaddition (CuAAC) conditions ($CuSO_4$, TCEP, TBTA) to append a biotin and TAMRA bi-functional handle. The biotinylated proteins were enriched on streptavidin magnetic beads (Invitrogen™ Dynabeads™ MyOne™ Streptavidin T1), and analysed by SDS-PAGE, followed by in-gel tryptic digest and liquid chromatography-tandem MS. Data are shown in figure 51.

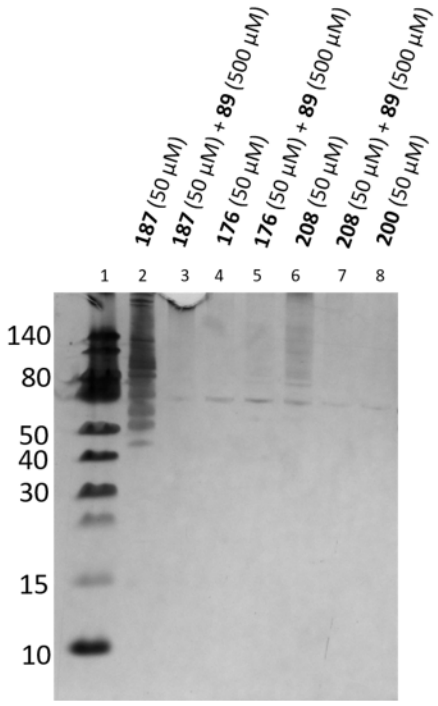


Figure 51: Gel electrophoresis of target engagement experiment in conditioned medium from MCF-7 cells. 4-12% Bis-Tris protein gel, MES SDS Running Buffer; PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kD in lane 1. Gel visualised by silver staining.

From the preliminary study, it appears that the disaccharide-based target engagement probes (figure 51, lanes 2 and 6) have caused labelling of proteins in the conditioned medium of MCF-7 cells. Furthermore, when incubated with a high concentration of inhibitor **89** (500 μM), which was a concentration determined to be at the saturation limit according to the biochemical study, the target engagement probes have been competed off

by **89**. This is evidence that the target engagement probes and **89** are occupying similar protein-ligand interactions, and therefore the target engagement probes are successfully mimicking inhibitor **89** in cells. Comparison of lanes 6 and 8 show the difference in labelling induced by iridum photocatalysts with and without the disaccharide ligand. Compound **200** was used as a control to ensure that the iridium complex does not cause any off-target protein labelling.

The experiment using the biphenyl-diazirine **176** (expected to be a non-selective Sulf-2 inhibitor), showed that it did not appear to engage with many proteins in the MCF-7 conditioned media and none that were competed off by **89**. This is further evidence that biphenyl **31** is not a good tool compound for studying the endosulfatases.

The protein bands on the gel were excised, subjected to a silver de-staining protocol, in-gel tryptic digest and submitted for LC-MS/MS in order to identify the proteins labelled by the target engagement probes. However, due to time restrictions the analysis of these samples will be included as future work.

5.13 Summary

This chapter describes the design and synthesis of three putative endosulfatase proximity-labelling probes, **176**, **187** and **208** (structures in figure 52). Two of these probes contain a routinely used 'minimalist' linker, which contains both the reactive group and the reporter group. Specifically, a diazirine, which is a photoaffinity tag that can be activated with UV-light to form a carbene, and a terminal alkyne that can be reacted via a CuAAC reaction post protein-ligand bonding for analysis. The third probe utilises a modern catalytic diazirine activation method, in which the biomolecule is conjugated to a photocatalyst that can initiate labelling of the target protein via Dexter energy transfer. Target engagement

probe **187** was evaluated as an inhibitor of Sulf-2 and it was found to have comparable activity to parent inhibitor **89**. Finally, the three target engagement probes were used in MCF-7 conditioned medium to investigate protein-ligand interactions in a cellular environment. Both disaccharide probes led to the labelling of proteins, which was off-competed when incubated with a high concentration of parent inhibitor **89**, indicating that these probes show promise for further development.

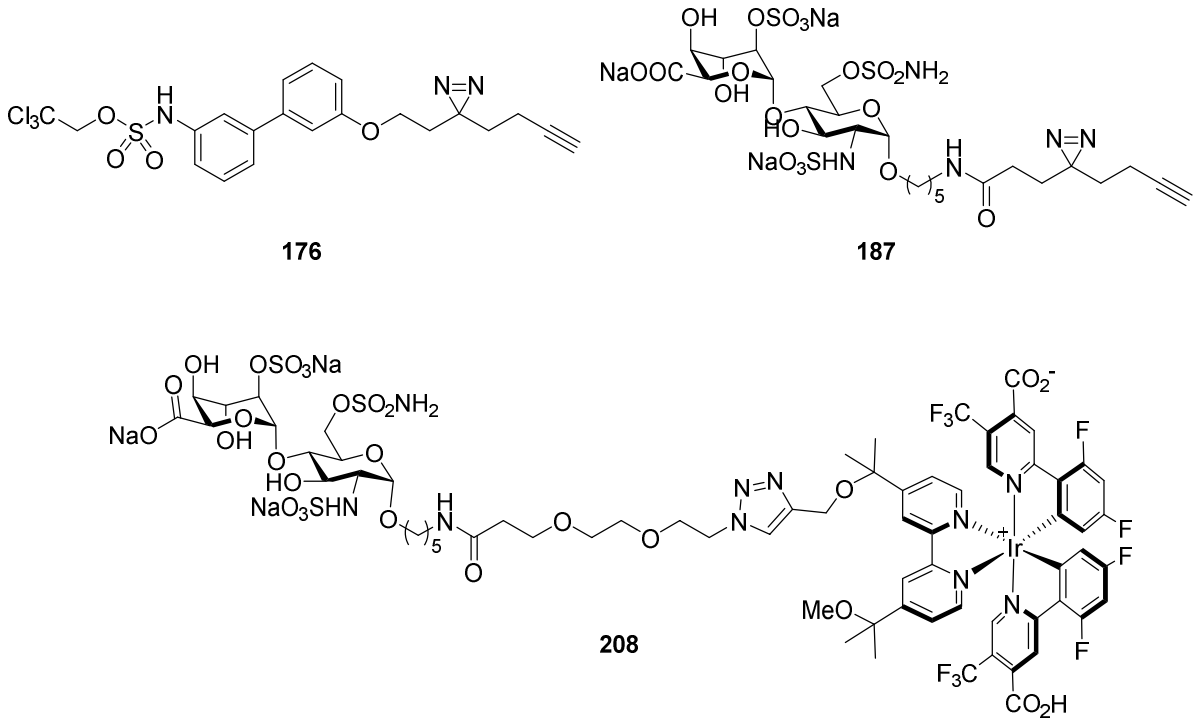


Figure 54: Summary of target engagement probes synthesised.

6. Synthesis of an IdoA(2S)-GlcNS(6Sulfamate) containing glycolpolymer

This chapter describes the efforts towards the synthesis of a multivalent ligand display of IdoA(2S)-GlcNS(6Sulfamate). The small molecule inhibitor was conjugated to a ROMP-derived polymer in attempt to enhance the potency of the disaccharide inhibitor **89** by mimicking the multivalent presentation of heparan sulfate.

6.1 Introduction

It was hypothesised that multivalent ligand display of the disaccharide inhibitor **89** would cause an increase in binding affinity to Sulf-1/2, because this would better mimic the dynamic nature of the binding interaction between Sulf-1/2 and heparan sulfate. It is somewhat difficult to measure the true binding affinity. Surface plasmon resonance (SPR) suggests that the HS/Sulf complex is extremely stable due to the strong avidity caused by multivalent interactions.[20] Milz et al used dynamic single-molecule force-spectroscopy to measure the association/dissociation kinetics of heparin with Sulf-1, and found that the average off-rate is in the range of 0.1 s^{-1}.[23] This value greatly differs from the binding affinity measured by SPR, demonstrating that single molecule analysis is required to resolve the kinetics of single dissociation events. While the authors could be confident that the highest number of interactions in the HS/Sulf-1 complex were between the hydrophilic domain (shown using atomic force microscopy), they were unable to quantify how many single interactions were formed simultaneously.

Considering the large difference between the binding affinity measured by SPR and AFM, it is likely that the HS/Sulf-2 binding interaction arises from simultaneous binding of multiple ligands within the HS chain. The heterogeneous structure of HS allows it to bind to a large number of proteins and thus participate in a large number of biological functions.

In order to mimic this binding mode, a multivalent display approach of inhibitor **89** was investigated. An advantage of this approach is that the sulfation pattern of the polymer can be controlled, therefore limiting the off-target bioactivity.

Multivalency uses multiple weak-binding saccharide ligands in high density to increase the number and cooperativity of possible binding events. Many multivalent display scaffolds have been used successfully to mimic the inhibitory and signal transduction properties of natural polysaccharides.[136] The commonly used scaffolds for multivalent carbohydrate display include dendrimers, polymers, cyclodextrins, calixarenes, nanoparticles and peptides. Multivalent display efficacy is based on the principle that altering the presentation of a molecule to its corresponding enzyme (i.e. increasing the local concentration of the ligand), can greatly influence its binding affinity. [137]

Synthetic multivalent ligands derived from ring-opening metathesis polymerisation (ROMP) have been utilised to study multivalent effects in a range of biological systems. These ROMP-derived polymers have several advantages over other synthetic methods, including well defined polymer length and low polydispersity. In this project it was decided that a norbornene monomer would be the easiest to manipulate into well-defined polymers with low dispersity. The length of the polymers synthesised could be controlled by the equivalents of initiator added, and other parameters of the reaction (e.g. solvent, temperature) could be altered to give the desired polymer. Utilising the norbornene-derived ROMP polymer as the multivalent display scaffold, two approaches to glycopolymer synthesis considered in this thesis (i) "Grafting through": the polymerisation of disaccharide-functionalised norbornene monomer and (ii) "Grafting on": the synthesis of a ROMP polymer with reactive side chains that can be subsequently reacted to attach the disaccharide ligand (shown in figure 55).

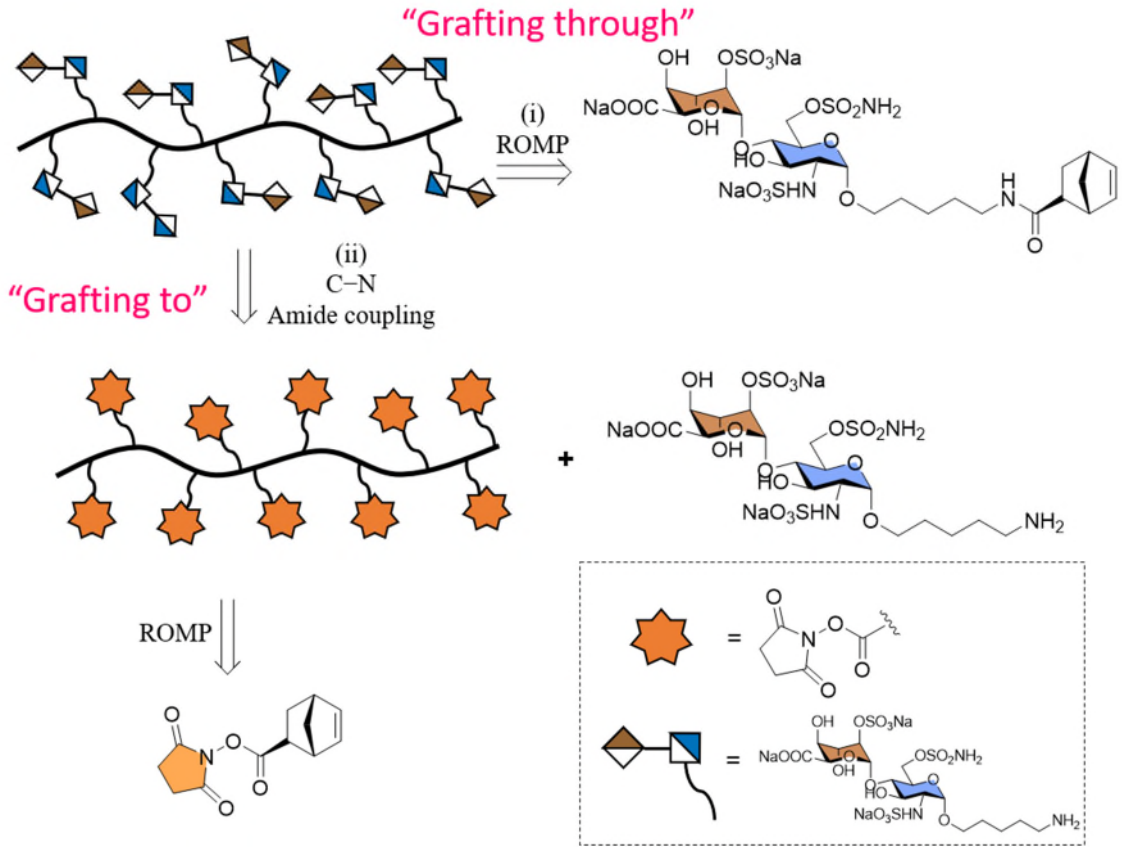
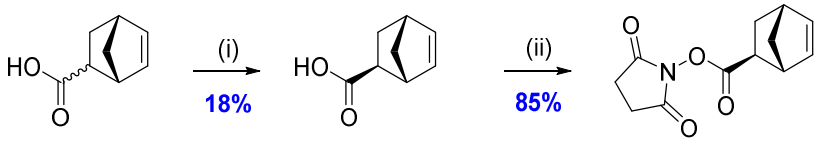


Figure 55: Schematic of two possible synthetic routes to a IdoA(2S)-GlcNS(6Sulfamate)-decorated glycolpolymer; (i) "Grafting through" - polymerisation of a saccharide-functionalised norbornene monomer; (ii) "Grafting to"- polymerisation of an activated ester-functionalised norbornene monomer and subsequent amide coupling with an amino-functionalised saccharide.

6.2 Polymerisation

The synthesis of the norbornenyl monomers began with the separation of the *endo* and *exo* diastereoisomers of commercially available 5-norbornene-4-carboxylic acid **215**. The *exo*-norbornene momomers typically react faster in ROMP reactions due to reduced steric hinderance at the Ru centre. This separation was achieved via iodolactionisation of the undesired *endo*-diastereomer with iodine and potassium iodide, followed by extraction of the unreacted *exo*-diastereomer **216** (isolated in 18% yield). The *exo*-5-norbornene-2-carboxylic acid was then converted into the activated ester by subjecting **216** to *N*-hydroxysuccinimide in the presence of EDC.HCl, which gave **217** in 85% yield (scheme 54).



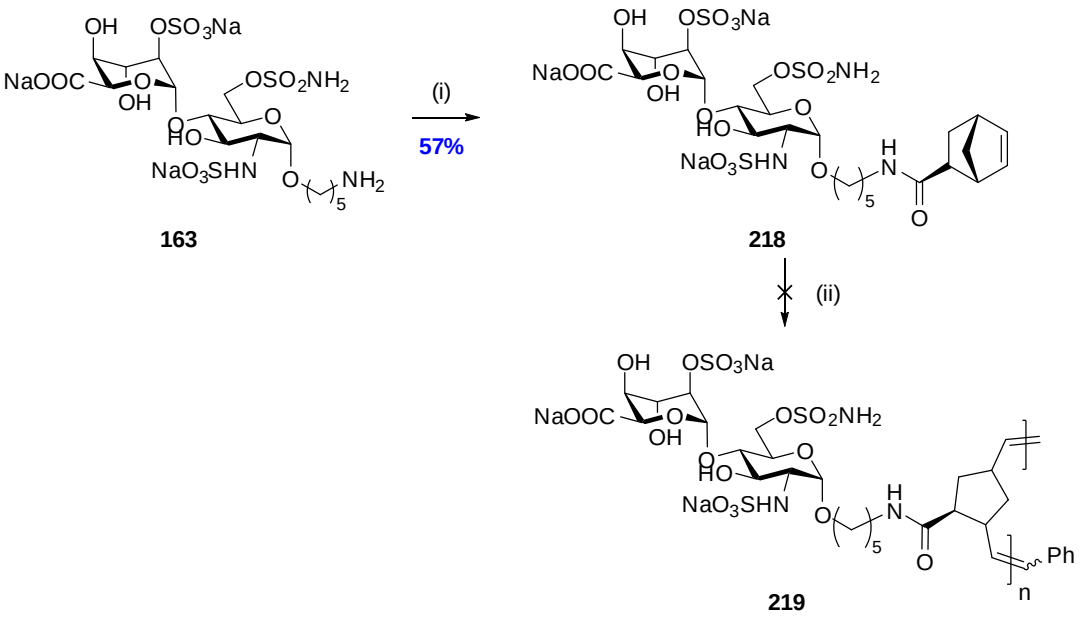
215
endo/exo
~8:2

216

217

Scheme 54: Synthesis of activated ester norbornene monomer **217**. Reagents and conditions: (i) I_2, KI, $NaHCO_3$, H_2O, 0 °C, 30 min, 18%; (ii) HOSu, EDC.HCl, MeCN, rt, 16 h, 85%.

Disaccharide-functionalised monomer **218** was synthesised by an amide coupling reaction of amine **163** and NHS-activated norbornene carboxylate monomer **217**, which proceeded in good yield to give **218** in 57% yield. Monomer **218** was subjected to ROMP-conditions using a Grubb's 3rd generation catalyst that was fast initiating, in a $CH_3OH/ClCH_2CH_2Cl$ (1:2.5) solvent system at 55 °C. Unfortunately, despite the fact that the reaction conditions were chosen to promote solubility of the sulfated monomer, the reaction mixture very quickly became opaque on addition of the catalyst. Nevertheless, the reaction was quenched by addition of ethyl vinyl ether after 1 h, and the polymer was precipitated into benzene/ether. Analysis of the precipitate via ^{1}H NMR spectroscopy confirmed that significant quantities of monomer **218** remained, indicating that the living polymerisation had stalled due to the insolubility of the growing polymer.



Scheme 55: Synthesis of polymer **217**. Reagents and conditions: (i) Na_2CO_3, CH_3OH, rt, 16 h, 57%; (ii) Grubb's catalyst® M300, $CH_3OH/ClCH_2CH_2Cl$, 55 °C.

The Grubb's third generation catalyst has reduced efficiency in methanol[138] and therefore it was not possible to increase the methanol volume further, therefore it was decided to redesign the monomer to incorporate some solubilising protecting groups.

6.3 Redesign of monomer

To overcome the insolubility of the extending living polymer, it was decided to investigate the ROMP reaction on a perbenzylated monomer (figure 56), which could undergo global deprotection at the polymer stage and simultaneously hydrogenate the polymer backbone alkenes.

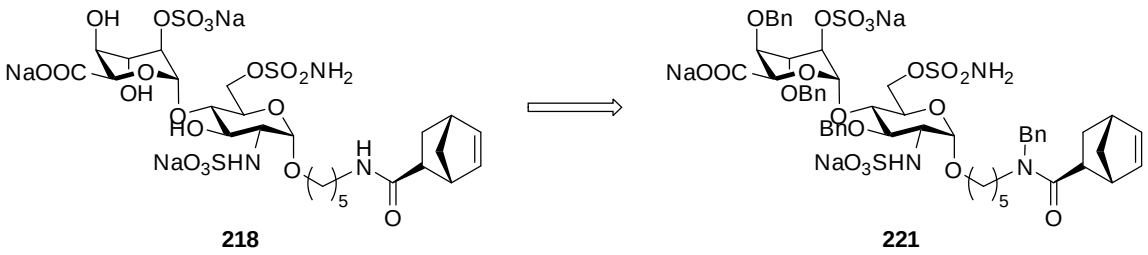
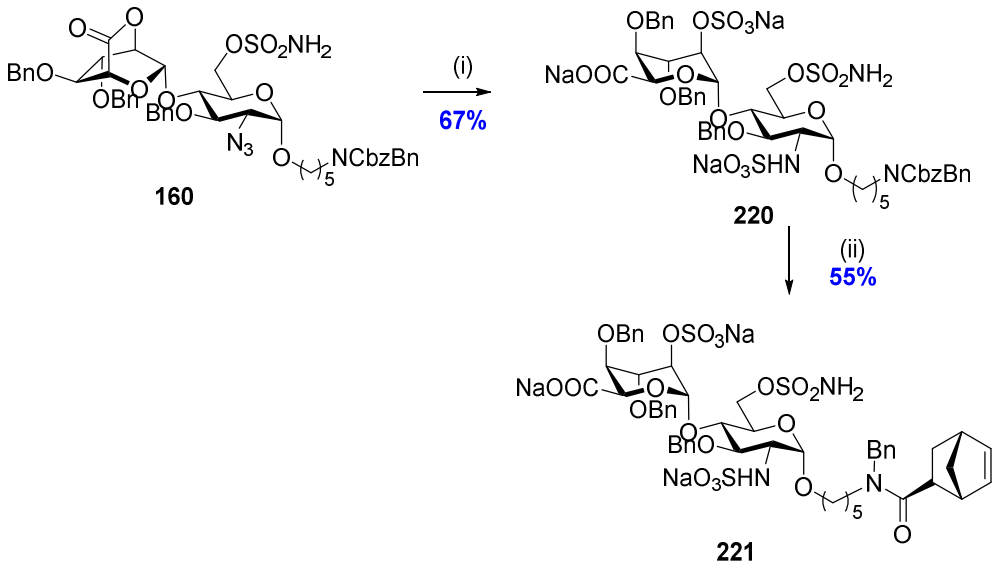


Figure 56: Redesign of norbornene monomer in an attempt to improve solubility

The synthesis of amine **163** was modified such that the 2'-*O*- and 2-*N*- sulfation could be done simultaneously, and so the lactone **160** (synthesis described in chapter 4, page 76), was subjected to a hydrolysis-Staudinger reduction-sulfation sequence of reactions using conditions previously discussed. The simultaneous *O,N*-sulfation reaction conditions were adapted from a protocol described by Xu et al.[139] These authors reported that for the simultaneous *O,N*-sulfation the optimal conditions were $SO_3 \cdot Et_3N$ in a pyridine/Et_3N (v/v 9:1) solvent system at 100 °C under microwave irradiation. The authors reported a significant reactivity difference between SO_3 as a pyridine complex vs the trimethylamine complex under these reaction conditions. When lactone **160** was subjected to lactone hydrolysis, Staudinger reduction and then Hu et al.'s sulfation conditions, the disulfated **220** was isolated in 67% yield after the three-step sequence (scheme 56).



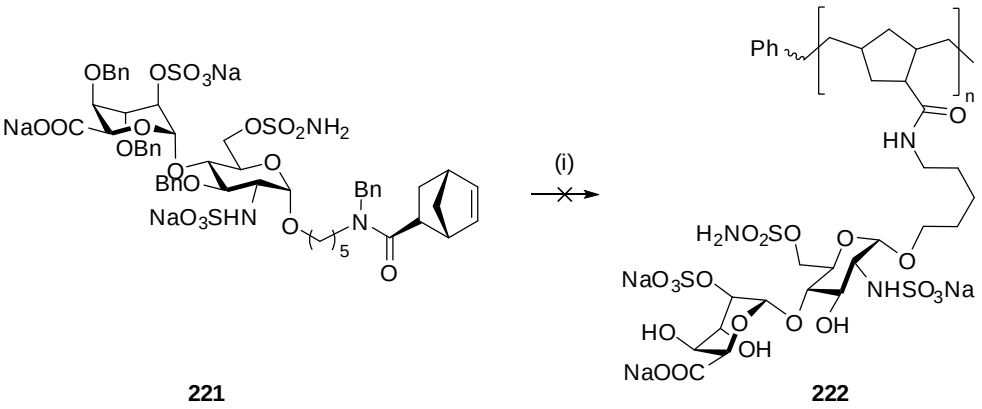
Scheme 56: Synthesis of monomer **221**. Reagents and conditions: (i) 1. LiOH, H_2O/THF, rt, 2 h; 2. PMe_3, 1M NaOH, THF, rt, 16 h; 3. $SO_3 \cdot Et_3N$, Py/Et_3N (v/v 9:1), 100 °C under microwave irradiation, 15 min; 67%; (ii) 1. H_2 (balloon), 10% Pd/C, CH_3OH, rt, 16 h, 10%; 2. **217**, N-methylmorpholine, DMF, 50 °C, microwave irradiation, 1 h, 55%.

The perbenzylated intermediate **220** then required selective *N*-Cbz deprotection to afford the secondary amine that could be reacted with **217**. Catalytic hydrogenation was an

attractive option, as it avoids the use of a large excess of hydrogen gas and it was hypothesised that if the in-situ generation of hydrogen could be controlled, then the chemoselectivity of the hydrogenation could be achieved. Indeed Sultane et al report the use of one equivalent of $NaBH_4$ as the hydrogen transfer catalyst which gave excellent yields of Cbz-deprotection in short reaction times (5-10 min).[140] When compound **220** was subjected to 10% Pd–C/$NaBH_4$ in CH_3OH, disappointingly there was minimal Cbz-removal, as analysed by ^{1}H NMR spectroscopy. Because of the lack of success with this protocol, it was then decided to trial the hydrogenation under standard conditions (H_2 balloon, 10% Pd/C in CH_3OH, rt), but to closely monitor the reaction by ^{1}H NMR. The reaction was filtered and concentrated for crude reaction analysis at 15 min intervals until no Cbz-remained by ^{1}H NMR. (45 min on 100 mg scale). The resulting secondary amine was reacted with NHS-activated ester **217** with Na_2CO_3 and methanol, however the desired product was only isolated in a low yield of 10% over two steps (scheme 56). It was hypothesised that this low yield was due to esterification of **217** competing with the amide coupling. To increase the conversion of this amide coupling, it was trialled under more forcing conditions (*N*-methylmorpholine, DMF, 50 °C under microwave irradiation), which resulted in an improved yield of 55%.

With monomer **221** in hand, it was then subjected to the ROMP reaction conditions using the $CH_3OH/ClCH_2CH_2Cl$ (v/v 1:2.5) solvent system to synthesise glycopolymer **222**. Crude analysis of the reaction by ^{1}H NMR indicated no remaining monomer (assigned by the disappearance of the norbornene alkene signals at δ_H (400 MHz, MeOH-d_4) 5.87 – 6.03 (m, 1H), 6.09 (q, J = 4.3 Hz, 1H)). Beyond this initial assessment, it was not possible to characterise the reaction further by ^{1}H NMR. The complexity of the aromatic region

masked the end-*Ph* and so end group analysis to determine the degree of polymerisation (*n*) was not possible.



221 **222**

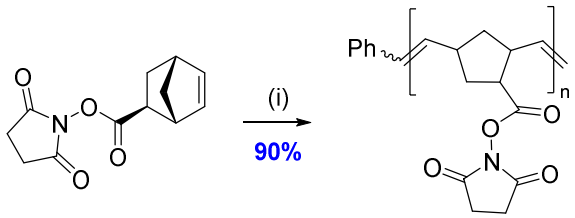
Scheme 57: Polymerisation of monomer **221**. Reagents and conditions: (i) 1. Grubb's catalyst® M300, degassed $ClCH_2CH_2Cl/CH_3OH$, 55 °C, 3 h; quenched by addition of ethyl vinyl ether; 2. H_2, $Pd(OH)_2/C$, CH_3OH/PBS, rt, 48 h.

This polymer was subjected to global debenzylation using $Pd(OH)_2/C$ in CH_3OH/PBS that produced **222**, which was analysed by ^1H NMR and it was determined that the polymerisation reaction had not been successful. The ^1H NMR showed well resolved multiplets (not characteristic of a polymer), and when the *Ph* was integrated for end group analysis the 'n' value was only 2-3. It was hypothesised that this polymerisation reaction failed to to the high charge density of the monomer causing electrostatic repulsion in addition to the high steric demand around the Ru-centre.

6.4 Activated ester ROMP-polymer

Following on from the difficulty in synthesis of the glycopolymer via 'graft through' approaches, it was decided to investigate 'graft to' methods. This involved the synthesis of a ROMP-derived polymer that was functionalised with an *N*-hydroxysuccinimide (NHS) ester that could be modified via amide coupling reaction with an amine-functionalised biological ligand of interest. The NHS-activated ester norbornene monomer **217** was

polymerised using Grubb's catalyst® M300 (generation 3) which proceeded to give polymers with narrow polydispersity (DP) (1.23-1.28, determined by GPC). Two length polymers were synthesised by varying the mol% Grubb's catalyst (table 16), to synthesise polymers with *n* = 10 **223** and *n* = 100 **224**.



Scheme 58: Synthesis of activated ester polymers **223** and **224**. Reagents and conditions: (i) Grubb's catalyst® M300, degassed CH_2Cl_2; quenched by addition of ethyl vinyl ether, rt, 1 h, 90%.

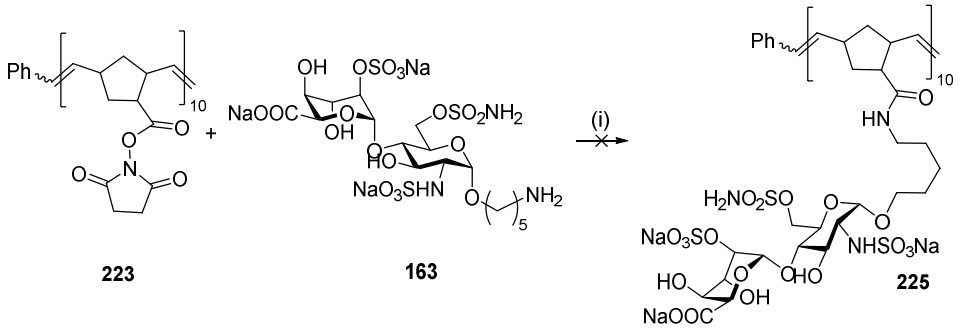
Table 16: Characterisation data for polymers **223** and **224**

Entry		*mol% Grubbs cat*	M_n	PD	*n*	yield
1	**223**	10	3069	1.28	12	91
2	**224**	1	20522	1.23	87	89

Polymers **223** and **224** were analysed using gel permeation chromatography (GPC), to determine the polymer molecular weight and polydispersity. The polydispersity index (1.23-1.28) was deemed acceptable, and indicates that the reaction was likely to proceed quickly, without chain transfer or premature chain termination, and without any aggregation or solubility issues. Furthermore, this amine nucleophile could be conjugated to the ROMP-derived polymer at different concentrations, allowing for control of the ligand density in the multivalent display.

An initial attempt was made to functionalise **223** with 10 equivalents of **163** in DMF with *N*-methylmorpholine (scheme 59). Unfortunately, this reaction did not proceed as smoothly

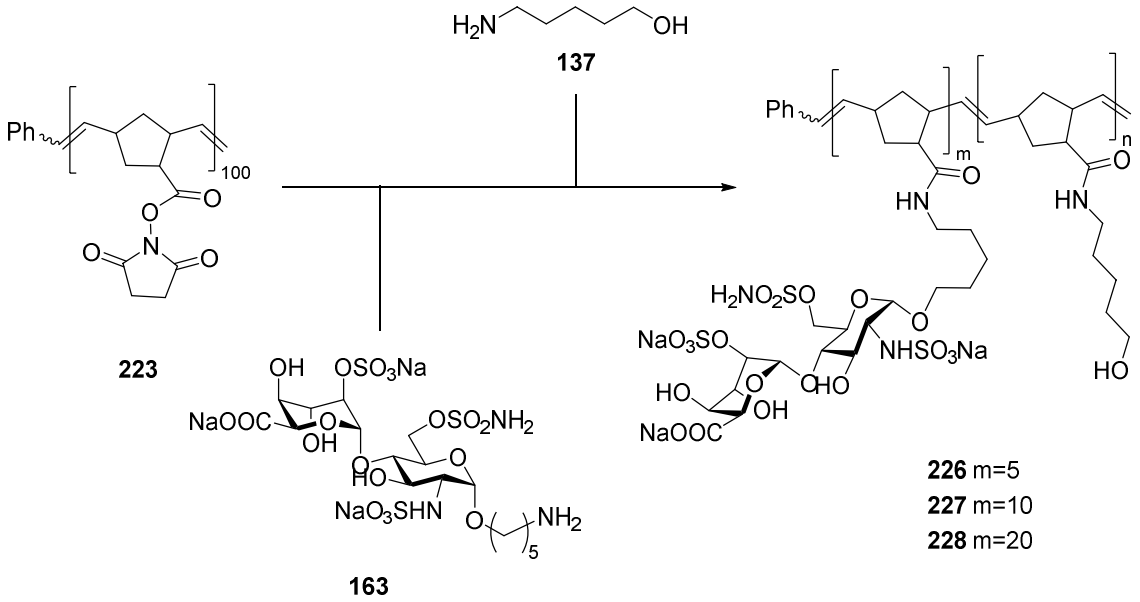
as desired. The first challenge encountered was the choice of solvent, the common amide coupling solvent (DMF) was undesirable due to its high boiling point and therefore difficulty in removing it from the reaction mixture in the absence of the opportunity to 'wash it out' with an aqueous work up. Secondly, monitoring the reaction progress proved to be challenging. Literature reports of similar reactions simply looked for the consumption of the starting material ligand however in this instance, the high polarity of the ligand meant this was difficult to monitor by TLC, and the complexity of the ^{1}H NMR spectrum rendered this technique impractical. Furthermore, the presence of DMF in the reaction only further complicated purification, as the reaction components proved to be too polar for 'common' chromatography. Size-exclusion chromatography was attempted but did not produce any clean material.



Scheme 59: Amide coupling of polymer **223** and amine **163**. Reagents and conditions: (i) N-methylmorpholine, DMF, rt, 16 h.

It was hypothesised that the reaction would be easier to monitor if the ligand equivalents were lowered, therefore producing a multivalent display where not every repeating unit contained the disaccharide. Because very little is known about the structures of the sugar-recognising site on Sulf-1/2 (specifically the distances between the sugar-binding sites and the number of sugar-binding sites), it was difficult to design polymers with the appropriate sugar density. The steric effects influencing binding of Sulf1/2 to this multivalent ligand are unknown, and therefore this 'graft-to' method of polymer diversification allowed the

optimal ligand density to be explored. Consequently, polymers containing 5, 10 or 20% IdoA(2S)-GlcNS(6Sulfamate) (i.e. 5, 10 and 20 average disaccharide units per polymer) were prepared, and unreacted activated ester groups were saturated with 5-amino-pentan-1-ol **137** to aid solubility (scheme 60).



Scheme 60: Synthesis of polymers **226**, **227** and **228**. Reagents and conditions: (i) Et_3N, DMF, rt, 16 h; then 5-aminopentan-1-ol **137**, rt, 24 h.

Polymer **224** was reacted with IdoA(2S)-GlcNS(6Sulfamate)-functionalised amine **163**, then quenched with excess 5-aminopentan-1-ol **137**. To circumvent the purification issues previously identified the polymers were purified via precipitation in Et_2O to give polymers **226**-**228** respectively (table 17, entries 2, 3 and 4).

Table 17: Characterisation data for polymers **225-228**

Entry	*SM polymer*	*Product polymer*	*Number of **89** units*	m *(^{1}H NMR)*	n *(^{1}H NMR)*
1	**223**	**225**	10	-	-
2	**224**	**226**	5	6	79
3	**224**	**227**	10	9	75
4	**224**	**228**	20	17	65

A key limitation of this 'graft-on' approach was that there was no control over the structural homogeneity, and it was not possible to characterise the modification positioning along the polymer chain. Because of this the characterisation was somewhat limited to ^{1}H NMR.

6.5 Biochemical evaluation of glycopolymers, 226, 227 and 228

In order to assess whether the increase in IdoA(2S)-GlcNS(6Sulfamate) **89** loading per polymer results in a true multivalency effect, the percentage inhibition was plotted against effective **89** molarity, and not polymer concentration. HSulf-2 inhibition was measuring using 4-MUS and monitoring the release of 4-MU (as described in chapter 2), using a single concentration of glycopolymers (effective [**89**] = 3 μM) (figure 55, A). At this single concentration, using the effective [**89**] it appeared that increasing the number of units of **89** per polymer led to increased HSulf-2 inhibition. At as low as effective [**89**] = 3 μM, polymers **227** and **228** inhibit HSulf-2 by greater than 20%. (This corresponds to a polymer molarity of 700 nM and 1.1 μM, respectively.)

Glycopolymer **228** was progressed for IC_{50} determination. Polymer **228** (containing on average 20 units of **89),** acts as an inhibitor of HSulf-2 with an IC_{50} of 9 μM ± 4 (figure 57, B). This value is approximately 5-fold more potent than the parent inhibitor **89**. This suggests a gain in avidity caused by the polymer construct, providing evidence that ligand multivalency has an effect on inhibition of HSulf-2.

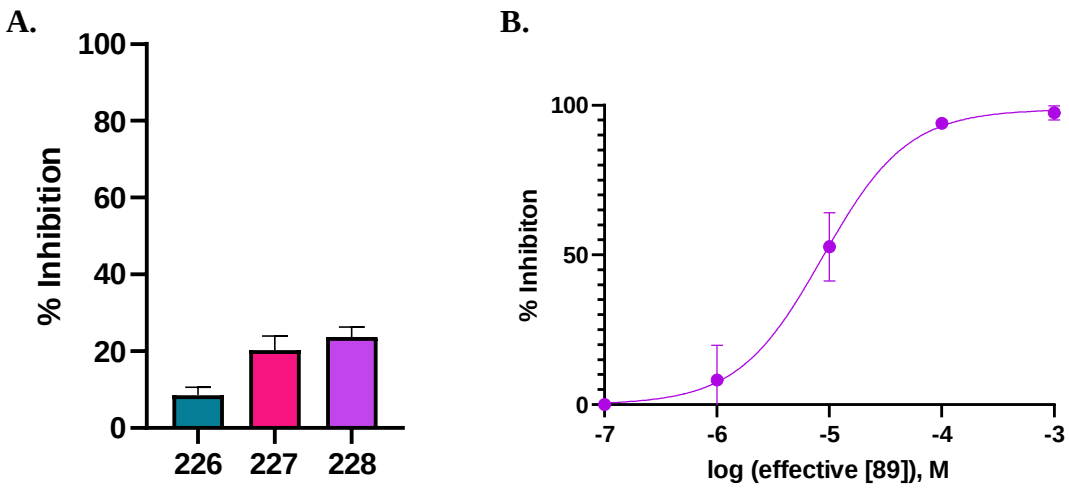
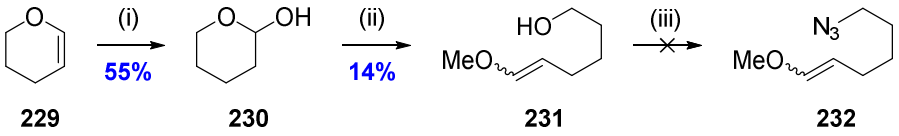


Figure 57: A. HSulf-2 inhibition data for glycopolymers **226, 227** and **228** at 3 μM. Data represented as the mean ± SD, (n = 2). **B.** IC_{50} curve for glycopolymer **228** against HSulf-2 (plotted as effective [**89**] rather than [**228**] to assess the multivalent affect). IC_{50} = 9 μM ± 4. Data presented as mean ±SD (n=2). 160 ng/μL HSulf-2, 10 mM 4-MUS, 50 mM Tris base, 10 mM $MgCl_2$, (pH 7.5), 37 °C, 4 h, then quenched by addition of 1 mM Tris base (pH 11.5) and analysed on a fluorescence plate reader (4-MU, λEx 350 nm, λEm 460 nm). Inhibition values are reported as percentages of the uninhibited control values.

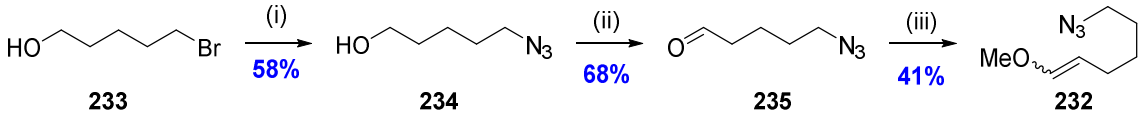
6.6 End functionalisation of living polymer

During the ROMP reaction, the ruthenium carbene only reacts with alkenes, and so end-functionalisation reagents must contain an alkene to form a Ru=C species that is inactive as a methathesis catalyst. The most commonly used approach to end-functionalisation is to use a substituted vinyl ether to terminate the chain by transferring a substituted methylene group onto the polymer, and thereby removing the Ru catalyst. This vinyl ether terminating approach has been successfully employed to append a variety of functional groups onto the end of a ROMP polymer, including trimethylsilyl groups[141], carboxylate groups/masked carboxylic acids[142], aldehydes[143], ketones[144], masked amines[145], azides[146] and activated esters[147]. For this thesis, it was decided that an azide would be most useful, as it would allow for a CuAAC reaction to append any desired functional group. This reaction is compatible with the functional groups present in the polymer, and importantly it is tolerant to water as a solvent.



Scheme 61: Synthesis of azide-containing capping agent **232**. Reagents and conditions: (i) 1. $Na_2S_2O_5$, H_2O, rt, 24 h; then 10% NaOH, 55%; 2. $ClPPh_3CH_2OCH_3$, n-BuLi, THF, −78 °C, 14%.

Following a modified protocol by Mangold et al.,[146] dihyropyran was first hydrated by treatment with aqueous sodium metabisulfite to form an intermediate bisulfate adduct which was then reacted with 1M NaOH to give **230** as a hemiacetal in 55% yield.[148] This hemiacetal was subjected to Wittig reaction with (methoxymethyl)triphenylphosphonium chloride to give enol ether **231** (E/Z, 8:2) in 14% yield. Finally, the primary alcohol was converted into an azide group by reaction with diphenylphosphoryl azide under mitsunobu-like conditions. However this reaction gave a complex reaction profile, and after column chromatography, no desired product was isolated. It was hypothesised that the enol ether had degraded, either during work up or on silica during purification. This disappointing result is far from the 85% yield reported in the literature.[146] Taking this in to consideration, in combination with the low yield obtained from the Wittig reaction (14%), it was decided to investigate an alternative route to **232** rather than optimising these reactions.



Scheme 62: Alternative synthetic route to azide-containing capping agent **232**. Reagents and conditions: (i) NaN_3, DMF, 90 °C, 4 h, 58%; (ii) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C, 4 h, 68%; (iii) $ClPPh_3CH_2OCH_3$, n-BuLi, THF, −78 °C, 41%.

In order to avoid the unsuccessful alcohol to azide transformation, it was decided to install the azide group via S_N2 reaction at the beginning of the synthesis. Reaction of 5-bromo-penan-1-ol **233** with sodium azide gave compound **234** in 58% yield, and the resulting alcohol was subjected to Swern oxidation to afford aldehyde **235** in 68% yield. Finally,

aldehyde **235** was subjected to a Wittig reaction with (methoxymethyl)triphenylphosphonium chloride (as in the previous reaction scheme) however with this substrate, the equivalents of ylide were reduced to 1.2 (compared to 2.4 eq. used previously) and this reaction proceeded efficiently to give **232** in 41% yield (scheme 62).

There are many advantages of end-labelling the glycopolymer. One practical reason is that an end-group can be added to allow for easier polymer characterisation (e.g. by ^{1}H NMR). A second reason is that if a reactive functional group is added, it allows for further downstream functionalisation. In the case of an azide-containing capping reagent, it allows for a CuAAC reaction with various coupling partners such as fluorophores for visualizing cellular internalisation or other ligands for targeted protein degradation (discussed in section 6.6).

An azide-functional group would easily installed by terminating the ROMP reaction with an excess of the azide-containing enol ether **232**.

6.7 Lysosome targeting chimeras (LYTAC)

Targeted protein degradation (TPD) is a promising new tool for probing biological pathways, and the therapeutic potential is evidenced by the development of proteolysis targeting chimeras (PROTACs) entering clinical trials. PROTACs induce the formation of a complex between a target protein and an E3 ligase, which results in ubiquitination of the target protein and subsequent degradation by the proteasome. A limitation of the PROTAC technology is that it can only be used to target intracellular proteins. Lysosome targeting chimeras (LYTACs) have been developed that use lysosomal degradation pathways to expand the scope of TPD technologies to allow for extracellular and membrane-bound

proteins. The principle is that a bifunctional molecule that is composed of one ligand for cell-surface lysosome-targeting receptors (LTRs), and a second ligand that binds to the desired target protein and when all three are in complex, the target protein is tansported to the lysosome for degredation. When the LTYAC molecule is bound to both proteins at the same time, the resulting complex is engulfed by the cell membrane to form a transport vesicle that is transported to the lysosome, where the target protein is degraded. To date, two LTRs have been targeted for LYTAC technologies: (1) the asialoglycoprotein receptor (ASGPr) which is uniquely expressed on hepatocytes, and (2) the cation-independent mannose-6-phosphate receptor (CI-M6PR), that is broadly expressed in most cells (figure 58).

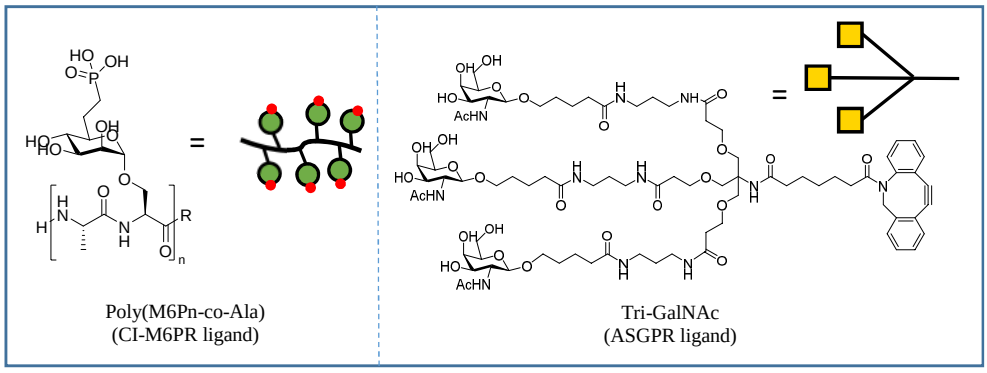


Figure 58: Schematic representation of the ligands developed for LTRs. Left, Poly(M6Pn-co-Ala) targets CI-M6PR. Right, Tri-GalNAc targets ASGPR.

It has long been recognised that CI-M6PR can be utilised to increase target-protein internalisation in disease models. Zhu et al. reported the conjugation of a high affinity CI-M6PR ligand (an oligosaccharide with high mannose-6-phosphate content), onto recombinant human acid α-glucosidase (rhGAA) for the treatment of Pompe disease.[149] The authors detected an increased delivery of rhGAA to the cardiac and skeletal mucscles, which was accompanied by an improvement of the disease pathology in mice treated with the modified enzyme compared to the control. This study demonstrated that CI-M6PR may

be used to induce target protein cellular uptake *in vivo*.[149] In addition to this study, the CI-M6PR has also been exploited to deliver therapeutic enzymes for the treatment of lysosomal storage disorders.[150]

In 2020, Banik et al reported first-generation LYTACs that used an *N*-carboxyanhydride (NCA)-derived glycopeptide bearing serine-*O*-mannose-6-phosphosphonates as a ligand for the cation-independent mannose-6-phosphate receptor (CI-M6PR), which endogenously transports proteins bearing *N*-glycans capped with mannose-6-phosphate (M6P) residues to lysosomes. The authors tethered the M6P glycopeptide to an antibody to target the extracellular protein of choice. However, in the absence of a reliable and specific Sulf-1 or Sulf-2 antibody it was hypothesised that the HS-mimetic glycopolymers developed in this thesis could be used instead, such that the LYTAC would be a block co-polymer (figure 59).

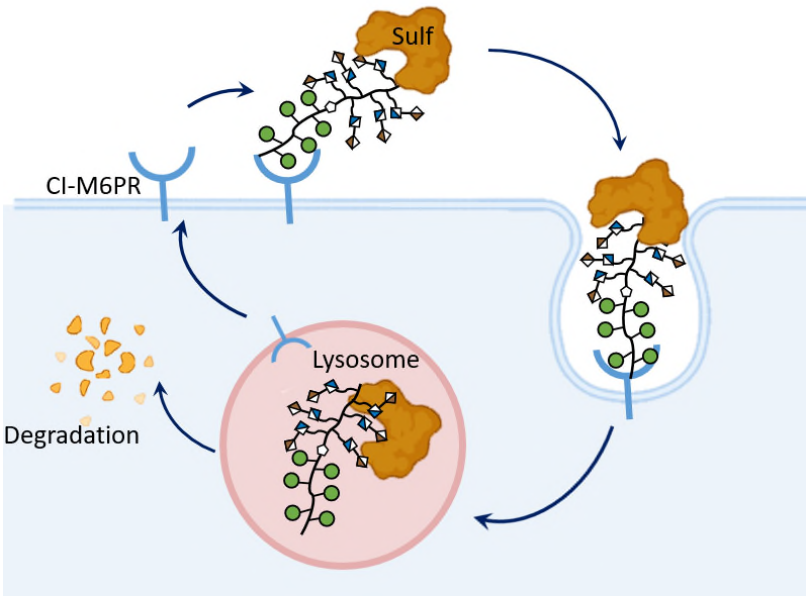
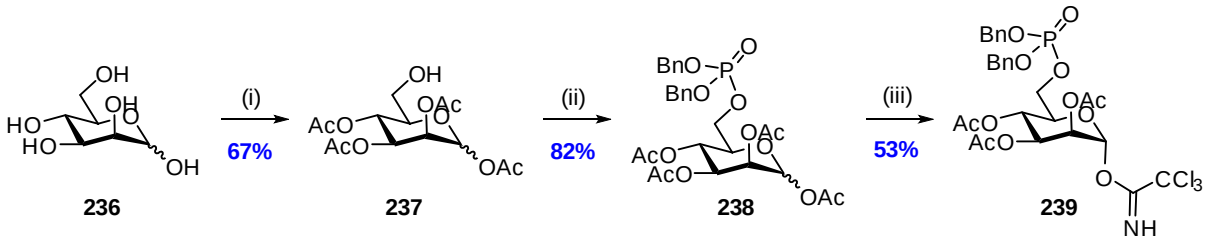


Figure 59: The concept of bis-polymer LYTAC proposed in this thesis, in which the poly(M6P) glycopolypeptide ligand for CI-M6PR is conjugated to the poly(IdoA(2S)-GlcNS(6Sulfamate)) to traffic endosulfatases to lysosomes for degredation. Figure made using BioRender.com.

6.8 Synthesis of poly(M6P) as a ligand CI-M6PR

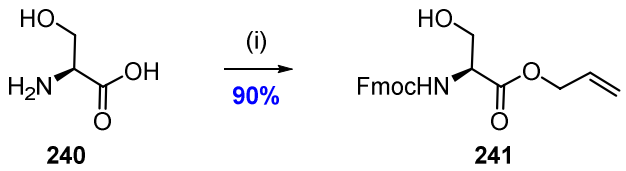
Firstly, 6-hydroxy-tetra-*O*-acetylmannopyranose **237** was prepared following a sequence of protecting group manipulations: regioselective tritylation of the 6-*O*H, peracetylation of the 1,2,3 and 4-*O*H and then deprotection of the 6-*O*-trityl was achieved in 67% over three steps. The product was obtained as an inconsequential mixture of anomers (α/β 2:3). Phosphorylation of primary alcohol **237** was accomplished using a 1*H*-tetrazole solution in acetonitrile and dibenzyl *N,N*-diisopropylphosphoramidite at 0 °C, followed by direct oxidation of the intermediate phosphite with *m*-chloroperbenzoic acid at −78 °C. This sequence of transformations proceeded effectively to afford dibenzyl phosphate **238** in excellent yield (82%).



Scheme 63: Synthesis of mannose-6-phosphate glycosyl donor **239**. Reagents and conditions: (i) 1. Ph_3CCl, Py, 50 °C, 4 h; then, Ac_2O, rt, 16 h; 2. TMSCl, NaI, MeCN, rt, 4 h, 67% (α/β 2:3); (ii) Dibenzyl N,N-diisopropylphosphoramidite, tetrazole, CH_2Cl_2, 0 °C; then, m-CPBA, −78 °C to rt, 16 h, 82%; (iii) 1. 3-(Dimethylamino)-1-propylamine, THF, 0 °C, 2 h; 2. Trichloroacetonitrile, DBU, CH_2Cl_2, 0 °C, 1 h, 53%.

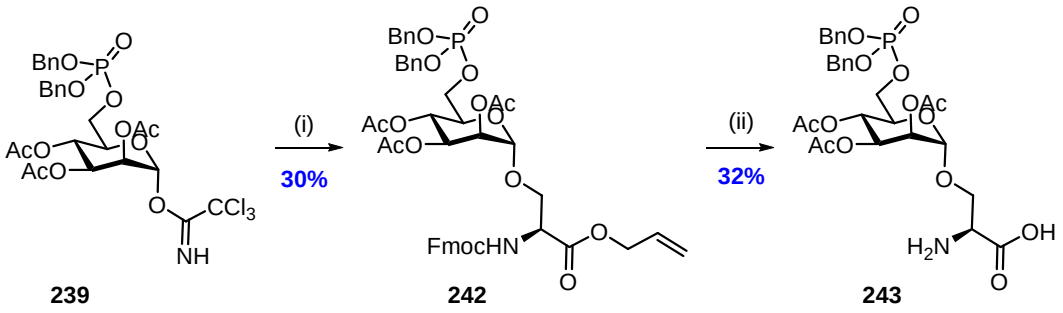
Subsequent regioselective anomeric deacetylation was achieved by subjecting compound **238** to 3-(dimethylamino)-1-propylamine at low temperature, and the crude alcohol was immediately converted into the trichloroacetimidate glycosyl acceptor **239** in 53% yield over two steps, and the product was obtained as a single anomer. The anomeric

configuration was assigned by ^{1}H NMR – C(1)*H* doublet at 6.27 ppm with a coupling constant of 1.9 Hz.[xii]



Scheme 64: Synthesis of N-(9-fluorenylmethoxycarbonyl)-L-serine allyl ester **241**. Reagents and conditions: (i) 1. FmocOSu, 10% aq $NaHCO_3$, 1,4-dioxane, rt, 16 h; 2. Allyl bromide, DIPEA, DMF, rt, 16 h, 90%.

N-Fmoc serine allyl ester was prepared from L-serine **240** as outlined in scheme 64. The amine was firstly protected by reaction with Fmoc *N*-hydroxysuccinimide ester, and secondly the acid was alkylated by reaction with allyl bromide and DIPEA to give alcohol **241** in 90% yield over two steps. Glycosyl acceptor **239** was subjected to *O*-chemical glycoylation reaction with *N*-Fmoc serine allyl ester **241** in the presence of catalytic TMSOTf, which proceeded in low yield to give the desired α-linked product (30%). Next, the deprotection of the *O*-allyl and *N*-Fmoc groups was accomplished by subjecting compound **239** to Tsuji Trost reaction conditions followed by morpholine initiated Fmoc cleavage to give amino acid **240** in 32% yield over two steps.

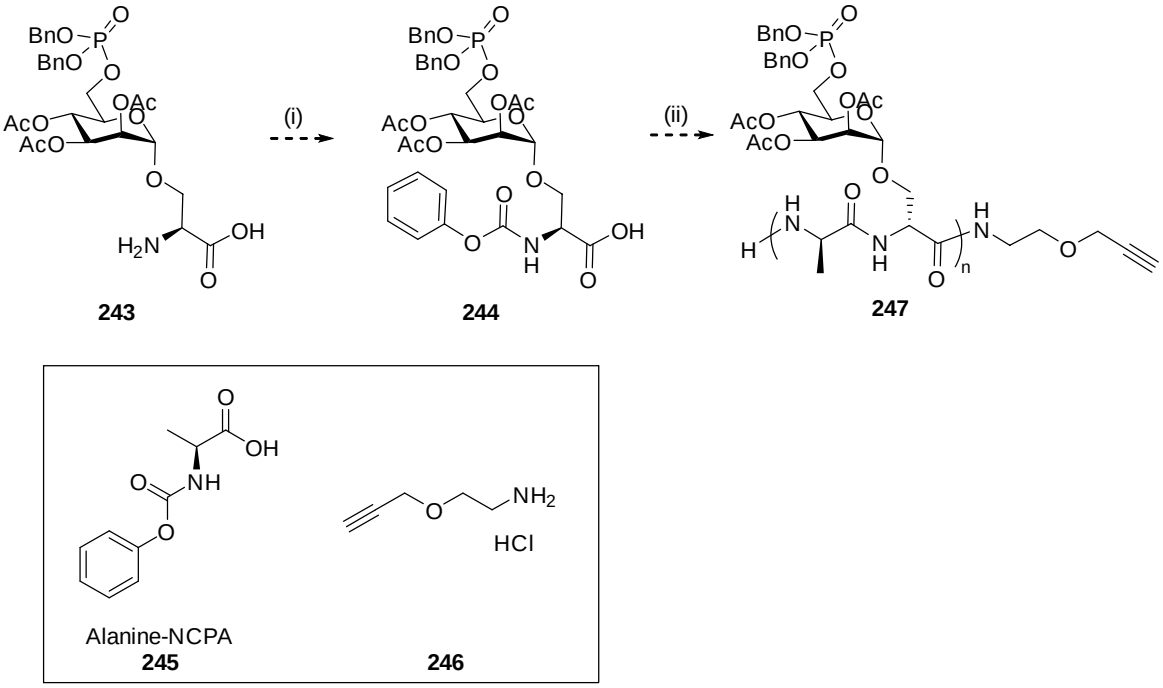


Scheme 65: Synthesis of amino acid **243**. Reagents and conditions: (i) **241**, cat. TMSOTf, 4Å molecular sieves, CH_2Cl_2/Et_2O, 0 °C to rt, 6 h, 30%; (v) 1. $Pd(PPh_3)_4$, N-methylaniline, THF, rt, 2 h; 2. Morpholine, DMF, rt, 1 h, 32%.

[xii] See chapter 7 (subsection ^{1}H NMR assignment of the anomeric configuration) for explanation

Commonly, synthetic polypeptides are accessed via the polymerisation of an α-amino acid *N*-carboxyanhydrides initiated by either a primary amine, a transition metal catalyst or an organocatalyst. However, due to the risk of spontaneous initiation by trace water these reactions are very moisture sensitive, and even when performed in a glovebox can lead to high polydispersity. In a recent publication by Li et al., an under-air procedure for controlled polypeptide synthesis has been developed using *N*-phenoxycarbonyl-functionalized α-amino acid (NPCA) as a masked NCA precursor.[151] This protocol was very desirable for several reasons (i) it avoids the use of toxic phosgene reagents, (ii) it can be performed under air and is moisture tolerant, (iii) it produces polymers with narrow polydispersity indices and (iv) it is tolerant to alkyne group that is required for end-functionalisation.

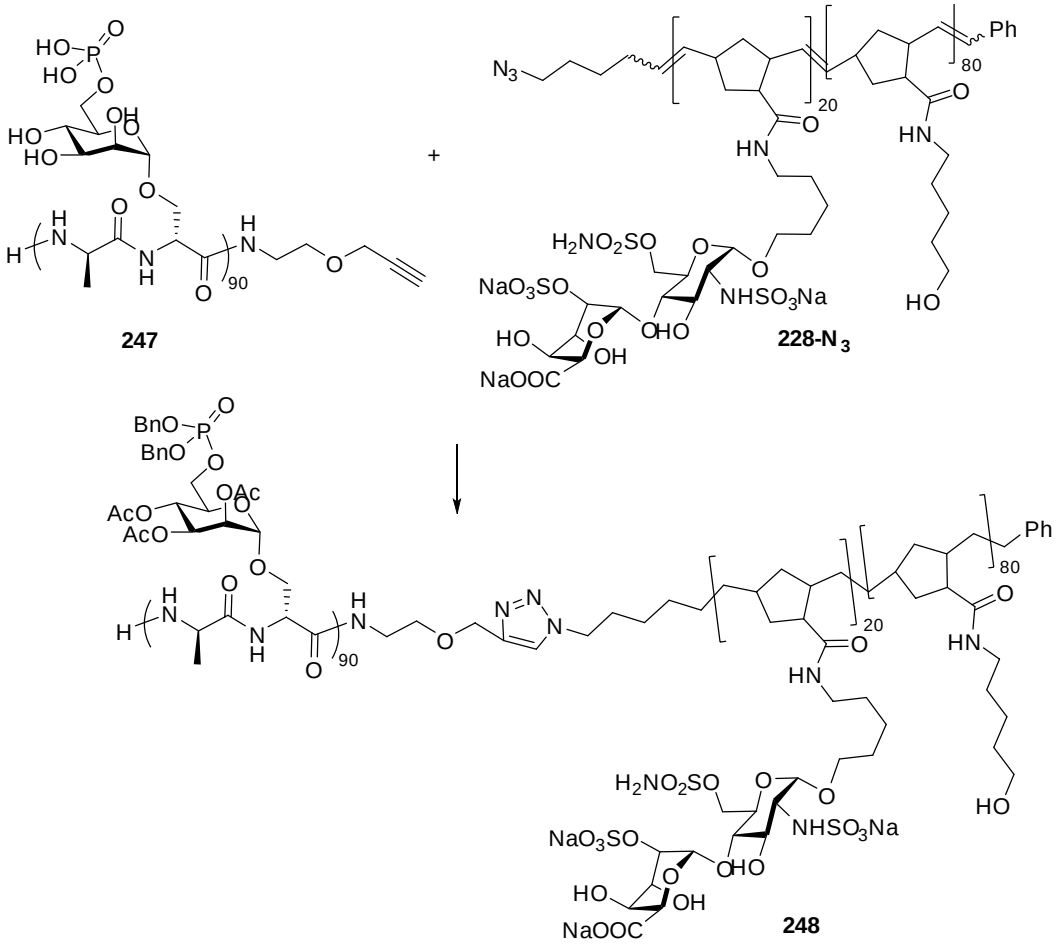
It was planned that the M6P-NPCA **244** could be synthesised by reaction of amino acid **243** with diphenyl carbonate and Et_3N in acetone/H_2O (scheme 66). The polymerisation could be attempted using alanine-NPCA as a co-monomer and 2-(Prop-2-yn-1-yloxy)ethan-1-amine hydrochloride **246** as the initator to give to give poly(M6P-Ala) **247** (scheme 66).



Scheme 66: Synthesis of glycopolypeptide poly(M6P) **242**. Reagents and conditions: (i) diphenyl carbonate, Et_3N, acetone/H_2O, rt, 16 h; (ii) Alanine-NCPA, 2-(Prop-2-yn-1-yloxy)ethan-1-amine hydrochloride, DMAc, 70 °C.

6.9 Future work: Construction of LYTAC

The construction of the endosulfatase degrading LYTAC would involve a CuAAC reaction between the azide-functionalised poly[(IdoA(2S)-GlcNS(6Sulfamate)] and the alkyne-functionalised poly(M6P-Ala), followed by global deacetylation and global debenzyltion under standard conditions (scheme 67).



Scheme 67: Synthesis of Sulf degrading LYTAC. Reagents and conditions: (i) DIPEA, $CuSO_4$, sodium ascorbate, H_2O, MeCN, rt, 16 h; DIPEA, $CuSO_4$, sodium ascorbate, H_2O, MeCN, rt, 16 h; (ii) CH_3ONa, CH_3OH, rt, 16 h; (iii) H_2 (balloon), $Pd(OH)_2/C$, CH_3OH, rt, 48 h.

6.10 Summary

This chapter describes two methods towards poly[IdoA(2S)-GlcNS(6Sulfamate)] glycopolymer in an attempt to increase the potency of the ligand by altering its presentation towards Sulf-1/2. Initial attempts used a disaccharide-functionalised norbornene monomer, however due to solubility issues the polymerisation was terminated prematurely. A more successful approach proved to be post-polymerisation functionalisation of a NHS-ester containing polymer. Three polymers were made, with varying loadings of IdoA(2S)-GlcNS(6Sulfamate) ligands. The highest disaccharide containing polymer was evaluated

as an inhibitor of HSulf-2 and found to have an IC_{50} value of 9 μM ± 4, which is approximately 5-fold more potent than the parent inhibitor **89**.

Secondly, end capping of **228** was investigated through the synthesis of an azide-containing enol ether. However, due to time constraints this portion of work was not completed.

Finally, it was envisioned that the azide-end labelled **228** could be tethered to poly(M6P) via a CuAAC reaction (a ligand for CI-M6PR, for targeted protein degredation via LYTAC technology). The construction of the LYTAC is also included in future work.

7. Thesis summary and future work

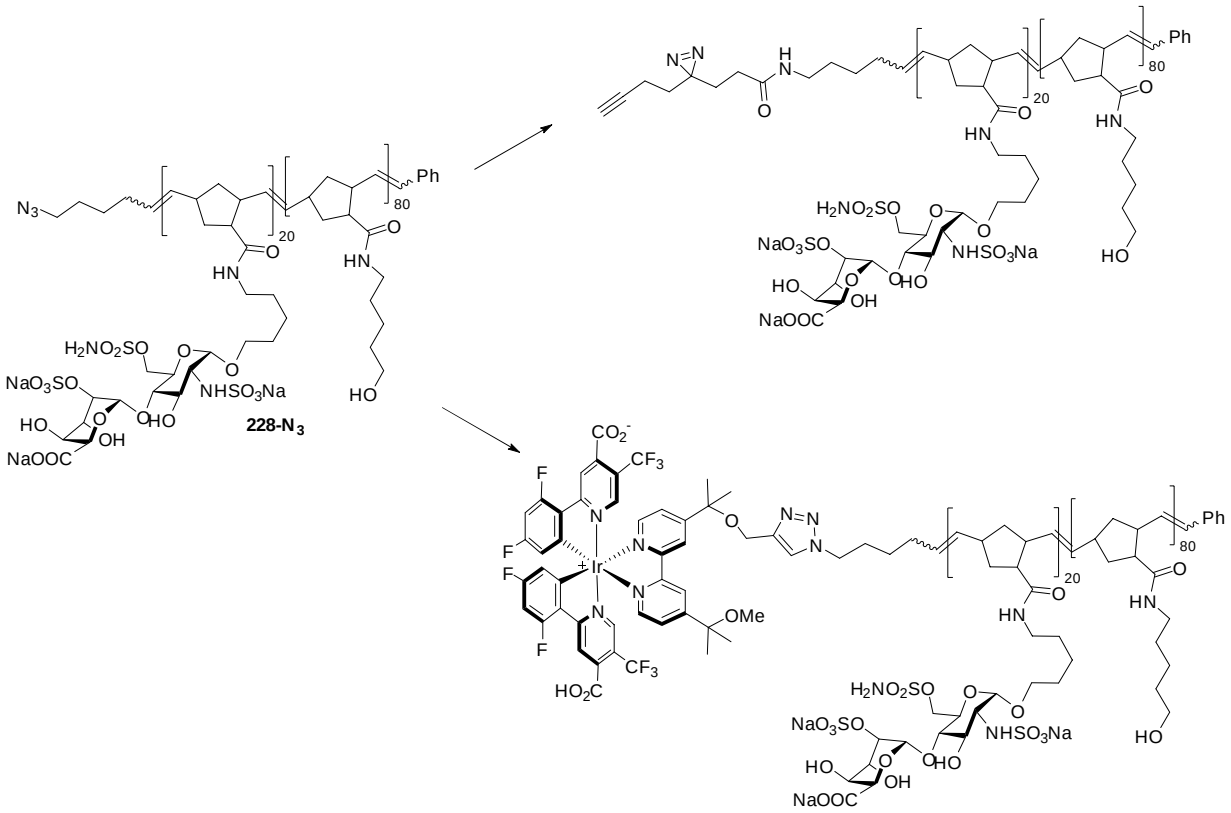
This thesis describes a multi-disciplinary project that aimed to develop chemical probes for studying endosulfatases in cells. The different approaches for tool development included small molecule inhibitors, target engagement probes via proximity labelling, and targeted degradation via LYTAC technology.

One of the major successes of this work is the development of a small molecule disaccharide inhibitor of endosulfatases, IdoA(2S)-GlcNS(6Sulfamate) **89**. Subsequent work focussed on incorporating a primary amine into this inhibitor so that it could be used for protein labelling and/or protein degradation. The synthesis of an amino-functionalised intermediate **163,** achieved over 17 steps, and its subsequent incorporation into target engagement probes was successfully implemented. The target engagement probes were evaluated in MCF-7 cells, and in a preliminary study it appears that the probes successfully labelled secreted proteins. Protein identification work is required to validate these probes as targeting endosulfatases.

A multivalent display of **89**, using a polynorbornene scaffold, was synthesised using a 'graft to' approach of polymer functionalisation. This glycopolymer was found to be approximately 5-fold more potent than **89** at inhibiting HSulf-2 in a biochemical assay, suggesting that the presentation of **89** towards HSulf-2 affects its inhibitory potential. Finally, it was envisioned that this glycopolymer could be tethered to a poly(M6P) ligand via a CuAAC reaction to produce a LYTAC compound for degradation of Sulfs in cells.

7.1 Future work

Following the successful development of an endosulfatase inhibiting glycopolymer (IC_{50} 9 μM), future work should include synthesis of the target engagement probes using this ligand as an alternative to the disaccharide **89**. The synthesis of these novel target engagement probes could be implemented following synthetic routes outlined in this thesis (scheme 54).

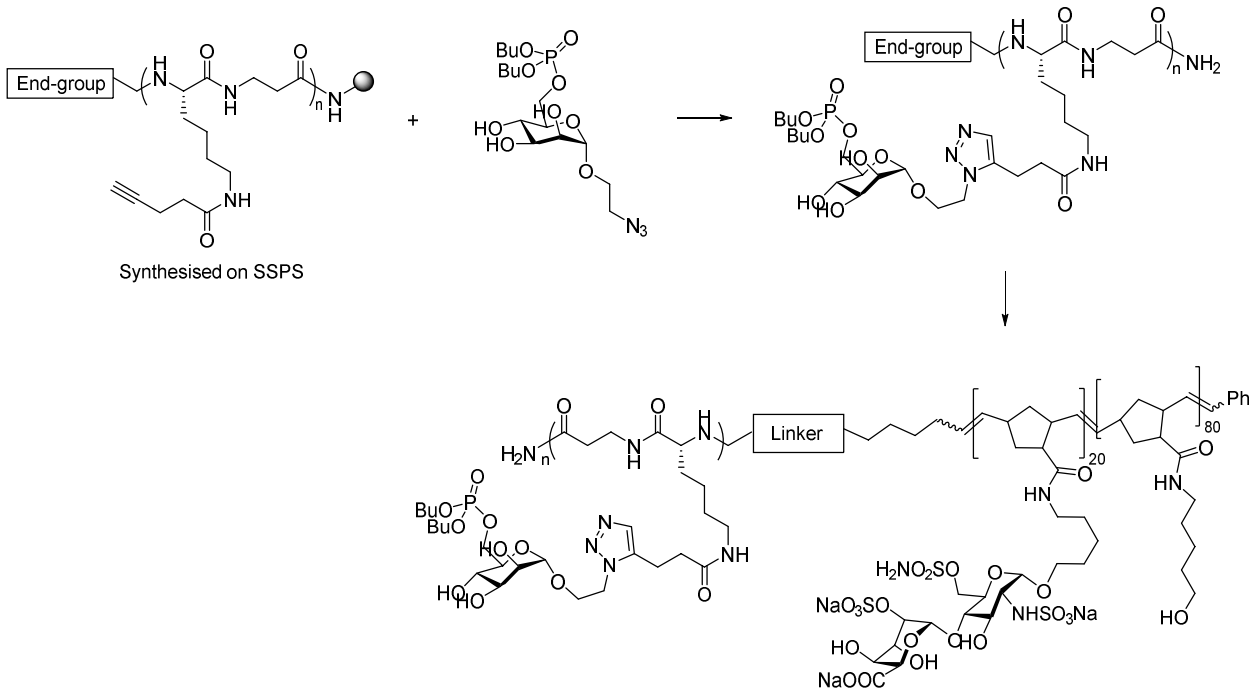


Scheme 54: Suggested target engagement probes based on glycopolymer **228**. (i) reduction of azide, then amide coupling with **174**; (ii) CuAAC with **200**.

The synthesis of the LYTAC compound should be completed. If the synthesis of the CI-M6PR targeting ligand, poly(M6P-Ala), proves too challenging, an alternative ligand has been reported by Hyun et al., [152] which involves the post-polymerisation CuAAC reaction between an alkyne-functionalised polypeptide and N_3-M6P(*t*-Bu)$_2$ (scheme 55). However,

a redesign of the ligand linkage unit would be required to append the endosulfatase targeting glycopolymer.

Finally, future work should include evaluation of the endosulfatase targeting tool compounds *in vitro* in cancer or regeneration assays to investigate the role of Sulfs in disease and to further confirm the selectivity and potency of these probes.



Scheme 55: Peptides containing M6P residues reported by Hyun et al, and proposed structure of alternative endosulfatase targeting LYTAC molecule.

8. Chemistry experimental

8.1 General Experimental

All reactions involving organometallic or other moisture-sensitive reagents were carried out under a nitrogen or argon atmosphere using standard vacuum line techniques and glassware that was flame dried and cooled under nitrogen before use. Solvents were dried according to the procedure outlined by Grubbs and co-workers. Water was purified by an Elix® UV-10 system. All other reagents were used as supplied (analytical or HPLC grade) without prior purification. Organic layers were dried over Na_2SO_4. Thin layer chromatography was performed on aluminium plates coated with 60 F254 silica. Plates were visualised using UV light (254 nm), 1% aq KMnO4, 10% ethanolic phosphomolybdic acid, 10% ethanolic *p*-anisaldehyde. Flash column chromatography was performed either on Kieselgel 60 silica on a glass column, or on a Biotage Isolera Four automated flash column chromatography platform.

Melting points were recorded on SRS MPA120 EZ-Melt Melting Point Apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a water-jacketed 10 cm cell. Specific rotations are reported in 10−1 deg cm2 g−1 and concentrations in g/100 mL. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer as a thin film on NaCl plates (film). Selected characteristic peaks are reported in cm–1. NMR spectra were recorded on Bruker Avance spectrometers in the deuterated solvent stated. Spectra were recorded at rt unless otherwise stated. The field was locked by external referencing to the relevant deuteron resonance. Low-resolution mass spectra were recorded on either an Agilent 6120 Single Quadrupole or a Waters LCT Premier spectrometer. Accurate mass measurements were run on either a Bruker MicroTOF internally calibrated with polyalanine, or a Micromass GCT instrument fitted with a

Scientific Glass Instruments BPX5 column (15 m × 0.25 mm) using amyl acetate as a lock mass. Analytical HPLC data were recorded on a PerkinElmer Flexar HPLC. GPC data were recorded on Agilent 1200 Series GPC-SEC System, PLgel MIXED GPC columns and $CHCl_3$ as the eluent.

8.2 General procedures

General Procedure 1: Chemoselective 6-*O*-sulfamoylation

Sulfamoyl chloride (3.0 eq.) in toluene (2 M) was added dropwise to the appropriate alcohol (1 eq.) in anhydrous DMF (2 mL/mmol) at −40 °C, under N_2 and the resulting solution was stirred at −40 °C for 16 h. The reaction was diluted with H_2O (5 mL) and extracted with EtOAc (10 mL). The organic layer was washed with brine (5 × 5 mL), dried over Na_2SO_4 and concentrated *in vacuo*.

General Procedure 2: Deprotection of benzyl carbamate via palladium catalysed hydrogenation

The appropriate benzyl carbamate (1 eq.) was dissolved in CH_3OH (1 M) under an atmosphere of N_2. Pd/C (10% w/w) was added and the flask was purged three times with N_2 before being sealed under an atmosphere of H_2. The reaction was stirred at rt for 16 h, filtered through Celite and concentrated *in vacuo.*

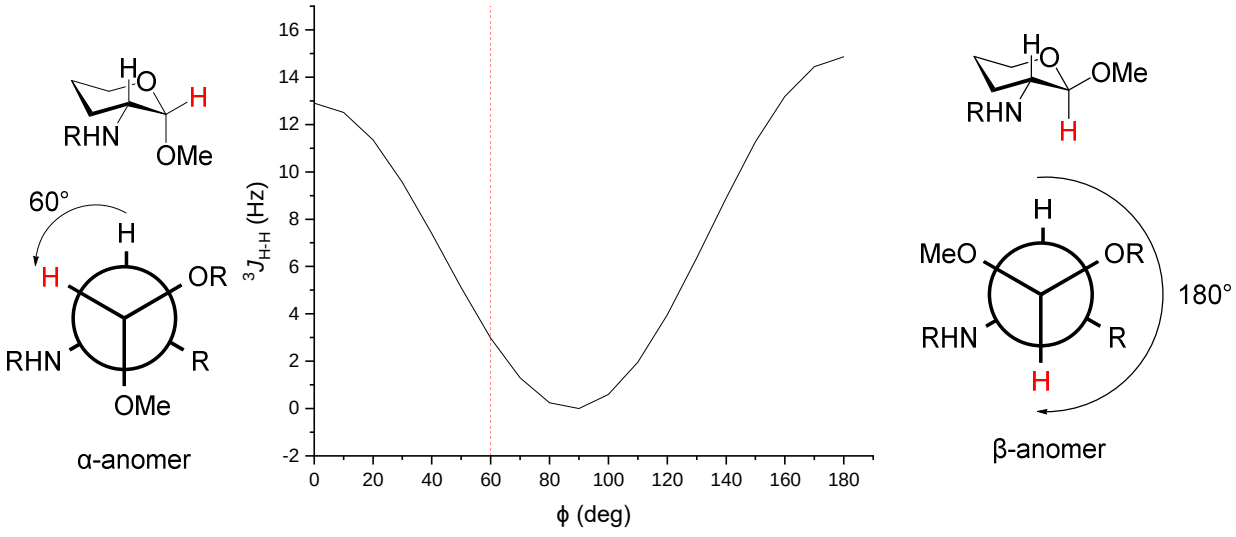
General Procedure 3: Chemoselective *N*-sulfation

The appropriate amine (1 eq.) was dissolved in H_2O (2 M) and the pH of the solution was adjusted to pH 9 - 10 with NaOH (2 M aq.). Pyridine-sulfur trioxide complex (1.1 eq.) was added portion-wise, and the pH of the solution was re-adjusted to pH 9-10 after each addition. The mixture was stirred at rt and retreated with pyridine-sulfur trioxide complex until complete (monitored by ^1H NMR spectroscopy). The solvent was removed *in vacuo*.

8.3 ^{1}H NMR assignment of the anomeric configuration

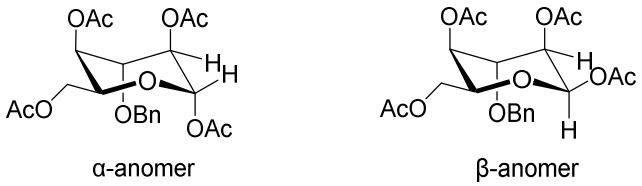
The anomeric configuration was identified using ^{1}H NMR spectroscopy based on chemical shifts of the C(1)*H* signal and the magnitude of its spin-spin coupling constant ($^{3}J_{H\text{-}H}$). In carbohydrates with an equatorial C(2)-OH (i.e. glucosamine), the C(1)*H* signal of the α-anomer is often more downfield than the β-anomer due to being more deshielded in the equatorial position. On the β-anomer, the C(1) axial-proton is more shielded by the proximity of the C(2)-hydroxyl. The magnitude of the coupling constant is determined by the dihedral angle between the two coupled protons, and this correlation is described by the Karplus equation:

$$J\,(\phi) = A\cos^2(\phi) + B\cos(\phi) + C$$

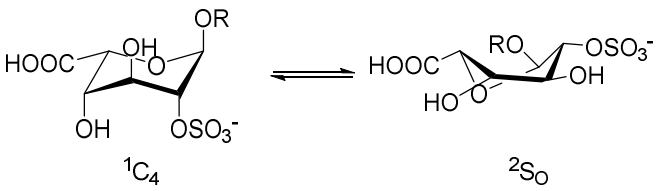


In glucosamine, the α-anomer has a dihedral angle of 60° and a coupling constant of 1-4 Hz, and the β-anomer has a dihedral angle of 180°, and while $^{3}J_{H\text{-}H}$ axial-axial has a coupling constant of 9-11 Hz, when it is axial- anomeric axial the coupling constant is 7-9 Hz.

In idose/iduronic acid, which exists primarily in the $^1C_4$ conformation, the α-anomer has a dihedral angle of <60° and a coupling constant of 0-2 Hz, and the β-anomer also has a dihedral angle of 60° and a coupling constant of 1-4 Hz.

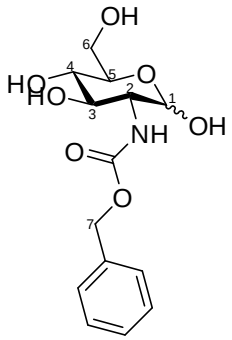


It should be noted, that when the IdoA has a sulfate in the C2 position, for example in compound **89**, the ^2S_O skew boat conformation becomes significant, which results in a coupling constant of 5-6 Hz.



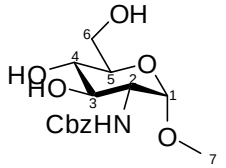
8.4 Compound preparations

Benzyl ((3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (37)



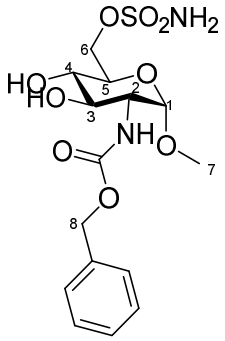
Benzyl chloroformate (21.8 mL, 153 mmol) was added dropwise to a solution of D-glucosamine hydrochloride (30.0 g, 139 mmol) and $NaHCO_3$ (35.1 g, 417 mmol) in H_2O (875 mL) at 0 °C. The resulting solution was stirred at rt for 16 h. The precipitate was collected via vacuum filtration and azeotroped with $PhCH_3$ (3 × 300 mL) to give **37,** as a mixture of anomers, as a white solid (41.8 g, 96%); mp 199-204 °C dec.; {lit.[85] mp 180 °C dec.}; ν_{max} (film) 3302 (br., O−H), 1681 (C=O), 1546 (C=O); δ_H (400 MHz, MeOH-d_4) 3.36 (t, *J* = 9.1 Hz, 1H, C(5)*H*), 3.57 (dd, *J* = 10.6, 3.4 Hz, 1H, C(2)*H*), 3.61 – 3.74 (m, 2H, C(3)*H*, C(4)*H*), 3.74 – 3.90 (m, 2H, C(6)H_2), 4.57 (d, *J* = 8.2 Hz, 0.2H, C(1β)*H*), 4.54 – 5.15 (m, 2.8H, C(1α)*H*, C(7)H_2), 7.22 – 7.43 (m, 5H, *Ph*); δ_C (126 MHz, MeOH-d_4) 57.7 (*C*(2)), 62.8 (*C*(6)), 67.6 (*C*(8)), 72.4 (*C*(5)), 72.9, 73.1 (*C*(3), *C*(4)), 92.9 (*C*(1α)), 97.3 (*C*(1β)), 128.9, 129.0 (*m*,*p*-Ph), 129.4 (*o*-Ph), 138.4 (*i*-Ph), 158.9 (*C*(7)); *m/z* (ESI^+) 336 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{14}H_{19}O_7NNa^+$ ($[M+Na]^+$) requires 336.1054; found 336.1055. Characterisation data are consistent with the literature.[85]

Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-4,5-dihydroxy-6-(hydroxymethyl)-2-methoxytetrahydro-2*H*-pyran-3-yl)carbamate (38)



37 (5.13 g, 16.4 mmol) was suspended in CH_3OH (40 mL) and HCl (4 M in 1,4-dioxane, 12.5 mL) and the reaction was heated at 80 °C for 16 h. The reaction was concentrated *in vacuo* and the residue was purified via column chromatography (eluent CH_3OH/CH_2Cl_2, 1:9) to give **38** as a white solid (3.07 g, 57%); mp 157-160 °C; {lit.[85] mp 159-160 °C}; $[\alpha]_{25}^{D}$ +95.7 (*c* 1.0 in CH_3OH); {lit.[85] $[\alpha]_{25}^{D}$ +84.8 (*c* 1.0 in CH_3OH)}; ν_{max} (film) 3313 (O–H), 2913 (C–H), 1671 (C=O), 1544 (C–O); δ_H (400 MHz, MeOH-d_4) 3.36 (s, 4H, C(4)*H*, C(7)H_3), 3.51 – 3.66 (m, 3H, C(2)*H*, C(3)*H*, C(5)*H*), 3.69 (dd, J = 11.88, 5.64 Hz, 1H, C(6)H_A), 3.82 (dd, J = 11.83, 2.36 Hz, 1H, C(6)H_B), 4.57 (s, 1H, N*H*), 4.68 (d, J = 3.05 Hz, 1H, C(1)*H*), 5.09 (s, 2H, CH_2Ph), 7.16 – 7.47 (m, 5H, *Ph*); δ_C (101 MHz, MeOH-d_4) 55.6 (*C*(7)), 57.2 (*C*(2)), 62.7 (*C*(6)), 67.6 (*C*H_2Ph), 72.2 (*C*(4)), 73.1 (*C*(3)), 73.6 (*C*(5)), 100.2 (*C*(1)), 128.9 (*o-Ph*), 129.0 (*p-Ph*), 129.4 (*m-Ph*), 138.2 (*i-Ph*), 158.8 (*C*=O); *m/z* (ESI^+) 350 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{15}H_{22}NO_7^+$ ($[M+H]^+$) requires 328.1391; found 328.1393. Characterisation is consistent with the literature.[85]

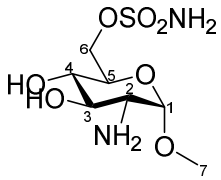
((2*R*,3*S*,4*R*,5*R*,6*S*)-5-(((Benzyloxy)carbonyl)amino)-3,4-dihydroxy-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (39)



Following *general procedure 1*, sulfamoyl chloride (212 mg, 1.83 mmol) in toluene (2 mL) was reacted with carbamate **38** (200 mg, 610 μmol) in DMF (2 mL). Purification via column chromatography (gradient elution, 0%→5% CH_3OH in CH_2Cl_2) gave **39** as a white solid (93 mg, 45%); mp 193-195 °C dec.; {lit.[85] mp 104-106 °C}; $[\alpha]_D^{25}$ +77.65 (*c* 0.43 in CH_3OH); {lit.[85] $[\alpha]_D^{22.6}$ +40.93 (*c* 0.43 in CH_3OH)}; δ_H (400 MHz, MeOH-d_4) 3.33 – 3.42

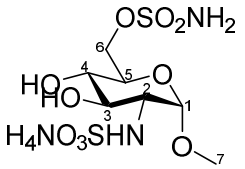
(m, 4H, C(4)*H*, C(7)H_3), 3.53 – 3.67 (m, 2H, C(2)*H*, C(3)*H*), 3.76 (ddd, *J* = 10.2, 6.0, 1.9 Hz, 1H, C(5)*H*), 4.25 (dd, *J* = 10.7, 6.0 Hz, 1H, C(6)H_A), 4.40 (dd, *J* = 10.7, 2.0 Hz, 1H, C(6)H_B), 4.68 (d, *J* = 3.2 Hz, 1H, C(1)*H*), 5.09 (s, 2H, C(8)H_2), 7.25 – 7.43 (m, 5H, *Ph*); δ_C (126 MHz, DMSO-d_6) 54.6 (*C*(7))), 55.7 (*C*(2)), 65.4 (*C*(8)), 68.6 (*C*(6)), 69.6 (*C*(5)), 70.4 (*C*(3)), 70.6 (*C*(4)), 98.1 (*C*(1)), 127.8, 127.8, 128.4 (*o,m,p-Ph*), 137.1 (*i-Ph*), 156.2 (*C*(7)); *m/z* (ESI$^+$) 429 ([M+Na]$^+$, 38%), 407 ([M+H]$^+$, 12%); HRMS (ESI$^+$) $C_{15}H_{23}N_2O_9S^+$ ([M+H]$^+$) requires 407.1119; found 407.1120. Characterisation data are consistent with the literature.[85]

((2*R*,3*S*,4*R*,5*R*,6*S*)-5-Amino-3,4-dihydroxy-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (40)



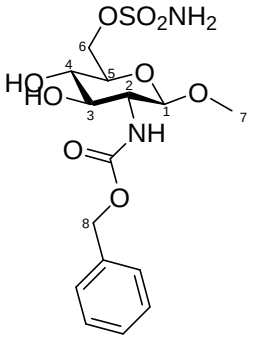
Following *general procedure 2*, **39** (630 mg, 1.55 mmol) and Pd/C (6 mg) were reacted in CH_3OH (6 mL), and the product was isolated without further purification to give **40** as a white solid (356 mg, 85%); mp 100-104 °C; {lit.[85] mp 104-112 °C}; $[\alpha]_D^{25}$ +105 (*c* 0.5 in CH_3OH); {lit.[85] $[\alpha]_D^{17.1}$ +59.2 (*c* 0.5 in CH_3OH)}; δ_H (400 MHz, MeOH-d_4) 2.61 (dd, *J* = 9.9, 3.6 Hz, 1H, C(2)*H*), 3.27 (dd, *J* = 10.1, 8.8 Hz, 1H, C(4)*H*), 3.41 (s, 3H, C(7)H_3), 3.44 (dd, *J* = 10.0, 8.8 Hz, 1H, C(3)*H*), 3.76 (ddd, *J* = 10.2, 5.8, 2.0 Hz, 1H, C(5)*H*), 4.25 (dd, *J* = 10.7, 5.9 Hz, 1H, C(6)H_A), 4.39 (dd, *J* = 10.7, 2.0 Hz, 1H, C(6)H_B), 4.68 (d, *J* = 3.6 Hz, 1H, C(1)*H*); δ_C (126 MHz, MeOH-d_4) 55.7 (*C*(7))), 57.0 (*C*(2)), 70.1 (*C*(6)), 71.5 (*C*(5)), 71.7 (*C*(4)), 76.1 (*C*(3)), 101.4 (*C*(1)); *m/z* (ESI$^+$) 273 ([M+H]$^+$, 62%), 295 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_7H_{17}N_2O_7S^+$ ([M+H]$^+$) requires 273.0751; found 273.0751. Characterisation data are consistent with the literature.[85]

Ammonium ((2*S*,3*R*,4*R*,5*S*,6*R*)-4,5-dihydroxy-2-methoxy-6-((sulfamoyloxy) methyl)tetrahydro-2*H*-pyran-3-yl)sulfamate (27)



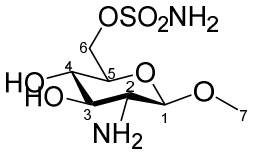
Following *general procedure 3*, amine **40** (100 mg, 370 μmol) was reacted with pyridine-sulfur trioxide complex (64.0 mg, 400 μmol) in H_2O (1 mL) at rt for 16 h. The reaction was retreated with with pyridine-sulfur trioxide complex (64.0 mg, 400 μmol) and stirred at rt for 2 h. Purification via column chromatography (eluent CH_3OH:CH_2Cl_2:NH_4OH 3:7:0.01) gave **27** as a white solid (22 mg, 16%); mp 173-183 °C (dec.); {lit.[85] mp 125-135 °C}; $[\alpha]_D^{25}$ +68.8 (*c* 0.32 in CH_3OH); {lit.[85] $[\alpha]_D^{16.9}$ +103.13 (*c* 0.32 in CH_3OH)}; δ_H (400 MHz, MeOH-d_4) 3.14 (dd, *J* = 10.2, 3.6 Hz, 1H, C(2)*H*), 3.26 (dd, *J* = 10.0, 8.8 Hz, 1H, C(4)*H*), 3.30 (s, 3H, C(7)H_3), 3.45 (dd, *J* = 10.2, 8.8 Hz, 1H, C(3)*H*), 3.66 (ddd, *J* = 9.9, 6.1, 1.8 Hz, 1H, C(5)*H*), 4.15 (dd, *J* = 10.7, 6.1 Hz, 1H, C(6)H_A), 4.30 (dd, *J* = 10.7, 1.9 Hz, 1H, C(6)H_B), 4.86 (d, *J* = 3.6 Hz, 1H, C(1)*H*); δ_C (126 MHz, D_2O) 55.5 (*C*(7)), 57.5 (*C*(2)), 68.8 (*C*(6)), 69.1 (*C*(5)), 69.5 (*C*(4)), 71.2 (*C*(3), 98.6 (*C*(1)); *m/z* (ESI^-) 351 ($[M\text{-}NH_4]^-$, 100%); HRMS (ESI^-) $C_7H_{15}N_2O_{10}S_2^-$ ($[M–NH_4]^-$) requires 351.0174; found 351.0173. Characterisation data are consistent with the literature.[85]

((2*R*,3*S*,4*R*,5*R*,6*R*)-5-(((Benzyloxy)carbonyl)amino)-3,4-dihydroxy-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (39-β)



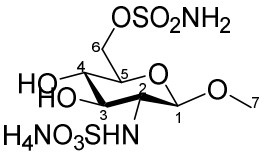
Following *general procedure 1*, sulfamoyl chloride (141 mg, 1.22 mmol) in $PhCH_3$ (2 mL) was reacted with carbamate **39-β** (200 mg, 610 μmol) in DMF (2 mL). Purification via flash column chromatography (eluent, EtOAc) gave **40-β** as a white solid (74 mg, 30%); mp 153-157 °C; {lit.[85] mp 155 °C (dec.)}; δ_H (400 MHz, MeOH-*d*$_4$) 3.32 – 3.53 (m, 7H, C(2)*H*, C(3)*H*, C(4)*H*, C(5)*H*, C(7)*H*), 4.19 – 4.34 (m, 2H, C(6)H_2), 4.45 (dd, *J* = 10.8, 1.9 Hz, 1H, C(1)*H*), 5.09 (s, 2H, C(8)H_2), 7.24 – 7.43 (m, 5H, *Ph*); *m/z* (ESI$^+$) 429 ([M+Na]$^+$). Characterisation data are consistent with the literature.[85]

((2*R*,3*S*,4*R*,5*R*,6*R*)-5-Amino-3,4-dihydroxy-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (40-β)



Following *general procedure 2*, **39-β** (90 mg, 0.221 mmol) and Pd/C (9 mg) in CH_3OH (2 mL) gave **40-β** as a colourless gum (57 mg, 98%); δ_H (400 MHz, D_2O) 2.95 (dd, *J* = 10.0, 8.4 Hz, 1H, C(2)*H*), 3.43 – 3.67 (m, 6H, C(3)*H*, C(4)*H*, C(5)*H*, C(7)H_3), 4.36 (dd, *J* = 11.2, 4.8 Hz, 1H, C(1)*H*), 4.42 – 4.48 (m, 2H, C(6)H_2); *m/z* (ESI$^+$) 273 ([M+H]$^+$, 62%), 295)[M+Na]$^+$, 100%). Characterisation data are consistent with the literature.[85]

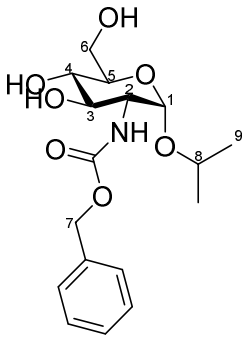
Ammonium ((2*R*,3*R*,4*R*,5*S*,6*R*)-4,5-dihydroxy-2-methoxy-6 ((sulfamoyloxy)methyl)tetrahydro-2*H*-pyran-3-yl)sulfamate (41)



Following *general procedure 3*, amine **40-β** (70.0 mg, 0.257 mmol) was reacted with pyridine-sulfur trioxide complex (49.0 mg, 0.309 mmol) in H_2O (1 mL). Purification via column chromatography (eluent CH_2Cl_2:CH_3OH:NH_4OH 7:3:0.01) gave **41** as a white solid (22 mg, 23%); mp 92-94 °C; {lit.[85] mp 130 °C (dec.)}; $[\alpha]_D^{25}$ –38.3 (*c* 0.37 in CH_3OH); δ_H (400 MHz, MeOH-*d*$_4$) 2.91 (dd, *J* = 9.8, 8.2 Hz, 1H, C(2)*H*), 3.26 (dd, *J* = 9.8,

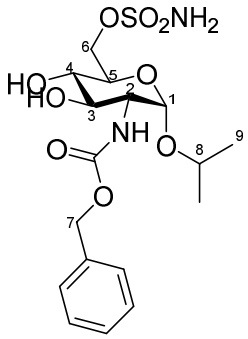
8.8 Hz, 1H, C(4)*H*), 3.40 (s, 3H, C(7)H_3), 3.44 (ddd, *J* = 9.9, 6.0, 1.9 Hz, 1H, C(5)*H*), 3.59 (dd, *J* = 9.7, 8.8 Hz, 1H, C(3)*H*), 4.15 (dd, *J* = 10.8, 6.0 Hz, 1H, C(6)H_A), 4.27 (d, *J* = 8.2 Hz, 1H, C(1)*H*), 4.34 (dd, *J* = 10.8, 1.9 Hz, 1H, C(6)H_B); Characterisation data are consistent with the literature.[85]

Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-4,5-dihydroxy-6-(hydroxymethyl)-2-isopropoxytetrahydro-2*H*-pyran-3-yl)carbamate (42)



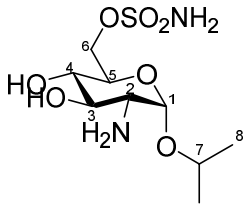
37 (2.00 g, 6.39 mmol) was suspended in 2-propanol (13.8 mL) and HCl (4M in dioxane, 6.2 mL) and the suspension was heated at 80 °C for 16 h. The solvent was removed *in vacuo* and purification via column chromatography (eluent CH_3OH:CH_2Cl_2 1:9) gave the α-anomer **42** as an off-white amorphous solid (420 mg, 18%); mp 156-157 °C; {lit.[85] mp 158-159 °C}; $[\alpha]_D^{25}$ + 116.4 (*c* 0.27 in EtOH); {lit.[85] $[\alpha]_D^{22.7}$ +114.1 (*c* 0.27 in EtOH)}; δ_H (400 MHz, MeOH-d_4) 0.99 (d, *J* = 6.1 Hz, 3H, C(9)H_3), 1.11 (d, *J* = 6.2 Hz, 3H, C(9)H_3), 3.22 – 3.28 (m, 1H, C(3)*H*), 3.43 – 3.64 (m, 4H, C(2)*H*, C(4)*H*, C(5)*H*, C(6)H_A), 3.66 – 3.72 (m, 1H, C(6)H_B), 3.78 (dt, *J* = 12.3, 6.2 Hz, 1H, C(8)*H*), 4.81 (d, *J* = 2.7 Hz, 1H, C(1)*H*), 4.99 (apparent q, *J* = 12.5 Hz, 2H, C(7)H_2), 7.11 – 7.45 (m, 5H, *Ph*). Data is consistent with the literature.[85]

((2*R*,3*S*,4*R*,5*R*,6*S*)-5-(((Benzyloxy)carbonyl)amino)-3,4-dihydroxy-6-isopropoxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (43)



Following *general procedure 1*, sulfamoyl chloride (390 mg, 3.37 mmol) in $PmCH_3$ (4 mL) was reacted with carbamate **42** (400 mg, 1.12 mmol) in DMF (4 mL). Purification via flash column chromatography (gradient elution, 0%→5% CH_3OH in CH_2Cl_2) gave **43** as a white solid (175 mg, 36%); mp 172-175 °C; {lit.[85] mp 166-168 °C}; $[\alpha]_D^{25}$ +27.3 (*c* 0.1 in CH_3OH); δ_H (400 MHz, MeOH-d_4) 1.09 (d, *J* = 6.1 Hz, 3H, C(9)H_3), 1.21 (d, *J* = 6.2 Hz, 3H, C(9)H_3), 3.33 – 3.36 (m, 1H, C(4)*H*), 3.53 – 3.63 (m, 2H, C(2)*H*, C(3)*H*), 3.78 – 3.95 (m, 2H, C(5)*H*, C(8)*H*), 4.24 (dd, *J* = 10.6, 5.9 Hz, 1H, C(6)H_A), 4.38 (dd, *J* = 10.6, 1.8 Hz, 1H, C(6)H_B), 4.91 (d, *J* = 2.6 Hz, 1H, C(1)*H*), 5.09 (apparent q, *J* = 12.5 Hz, 2H, C(7)H_2), 7.14 – 7.48 (m, 5H, *Ph*); Characterisation data are consistent with the literature.[85]

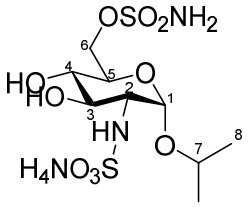
((2*R*,3*S*,4*R*,5*R*,6*S*)-5-Amino-3,4-dihydroxy-6-isopropoxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (44)



Following *general procedure 2*, benzyl carbamate **43** (140 mg, 0.344 mmol) and Pd/C (2 mg) in CH_3OH (2 mL) gave **44** as a colourless gum (91 mg, 88%); $[\alpha]_D^{25}$ +172 (*c* 0.1 in CH_3OH); δ_H (400 MHz, MeOH-d_4) 1.10 (d, *J* = 6.1 Hz, 3H, C(8)H_3), 1.16 (d, *J* = 6.1 Hz, 3H, C(8)H_3), 2.68 (dd, *J* = 10.2, 3.7 Hz, 1H, C(2)*H*), 3.45 (dd, *J* = 10.1, 8.9 Hz, 1H, C(4)*H*), 3.77 (dtt, *J* = 11.3, 5.8, 2.8 Hz, 1H, C(7)*H*), 3.85 (dt, *J* = 12.4, 6.2 Hz, 1H, C(5)*H*), 4.15

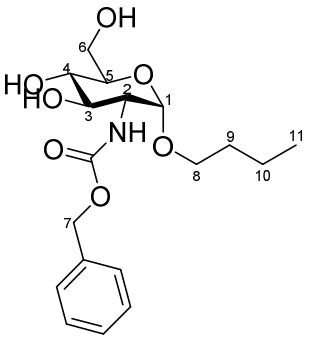
(dd, *J* = 10.7, 5.9 Hz, 1H, C(6)H_A), 4.28 (dd, *J* = 10.7, 2.0 Hz, 1H, C(6)H_B), 4.88 (d, *J* = 3.6 Hz, 1H, C(1)*H*); *m/z* (ESI^+) 301 ($[M+H]^+$, 100%). Characterisation data are consistent with the literature.[85]

Ammonium ((2*S*,3*R*,4*R*,5*S*,6*R*)-4,5-dihydroxy-2-isopropoxy-6-((sulfamoyloxy)methyl)tetra-hydro-2*H*-pyran-3-yl)sulfamate (45)



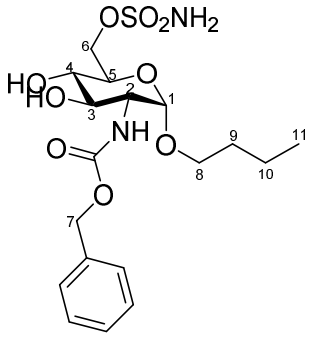
Following *general procedure 3*, **44** (20.0 mg, 67.0 μmol) was reacted with pyridine-sulfur trioxide complex (17.0 mg, 110 μmol) in H_2O (1 mL). The reaction was retreated with pyridine-sulfur trioxide complex (4 mg, 0.028 mmol). Purification via column chromatography (eluent CH_2Cl_2:CH_3OH:NH_4OH 7:3:0.01) gave **45** as a white solid (3 mg, 11%); mp 40-43 °C; {lit.[85] mp 50-70 °C}; δ_H (400 MHz, D_2O) 1.15 (d, *J* = 6.2 Hz, 3H, C(8)H_3), 1.19 (d, *J* = 6.3 Hz, 3H, C(8)H_3), 3.18 (dd, *J* = 10.3, 3.8 Hz, 1H, C(2)*H*), 3.47 (dd, *J* = 9.9, 9.1 Hz, 1H, C(4)*H*), 3.56 (dd, *J* = 10.2, 9.0 Hz, 1H, C(3)*H*), 3.86 – 3.99 (m, 2H, C(5)*H*, C(7)*H*), 4.31 – 4.45 (m, 2H, C(6)H_2), 5.22 (d, *J* = 3.7 Hz, 1H, C(1)*H*); *m/z* (ESI^-) 379 ($[M–NH_4]^-$, 100%). Characterisation data are consistent with the literature.[85]

Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-2-butoxy-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)-carbamate (46)



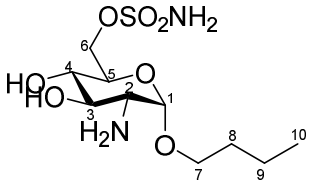
37 was suspended in butan-1-ol (6.9 mL) and HCl (4M in dioxane, 3.1 mL) and heated at 60 °C for 3 h. The resulting solution was diluted with satd aq $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→5% CH_3OH in EtOAc) gave the α-anomer **46** as a white solid (814 mg, 69%); mp 143-144 °C; $[\alpha]_D^{25}$ +118 (*c* 0.1 in CH_3OH); ν_{max} (film) 3313 br (O-H), 2931, 2885, 2160, 2033, 1688 (C=O), 1537 (C=O); δ_H (500 MHz, $(CD_3)_2CO$) 0.89 (t, *J* = 7.4 Hz, 3H, C(11)H_3), 1.33 – 1.44 (m, 2H, C(10)H_2), 1.47 – 1.62 (m, 2H, C(9)H_2), 3.36 (dt, *J* = 9.7, 6.3 Hz, 1H, C(8)H_A), 3.39 – 3.47 (m, 1H, C(4)*H*), 3.53 (t, *J* = 6.3 Hz, 1H, O*H*), 3.55 – 3.65 (m, 2H, C(2)*H*, C(5)*H*), 3.65 – 3.73 (m, 3H, C(3)*H*, C(6)H_A, C(8)H_B), 3.74 – 3.83 (m, 1H, C(6)H_B), 4.09 (d, *J* = 5.0 Hz, 1H, O*H*), 4.24 (d, *J* = 4.4 Hz, 1H, O*H*), 4.79 (d, *J* = 3.5 Hz, 1H, C(1)*H*), 5.02 – 5.11 (m, 2H, C(7)H_2), 6.04 (d, *J* = 8.6 Hz, 1H, N*H*), 7.26 – 7.43 (m, 5H, *Ph*); δ_C (126 MHz, $(CD_3)_2CO$) 14.2 (*C*(12)), 20.0 (*C*(11)), 32.3 (*C*(10)), 56.9 (*C*(2)), 62.9 (*C*(6)), 66.6 (*C*(7)), 68.0 (*C*(9)), 72.6 (*C*(4)), 73.0 (*C*(3)), 73.4 (*C*(5)), 98.5 (*C*(1)), 128.6, 128.6, 129.2 (*o,m,p-Ph*), 138.3 (*i-Ph*), 157.2 (*C*O); *m/z* (ESI$^+$) 392 ([M+Na]$^+$, 100%), 252 (20%); HRMS (ESI$^+$) $C_{18}H_{27}NNaO_7^+$ ([M+Na]$^+$) requires 392.1680; found 392.1684.

((2*R*,3*S*,4*R*,5*R*,6*S*)-5-(((Benzyloxy)carbonyl)amino)-6-butoxy-3,4-dihydroxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (47)



Following *general procedure 1*, sulfamoyl chloride (469 mg, 4.06 mmol) in $PhCH_3$ (2.5 mL) was reacted with **46** (500 mg, 1.35 mmol) in DMF (2.5 mL). Purification via column chromatography (gradient elution, 0%→5% CH_3OH in CH_2Cl_2) gave **47** as a white solid (213 mg, 35%); mp 159-162 °C; $[\alpha]_D^{25}$ +95 (*c* 0.1 in CH_3OH); ν_{max} (film) 3414, 3377, 3341, 3239, 1694 (C=O), 1540 (C=O), 1372 (S=O), 1290 (S=O); δ_H (500 MHz, $(CD_3)_2CO$) 0.89 (t, *J* = 7.4 Hz, 3H, C(11)*H*$_3$), 1.33 – 1.46 (m, 2H, C(10)*H*$_2$), 1.48 – 1.64 (m, 2H, C(9)*H*$_2$), 3.33 – 3.47 (m, 2H, C(4)*H*, C(8)*H*$_A$), 3.60 – 3.67 (m, 1H, C(2)*H*), 3.67 – 3.78 (m, 2H, C(8)*H*$_B$, C(3)*H*), 3.83 (ddd, *J* = 10.3, 5.9, 2.0 Hz, 1H, C(5)*H*), 4.22 (d, *J* = 5.1 Hz, 1H, O*H*), 4.29 (dd, *J* = 10.7, 5.9 Hz, 1H, C(6)*H*$_A$), 4.44 (dd, *J* = 10.8, 2.0 Hz, 1H, C(6)*H*$_B$), 4.48 (d, *J* = 4.8 Hz, 1H, O*H*), 4.82 (d, *J* = 3.5 Hz, 1H, C(1)*H*), 5.01 – 5.13 (m, 2H, C(7)*H*$_2$), 6.10 (d, *J* = 8.6 Hz, 1H, N*H*), 6.65 (d, *J* = 5.2 Hz, 2H, N*H*$_2$), 7.26 – 7.46 (m, 5H, *Ph*); δ_C (126 MHz, $(CD_3)_2CO$) 13.3 (*C*(12)), 19.1 (*C*(11)), 31.4 (*C*(10)), 55.9 (*C*(2)), 65.8 (*C*(7)), 67.4 (*C*(10)), 69.0 (*C*(6)), 70.1 (*C*(5)), 71.0 (*C*(4)), 72.0 (*C*(3)), 97.6 (*C*(1)), 127.7, 127.7, 128.3 (*o,m,p-Ph*), 137.4 (*i-Ph*), 156.3 (*C*O); *m/z* (ESI$^+$) 471 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{18}H_{29}N_2O_9S^+$ ([M+H]$^+$) requires 449.1588; found 449.1589.

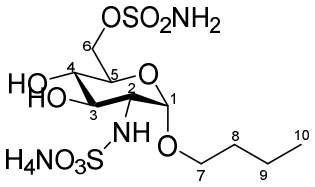
((2*R*,3*S*,4*R*,5*R*,6*S*)-5-Amino-6-butoxy-3,4-dihydroxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (48)



Following *general procedure 2*, **47** (200 mg, 0.446 mmol) and Pd/C (20 mg) in CH_3OH (3 mL). Purification via column chromatography (eluent gradient, 0%→10% CH_3OH in CH_2Cl_2) gave **48** as a colourless gum (36 mg, 26%); $[\alpha]_D^{25}$ +73.1 (*c* 0.45 in CH_3OH); ν_{max} (film) 3317 (O–H), 2958 (C–H), 2930 (C–H), 2874 (C–H), 1464 (C=O), 1350 (S=O), 1181 (S=O); δ_H (400 MHz, D_2O) 0.86 (t, *J* = 7.4 Hz, 3H, C(10)*H*$_3$), 1.26 – 1.40 (m, 2H, C(9)*H*$_2$),

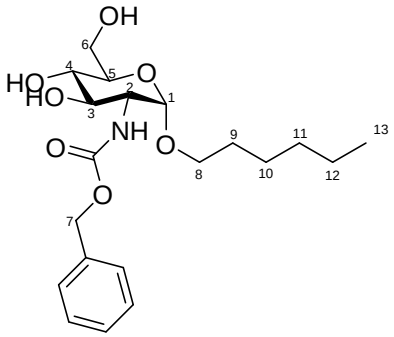
1.48 – 1.63 (m, 2H, C(8)H_2), 3.01 (dd, *J* = 10.3, 3.7 Hz, 1H, C(2)*H*), 3.42 – 3.54 (m, 2H, C(4)*H*, C(7)H_A), 3.64 – 3.77 (m, 2H, C(3)*H*, C(7)H_B), 3.91 (ddd, *J* = 10.0, 4.5, 2.4 Hz, 1H, C(5)*H*), 4.32 – 4.45 (m, 2H, C(6)H_2), 4.96 (d, *J* = 3.7 Hz, 1H, C(1)*H*); δ_C (126 MHz, D_2O) 13.0 (*C*(10)), 18.7 (*C*(9)), 30.7 (*C*(8)), 54.3 (*C*(2)), 68.4 (*C*(7)), 68.6 (*C*(6)), 69.2 (*C*(4)), 69.7 (*C*(3)), 71.8 (*C*(5)), 96.8 (*C*(1)); *m/z* (ESI^-) 314 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_{10}H_{23}N_2O_7S^+$ ($[M+H]^+$) requires 315.1221; found 315.1220.

((2*S*,3*R*,4*R*,5*S*,6*R*)-2-Butoxy-4,5-dihydroxy-6-((sulfamoyloxy)methyl)tetrahydro-2*H*-pyran-3-yl)sulfamic acid (49)



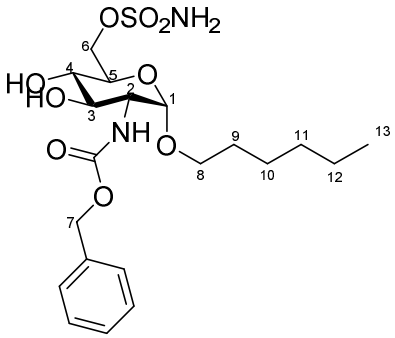
Following *general procedure 3*, **48** (30 mg, 0.095 mmol) was reacted with pyridine-sulfur trioxide complex (17 mg, 0.105 mmol) in H_2O (1 mL) and THF (0.3 mL). The solvent was removed *in vacuo* and the residue was triturated in CH_3OH, filtered and the filtrate was concentrated *in vacuo.* Purification via column chromatography (eluent CH_3OH:CH_2Cl_2 3:7 with 1% NH_4OH) gave **49** as a white solid (14 mg, 36%); $[\alpha]_D^{25}$ +100 (*c* 0.1 in CH_3OH); δ_H (400 MHz, D_2O) 0.92 (t, J = 7.4 Hz, 3H, C(10)H_3), 1.32 – 1.45 (m, 2H, C(9)H_2), 1.56 – 1.70 (m, 2H, C(8)H_2), 3.38 (dd, J = 10.6, 3.7 Hz, 1H, C(2)*H*), 3.53 – 3.62 (m, 2H, C(4)*H*, C(7)H_A), 3.79 (dt, J = 9.7, 6.8 Hz, 1H, C(7)H_B), 3.90 (dd, J = 10.6, 9.0 Hz, 1H, C(3)*H*), 3.99 (ddd, J = 10.2, 4.5, 2.5 Hz, 1H, C(5)*H*), 4.39 – 4.56 (m, 2H, C(6)H_2), 5.15 (d, J = 3.7 Hz, 1H, C(1)*H*), δ_C (101 MHz, D_2O) 13.0 (*C*(10)), 18.7 (*C*(9)), 30.7 (*C*(8)), 53.8 (*C*(2)), 68.5 (*C*(6)), 68.6 (*C*(7)), 69.1 (*C*(4)), 69.7 (*C*(5)), 69.9 (*C*(3)), 95.2 (*C*(1)); *m/z* (ESI^-) 393 ($[M–H]^-$, 100%); HRMS (ESI^-) $C_{10}H_{21}N_2O_{10}S_2^-$ ($[M–NH_4)^-$) requires 393.0643; found 393.0641.

Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-2-(hexyloxy)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (50)



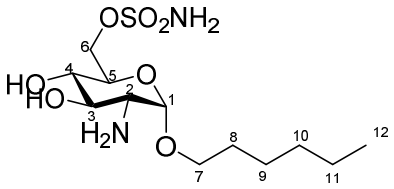
37 (800 mg, 2.56 mmol) was suspended in 1-hexanol (6.9 mL) and HCl (4M in dioxane, 3.1 mL) and heated at 60 ºC for 3 h. The resulting solution was diluted with satd aq $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→100% EtOAc in pentane followed by 0%→5% CH_3OH in EtOAc) gave the α-anomer **50** as a white solid (793 mg, 63%); mp 131-133 °C; $[\alpha]_D^{25}$ +102 (*c* 0.1 in CH_3OH); ν_{max} (film) 3315 br (O-H), 1689 (C=O), 1537 (C=O); δ_H (500 MHz, $(CD_3)_2CO$) 0.83 – 0.90 (m, 3H, C(13)H_3), 1.26 – 1.32 (m, 4H, C(11)H_2, C(12)H_2), 1.33 – 1.42 (m, 2H, C(10)H_2), 1.49 – 1.63 (m, 2H, C(9)H_2), 3.37 (dt, *J* = 9.7, 6.4 Hz, 1H, C(8)H_A), 3.40 – 3.47 (m, 1H, C(4)*H*), 3.52 (t, *J* = 6.3 Hz, 1H, O*H*), 3.55 – 3.66 (m, 2H, C(2)*H*, C(3)*H*), 3.66 – 3.73 (m, 3H, C(5)*H*, C(8)H_B, C(6)H_A), 3.76 – 3.83 (m, 1H, C(6)H_B), 4.08 (d, *J* = 5.0 Hz, 1H, O*H*), 4.23 (d, *J* = 4.4 Hz, 1H, O*H*), 4.79 (d, *J* = 3.5 Hz, 1H, C(1)*H*), 5.06 (s, 2H, C(7)H_2), 6.03 (d, *J* = 8.7 Hz, 1H, N*H*), 7.27 – 7.41 (m, 5H, *Ph*); δ_C (126 MHz, $(CD_3)_2CO$) 14.3 (*C*(13)), 23.3 (*C*(12)), 26.6 (*C*(10)), 32.4 (*C*(11)), 56.9 (*C*(2)), 62.9 (*C*(6)), 66.6 (*C*(7)), 68.3 (*C*(8)), 72.6 (*C*(4)), 73.0 (*C*(5)), 73.4 (*C*(2)), 98.5 (*C*(1)), 128.6, 128.6, 129.2 (*o,m,p-Ph*), 138.3 (*i-Ph*), 157.2 (*C*O), (*C*(9) under acetone); *m/z* (ESI^+) 420 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{20}H_{31}NNaO_7^+$ ($[M+Na]^+$) requires 420.1998; found 420.1989.

((2*R*,3*S*,4*R*,5*R*,6*S*)-5-(((Benzyloxy)carbonyl)amino)-6-(hexyloxy)-3,4-dihydroxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (51)



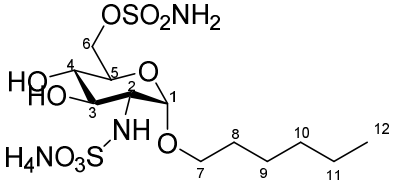
Following *general procedure 1*, sulfamoyl chloride (426 mg, 3.77 mmol) in $PhCH_3$ (2.5 mL) was reacted with **50** (500 mg, 1.26 mmol) in DMF (2.5 mL). Purification via flash column chromatography (gradient elution, 0%→5% CH_3OH in CH_2Cl_2) gave **51** as a white solid (259 mg, 43%); mp 165-167 °C; $[\alpha]_D^{25}$ +94.0 (*c* 0.1 in CH_3OH); ν_{max} (film) 3334 (SN-H), 3232 (O-H), 2955 (O-H), 1687 (C=O), 1545 (C=O); 1373 (S=O), 1227 (S=O); δ_H (500 MHz, $(CD_3)_2CO$) 0.86 – 0.92 (m, 3H, C(13)H_3), 1.25 – 1.35 (m, 4H, C(212)H_2, C(13)H_2), 1.35 – 1.44 (m, 2H, C(10)H_2), 1.51 – 1.65 (m, 2H, C(9)H_2), 3.37 – 3.47 (m, 2H, C(4)*H*, C(8)H_A), 3.62 – 3.77 (m, 3H, C(2)*H*, C(3)*H*, C(8)H_B), 3.85 (ddd, *J* = 10.2, 5.9, 1.9 Hz, 1H, C(5)*H*), 4.26 (d, *J* = 4.9 Hz, 1H, O*H*), 4.31 (dd, *J* = 10.7, 5.9 Hz, 1H, C(6)H_A), 4.45 (dd, *J* = 10.8, 2.0 Hz, 1H, C(6)H_B), 4.52 (s, 1H, O*H*), 4.84 (d, *J* = 3.4 Hz, 1H, C(1)*H*), 5.08 (s, 2H, C(7)H_2), 6.11 (d, *J* = 8.7 Hz, 1H, N*H*), 6.68 (s, 2H, SO_2NH_2), 7.28 – 7.46 (m, 5H, *Ph*); δ_C (126 MHz, $(CD_3)_2CO$) 13.4 (*C*(22)), 22.4 (*C*(21)), 25.6 (*C*(19)), 31.5 (*C*(20)), 55.9 (*C*(3)), 65.8 (*C*(29)), 67.7 (*C*(10)), 69.0 (*C*(7)), 70.1 (*C*(6)), 71.0 (*C*(1)), 72.0 (*C*(2)), 97.6 (*C*(4)), 127.7, 127.8, 128.3 (*o,m,p-Ph*), 137.4 (*i-Ph*), 156.4 (*C*O) (*C*(9) under acetone); *m/z* (ESI$^+$) 499 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{20}H_{33}N_2O_9S^+$ ([M+H]$^+$) requires 477.1901; found 477.1903.

((2*R*,3*S*,4*R*,5*R*,6*S*)-5-Amino-6-(hexyloxy)-3,4-dihydroxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (52)



Following *general procedure 2*, **51** (180 mg, 0.378 mmol) and Pd/C (18 mg) in CH_3OH (2 mL) gave **52** as a colourless gum (120 mg, 93%); $[\alpha]_D^{25}$ +55.0 (*c* 0.1 in CH_3OH); ν_{max} (film) 3360, 3301, 2928, 1636 (C=O), 1587 (C=O), 1363 (S=O), 1179 (S=O); δ_H (500 MHz, D_2O) 0.83 – 0.92 (m, 3H, C(12)H_3), 1.26 – 1.34 (m, 4H, C(10)H_2, C(11)H_2), 1.34 – 1.44 (m, 2H, C(9)H_2), 1.57 – 1.68 (m, 2H, C(8)H_2), 2.83 (dd, *J* = 10.2, 3.7 Hz, 1H, C(2)*H*), 3.46 (dd, *J* = 10.1, 9.1 Hz, 1H, C(4)*H*), 3.53 (dt, *J* = 9.8, 6.3 Hz, 1H, C(7)H_A), 3.61 (dd, *J* = 10.2, 9.0 Hz, 1H, C(3)*H*), 3.75 (dt, *J* = 9.8, 6.6 Hz, 1H, C(7)H_B), 3.94 (ddd, *J* = 10.1, 4.6, 2.4 Hz, 1H, C(5)*H*), 4.36 – 4.44 (m, 2H, C(6)H_2), 4.91 (d, *J* = 3.7 Hz, 1H, C(1)*H*); δ_C (126 MHz, D_2O) 13.4 (*C*(12)), 22.0 (*C*(11)), 25.2 (*C*(9)), 28.6 (*C*(8)), 30.8 (*C*(10)), 54.8 (*C*(2)), 68.5 (*C*(6)), 68.8 (*C*(7)), 69.5 (*C*(4)), 69.8 (*C*(5)), 73.5 (*C*(3)), 98.5 (*C*(1)); *m/z* (ESI–) 377 (82%), 341 ($[M–H]^-$, 100%); HRMS (ESI^-) $C_{12}H_{25}N_2O_7S^-$ ($[M–H]^-$) requires 341.1388; found 341.1388.

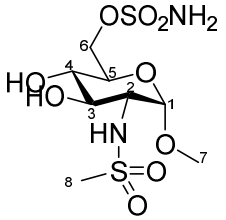
Ammonium ((2*S*,3*R*,4*R*,5*S*,6*R*)-2-(hexyloxy)-4,5-dihydroxy-6-((sulfamoyloxy)methyl)tetrahydro-2*H*-pyran-3-yl)sulfamate (53)



Following *general procedure 3*, **52** (100 mg, 0.292 mmol) was reacted with pyridine-sulfur trioxide complex (51.1 mg, 0.321 mmol) in H_2O (1 mL). Purification via reverse phase column chromatography (eluent H_2O 100%) gave **53** as a white solid (86 mg, 67%); mp 140-142 °C; $[\alpha]_D^{25}$ +77.1 (*c* 0.34 in CH_3OH); ν_{max} (film) 3301 (O–H), 2956 (C–H), 2929 (C–H), 2860 (C–H), 1362 (C=O), 1177 (S=O), 1039 (S=O); δ_H (500 MHz, D_2O) 0.79 (t, *J*

= 7.0 Hz, 3H, C(12)H_3), 1.17 – 1.27 (m, 4H, C(10)H_2, C(11)H_2), 1.27 – 1.37 (m, 2H, C(9)H_2), 1.48 – 1.62 (m, 2H, C(8)H_2), 3.17 (dd, *J* = 10.3, 3.6 Hz, 1H, C(2)*H*), 3.42 – 3.52 (m, 2H, C(4)*H*, C(7)H_A), 3.54 (dd, *J* = 10.1, 9.2 Hz, 1H, C(3)*H*), 3.64 (dt, *J* = 9.7, 6.9 Hz, 1H, C(7)H_B), 3.86 (ddd, *J* = 10.0, 4.9, 2.2 Hz, 1H, C(5)*H*), 4.35 (dd, *J* = 11.1, 4.9 Hz, 1H, C(6)H_A), 4.38 (dd, *J* = 11.1, 2.2 Hz, 1H, C(6)H_B), 5.06 (d, *J* = 3.6 Hz, 1H, C(1)*H*); δ_C (126 MHz, D_2O) 13.3 (*C*(12)), 21.9 (*C*(11)), 25.1 (*C*(9)), 28.5 (*C*(8)), 30.8 (*C*(10)), 57.6 (*C*(2)), 68.9 (*C*(6), *C*(7)), 69.3 (*C*(5)), 69.6 (*C*(4)), 71.4 (*C*(3)), 97.5 (*C*(1)); *m/z* (ESI^-) 865 (3%), 843 ($[2M-H]^-$, 7%), 421 ($[M-H]^-$, 100%); HRMS (ESI^-) $C_{12}H_{25}N_2O_{10}S_2^-$ requires 421.0956; found 421.0957.

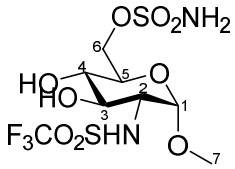
((2*R*,3*S*,4*R*,5*R*,6*S*)-3,4-Dihydroxy-6-methoxy-5-(methylsulfonamido)tetrahydro-2*H*-pyran-2-yl)methyl sulfamate (54)



Methanesulfonyl chloride (15 µL, 0.19 mmol) was added dropwise to a suspension of **41** (50 mg, 0.18 mmol) in CH_2Cl_2 (0.5 mL) and pyridine (0.5 mL) at 0 °C. The resulting solution was stirred at 0 °C for 1 h. The reaction was retreated with methanesulfonyl chloride (5 µL, 0.07 mmol) and stirred at 0 °C for 1 h. The reaction was quenched with H_2O (1 mL) and extracted with IPA:$CHCl_3$ 1:1 (3 × 10 mL). The combined organic layers were eluted through a phase separator and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0→8% CH_3OH in EtOAc) gave **54** as a colourless gum (8 mg, 12%); $[\alpha]_D^{25}$ +43.1 (*c* 0.3 in CH_3OH) {lit.[85] $[\alpha]_D^{22.6}$ +41.5 (*c* 0.3 in CH_3OH)}; ν_{max} (film) 3329 (O–H), 1406 (S=O), 1379 (S=O), 1224 (S=O), 1160 (S=O); δ_H (400 MHz,

MeOH-d_4) 3.03 (s, 3H, C(8)H_3), 3.41 (s, 3H, C(7)H_3), 3.59 (dd, J = 10.5, 8.7 Hz, 1H, C(3)*H*), 3.76 (ddd, J = 10.0, 6.0, 1.8 Hz, 1H, C(5)*H*), 4.25 (dd, J = 10.7, 5.9 Hz, 1H, C(6)H_A), 4.40 (dd, J = 10.7, 2.0 Hz, 1H, C(6)H_B), 4.68 (d, J = 3.6 Hz, 1H, C(1)*H*); δ_C (126 MHz, MeOH-d_4) 42.0 **(***C*(8)), 55.9 (*C*(7)), 59.3 (*C*(2)), 70.0 (*C*(6)), 71.1 (*C*(5)), 72.2 (*C*(4)), 73.4 (*C*(3)), 101.5 (*C*(1)); *m/z* (ESI$^-$) 349 ([M−H]$^-$, 100%); HRMS (ESI$^-$) $C_8H_{17}N_2O_9S_2^-$ ([M−H]$^-$) requires 349.0381; found 349.0380. Characterisation data are consistent with the literature.[85]

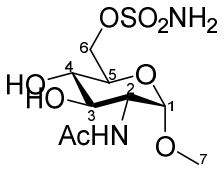
((2*R*,3*S*,4*R*,5*R*,6*S*)-3,4-Dihydroxy-6-methoxy-5-((trifluoromethyl)sulfonamido)tetrahydro-2*H*-pyran-2-yl)methyl sulfamate (55)



Trifluoromethanesulfonic anhydride (34 µL, 0.20 mmol) was added to a solution of **41** (50 mg, 0.18 mmol) in CH_2Cl_2 (0.5 mL) and pyridine (0.5 mL) at 0 °C. The resulting solution was stirred at 0 °C for 1 h. Trifluoromethanesulfonic anhydride (20 µL, 0.12 mmol) was added and the reaction was stirred at 0 °C for 1 h. The reaction was quenched with H_2O (0.5 mL) and extracted with IPA:$CHCl_3$ (3 × 10 mL), the combined organic layers were eluted through a phase separator and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 2%→10% CH_3OH in CH_2Cl_2) gave **55** as a white solid (5 mg, 7%); mp 63-65 °C {lit. mp 55-65 °C}; $[\alpha]_D^{25}$ +86.2 (*c* 0.2 in CH_3OH) {lit. $[\alpha]_D^{22.6}$ +95.5 (*c* 0.2 in CH_3OH)}; ν_{max} (film) 3314 (O–H), 1458 (S=O), 1228 (S=O), 1372 (S=O), 1029 (S=O); δ_H (400 MHz, MeOH-d_4) 3.34 – 3.39 (m, 2H, C(2)*H*, C(4)*H*), 3.42 (s, 3H, C(7)H_3), 3.61 (dd, J = 10.4, 8.7 Hz, 1H, C(3)*H*), 3.77 (ddd, J = 9.8, 5.9, 1.8 Hz, 1H, C(5)*H*), 4.25 (dd, J = 10.7, 5.9 Hz, 1H, C(6)H_A), 4.40 (dd, J = 10.7, 2.0 Hz, 1H, C(6)H_B), 4.69 (d, J = 3.4 Hz, 1H, C(1)*H*); δ_C (126 MHz, MeOH-d_4) 55.9 (*C*(7)), 60.4 (*C*(4)), 69.9 (*C*(6)),

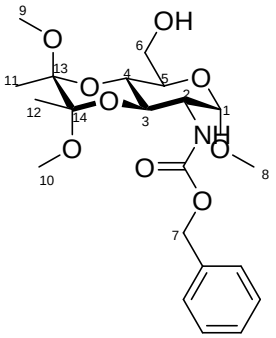
71.2 (*C*(5)), 72.0 (*C*(2)), 72.5 (*C*(3)), 100.8 (*C*(1)), 126.7 (d, *J* =1039.0, *C*F_3); *m/z* (ESI^+) 417 ($[M+Na]^+$, 100%); HRMS (ESI^-) $C_8H_{14}F_3N_2O_9S_2^-$ ($[M–H]^-$) requires 403.0098; found 403.0098. Characterisation data are consistent with the literature.[85]

((2*R*,3*S*,4*R*,5*R*,6*S*)-5-Acetamido-3,4-dihydroxy-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (56)



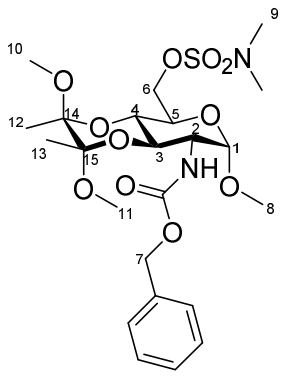
Acetyl chloride (8 µL, 0.11 mmol) was added dropwise to a solution of **41** (30 mg, 0.11 mmol) in pyridine (300 µL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched with CH_3OH (300 µL) and it was concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→20% CH_3OH in CH_2Cl_2) gave **56** as a colourless oil (10 mg, 29%); δ_H (400 MHz, MeOH-d_4) 1.98 (s, 3H), 3.38 (s, 3H), 3.64 (dd, *J* = 10.7, 8.8 Hz, 1H), 3.78 (ddd, *J* = 10.0, 6.0, 1.8 Hz, 1H), 3.91 (dd, *J* = 10.7, 3.6 Hz, 1H), 4.26 (dd, *J* = 10.7, 6.0 Hz, 1H), 4.41 (dd, *J* = 10.7, 2.0 Hz, 1H), 4.65 (d, *J* = 3.6 Hz, 1H); δ_C (101 MHz, MeOH-d_4) 22.5, 55.2, 55.7, 70.1, 71.3, 72.1, 72.9, 99.9, 173.7; *m/z* (ESI^+) 337 ($[M+Na]^+$, 100%). Characterisation data are consistent with the literature.[2]

Benzyl ((2*S*,3*S*,4a*S*,5*R*,7*S*,8*R*,8a*R*)-5-(hydroxymethyl)-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-b][1,4]dioxin-8-yl)carbamate (57)



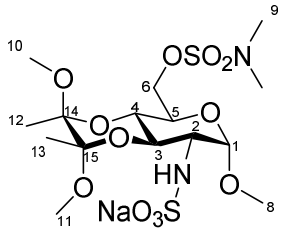
Carbamate **38** (1.75 g, 5.35 mmol), (1*S*)-(+)-10-camphorsulfonic acid (190 mg, 0.535 mmol), trimethyl orthoformate (1.75 mL, 16.05 mmol) and 2,3-butanedione (516 µL, 5.89 mmol) were suspended in CH_3OH (20 mL) and the resulting solution was heated at 75 °C for 72 h. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc (20 mL) and satd aq $NaHCO_3$ (20 mL). The organic layer was washed with brine (10 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent pentanes/EtOAc 1:1) gave **57** as a white solid (1.95 g, 83%); mp 65-68 °C; $[\alpha]_D^{25}$ + 194 (*c* 0.2 in CH_3OH); ν_{max} (film) 2948 br (O–H), 2160 (C–H), 2034 (C–H), 1977 (C–H), 1707 (C=O), 1512 (C=O); δ_H (400 MHz, $CDCl_3$) 1.27 (s, 3H, C(11)H_3 or C(12)H_3), 1.27 (s, 3H, C(11)H_3 or C(12)H_3), 3.19 (s, 3H, C(9)H_3 or C(10)H_3), 3.25 (s, 3H, C(9)H_3 or C(10)H_3), 3.34 (s, 3H, C(8)H_3), 3.69 – 3.78 (m, 3H, C(4)*H*, C(5)*H*, C(6)H_A), 3.79 – 3.88 (m, 2H, C(3)*H*, C(6)H_B), 3.89 – 3.99 (m, 1H, C(2)*H*), 4.79 (d, *J* = 3.0 Hz, 1H, C(1)*H*), 4.87 (d, *J* = 8.5 Hz, 1H, N*H*), 5.03 – 5.21 (m, 2H, C(7)H_2), 7.28 – 7.45 (m, 5H, *Ph*); δ_C (101 MHz, $CDCl_3$) 17.8, 17.9 (*C*(11), *C*(12)), 48.0 (*C*(9), *C*(10)), 53.0 (*C*(2)), 55.3 (*C*(8)), 61.5 (*C*(6)), 66.9 (*C*(4)), 67.0 (*C*(7)), 68.0 (*C*(3)), 69.9 (*C*(5)), 99.0 (*C*(1)), 99.8, 100.0 (*C*(13), *C*(14)), 128.3, 128.6 (*o,m,p-Ph*), 136.6 (*i-Ph*), 156.2 (C=O); *m/z* (ESI^+) 464 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{21}H_{31}NNaO_9^+$ ($[M+Na]^+$) requires 464.1891; found 464.1887.

((2*S*,3*S*,4a*S*,5*R*,7*S*,8*R*,8a*R*)-8-(((benzyloxy)carbonyl)amino)-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-b][1,4]dioxin-5-yl)methyl dimethylsulfamate (58)



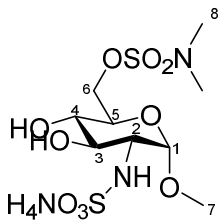
57 (500 mg, 1.13 mmol) in THF (5 mL) was added to NaH (60% in mineral oil, 100 mg, 2.49 mmol) under N_2 at 0 ºC and the resulting suspension was stirred at rt for 1 h. *N*,*N*'-Dimethylsulfamoyl chloride (146 µL, 1.36 mmol) was added and the resulting solution was stirred at rt for 16 h. The reaction was retreated with NaH (60% in mineral oil, 100 mg, 2.49 mmol) and *N*,*N*'-dimethylsulfamoyl chloride (146 µL, 1.36 mmol) and the resulting solution was stirred at rt for 16 h. The reaction was quenched with satd aq $NaHCO_3$ (5 mL) and extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→55% EtOAc in pentanes) gave **58** as a white solid (351 mg, 57%); mp 63-73 °C; $[\alpha]_D^{25}$ + 180 (*c* 0.1 in CH_3OH); ν_{max} (film) 2951 (C–H), 2181 (C–H), 1716 (C=O), 1512 (C=O), 1455 (S=O), 1358 (S=O); δ_H (500 MHz, $(CD_3)_2CO$) 1.22, 1.25 (2 × s, 2 × 3H, C(12)H_3, C(13)H_3), 2.91 (s, 6H, 2 × C(9)H_3), 3.22, 3.25 (2 × s, 2 × 3H, C(10)H_3, C(11)H_3), 3.36 (s, 3H, C(8)H_3), 3.64 (t, *J* = 9.4 Hz, 1H, C(4)*H*), 3.83 – 3.97 (m, 3H, C(2)*H*, C(3)*H*, C(5)*H*), 4.27 (dd, *J* = 10.9, 5.4 Hz, 1H, C(6)H_A), 4.39 (dd, *J* = 10.9, 2.0 Hz, 1H, C(6)H_B), 4.75 (d, *J* = 3.0 Hz, 1H, C(1)*H*), 4.99 – 5.15 (dd, *J* = 34.5, 12.6 Hz, 2H, C(7)H_2), 6.25 (d, *J* = 8.5 Hz, 1H, N*H*), 7.27 – 7.45 (m, 5H, *Ph*); δ_C (126 MHz, $(CD_3)_2CO$) 18.0, 18.1 (*C*(12), *C*(13)), 38.7 (*C*(9)), 48.2, 48.3 (*C*(10), *C*(11)), 53.9 (*C*(2)), 55.6 (*C*(8)), 66.6 (*C*(7)), 67.7 (*C*(4)), 68.3 (*C*(3)), 68.7 (*C*(6)), 68.8 (*C*(5)), 100.1 (*C*(1)), 100.5, 100.6 (*C*(14), *C*(15)), 128.55, 128.60, 129.18 (*o*,*m*,*p*-*Ph*), 138.35 (*i*-*Ph*), 157.07 (*C*O); *m/z* (ESI^+) 571 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{23}H_{36}N_2NaO_{11}S^+$ requires 571.1932; found 571.1934.

Sodium((2*S*,3*S*,4a*S*,5*R*,7*S*,8*R*,8a*R*)-5-(((*N*,*N*-dimethylsulfamoyl)oxy)methyl)-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-b][1,4]dioxin-8-yl)sulfamate (60)



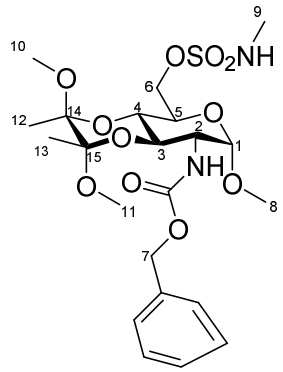
(1) Cbz-deprotection. Following *general procedure 2*, **58** (350 mg, 0.638 mmol) and Pd/C (35 mg) in CH_3OH (4 mL) gave **59** as a crude white solid (233 mg, 88%); the crude product was used in the subsequent step without any purification. **(2) *N*-sulfation.** Following *general procedure 3*, the abovementioned amine **59** intermediate (100 mg, 0.241 mmol) was reacted with pyridine-sulfur trioxide complex (42.0 mg, 0.266 mmol) in H_2O (1 mL). The solvent was removed *in vacuo* and the residue was triturated in CH_3OH, filtered and the filtrate was concentrated *in vacuo* to give **60** as a white gum (118 mg, 96%); $[\alpha]_D^{25}$ + 9 (*c* 0.1 in CH_3OH); δ_H (500 MHz, D_2O) 1.35, 1.36 (2 × s, 2 × 3H, C(11)H_3, C(12)H_3), 2.95 (s, 6H, 2 × C(8)H_3), 3.30, 3.33 (2 × s, 2 × 3H, C(9)H_3, C(10)H_3), 3.41 – 3.48 (m, 4H, C(2)*H*, C(7)H_3), 3.74 (t, *J* = 9.9 Hz, 1H, C(4)*H*), 3.85 (t, *J* = 10.3 Hz, 1H, C(3)*H*), 4.02 (dt, *J* = 10.0, 3.0 Hz, 1H, C(5)*H*), 4.47 (d, *J* = 2.7 Hz, 2H, C(6)H_2), 5.14 (d, *J* = 3.5 Hz, 1H, C(1)*H*); δ_C (126 MHz, D_2O) 16.75, 16.80, (*C*(11), *C*(12)), 37.88(*C*(8)), 47.90, 48.10 (*C*(9), *C*(10)), 54.93 (*C*(2)), 55.71 (*C*(7)), 66.43 (*C*(4)), 67.18, 67.19 (*C*(3), *C*(5)), 68.08 (*C*(6)), 98.87 (*C*(1)), 100.21, 100.53 (*C*(13), *C*(14)); *m/z* (ESI^+) 539 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{15}H_{29}N_2Na_2O_{12}S_2^+$ ($[M+Na]^+$) requires 539.0976; found 539.0953.

Ammonium ((2*S*,3*R*,4*R*,5*S*,6*R*)-6-(((*N*,*N*-dimethylsulfamoyl)oxy)methyl)-4,5-dihydroxy-2-methoxytetrahydro-2*H*-pyran-3-yl)sulfamate (61)



F_3CCO_2H (0.8 mL) was added to a solution of **60** (118 mg, 0.23 mmol) in H_2O (0.8 mL) and the resulting solution was stirred at rt for 2 h. The solvent was removed *in vacuo* and purification via column chromatography (eluent CH_3OH:CH_2Cl_2:NH_4OH 3:7:0.01) gave **61** as a white solid (10 mg, 11%); mp 95-109 °C; $[\alpha]_D^{25}$ + 108 (*c* 0.1 in CH_3OH); δ_H (500 MHz, D_2O) 2.86 (s, 6H, 2 × C(8)*H*$_3$), 3.18 (dd, *J* = 10.2, 3.6 Hz, 1H, C(2)*H*), 3.35 (s, 3H, C(7)*H*$_3$), 3.41 – 3.48 (m, 1H, C(4)*H*), 3.48 – 3.56 (m, 1H, C(3)*H*), 3.81 (ddd, *J* = 10.0, 4.8, 2.1 Hz, 1H, C(5)*H*), 4.37 (dd, *J* = 11.3, 4.9 Hz, 1H, C(6)*H*$_A$), 4.41 (dd, *J* = 11.2, 2.1 Hz, 1H, C(6)*H*$_B$), 4.96 (d, *J* = 3.6 Hz, 1H, C(1)*H*); δ_C (126 MHz, D_2O) 37.9 (*C*(8)), 55.5 (*C*(7)), 57.5 (*C*(2)), 69.2 (*C*(5)), 69.4 (*C*(6)), 69.5 (*C*(4)), 71.3 (*C*(3)), 98.6 (*C*(1)); *m/z* (ESI^-) 781 (7%), 759 ($[2M–H]^-$, 18%) 379 ($[M–H]^-$, 100%); HRMS (ESI^-) $C_9H_{19}N_2O_{10}S_2^-$ requires 379.0487; found 379.0483.

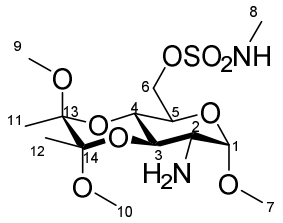
((2*S*,3*S*,4a*S*,5*R*,7*S*,8*R*,8a*R*)-8-(((Benzyloxy)carbonyl)amino)-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-b][1,4]dioxin-5-yl)methyl methylsulfamate (62)



N-methylsulfamoyl chloride (92 µL, 1.061 mmol) was added to a suspension of carbamate **57** (390 mg, 0.884 mmol) and Cs_2CO_3 (576 mg, 1.768 mmol) in DMF (4 mL) and the resulting suspension was stirred at rt for 48 h. The reaction was diluted with EtOAc (10 mL) and the organic layer was washed with brine (5 × 5 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→100% EtOAc in pentanes) gave **62** as a white solid (218 mg, 46%); mp 77-82 °C;

$[\alpha]_D^{25}$ +174 (*c* 0.1 in CH_3OH); ν_{max} (film) 3303 (N–H), 2951 (C–H), 1703 (C=O), 1515 (C=O), 1455 (S=O), 1351 (S=O); δ_H (500 MHz, $(CD_3)_2CO$) 1.22, 1.24 (2 × s, 2 × 3H, C(12)*H*$_3$, C(13)*H*$_3$), 2.76 (s, 3H, C(9)*H*$_3$), 3.22, 3.24 (2 × s, 2 × 3H, C(10)H$_3$, C(11)*H*$_3$), 3.38 (s, 3H, C(8)*H*$_3$), 3.59 – 3.67 (m, 1H, C(4)*H*), 3.83 – 3.94 (m, 3H, C(2)*H*, C(3)*H*, C(5)*H*), 4.21 (dd, *J* = 11.0, 5.5 Hz, 1H, C(6)H_A), 4.28 – 4.36 (m, 1H, C(6)H_B), 4.74 (d, *J* = 3.2 Hz, 1H, C(1)*H*), 5.00 – 5.15 (m, 2H, C(7)*H*$_2$), 6.26 (d, *J* = 8.6 Hz, 1H, N*H*), 6.58 – 6.69 (m, 1H, N*H*), 7.24 – 7.47 (m, 5H, *Ph*); δ_C (126 MHz, $(CD_3)_2CO$) 18.0, 18.1 (*C*(12), *C*(13)), 48.2, 48.2 (*C*(10), *C*(11)), 54.0 (*C*(2)), 55.7 (*C*(8)), 66.6 (*C*(7)), 67.8 (*C*(4)), 68.3 (*C*(6)), 68.4, 68.6 (*C*(3), *C*(5)), 100.1 (*C*(1)), 100.5, 100.6 (*C*(14)), (*C*(15)), 128.6, 128.6, 129.2 (*o,m,p-Ph*), 138.4 (*i-Ph*), 157.1 (*C*O)), (*C*(9)) under acetone); *m/z* (ESI^+) 557 ([M+Na]+, 100%); HRMS (ESI^+) $C_{22}H_{34}N_2NaO_{11}S^+$ ($[M+Na]^+$) requires 557.1776; found 557.1776.

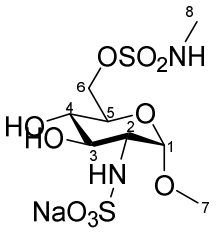
((2*S*,3*S*,4a*S*,5*R*,7*S*,8*R*,8a*R*)-8-Amino-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-b][1,4]dioxin-5-yl)methyl methylsulfamate (63)



Following *general procedure 2*, **62** (165 mg, 0.374 mmol) and Pd/C (17 mg) in CH_3OH (2 mL) gave **63** as a colourless gum (112 mg, 91%); $[\alpha]_D^{25}$ +236 (*c* 0.1 in CH_3OH); ν_{max} (film) 3303 (N–H), 2932 (C–H), 2161 (C–H), 1687 (C=O), 1534 (C=O), 1456 (S=O), 1356 (S=O); δ_H (500 MHz, D_2O) 1.34, 1.35 (2 × s, 2 × 3H, C(11)*H*$_3$, C(12)*H*$_3$), 2.75 (s, 3H, C(8)*H*$_3$), 2.94 (dd, *J* = 10.6, 3.7 Hz, 1H, C(2)*H*), 3.30, 3.31 (2 × s, 2 × 3H, C(9)*H*$_3$, C(10)*H*$_3$), 3.44 (s, 3H, C(7)*H*$_3$), 3.67 (t, *J* = 10.0 Hz, 1H, C(3)*H*), 3.77 (t, *J* = 10.3 Hz, 1H, C(4)*H*), 4.03 (dt, *J* = 10.2, 3.1 Hz, 1H, C(5)*H*), 4.33 – 4.42 (m, 2H, C(6)*H*$_2$) (C(1)*H* under H_2O); δ_C (126 MHz, D_2O) 16.8, 16.9 (*C*(11), *C*(12)), 28.6 (*C*(8)), 47.7, 48.0 (*C*(9), *C*(10)),

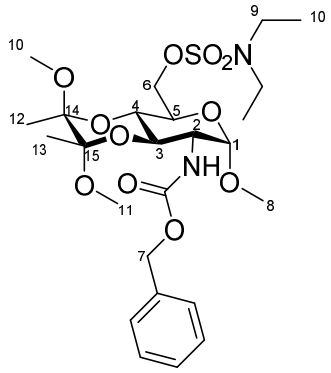
52.3 (*C*(2)), 55.6 (*C*(7)), 66.2 (*C*(4)), 67.4 (*C*(5)), 67.6 (*C*(6)), 69.9 (*C*(3)), 100.2 (*C*(13), *C*(14)), 100.6 (*C*(1); *m/z* (ESI$^+$) 423 ([M+Na]$^+$, 61%), 401 ([M+H]$^+$, 14%), 369 ([M-CH_4O]$^+$, 15%), 337 (79%), 237 (100%); HRMS (ESI$^+$) $C_{14}H_{29}N_2O_9S^+$ ([M+H]$^+$) requires 401.1588; found 401.1592.

((2S,3R,4R,5S,6R)-4,5-dihydroxy-2-methoxy-6-(((N-methylsulfamoyl)oxy)methyl)tetrahydro-2H-pyran-3-yl)sulfamic acid (65)



(1) *N*-sulfation. Following *general procedure 3*, **63** (100 mg, 0.250 mmol) and pyridine-sulfur trioxide complex (44.0 mg, 0.276 mmol) in H_2O (1 mL). The solvent was removed *in vacuo* and the residue was triturated in CH_3OH, filtered and the filtrate was concentrated *in vacuo* to give **64** as a white solid (116 mg, 96%); the crude was used in the subsequent step without any purification. **(2) Bis-acetal hydrolysis.** F_3CCO_2H (0.8 mL) was added to a solution of sulphate **64** (104 mg, 0.21 mmol) in H_2O (0.8 mL) and the resulting solution was stirred at rt for 2 h. The solvent was removed *in vacuo* and purification via column chromatography (eluent CH_3OH:CH_2Cl_2:NH_4OH 3:7:0.01) gave **65** as a white gum (27 mg, 33%); $[\alpha]_D^{25}$ +42.5 (*c* 0.16 in CH_3OH); δ_H (500 MHz, D_2O) 2.67 (s, 3H, C(8)H_3), 3.15 (dd, *J* = 10.5, 3.6 Hz, 1H, C(2)*H*), 3.37 (s, 3H, C(7)H_3), 3.42 – 3.49 (m, 1H, C(4)*H*), 3.68 – 3.75 (m, 1H, C(3)*H*), 3.86 (ddd, *J* = 10.0, 4.0, 2.5 Hz, 1H, C(5)*H*), 4.29 – 4.38 (m, 2H, C(6)H_2), 4.90 (d, *J* = 3.6 Hz, 1H, C(1)*H*); δ_C (126 MHz, D_2O) 28.5 (*C*(8)), 53.9 (*C*(2)), 55.4 (*C*(7)), 68.5 (*C*(6)), 69.1 (*C*(4)), 69.5 (*C*(5)), 70.7 (*C*(3)), 97.1 (*C*(1)). *m/z* (ESI$^-$) 365 ([M-Na]$^-$, 100%).

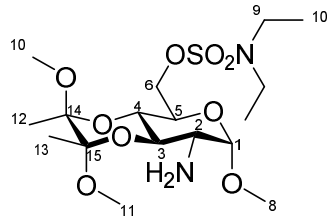
((2*S*,3*S*,4a*S*,5*R*,7*S*,8*R*,8a*R*)-8-(((Benzyloxy)carbonyl)amino)-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-b][1,4]dioxin-5-yl)methyl diethylsulfamate (66)



57 (500 mg, 1.13 mmol) in THF (5 mL) was added to NaH (60% in mineral oil, 100 mg, 2.49 mmol) under N_2 at 0 °C and the resulting suspension was stirred at rt for 1 h. *N,N'*-diethylsulfamoyl chloride (190 µL, 1.36 mmol) was added and the resulting suspension was stirred at rt for 16 h. The reaction was retreated with NaH (60% in mineral oil, 100 meg, 2.49 mmol) and *N,N'*-diethylsulfamoyl chloride (190 µL, 1.36 mmol) and the resulting suspension was stirred at rt for 16 h. The reaction was quenched with satd aq $NaHCO_3$ (5 mL) and extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→50% EtOAc in pentanes) gave **66** as a white solid (324 mg, 50%); mp 136-138 °C; $[\alpha]_D^{25}$ + 153 (*c* 0.1 in CH_3OH); ν_{max} (film) 3306 (N–H), 2945 (br., O–H), 1715 (C=O), 1512 (C=O), 1455 (S=O), 1361 (S=O); δ_H (500 MHz, $(CD_3)_2CO$) 1.17 – 1.26 (m, 12H, C(13)H_3, C(14)H_3, 2 × C(10)H_3), 3.22, 3.25 (2 × s, 2 × 3H, C(11)H_3, C(12)H_3), 3.32 – 3.39 (m, 7H, C(8)H_3, 2 × C(9)H_3), 3.62 (t, *J* = 9.5 Hz, 1H, C(4)*H*), 3.81 – 3.95 (m, 3H, C(2)*H*, C(3)*H*, C(5)*H*), 4.20 (dd, *J* = 10.8, 5.5 Hz, 1H, C(6)H_A), 4.33 (dd, *J* = 10.8, 2.0 Hz, 1H, C(6)H_B), 4.73 (d, *J* = 3.3 Hz, 1H, C(1)*H*), 5.00 – 5.15 (m, 2H, C(7)*H*), 6.25 (d, *J* = 8.9 Hz, 1H, N*H*), 7.25 – 7.46 (m, 5H, *Ph*); δ_C (126 MHz, $(CD_3)_2CO$) 13.8 (*C*(10)), 18.0, 18.1 (*C*(13), *C*(14)), 43.5 (*C*(9)), 48.2, 48.3 (*C*(11), *C*(12)), 53.9 (*C*(2)), 55.6 (*C*(8)), 66.6 (*C*(7)), 67.8 (*C*(4)), 68.2 (*C*(6)), 68.3, 68.7 (*C*(3), *C*(5)), 100.1

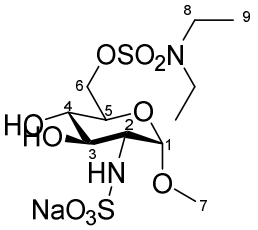
(*C*(1)), 100.5, 100.6 (*C*(15), *C*(16)), 128.6, 128.6, 129.2 (*o,m,p-Ph*), 138.4 (*i-Ph*), 157.1 (*C*O); *m/z* (ESI$^+$) 599 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{25}H_{40}N_2NaO_{11}S^+$ ([M+Na]$^+$) requires 599.2245; found 599.2244.

((2*S*,3*S*,4a*S*,5*R*,7*S*,8*R*,8a*R*)-8-Amino-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-b][1,4]dioxin-5-yl)methyl diethylsulfamate (67)



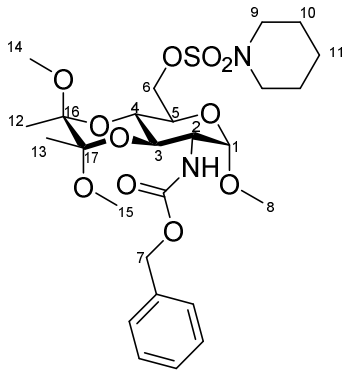
Following *general procedure 2*, **66** (300 mg, 0.521 mmol) and Pd/C (30 mg) in CH_3OH (3 mL) gave **67** as a colourless glass (225 mg, 98%); mp 57-60 °C; $[\alpha]_D^{25}$ + 208 (*c* 0.1 in CH_3OH); ν_{max} (film) 2947 (br., O–H), 1455 (C=O), 1361 (C=O), 1206 (S=O), 1026 (S=O); δ_H (400 MHz, $CDCl_3$) 1.13 (t, *J* = 7.2 Hz, 6H, 2 × C(9)*H*$_3$), 1.23, 1.26 (2 × s, 2 × 3H, C(12)*H*$_3$, C(13)*H*$_3$), 2.83 (dd, *J* = 10.4, 3.6 Hz, 1H, C(2)*H*), 3.19, 3.21 (2 × s, 2 × 3H, C(10)*H*$_3$, C(11)*H*$_3$), 3.22 – 3.28 (m, 4H, 2 × C(8)*H*$_2$), 3.31 (s, 3H, C(7)*H*$_3$), 3.48 (t, *J* = 9.9 Hz, 1H, C(4)*H*), 3.67 (t, *J* = 10.1 Hz, 1H, C(3)*H*), 3.88 (ddd, *J* = 10.1, 5.5, 1.9 Hz, 1H, C(5)*H*), 4.14 (dd, *J* = 10.5, 5.6 Hz, 1H, C(6)*H*$_A$), 4.26 (dd, *J* = 10.5, 2.0 Hz, 1H, C(6)*H*$_B$), 4.65 (d, *J* = 3.6 Hz, 1H, C(1)*H*); δ_C (101 MHz, $CDCl_3$) 13.5 (2 × *C*(9)), 17.8, 18.0 (*C*(12), *C*(13), 42.8 (*C*(7)), 48.0, 48.3 (*C*(10), *C*(11)), 53.8 (*C*(2)), 55.4 (2 × *C*(8)), 66.8 (*C*(4)), 67.6 (*C*(6)), 68.2 (*C*(5)), 71.0 (*C*(3)), 99.9, 100.0 (*C*(14), *C*(15)), 100.8 (*C*(1)); *m/z* (ESI$^+$) 465 ([M+Na]$^+$, 96%), 443 ([M+H]$^+$, 39%), 411 ([M-OCH_4]$^+$, 42%), 379 (100%); HRMS (ESI$^+$) $C_{17}H_{35}N_2O_9S^+$ ([M+H]$^+$) requires 443.2058; found 443.2053.

Sodium ((2*S*,3*R*,4*R*,5*S*,6*R*)-6-(((*N*,*N*-diethylsulfamoyl)oxy)methyl)-4,5-dihydroxy-2-methoxytetrahydro-2*H*-pyran-3-yl)sulfamate (69)



(1) *N*-sulfation. Following *general procedure 3*, amine **67** (100 mg, 0.225 mmol) was reacted with pyridine-sulfur trioxide complex (40 mg, 0.249 mmol) in H_2O (1 mL). The solvent was removed *in vacuo* and the residue was triturated in CH_3OH, filtered and the filtrate was concentrated *in vacuo;* the crude product was used in the subsequent step without any purification. **(2) Bis-acetal hydrolysis.** F_3CCO_2H (0.8 mL) was added to a solution of **68** (100 mg, 0.18 mmol) in H_2O (0.8 mL) and the resulting solution was stirred at rt for 2 h. The solvent was removed *in vacuo* and purification via column chromatography (eluent CH_3OH:CH_2Cl_2:NH_4OH 3:7:0.01) gave **69** as a white solid (10 mg, 13%); mp 115-128 °C; $[\alpha]_D^{25}$ +54 (*c* 0.1 in CH_3OH); δ_H (500 MHz, D_2O) 1.12 (t, *J* = 7.2 Hz, 6H, 2 × C(9)H_3), 3.17 (dd, *J* = 10.3, 3.6 Hz, 1H, C(2)*H*), 3.29 (q, *J* = 7.2 Hz, 4H, 2 × C(8)H_2), 3.34 (s, 3H, C(7)H_3), 3.38 – 3.45 (m, 1H, C(4)*H*), 3.51 (dd, *J* = 10.2, 9.2 Hz, 1H, C(3)*H*), 3.80 (ddd, *J* = 10.0, 5.0, 2.0 Hz, 1H, C(5)*H*), 4.30 (dd, *J* = 11.1, 5.1 Hz, 1H, C(6)H_A), 4.35 (dd, *J* = 11.1, 2.1 Hz, 1H, C(6)H_B), 4.95 (d, *J* = 3.6 Hz, 1H, C(1)*H*); δ_C (126 MHz, D_2O) 12.7 (*C*(9)), 43.0 (*C*(8)), 55.5 (*C*(7)), 57.5 (*C*(2)), 68.8 (*C*(6)), 69.2 (*C*(5)), 69.6 (*C*(4)), 71.3 (*C*(3)), 98.6 (*C*(1)); *m/z* (ESI^-) 837 (5%), 816 ($[2M–H]^-$, 17%), 407 ($[M–H]^-$, 100%); HRMS (ESI^-) $C_{11}H_{23}N_2O_{10}S_2^-$ requires 407.0800; found 407.0797.

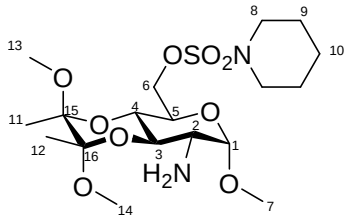
((2*S*,3*S*,4a*S*,5*R*,7*S*,8*R*,8a*R*)-8-(((Benzyloxy)carbonyl)amino)-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-b][1,4]dioxin-5-yl)methyl piperidine-1-sulfonate (70)



57 (500 mg, 1.13 mmol) in THF (5 mL) was added to NaH (60% in mineral oil, 100 mg, 2.49 mmol) under N_2 at 0 ºC and the resulting suspension was stirred at rt for 1 h. 1-Piperidinesulfonyl chloride (168 µL, 1.36 mmol) was added and the resulting solution was stirred at rt for 16 h. The reaction was quenched with satd aq $NaHCO_3$ (5 mL) and extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→35% EtOAc in pentanes) gave **70** as a white solid (325 mg, 49%); mp 75-79 °C; $[\alpha]_D^{25}$+171 (*c* 0.1 in CH_3OH); ν_{max} (film) 2946 (br., O–H), 1717 (C=O), 1512 (C=O); δ_H (500 MHz, $(CD_3)_2CO$) 1.22 (s, 3H, C(12)H_3 or C(13)H_3), 1.25 (s, 3H, C(12)H_3 or C(13)H_3), 1.54 – 1.61 (m, 2H, C(11)H_2), 1.61 – 1.69 (m, 4H, C(10)H_2), 3.22 (s, 3H, C(14)H_3 or C(15)H_3), 3.25 (s, 3H, C(14)H_3 or C(15)H_3), 3.30 (t, *J* = 5.5 Hz, 4H, C(9)H_2), 3.39 (s, 3H, C(8)H_3), 3.60 – 3.67 (m, 1H, C(4)*H*), 3.83 – 3.95 (m, 3H, C(2)*H*, C(3)*H*, C(5)*H*), 4.25 (dd, *J* = 10.9, 5.4 Hz, 1H, C(6)H_A), 4.38 (dd, *J* = 10.8, 2.0 Hz, 1H, C(6)H_B), 4.74 (d, *J* = 3.1 Hz, 1H, C(1)*H*), 5.01 – 5.15 (m, 2H, C(7)H_2), 6.25 (d, *J* = 8.6 Hz, 1H, N*H*), 7.28 – 7.40 (m, 5H, *Ph*); δ_C (126 MHz, $(CD_3)_2CO$) 17.1, 17.3 (*C*(14) and *C*(15)), 23.3 (*C*(11)) and 24.9 (*C*(10)), 47.3 (*C*(14) or *C*(15)), 47.4 (*C*(9)), 47.4 (*C*(14) or *C*(15)), 53.0 (*C*(2)), 54.8 (*C*(10)), 65.8 (*C*(7)), 66.8 (*C*(4)), 67.4 (*C*(5)), 67.7 (*C*(3)), 67.8 (*C*(6)), 99.2 (*C*(1)), 99.7, 99.7 (*C*(16) and *C*(17)), 127.7, 127.7, 128.3 (*o,m,p-Ph*), 137.5 (*i-Ph*), 156.2

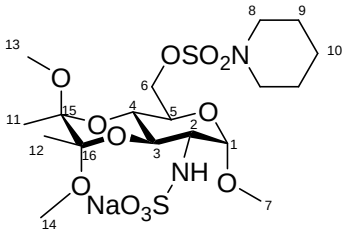
(*C*O); *m/z* (ESI$^+$) 611 ([M+Na]$^+$, 57%), 372 (100%); HRMS (ESI$^+$) $C_{26}H_{40}N_2NaO_{11}S^+$ ([M+Na]$^+$) requires 611.2245; found 611.2239.

((2*S*,3*S*,4a*S*,5*R*,7*S*,8*R*,8a*R*)-8-Amino-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-b][1,4]dioxin-5-yl)methyl piperidine-1-sulfonate (71)



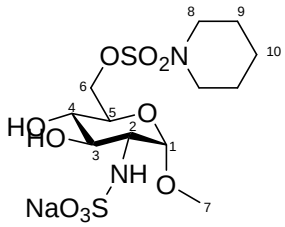
Following *general procedure 2*, benzyl carbamate **70** (315 mg, 0.536 mmol) and Pd/C (32 mg) in CH_3OH (4 mL) gave **71** as a colourless gum (220 mg, 90%); $[\alpha]_D^{25}$+171 (*c* 0.64 in CH_3OH); ν_{max} (film) 2917 (br. O–H), 1454 (S=O), 1367 (C=O), 1180 (C=O), 1028 (S=O); δ_H (500 MHz, D_2O) 1.34, 1.35 (2 × s, 2 × 3H, C(11)*H*$_3$, C(12)*H*$_3$), 1.56 – 1.61 (m, 2H, C(10)*H*$_2$), 1.61 – 1.69 (m, 4H, 2 × *C*(9)*H*$_2$), 2.93 (dd, *J* = 10.6, 3.4 Hz, 1H, C(2)*H*), 3.30, 3.31 (2 × s, 2 × 3H, C(13)*H*$_3$, C(14)*H*$_3$), 3.31 – 3.35 (m, 4H, 2 × C(8)*H*$_2$), 3.44 (s, 3H, C(7)*H*$_3$), 3.65 (t, *J* = 10.0 Hz, 1H, C(4)*H*), 3.77 (t, *J* = 10.3 Hz, 1H, C(3)*H*), 3.98 – 4.04 (m, 1H, C(5)*H*), 4.39 – 4.47 (m, 2H, C(6)*H*$_2$); δ_C (126 MHz, D_2O) 16.8, 17.0 (*C*(11), (*C*(12)), 22.8 (*C*(10)), 24.7 (*C*(9)), 47.7 (*C*(8)), 47.8, 48.1 (*C*(13), (*C*(14)), 52.4 (*C*(2)), 55.6 (*C*(7)), 66.2 (*C*(4)), 67.6 (*C*(5)), 68.1 (*C*(6)), 69.9 (*C*(3)), 100.3, 100.3 (*C*(15), (*C*(16)), 100.6 (*C*(1)); *m/z* (ESI$^+$) 477 ([M+Na]$^+$, 34%), 423 ([M-CH_4O]$^+$, 23%), 391 (100%), 359 (16%), 291 (51%); HRMS (ESI$^+$) $C_{18}H_{34}N_2NaO_9S^+$ ([M+Na]$^+$) requires 477.1877; found 477.1870.

Sodium ((2*S*,3*S*,4a*S*,5*R*,7*S*,8*R*,8a*R*)-2,3,7-trimethoxy-2,3-dimethyl-5-(((piperidin-1-ylsulfonyl)oxy)methyl)hexahydro-5*H*-pyrano[3,4-b][1,4]dioxin-8-yl)sulfamic acid (72)



Following *general procedure 3*, **71** (100 mg, 0.220 mmol) and pyridine-sulfur trioxide complex (39 mg, 0.242 mmol) in H_2O (1 mL). Purification via column chromatography (eluent CH_3OH:CH_2Cl_2 3:7) gave **72** as a white solid (63 mg, 54%); mp 71-73 ºC; $[\alpha]_D^{25}$ + 97.0 (*c* 0.1 in CH_3OH); ν_{max} (film) 3387 (br., O–H), 1632 (C=O), 1556 (C=O), 1370 (S=O), 1234 (S=O); δ_H (500 MHz, $(CD_3)_2CO$) 1.25, 1.31 (2 × s, 2 × 3H, C(11)H_3, C(12)H_3), 1.54 – 1.61 (m, 2H, C(10)H_2), 1.61 – 1.69 (m, 4H, 2 × C(9)H_2), 3.25, 3.26 (2 × s, 2 × 3H, C(13)H_3, C(14)H_3), 3.27 – 3.31 (m, 4H, 2 × C(8)H_2), 3.40 – 3.46 (m, 4H, C(2)*H*, C(7)H_3), 3.60 (t, *J* = 9.8 Hz, 1H, C(4)*H*), 3.77 (dd, *J* = 11.1, 9.5 Hz, 1H, C(3)*H*), 3.86 (ddd, *J* = 10.3, 5.2, 2.0 Hz, 1H, C(5)*H*), 4.23 (dd, *J* = 10.8, 5.3 Hz, 1H, C(6)H_A), 4.35 (dd, *J* = 10.7, 2.0 Hz, 1H, C(6)H_B), 5.22 (d, *J* = 3.0, Hz, 1H, C(1)*H*); δ_C (126 MHz, $(CD_3)_2CO$) 18.0, 18.1 (*C*(11), *C*(12)), 24.2 (*C*(10)), 25.8 (*C*(9)), 48.2, 48.3 (*C*(13), *C*(14)), 48.5 (*C*(8)), 56.0 (*C*(7)), 56.1 (*C*(2)), 67.8 (*C*(4)), 68.3 (*C*(3)), 68.5 (*C*(5)), 68.9 (*C*(6)), 100.1 (*C*(1)), 100.4, 100.9 *C*(15), *C*(16)); *m/z* (ESI^-) 533 ($[M–H]^-$, 100%); HRMS (ESI^-) $C_{18}H_{33}N_2O_{12}S_2^-$ ($[M–H]^-$) requires 533.1480; found 533.1476.

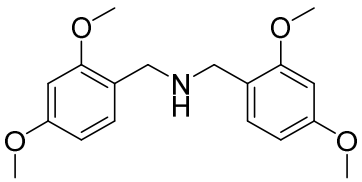
((2*S*,3*R*,4*R*,5*S*,6*R*)-4,5-Dihydroxy-2-methoxy-6-(((piperidin-1-ylsulfonyl)oxy)methyl)-tetrahydro-2*H*-pyran-3-yl)sulfamic acid (73)



F_3CCO_2H (1 mL) was added to a solution of **72** (63 mg, 0.12 mmol) in H_2O (1 mL) and the resulting solution was stirred at rt for 2 h. The solvent was removed *in vacuo* and

purification via column chromatography (eluent CH_3OH:CH_2Cl_2:NH_4OH 3:7:0.01) gave **73** as a white solid (19 mg, 36%); mp 155-167 °C; $[\alpha]_D^{25}$ +74.9 (*c* 0.1 in CH_3OH); δ_H (500 MHz, D_2O) 1.45 – 1.54 (m, 2H, C(10)H_2), 1.54 – 1.62 (m, 4H, 2 × C(9)H_2), 3.17 (dd, *J* = 10.3, 3.6 Hz, 1H, C(2)*H*), 3.21 – 3.29 (m, 4H, 2 × C(8)H_2), 3.35 (s, 3H, C(7)H_3), 3.39 – 3.47 (m, 1H, C(3)*H*), 3.51 (dd, *J* = 10.1, 9.2 Hz, 1H, C(4)*H*), 3.81 (ddd, *J* = 10.0, 5.0, 2.0 Hz, 1H, C(5)*H*), 4.35 (dd, *J* = 11.2, 5.0 Hz, 1H, C(6)H_A), 4.40 (dd, *J* = 11.2, 2.1 Hz, 1H, C(6)H_B), 4.95 (d, *J* = 3.6 Hz, 1H, C(1)*H*); δ_C (126 MHz, D_2O) 22.7 (*C*(10)), 24.5 (*C*(9)), 47.6 (*C*(8)), 55.5 (*C*(7)), 57.5 (*C*(2)), 69.2 (*C*(5)), 69.3 (*C*(6)), 69.5 (*C*(4)), 71.2 (*C*(3)), 98.6 (*C*(1)); *m/z* (ESI^-) 861 (5%), 839 ($[2M–H]^-$, 18%), 419 ($[M–H]^-$, 100%); HRMS (ESI^-) $C_{12}H_{23}N_2O_{10}S_2^-$ requires 419.0800; found 419.0799.

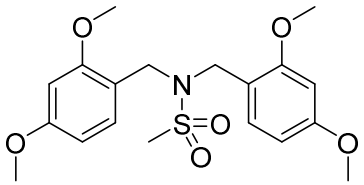
Bis(2,4-dimethoxybenzyl)amine (77)



2,4-dimethoxybenzylamine (1.09 mL, 7.28 mmol), 2,4-dimethoxybenzaldehyde (1.21 g, 7.28 mmol) and anhydrous Na_2SO_4 (190 mg, 1.33 mmol) were suspended in anhydrous CH_3OH (4 mL) and the reaction was heated at 65 °C for 4 h. The reaction was cooled to 0 °C, and $NaBH_4$ (303 mg, 8.00 mmol) was added portion-wise, and the resulting suspension was stirred at rt for 16 h. The reaction was quenched with satd aq $NaHCO_3$ (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→100%, EtOAc in pentanes) gave **77** as a colourless oil (1.63 g, 71%); δ_H (400 MHz, $CDCl_3$) 3.71 (s, 4H), 3.79 (s, 6H), 3.80 (s, 6H), 6.41 – 6.47 (m, 4H), 7.16 (d, *J* = 8.3 Hz, 2H); δ_C (101 MHz, $CDCl_3$) 45.9, 55.5, 55.6, 98.5, 104.7, 111.9, 133.0,

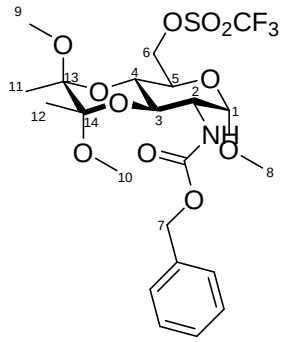
158.8, 162.1; *m/z* (ESI$^+$) 318 ([M+H]$^+$, 100%). Characterisation data are consistent with the literature.[86]

_N_,_N_-Bis(2,4-dimethoxybenzyl)methanesulfonamide (78)



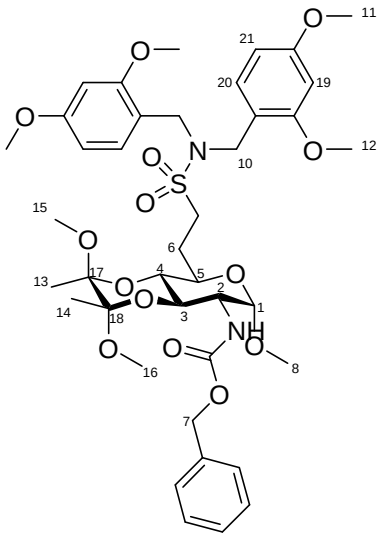
Methanesulfonyl chloride (122 μL, 1.57 mmol) was added dropwise to a stirred solution of **77** (500 mg, 1.57 mmol) and triethylamine (220 μL, 1.57 mmol) in toluene (3.5 mL) at 0 °C. The reaction was then warmed to rt and stirred for 1 h. The reaction was quenched with satd aq $NaHCO_3$ (2 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→35% EtOAc in pentanes) gave **78** as a white gum (451 mg, 73%); mp 80-81 °C {lit. mp 76-77 °C}; δ_H (400 MHz, $CDCl_3$) 2.73 (s, 3H), 3.76 (s, 6H), 3.80 (s, 6H), 4.37 (s, 4H), 6.41 – 6.49 (m, 4H), 7.23 (d, *J* = 8.3 Hz, 2H); *m/z* (ESI$^+$) 418 ([M+Na]$^+$, 100%). Characterisation data are consistent with the literature.[98]

((2*S*,3*S*,4a*S*,5*R*,7*S*,8*R*,8a*R*)-8-(((Benzyloxy)carbonyl)amino)-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-b][1,4]dioxin-5-yl)methyl trifluoromethanesulfonate (80)



Trifluoromethanesulfonic anhydride (46 µL, 0.272 mmol) was added to a solution of **57** (100 mg, 0.227 mmol) and *N*,*N*-diisopropylethylamine (47 µL, 0.272 mmol) in CH_2Cl_2 (1 mL) at 0 °C. The resulting solution was stirred at 0 °C for 1 h. The reaction was retreated with *N*,*N*-diisopropylethylamine (47 µL, 0.272 mmol) and trifluoromethanesulfonic anhydride (46 µL, 0.272 mmol) and the reaction was stirred at rt for an additional 1 h. The reaction was quenched with H_2O (1 mL) and extracted with CH_2Cl_2 (3 × 3 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→20% EtOAc in pentanes) gave **80** as a pale yellow solid (75 mg, 58%); mp 58-61 °C; $[\alpha]_D^{25}$ +82.7 (*c* 0.03 in CH_3OH); ν_{max} (film) 1717 (C=O), 1514 (C=O), 1515 (S=O), 1246 (S=O); δ_H (400 MHz, $CDCl_3$) 1.27 (s, 3H, C(11)*H*$_3$ or C(12)*H*$_3$), 1.28 (s, 3H, C(11)*H*$_3$ or C(12)*H*$_3$), 3.19 (s, 3H, C(9)*H*$_3$ or C(10)*H*$_3$), 3.22 (s, 3H, C(9)*H*$_3$ or C(10)*H*$_3$), 3.36 (s, 3H, C(8)*H*$_3$), 3.69 (t, *J* = 9.8 Hz, 1H, C(4)*H*), 3.83 (dd, *J* = 10.8, 9.5 Hz, 1H, C(3)*H*), 3.98 (ddd, *J* = 10.1, 5.3, 1.9 Hz, 2H, C(2)*H*, C(5)*H*), 4.60 (dd, *J* = 10.7, 5.4 Hz, 1H, C(6)*H*$_A$), 4.65 – 4.75 (m, 1H, C(6)*H*$_B$), 4.84 (d, *J* = 8.9 Hz, 1H, N*H*), 5.02 – 5.20 (m, 2H, C(7)*H*$_2$), 7.28 – 7.45 (m, 5H, *Ph*); δ_C (126 MHz, $CDCl_3$) 17.5, 17.7 (*C*(11), *C*(12)), 48.0, 48.1, (*C*(9), *C*(10)), 52.46 (*C*(3)), 55.48 (*C*(8)), 66.32 (*C*(4)), 66.99 (*C*(7)), 67.34 (*C*(5)), 67.81 (*C*(3)), 73.83 (*C*(6)), 98.95 (*C*(1)), 99.96, 100.05 (*C*(13), *C*(14)), 118.61 (q, *J* = 319.5 Hz, *C*F_3), 128.26, 128.51 (*o*,*m*,*p*-*Ph*), 136.38 (*i*-*Ph*), 155.88 (*C*O); δ_F (376 MHz, $CDCl_3$) –74.53; *m/z* (ESI$^+$) 596 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{22}H_{30}F_3NNaO_{11}S^+$ ([M+Na]$^+$) requires 596.1384; found 596.1386.

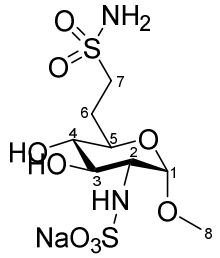
Benzyl ((2*S*,3*S*,4a*R*,5*R*,7*S*,8*R*,8a*R*)-5-(2-(*N*,*N*-bis(2,4-dimethoxybenzyl)sulfamoyl)ethyl)-2,3,7-trimethoxy-2,3 dimethylhexa-hydro-5*H*-pyrano[3,4-b][1,4]dioxin-8-yl)carbamate (81)



n-BuLi (1.6M in hexanes, 445 µL, 0.712 mmol) was added dropwise to a solution of **78** (268 mg, 0.678 mmol) in THF (3 mL) at –78 °C. The resulting solution was stirred for 15 min before a solution of **80** (194 mg, 0.339 mmol) in THF (2 mL) was added dropwise and the reaction was stirred for 5 h; the temperature was maintained at –78 °C throughout. The reaction was quenched with H_2O (5 mL) and extracted with EtOAc (3 × 5 mL). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentanes 4:6) gave **81** as a white solid (180 mg, 65%); mp 85-91 °C; $[\alpha]_D^{25}$ +113 (*c* 0.1 in CH_3OH); ν_{max} (film) 2927 (C–H), 2836 (C–H), 1721, 1613 (C=O), 1588, 1507 (C=O), 1456, 1328, 1293, 1261, 1208 (S=O), 1115, 1032 (S=O); δ_H (500 MHz, $CDCl_3$) 1.25 (s, 6H, C(13)H_3, C(14)H_3), 1.73 – 1.84 (m, 1H, C(6)H_A), 2.24 – 2.34 (m, 1H, C(6)H_B), 2.73 – 2.83 (m, 1H, C(7)H_A), 2.95 – 3.05 (m, 1H, C(7)H_B), 3.16 (s, 3H, C(15)H_3 or C(16)H_3), 3.21 (s, 6H, C(9)H_3, C(15)H_3 or C(16)H_3), 3.31 – 3.42 (m, 1H, C(4)*H*), 3.55 (t, *J* = 8.7 Hz, 1H, C(5)*H*), 3.71 – 3.78 (m, 7H, C(3)*H*, 2 × C(11)H_3), 3.80 (s, 6H, 2 × C(12)H_3), 3.84 – 3.94 (m, 1H, C(2)*H*), 4.33 – 4.42 (m, 4H, 2 × C(10)H_2), 4.66 (d, *J* = 2.8 Hz, 1H, C(1)*H*), 4.83 (d, *J* = 9.0 Hz, 1H, N*H*), 5.03 – 5.21 (m, 2H, C(8)H_2), 6.37 – 6.42 (m, 2H, 2 × C(19)*H*), 6.44 (dd, *J* = 8.3, 2.3 Hz, 2H, 2 × C(21)*H*), 7.22 (d, *J* = 8.3 Hz, 2H, 2 × C(20)*H*), 7.28 – 7.42 (m, 5H, *Ph*); δ_C (126 MHz, $CDCl_3$) 17.6, 17.7 (*C*(13),

C(14)), 24.9 (*C*(6)), 45.5 (*C*(10)), 47.8, 47.9 (*C*(15), *C*(16)), 49.5 (*C*(13)), 52.8 (*C*(2)), 54.9 (*C*(9)), 55.1, 55.4 (2 × *C*(11), 2 × *C*(12)), 66.9 (*C*(8)), 67.8 (*C*(5)), 67.9 (*C*(3)), 70.4 (*C*(4)), 98.3 (2 × *C*(19)), 98.5 (*C*(1)), 99.7, 99.8 (*C*(17), *C*(18)), 103.9 (2 × *C*(21)), 117.1 (2 × PMB-*i-Ph*), 128.2, 128.5 (*o,m,p-Ph*), 131.0 (2 × *C*(20)), 136.5 (*i-Ph*), 156.0 (*C*O), 158.4 (2 × PMB *m-Ph*), 160.5 (2 × PMB *o-Ph*); *m/z* (ESI$^+$) 841 (100%, [M+Na]$^+$); HRMS (ESI$^+$) $C_{40}H_{54}N_2NaO_{14}S^+$ [M+Na]$^+$ requires 841.3188; found 841.3187.

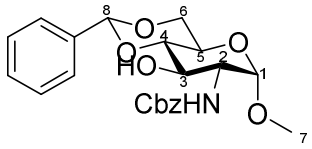
((2*S*,3*R*,4*R*,5*S*,6*R*)-4,5-Dihydroxy-2-methoxy-6-(2-sulfamoylethyl)tetrahydro-2*H*-pyran-3-yl)sulfamic acid (74)



(1) Cbz-deprotection. Following *general procedure 2*, **81** (170 mg, 0.210 mmol) was reacted with Pd/C (8 mg) in CH_3OH (3 mL). **(2) *N*-sulfation.** Following *general procedure 3*, the intermediate amine (65.0 mg, 79.5 μmol) was reacted with pyridine-sulfur trioxide complex (17.0 mg, 107 μmol) in H_2O (1 mL). The solvent was removed *in vacuo*, and the residue was triturated in chloroform, filtered and the filtrate was concentrated *in vacuo.* **(3) Bis-acetal hydrolysis.** F_3CCO_2H (1 mL) was added to the intermediate bisacetal (63 mg, 0.09 mmol) in H_2O (1 mL) and the resulting solution was stirred at rt for 2 h. The solvent was removed *in vacuo* and purification via column chromatography (eluent CH_3OH:CH_2Cl_2:NH_4OH 3:7:0.01) gave **74** as a white gum (5 mg, 7% over 3 steps) $[\alpha]_D^{25}$+12.5 (*c* 0.4 in H_2O); δ_H (500 MHz, D_2O) 1.85 – 1.94 (m, 1H, C(6)H_A), 2.26 – 2.34 (m, 1H, C(6)H_B), 3.17 (dd, *J* = 10.4, 3.7 Hz, 1H, C(2)*H*), 3.20 – 3.27 (m, 1H, C(4)*H*), 3.28 – 3.41 (m, 5H, C(7)H_2, C(8)H_3), 3.46 (dd, *J* = 10.3, 9.1 Hz, 1H, C(3)*H*), 3.64 (td, *J* = 9.6, 2.7 Hz, 1H, C(5)*H*), 4.92 (d, *J* = 3.6 Hz, 1H, C(1)*H*); δ_C (126 MHz, D_2O) 25.38 (*C*(6)),

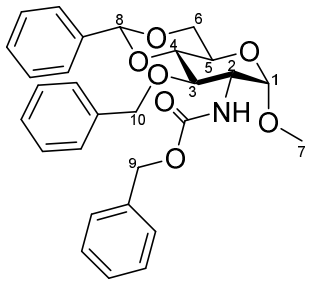
50.6 (*C*(7)), 55.4 (*C*(8)), 57.8 (*C*(2)), 69.0 (*C*(5)), 71.2 (*C*(3)), 73.5 (*C*(4)), 98.5 (*C*(1)); *m/z* (ESI^-) 349 (100%, $[M-H]^-$); HRMS (ESI^-) $C_8H_{17}N_2O_9S_2^-$ ($[M–NH_4]^-$) requires 349.0381; found 349.0384.

Methyl-4,6-*O*-benzylidene-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (90)



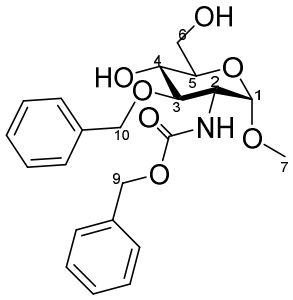
(1*S*)-(+)-10-Camphorsulfonic acid (180 mg, 0.776 mmol) and benzaldehyde dimethyl acetal (1.70 mL, 11.3 mmol) were sequentially added to a solution of **38** (2.54 mg, 7.77 mmol) in DMF (35 mL) at 0 °C, and the reaction was stirred at rt for 16 h. The reaction was concentrated *in vacuo* and the residue was purified via to column chromatography (eluent CH_3OH/CH_2Cl_2, 2:98) to give **90** as a white solid (2.27 g, 70%); mp 206-209 °C; {lit.[153] mp 207-208 °C}; $[\alpha]_D^{25}$ +37.0 (*c* 1.0 in $CHCl_3$); {lit.[153] $[\alpha]_D^{21}$ +53 (*c* 0.55 in $CHCl_3$)}; ν_{max} (film) 3312 (O–H), 3069 (C–H), 1685 (C=O), 1545; δ_H (400 MHz, DMSO-d_6) 3.29 (s, 3H, C(7)H_3), 3.44 – 3.81 (m, 6H, C(2)*H*, C(3)*H*, C(4)*H*, C(5)*H*, C(6)H_A, N*H* or O*H*), 4.17 (dd, J = 9.95, 4.76 Hz, 1H, C(6)H_B), 4.68 (d, J = 3.57 Hz, 1H, C(1)*H*), 5.04 (s, 2H, CH_2Ph), 5.24 (s, 1H, N*H* or O*H*), 5.61 (s, 1H, C(8)*H*), 7.23 – 7.51 (m, 10H, 2 × *Ph*); δ_C (101 MHz, DMSO-d_6) 55.3 (*C*(7)), 56.9 (*C*(2)), 62.9 (*C*(5)), 65.9 (*C*H_2Ph), 67.7 (*C*(3)), 68.5 (*C*(6)), 82.4 (*C*(4)), 99.3 (*C*(1)), 101.4 (*C*(8)), 126.9 (*Ph*), 128.3 (*Ph*), 128.5 (*Ph*), 128.8 (*Ph*), 129.3 (*Ph*), 137.5 (*i-Ph*), 138.2 (*i-Ph*), 156.7 (*C*=O); *m/z* (ESI^+) 438 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{22}H_{25}NNaO_7^+$ ($[M+Na]^+$) requires 438.1523; found 438.1525. Characterisation is consistent with the literature.[153]

Benzyl ((2*R*,4a*R*,6*S*,7*R*,8*R*,8a*S*)-8-(benzyloxy)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)carbamate (91)



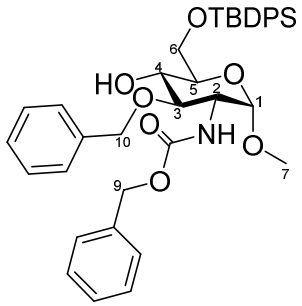
Sodium hydride (60% dispersion in mineral oil, 560 mg, 14.0 mmol) was added to **90** (4.52 g, 10.9 mmol) and benzyl bromide (1.38 mL, 11.6 mmol) in DMF (34 mL) under N_2, at 0 °C, and the reaction was stirred at rt for 16 h. The reaction was quenched with H_2O (200 mL) and the aqueous layer was extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with ½ sat brine (3 × 30 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→20% EtOAc in pentanes) gave **91** as a white solid (4.41 mg, 84%); mp 202-204 °C; {lit.[154] mp 202-203 °C}; $[\alpha]_D^{25}$ +56.8 (*c* 1.0 $CHCl_3$); {lit.[154] $[\alpha]_D^{23}$+46 (*c* 1.0 in $CHCl_3$)}; ν_{max} (film) 3313 (N–H), 3065 (C–H), 3033 (C–H), 2916 (C–H), 1690 (C=O), 1545 (C=O); δ_H (400 MHz, $CDCl_3$) 3.27 (s, 3H, C(7)*H*$_3$), 3.61 (dd, J = 9.85, 8.33 Hz, 1H, C(3)*H*), 3.66 – 3.76 (m, 3H, C(4)*H*, C(5)*H*, C(6)*H*$_A$), 3.95 (td, J = 9.66, 3.48 Hz, 1H, C(2)*H*), 4.16 – 4.25 (m, 1H, C(6)*H*$_B$), 4.58 (d, J = 11.90 Hz, 1H, C(10)*H*$_A$), 4.61 – 4.67 (m, 1H, C(1)*H*), 4.74 – 4.85 (m, 2H, C(10)*H*$_B$, N*H*), 5.01 (d, J = 12.16 Hz, 1H, C(9)*H*$_A$), 5.09 (d, J = 12.15 Hz, 1H, C(9)*H*$_B$), 5.51 (s, 1H, C(8)*H*), 7.11 – 7.47 (m, 15H, *Ar*); δ_C (101 MHz, $CDCl_3$) 54.8 (*C*(2)), 55.4 (*C*(7)), 62.8 (*C*(5)), 67.1 (*C*(9)), 69.1 (*C*(6)), 74.4 (*C*(10)), 76.7 (*C*(3)), 82.8 (*C*(4)), 99.7 (*C*(1)), 101.4 (*C*(8)), 126.2 (*Ar*), 127.7 (*Ar*), 128.0 (*Ar*), 128.4 (*Ar*), 128.7 (*Ar*), 129.1 (*Ar*), 136.5 (*Ar*), 137.6 (*Ar*), 138.6 (*Ar*), 156.2 (*C*=O); *m/z* (ESI$^+$) 528 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{29}H_{32}NO_7^+$ ([M+H]$^+$) requires 506.2173; found 506.2167. Characterisation data are consistent with the literature.[154]

Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-4-(benzyloxy)-5-hydroxy-6-(hydroxymethyl)-2-methoxytetrahydro-2*H*-pyran-3-yl)carbamate (92)



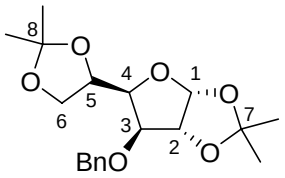
91 (2.22 g, 4.40 mmol) was suspended in 70% AcOH (30 mL) and the reaction was heated at 65 °C for 16 h. The reaction was cooled to rt and neutralised by addition of 2M NaOH. The aqueous layer was extracted with EtOAc (3 × 100 mL), and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Purification via column chromatography (gradient elution, 0%→70% EtOAc in pentane) gave **92** as a white solid (1.34 g, 73%); mp 144-146 °C; {lit.[154] mp 151-152 °C (IPA)}; $[\alpha]_D^{25}$ +84.4 (*c* 1.0 in CH_3OH); {lit.[154] $[\alpha]_D^{23}$ +94 (*c* 1.0 in CH_3OH)}; ν_{max} (film) 3324 (O−H), 2933 (C−H), 2900 (C−H), 2837 (C−H), 1688 (C=O); δ_H (400 MHz, $CDCl_3$) 3.36 (s, 3H, C(7)*H*$_3$), 3.54 (dd, J = 10.3, 8.5 Hz, 1H, C(3)*H*), 3.58 – 3.71 (m, 2H, C(4)*H*, C(5)*H*), 3.81 (qd, J = 11.7, 3.8 Hz, 2H, C(6)*H*$_2$), 3.98 (td, J = 9.9, 3.5 Hz, 1H, C(2)*H*), 4.62 (d, J = 11.5 Hz, 1H, C(10)*H*$_A$), 4.68 (d, J = 3.6 Hz, 1H, C(1)*H*), 4.75 (d, J = 11.5 Hz, 1H, C(10)*H*$_B$), 4.97 (d, J = 10.0 Hz, 1H, N*H*), 5.08 (d, J = 12.2 Hz, 1H, C(9)*H*$_A$), 5.15 (d, J = 12.1 Hz, 1H, C(9)*H*$_B$), 7.26 – 7.38 (m, 10H); δ_C (101 MHz, $CDCl_3$) 54.6 (*C*(2)), 55.4 (*C*(7)), 62.7 (*C*(6)), 67.2 (*C*(9)), 70.9 (*C*(4)), 71.3 (*C*(5)), 74.5 (*C*(10)), 81.0 (*C*(3)), 99.3 (*C*(1)), 128.1 (*Ph*), 128.1 (*Ph*), 128.4 (*Ph*), 128.7 (*Ph*), 128.8 (*Ph*), 135.4 (*i-Ph*), 138.3 (*i-Ph*), 156.0 (*C*=O); *m/z* (ESI^+) 440 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{22}H_{27}NNaO_7^+$ ($[M+Na]^+$) requires 418.1860; found 418.1862. Characterisation data are consistent with the literature.[154]

Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-4-(benzyloxy)-6-(((tert-butyldiphenylsilyl)oxy)methyl)-5-hydroxy-2-methoxytetrahydro-2*H*-pyran-3-yl)carbamate (93)



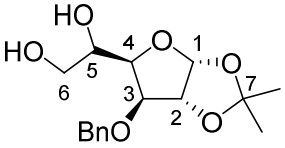
TBDPSCl (1.17 mL, 4.51 mmol) was added to a solution of **92** (1.34 g, 3.22 mmol), DMAP (78 mg, 0.639 mmol) and Et_3N (2.80 mL, 20.0 mmol) in CH_2Cl_2 (16 mL) and the reaction was stirred at rt for 16 h. The reaction was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (50 mL). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→20% EtOAc in pentane) gave **93** as a colourless viscous oil (1.82 g, 86%); $[\alpha]_D^{25}$ +32.3 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3439 (O−H), 2930 (C−H), 2897 (C−H), 2855 (C−H), 2837 (C−H), 1707 (C=O); δ_H (400 MHz, $CDCl_3$) 1.07 (s, 9H, C(C*H*$_3$)$_3$), 3.31 (s, 3H, C(7)*H*$_3$), 3.53 (dd, J = 10.4, 8.7 Hz, 1H, C(3)*H*), 3.62 (dt, J = 9.4, 4.4 Hz, 1H, C(5)*H*), 3.76 (t, J = 9.2 Hz, 1H, C(4)*H*), 3.88 (d, J = 4.4 Hz, 2H, C(6)*H*$_2$), 3.96 (td, J = 10.1, 3.4 Hz, 1H, C(2)*H*), 4.67 (d, J = 3.6 Hz, 1H, C(1)*H*), 4.73 (d, J = 2.0 Hz, 2H, C(9)*H*$_2$), 4.92 (d, J = 9.9 Hz, 1H, N*H*), 5.08 (d, J = 12.2 Hz, 1H, C(10)*H*$_A$), 5.15 (d, J = 12.2 Hz, 1H, C(10)*H*$_B$), 7.27 – 7.48 (m, 16H, *Ph*), 7.70 (ddt, J = 5.9, 4.2, 1.7 Hz, 4H, *Ph*); δ_C (101 MHz, $CDCl_3$) 19.4 (*C*(CH$_3$)$_3$), 27.0 (C(*C*H$_3$)$_3$), 54.4 (*C*(2)), 55.1 (*C*(7)), 64.6 (*C*(6)), 67.1 (*C*(9)), 71.2 (*C*(5)), 72.2 (*C*(4)), 74.5 (*C*(10)), 80.8 (*C*(3)), 99.1 (*C*(1)), 127.9 (*Ph*), 127.9 (*Ph*), 128.1 (*Ph*), 128.3 (*Ph*), 128.4 (*Ph*), 128.6 (*Ph*), 128.7 (*Ph*), 129.9 (*Ph*), 129.9 (*Ph*), 133.2 (*Ph*), 133.3 (*Ph*), 135.8 (*Ph*), 135.8 (*Ph*), 136.6 (*Ph*), 138.6 (*Ph*), 156.1 (*C*=O); *m/z* (ESI^+) 678 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{38}H_{45}NNaO_7Si^+$ ($[M+Na]^+$) requires 678.2858; found 678.2854.

3-*O*-Benzyl-1,2-5,6-*O*-diisopropylidene-α-D-glucofuranose (95)



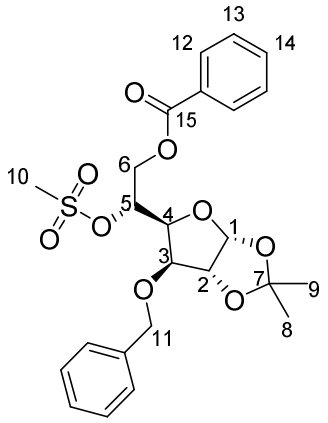
NaH (60% in mineral oil, 4.60 g, 116 mmol) was added to a solution of diacetone-D-glucose (10.0 g, 38.5 mmol) in anhydrous DMF (40 mL) at 0 °C under Ar. The reaction was stirred at 0 °C for 30 min, before benzyl bromide (5.85 mL, 57.7 mmol) was added and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of H_2O (20 mL) and extracted with EtOAc (50 mL). The organic layer was washed with aq LiCl (5% w/v, 5 × 20 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentane, 1:9) gave **95** as a colourless oil (13.5 g, 100%); $[\alpha]_D^{25}$ −28.9 (*c* 1.0 in $CHCl_3$); {lit.[155] $[\alpha]_D^{25}$ −26 (*c* 1.0 in $CHCl_3$)}; ν_{max} (film) 2987 (C–H), 2934 (C–H); δ_H (400 MHz, $CDCl_3$) 1.31 (s, 4H, CH_3), 1.38 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 3.94 – 4.07 (m, 2H, C(3)*H*, C(6)H_A), 4.06 – 4.24 (m, 2H, C(4)*H*, C(6)H_B), 4.37 (dt, *J* = 7.70, 5.99 Hz, 1H, C(5)*H*), 4.59 (d, *J* = 3.69 Hz, 1H, C(2)*H*), 4.62 – 4.74 (m, 2H, CH_2Ph), 5.90 (d, *J* = 3.66 Hz, 1H, C(1)*H*), 7.27 – 7.40 (m, 5H, *Ph*); δ_C (101 MHz, $CDCl_3$) 25.6, 26.4, 26.9, 27.0 (4 × *C*H_3), 67.6 (*C*(6)), 72.5 (*C*H_2Ph), 72.7 (*C*(5)), 81.5 (*C*(4)), 81.9 (*C*(3)), 82.8 (*C*(2)), 105.5 (*C*(1)), 109.1 (*C*(8)), 112.0 (*C*(7)), 127.8 (2 × *o-Ph*), 128.0 (*p-Ph*), 128.6 (2 × *m-Ph*), 137.8 (*i-Ph*); *m/z* (ESI$^+$) 373 ([M+Na]$^+$, 45%), 723 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{19}H_{26}NaO_6^+$ ([M+Na]$^+$) requires 373.1622; found 373.1621. Characterisation data are consistent with the literature.[155]

1,2-*O*-isopropylidene-3-*O*-benzyl-α-D-glucofuranose (96)



A solution of **95** (13.5 g, 38.6 mmol) in AcOH/H_2O (1:1, 50 mL) was heated at 45 °C for 16 h. The reaction was concentrated *in vacuo* and the residue was partitioned between satd aq $NaHCO_3$ (20 mL) and EtOAc (20 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give **96** as a colourless oil (12.0 g, 100%); $[\alpha]_D^{25}$ –47.0 (*c* 1.0 in $CHCl_3$); {lit.[156] $[\alpha]_D^{25}$ −39.4 (*c* 1.08 in $CHCl_3$); ν_{max} (film) 3423 (O–H), 2986 (C–H), 2934 (C–H); δ_H (400 MHz, $CDCl_3$) 1.32 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 3.69 (dd, J = 11.50, 5.50 Hz, 1H, C(6)H_A), 3.81 (dd, J = 11.44, 3.45 Hz, 1H, C(6)H_B), 4.02 (ddd, J = 7.68, 5.52, 3.44 Hz, 1H, C(5)*H*), 4.09 – 4.18 (m, 2H, C(3)*H*, C(4)*H*), 4.55 (d, J = 11.69 Hz, 1H, CH_APh), 4.63 (d, J = 3.83 Hz, 1H, C(2)*H*), 4.73 (d, J = 11.72 Hz, 1H, CH_BPh), 5.94 (d, J = 3.79 Hz, 1H, C(1)*H*), 7.28 – 7.47 (m, 5H, *Ph*); δ_C (101 MHz, $CDCl_3$) 26.4 (*C*H_3), 26.9 (*C*H_3), 64.5 (*C*(6)), 69.4 (*C*(5)), 72.3 (*C*H_2Ph), 80.1 (*C*(4)), 82.1 (*C*(3)), 82.2 (*C*(2)), 105.3 (*C*(1)), 112.0 (*C*(7)), 128.0 (2 × *o-Ph*), 128.4 (*p-Ph*), 128.9 (2 × *m-Ph*), 137.3 (*i-Ph*); *m/z* (ESI^+) 333 ($[M+Na]^+$, 28%), 643 ($[2M+Na]^+$, 100%), 953 ($[3M+Na]^+$, 100%); HRMS (ESI^+) $C_{16}H_{22}NaO_6^+$ ($[M+Na]^+$) requires 333.1309; found 333.1307. Characterization data is consistent with the literature.[156]

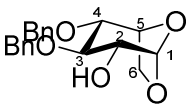
(*R*)-2-((3a*R*,5*S*,6*S*,6a*R*)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-2-((methylsulfonyl)oxy)ethyl benzoate (97)



Benzoyl chloride (5.20 mL, 45.0 mmol) was added dropwise to a solution of **96** (12.7 g, 40.9 mmol) and pyridine (32.9 mL, 409 mmol) in CH_2Cl_2 (205 mL) under N_2 at 0 °C. The reaction was stirred at 0 °C for 4 h, before methanesulfonyl chloride (4.72 mL, 61.3 mmol) was added and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of satd aq $NaHCO_3$ (200 mL) and the organic layer was separated, washed with satd aq NH_4Cl, brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentane, 2:8) gave **97** as a white solid (13.1 g, 65%); $[\alpha]_D^{25}$ –9.92 (*c* 1.0 in $CHCl_3$); {lit.[105] $[\alpha]_D^{26}$ −14.52 (*c* 0.65 in $CHCl_3$)}; mp 94-97 °C; {lit.[105] mp 94-95 °C}; ν_{max} (film) 2937 (C–H), 1723 (C=O), 1357 (S=O), 1272 (S=O); δ_H (400 MHz, $CDCl_3$) 1.33 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 3.00 (s, 3H, C(10)H_3), 4.15 (d, *J* = 3.11 Hz, 1H, C(3)*H*), 4.44 – 4.55 (m, 2H, C(4)*H*, C(6)H_A), 4.58 – 4.65 (m, 2H, C(2)*H*, C(11)H_A), 4.72 (d, *J* = 11.05 Hz, 1H, C(11)H_B), 4.93 (dd, *J* = 12.73, 2.07 Hz, 1H, C(6)H_B), 5.42 (ddd, *J* = 8.19, 6.24, 2.06 Hz, 1H, C(5)*H*), 5.93 (d, *J* = 3.59 Hz, 1H, C(1)*H*), 7.27 – 7.51 (m, 7H, *Ph*, 2 × C(13)*H*), 7.53 – 7.62 (m, 1H, C(14)*H*), 8.04 – 8.14 (m, 2H, 2 × C(12)*H*); δ_C (101 MHz, $CDCl_3$) 26.4 (*C*H_3), 27.0 (*C*H_3), 39.3 (*C*(10)), 64.2 (*C*(11)), 72.5 (*C*(6)), 75.5 (*C*(5)), 78.4 (*C*(4)), 81.3 (*C*(3)), 81.7 (*C*(2)), 105.5 (*C*(1)), 112.4 (*C*(7)), 128.2, 128.2, 128.6, 129.8, 129.9, 133.4, 137.3, 166.1 (*C*(15)); *m/z* (ESI^+) 493 ($[M+H]^+$, 35%), 515 ($[M+Na]^+$, 65%),

1007 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{24}H_{28}NaO_9S^+$ ([M+Na]$^+$) requires 515.1346; found 515.1343. Characterization data is consistent with the literature.[105]

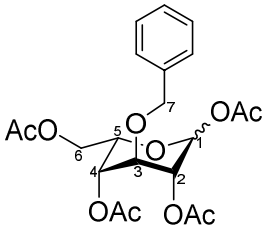
1,6-Anhydro-3-*O*-benzyl-β-L-idopyranose (98)



KO^{t}Bu (1.68 g, 15.0 mmol) was added to a solution of **97** (3.35 g, 6.41 mmol) in CH_2Cl_2:*t*-BuOH [1:1 (v/v), 70 mL] at 0 °C and the reaction was stirred at 0 °C for 2 h. The reaction was quenched by addition of 0.6 N H_2SO_4 (ca. 9 mL) and concentrated *in vacuo*. The residue was dissolved in 1,4-dioxane (120 mL) and 3 N H_2SO_4 (60 mL) and heated at 120 °C for 16 h. The reaction was neutralised by addition of NaOH (10N aq, ca. 2 mL) and concentrated *in vacuo*. The residue was partitioned between EtOAc (50 mL) and H_2O (50 mL), separated and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent pentane:EtOAc, 4:6) gave the title compound **98** as an off-white solid (1.07 g, 66%); mp 158-160 °C ; {lit.[106] mp 158-159 °C (CH_2Cl_2:hexane};$[\alpha]_D^{25}$ +50.8 (*c* 1.0 in CH_3OH); {lit.[106] $[\alpha]_D^{27}$ +69.2 (*c* 1.0 in CH_3OH)}; ν_{max} (film) 3477 (O–H), 3308 (O–H), 2955 (O–H), 2893 (O–H); δ_H (400 MHz, DMSO-*d*6) 3.29 – 3.36 (m, 2H, C(2)*H*, C(3)*H*), 3.56 (ddd, *J* = 7.7, 5.2, 1.1 Hz, 1H, C(6)H_A), 3.62 (dd, *J* = 7.4, 4.3 Hz, 1H, C(4)*H*), 3.93 (dd, *J* = 7.4, 0.9 Hz, 1H, C(6)H_B), 4.24 – 4.34 (m, 1H, C(5)*H*), 4.77 (s, 2H, OCH_2Ph), 5.12 (d, *J* = 1.7 Hz, 1H, C(1)*H*), 5.24 (d, *J* = 5.1 Hz, 1H, C(2)O*H*), 5.41 (s, 1H, C(4)O*H*), 7.21 – 7.42 (m, 5H, *Ph*); δ_C (100 MHz, $CDCl_3$) 64.2 (*C*(6)), 70.7(*C*(4)), 73.6 (O*C*H_2Ph), 74.3 (*C*(2)), 75.3 (*C*(5)), 83.4 (*C*(3)), 101.9 (*C*(1)), 127.1, 127.4, 128.0 (*o,m,p-Ph),* 139.4 (*i-Ph*); *m/z* (ESI$^+$) 275 ([M+Na]$^+$, 100%); HRMS (ESI$^+$)

$C_{13}H_{16}NaO_5^+$ ([M+Na]$^+$) requires 275.0890; found 275.0891. Characterisation data are consistent with the literature.[106]

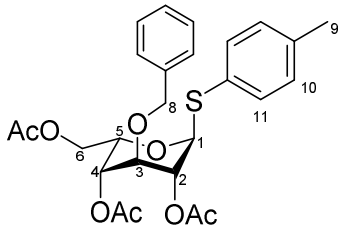
(3*R*,4*S*,5*R*,6*S*)-6-(acetoxymethyl)-4-(benzyloxy)tetrahydro-2*H*-pyran-2,3,5-triyl triacetate (99)



TMSOTf (107 μL, 560 μmol) was added to a solution of **98** (3.00 g, 12.0 mmol) and Ac_2O (22.5 mL, 238 mmol) in CH_2Cl_2 (48 mL) at 0 °C under N_2 and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of satd aq $NaHCO_3$ (50 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Purification via column chromatography (eluent pentane/EtOAc, 4:1) gave **99** as a colourless oil (4.67 g, 90%, α/β, 3:2); ν_{max} (film) 2936 (C−H), 2876 (C−H), 1743 (C=O); δ_H (400 MHz, $CDCl_3$) δ 2.05 (d, J = 1.3 Hz, 3H, *Ac*), 2.07 – 2.12 (m, 12H, 3 × *Ac*), 3.78 (td, J = 2.9, 1.3 Hz, 0.6H, C(3α)*H*), 3.89 (t, J = 3.2 Hz, 0.4H, C(3β)*H*), 4.18 (d, J = 6.5 Hz, 1.2H, C(6α)H_2), 4.20 – 4.24 (m, 0.8H, C(6β)H_2), 4.39 (dddd, J = 8.3, 6.3, 4.4, 2.6 Hz, 0.4H, C(5β)*H*), 4.54 (td, J = 6.4, 1.8 Hz, 1H, C(5α)*H*), 4.69 – 4.74 (m, 2H, C(7)H_2), 4.86 (ddd, J = 3.0, 2.0, 0.9 Hz, 0.4H, C(4β)*H*), 4.92 (ddd, J = 2.8, 1.9, 0.9 Hz, 0.6H, C(4)*H*), 4.95 (dt, J = 2.6, 1.2 Hz, 0.6H, C(2α)*H*), 5.05 (ddd, J = 3.4, 1.8, 0.9 Hz, 0.4H, C(2β)*H*), 6.07 (td, J = 1.4, 0.7 Hz, 0.6H, C(1α)*H*), 6.09 (d, J = 1.9 Hz, 0.4H, C(1β)*H*), 7.28 – 7.40 (m, 5H, *Ph*); δ_C (101 MHz, $CDCl_3$) 20.9, 20.9, 21.0, 21.0 (4 × *Ac*), 62.3 (*C*(6)), 66.4 (*C*(4)), 66.9 (*C*(2)), 72.0 (*C*(5)), 73.2 (*C*(7)), 73.5 (*C*(3)), 90.3 (*C*(1)), 127.9 (*o-Ph*), 128.4 (*p-Ph*), 128.7 (*m-Ph*), 137.0 (*i-Ph*), 168.7, 170.0, 170.1, 170.7 (4 × *C*=O); *m/z* (ESI$^+$) 401 ([M−HOAc+Na]$^+$,

100%) 461 ([M+Na]$^+$, 35%); HRMS (ESI$^+$) $C_{21}H_{26}NaO_{10}^+$ ([M+Na]$^+$) requires 461.1418; found 461.1418. Characterisation data are consistent with the literature.[157]

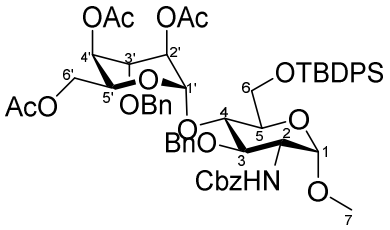
(2*S*,3*R*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-4-(benzyloxy)-6-(p-tolylthio)tetrahydro-2*H*-pyran-3,5-diyl diacetate (100)



$BF_3{\cdot}OEt_2$ (377 μL, 3.05 mmol) was added to a solution of **99** (3.34 g, 7.63 mmol) and 4-methylbenzenethiol (1.42 g, 11.5 mmol) in CH_2Cl_2 (38 mL) at 0 °C under N_2. The reaction was stirred at rt for 16 h. The reaction was quenched by addition of satd aq $NaHCO_3$ (100 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→20% EtOAc in pentane) gave **100** as a viscous, orange oil (2.76 g, 72%); $[\alpha]_D^{25}$ −111 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 2921 (C−H), 2871 (C−H), 1741 (C=O); δ_H (400 MHz, $CDCl_3$) 2.05 (s, 3H, *Ac*), 2.06 (s, 3H, *Ac*), 2.09 (s, 3H, *Ac*), 2.33 (s, 3H, C(9)H_3), 3.78 (td, J = 2.7, 1.2 Hz, 1H, C(3)*H*), 4.19 (dd, J = 11.5, 5.0 Hz, 1H, C(6)H_A), 4.26 (dd, J = 11.5, 7.7 Hz, 1H, C(6)H_B), 4.70 (d, J = 11.9 Hz, 1H, C(8)H_A), 4.84 (d, J = 11.8 Hz, 1H, C(8)H_B), 4.89 (ddt, J = 2.8, 1.8, 0.9 Hz, 1H, C(4)*H*), 5.02 (ddd, J = 7.3, 5.0, 1.7 Hz, 1H, C(5)*H*), 5.17 (dt, J = 2.4, 1.1 Hz, 1H, C(2)*H*), 5.42 (s, 1H, C(1)*H*), 7.04 – 7.14 (m, 2H, 2 × C(10)*H*), 7.28 – 7.35 (m, 1H, *Ph*), 7.35 – 7.41 (m, 2H, *Ph*), 7.44 (dq, J = 8.4, 2.1 Hz, 4H, 2 × C(11)*H*, *Ph*); δ_C (101 MHz, $CDCl_3$) 20.9 (*Ac*), 20.9 (*Ac*), 21.0 (*Ac*), 21.2 (*C*(9)), 63.0 (*C*(6)), 64.6 (*C*(5)), 67.3 (*C*(4)), 68.9 (*C*(5)), 71.6 (*C*(3)), 72.7 (*C*(8)), 86.4 (*C*(1)), 127.8 (*Ph*), 128.1 (*Ph*), 128.6 (*Ph*), 129.8 (*Ph*), 132.2 (*Ph*), 132.2 (*Ph*), 137.2 (*Ph*), 137.8 (*Ph*), 169.6 (*C*=O), 170.2 (*C*=O), 170.7 (*C*=O); *m/z* (ESI$^+$) 525 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{26}H_{30}NaO_8S^+$

([M+Na]$^+$) requires 525.1554; found 525.1556. Characterisation data are consistent with the literature.[158]

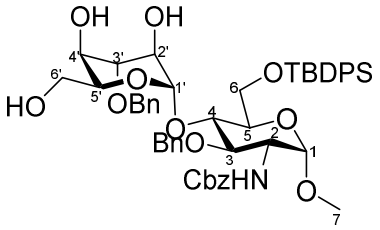
(2*S*,3*R*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-4-(benzyloxy)-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-4-(benzyloxy)-5-(((benzyloxy)carbonyl)amino)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-6-methoxytetrahydro-2*H*-pyran-3-yl)oxy)tetrahydro-2*H*-pyran-3,5-diyl diacetate (101)



93 (2.35 g, 3.58 mmol) and **100** (1.50 g, 2.98 mmol) were dissolved in CH_2Cl_2 (60 mL), under N_2, and dried over 4Å molecular sieves (2.0 g, freshly activated) for 1 h at rt. The reaction was cooled to −60 °C and NIS (873 mg, 3.88 mmol) and TfOH (1M in Et_2O, 895 μL, 0.895 mmol) were added in quick succession. The reaction was allowed to warm to −20 °C over 3 h. The reaction was quenched by addition of $NaHCO_3$ (30 mL) and filtered through Celite®. The organic layer was washed with satd aq $Na_2S_2O_3$ (30 mL), brine, dried over Na_2SO_4 and concentrated in vacuo. Purification via column chromatography (gradient elution, 10%→30% EtOAc in pentane) gave **101** as a white solid (2.60 g, 84%); mp 64-66 °C; $[\alpha]_D^{25}$+13.7 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 2933 (C−H), 2900 (C−H), 2858 (C−H), 1739 (C=O); δ_H (400 MHz, $CDCl_3$) 1.07 (s, 9H, C(C*H*$_3$)$_3$), 1.93 (s, 3H, *Ac*), 1.94 (s, 3H, *Ac*), 2.04 (s, 3H, *Ac*), 3.25 (s, 3H, C(7)*H*$_3$), 3.51 (dd, J = 10.3, 9.2 Hz, 1H, C(3)*H*), 3.62 (ddd, J = 9.7, 3.5, 1.9 Hz, 1H, C(5)*H*), 3.69 (td, J = 2.9, 1.0 Hz, 1H, C(3)*H*), 3.81 – 3.92 (m, 3H, C(6)*H*$_2$, C(6')*H*$_A$), 3.96 (dq, J = 10.3, 4.3 Hz, 2H, C(6')*H*$_B$, C(2)*H*), 4.13 (t, J = 9.5 Hz, 1H, C(4)*H*), 4.43 (d, J = 11.3 Hz, 1H, C(3')OC*H*$_A$Ph), 4.58 – 4.64 (m, 2H, C(1)*H*, C(5')*H*), 4.65 – 4.69 (m, 2H, C(3')OC*H*$_B$, C(3)OC*H*$_A$), 4.72 (d, J = 11.5 Hz, 1H, C(3)OC*H*$_B$), 4.75 (t, J = 2.5 Hz, 1H, C(4')*H*), 4.79 (d, J = 10.0 Hz, 1H, N*H*), 4.87 – 4.94 (m, 1H, C(2')*H*),

5.01 (d, J = 2.6 Hz, 2H, C(9)H_2), 5.18 (s, 1H, C(1')*H*), 7.10 (dd, J = 7.3, 2.3 Hz, 2H, *Ph*), 7.19 (dd, J = 5.6, 1.8 Hz, 3H, *Ph*), 7.28 (td, J = 8.9, 5.7 Hz, 5H, *Ph*), 7.31 – 7.45 (m, 11H, *Ph*), 7.67 – 7.71 (m, 2H, *Ph*), 7.71 – 7.75 (m, 2H, *Ph*); δ_C (101 MHz, $CDCl_3$) 19.6 (*C*(CH_3)$_3$), 20.9 (*Ac*), 20.9 (*Ac*), 21.0 (*Ac*), 26.9 (C(*C*H_3)$_3$), 55.0 (*C*(7)), 55.5 (*C*(2)), 62.4 (*C*(6')), 62.9 (*C*(6)), 63.6 (*C*(5')), 67.0 (*C*(9)), 67.2 (*C*(4')), 67.5 (*C*(2')), 72.2 (*C*(5)), 72.5 (C(3')O*C*H_2Ph), 72.9 (*C*(3')), 73.1 (*C*(4)), 75.3 (C(3)O*C*H_2Ph), 79.3 (*C*(3)), 96.6 (*C*(1')), 98.8 (*C*(1)), 127.4 (*Ph*), 127.6 (*Ph*), 127.7 (*Ph*), 128.1 (*Ph*), 128.3 (*Ph*), 128.3 (*Ph*), 128.5 (*Ph*), 128.6 (*Ph*), 129.6 (*Ph*), 129.7 (*Ph*), 133.5 (*Ph*), 134.1 (*Ph*), 135.8 (*Ph*), 136.1 (*Ph*), 136.4 (*Ph*), 137.5 (*Ph*), 138.4 (*Ph*), 156.0 (C_{Cbz}=O), 169.7 (C_{Ac}=O), 170.4 (C_{Ac}=O), 170.8 (C_{Ac}=O); *m/z* (ESI^+) 1056 ($[M+Na]^+$, 100%) ; HRMS (ESI^+) $C_{57}H_{68}NO_{15}Si^+$ ($[M+H]^+$) requires 1034.4353; found 1034.4347.

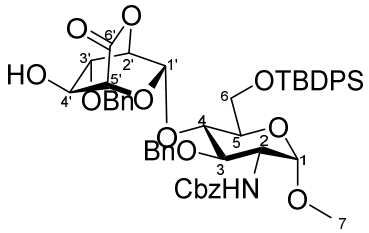
Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-4-(benzyloxy)-5-(((2*S*,3*R*,4*S*,5*R*,6*S*)-4-(benzyloxy)-3,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-6-(((tert-butyldiphenylsilyl)oxy)methyl)-2-methoxytetrahydro-2*H*-pyran-3-yl)carbamate (102)



CH_3ONa (126 mg, 2.34 mmol) was added to a solution of **101** (4.85 g, 4.69 mmol) in CH_3OH/CH_2Cl_2 (1:1, 60 mL) and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of 1M HCl, and concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 and H_2O (1:1, 60 mL), the organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give **102** as a white solid (4.18 g, 98%); mp 74-77 °C; $[\alpha]_D^{25}$ +23.1 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3437 (O−H), 2932 (C−H), 2895 (C−H), 2858 (C−H), 1713 (C=O); δ_H (400 MHz, $CDCl_3$) 1.07 (s, 9H, C(C*H*$_3$)$_3$), 2.99 (dd, J = 12.5,

2.2 Hz, 1H, C(6')H_A), 3.32 (s, 3H, C(7)H_3), 3.42 – 3.53 (m, 2H, C(3)*H*, C(6')H_B), 3.60 – 3.73 (m, 3H, C(5)*H*, C(2')*H*, C(3')*H*), 3.82 (d, J = 3.4 Hz, 1H, C(4')*H*), 3.87 (d, J = 2.8 Hz, 2H, C(6)H_2), 4.02 – 4.11 (m, 3H, C(2)*H*, C(4)*H*, C(5')*H*), 4.41 – 4.50 (m, 2H, C(3)O*C*H_2Ph), 4.55 (d, J = 11.3 Hz, 1H, C(3')O*C*H_APh), 4.63 – 4.74 (m, 2H, C(1)*H*, C(3')O*C*H_BPh), 4.96 (d, J = 10.1 Hz, 1H, N*H*), 5.06 (s, 2H, C(9)H_2), 5.19 (s, 1H, C(1')*H*), 7.05 – 7.15 (m, 2H, *Ph*), 7.22 (dd, J = 5.1, 2.0 Hz, 3H, *Ph*), 7.28 – 7.46 (m, 16H, *Ph*), 7.75 (ddt, J = 14.1, 6.4, 1.8 Hz, 4H, *Ph*); δ_C (101 MHz, $CDCl_3$) 19.5 (*C*$(CH_3)_3$), 26.9 (C(*C*$H_3)_3$), 55.1 (*C*(7)), 55.5 (*C*(2)), 62.9 (*C*(6)), 64.8 (*C*(5')), 65.2 (*C*(6')), 66.4 (*C*(2')), 67.1 (*C*(9)), 71.5 (*C*(4')), 72.0 (C(3')O*C*H_2Ph), 72.4 (*C*(5)), 73.5 (*C*(4)), 75.2 (C(3)O*C*H_2Ph), 75.6 (*C*(3')), 79.8 (*C*(3)), 98.9 (*C*(1)), 101.1 (*C*(1')), 127.5 (*Ph*), 127.7 (*Ph*), 127.8 (*Ph*), 128.0 (*Ph*), 128.2 (*Ph*), 128.3 (*Ph*), 128.3 (*Ph*), 128.3 (*Ph*), 128.5 (*Ph*), 128.6 (*Ph*), 129.8 (*Ph*), 129.9 (*Ph*), 133.2 (*Ph*), 133.5 (*Ph*), 135.9 (*Ph*), 136.1 (*Ph*), 136.5 (*Ph*), 138.5 (*Ph*), 156.1 (*C*=O); *m/z* (ESI^+) 930 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{51}H_{61}NNaO_{12}Si^+$ ($[M+Na]^+$) requires 930.3855; found 930.3857.

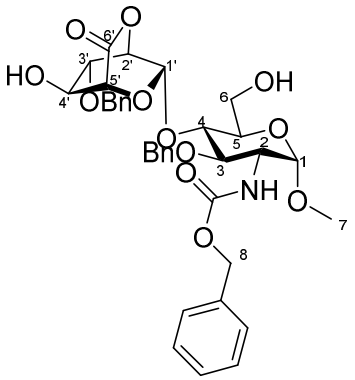
Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-4-(benzyloxy)-5-(((1*R*,3*R*,4*R*,7*S*,8*S*)-8-(benzyloxy)-7-hydroxy-6-oxo-2,5-dioxabicyclo[2.2.2]octan-3-yl)oxy)-6-(((tert-butyldiphenylsilyl)oxy)methyl)-2-methoxytetrahydro-2*H*-pyran-3-yl)carbamate (103)



TEMPO (28 mg, 179 μmol) and (diacetoxyiodo)benzene (567 mg, 1.76 mmol) were added in quick succession to a solution of **102** (800 mg, 882 μmol) in CH_2Cl_2 (8.8 mL) and the reaction was stirred at rt for 16 h. The reaction was diluted with CH_2Cl_2 (10 mL), washed with satd aq $NaHCO_3$ (10 mL), 10% $Na_2S_2O_3$ (10 mL) and brine, dried over Na_2SO_4 and

concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→30% EtOAc in pentane) gave **103** as a colourless glass (485 mg, 61%); mp 77-81 °C; $[\alpha]_D^{25}$ +13.6 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3437 (O−H), 2932 (C−H), 2895 (C−H), 2858 (C−H), 1792 ($C_{Lactone}$=O), 1715 (C_{Cbz}=O); δ_H (400 MHz, $CDCl_3$) 1.06 (s, 9H, C(C*H*$_3$)$_3$), 3.30 (s, 3H, C(7)*H*$_3$), 3.55 (dt, J = 9.7, 2.4 Hz, 1H, C(5)*H*), 3.59 – 3.67 (m, 2H, C(3)*H*, C(3')*H*), 3.71 (dd, J = 11.5, 3.1 Hz, 1H, C(6)*H*$_A$), 3.79 (dd, J = 11.7, 1.8 Hz, 1H, C(6)*H*$_B$), 3.98 (d, J = 3.3 Hz, 1H, C(5')*H*), 3.99 – 4.10 (m, 2H, C(2)*H*, C(4)*H*), 4.14 (dd, J = 5.0, 3.0 Hz, 1H, C(4')*H*), 4.27 (d, J = 2.8 Hz, 1H, C(2')*H*), 4.48 (s, 2H, C(3)OC*H*$_2$Ph), 4.65 (d, J = 11.2 Hz, 1H, C(3')OC*H*$_A$Ph), 4.71 (d, J = 3.7 Hz, 1H, C(1)*H*), 4.83 (t, J = 11.5 Hz, 2H, N*H*, C(3')OC*H*$_B$Ph), 5.08 (q, J = 12.2 Hz, 2H, C(9)*H*$_2$), 5.41 (dd, J = 2.6, 1.3 Hz, 1H, C(1')*H*), 7.26 – 7.34 (m, 12H, *Ph*), 7.41 (dtd, J = 16.5, 7.6, 6.0 Hz, 8H, *Ph*), 7.67 (dp, J = 9.1, 2.1 Hz, 5H, *Ph*); δ_C (101 MHz, $CDCl_3$) 19.3 (*C*(CH$_3$)$_3$), 27.0 (C(*C*H$_3$)$_3$), 54.8 (*C*(2)), 55.2 (*C*(7)), 62.4 (*C*(6)), 67.1 (*C*(9)), 71.3 (*C*(5')), 71.3 (*C*(5)), 71.5 (*C*(4')), 72.3 (C(3)O*C*H$_2$Ph), 72.5 (*C*(2')), 74.2 (C(3')O*C*H$_2$Ph), 77.4 (*C*(4)), 79.1 (*C*(3)), 81.3 (*C*(3')), 97.6 (*C*(1')), 99.0 (*C*(1)), 127.7 (*Ph*), 127.8 (*Ph*), 127.9 (*Ph*), 127.9 (*Ph*), 128.0 (*Ph*), 128.2 (*Ph*), 128.4 (*Ph*), 128.6 (*Ph*), 128.6 (*Ph*), 129.9 (*Ph*), 130.1 (*Ph*), 132.9 (*Ph*), 135.8 (*Ph*), 136.1 (*Ph*), 136.5 (*Ph*), 137.5 (*Ph*), 138.7 (*Ph*), 156.0 (*C*$_{Cbz}$=O), 168.0 (*C*$_{Lactone}$=O); *m/z* (ESI$^+$) 926 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{51}H_{58}NO_{12}Si^+$ ([M+H]$^+$) requires 904.3723; found 904.3717.

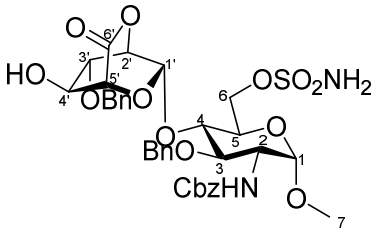
Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-4-(benzyloxy)-5-(((1*R*,3*R*,4*R*,7*S*,8*S*)-8-(benzyloxy)-7-hydroxy-6-oxo-2,5-dioxabicyclo[2.2.2]octan-3-yl)oxy)-6-(hydroxymethyl)-2-methoxytetrahydro-2*H*-pyran-3-yl)carbamate (104)



Tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF, 110 mg, 399 μmol) was added to a solution of **103** (380 mg, 421 μmol) in DMF (4 mL) under N_2 and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of satd aq NH_4Cl (5 mL) and extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with ½ sat brine (5 × 5 mL), brine, dried over Na_2SO_4 and concentrated in vacuo. Purification via column chromatography (gradient elution, 0%→60% EtOAc in pentane) gave **104** as a white solid (130 mg, 75%); mp 86-88 °C; $[\alpha]_D^{25}$ +24.7 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3424 (O−H), 2936 (C−H), 2922 (C−H), 2865 (C−H), 2844 (C−H), 1790 ($C_{Lactone}$=O), 1702 (C_{Cbz}=O); δ_H (400 MHz, $CDCl_3$) 3.28 (s, 3H, C(7)*H*$_3$), 3.48 – 3.59 (m, 1H, C(5)*H*), 3.59 – 3.71 (m, 4H, C(3)*H*, C(3')*H*, C(6)*H*$_2$), 3.79 (t, J = 9.4 Hz, 1H, C(4)*H*), 3.93 (d, J = 3.4 Hz, 2H, C(2)*H*, C(5')*H*), 4.13 (s, 1H, C(4)*H*), 4.46 (t, J = 2.7 Hz, 1H, C(2')*H*), 4.61 (s, 4H, C(1)*H*, C(3)OC*H*$_2$Ph, C(3')OC*H*$_A$Ph), 4.71 (d, J = 10.8 Hz, 1H, C(3')OC*H*$_B$Ph), 4.81 (d, J = 9.9 Hz, 1H, N*H*), 5.00 (q, J = 12.2 Hz, 2H, C(9)*H*$_2$), 5.34 (dd, J = 2.6, 1.3 Hz, 1H, C(1')*H*), 7.14 – 7.33 (m, 15H, *Ph*); δ_C (151 MHz, $CDCl_3$) 54.9 (*C*(2)), 55.4 (*C*(7)), 61.9 (*C*(6)), 67.2 (*C*(8)), 70.9 (*C*(5)), 71.3 (*C*(5')), 71.5 (*C*(4')), 72.7 (*C*(2')), 74.0, 74.2 (C(3)O*C*H$_2$Ph and C(3')O*C*H$_2$Ph), 77.5 (*C*(4)), 79.1 (*C*(3)), 81.2 (*C*(3')), 97.5 (*C*(1')), 99.1 (*C*(1)), 127.8 (*Ph*), 127.9 (*Ph*), 128.2 (*Ph*), 128.4 (*Ph*), 128.4 (*Ph*), 128.5 (*Ph*), 128.7 (*Ph*), 128.8 (*Ph*), 136.4

(*Ph*), 137.3 (*Ph*), 138.5 (*Ph*), 156.0 (C_{Cbz}=O), 167.7 ($C_{Lactone}$=O); *m/z* (ESI^+) 688 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{35}H_{40}NO_{12}^+$ ($[M+H]^+$) requires 666.2545; found 666.2544.

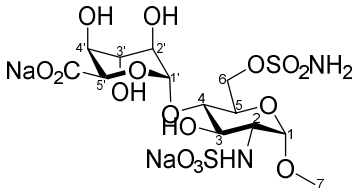
((2*R*,3*S*,4*R*,5*R*,6*S*)-4-(benzyloxy)-3-(((1*R*,3*R*,4*R*,7*S*,8*S*)-8-(benzyloxy)-7-hydroxy-6-oxo-2,5-dioxabicyclo[2.2.2]octan-3-yl)oxy)-5-(((benzyloxy)carbonyl)amino)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (105)



$ClSO_2NH_2$ (52.0 mg, 401 μmol) in $PhCH_3$ (220 μL) was added to a solution of **104** (150 mg, 225 μmol) in DMF (1.35 mL) at −20 °C under N_2 and the reaction was stirred at −20 °C for 16 h. The reaction was quenched by addition of satd aq $NaHCO_3$ (5 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with ½ sat brine (5 × 5 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→60% EtOAc in pentane) gave **105** as a white solid (111 mg, 66%); mp 164-167 °C; $[\alpha]_D^{25}$ −3.5 (*c* 1.0 in CH_3OH); ν_{max} (film) 3378 (O−H), 2918 (C−H), 2877 (C−H), 2851 (C−H), 2838 (C−H), 1787 ($C_{Lactone}$=O), 1702 (C_{Cbz}=O); δ_H (400 MHz, MeOH-*d*$_4$) 3.4 (s, 3H, C(7)H_3), 3.7 – 3.8 (m, 5H, C(2)*H*, C(3)*H*, C(4)*H*, C(5)*H*, C(3')*H*), 4.0 (d, J = 3.5 Hz, 1H, C(2')*H*), 4.2 (dd, J = 10.9, 1.7 Hz, 1H, C(6)H_A), 4.3 (dd, J = 4.6, 3.5 Hz, 1H, C(5')*H*), 4.3 (dd, J = 11.0, 3.3 Hz, 1H, C(6)H_B), 4.6 (d, J = 3.5 Hz, 2H, C(1)*H*, C(3')OCH_A), 4.7 (s, 2H, C(3)OCH_2), 4.9 – 4.9 (m, 2H, C(5')*H*, C(3')OCH_B), 5.0 – 5.1 (m, 2H, NHCOOCH_2Ph), 5.4 (dd, J = 2.7, 1.3 Hz, 1H, C(1')*H*), 7.2 – 7.5 (m, 15H, 3 × *Ph*); δ_C (101 MHz, MeOH-*d*$_4$) 55.8 (*C*(7)), 56.6 (*C*(2)), 67.7 (NHCOO*C*H_2Ph), 68.8 (*C*(6)), 69.9 (*C*(5)), 72.3 (*C*(4')), 72.9 (*C*(2')), 73.0 (C(3)O*C*H_2Ph), 73.6 (*C*(5')), 76.0 (C(3')O*C*H_2Ph), 80.2, 80.5 (*C*(3) and *C*(4)), 82.5 (*C*(3')), 100.2 (*Ar*),

100.5 (*Ar*), 128.5 (*Ar*), 128.9 (*Ar*), 129.0 (*Ar*), 129.0 (*Ar*), 129.2 (*Ar*), 129.4 (*Ar*), 129.5 (*Ar*), 139.2 (*Ar*), 139.9 (*Ar*), 158.6 (C_{Cbz}=O), 170.1 ($C_{lactone}$=O); *m/z* (ESI^+) 767 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{35}H_{41}N_2O_{14}S^+$ ($[M+H]^+$) requires 745.2273; found 745.2273.

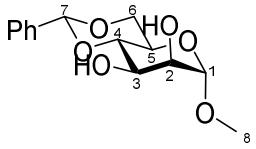
Sodium (2*R*,3*S*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-4-hydroxy-6-methoxy-2-((sulfamoyloxy)methyl)-5-(sulfonatoamino)tetrahydro-2*H*-pyran-3-yl)oxy)tetrahydro-2*H*-pyran-2-carboxylate (88)



(1) Hydrogenation. A suspension of **105** (100 mg, 0.134 mmol), $Pd(OH)_2$/C (200 mg) and cyclohexene (4 mL) in CH_3OH (10 mL) and H_2O (0.75 mL) was heated at 80 °C for 16 h. The reaction was filtered through a PTFE membrane filter and concentrated *in vacuo* to give **107** as a colourless gum (36 mg, 58%). **(2) *N*-sulfation.** SO_3·Py (12.0 mg, 71.0 μmol) was added portion-wise to a solution of **107** (30.0 mg, 65.0 μmol) in H_2O (0.5 mL, pH 9-10) at rt and pH was readjusted after each portion by addition of 1M NaOH. The reaction was stirred at rt for 16 h, concentrated in vacuo and purification via ion-paired reversed phase chromatography (gradient elution, 0→100% MeCN in triethylammonium bicarbonate (1M)). The product containing fractions were concentrated *in vacuo* then eluted from a Dowex® 50X8 Na^+-form column (eluent H_2O) and lyophilised to give **88** as a white solid (8 mg, 22%); mp 248-254 °C (dec.); $[\alpha]_D^{25}$ +36.1 (*c* 1.0 in CH_3OH); ν_{max} (film) 3347 (br., O–H), 3066 (C–H), 3032 (C–H), 2917 (C–H), 2852 (C–H), 1675 (C=O); δ_H (500 MHz, D_2O) 3.34 (s, 3H, C(7)H_3), 3.36 – 3.42 (m, 1H, C(2)*H*), 3.54 – 3.62 (m, 2H, C(3)*H*, C(8)*H*), 3.69 – 3.77 (m, 2H, C(4)*H*, C(9)*H*), 3.95 (ddd, J = 10.09, 3.82, 2.21 Hz, 1H, C(5)*H*), 4.43 (tdd, J = 11.05, 8.77, 2.98 Hz, 3H, C(6)H_2, C(10)*H*), 4.74 (d, J = 5.97 Hz, 1H, C(1)*H*), 4.97 (d, J = 3.64 Hz, 1H, C(6)*H*); δ_C (126 MHz, D_2O) 8.2, 46.6, 55.5 (*C*(11)),

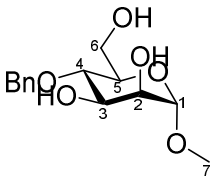
57.6 (*C*(7)), 68.1 (*C*(5)), 68.1 (*C*(6)), 69.5 (*C*(3)), 71.0 (*C*(4)), 71.6 (*C*(2)), 71.6 (*C*(10)) 72.5 (*C*(8)), 77.4 (*C*(9)), 98.4 (*C*(6)), 100.8 (*C*(1)), 175.8 (*C*=O); HRMS (ESI^-) $C_{13}H_{23}N_2O_{16}S_2^-$ ($[M–2Na+H]^-$) requires Exact Mass: 527.0494; found 527.0494.

(2*R*,4a*R*,6*S*,7*S*,8*R*,8a*S*)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxine-7,8-diol (113)



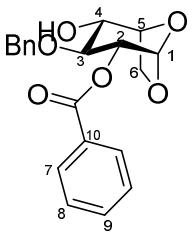
p-TsOH (100 mg, 516 μmol) was added to a solution of methyl α-D-mannopyranoside (2.00 g, 10.3 mmol) and benzaldehyde dimethyl acetal (4.64 mL, 30.9 mmol) in DMF (20 mL) under N_2 and the reaction was stirred at rt for 16 h. The reaction was diluted with EtOAc (100 mL) and washed with ½ sat brine (5 × 25 mL), brine, dried over Na_2SO_4 and concentrated in vacuo. Purification via column chromatography (eluent pentane/EtOAc, 1:1) gave **113** as a white solid (1.18 g, 41%); mp 133-135 °C; {lit.[159] mp 131.1-133.7 °C ($CHCl_3$)}; $[\alpha]_D^{25}$ +56.1 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3367 (O−H), 3234 (O−H), 2903 (C−H), 2831 (C−H); δ_H (400 MHz, $CDCl_3$) 3.36 (s, 3H, C(8)H_3), 3.72 – 3.85 (m, 2H, C(5)*H*, C(6)H_A), 3.86 – 3.92 (m, 1H, C(4)*H*), 3.94 (dd, J = 3.5, 1.5 Hz, 1H, C(2)*H*), 4.00 (dd, J = 9.5, 3.5 Hz, 1H, C(3)*H*), 4.22 – 4.28 (m, 1H, C(6)H_B), 4.68 (d, J = 1.4 Hz, 1H, C(1)*H*), 5.53 (s, 1H, C(7)*H*), 7.36 (qd, J = 3.8, 1.6 Hz, 3H, *Ph*), 7.43 – 7.58 (m, 2H, *Ph*); δ_C (101 MHz, $CDCl_3$) 55.1 (*C*(7)), 63.2 (*C*(5)), 68.7 (*C*(3)), 68.9 (*C*(6)), 71.0 (*C*(2)), 78.9 (*C*(4)), 101.5 (*C*(1)), 102.3 (*C*(7)), 126.4 (*Ph*), 126.4 (*Ph*), 128.4 (*Ph*), 137.4 (*Ph*); *m/z* (ESI^+) 305 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{14}H_{18}NaO_6^+$ ($[M+Na]^+$) requires 305.0996; found 305.0995. characterisation data are consistent with the literature.[160]

(2*S*,3*S*,4*R*,5*S*,6*R*)-5-(benzyloxy)-6-(hydroxymethyl)-2-methoxytetrahydro-2*H*-pyran-3,4-diol (114)



BH_3·THF (1 M in THF, 8.80 mL, 8.87 mmol) and TMSOTf (65.0 µL, 355 µmol) were added in quick succession to a solution of **113** (500 mg, 1.77 mmol) in CH_2Cl_2 (9 mL) under N_2 at 0 °C. The reaction was stirred at rt for 16 h, and was quenched by addition of H_2O (10 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Purification via column chromatography (eluent pentane/EtOAc, 12:88) gave **114** as a white solid (240 mg, 48%); mp 68-70 °C; $[\alpha]_D^{25}$ +59.5 (*c* 1.0 in $CHCl_3$); ν_{max} (film) (3402 (O−H), 2932 (C−H), 2835 (C−H); δ_H (400 MHz, $CDCl_3$) δ 2.17 – 2.24 (m, 1H, O*H*), 2.37 (d, J = 4.9 Hz, 1H, O*H*), 2.66 (d, J = 3.5 Hz, 1H, O*H*), 3.36 (s, 3H, C(7)H_3), 3.62 (dddd, J = 9.8, 3.4, 2.6, 0.6 Hz, 1H, C(5)*H*), 3.74 (dd, J = 9.8, 8.7 Hz, 1H, C(4)*H*), 3.78 – 3.88 (m, 2H, C(6)H_2), 3.88 – 3.97 (m, 1H, C(2)*H*), 4.73 (d, J = 1.3 Hz, 1H, C(1)*H*), 4.76 (s, 2H, C(8)H_2), 7.28 – 7.40 (m, 5H, *Ph*); δ_C (101 MHz, $CDCl_3$) 55.2 (*C*(7)), 62.1 (*C*(6)), 71.4 (*C*(2)), 71.7 (*C*(5)), 74.9 (*C*(8)), 75.4 (*C*(4)), 100.8 (*C*(1)), 128.2 (*Ph*), 128.2 (*Ph*), 128.8 (*Ph*), 138.4 (*i-Ph*); *m/z* (ESI^+) 307 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{14}H_{20}NaO_6^+$ ($[M+Na]^+$) requires 307.1152; found 307.1151.

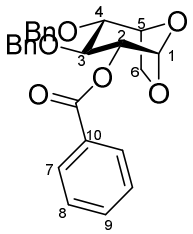
1,6-Anhydro-3-*O*-benzyl-4-*O*-benzoyl-β-L-idopyranose (115)



Benzoyl chloride (622 µL, 5.38 mmol) was added to a solution of **98** (1.13 g, 4.48 mmol) in pyridine (1.10 mL, 13.4 mmol) and CH_2Cl_2 (10 mL) at 0 °C. The reaction was stirred at

rt for 2 h before being quenched by addition of H_2O (10 mL). The organic layer was washed with satd aq $NaHCO_3$ (10 mL), satd aq NH_4Cl (10 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent pentane:EtOAc, 3:1) gave **115** as an amorphous white solid (818 mg, 84%); mp 136-140 °C; {lit.[106] mp 140-141 °C (ethanol)}; $[\alpha]_D^{25}$ +121.6 (*c* 1.0 in $CHCl_3$); {lit.[106] $[\alpha]_D^{26}$ +118.8 (*c* 1.7 in $CHCl_3$)}; ν_{max} (film) 3476 (O−H), 2964 (O−H), 2904 (O−H), 1720 (C=O); δ_H (400 MHz, $CDCl_3$) 2.27 (d, *J* = 3.21 Hz, 1H, O*H*), 3.74 (ddd, *J* = 7.91, 5.02, 1.11 Hz, 1H, C(6)H_A), 3.86 (t, *J* = 8.20 Hz, 1H, C(3)*H*), 3.95 – 4.04 (m, 1H, C(4)*H*), 4.14 (dd, *J* = 7.70, 0.81 Hz, 1H, C(6)H_B), 4.48 (t, *J* = 4.60 Hz, 1H, C(5)*H*), 4.64 (d, *J* = 11.56 Hz, 1H, CH_APh), 4.79 (d, *J* = 11.57 Hz, 1H, CH_BPh), 5.05 (dd, *J* = 8.29, 1.77 Hz, 1H, C(2)*H*), 5.52 (d, *J* = 1.74 Hz, 1H, C(1)*H*), 7.19 – 7.30 (m, 5H, *Ph*), 7.40 – 7.49 (m, 2H, 2 × C(8)*H*), 7.53 – 7.63 (m, 1H, C(9)*H*), 8.01 – 8.11 (m, 2H, 2 × C(7)*H*); δ_C (101 MHz, $CDCl_3$) 65.5 (*C*(6)), 71.5 (*C*(4)), 74.8 (*C*H_2Ph), 75.2 (*C*(5)), 76.9 (*C*(2)), 80.4 (*C*(3)), 99.6 (*C*(1)), 128.0 (*o-Ph*), 128.2 (*p-Ph*), 128.6 (*m-Ph*), 128.8 (2 × *C*(8)), 129.6 (*C*(10)), 130.0 (2 × *C*(7)), 133.6 (C(9)), 138.1 (*i-Ph*), 165.9 (*C*=O); *m/z* (ESI^+) 379 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{20}H_{21}O_6^+$ ($[M+H]^+$) requires 357.1338; found 357.1334. Characterization data is consistent with the literature.[106]

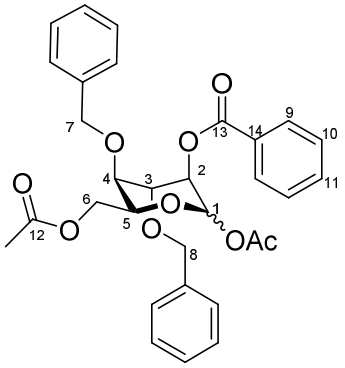
1,6-Anhydro-3-*O*-benzyl-4-*O*-benzoyl-5-*O*-benzyl-β-L-idopyranose (116)



Benzyl bromide (625 µL, 5.26 mmol) was added to a suspension of **115** (1.56 g, 4.38 mmol), Ag_2O (2.03 g, 8.76 mmol) and 4 Å molecular sieves (freshly pre-activated, 1.5 g)

in CH_2Cl_2 (15.5 mL). The reaction was protected from light and was stirred at rt for 2 d. The reaction contained remaining starting material, indicated by TLC, and benzyl bromide (625 µL, 5.26 mmol) was added and the reaction was stirred at rt for 2 d. The reaction was filtered through Celite® and the filtrate was concentrated *in vacuo.* Purification via column chromatography (gradient elution, 0%→15% EtOAc in pentane) gave **116** as a colourless oil (1.45 g, 74%); $[\alpha]_D^{25}$ +133.7 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 1721 (C=O); δ_H (400 MHz, $CDCl_3$) 3.73 (ddd, *J* = 7.79, 5.14, 1.20 Hz, 1H), 3.87 (ddd, *J* = 8.12, 4.24, 1.14 Hz, 1H), 3.99 (t, *J* = 8.19 Hz, 1H), 4.20 (dd, *J* = 7.62, 0.82 Hz, 1H), 4.49 (t, *J* = 4.50 Hz, 1H), 4.67 (d, *J* = 11.77 Hz, 1H), 4.71 – 4.84 (m, 3H), 5.04 (dd, *J* = 8.22, 1.81 Hz, 1H), 5.52 (d, *J* = 1.80 Hz, 1H), 7.18 – 7.25 (m, 5H), 7.28 – 7.40 (m, 5H), 7.40 – 7.49 (m, 2H), 7.54 – 7.63 (m, 1H), 7.94 – 8.11 (m, 2H); δ_C (101 MHz, $CDCl_3$) 65.7(*C*(6)), 73.4 (*C*(5)), 73.5 (*C*H_2Ph), 75.0 (*C*H_2Ph), 77.0 (*C*(2)), 79.5 (*C*(3)), 79.6 (*C*(4)), 99.4 (*C*(1)), 127.8 (*Ph*), 127.9 (*Ph*), 128.0(*Ph*), 128.2(*Ph*), 128.5(*Ph*), 128.5(*Ph*), 128.6 (*Ph*), 128.7 (*Ph*), 129.7 (*Ph*), 130.0 (*Ph*), 133.4 (*Ph*), 138.1 (*Ph*), 138.3 (*Ph*), 166.0 (*C*=O); *m/z* (ESI^+) 469 ($[M+Na]^+$, 60%), 915 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{27}H_{26}NaO_6^+$ ($[M+Na]^+$) requires 469.1622; found 469.1622.

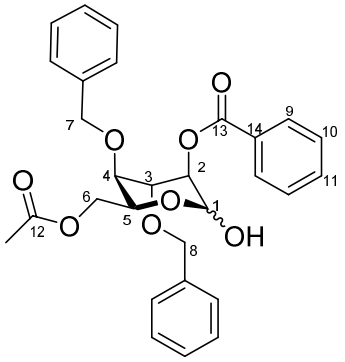
(1*S*,2*R*,3*S*,4*R*,5*S*)-2,3-bis(benzyloxy)-6,8-dioxabicyclo[3.2.1]octan-4-yl benzoate (117)



$Cu(OTf)_2$ (60.0 mg, 0.162 mmol) was added to a solution of **116** (1.45 g, 3.25 mmol) in Ac_2O (7 mL) under N_2 at 0 °C and the reaction was stirred at rt for 16 h. The reaction was

quenched by addition of satd aq $NaHCO_3$ (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentanes, 3:17) gave **117** as an inseparable mixture of anaomers as a pale yellow oil (1.51 g, 85%, α/β; 4:1); ν_{max} (film) 2924 (C–H), 1742 (C=O), 1721 (C=O); δ_H (400 MHz, $CDCl_3$) 1.95 (s, 2.4H, *Ac*), 1.96 (s, 0.6H, *Ac*), 1.97 (s, 0.6H, *Ac*), 2.00 (s, 2.4H, *Ac*), 3.44 (t, J = 3.41 Hz, 0.2H, C(4-β)*H*), 3.48 (t, J = 2.88 Hz, 0.8H, C(4-α)*H*), 3.90 (td, J = 3.28, 1.16 Hz, 0.8H, C(3-α)*H*), 4.01 (t, J = 4.26 Hz, 0.2H, C(3-β)*H*), 4.16 – 4.34 (m, 3.2H, C(5-β)*H*, C(6)H_2, C(8)H_A), 4.34 – 4.46 (m, 1.8H, C(5-α)*H*, C(8)H_B), 4.54 – 4.61 (m, 1H, C(7)H_A), 4.68 – 4.77 (m, 1H, C(7)H_B), 5.12 – 5.19 (m, 0.8H, C(2-α)*H*), 5.24 (dd, J = 4.55, 2.11 Hz, 0.2H, C(2-β)*H*), 6.13 (br s, 1H, C(1)*H*), 7.01 – 7.09 (m, 2H, *Ph*), 7.10 – 7.19 (m, 3H, *Ph*), 7.20 – 7.31 (m, 7H, *Ph*, 2 × C(10)*H*), 7.41 – 7.49 (m, 1H, C(11)*H*), 7.90 – 7.96 (m, 2H, 2 × C(9)*H*); δ_C (101 MHz, $CDCl_3$) 21.0 (*Ac*), 21.1 (*Ac*), 21.1 (*Ac*), 63.2 (*C*(6-α)), 63.6 (*C*(6-β)), 66.9 (*C*(2-α)), 67.7 (*C*(5-α)), 67.7 (*C*(2-β)), 71.2 (*C*(3-α)), 72.4 (*C*(8-α)), 72.7 (*C*(8-β)), 72.8 (*C*(3-β)), 73.1 (*C*(4-α)), 73.4 (*C*(7-β)), 73.5 (*C*(4-β)), 73.8 (*C*(5-β)), 90.7 (*C*(1-β)), 91.8 (*C*(1-α)), 127.6 (*Ar*), 127.9 (*Ar*), 128.1 (*Ar*), 128.1 (*Ar*), 128.1 (*Ar*), 128.3 (*Ar*), 128.5 (*Ar*), 128.5 (*Ar*), 128.5 (*Ar*), 128.6 (*Ar*), 128.7 (*Ar*), 129.4 (*Ar*), 129.6 (*Ar*), 129.9 (*Ar*), 130.1 (*C*(9-α)), 130.2 (*C*(9-β)), 133.3 (*C*(11-β)), 133.5 (*C*(11-α)), 137.3 (*Ar*), 137.7 (*Ar*), 165.6 (*C*(Bz-α)=O), 166.0 (*C*(Bz-β)=O), 169.0 (*C*(Ac-β)=O), 169.1 (*C*(Ac-α)=O), 170.8 (*C*(Ac-α)=O), 170.9 (*C*(Ac-β)=O); *m/z* (ESI+) 571 ($[M+Na]^+$, 58%), 1119 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{31}H_{32}NaO_9^+$ ($[M+Na]^+$) requires 571.1939; found 571.1937.

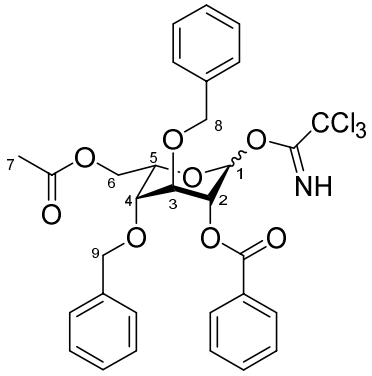
(3*R*,4*S*,5*R*,6*S*)-6-(acetoxymethyl)-4,5-bis(benzyloxy)-2-hydroxytetrahydro-2*H*-pyran-3-yl benzoate (118)



NH_3 (7 N in CH_3OH, 15 mL) was added to a solution of **117** (1.50 g, 2.73 mmol) in THF (160 mL) at 0 °C under N_2 and the reaction was stirred at 0 °C for 16 h. The reaction was concentrated *in vacuo* and the crude residue was purified via column chromatography (eluent EtOAc/pentane, 3:17) to give **118** as an inseparable mixture of anomers as a colourless oil (780 mg, 56%, 75% b.r.s.m., α/β, 0.45:0.55); ν_{max} (film) 3457 (O–H), 1717 (C=O); δ_H (400 MHz, $(CD_3)_2CO$) 2.00 (s, 1.4H, CH_3), 2.00 (s, 1.4H, CH_3), 3.63 (ddd, J = 3.42, 2.47, 0.84 Hz, 0.45H, C(4)*H*), 3.74 – 3.79 (m, 0.55H, C(4)*H*), 4.15 (td, J = 4.53, 0.93 Hz, 0.45H, C(3)*H*), 4.21 – 4.23 (m, 0.55H, C(3)*H*), 4.29 – 4.41 (m, 2H, C(6)H_2), 4.46 (d, J = 11.47 Hz, 0.6H, C(8)H_A), 4.52 – 4.61 (m, 1.45H, C(5)*H*, C(8)H_B, C(8)H_A), 4.69 (d, J = 11.47 Hz, 0.45H, C(8)H_B), 4.77 (dd, J = 11.76, 1.73 Hz, 1H, C(7)H_A), 4.84 (dd, J = 11.76, 4.49 Hz, 1H, C(7)H_B), 5.17 – 5.28 (m, 2H, C(1)*H*, C(2)*H*), 5.59 (d, J = 6.37 Hz, 0.45H, O*H*), 5.89 (d, J = 8.44 Hz, 0.55H, O*H*), 7.15 – 7.45 (m, 12H, 2 × *Ph*, 2 × C(10)*H*), 7.55 – 7.64 (m, 1H, C(11)*H*), 7.97 – 8.04 (m, 2H, 2 × C(9)*H*); δ_C (101 MHz, $(CD_3)_2CO$) 20.8 (*C*H_3), 20.8 (*C*H_3), 63.6 (*C*(6)), 64.5 (*C*(6)), 67.2 (*C*(5)), 70.2 (*C*(2)), 71.1 (*C*(2)), 72.8 (*C*(8)), 73.0 (*C*(8)), 73.4 (*C*(7)), 73.5 (*C*(7)), 73.9 (*C*(3)), 74.3 (*C*(4)), 74.7 (*C*(3)), 75.6 (*C*(4)), 92.9 (*C*(1)), 93.1 (*C*(1)), 128.4 (*Ph*), 128.5 (*Ph*), 128.6 (*Ph*), 128.7 (*Ph*), 128.7 (*Ph*), 128.8 (*Ph*), 129.0 (*Ph*), 129.1 (*Ph*), 129.2 (*Ph*), 129.3 (*Ph*), 130.5 (2 × *C*(9)), 130.7 (2 × *C*(9)), 131.0 (*C*(14)), 131.2 (*C*(14)), 133.8 (*C*(11)), 134.0 (*C*(11)), 139.0 (*i-Ph*), 139.1 (*i-*

Ph), 139.2 (*i-Ph*), 139.2 (*i-Ph*), 166.0 (*C*(13)), 166.6 (*C*(13)), 170.8 (*C*(12)), 170.9 (*C*(12)); HRMS (ESI+) $C_{29}H_{30}NaO_8^+$ ($[M+Na]^+$) requires 529.1838; found 529.1837.

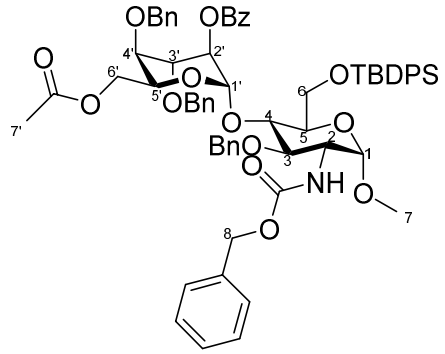
(3*R*,4*S*,5*R*,6*S*)-6-(Acetoxymethyl)-4,5-bis(benzyloxy)-2-(2,2,2-trichloro-1-iminoethoxy)-tetrahydro-2*H*-pyran-3-yl benzoate (119)



Trichloroacetonitrile (200 µL, 2.00 mmol) was added to a suspension of **118** (100 mg, 200 µmol) and K_2CO_3 (138 mg, 1.00 mmol) in CH_2Cl_2 (1.0 mL) and the reaction was stirred at rt for 16 h. The reaction was filtered, and the filtrate was concentrated *in vacuo* to give **119** as a pale yellow oil as a mixture of anomers (149 mg, quant., α:β, 1/1) that was used without further purification; ν_{max} (film) 3332 (=NH), 2923 (C–H), 2855 (C–H), 1724 (C=O), 1673 (C=O); δ_H (400 MHz, CD_2Cl_2) 1.98 (s, 1.5H, C(7)H_3), 2.01 (s, 1.5H, C(7)H_3), 3.64 (qd, J = 1.82, 0.83 Hz, 0.5H, C(4)*H*), 3.77 (dd, J = 6.13, 4.09 Hz, 0.5H, C(4)*H*), 4.05 (td, J = 2.92, 1.28 Hz, 0.5H, C(3)*H*), 4.21 (t, J = 6.39 Hz, 0.5H, C(3)*H*), 4.25 – 4.34 (m, 1.5H, C(6)H_2, C(6)H_A), 4.34 – 4.39 (m, 0.5H, C(6)H_B), 4.39 – 4.49 (m, 1H, C(5)*H*, CH_APh), 4.49 – 4.69 (m, 1.5H, C(5)*H*, CH_APh, CH_BPh), 4.71 – 4.82 (m, 2H, CH_2Ph), 4.84 – 4.91 (m, 0.5H, CH_BPh), 5.40 – 5.47 (m, 1H, C(2)*H*, C(2)*H*), 6.38 (s, 0.5H, C(1)*H*), 6.44 (d, J = 2.81 Hz, 1H, C(1)*H*), 7.13 – 7.45 (m, 12H, *Ar*), 7.54 – 7.62 (m, 1H, *Ar*), 7.92 – 8.04 (m, 2H, *Ar*), 8.59 (s, 0.5H, N*H*), 8.71 (s, 0.5H, N*H*); δ_C (101 MHz, CD_2Cl_2) 21.0 (*C*(7)), 21.0 (*C*(7)), 63.7 (*C*(6)), 64.0 (*C*(6)), 66.5 (*C*(2)), 68.4 (*C*(5)), 69.4 (*C*(2)), 71.3 (*C*(3)), 72.6 (*C*(8)), 72.7 (*C*(8)), 73.4 (*C*(4)), 73.8 (*C*(3)), 74.1 (*C*(5))), 74.5 (*C*(9)), 75.9 (*C*(4)), 95.5 (*C*(1)), 95.6

(*C*(1)), 128.1 (*Ar*), 128.2 (*Ar*), 128.3 (*Ar*), 128.3 (*Ar*), 128.5 (*Ar*), 128.5 (*Ar*), 128.8 (*Ar*), 128.8 (*Ar*), 128.8 (*Ar*), 128.9 (*Ar*), 129.8 (*Ar*), 130.2 (*Ar*), 130.3 (*Ar*), 133.7 (*Ar*), 133.8 (*Ar*), 137.8 (*Ar*), 138.0 (*Ar*), 138.1 (*Ar*), 138.1 (*Ar*), 160.9 (*C*=N), 160.9 (*C*=N), 165.7 (C_{Bz}=O), 166.0 (C_{Bz}=O), 170.8 (C_{Ac}=O), 170.9 (C_{Ac}=O); *m/z* (ESI$^+$) 672 ([M(^{35}Cl)$_3$+Na]$^+$, 96%), 674 ([M(^{35}Cl)$_2$(^{37}Cl)+Na]$^+$, 100%), 676 ([M(^{35}Cl)(^{37}Cl)$_2$+Na]$^+$, 38%), 678 ([M(^{37}Cl)$_3$+Na]$^+$, 7%); HRMS (ESI$^+$) $C_{31}H_{30}Cl_3NNaO_8^+$ ([M(^{35}Cl)$_2$(^{37}Cl)+Na]$^+$) requires 674.0904; found 674.0888.

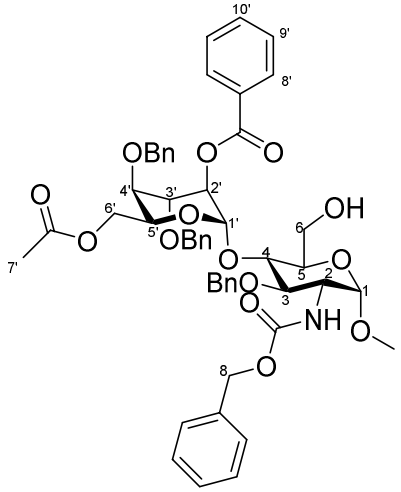
(2*S*,3*R*,4*S*,5*R*,6*S*)-6-(acetoxymethyl)-4,5-bis(benzyloxy)-2-(((2*R*,3*S*,4*R*,5*R*,6*S*)-4-(benzyloxy)-5-(((benzyloxy)carbonyl)amino)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-6-methoxytetrahydro-2*H*-pyran-3-yl)oxy)tetrahydro-2*H*-pyran-3-yl benzoate (120)



93 (875 mg, 1.04 mmol) and **119** (450 mg, 0.690 mmol) in CH_2Cl_2/Et_2O (1:4, 7 mL) were dried over 4Å molecular sieves (700 mg) under N_2 for 1 h and the reaction was cooled to −4 °C and TMSOTf (50 μL, 270 μmol) was added dropwise. The reaction was stirred at 0 °C for 1 h before being quenched by addition of satd aq $NaHCO_3$ (4 mL). The reaction was filtered through Celite® and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent acetone/pentane, 1:4) gave **120** as a white solid (757 mg, 96%); mp 59-61 °C; $[\alpha]_{25}^{D}$ +35.8 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3066 (C–H), 3032 (C–H), 2931 (C–H), 2894 (C–H), 2858 (C–H), 1718 (C=O); δ_H (400 MHz, $CDCl_3$) 0.96 (s, 9H, C(C*H*$_3$)$_3$), 1.90 (s, 3H, C(7')*H*$_3$), 3.20 (s, 3H, C(7)*H*$_3$), 3.37 (t, J = 2.9 Hz, 1H, C(3')*H*),

3.50 (t, J = 9.7 Hz, 1H, C(3)*H*), 3.54 – 3.59 (m, 1H, C(5)*H*), 3.79 (d, J = 11.6 Hz, 1H, C(6)H_A), 3.85 – 3.89 (m, 2H, C(6)H_B, C(4')*H*), 3.90 (td, J = 10.1, 3.5 Hz, 1H, C(2)*H*), 4.07 (d, J = 6.3 Hz, 2H, C(6')H_2), 4.15 (t, J = 9.4 Hz, 1H, C(4)*H*), 4.26 (d, J = 11.5 Hz, 1H, C(3')OH_APh), 4.40 – 4.47 (m, 2H, C(3)OH_APh C(3')OH_BPh), 4.50 (dt, J = 7.9, 3.8 Hz, 1H, C(5')*H*), 4.56 – 4.60 (m, 2H, C(1)*H*, C(4')OH_APh), 4.69 (d, J = 9.9 Hz, 1H, N*H*), 4.71 – 4.79 (m, 2H, C(3)OH_APh, C(4')OH_BPh), 4.93 – 5.03 (m, 2H, C(8)H_2), 5.16 (s, 1H, C(2')*H*), 5.28 (s, 1H, C(1')*H*), 7.08 – 7.16 (m, 9H, *Ar*), 7.17 – 7.33 (m, 18H, *Ar*), 7.48 (td, J = 7.4, 1.4 Hz, 1H, *Ar*), 7.57 (d, J = 7.8 Hz, 2H, *Ar*), 7.65 (d, J = 7.3 Hz, 2H, *Ar*), 7.88 (d, J = 8.2 Hz, 2H, *Ar*); δ_C (176 MHz, $CDCl_3$) 19.5 (*C*$(CH_3)_3$), 21.0 (*C*(7')), 26.9 (C(*C*$H_3)_3$), 55.0 (*C*(7)), 55.3 (*C*(2)), 62.9 (*C*(6)), 63.4 (*C*(6')), 65.6 (*C*(5')), 67.0 (*C*(8)), 68.4 (*C*(2')), 72.1 (*C*(4')), 72.2 (C(3')O*C*H_2Ph), 72.2 (*C*(5)), 72.5 (C(4')O*C*H_2Ph), 73.2 (*C*(4)), 73.6 (*C*(3')), 75.2 (C(3)O*C*H_2Ph), 79.2 (*C*(3)), 96.8 (*C*(1')), 98.8 (*C*(1)), 127.3 (*Ar*), 127.5 (*Ar*), 127.6 (*Ar*), 127.8 (*Ar*), 127.9 (*Ar*), 128.0 (*Ar*), 128.1 (*Ar*), 128.2 (*Ar*), 128.3 (*Ar*), 128.4 (*Ar*), 128.4 (*Ar*), 128.4 (*Ar*), 128.5 (*Ar*), 128.6 (*Ar*), 129.6 (*Ar*), 129.6 (*Ar*), 129.7 (*Ar*), 130.1 (*Ar*), 133.3 (*Ar*), 133.4 (*Ar*), 133.8 (*Ar*), 135.8 (*Ar*), 136.1 (*Ar*), 136.5 (*Ar*), 137.7 (*Ar*), 137.7 (*Ar*), 137.8 (*Ar*), 138.7 (*Ar*), 156.0 (C_{Cbz}=O), 165.8 (C_{Bz}=O), 171.0 (C_{Ac}=O); *m/z* (ESI^+) 1145 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{67}H_{73}NO_{14}NaSi^+$ ($[M+Na]^+$) requires 1166.4693; found 1166.4638.

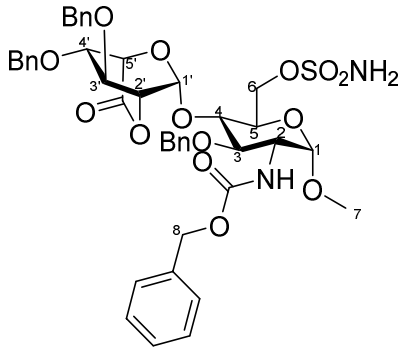
(2*S*,3*R*,4*S*,5*R*,6*S*)-6-(Acetoxymethyl)-4,5-bis(benzyloxy)-2-(((2*R*,3*S*,4*R*,5*R*,6*S*)-4-(benzyloxy)-5-(((benzyloxy)carbonyl)amino)-2-(hydroxymethyl)-6-methoxytetrahydro-2*H*-pyran-3-yl)oxy)tetrahydro-2*H*-pyran-3-yl benzoate (121)



Acetic acid (100 μL, 1.75 mmol) and TBAF (1 M in THF, 1.65 mL, 1.65 mmol) were sequentially added to a solution of **120** (940 mg, 822 μmol) in THF (23.5 mL) at rt and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of satd aq $NaHCO_3$ (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→30% acetone in pentane) gave **121** as a white solid (475 mg, 64%); mp 117-120 °C; $[\alpha]_{25}^{D}$ +32.0 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3443 (OH), 3064 (CH), 3031 (CH), 2952 (CH), 2924 (CH), 1718 (C=O); δ_H (400 MHz, $CDCl_3$) 1.96 (s, 3H, C(7')H_3), 3.31 (s, 3H, C(7)H_3), 3.51 (t, J = 3.5 Hz, 1H, (C(4')*H*), 3.56 (dd, J = 10.4, 9.1 Hz, 1H, C(3)*H*), 3.64 (dt, J = 10.0, 3.2 Hz, 1H, C(5)*H*), 3.82 (d, J = 3.4 Hz, 2H, C(6)H_2), 3.87 – 3.97 (m, 3H, C(2)*H*, C(4)*H*, C(3')*H*), 4.14 (dd, J = 11.5, 4.9 Hz, 1H), 4.22 (dd, J = 11.5, 7.6 Hz, 1H), 4.37 (d, J = 11.6 Hz, 1H, C(4')OCH_APh), 4.43 – 4.52 (m, 2H, C(5')*H*, C(3)OCH_APh), 4.55 (d, J = 11.5 Hz, 1H, C(4')OCH_BPh), 4.61 – 4.67 (m, 2H, C(1)*H*, C(3')OCH_APh), 4.69 (d, J = 9.6 Hz, 1H), 4.75 – 4.87 (m, 2H, C(3)OCH_BPh, C(3')OCH_BPh), 5.01 (s, 2H, C(8)H_2), 5.20 (d, J = 3.1 Hz, 2H, C(1')*H*, C(2')*H*), 7.13 – 7.39 (m, 22H, 4 × *Ph*, 2 × C(9')*H*), 7.49 – 7.57 (m, 1H, C(10')*H*), 7.89 – 8.00 (m, 2H, 2 ×

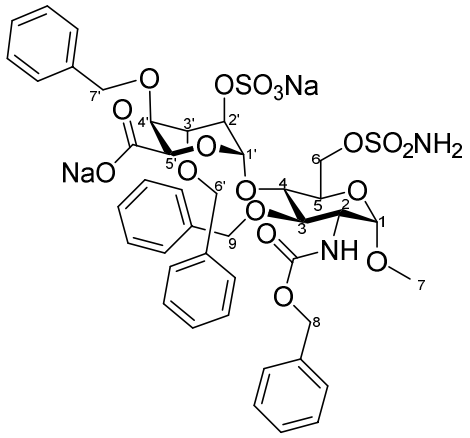
C(8')*H*); δ_C (151 MHz, $CDCl_3$) 21.1 (*C*(7')), 55.1 (*C*(2)), 55.3 (*C*(7)), 61.7 (*C*(6)), 63.0 (*C*(6')), 66.7 (*C*(5')), 67.0 (*C*(8)), 69.2 (*C*(5)), 71.7 (*C*(2')), 72.5 (C(4')O*C*H_2Ph), 73.0 (C(3')O*C*H_2Ph), 73.0 (*C*(3')), 74.1 (*C*(4')), 74.7 (*C*(4)), 75.1 (C(3)O*C*H_2Ph), 78.9 (*C*(3)), 97.4 (*C*(1')), 99.1 (*C*(1)), 127.5 (*Ar*), 128.0 (*Ar*), 128.0 (*Ar*), 128.1 (*Ar*), 128.2 (*Ar*), 128.2 (*Ar*), 128.3 (*Ar*), 128.3 (*Ar*), 128.4 (*Ar*), 128.5 (*Ar*), 128.5 (*Ar*), 128.6 (*Ar*), 128.6 (*Ar*), 129.6 (*Ar*), 130.1 (2 × *C*(8')), 133.4 (*C*(10')), 136.5 (*i-Ph*), 137.6 (*i-Ph*), 137.7 (*i-Ph*), 138.5 (*i-Ph*), 155.9 (C_{Cbz}=O), 165.9 (C_{Bz}=O), 171.1 (C_{Ac}=O); *m/z* (ESI^+) 928 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{51}H_{55}NNaO_{14}^+$ ($[M+Na]^+$) requires 928.3515; found 928.3509.

((2*R*,3*S*,4*R*,5*R*,6*S*)-4-(Benzyloxy)-5-(((benzyloxy)carbonyl)amino)-3-(((2*S*,3*R*,4*R*,5*R*,6*S*)-4,5-bis(benzyloxy)-3-hydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl sulfamate (124)


BnO
BnO
OSO2NH2
BnO
NH
O
O
O

and concentrated *in vacuo* to give the intermediate **123** (960 mg, 83 mmol) that was used without further purification. **(3) Lactone formation.** TEMPO (29 mg, 187 μmol) and (bisacetoxy)iodobenzene (723 mg, 2.25 mmol) were consecutively added to a solution of **123** (960 mg, 936 μmol) in CH_2Cl_2/H_2O (2:1, 15.6 mL) at rt and the reaction was stirred vigorously for 16 h. The reaction was diluted with EtOAc (20 mL) and washed with 10% aq $Na_2S_2O_3$ (20 mL), satd aq $NaHCO_3$ (20 mL), brine, then dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent acetone/pentane, 3:7) gave **124** as a white solid (925 mg, 96%); mp 80-82 °C; $[\alpha]_{25}^{D}$ +2.5 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3370 (br., N–H), 3033 (C–H), 2936 (C–H), 1789 (C=O), 1715 (C=O), 1518 (N–H), 1454 (S=O), 1369 (S=O); δ_H (400 MHz, $CDCl_3$) 3.33 (s, 3H, C(7)H_3), 3.66 – 3.79 (m, 2H, C(3)*H*, C(5)*H*), 3.81 (d, J = 9.4 Hz, 1H, C(4)*H*), 3.87 (ddd, J = 4.4, 3.0, 1.2 Hz, 1H, C(3')*H*), 3.99 (td, J = 9.9, 3.5 Hz, 1H, C(2)*H*), 4.07 (t, J = 4.0 Hz, 1H, C(4')*H*), 4.22 (d, J = 11.1 Hz, 1H, C(6)H_A), 4.29 (d, J = 3.3 Hz, 1H, C(5')*H*), 4.35 – 4.42 (m, 1H, C(6)H_B), 4.43 (d, J = 11.8 Hz, H, C(4')OH_APh), 4.55 (d, J = 1.7 Hz, 1H, C(4')OH_BPh), 4.57 – 4.61 (m, 3H, C(3)OH_APh, C(3')OH_2Ph), 4.62 – 4.64 (m, 1H, C(2')*H*), 4.67 (d, J = 3.5 Hz, 1H, C(1)*H*), 4.76 (d, J = 10.7 Hz, 1H, C(3)OH_BPh), 4.89 (d, J = 9.9 Hz, 1H, N*H*), 4.97 (s, 2H, SO_2NH_2), 5.01 – 5.11 (m, 2H, C(8)H_2), 5.42 (s, 1H, C(1')*H*), 7.13 – 7.43 (m, 20H, 4 × *Ph*); δ_C (151 MHz, $CDCl_3$) 54.7 (*C*(2)), 55.7 (*C*(7)), 67.3 (*C*(8)), 68.7 (*C*(5')), 68.8 (*C*(5)), 68.9 (*C*(6)), 71.8 (*C*(4')), 72.2 (*C*(2')), 72.6 (C(3')O*C*H_2Ph), 74.5 (C(3)O*C*H_2Ph), 77.2 (*C*(4)), 77.9 (*C*(4')), 78.7 (*C*(3)), 80.0 (*C*(3')), 97.9 (*C*(1')), 99.1 (*C*(1)), 128.0 (*Ph*), 128.0 (*Ph*), 128.1 (*Ph*), 128.3 (*Ph*), 128.4 (*Ph*), 128.4 (*Ph*), 128.4 (*Ph*), 128.6 (*Ph*), 128.7 (*Ph*), 128.7 (*Ph*), 136.2 (*i-Ph*), 137.0 (*i-Ph*), 137.1 (*i-Ph*), 137.9 (*i-Ph*), 156.0 (C_{Cbz}=O), 167.5 ($C_{lactone}$=O); *m/z* (ESI$^+$) 835 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{42}H_{47}N_2O_{14}S^+$ ([M+H]$^+$) requires 835.2720; found 835.2738.

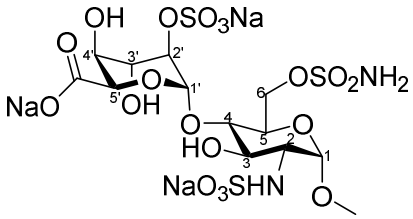
Sodium (2*R*,3*R*,4*S*,5*S*,6*R*)-4,5-bis(benzyloxy)-2-(((2*R*,3*S*,4*R*,5*R*,6*S*)-4-(benzyloxy)-5-(((benzyloxy)carbonyl)amino)-6-methoxy-2-((sulfamoyloxy)methyl)tetrahydro-2*H*-pyran-3-yl)oxy)-6-carboxytetrahydro-2*H*-pyran-3-yl sulfate (125)



(1) Lactone hydrolysis. LiOH (1 M aq, 1.65 mL, 1.65 mmol) was added to **124** (230 mg, 276 μmol) in THF (15 mL) and stirred at rt for 1 h. The reaction was neutralised by addition of Dowex® 50WX4-200R acidic resin, filtered and concentrated *in vacuo*. **(2) *O*-Sulfation.** The residue was dissolved in DMF (3.45 mL) under N_2 and sulfur trioxide-pyridine complex (263 mg, 1.65 mmol) was added. The reaction was heated at 60 °C for 18 h. The reaction was quenched by addition of $NaHCO_3$ (800 mg) and H_2O (1 mL), and was stirred at rt for 1 h. The reaction was concentrated *in vacuo* and the residue was azeotroped with H_2O (2 × 15 mL), then PhMe (1 × 15 mL). Purification via column chromatography (eluent, EtOAc/IPA/H_2O = 82:16:2) gave **125** as a white solid (178 mg, 66% over two steps); mp 87-89 °C; $[\alpha]_{25}^{D}$ +23.2 (*c* 1.0 in CH_3OH); ν_{max} (film) 3437 (O–H), 3064 (C–H), 3033 (C–H), 2954 (C–H), 1714 ($C_{carbamate}$=O), 1615 ($C_{carboxylate}$=O), 1517 ($C_{carbamate}$=O), 1455 ($S_{sulfate}$=O), 1368 ($S_{sulfamate}$=O), ; δ_H (400 MHz, MeOH-d_4) 3.38 (s, 3H, C(7)H_3), 3.64 – 3.84 (m, 3H, C(2)*H*, C(3)*H*, C(4)*H*), 3.88 – 3.96 (m, 1H, C(5)*H*), 4.05 (s, 1H, C(3')*H*), 4.20 (t, J = 2.5 Hz, 1H, C(4')*H*), 4.33 (dd, J = 11.1, 5.9 Hz, 1H, C(6)H_A), 4.44 – 4.56 (m, 3H, C(6)H_B, C(6')H_A, C(9)H_A), 4.57 (d, J = 3.5 Hz, 1H, C(1)*H*), 4.62 – 4.71 (m, 3H, C(2')*H*, C(6')H_B, C(7')H_A), 4.78 (t, J = 11.4 Hz, 2H, C(7')H_B, C(9')H_B), 4.90 (d, J = 1.9 Hz, 1H,

C(5')*H*), 4.92 – 5.03 (m, 2H, C(8)H_2), 5.36 (s, 1H, C(1')*H*), 7.11 – 7.37 (m, 16H, *Ar*), 7.37 – 7.47 (m, 4H, *Ar*); δ_C (126 MHz, MeOH-d_4) 55.7 (*C*(7)), 56.9 (*C*(2)), 67.7 (*C*(8)), 69.6 (*C*(6)), 71.1 (*C*(5')), 71.2 (*C*(5)), 71.8 (*C*(2')), 72.6 (*C*(4')), 72.9 (*C*(7')), 73.6 (*C*(6')), 76.1 (*C*(3')), 77.2 (*C*(9)), 78.6 (*C*(3)), 80.4 (*C*(4)), 100.1 (*C*(1)), 101.0 (*C*(1')), 128.6 (*Ar*), 128.8 (*Ar*), 128.9 (*Ar*), 129.0 (*Ar*), 129.0 (*Ar*), 129.3 (*Ar*), 129.3 (*Ar*), 129.4 (*Ar*), 129.4 (*Ar*), 129.6 (*Ar*), 130.0 (*Ar*), 138.0 (*i-Ph*), 138.7 (*i-Ph*), 139.0 (*i-Ph*), 139.3 (*i-Ph*), 158.4 (C_{Cbz}=O), 176.3 (C_{Acid}=O); *m/z* (ESI$^-$) 931 (100%, [M–Na]$^-$); HRMS (ESI$^-$) $C_{42}H_{47}N_2O_{18}S_2^-$ ([M–Na]$^-$) requires 931.2271; found 931.2266.

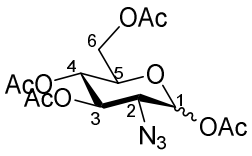
Sodium (2*R*,3*S*,4*S*,5*R*,6*R*)-3,4-dihydroxy-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-4-hydroxy-6-methoxy-2-((sulfamoyloxy)methyl)-5-(sulfonatoamino)tetrahydro-2*H*-pyran-3-yl)oxy)-5-(sulfonatooxy)tetrahydro-2*H*-pyran-2-carboxylate (89)



(1) Global deprotection. Pd(OH)$_2$/C (120 mg) was added to a solution of **125** (160 mg, 171 μmol) in phosphate buffer (20 mM, pH = 7.0)/CH_3OH (17 mL, 1:9) under N_2. The reaction flask was evacuated and back filled with N_2 (× 3) before being put under an atmosphere of H_2 and the reaction was stirred at rt for 16 h. The reaction was filtered through celite and concentrated *in vacuo*. **(2) *N*-Sulfation**. The crude residue was dissolved in H_2O (3 mL), and the solution was adjusted to pH 9-10 by addition of 2 N NaOH(aq). Sulfur trioxide-pyridine complex (40.0 mg, 257 μmol) was added in four equal portions in half-hour intervals at rt, and the pH value was re-adjusted to pH 9-10 using of 2 N NaOH(aq) after each addition. The reaction was stirred at rt for 3 h, then concentrated *in vacuo* and purification via column chromatography on Sephadex G-25 (eluent H_2O), followed by a column of DOWEX 50WX8-Na^+ (eluent H_2O) gave **89** as a white solid (45

mg, 39%); mp 269-277 °C; $[\alpha]_D^{25}$+ 13.2 (*c* 1.0 in H_2O); ν_{max} (film) 3348 (br. O–H), 2918 (C–H), 2852 (C–H), 1676 ($C_{carboxylate}$=O), 1365 (S=O), 1180 (S=O); δ_H (400 MHz, D_2O) 3.28 (dd, J = 10.2, 3.6 Hz, 1H, C(2)*H*), 3.42 (s, 3H, C(7)H_3), 3.68 (dd, J = 10.2, 8.8 Hz, 1H, C(3)*H*), 3.75 (t, J = 9.4 Hz, 1H, C(4)*H*), 3.97 (t, J = 3.7 Hz, 2H, C(5)*H*, C(4')*H*), 4.00 – 4.07 (m, 1H, C(3')*H*), 4.25 (ddd, J = 4.2, 2.6, 0.8 Hz, 1H, C(2')*H*), 4.36 (d, J = 3.1 Hz, 2H, C(6)H_2), 4.6 – 4.7 (m, 1H, C(5')*H*) 5.02 (d, J = 3.6 Hz, 1H, C(1)*H*), 5.15 (d, J = 2.6 Hz, 1H, C(1')*H*); δ_C 13C NMR (151 MHz, D_2O) δ 55.5 (*C*(7)), 57.8 (*C*(2)), 67.1 (*C*(6)), 68.4 (*C*(5)), 69.3 (*C*(5')), 69.3 (*C*(4')), 69.5 (*C*(3')), 69.8 (*C*(3)), 75.0 (*C*(2')), 77.8 (*C*(4)), 98.3 (*C*(1)), 99.3 (*C*(1')), 176.1(*C*=O); *m/z* (ESI^+) 675 (100%, $[M–H]^–$); HRMS ($ESI^–$) $C_{13}H_{23}N_2O_{19}S_3^–$ ($[M–3Na+2H]^–$) requires 607.0063; found 607.0055.

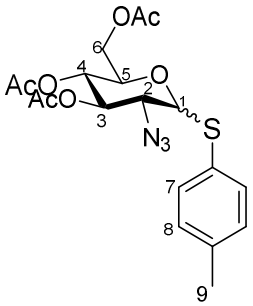
1,3,4,6-tetra-*O*-Acetyl-2-azido-2-deoxy-D-glucopyranose (130)



(1) Diazotransfer. Tf_2O (1.045 mL, 62.1 mmol) was added to a biphasic mixture of NaN_3 (19.5 g, 300 mmol) in H_2O (50 mL) and CH_2Cl_2 (85 mL) at 0 °C. The reaction was stirred at 0 °C for 2 h. The reaction was basified by addition of Na_2SO_4 and the CH_2Cl_2 was separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were added into a solution of glucosamine hydrochloride (6.45 g, 30.0 mmol), $CuSO_4$ (48 mg, 300 μmol) and K_2CO_3 (6.20 g, 45.0 mmol) in H_2O (100 mL). CH_3OH (approx. 100 mL) was added until the reaction was homogenous, and the reaction was stirred at rt for 24 h. The reaction was quenched by addition of glycine (24.0 g) and the reaction was stirred at rt for 24 h. The glycine was removed by vacuum filtration and the filtrate was concentrated *in vacuo*. The residue was azeotroped with $PhCH_3$ (2 × 50 mL) and dried under high vacuum (2 h) to give crude 2-azido-2-deoxy-D-glucose that was used

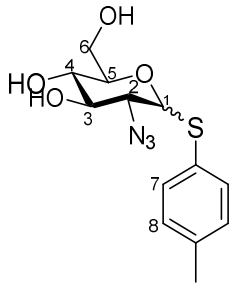
without purification. **(2) Peracetylation.** DMAP (176 mg, 1.44 mmol) was added to a solution of crude 2-azido-2-deoxy-D-glucose in acetic anhydride (29.0 mL, 263 mmol) and pyridine (32 mL) at 0 °C under Ar, before being stirred at rt for 16 h. The reaction was concentrated *in vacuo*, and the residue was partitioned between satd aq $NaHCO_3$ (20 mL) and EtOAc (20 mL). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→40% EtOAc in pentane) gave **130** as a mixture of anomers a colourless oil (8.65 g, 79%, α/β, 0.4:0.6); ν_{max} (film) 2112 (N=N=N), 1745 (C=O); δ_H (400 MHz, $CDCl_3$) 2.02, 2.04, 2.07, 2.09, 2.10, 2.19 (6 × s, 12H, 4 × CH_3), 3.63 – 3.71 (m, 1H, C(2)*H*), 3.77 – 3.84 (m, 0.6H, C(5-β)*H*), 4.01 – 4.13 (m, 1.6H, C(5-α)H, C(6)H_A), 4.29 (ddd, J = 12.59, 4.32, 3.20 Hz, 1H, C(6)H_B), 4.97 – 5.18 (m, 1.6H, C(3-β)*H*, C(4)*H*), 5.45 (dd, J = 10.54, 9.40 Hz, 0.4H, C(3-α)*H*), 5.55 (d, J = 8.56 Hz, 0.6H, C(1-β)*H*), 6.29 (d, *J* = 3.65 Hz, 0.4H, C(1-α)*H*); δ_C (101 MHz, $CDCl_3$) 20.7, 20.7, 20.8, 20.8, 21.0, 21.0 (4 × *C*H_3), 60.4 (*C*(2)), 61.5(*C*(6)), 61.6 (*C*(6)), 62.7 (*C*(2)), 67.9 (*C*(4)), 68.0 (*C*(4)), 69.9 (*C*(5)), 70.9 (*C*(3)), 72.8 (*C*(3)), 72.9 (*C*(5)), 90.1 (*C*(1-α)), 92.7 (*C*(1-β)), 168.6, 168.7, 169.7, 169.7, 169.9, 170.2, 170.7 (4 × *C*=O); *m/z* (ESI$^+$) 396 ([M+Na]$^+$, 75%), 769 ([2M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{14}H_{19}N_3NaO_9^+$ ([M+Na]$^+$) requires 396.1014; found 396.1015.

(2*R*,3*S*,4*R*,5*R*)-2-(Acetoxymethyl)-5-azido-6-(p-tolylthio)tetrahydro-2*H*-pyran-3,4-diyl diacetate (131)



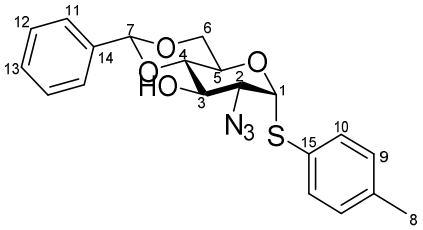
$BF_3.OEt_2$ (3.0 mL, 24.7 mmol) was added to a solution of **130** (2.30 g, 6.17 mmol) and 4-methylbenzenethiol (920 mg, 7.40 mmol) in anhydrous CH_2Cl_2 (30 mL) under N_2 and the reaction was heated at 40 °C for 8 h. The reaction was quenched by addition of satd aq $NaHCO_3$ (10 mL) and the organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentane, 1:5) gave **131** as an inseparable mixture of anomers as an orange oil (1.75 g, 65%, α/β, 3:1); ν_{max} (film) 2110 (N=N=N), 1750 (C=O); δ_H (400 MHz, $CDCl_3$) 2.01 (s, 0.75H, β-C*H*$_3$), 2.04 (s, 2.25H, α-C*H*$_3$), 2.05 (s, 2.25H, α-C*H*$_3$), 2.09 (s, 0.75H, β-C*H*$_3$), 2.10 (s, 2.25H, α-C*H*$_3$), 2.33 (s, 2.25H, C(9-α)*H*), 2.38 (s, 0.75H, C(9-β)*H*), 3.36 (t, J = 9.91 Hz, 0.25H, C(2-β)*H*), 3.67 (ddd, J = 10.08, 4.76, 2.50 Hz, 0.25H, C(6-β)*H*), 4.00 – 4.10 (m, 1.5H, C(2-α)*H*, C(6-α)*H*$_A$), 4.17 – 4.22 (m, 0.5H, C(6-β)*H*$_2$), 4.29 (dd, J = 12.35, 5.13 Hz, 0.75H, C(6-α)*H*$_B$), 4.42 (d, J = 10.13 Hz, 0.25H, C(1-β)*H*), 4.57 – 4.67 (m, 0.75H, C(6-α)*H*), 4.90 (t, J = 9.76 Hz, 0.25H, C(4-β)*H*), 4.99 – 5.10 (m, 1H, C(4-α)*H*, C(3-β)*H*), 5.33 (dd, J = 10.56, 9.20 Hz, 0.75H, C(3-α)*H*), 5.56 (d, J = 5.60 Hz, 0.75H, C(1-α)*H*), 7.09 – 7.19 (m, 2H, 2 × C(7)*H*), 7.33 – 7.42 (m, 1.5H, 2 × C(8-α)*H*), 7.45 – 7.51 (m, 0.5H, 2 × C(8-β)*H*); δ_C (101 MHz, $CDCl_3$) 20.7 (*C*H$_3$), 20.8 (*C*H$_3$), 20.8 (*C*H$_3$), 21.3 (*C*H$_3$), 21.4 (*C*H$_3$), 61.8 (*C*(6-α)), 62.1 (*C*(6-β)), 62.2 (*C*(2-β)), 62.6 (*C*(4-β)), 68.6 (*C*(5-α)), 68.9 (*C*(4-α)), 72.2 (*C*(3-α)), 74.6 (*C*(3-β)), 75.9 (*C*(5-β)), 86.0 (*C*(1-β)), 87.1 (*C*(1-α)), 128.7 (2 × *C*(7-β)), 130.0 (2 × *C*(7-α)), 130.2 (*i-Ph*), 133.0 (2 × *C*(8-α)), 134.8 (2 × *C*(8-β)), 138.6 (*p-Ph*), 169.8 (*C*=O), 169.9 (*C*=O), 170.6 (*C*=O); *m/z* (ESI$^+$) 460 ([M+Na]+, 100%); HRMS (ESI+) $C_{19}H_{23}N_3NaO_7S^+$ ([M+Na]$^+$) requires 460.1149; found 460.1144.

(2*R*,3*S*,4*R*,5*R*)-5-Azido-2-(hydroxymethyl)-6-(p-tolylthio)tetrahydro-2*H*-pyran-3,4-diol (132)



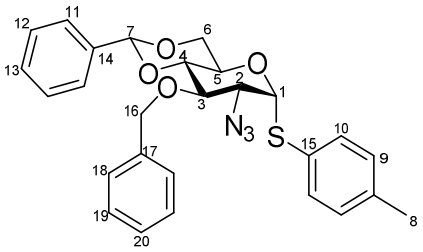
$NaOCH_3$ (45 mg, 800 μmol) was added to a solution of **131** (1.75 g, 4.00 mmol) in CH_3OH (10 mL) and the reaction was stirred at rt for 1 h. The reaction was quenched by addition of Amberlite® IR-120, H^+ form (750 mg), filtered and concentrated *in vacuo* to give an orange oil that was used without further purification (1.25 g, 100%). An analytical sample was prepared by purification via column chromatography (gradient elution, 0→10% CH_3OH in CH_2Cl_2) that gave **132** as an inseparable mixture of anomers as a colourless oil (α/β, 2:1); ν_{max} (film) 3362 (O–H), 2110 (N=N=N); δ_H (500 MHz, Chloroform-d) 2.33 (s, 2H, C*H*$_3$), 2.34 (s, 1H, C*H*$_3$), 3.27 (t, J = 9.62 Hz, 0.35H, C(2-β)*H*), 3.33 (dt, J = 9.44, 3.75 Hz, 0.35H, C(5-β)*H*), 3.48 (t, J = 9.12 Hz, 0.35H, C(3-β)*H*), 3.56 (t, J = 9.27 Hz, 0.35H, C(4-β)*H*), 3.63 – 3.73 (m, 0.65H, C(4-α)*H*), 3.76 – 3.94 (m, 3.3H, C(2-α)*H*, C(5-α)*H*, C(6)*H*$_2$), 4.16 – 4.25 (m, 0.65H, C(3-α)*H*), 4.46 (d, J = 10.08 Hz, 0.35H, C(1-β)*H*), 5.48 (dd, J = 3.38, 1.43 Hz, 0.65H, C(1-α)*H*), 7.13 (t, J = 8.47 Hz, 2H, 2 × C(7)*H*), 7.33 – 7.39 (m, 1.3H, 2 × C(8-α)*H*), 7.42 – 7.45 (m, 0.7H, 2 × C(8-β)*H*); δ_C (126 MHz, Chloroform-d) 21.3 (*C*H$_3$), 21.3 (*C*H$_3$), 61.9 (*C*(6-α)), 62.3 (*C*(6-β)), 63.8 (*C*(2-α)), 65.2 (*C*(3-α)), 70.0 (*C*(4-β)), 70.8 (*C*(4-α)) , 72.2 (*C*(3-α), 73.7 (*C*(5-α)), 79.3 (*C*(5-β)), 86.9 (*C*(1-β)), 87.8 (*C*(1-α)), 127.6 (*i-Ph*), 129.4 (*i-Ph*), 130.1 (*C*(7)), 130.2 (*C*(7)), 133.2 (*C*(8-α)), 133.8 (*C*(8-β)), 138.5 (*p-Ph*), 139.1 (*p-Ph*); *m/z* (ESI^+) 334 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{13}H_{17}N_3NaO_4S^+$ ($[M+Na]^+$) requires 334.0832; found 334.0834.

(2*R*,4a*R*,6*R*,7*R*,8*R*,8a*S*)-7-Azido-2-phenyl-6-(p-tolylthio)hexahydropyrano[3,2-d][1,3]dioxin-8-ol (133)



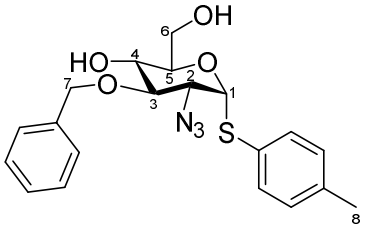
(1*S*)-(+)-10-Camphorsulfonic acid (180 mg, 780 μmol) was added to a suspension of **132** (800 mg, 2.57 mmol) and benzaldehyde dimethyl acetal (770 μL, 5.34 mmol) in CH_3CN (20 mL), under N_2 at rt. The reaction was stirred at rt for 1 h before being concentrated *in vacuo*. Purification via column chromatography (eluent Et_2O/pentane, 2:8) gave **133** as a white solid (775 mg, 76%); mp 149-151 °C; $[\alpha]_{29}^{D}$ +230 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3385 (O–H), 2917 (C–H), 2871 (C–H), 2106 (N=N=N); δ_H (400 MHz, $CDCl_3$) 2.35 (s, 3H, C(8)H_3), 3.57 (t, J = 9.36 Hz, 1H, C(4)*H*), 3.75 (t, J = 10.32 Hz, 1H, C(6)H_A), 3.90 (dd, J = 9.96, 5.59 Hz, 1H, C(2)*H*), 4.08 (t, J = 9.53 Hz, 1H, C(3)*H*), 4.24 (dd, J = 10.33, 4.98 Hz, 1H, C(6)H_B), 4.42 (td, J = 9.94, 4.98 Hz, 1H, C(5)*H*), 5.50 (d, J = 5.54 Hz, 1H, C(1)*H*), 5.56 (s, 1H, C(7)*H*), 7.06 – 7.21 (m, 2H, 2 × C(9)*H*), 7.35 – 7.44 (m, 5H, 2 × C(10)*H*, 2 × C(12)*H*, C(13)*H*), 7.47 – 7.55 (m, 2H, 2 × C(11)*H*); δ_C (101 MHz, $CDCl_3$) 21.3 (*C*(8)), 63.5 (*C*(5)) , 64.1 (*C*(2)), 68.7 (*C*(6)), 70.9 (*C*(3)), 81.9 (*C*(4)), 88.2 (*C*(1)), 102.3 (*C*(7)), 126.4 (2 × *C*(11)), 128.6 (2 × *C*(12)), 129.3 (*C*(13)), 129.6 (*C*(15)), 130.2 (2 × *C*(9)), 133.3 (2 × *C*(10)), 136.9 (*C*(14)), 138.6 (*C*(15)); *m/z* (ESI^+) 422 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{20}H_{21}N_3NaO_4S^+$ ($[M+Na]^+$) requires 422.1145; found 422.1145.

(2*R*,4a*R*,6*R*,7*R*,8*R*,8a*S*)-7-Azido-8-(benzyloxy)-2-phenyl-6-(p-tolylthio)hexahydropyrano[3,2-d][1,3]dioxine (134)



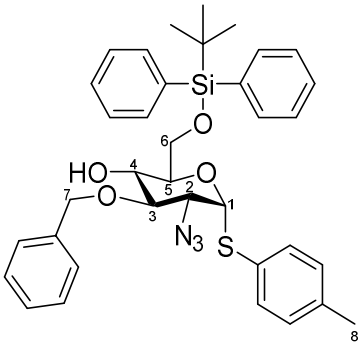
133 (180 mg, 450 μmol) in DMF (2 mL) was added to a suspension of NaH (13 mg, 540 μmol), Bu_4NI (17 mg, 45.0 μmol), and BnBr (110 μL, 900 μmol) in DMF (3 mL) at 0 °C under N_2. The reaction was stirred at rt for 16 h and was quenched by addition of brine. The aqueous layer was extracted with Et_2O (3 × 10 mL) and the combined organic layers were washed with ½ sat. brine (3 × 10 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→20% EtOAc in pentanes) gave **134** as an amorphous white solid (150 mg, 68%); mp 163-165 °C; $[\alpha]_{25}^{D}$ +137 (*c* 1.0 in $CHCl_3$); {Lit. $[\alpha]_D$ +130.6 (*c* 0.75 in $CHCl_3$)}; ν_{max} (film) 2900 (C–H), 2867 (C–H), 2106 (N=N=N); δ_H (400 MHz, $CDCl_3$) 2.34 (s, 3H, C(8)H_3), 3.70 – 3.82 (m, 2H, C(4)*H*, C(6)H_A), 3.92 – 4.01 (m, 2H, C(2)*H*, C(3)*H*), 4.23 (dd, J = 10.37, 4.93 Hz, 1H, C(6)H_B), 4.45 (td, J = 9.95, 4.95 Hz, 1H, C(5)*H*), 4.83 (d, J = 10.87 Hz, 1H, C(16)H_A), 4.98 (d, J = 10.88 Hz, 1H, C(16)H_B), 5.49 (d, J = 4.88 Hz, 1H, C(1)*H*), 5.60 (s, 1H, C(7)*H*), 7.14 (d, J = 8.00 Hz, 2H, 2 × C(9)*H*), 7.27 – 7.45 (m, 10H, 2 × C(10)*H*, 2 × C(12)*H*, C(13)*H*, 2 × C(18)*H*, 2 × C(19)*H*, C(20)*H*), 7.46 – 7.54 (m, 2H, 2 × C(11)*H*); δ_C (101 MHz, $CDCl_3$) 21.2 (*C*(8)), 63.6 (*C*(2)), 63.7 (*C*(5)), 68.6 (*C*(6)), 75.2 (*C*(16)), 77.8 (*C*(3)), 82.8 (*C*(4)), 88.2 (*C*(1)), 101.5 (*C*(7)), 126.0 (*Ar*), 128.0 (*Ar*), 128.3 (*Ar*), 128.3 (*Ar*), 128.5 (*Ar*), 129.1 (*Ar*), 129.1 (*Ar*), 130.0 (*Ar*), 133.1 (*Ar*), 137.1 (*Ar*), 137.7 (*Ar*), 138.4 (*Ar*); *m/z* (ESI^+) 512 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{27}H_{27}N_3NaO_4S^+$ ($[M+Na]^+$) requires 512.1614; found 512.1614.

(2*R*,3*S*,4*R*,5*R*)-5-Azido-4-(benzyloxy)-2-(hydroxymethyl)-6-(p-tolylthio)tetrahydro-2*H*-pyran-3-ol (135)



134 (640 g, 1.29 mmol) was suspended in 80% AcOH (10 mL) and the reaction was heated at 90 °C for 2 h. The reaction was neutralised by addition of aq. 2M NaOH and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→50% EtOAc in pentane) gave **135** as a white crystalline solid (458 mg, 89%); mp 104-105 °C; $[\alpha]_D^{25}$ +137 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3410 (O–H), 2921 (C–H), 2883 (C–H), 2107 (N=N=N); δ_H (400 MHz, $CDCl_3$) 2.34 (s, 3H, C(8)H_3), 3.60 – 3.71 (m, 2H, C(3)*H*, C(4)*H*), 3.75 – 3.84 (m, 2H, C(6)H_2), 3.83 – 3.92 (m, 1H, C(2)*H*), 4.22 (dt, J = 9.89, 3.80 Hz, 1H, C(5)*H*), 4.77 (d, J = 11.32 Hz, 1H, C(7)H_A), 4.99 (d, J = 11.25 Hz, 1H, C(7)H_B), 5.48 (d, J = 5.39 Hz, 1H, C(1)*H*), 7.07 – 7.19 (m, 2H, *Ar*), 7.30 – 7.47 (m, 7H, *Ar*); δ_C (101 MHz, $CDCl_3$) 21.2 (*C*(8)), 62.1 (*C*(6)), 63.8 (*C*(2)), 71.0 (*C*(4)), 72.2 (*C*(5)), 75.5 (*C*(7)), 81.5 (*C*(3)), 87.6 (*C*(1)), 128.2 (*Ar*), 128.3 (*Ar*), 128.8 (*Ar*), 129.1 (*Ar*), 130.0 (*Ar*), 133.2 (*Ar*), 137.9 (*Ar*), 138.4 (*Ar*); *m/z* (ESI$^+$) 424 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{20}H_{23}N_3NaO_4S^+$ ([M+Na]$^+$) requires 424.1301; found 424.1299.

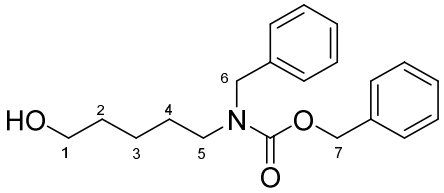
(2*R*,3*S*,4*R*,5*R*,6*R*)-5-Azido-4-(benzyloxy)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-6-(p-tolylthio)tetrahydro-2*H*-pyran-3-ol (136)



tert-Butyldiphenylchlorosilane (195 μL, 750 μmol) was added to a solution of 135 (200 mg, 500 μmol), Et_3N (280 μL, 2.00 mmol) and DMAP (12.0 mg, 100 μmol) in CH_2Cl_2 (2.5 mL), under N_2 at 0 °C. The reaction was stirred at rt for 16 h and was concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→15% EtOAc in pentane)

gave **136** as a colourless oil (300 mg, 94%); $[\alpha]_D^{25}$+62.3 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3480 (O–H), 2931 (C–H), 2857 (C–H), 2108 (N=N=N); δ_H (400 MHz, $CDCl_3$) 1.08 (s, 9H, C(C*H*$_3$)$_3$), 2.32 (s, 3H, C(8)*H*$_3$), 2.62 (d, J = 2.68 Hz, 1H, C(4)O*H*), 3.70 (dd, J = 10.23, 8.61 Hz, 1H, C(3)*H*), 3.80 (t, J = 9.02 Hz, 1H, C(4)*H*), 3.83 – 3.96 (m, 3H, C(2)*H*, C(6)*H*$_2$), 4.28 (dt, J = 9.17, 4.34 Hz, 1H, C(5)*H*), 4.90 (d, J = 11.02 Hz, 1H, C(7)*H*$_A$), 4.95 (d, J = 11.05 Hz, 1H, C(7)*H*$_B$), 5.48 (d, J = 5.32 Hz, 1H, C(1)*H*), 7.01 – 7.09 (m, 2H, *Ar*), 7.33 – 7.48 (m, 13H, *Ar*), 7.65 – 7.73 (m, 4H, *Ar*); δ_C (101 MHz, $CDCl_3$) 19.4 (*C*(CH$_3$)$_3$), 21.2 (*C*(8)), 27.0 (C(*C*H$_3$)$_3$), 63.7 (*C*(2)), 64.4 (*C*(6)), 72.0 (*C*(5)), 73.0 (*C*(4)), 75.6 (*C*(7)), 81.4 (*C*(3)), 87.8 (*C*(1)), 127.9 (*Ar*), 128.0 (*Ar*), 128.2 (*Ar*), 128.4 (*Ar*), 128.8 (*Ar*), 129.9 (*Ar*), 130.0 (*Ar*), 130.0 (*Ar*), 132.8 (*Ar*), 132.9 (*Ar*), 133.2 (*Ar*), 135.7 (*Ar*), 135.8 (*Ar*), 138.1 (*Ar*); *m/z* (ESI$^+$) 662 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{36}H_{41}N_3NaO_4SSi^+$ ([M+Na]$^+$) requires 662.2479; found 662.2474.

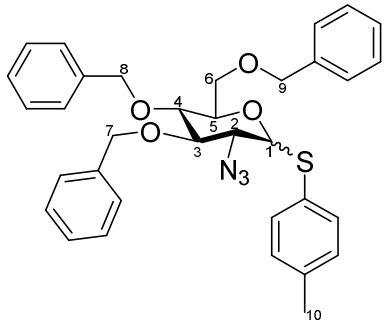
Benzyl benzyl(5-hydroxypentyl)carbamate (138)



5-Amino-1-pentanol (20.0 g, 194 mmol) and benzaldehyde (19.7 mL, 194 mmol) were dissolved in EtOH (300 mL) and the reaction was heated under reduced pressure at 50 °C, slowly evaporating to dryness, and then was co-evaporated with toluene (2 × 150 mL). The crude residue was dissolved in CH_3OH (200 mL) and cooled to 0 °C, and $NaBH_4$ (8.85 g, 233 mmol) was added portion-wise. The reaction was stirred at rt for 2 h. The reaction concentrated *in vacuo* and the residue was partitioned between Et_2O (200 mL) and sat aq $NaHCO_3$ (200 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude was dissolved in Et_2O (100 mL) and sat aq $NaHCO_3$

(100 mL) and benzyl chloroformate (30.6 mL, 214 mmol) was added and the reaction was stirred at rt for 16 h. The reaction was extracted in to Et_2O (200 mL), the organic phase was washed successively with 1M HCl, H_2O and brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent, pentane:EtOAc, 1/1) gave **138** as a colourless oil as a mixture of conformers (55.6 g, 88%); ν_{max} 3426 (O–H), 2934 (C–H), 2860 (C–H), 1694 (carbamate); δ_H (400 MHz, $CDCl_3$) 1.16 – 1.39 (m, 2H, C(3)H_2), 1.44 – 1.61 (m, 4H, C(2)H_2, C(4)H_2), 3.11 – 3.39 (m, 2H, C(5)H_2), 3.41 – 3.70 (m, 2H, C(1)H_2), 4.50 (d, J = 5.56 Hz, 2H, C(6)H_2), 5.18 (d, J = 9.65 Hz, 2H, C(7)H_2), 7.07 – 7.50 (m, 10H, *Ar*); δ_C (126 MHz, $CDCl_3$) 23.0 (*C*(3)), 27.5 (*C*(4)), 28.0 (*C*(4)), 32.4 (*C*(2)), 46.3 (*C*(5)), 47.1 (*C*(5)), 50.3 (*C*(6)), 50.6 (*C*(6)), 62.8 (*C*(1)), 67.3 (*C*(7)), 127.3 (*Ar*), 127.4 (*Ar*), 128.0 (*Ar*), 128.6 (*Ar*), 128.7 (*Ar*), 136.8 (*Ar*), 137.0 (*Ar*), 138.0 (*Ar*), 156.4 (*C*=O), 156.9 (*C*=O); *m/z* (ESI$^+$) 350 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{20}H_{25}NNaO_3^+$ ([M+Na]$^+$) requires 350.1727; found 350.1728.

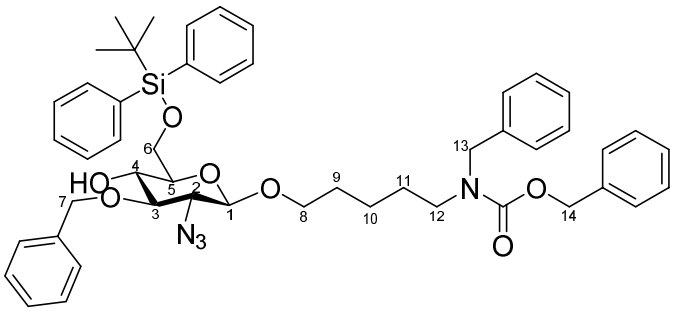
(3*R*,4*R*,5*S*,6*R*)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(p-tolylthio)tetrahydro-2*H*-pyran (139)



NaH (60% in mineral oil, 385 mg, 9.63 mmol) was added to a solution of **132** (500 mg, 1.61 mmol) and BnBr (685 µL, 5.76 mmol) in DMF (6.4 mL) at 0 °C under N_2, and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of ½ saturated brine (10 mL) and was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with ½ saturated brine (5 × 10 mL), brine, dried over Na_2SO_4 and

concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→10% EtOAc in pentanes) gave **139** as a colourless oil as an inconsequential mixture of anomers (720 mg, 77%, α/β, 7:3); ν_{max} (film) 3031 (C–H), 2910 (C–H), 2867 (C–H), 2108 (N=N=N); δ_H (500 MHz, $CDCl_3$) 2.35 (s, 3H, C(10)H_3), 3.33 (dd, J = 10.09, 9.28 Hz, 0.3H, C(2β)*H*), 3.48 (ddd, J = 9.77, 4.00, 2.16 Hz, 0.3H, C(5β)*H*), 3.53 (t, J = 9.13 Hz, 0.3H, C(3β)*H*), 3.58 – 3.64 (m, 0.3H, C(4β)*H*), 3.67 (dd, J = 10.86, 2.04 Hz, 0.7H, C(6α)H_A), 3.72 – 3.89 (m, 3H, C(3α)*H*, C(4α)*H*, C(6α)H_B, C(6β)H_2), 3.96 (dd, J = 10.20, 5.38 Hz, 0.7H, C(2α)*H*), 4.38 (d, J = 10.10 Hz, 0.3H, C(1β)*H*), 4.42 (ddd, J = 9.95, 3.89, 1.98 Hz, 0.7H, C(5α)*H*), 4.47 (d, J = 11.95 Hz, 0.7H, C(9α)H_A), 4.55 – 4.59 (m, 1H, C(8α)H_B, C(9β)H_A), 4.59 – 4.68 (m, 1.3H, C(9α)H_B, C(8β)H_B, C(9β)H_B), 4.79 – 4.88 (m, 1.6H, C(7β)H_2, C(8α)H_B, C(8β)H_B), 4.91 (d, J = 10.48 Hz, 1H, C(7α)H_A), 4.95 (d, J = 10.59 Hz, 1H, C(7α)H_B), 5.57 (d, J = 5.36 Hz, 0.7H, C(1α)*H*), 7.06 – 7.12 (m, 2H, *Ar*), 7.18 – 7.25 (m, 2H, *Ar*), 7.29 – 7.45 (m, 15H, *Ar*), 7.51 – 7.55 (m, 0.6H, *Ar*); δ_C (126 MHz, $CDCl_3$) 21.2 (*C*(10α)), 21.2 (*C*(10β)), 64.2 (*C*(2α)), 64.9 (*C*(2β)), 68.4 (*C*(6α)), 68.8 (*C*(6β)), 71.7 (*C*(5α)), 73.4 (*C*(9β)), 73.5 (*C*(9α)), 75.1 (*C*(8β)), 75.1 (*C*(8α)), 75.8 (*C*(7α)), 75.9 (*C*(7β)), 77.6 (*C*(4β)), 78.3 (*C*(4α)), 79.3 (*C*(5β)), 81.8 (*C*(3α)), 85.1 (*C*(3β)), 85.9 (*C*(1β)), 87.6 (*C*(1α)), 127.0 (*Ar*), 127.6 (*Ar*), 127.7 (*Ar*), 127.8 (*Ar*), 127.9 (*Ar*), 127.9 (*Ar*), 128.0 (*Ar*), 128.0 (*Ar*), 128.2 (*Ar*), 128.3 (*Ar*), 128.4 (*Ar*), 128.5 (*Ar*), 128.5 (*Ar*), 128.6 (*Ar*), 129.6 (*Ar*), 129.8 (*Ar*), 129.9 (*Ar*), 132.7 (*Ar*), 134.4 (*Ar*), 137.6 (*Ar*), 137.7 (*Ar*), 137.8 (*Ar*), 137.9 (*Ar*), 137.9 (*Ar*), 138.3 (*Ar*), 138.8 (*Ar*); *m/z* (ESI^+) 604 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{34}H_{35}N_3NaO_4S^+$ ($[M+Na]^+$) requires 604.2265; found 604.2238.

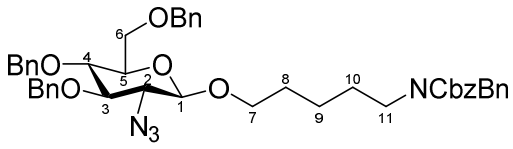
Benzyl (5-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-azido-4-(benzyloxy)-6-(((tert-butyldiphenylsilyl)oxy)methyl)-5-hydroxytetrahydro-2*H*-pyran-2-yl)oxy)pentyl)(benzyl)carbamate (140)



A suspension of **136** (200 mg, 0.313 mmol), **138** (128 mg, 0.376 mmol) and 4Å molecular sieves (freshly activated, 780 mg) in CH_2Cl_2 (7.8 mL) was stirred at rt for 1 h. The reaction was cooled to –78 °C and NIS (85 mg, 0.376 mmol) and TMSOTf (23 µL, 0.125 mmol) were added sequentially. The reaction was allowed to warm to –40 °C, over 3 h. The reaction was quenched by addition of satd aq $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (10 mL). The organic layer was washed with $Na_2S_2O_3$ (10 w/v%), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→20% EtOAc in pentane) gave **140** as a colourless oil (120 mg, 46%); $[\alpha]_D^{25}$ –11.9 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 2934 (CH), 2864 (CH), 2107 (N=N=N), 1698 (C=O); δ_H (500 MHz, DMSO, 338K) 1.02 (s, 9H, C(CH_3)$_3$), 1.24 – 1.36 (m, 2H, C(10)H_2), 1.45 – 1.62 (m, 4H, C(9)H_2, C(11)H_2), 3.21 (t, *J* = 7.38 Hz, 2H, C(12)H_2), 3.25 – 3.38 (m, 2H, C(2)*H*, C(3)*H*), 3.38 – 3.56 (m, 3H, C(4)*H*, C(5)*H*, C(8)H_A), 3.73 – 3.80 (m, 1H, C(8)H_B), 3.84 (dd, *J* = 11.07, 5.56 Hz, 1H, C(6)H_A), 4.01 (dd, *J* = 11.17, 1.86 Hz, 1H, C(6)H_B), 4.39 (d, *J* = 7.63 Hz, 1H, C(1)*H*), 4.45 (s, 2H, C(13)H_2), 4.73 (d, *J* = 11.49 Hz, 1H, C(7)H_A), 4.90 (d, *J* = 11.51 Hz, 1H, C(7)H_B), 5.12 (s, 2H, C(14)H_2), 5.32 (d, *J* = 6.48 Hz, 1H, C(4)O*H*), 7.18 – 7.37 (m, 12H, *Ar*), 7.37 – 7.49 (m, 8H, *Ar*), 7.64 – 7.74 (m, 4H, *Ar*); δ_C (126 MHz, DMSO, 338K) 18.6 (*C*(CH$_3$)$_3$), 22.4 (*C*(10)), 26.3 (C(*C*H$_3$)$_3$), 28.5 (*C*(11)), 27.4 (*C*(9)), 46.2 (*C*(12)), 49.6 (*C*(13)), 63.1(*C*(6)), 65.2 (*C*(2)), 66.0 (*C*(14)), 68.2 (*C*(8), 69.6 (*C*(4)), 73.4 (*C*(7)), 76.4 (*C*(5)), 82.3 (*C*(3)), 100.5 (*C*(1)), 126.7 (*Ar*), 127.0 (*Ar*), 127.1 (*Ar*), 127.3 (*Ar*), 127.4 (*Ar*), 127.4 (*Ar*), 127.7 (*Ar*), 128.0 (*Ar*), 128.1 (*Ar*), 129.3 (*Ar*), 129.4 (*Ar*),

133.1 (*Ar*), 133.2 (*Ar*), 134.8 (*Ar*), 134.8 (*Ar*), 136.7 (*Ar*), 137.9 (*Ar*), 138.5 (*Ar*), 155.4 (*C*=O); *m/z* (ESI$^+$) 866 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{49}H_{59}N_4O7Si^+$ ([M+H]$^+$) requires 843.4148; found 843.4139. The α-anomer was also isolated as a white solid (26 mg, 10%); $[\alpha]_D^{25}$ + 38.2 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 2934 (CH), 2864 (CH), 2107 (N=N=N), 1698 (C=O); δ_H (400 MHz, $CDCl_3$) 1.07 (s, 9H, C(CH_3)$_3$), 1.24 – 1.43 (m, 2H, C(9)H_2), 1.48 – 1.69 (m, 4H, C(8)H_2, C(10)H_2), 3.25 (dd, J = 10.2, 3.6 Hz, 3H, C(2)*H*, C(11)H_2), 3.31 – 3.49 (m, 1H, C(7)H_A), 3.71 (t, J = 8.2 Hz, 3H, C(4)*H*, C(5)*H*, C(7)H_B), 3.79 – 4.00 (m, 3H, C(3)*H*, C(6)H_2), 4.44 – 4.55 (m, 2H, C(12)H_2), 4.78 – 4.95 (m, 3H, C(1)*H*, C(14)H_2), 5.07 – 5.25 (m, 2H, C(13)H_2), 7.13 – 7.48 (m, 21H, *Ar*), 7.69 (ddd, J = 7.9, 4.8, 1.5 Hz, 4H, *Ar*); δ_C (151 MHz, $CDCl_3$) 19.4 (*C*(CH$_3$)$_3$), 23.5 (*C*(9)), 23.7 (*C*(9)), 27.0 (C(*C*H$_3$)$_3$), 27.6 (*C*(10)), 28.0 (*C*(10)), 29.1 (*C*(8)), 29.1 (*C*(8)), 46.2 (*C*(11)), 47.4 (*C*(11)), 50.5 (*C*(12)), 50.7 (*C*(12)), 62.9 (*C*(2)), 64.4 (*C*(6)), 67.3 (*C*(13)), 67.9 (*C*(7)), 71.3 (*C*(4)), 71.5 (*C*(4)), 72.7 (*C*(5)), 75.2 (*C*(14)), 80.0 (*C*(3)), 97.8 (*C*(1)), 127.3 (*Ph*), 127.4 (*Ph*), 127.9 (*Ph*), 127.9 (*Ph*), 128.0 (*Ph*), 128.1 (*Ph*), 128.2 (*Ph*), 128.6 (*Ph*), 128.7 (*Ph*), 130.0 (*Ph*), 133.2 (*i-Ph*), 135.7 (*i-Ph*), 135.8 (4 × *C*(15)), 136.8 (*i-Ph*), 137.0 (*i-Ph*), 138.0 (*i-Ph*), 138.4 (*i-Ph*), 156.4 (*C*=O), 156.9 (*C*=O); *m/z* (ESI$^+$) 865 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{49}H_{58}N_4NaO_7Si^+$ ([M+Na]$^+$) requires 865.3967; found 865.3962.

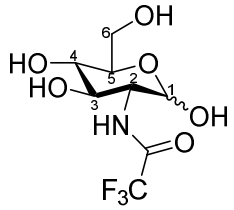
Benzyl (5-(((3*R*,4*R*,5*S*,6*R*)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2*H*-pyran-2-yl)oxy)pentyl)(benzyl)carbamate (141)



A suspension of **139** (162 mg, 280 μmol), **138** (100 mg, 0.308 mmol) and 4 Å molecular sieves (700 mg, freshly activated) in CH_2Cl_2 (7 mL) was stirred at rt for 1 h. The reaction was cooled to – 78 °C, and NIS (75.0 mg, 0.340 mmol) and TMSOTf (20 μL, 0.112 mmol) were added in quick succession. The reaction was allowed to warm to – 40 °C for 4 h, and

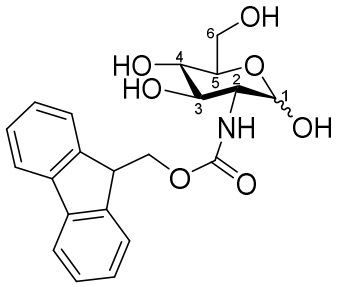
quenched by addition of satd. aq. $NaHCO_3$ (10 mL) and the organic layer was separated, washed with 10 w/v% $Na_2S_2O_3$ (15 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→50% EtOAc in pentanes) gave **141** as a pale yellow oil (138 mg, 63%); $[\alpha]_D^{25}$ –6.21 (*c* 0.55 in $CHCl_3$); ν_{max} (film) 3031 (C–H), 2924 (C–H), 2866 (C–H), 1699 (N=N=N), 1699 (C=O); δ_H (500 MHz, $CDCl_3$) 1.35 (s, 2H, C(9)H_2), 1.53 – 1.71 (m, 4H, C(8)H_2, C(10)H_2), 3.18 – 3.34 (m, 2H, C(11)H_2), 3.39 – 3.47 (m, 3H, C(2)*H*, C(3)*H*, C(5)*H*), 3.47 – 3.57 (m, 1H, C(7)H_A), 3.63 (tt, J = 9.9, 7.3 Hz, 1H, C(4)*H*), 3.73 (qd, J = 10.9, 3.3 Hz, 2H, C(6)H_2), 3.86 – 3.98 (m, 1H, C(7)H_B), 4.25 (d, J = 10.7 Hz, 1H, C(1)*H*), 4.45 – 4.59 (m, 4H, NCH_2Ph, OCH_2Ph), 4.63 (d, J = 12.5 Hz, 1H, OC*H*Ph), 4.82 (dd, J = 10.8, 5.3 Hz, 2H, OCH_2Ph), 4.91 (d, J = 10.8 Hz, 1H, OC*H*Ph), 5.21 (d, J = 14.4 Hz, 2H, COOCH_2Ph), 7.15 – 7.43 (m, 25H, 5 × *Ph*); δ_C (126 MHz, $CDCl_3$) 23.3 (*C*(9)), 27.6 (*C*(10)), 28.0 (*C*(10)), 29.4 (*C*(8)), 46.3 (*C*(11)), 47.2 (*C*(11)), 50.3 (N*C*H_2Ph), 50.7 (N*C*H_2Ph), 66.5 (*C*(2)), 67.3 (COO*C*H_2Ph), 68.8 (*C*(6)), 70.0 (*C*(7)), 73.6 (O*C*H_2Ph), 75.1 (O*C*H_2Ph), 75.2 (*C*(5)), 75.6 (O*C*H_2Ph), 77.9 (*C*(4)), 83.2 (*C*(3)), 102.3 (*C*(1)), 127.4 (*Ar*), 127.8 (*Ar*), 127.9 (*Ar*), 128.0 (*Ar*), 128.0 (*Ar*), 128.0 (*Ar*), 128.0 (*Ar*), 128.2 (*Ar*), 128.5 (*Ar*), 128.6 (*Ar*), 128.6 (*Ar*), 128.7 (*Ar*), 136.9 (*Ar*), 137.1 (*Ar*), 138.0 (*Ar*), 138.1 (*Ar*), 138.1 (*Ar*), 156.3 (*C*=O), 156.9 (*C*=O); *m/z* (ESI^+) 807 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{47}H_{52}N_4NaO_7^+$ ($[M+Na]^+$) requires 807.3752; found 807.3727.

2,2,2-trifluoro-*N*-((3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)acetamide (143)



Ethyl trifluoroacetate (1.34 mL, 9.46 mmol) was added to a suspension of glucosamine hydrochloride (1.02 g, 4.73 mmol) and Na_2CO_3 (1.00 g, 9.46 mmol) in anhydrous CH_3OH (10 mL). The reaction was stirred at rt for 24 h, filtered, and the filtrate was concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/MeOH/H_2O, 10:2:0.2) gave **143** as a mixture of anomers as a white solid (1.16 g, 89%, α:β, 4/6); mp 153–155 °C; ν_{max} (film) 3265 (O–H), 2892 (C–H), 2860 (C–H), 1717 (C=O), 1653 (C=O); δ_H (500 MHz, D_2O) 3.41 – 3.57 (m, 1.6H, C(3β)*H*, C(4α)*H*, C(4β)*H*), 3.63 (dq, J = 10.99, 5.68 Hz, 0.6H, C(5β)*H*), 3.71 – 3.83 (m, 1.6H, C(2β)*H*, C(6α)H_A, C(6β)H_A), 3.83 – 3.96 (m, 1.8H, C(3α)*H*, C(5α)*H*, C(6α)H_B, C(6β)H_B), 4.00 (dd, J = 10.77, 3.61 Hz, 0.4H, C(2α)*H*), 4.82 (d, J = 8.36 Hz, 0.6H, C(1β)*H*), 5.28 (d, J = 3.58 Hz, 0.4H, C(1α)*H*); δ_C (126 MHz, D_2O) 54.7 (*C*(2α)), 57.2 (*C*(2β)), 60.5 (*C*(6α)), 60.6 (*C*(6β)), 69.7 (*C*(4β)), 70.0 (*C*(3α)), 70.1 (*C*(4α)), 71.5 (*C*(5α)), 73.2 (*C*(5β)), 76.0 (*C*(3β)), 90.3 (*C*(1α)), 94.2 (*C*(1β)), 115.7 (q, J = 286.10 Hz, *C*F_3), 115.8 (q, J = 286.10 Hz, *C*F_3), 159.2 (q, J = 37.43 Hz, *C*=O), 159.6 (q, J = 37.43 Hz, *C*=O); δ_F NMR (377 MHz, D_2O) δ -75.71 (C*F*$_3$), -75.62 (C*F*$_3$); *m/z* (ESI$^+$) 298 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_8H_{12}F_3NNaO_6^+$ ([M+Na]$^+$) requires 298.0509; found 298.0508.

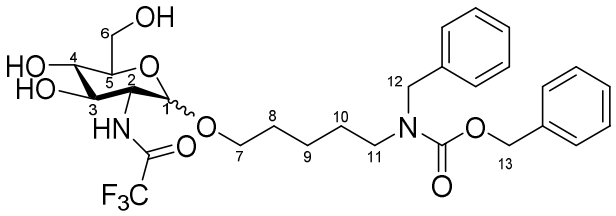
(9*H*-fluoren-9-yl)methyl ((3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (144)



FmocCl (13.3 g, 51.4 mmol) was added to a solution of glucosamine hydrochloride (10.0 g, 46.5 mmol) and $NaHCO_3$ (7.00 g, 83.3 mmol) in H_2O/acetone (1:1, 500 mL). The reaction was stirred at rt for 16 h and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/CH_3OH, 4:1) gave **144** as a white solid (16.4 g, 88%, α/β,

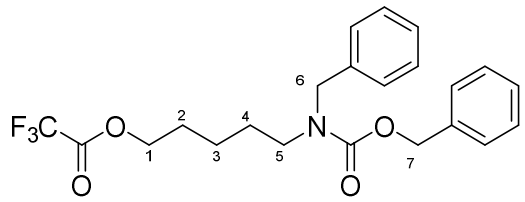
3:7); δ_H (400 MHz, DMSO) 3.03 – 3.21 (m, 2H), 3.25 – 3.35 (m, 1H), 3.39 – 3.71 (m, 4H), 4.10 – 4.28 (m, 3H), 4.43 – 4.60 (m, 1H), 4.78 (d, J = 5.3 Hz, 1H), 4.91 – 5.05 (m, 2H), 6.45 (dd, J = 4.7, 1.1 Hz, 1H), 7.10 (d, J = 8.2 Hz, 1H), 7.33 (dddq, J = 7.5, 4.0, 2.8, 1.2 Hz, 2H), 7.41 (td, J = 7.5, 1.2 Hz, 2H), 7.71 – 7.78 (m, 2H), 7.88 (dt, J = 7.6, 1.0 Hz, 2H); δ_C (101 MHz, DMSO) 30.7, 46.7, 48.6, 54.9, 56.4, 58.7, 61.1, 65.6, 70.2, 71.1, 72.0, 76.7, 90.7, 120.1, 121.4, 125.3, 125.5, 127.1, 127.3, 127.6, 128.9, 140.7, 143.9, 144.0, 144.0, 156.2; *m/z* (ESI^+) 402 ($[M+H]^+$, 100%). Characterisation data are consistent with the literature.[161]

Benzyl benzyl(5-(((2*R*,3*R*,4*R*,5*S*,6*R*)-4,5-dihydroxy-6-(hydroxymethyl)-3-(2,2,2-trifluoroacetamido)tetrahydro-2*H*-pyran-2-yl)oxy)pentyl)carbamate (145)



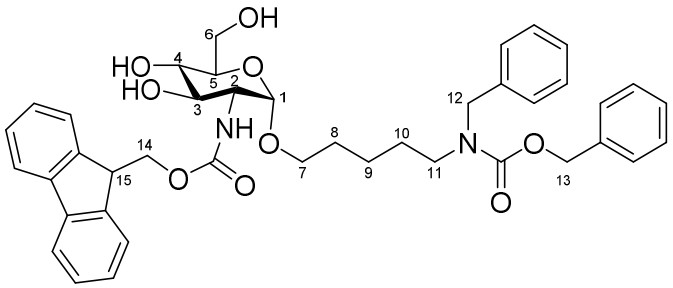
HCl (4M in 1,4-dioaxne, 0.5 mL) was added to a suspension of **144** (200 mg, 0.727 mmol) and **138** (1.18 g, 3.61 mmol) under N_2, and the reaction was heated at 80 °C for 2 h. Reaction stopped due to rapid decomposition of the reaction mixture. Purification via column chromatography (gradient elution, 50%→100% EtOAc in pentanes) **145-α** (27 mg, 6%); $[\alpha]_D^{25}$+85.5 (*c* 1.0 in CH_3OH); mp 103–105°C; ν_{max} (film) 3334 (O–H), 3033 (C–H), 2935 (C–H), 1701 (C=O); δ_H (400 MHz, DMSO) 1.24 (s, 2H, C(9)H_2), 1.45 (s, 4H, C(8)H_2, C(10)H_2), 3.04 – 3.29 (m, 4H,), 3.35 – 3.42 (m, 1H), 3.43 – 3.59 (m, 2H), 3.59 – 3.71 (m, 3H), 4.45 (s, 2H), 4.51 (t, J = 5.88 Hz, 1H), 4.71 (s, 1H), 4.85 (d, J = 4.50 Hz, 1H), 4.99 – 5.21 (m, 3H, C(x)*H*, C(13)H_2), 6.97 – 7.62 (m, 10H, *Ar*), 9.31 (s, 1H); δ_F (376 MHz, DMSO) -73.8; *m/z* (ESI^+) 607 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{28}H_{36}F_3N_2O_8^+$ ($[M+H]^+$) requires 585.2418; found 585.2419. Also isolated **145-β** as a white solid (24 mg, 5.5%);

$[\alpha]_D^{25}$+15.6 (*c* 1.0 in CH_3OH); mp 102–104 °C; ν_{max} (film) 3308 (O–H), 2938 (C–H), 2866 (C–H), 1704 (C=O), 1677 (C=O); δ_H (400 MHz, DMSO) 1.05 – 1.29 (m, 2H, C(9)H_2), 1.29 – 1.55 (m, 4H, C(8)H_2, C(10)H_2), 3.01 – 3.20 (m, 4H, C(2)*H*, C(5)*H*, C(11)H_2), 3.27 (br s, 1H, C(7)H_A), 3.35 – 3.54 (m, 3H, C(3)*H*, C(4)*H*, C(6)H_A), 3.59 – 3.78 (m, 2H, C(6)H_B, C(7)H_B), 4.34 (d, J = 7.93 Hz, 1H, C(1)*H*), 4.44 (s, 2H, C(12)H_2), 4.55 (t, J = 5.90 Hz, 1H, C(6)O*H*), 5.04 – 5.15 (m, 3H, C(13)H_2, O*H*), 5.18 (d, J = 5.38 Hz, 1H, N*H*), 7.05 – 7.51 (m, 10H, *Ar*), 9.15 (d, J = 8.74 Hz, 1H, O*H*); δ_C (126 MHz, DMSO) 22.5, 26.9, 27.5, 28.6, 45.9, 46.6, 49.5, 49.8, 56.1, 60.8, 66.2, 68.3, 70.4, 73.1, 77.0, 100.1, 116.0 (q, J = 288.76 Hz), 127.0 (d, J = 5.97 Hz), 127.3, 127.4, 127.6, 128.2, 128.3, 136.9, 138.1, 155.2, 155.7, 156.2 (q, J = 36.27 Hz); δ_F NMR (377 MHz, DMSO) – 74.4 (s, CF_3); *m/z* (ESI^+) 607 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{28}H_{35}F_3N_2NaO_8^+$ ($[M+Na]^+$) requires 607.2238; found 607.2235.



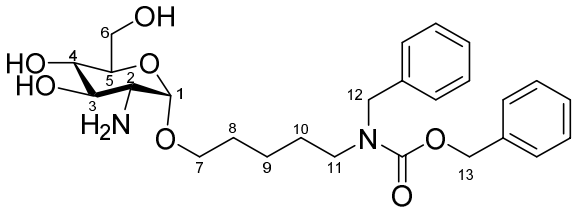
Also isolated **5-(benzyl((benzyloxy)carbonyl)amino)pentyl 2,2,2-trifluoroacetate**; δ_H (400 MHz, $CDCl_3$) δ 1.18 – 1.41 (m, 2H, C(3)H_2), 1.41 – 1.83 (m, 4H, C(2)H_2, C(4)H_2), 3.08 – 3.36 (m, 2H, C(5)H_2), 4.13 – 4.36 (m, 2H, C(1)H_2), 4.50 (d, J = 5.1 Hz, 2H, C(6)H_2), 5.18 (d, J = 6.7 Hz, 2H, C(7)H_2), 7.02 – 7.55 (m, 10H, 2 × *Ph*); δ_F (377 MHz, $CDCl_3$) -75.1 (s, 3F); *m/z* (ESI^+) 392 (100%), 446 ($[M+Na]^+$, 40%).

Benzyl (5-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)pentyl)(benzyl)carbamate (146)



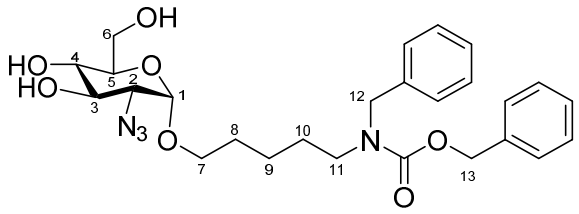
HCl (4M in 1,4-dioaxne, 0.5 mL) was added to a suspension of **144** (200 mg, 0. mmol) and **138** (1.18 g, 3.61 mmol) under N_2, and the reaction was heated at 80 °C for 16 h. Purification via column chromatography (gradient elution, 0%→5% CH_3OH in $CHCl_3$) gave **146** as a white solid (145 mg, 41%); $[\alpha]_D^{25}$+57.9 (*c* 1.0 in CH_3OH); mp 102-106 °C; ν_{max} (film) 3366 (OH), 3087 (CH), 3064 (CH), 2934 (CH), 2868 (CH), 1697 (C=O); δ_H (400 MHz, DMSO, 65 °C) 1.26 (s, 2H, C(9)H_2), 1.50 (dq, J = 12.4, 7.2 Hz, 4H, C(8)H_2, C(10)H_2), 2.39 (dd, J = 9.8, 3.6 Hz, 1H, C(2)*H*), 3.21 (dt, J = 14.4, 7.4 Hz, 3H, (C(11)H_2,), 3.28 (t, J = 8.0 Hz, 1H, C(7)H_A),3.45 – 3.62 (m, 2H, C(14)H_2), 3.64 (d, J = 6.4 Hz, 1H, C(6)H_A) 4.15 – 4.29 (m, 1H, C(6)H_A), 4.32 (dd, J = 10.2, 7.1 Hz, 1H, C(6)H_B), 4.46 (d, J = 14.0 Hz, 2H, C(12)H_2), 4.54 (d, J = 4.2 Hz, 1H, O*H*), 4.60 (d, J = 3.6 Hz, 1H, C(1)*H*), 5.12 (d, J = 8.2 Hz, 2H, C(13)H_2), 7.11 – 7.48 (m, 12H, *Ar*), 7.70 (dd, J = 7.5, 3.5 Hz, 1H, *Ar*), 7.78 – 7.93 (m, 2H, *Ar*); *m/z* (ESI^+) 733 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{41}H_{46}N_2NaO_9^+$ ($[M+Na]^+$) requires 733.3096; found 733.3090. Unable to obtain ^{13}C at 338K due to decomposition of the sample at this temperature for the time required to acquire the data.

Benzyl (5-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-amino-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)pentyl)(benzyl)carbamate (147)



Piperidine (0.70 mL, 7.0 mmol) was added to a solution of **146** (500 mg, 0.70 mmol) in CH_2Cl_2 (5.0 mL) at rt and the reaction was stirred for 1 h. Purification via column chromatography (eluent, 7 N NH_3 in $CH_3OH/CHCl_3$, 1:9, then 3:7) gave **147** as a colourless oil (190 mg, 56%; $[\alpha]_D^{25}$+53.4 (*c* 1.0 in CH_3OH); ν_{max} (film) 3367 (O–H), 2930 (C–H), 1695 (C=O); δ_H (400 MHz, MeOH-*d*$_4$) 1.17 – 1.45 (m, 2H, C(9)*H*$_2$), 1.45 – 1.62 (m, 4H, C(8)*H*$_2$, C(10)*H*$_2$), 2.60 (dd, J = 9.9, 3.6 Hz, 1H, C(2)*H*), 2.87 – 2.99 (m, 1H, C(11)*H*$_A$), 3.21 – 3.38 (m, 3H, C(4)*H*, C(7)*H*$_A$, C(11)*H*$_B$), 3.42 – 3.53 (m, 1H, C(3)*H*), 3.53 – 3.63 (m, 1H, C(5)*H*), 3.72 (dd, J = 11.8, 5.5 Hz, 2H, C(6)*H*$_A$, C(7)*H*$_B$), 3.83 (dd, J = 11.8, 2.4 Hz, 1H, C(6)*H*$_B$), 4.52 (s, 2H, C(12)*H*$_2$), 4.78 (s, 1H, C(1)*H*), 5.18 (d, J = 13.9 Hz, 2H, C(13)*H*$_2$), 7.04 – 7.56 (m, 10H, 2 × *Ph*); δ_C (101 MHz, MeOH-*d*$_4$) 24.5 (*C*(9)), 28.5 (*C*(10)), 29.0 (*C*(10)), 30.2 (*C*(8)), 46.7 (*C*(11)), 47.5 (*C*(11)), 51.3 (*C*(12)), 51.5 (*C*(12)), 57.1 (*C*(2)), 62.7 (*C*(6)), 68.4 (*C*(13)), 68.6 (*C*(7)), 72.0 (*C*(4)), 74.0 (*C*(5)), 76.3 (*C*(3)), 100.1 (*C*(1)), 128.3 (*Ph*), 128.7 (*Ph*), 128.9 (*Ph*), 129.1 (*Ph*), 129.5 (*Ph*), 129.6 (*Ph*), 138.1 (*i-Ph*), 139.2 (*i-Ph*), 157.9 (*C*=O), 158.4 (*C*=O); *m/z* (ESI$^+$) 489 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{26}H_{37}N_2O_7^+$ ([M+H]$^+$) requires 489.2595; found 489.2586.

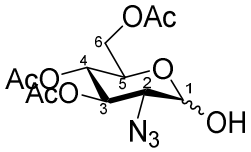
Benzyl (5-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-azido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)pentyl)(benzyl)carbamate (148)



$NaOCH_3$ (80 mg, 1.48 mmol) was added to a solution of **147** (4.97 g, 7.77 mmol) in CH_3OH (40 mL) at rt and the reaction was stirred for 16 h. The reaction was quenched by addition of Amberlite® IR-120 resin (2.0 g), filtered and concentrated *in vacuo* to give **148** as a colourless oil (3.95 g, 99%); $[\alpha]_D^{25}$ +59.7 (*c* 1.0 in CH_3OH); ν_{max} (film) 3379 (O−H), 2939 (C−H), 2868 (C−H) 2108 (N=N=N), 1681 (C=O); δ_H (400 MHz, $CDCl_3$) 1.28 – 1.43 (m,

2H, C(9)H_2), 1.43 – 1.80 (m, 4H, C(8)H_2, C(10)H_2), 3.14 (dd, J = 10.3, 3.5 Hz, 1H), 3.17 – 3.34 (m, 3H), 3.34 – 3.42 (m, 1H), 3.42 – 3.51 (m, 1H), 3.51 – 3.77 (m, 3H), 3.77 – 3.92 (m, 2H), 3.94 – 4.05 (m, 1H), 4.49 (s, 2H, C(12)H_2), 4.85 (d, J = 3.8 Hz, 1H, C(1)*H*), 5.10 – 5.22 (m, 2H, C(13)H_2), 7.08 – 7.45 (m, 10H, 2 × *Ph*); δ_C (126 MHz, $CDCl_3$) 23.4 (*C*(9)), 23.6 (*C*(9)), 27.3 (*C*(10)), 27.9 (*C*(10)), 28.8 (*C*(8)), 29.1 (*C*(8)), 46.2 (*C*(11)), 47.5 (*C*(11)), 50.5 (*C*(12)), 61.9 (*C*(6)), 62.2 (*C*(6)), 62.9 (*C*(2)), 67.5 (*C*(13)), 68.0 (*C*(7)), 68.2 (*C*(7)), 71.4 (*C*(4)), 71.7 (*C*(3)), 77.4 (*C*(5)), 98.0 (*C*(1)), 98.2 (*C*(1)), 127.3 (*Ph*), 127.5 (*Ph*), 127.9 (*Ph*), 128.1 (*Ph*), 128.6 (*Ph*), 128.7 (*Ph*), 136.5 (*i-Ph*), 136.8 (*i-Ph*), 137.7 (*i-Ph*), 156.6 (*C*=O), 157.1 (*C*=O); *m/z* (ESI^+) 515 ($[M+H]^+$, 10%) 537 ($[M+Na]^+$, 66%), 1051 ($[2M+Na]^+$, 100%); HRMS (ESI^+) $C_{26}H_{35}N_4O_7^+$ ($[M+H]^+$) requires 515.2500; found 515.2498.

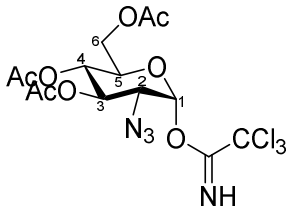
(2*R*,3*S*,4*R*,5*R*)-2-(Acetoxymethyl)-5-azido-6-hydroxytetrahydro-2*H*-pyran-3,4-diyl diacetate (149)



$(CH_3)_2NH$ (5.6 M in EtOH, 4.80 mL, 26.8 mmol) was added to a solution of **130** (4.99 g, 13.4 mmol) in THF (30 mL) at 0 °C, and the reaction was stirred for 1 h at 0 °C. The reaction was concentrated *in vacuo* and purification via column chromatography (gradient elution, 0%→50% EtOAc in pentane) gave **149** as a mixture of anomers as a colourless oil (4.10 g, 92%, α/β, 3:2); ν_{max} (film) 3429 (O−H), 2954 (C−H), 2916 (C−H), 2111 (N=N=N), 1744 (C=O); δ_H (400 MHz, $CDCl_3$) 2.04 (s, 3H, *Ac*), 2.06 – 2.13 (m, 6H, 2 × *Ac*), 3.42 (dd, J = 10.5, 3.4 Hz, 0.6H, C(2-α)*H*), 3.45 – 3.51 (m, 0.4H, C(2-β)*H*), 3.68 – 3.74 (m, 0.4H, C(5-β)*H*), 4.06 – 4.18 (m, 1H, C(6)H_A), 4.18 – 4.31 (m, 1.6H, C(6)H_B, C(5-α)*H*), 4.73 (d, J = 8.0 Hz, 0.4H, C(1-β)*H*), 4.98 – 5.10 (m, 1.4H, C(3-β)*H*, C(4-α)*H*, C(4-β)*H*), 5.39 (d, J

= 3.4 Hz, 0.6H, C(1-α)*H*), 5.52 (dd, J = 10.6, 9.3 Hz, 0.6H, C(3-α)*H*); δ_C (101 MHz, $CDCl_3$) 20.7 (*Ac*), 20.7 (*Ac*), 20.8 (*Ac*), 20.8 (*Ac*), 20.8 (*Ac*), 20.9 (*Ac*), 61.6 (*C*(2-α)), 62.1 (*C*(6)), 65.0 (*C*(2-β)), 67.7 (*C*(5-α)), 68.5 (*C*(4-β)), 68.7 (*C*(4-α)), 70.6 (*C*(3-α)), 72.1 (*C*(5-β)), 72.7 (*C*(3-β)), 92.2 (*C*(1-α)), 96.3 (*C*(1-β)), 169.9 (*C*=O), 170.0 (*C*=O), 170.2 (*C*=O), 170.3 (*C*=O), 171.0 (*C*=O), 171.0 (*C*=O); *m/z* (ESI^+) 354 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{12}H_{17}N_3NaO_8^+$ ($[M+Na]^+$) requires 354.0908; found 354.0907.

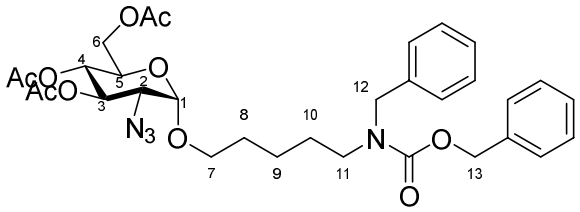
(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(acetoxymethyl)-5-azido-6-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2*H*-pyran-3,4-diyl diacetate (150)



DBU (10.0 μL, 68.0 μmol) was added to a solution of **149** (225 mg, 680 μmol) and trichloroacetonitrile (680 μL, 6.80 mmol) in CH_2Cl_2 (4.5 mL) under N_2 at rt. The reaction was stirred at rt for 4 h and then concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentane, 3:7) gave **150** as a white crystalline solid (200 mg, 62%); mp 127-129 °C; $[\alpha]_{25}^{D}$ +118 (*c* 1.0 in CH_2Cl_2); ν_{max} (film) 3321 (N−H), 2691 (C−H), 2924 (C−H), 2113 (N=N=N), 1748 (C=O), 1677 (C=N); δ_H (400 MHz, CD_2Cl_2) 2.03 (s, 6H, 2 × *Ac*), 2.09 (s, 3H, *Ac*), 3.79 (dd, J = 10.6, 3.6 Hz, 1H, C(2)*H*), 4.03 – 4.15 (m, 1H, C(6)H_A), 4.16 – 4.26 (m, 2H, C(5)*H*, C(6)H_B), 5.17 (td, J = 10.1, 2.6 Hz, 1H, C(4)*H*), 5.49 (dd, J = 10.6, 9.4 Hz, 1H, C(3)*H*), 6.48 (d, J = 3.6 Hz, 1H, C(1)*H*), 8.89 (s, 1H, N*H*); δ_C (151 MHz, CD_2Cl_2) δ 20.8 (*Ac*), 20.8 (*Ac*), 20.9 (*Ac*), 61.2 (*C*(2)), 61.8 (*C*(6)), 68.4 (*C*(4)), 70.7 (*C*(5)), 70.8 (*C*(3)), 91.0 (CCl_3), 94.5 (*C*(1)), 160.9 (*C*=N), 169.9 (*C*=O),

170.1 (*C*=O), 170.7 (*C*=O); *m/z* (ESI$^+$) 497 ([M(^{35}Cl)$_3$+Na]$^+$, 100%), 499 ([M(^{35}Cl)$_2$(^{37}Cl)+Na]$^+$, 88%), 501 ([M(^{35}Cl)(^{37}Cl)$_2$+Na]$^+$, 11%), 971 ([2M(^{35}Cl)$_3$+Na]$^+$, 9%), 973 (2[M(^{35}Cl)(^{37}Cl)$_2$+Na]$^+$, 41%), 975 (2[M(^{35}Cl)$_2$(^{37}Cl)+Na]$^+$, 22%), 977 (2[M(^{37}Cl)$_3$+Na]$^+$, 2%); HRMS (ESI$^+$) $C_{14}H_{17}(^{35}Cl)_3N_4NaO_8^+$ ([M(^{35}Cl)$_3$+Na]$^+$) requires 497.0004; found 497.0004.

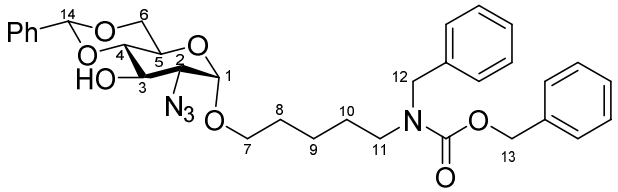
(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(acetoxymethyl)-5-azido-6-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2*H*-pyran-3,4-diyl diacetate (151)



150 (200 mg, 0.422 mmol) and **138** (166 mg, 0.506 mmol) were dried by azeotroping with $PhCH_3$ (2 × 5 mL) and then dissolved in CH_2Cl_2/Et_2O (1:4, 4.25 mL) and dried over 4Å molecular sieves (freshly activated, 450 mg) at rt for 1 h. The reaction was cooled to 0 °C, TMSOTf (4 μL, 21 μmol) was added, and the reaction was stirred at 0 °C for 10 min. The reaction was quenched by addition of pyridine (~15 μL), filtered through Celite® and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentane, 1:4) gave **151** as a colourless oil (150 mg, 56%); ν_{max} (film) 3031 (CH), 2946 (CH), 2874 (CH), 2109 (N=N=N), 1750 (C=O), 1698 (C=O); $[\alpha]_D^{25}$ + 92.4 (*c* 1.0 in $CHCl_3$); δ_H (600 MHz, $CDCl_3$) 1.27 – 1.41 (m, 2H, C(9)H_2), 1.47 – 1.69 (m, 4H, C(8)H_2, C(10)H_2), 2.03 (s, 3H, *Ac*), 2.08 (s, 3H, *Ac*), 2.08 (s, 3H, *Ac*), 3.14 – 3.32 (m, 3H, C(2)*H*, C(11)H_2), 3.35 – 3.52 (m, 1H, C(7)H_A), 3.59 – 3.74 (m, 1H, C(7)H_B), 3.94 – 4.03 (m, 1H, C(5)*H*), 4.06 (dd,

J = 12.2, 2.4 Hz, 1H, C(6)H_A), 4.22 – 4.33 (m, 1H, C(6)H_B), 4.50 (d, J = 9.4 Hz, 2H, C(12)H_2), 4.86 – 4.98 (m, 1H, C(1)*H*), 5.03 (dd, J = 10.2, 9.2 Hz, 1H, C(4)*H*), 5.12 – 5.24 (m, 2H, C(13)H_2), 5.47 (dd, J = 10.6, 9.2 Hz, 1H, C(3)*H*), 7.10 – 7.41 (m, 10H, 2 × *Ph*); δ_C (151 MHz, $CDCl_3$) 20.8 (*Ac*), 20.9 (*Ac*), 23.5 (*C*(9)), 27.6 (*C*(10)), 28.0 (*C*(10)), 29.1 (*C*(8)), 46.3 (*C*(11)), 47.2 (*C*(11)), 50.4 (*C*(12)), 50.7 (*C*(12)), 61.0 (*C*(2)), 62.0 (*C*(6)), 67.3 (*C*(13)), 67.7 (*C*(5)), 68.8 (*C*(4)), 68.8 (*C*(7)), 70.5 (*C*(3)), 98.0 (*C*(1)), 127.4 (*Ar*), 128.0 (*Ar*), 128.6 (*Ar*), 128.7 (*Ar*), 136.9 (*i-Ph*), 137.1 (*i-Ph*), 138.1 (*i-Ph*), 156.4 (C_{Cbz}=O), 156.9 (C_{Cbz}=O), 169.8 (*C*=O), 170.2 (*C*=O), 170.7 (*C*=O); *m/z* (ESI^+) 663 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{32}H_{41}N_4O_{10}^+$ ($[M+H]^+$) requires 641.2817; found 641.2826.

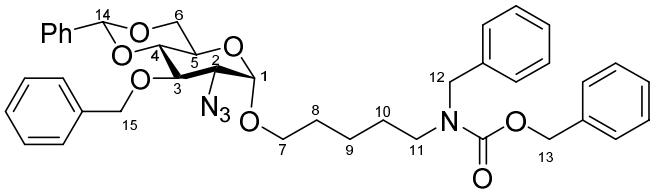
Benzyl (5-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-azido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)pentyl)(benzyl)carbamate (152)



CSA (50.0 mg, 220 μmol) was added to a solution of **148** (220 mg, 430 μmol) and benzaldehyde dimethyl acetal (96.0 μL, 640 μmol) in CH_3CN (2 mL) and the reaction was heated at 60 °C for 6 h. The reaction was concentrated *in vacuo* and the residue was partitioned between EtOAc (10 mL) and sat aq $NaHCO_3$ (10 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent acetone/pentane, 2:8) gave **152** as a colourless oil (180 mg, 70%); $[\alpha]_D^{25}$ + 73.0 (*c* 1.0 in $CHCl_3$); ν_{max} 3420 (O–H), 3064 (C–H), 3032 (C–H), 2939 (C–H), 2867 (C–H), 2107 (N=N=N), 1697 (C=O), 1685 (C=O); δ_H (400 MHz, Acetone) 1.33 – 1.46 (m, 2H, C(9)H_2), 1.49 – 1.71 (m, 4H, C(8)H_2, C(10)H_2), 3.20 – 3.34 (m, 3H, C(2)*H*, C(11)H_2), 3.44 (s, 1H, C(7)H_A), 3.56 (t, J = 9.1 Hz, 1H, C(4)*H*), 3.66 – 3.84 (m, 3H, C(5)*H*, C(6)H_A, C(7)H_B), 4.05 – 4.17 (m, 1H, C(3)*H*), 4.17 – 4.27 (m, 1H, C(6)H_B),

4.54 (s, 2H, C(12)H_2), 4.92 (s, 1H, C(1)*H*), 5.11 (d, J = 4.5 Hz, 1H, O*H*), 5.17 (d, J = 8.2 Hz, 2H, C(13)H_2), 5.62 (s, 1H, C(14)*H*), 7.19 – 7.46 (m, 13H, *Ar*), 7.47 – 7.56 (m, 2H, *Ar*); δ_C (101 MHz, Acetone) 24.0 (*C*(9)), 28.1 (*C*(10)), 28.7 (*C*(10)), 47.0 (*C*(11)), 47.7 (*C*(11)), 51.0 (*C*(12)), 63.8 (*C*(5)), 64.6 (*C*(2)), 68.7 (*C*(7)), 69.1 (*C*(3)), 69.3 (*C*(6)), 83.0 (*C*(4)), 99.6 (*C*(1)), 102.4 (*C*(14)), 127.3 (*Ar*), 128.0 (*Ar*), 128.6 (*Ar*), 128.8 (*Ar*), 129.2 (*Ar*), 129.3 (*Ar*), 129.6 (*Ar*), 138.3 (*i-Ph*), 139.0 (*i-Ph*), 139.5 (*i-Ph*), 157.1 (*C*=O), (*C*(8) under acetone); *m/z* (ESI$^+$) 625 (100%, [M+Na]$^+$); HRMS (ESI$^+$) $C_{33}H_{38}N_4NaO_7^+$ ([M+Na]$^+$) requires 625.2633; found 625.2628.

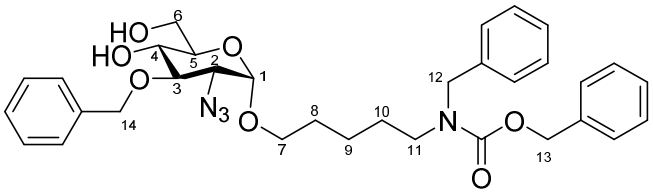
Benzyl (5-(((2*R*,4a*R*,6*S*,7*R*,8*R*,8a*S*)-7-azido-8-(benzyloxy)-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-6-yl)oxy)pentyl)(benzyl)carbamate (153)



NaH (60% in mineral oil, 270 mg, 6.67 mmol) was added to a solution of **152** (3.35 g, 5.56 mmol) and benzyl bromide (990 μL, 8.34 mmol) in DMF (15 mL) at 0 °C under N_2. The reaction was stirred at rt for 16 h before being quenched by addition of ½ sat brine (10 mL) and extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with ½ sat brine (5 × 10 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent acetone/pentane, 1:9) gave **153** as a colourless oil (3.72 g, 96%); $[\alpha]_D^{25}$+40.0 (*c* 1.0 in $CHCl_3$); ν_{max} 3064 (CH), 2032 (CH), 2937 (CH), 2866 (CH), 2106 (N=N=N), 1698 (C=O); δ_H (400 MHz, Acetone) 1.40 (s, 2H, C(9)H_2), 1.52 – 1.71 (m, 4H, C(8)H_2, C(10)H_2), 3.20 – 3.34 (m, 2H, C(11)H_2), 3.38 – 3.53 (m, 2H, C(2)*H*, C(7)H_A), 3.73 (s, 1H, C(7)H_B), 3.77 – 3.88 (m, 3H, C(4)*H*, C(5)*H*, C(6)H_A), 4.03 (t, J = 9.2 Hz, 1H, C(3)*H*)), 4.20 – 4.30 (m, 1H, C(6)H_B), 4.54 (s, 2H, C(12)H_2), 4.78 (d, J = 11.4 Hz, 1H, C(15)H_A), 4.91 – 5.01 (m, 2H, C(1)*H*, C(15)H_B), 5.09 – 5.24 (m, 2H, C(13)H_2), 5.72

(s, 1H, C(14)*H*), 7.19 – 7.46 (m, 18H, *Ar*), 7.50 – 7.57 (m, 2H, *Ar*); δ_C (151 MHz, Acetone) 24.0 (*C*(9)), 28.1 (*C*(10)), 28.7 (*C*(10)),)), 47.0 (*C*(11)), 47.7 (*C*(11)), 50.8 (*C*(12)), 51.0 (*C*(12)), 67.4 (*C*(13)), 68.8 (*C*(7)), 69.4 (*C*(6)), 75.0 (*C*(15)), 76.9 (*C*(3)), 83.5 (*C*(1)), 99.4 (*C*(14)), 102.0 (*C*(1)), 127.1 (*C*(14)), 128.0 (*Ar*), 128.2 (*Ar*), 128.4 (*Ar*), 128.6 (*Ar*), 128.7 (*Ar*), 128.7 (*Ar*), 128.9 (*Ar*), 129.0 (*Ar*), 129.0 (*Ar*), 129.2 (*Ar*), 129.3 (*Ar*), 129.6 (*Ar*), 138.4 (*Ar*), 138.9 (*Ar*), 139.5, 139.5, 156.6 (*C*=O), 157.2 (*C*=O); *m/z* (ESI^+) 715 (100%, $[M+Na]^+$); HRMS (ESI^+) $C_{40}H_{45}N_4O_7^+$ ($[M+H]^+$) requires 693.3283; found 693.3281.

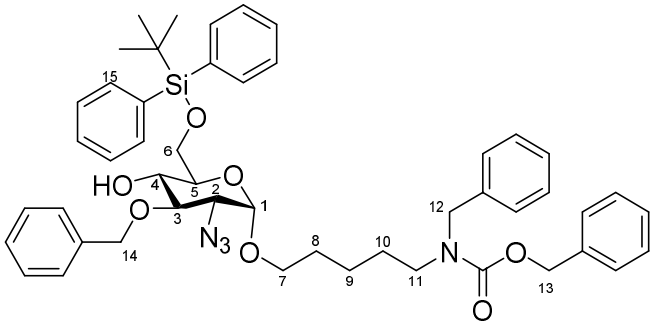
Benzyl (5-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-azido-4-(benzyloxy)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)pentyl)(benzyl)carbamate (154)



153 (1.00 g, 1.45 mmol) was dissolved in 70% AcOH (15 mL) and the reaction was heated at 65 °C for 6 h. The reaction was cooled to rt and neutralised by addition of NaOH, then extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 30%→50% EtOAc in pentane) gave **154** as a colourless oil (756 mg, 86%); $[\alpha]_D^{25}$+ 79.8 (*c* 1.0 in CH_3OH); ν_{max} (film) 3427 (O−H), 2936 (C−H), 2873 (C−H), 2106 (N=N=N), 1679 (C=O); δ_H (400 MHz, $CDCl_3$) 1.20 – 1.48 (m, 2H), 1.48 – 1.66 (m, 4H), 1.72 (dt, J = 13.9, 7.0 Hz, 1H), 3.13 – 3.52 (m, 4H), 3.57 – 3.74 (m, 3H), 3.74 – 3.94 (m, 3H), 4.50 (d, J = 13.0 Hz, 2H), 4.69 – 4.90 (m, 2H), 4.90 – 5.02 (m, 1H), 5.08 – 5.30 (m, 2H), 6.55 – 7.89 (m, 14H); δ_C (151 MHz, MeOH-d_4) 24.5 (*C*(9)), 28.5 (*C*(10)), 28.9 (*C*(10)), 30.1 (*C*(8)), 47.5 (*C*(11)), 48.4 (*C*(11)), 51.4 (*C*(12)), 51.6 (*C*(12)), 62.4 (*C*(6)), 64.2 (*C*(2)), 68.5 (*C*(13)), 68.8 (*C*(7)), 72.3 (*C*(4)), 74.1 (*C*(4)), 75.9 (*C*(14)), 80.9 (*C*(3)), 99.2 (*C*(1)), 128.4 (*Ph*), 128.7 (*Ph*), 128.7 (*Ph*), 129.0 (*Ph*), 129.1 (*Ph*), 129.2 (*Ph*), 129.3

(*Ph*), 129.5 (*Ph*), 129.6 (*Ph*), 138.1 (*i-Ph*), 138.2 (*i-Ph*), 139.3 (*i-Ph*), 139.4 (*i-Ph*), 139.9 (*i-Ph*), 158.0 (*C*=O), 158.5 (*C*=O); *m/z* (ESI$^+$) 627 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{33}H_{41}N_4O_7^+$ ([M+H]$^+$) requires 605.2970; found 605.2974.

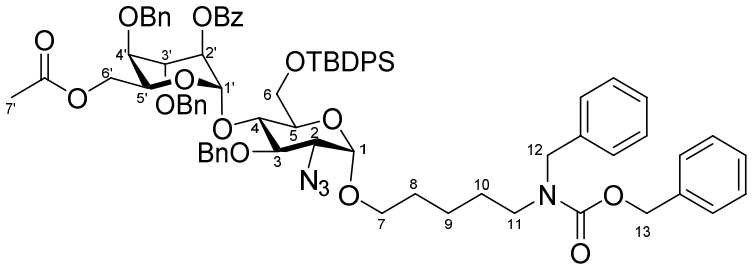
Benzyl (5-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-azido-4-(benzyloxy)-6-(((tert-butyldiphenylsilyl)oxy)methyl)-5-hydroxytetrahydro-2*H*-pyran-2-yl)oxy)pentyl)(benzyl)carbamate (155)



DMAP (30.0 mg, 250 μmol) was added to a solution of **154** (756 mg, 1.25 mmol), Et3N (700 μL, 5.0 mmol) and TBDPSCl (485 μL, 1.88 mmol) in CH_2Cl_2 (6.25 mL) at rt under N_2. The reaction was stirred at rt for 16 h, then quenched by addition of sat aq NH_4Cl (20 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent acetone/pentane, 2:8) gave **155** as a colourless oil (930 mg, 88%); $[\alpha]_D^{25}$+ 38.2 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 2934 (CH), 2864 (CH), 2107 (N=N=N), 1698 (C=O); δ_H (400 MHz, $CDCl_3$) 1.07 (s, 9H, C(C*H*$_3$)$_3$), 1.24 – 1.43 (m, 2H, C(9)*H*$_2$), 1.48 – 1.69 (m, 4H, C(8)*H*$_2$, C(10)*H*$_2$), 3.25 (dd, J = 10.2, 3.6 Hz, 3H, C(2)*H*, C(11)*H*$_2$), 3.31 – 3.49 (m, 1H, C(7)*H*$_A$), 3.71 (t, J = 8.2 Hz, 3H, C(4)*H*, C(5)*H*, C(7)*H*$_B$), 3.79 – 4.00 (m, 3H, C(3)*H*, C(6)*H*$_2$), 4.44 – 4.55 (m, 2H, C(12)*H*$_2$), 4.78 – 4.95 (m, 3H, C(1)*H*, C(14)*H*$_2$), 5.07 – 5.25 (m, 2H, C(13)*H*$_2$), 7.13 – 7.48 (m, 21H, *Ar*), 7.69 (ddd, J = 7.9, 4.8, 1.5 Hz, 4H, *Ar*);

δ_C (151 MHz, $CDCl_3$) 19.4 (*C*(CH_3)$_3$), 23.5 (*C*(9)), 23.7 (*C*(9)), 27.0 (C(*C*H_3)$_3$), 27.6 (*C*(10)), 28.0 (*C*(10)), 29.1 (*C*(8)), 29.1 (*C*(8)), 46.2 (*C*(11)), 47.4 (*C*(11)), 50.5 (*C*(12)), 50.7 (*C*(12)), 62.9 (*C*(2)), 64.4 (*C*(6)), 67.3 (*C*(13)), 67.9 (*C*(7)), 71.3 (*C*(4)), 71.5 (*C*(4)), 72.7 (*C*(5)), 75.2 (*C*(14)), 80.0 (*C*(3)), 97.8 (*C*(1)), 127.3 (*Ph*), 127.4 (*Ph*), 127.9 (*Ph*), 127.9 (*Ph*), 128.0 (*Ph*), 128.1 (*Ph*), 128.2 (*Ph*), 128.6 (*Ph*), 128.7 (*Ph*), 130.0 (*Ph*), 133.2 (*i-Ph*), 135.7 (*i-Ph*), 135.8 (4 × *C*(15)), 136.8 (*i-Ph*), 137.0 (*i-Ph*), 138.0 (*i-Ph*), 138.4 (*i-Ph*), 156.4 (*C*=O), 156.9 (*C*=O); *m/z* (ESI^+) 865 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{49}H_{58}N_4NaO_7Si^+$ ($[M+Na]^+$) requires 865.3967; found 865.3962.

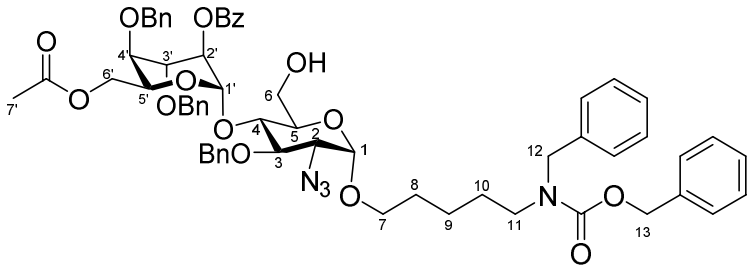
(2*S*,3*R*,4*S*,5*R*,6*S*)-6-(Acetoxymethyl)-2-(((2*R*,3*S*,4*R*,5*R*,6*S*)-5-azido-6-((5-(benzyl((benzyloxy)carbonyl)amino)pentyl)oxy)-4-(benzyloxy)-2-(((tert-butyldiphenylsilyl)oxy)methyl)tetrahydro-2*H*-pyran-3-yl)oxy)-4,5-bis(benzyloxy)tetrahydro-2*H*-pyran-3-yl benzoate (156)



119 (1.38 g, 2.12 mmol) and **155** (2.68 g, 3.19 mmol) were combined and azeotroped with $PhCH_3$ (2 × 20 mL), then dissolved in CH_2Cl_2 (4.25 mL) and Et_2O (17 mL) under N_2 and dried over 4Å molecular sieves (2 g, freshly activated) for 1 h. The reaction was cooled to 0 °C and TMSOTf (150 μL, 830 μmol) was added. The reaction was allowed to warm to rt over 16 h. The reaction was filtered through celite, washed with $NaHCO_3$ then brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentanes, 3:17) gave **156** as a colourless oil (2.67 g, 96%); $[\alpha]_D^{25}$+2.6 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 2907 (C–H), 2889 (C–H), 2106 (N=N=N), 1740 (C=O), 1719 (C=O); δ_H (400 MHz, $CDCl_3$) 0.97 (s, 9H, C (C*H*$_3$)$_3$), 1.22 – 1.36 (m, 2H, C(9)*H*$_2$), 1.46 – 1.64 (m, 4H, C(8)*H*$_2$, C(10)*H*$_2$), 1.81 (s, 3H, C(7')*H*$_3$), 3.14 – 3.21 (m, 1H, C(11)*H*$_A$), 3.22 – 3.36

(m, 3H, C(2)*H*, C(7)H_A, C(11)H_B,), 3.40 (t, J = 3.0 Hz, 1H, C(4')*H*), 3.44 – 3.58 (m, 1H, C(7)H_B), 3.64 (d, J = 9.7 Hz, 1H, C(5)*H*), 3.76 – 3.88 (m, 2H, C(3)*H*, C(6)H_A), 3.88 – 3.96 (m, 2H, C(3')*H*, C(6)H_B), 4.02 – 4.10 (m, 2H, C(6')H_2), 4.10 – 4.18 (m, 1H, C(4)*H*), 4.29 (d, J = 11.5 Hz, 1H, C(4')OCH_APh), 4.43 – 4.52 (m, 4H, C(5')*H*, C(12)H_2, C(4')CH_BPh), 4.55 – 4.67 (m, 2H, C(3)OCH_APh, C(3')OCH_APh), 4.74 – 4.84 (m, 2H, C(1)*H*, C(3')OCH_BPh), 4.89 (d, J = 11.0 Hz, 1H, C(3)OCH_BPh), 5.17 (d, J = 8.9 Hz, 2H, C(13)H_2), 5.22 (t, J = 2.7 Hz, 1H, C(2')*H*), 5.29 – 5.34 (m, 1H, C(1')*H*), 7.10 – 7.18 (m, 4H, *Ar*), 7.20 – 7.41 (m, 29H, *Ar*), 7.47 – 7.56 (m, 1H, *Ar*), 7.56 – 7.63 (m, 2H, *Ar*), 7.63 – 7.72 (m, 2H, *Ar*), 7.87 – 7.94 (m, 2H, *Ar*); δ_C (176 MHz, $CDCl_3$) 19.5 (*C*$(CH_3)_3$), 20.9 (*C*(7')), 23.4 (*C*(9)), 26.9 (C(*C*$H_3)_3$), 27.6 (*C*(10)), 28.1 (*C*(10)), 29.1 (*C*(8)), 29.2 (*C*(8)), 46.3 (*C*(11)), 47.2 (*C*(11)), 50.4 (*C*(12)), 50.7 (*C*(12)), 62.8 (*C*(6)), 63.0 (*C*(6')), 64.2 (*C*(2)), 65.6 (*C*(5')), 67.3 (*C*(13)), 67.9 (*C*(7)), 68.5 (*C*(2')), 72.0 (*C*(3')), 72.1 (*C*(5)), 72.2 (C(4')O*C*H_2Ph), 72.5 (C(4')O*C*H_2Ph), 73.5 (*C*(4)), 73.5 (*C*(4')), 75.0 (C(3)O*C*H_2Ph), 78.8 (*C*(3)), 96.9 (*C*(1')), 97.5 (*C*(1)), 127.3 (*Ar*), 127.4 (*Ar*), 127.5 (*Ar*), 127.6 (*Ar*), 127.7 (*Ar*), 127.9 (*Ar*), 128.0 (*Ar*), 128.0 (*Ar*), 128.1 (*Ar*), 128.2 (*Ar*), 128.4 (*Ar*), 128.4 (*Ar*), 128.5 (*Ar*), 128.6 (*Ar*), 128.7 (*Ar*), 129.6 (*Ar*), 129.7 (*Ar*), 130.1 (*Ar*), 133.3 (*Ar*), 133.5 (*Ar*), 135.7 (*Ar*), 136.1 (*Ar*), 137.6 (*Ar*), 137.7 (*Ar*), 138.1 (*Ar*), 138.5 (*Ar*), 156.3 (C_{Cbz}=O), 156.9 (C_{Cbz}=O), 165.7 (C_{Bz}=O), 170.6 (C_{Ac}=O); *m/z* (ESI^+) 1354 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{78}H_{86}N_4NaO_{14}Si^+$ ($[M+Na]^+$) requires 1353.5802; found 1353.5755.

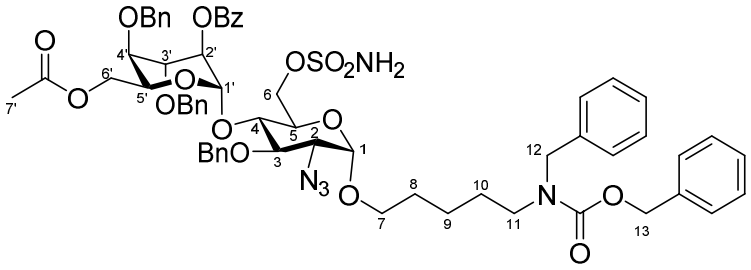
(2*S*,3*R*,4*S*,5*R*,6*S*)-6-(Acetoxymethyl)-2-(((2*R*,3*S*,4*R*,5*R*,6*S*)-5-azido-6-((5-(benzyl((benzyloxy)carbonyl)amino)pentyl)oxy)-4-(benzyloxy)-2-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)oxy)-4,5-bis(benzyloxy)tetrahydro-2*H*-pyran-3-yl benzoate (157)



TBAF (1 M in THF, 11.3 mL, 11.3 mmol) was added to a solution of **156** (3.71 g, 2.83 mmol) and AcOH (712 μL, 12.4 mmol) in THF (14 mL) under N_2 at rt. The reaction was stirred at rt for 16 h. The reaction was quenched by addition of $NaHCO_3$ (25 mL), and extracted into CH_2Cl_2 (3 × 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent $(CH_3)_2CO$/pentane, 3:7) gave **157** as a colourless oil (2.30 g, 74%); $[\alpha]_D^{25}$+38.4 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3502 (O−H), 2930 (C−H), 2867 (C−H), 2107 (N=N=N), 1735 (C=O), 1715 (C=O), 1701 (C=O); δ_H (600 MHz, $CDCl_3$) 1.27 – 1.40 (m, 2H, C(9)H_2), 1.49 – 1.65 (m, 4H, C(8)H_2, C(10)H_2), 1.88 (s, 3H, C(7')H_3), 3.17 – 3.27 (m, 3H, C(2)*H*, C(11)H_2), 3.27 – 3.45 (m, 1H, C(7)H_A), 3.52 (t, J = 3.8 Hz, 1H,C(4')*H*), 3.54 – 3.72 (m, 2H, C(5)*H*, C(7)H_B), 3.74 – 3.81 (m, 1H, C(6)H_A), 3.83 (dd, J = 12.2, 3.6 Hz, 1H, C(6)H_B), 3.88 (dd, J = 7.4, 2.6 Hz, 2H, C(3)*H*, C(4)*H*), 3.91 – 3.95 (m, 1H, C(3')*H*), 4.09 (dd, J = 11.5, 5.1 Hz, 1H, C(6')H_A), 4.21 – 4.27 (m, 1H, C(6')H_B), 4.37 (d, J = 11.5 Hz, 1H, C(4')OCH_APh), 4.39 – 4.43 (m, 1H, C(5')*H*), 4.47 – 4.53 (m, 2H, C(12)H_2), 4.55 (d, J = 11.6 Hz, 1H, C(4')OCH_BPh), 4.65 (d, J = 6.1 Hz, 1H, C(3)OCH_APh. C(3')OCH_APh), 4.67 (d, J = 5.1 Hz, 1H, C(3')OCH_BPh), 4.74 – 4.87 (m, 1H, C(1)*H*), 4.94 (d, J = 10.6 Hz, 1H, C(3)OCH_BPh), 5.12 – 5.20 (m, 2H, C(13)H_2), 5.21 (d, J = 2.9 Hz, 2H, C(1')*H*, C(2')*H*), 7.13 – 7.41 (m, 27H, 5 × *Ph*, *o*-Ph_{Bz}), 7.53 (tt, J = 7.3, 1.4 Hz, 1H, *p*-Ph_{Bz}), 7.87 – 8.03 (m, 2H, *m*-Ph_{Bz}); δ_C (151 MHz, $CDCl_3$) δ 21.0 (*C*(7')), 23.4 (*C*(9)), 27.5 (*C*(10)), 28.0 (*C*(10)), 29.1 (*C*(8)), 46.3 (*C*(11)), 47.2 (*C*(11)), 50.4 (*C*(12)), 50.7 (*C*(12)), 61.6 (*C*(6)), 62.8

(*C*(6')), 63.9 (*C*(2)), 67.0 (*C*(5)), 67.1 (*C*(5')), 67.3 (*C*(13)), 68.2 (*C*(7)), 69.5 (*C*(2')), 69.6 (*C*(2')), 71.6 (*C*(5)), 72.6 (C(4')O*C*H_2Ph), 73.1 (C(3')O*C*H_2Ph), 73.2 (*C*(3')), 74.1 (*C*(4')), 75.1 ((C(3)O*C*H_2Ph), 75.2 (*C*(4)), 78.6 (*C*(3)), 97.6 (*C*(1')), 97.9 (*C*(1)), 127.4 (*Ar*), 127.4 (*Ar*), 127.6 (*Ar*), 128.0 (*Ar*), 128.0 (*Ar*), 128.0 (*Ar*), 128.2 (*Ar*), 128.4 (*Ar*), 128.5 (*Ar*), 128.6 (*Ar*), 128.6 (*Ar*), 128.7 (*Ar*), 129.6 (*Ar*), 130.1 (*m-Ph*$_{Bz}$), 133.5 (*p-Ph*$_{Bz}$), 136.9 (*i-Ph*$_{Bz}$), 137.1 (*i-Ph*$_{Cbz}$), 137.6 (*i-Ph*$_{Cbz}$), 137.6 (*i-Ph*), 138.1 (*i-Ph*), 138.3 (*i-Ph*), 156.4 (*C*$_{Cbz}$=O), 156.9 (*C*$_{Cbz}$=O), 165.8 (*C*$_{Bz}$=O), 170.8 (*C*$_{Ac}$=O); *m/z* (ESI$^+$) 1115 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{62}H_{68}N_4NaO_{14}^+$ ([M+Na]$^+$) requires 1115.4624; found 1115.4583.

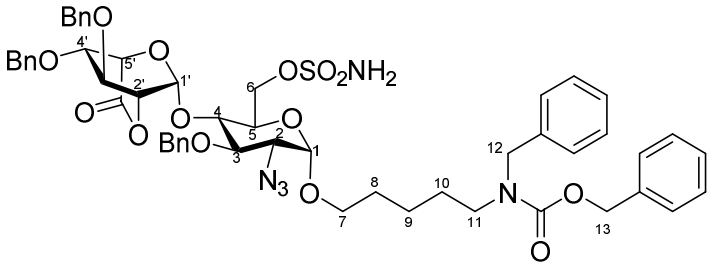
(2*S*,3*R*,4*S*,5*R*,6*S*)-6-(Acetoxymethyl)-2-(((2*R*,3*S*,4*R*,5*R*,6*S*)-5-azido-6-((5-(benzyl((benzyloxy)carbonyl)amino)pentyl)oxy)-4-(benzyloxy)-2-((sulfamoyloxy)methyl)tetrahydro-2*H*-pyran-3-yl)oxy)-4,5-bis(benzyloxy)tetrahydro-2*H*-pyran-3-yl benzoate (158)



Sulfamoyl chloride (490 mg, 4.3 mmol) was added to a solution of **157** (2.3 g, 2.1 mmol) in DMF (10 mL) at 0 °C and the reaction was allowed to warm to rt over 16 h. The reaction was quenched by addition of satd aq $NaHCO_3$ (20 mL) and extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with ½ sat brine (5 × 20 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent $(CH_3)_2CO$/pentane, 3:7) gave **158** as a white solid (2.12 g, 86%); mp 50-52 °C; $[\alpha]_D^{28}$ +28.9 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3345 (N−H), 2936 (C−H), 2876 (C−H), 2107 (N=N=N), 1729 (C=O), 1716 (C=O), 1699 (C=O); δ_H (400 MHz, $CDCl_3$) 1.13 – 1.31 (m, 2H, C(9)H_2), 1.36 – 1.60 (m, 4H, C(8)H_2, C(10)H_2), 1.82 (s, 3H, C(7')H_3), 3.05 – 3.39 (m, 4H, C(7)H_A,

C(11)H_2, C(2)*H*), 3.54 (s, 2H, C(7)H_B, C(4')*H*), 3.65 – 3.91 (m, 3H, C(3)*H*, C(4)*H*, C(5)*H*), 3.88 (td, J = 5.1, 2.4 Hz, 1H, C(3')*H*), 3.99 (dd, J = 11.6, 4.4 Hz, 1H, C(6')H_A), 4.17 – 4.28 (m, 1H, C(6)H_A), 4.32 (dt, J = 8.2, 3.7 Hz, 1H, C(5')*H*), 4.33 – 4.50 (m, 5H, C(6)H_B, C(4')OCH_APh, C(6')H_B, C(12)H_2), 4.50 – 4.62 (m, 3H, C(3')OCH_APh, C(3)OCH_APh, C(4')OCH_BPh), 4.62 – 4.79 (m, 2H, C(1')*H*, C(3')OCH_BPh), 4.92 (d, J = 10.3 Hz, 1H, C(3)OCH_BPh), 5.04 – 5.17 (m, 4H, C(1)*H*, C(2')*H*, C(13)H_2), 5.42 – 5.66 (m, 2H, OSO_2NH_2), 7.06 – 7.33 (m, 27H, *Ar*), 7.45 (t, J = 7.4 Hz, 1H, *Ar*), 7.84 – 7.95 (m, 2H, *Ar*); δ_C (151 MHz, $CDCl_3$) 21.1 (*C*(7')), 23.3 (*C*(9)), 27.4 (*C*(10)), 28.0 (*C*(10)), 29.0 (*C*(8)), 29.1 (*C*(8)), 46.2 (*C*(11)), 47.2 (*C*(11)), 50.3 (*C*(12)), 50.7 (*C*(12)), 62.3 (*C*(6')), 62.4 (*C*(6')), 63.4 (*C*(2)), 63.5 (*C*(2)), 67.4 (*C*(13)), 67.4 (*C*(6)), 67.6 (*C*(6)), 68.3 (*C*(5')), 68.6 (*C*(7)), 68.8 (*C*(7)), 69.2 (*C*(5)), 69.3 (*C*(5)), 70.3 (*C*(2')), 70.6 (*C*(2')), 72.8 (*C*(4')OCH_2Ph), 73.6 (*C*(3')OCH_2Ph), 73.7 (*C*(3')OCH_2Ph), 74.1 (*C*(3')), 74.5 (*C*(4')), 75.2 (*C*(3)OCH_2Ph), 76.0 (*C*(4)), 78.4 (*C*(3)), 78.5 (*C*(3)), 97.8 (*C*(1)), 97.9 (*C*(1)), 98.0 (*C*(1')), 127.3 (*Ar*), 127.4 (*Ar*), 127.7 (*Ar*), 128.0 (*Ar*), 128.0 (*Ar*), 128.1 (*Ar*), 128.2 (*Ar*), 128.5 (*Ar*), 128.6 (*Ar*), 128.7 (*Ar*), 128.7 (*Ar*), 129.2 (*Ar*),130.1 (*i-Ph*$_{Bz}$), 133.7 (*p-Ph*$_{Bz}$), 136.8 (*i-Ph*), 137.0 (*i-Ph*), 137.5 (*i-Ph*), 138.0 (*i-Ph*), 138.2 (*i-Ph*), 156.4 (C_{Cbz}=O), 156.9 (C_{Cbz}=O), 166.1 (C_{Bz}=O), 166.2 (C_{Bz}=O), 171.4 (C_{Ac}=O), 171.5 (C_{Ac}=O); *m/z* (ESI$^+$) 1194 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{62}H_{69}N_5NaO_{16}S^+$ ([M+Na]$^+$) requires 1194.4352; found 1194.4300.

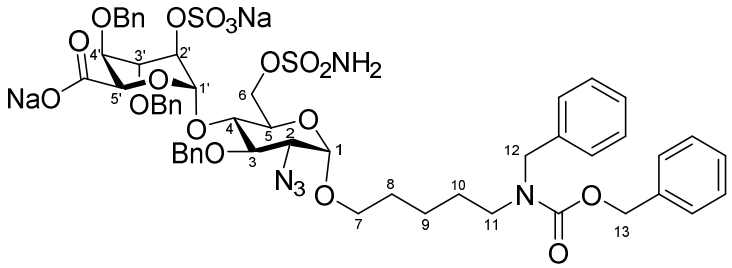
((2*R*,3*S*,4*R*,5*R*,6*S*)-5-Azido-6-((5-(benzyl((benzyloxy)carbonyl)amino)pentyl)oxy)-4-(benzyloxy)-3-(((1*R*,3*S*,4*R*,7*S*,8*S*)-7,8-bis(benzyloxy)-6-oxo-2,5-dioxabicyclo[2.2.2]octan-3-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl sulfamate (160)



(1) Ester deprotection. CH_3ONa (125 mg, 2.27 mmol) was added to a solution of **158** (1.33 g, 1.135 mmol) in CH_3OH (10 mL) and the reaction was heated at 35 °C for 16 h. The reaction was quenched by addition of Amberlite® IR 120 (750 mg), filtered and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→40% $(CH_3)_2CO$ in pentane) gave the intermediate diol **159** as a colourless oil (960 mg, 83%). **(2) Lactone formation.** TEMPO (29 mg, 187 μmol) and PIDA (723 mg, 2.25 mmol) were added in quick succession to a vigorously stirred solution of the above intermediate (960 mg, 936 μmol) in CH_2Cl_2/H_2O (2:1, 15.6 mL) at rt. The reaction was stirred at rt for 16 h, before being quenched by addition of 10% aq $Na_2S_2O_3$ (3 mL) and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with sat aq $NaHCO_3$ (10 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→40% $(CH_3)_2CO$ in pentane) gave **160** as a colourless oil (925 mg, 96%); $[\alpha]_D^{28}$ −1.11 (*c* 1.0 in $CHCl_3$); ν_{max} (film) 3283 (N−H), 2940 (C−H), 2868 (C−H), 2107 (N=N=N), 1791 (C=O, lactone), 1696 (C=O), 1675 (C=O); δ_H (400 MHz, $CDCl_3$)1.17 – 1.37 (m, 2H, C(9)H_2), 1.41 – 1.62 (m, 4H, C(8)H_2, C(10)H_2), 3.08 – 3.40 (m, 3H, C(2)*H*, C(11)H_2), 3.40 – 3.62 (m, 2H, C(7)H_2), 3.61 – 3.73 (m, 1H, C(4)*H*), 3.82 (ddd, J = 4.3, 3.0, 1.3 Hz, 2H, C(5)*H*, C(3')*H*), 3.91 (dd, J = 10.1, 8.5 Hz, 1H, C(3)*H*), 3.95 – 4.03 (m, 1H, C(4')*H*), 4.15 – 4.26 (m, 2H, C(6)H_A, C(5')*H*), 4.26 – 4.35 (m, 2H, C(6)H_B, C(4')CH_APh), 4.39 – 4.59 (m, 5H, C(4')CH_BPh, C(3')CH_2Ph, C(12)H_2), 4.62 (t, J = 2.7 Hz, 1H, C(2')*H*), 4.74 (d, J = 10.4 Hz, 1H, C(3)CH_APh), 4.77 – 4.82 (m,

1H, C(1)*H*), 4.84 (d, J = 10.4 Hz, 1H, C(3)CH_BPh), 4.98 (s, 1H, NH_A) 5.12 (d, J = 17.2 Hz, 2H, C(13)H_2), 5.21 (s, 1H, NH_B), 5.34 (dd, J = 2.5, 1.3 Hz, 1H, C(1')*H*), 6.39 – 7.72 (m, 25H, 5 × *Ph*); δ_C (151 MHz, $CDCl_3$) 23.2 (*C*(9)), 23.3 (*C*(9)), 27.2 (*C*(10)), 27.9 (*C*(10)), 29.0 (*C*(8)), 46.2 (*C*(11)), 47.1 (*C*(11)), 50.4 (*C*(12)), 50.7 (*C*(12)), 63.2 (*C*(2)), 67.4 (C(13)), 68.6 (*C*(6)), 68.7 (*C*(5')), 68.7 (*C*(5)), 68.9 (*C*(7)), 71.8 (C(4')*C*H_2Ph), 72.2 (*C*(2')), 72.3 ((C(3')*C*H_2Ph), 74.6 ((C(3)*C*H_2Ph), 77.9 (*C*(4')), 78.1 (*C*(4)), 78.1 (*C*(3)), 79.7 (*C*(3')), 98.0 (*C*(1)), 98.3 (*C*(1')), 127.3 (*Ph*), 127.5 (*Ph*), 127.8 (*Ph*), 127.9 (*Ph*), 127.9 (*Ph*), 128.1 (*Ph*), 128.2 (*Ph*), 128.3 (*Ph*), 128.6 (*Ph*), 128.6 (*Ph*), 128.7 (*Ph*), 136.7 (*i-Ph*), 136.9 (*i-Ph*), 137.2 (*i-Ph*), 137.9 (*i-Ph*), 137.9 (*i-Ph*), 156.5 (C_{Cbz}=O), 156.9 (C_{Cbz}=O), 167.6 ($C_{lactone}$=O); *m/z* (ESI$^+$) 1044 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{53}H_{59}N_5NaO_{14}S^+$ ([M+Na]$^+$) requires 1044.3671; found 1044.3635.

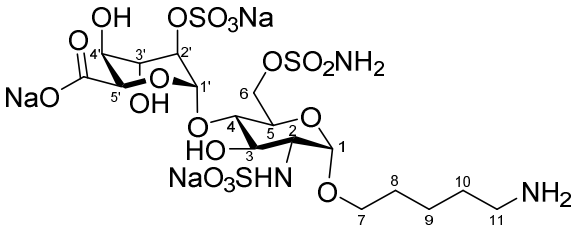
Sodium (2*R*,3*S*,4*S*,5*R*,6*R*)-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-5-azido-6-((5-(benzyl((benzyloxy)carbonyl)-amino)pentyl)oxy)-4-(benzyloxy)-2-((sulfamoyloxy)methyl)tetrahydro-2*H*-pyran-3-yl)oxy)-3,4-bis(benzyloxy)-5-(sulfonatooxy)tetrahydro-2*H*-pyran-2-carboxylate (161)



(1) Lactone hydrolysis. LiOH (1 M aq, 1.6 mL, 1.6 mmol) was added to a solution of **160** (275 mg, 0.270 mmol) in THF (15 mL) at rt and the reaction was stirred at rt for 2 h. The reaction was quenched by addition of Dowex® 50WX4-200R ion-exchange resin, filtered and concentrated *in vacuo*. The residue was azeotroped with PhMe (2 × 15 mL) and dried under high vacuum for 2 h. (**2) *O*-sulfation.** The crude residue was dissolved in DMF (3.45 mL) under N_2 and sulfur trioxide pyridine complex (263 mg, 1.60 mmol) was added. The reaction was heated at 60 °C for 18 h. The reaction was quenched by addition of $NaHCO_3$

(800 mg) and H_2O (1 mL), and was stirred at rt for 1 h. The reaction was concentrated *in vacuo* and the residue was azeotroped with H_2O (2 × 15 mL), then PhMe (1 × 15 mL). Purification via column chromatography (eluent, EtOAc/IPA/H_2O = 82:16:2) gave **161** as a white solid (163 mg, 52% over two steps); mp 207-208 °C (dec.); $[\alpha]_D^{25}$ +35.1 (*c* 1.0 in CH_3OH); ν_{max} (film) 3414 (N–H), 2940 (C–H), 2872 (C–H), 2108 (N=N=N), 1696 (C=O), 1611 (COO^-) 1368 (S=O), 1226 (S=O); δ_H (600 MHz, MeOH-*d*$_4$) 1.23 – 1.45 (m, 2H, C(9)H_2), 1.45 – 1.68 (m, 4H, C(8)H_2, C(10)H_2), 3.10 – 3.19 (m, 1H, C(2)*H*), 3.19 – 3.29 (m, 2H, C(11)H_2), 3.32 – 3.49 (m, 1H, C(7)H_A), 3.72 (t, J = 9.3 Hz, 2H, C(4)*H*, C(7)H_B), 3.75 – 3.85 (m, 1H, C(3)*H*), 3.92 – 4.02 (m, 1H, C(5)*H*), 4.06 (d, J = 2.8 Hz, 1H, C(4')*H*), 4.21 (t, J = 2.7 Hz, 1H, C(3')*H*), 4.34 – 4.44 (m, 2H, C(6)H_A, C(3')CH_APh), 4.47 – 4.52 (m, 4H, C(6)H_B, C(12)H_2, C(4')CH_APh), 4.52 – 4.58 (m, 1H), 4.58 – 4.64 (m, 1H, C(3)CH_APh), 4.67 – 4.73 (m, 2H, C(2')*H*, C(4')CH_BPh), 4.73 – 4.82 (m, 3H, C(1)*H*, C(3)CH_BPh, C(3')CH_BPh), 4.94 (d, J = 2.4 Hz, 1H, C(5')*H*), 5.15 (appt d, 2H, C(13)H_2), 5.45 (s, 1H, C(1')*H*), 7.13 – 7.45 (m, 25H, 5 × *Ph*); δ_C (151 MHz, MeOH-*d*$_4$) 24.5 (*C*(9)), 28.4 (*C*(10)), 28.9 (*C*(10)), 30.1 (*C*(8)), 30.2 (*C*(8)), 47.6 (*C*(11)), 51.3 (*C*(12)), 51.6 (*C*(12)), 64.8 (*C*(2)), 68.4 (*C*(13)), 68.5 (*C*(13)), 69.2 (*C*(7)), 69.5 (*C*(6)), 71.4 (*C*(5')), 71.5 (*C*(2'), *C*(5)), 72.4 (*C*(3')), 72.8 (*C*(4')OCH_2Ph), 73.7 (*C*(3)OCH_2Ph), 76.4 (*C*(4')), 76.9 (*C*(3')OCH_2Ph), 78.3 (*C*(4)), 79.8 (*C*(3)), 98.8 (*C*(1)), 100.7 (*C*(1')), 128.4 (*Ar*), 128.8 (*Ar*), 128.9 (*Ar*), 129.0 (*Ar*), 129.3 (*Ar*), 129.4 (*Ar*), 129.4 (*Ar*), 129.5 (*Ar*), 129.6 (*Ar*), 129.7 (*Ar*), 130.1 (*Ar*), 138.1 (*i-Ph*), 138.7 (*i-Ph*), 138.7 (*i-Ph*), 139.3 (*i-Ph*), 139.3 (*i-Ph*), 158.0 (C_{Cbz}=O), 158.5 (C_{Cbz}=O), 176.4 (C_{Acid}=O); *m/z* (ESI^-) 1118 ([M−2Na+H]$^-$, 100%); HRMS (ESI^-) $C_{53}H_{60}N_5O_{18}S_{22}^-$ ([M−2Na+H]$^-$) requires 1118.3380; found 1118.3403.

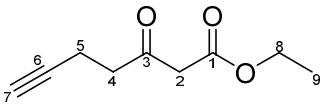
Sodium (2*R*,3*S*,4*S*,5*R*,6*R*)-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-6-((5-(benzyl((benzyloxy)carbonyl)amino)-pentyl)oxy)-4-(benzyloxy)-2-((sulfamoyloxy)methyl)-5-(sulfonatoamino)tetrahydro-2*H*-pyran-3-yl)oxy)-3,4-bis(benzyloxy)-5-(sulfonatooxy)tetrahydro-2*H*-pyran-2-carboxylate (163)



(1) Staudinger reaction. PMe_3 (1 M in THF, 336 μL, 0.336 mmol) was added to a solution of **161** (125 mg, 0.168 mmol) in THF (14 mL) and NaOH solution (0.1 M in H_2O, 1.5 mL) under N_2 at rt and the reaction was stirred for 16 h. The reaction was naturalised by addition of CH_3COOH (pH 8-9) and concentrated *in vacuo*. Purification via reverse phase chromatography (eluent H_2O) gave the intermediate amine as a colourless oil. **(2) *N*-sulfation.** The above intermediate was dissolved in THF/H_2O (1:1, 2 mL) and the pH was adjusted to pH 9-10 by addition of NaOH (1 M in H_2O). Sulfur trioxide pyridine complex (60 mg, 0.37 mmol) was added portion-wise, with careful adjustment of the pH after each addition. The reaction was stirred at rt for 4 h and was concentrated *in vacuo*. Purification via column chromatography (eluent CH_3OH/EtOAc, 1:4) gave the intermediate sulfamate **162** as a colourless oil. **(3) Global debenzylation.** $Pd(OH)_2$/C (20% weight loading, 180 mg) was added to a solution of the above intermediate in CH_3OH/Phosphate buffered saline solution (20 mM) (9:1, 5 mL) under N_2 and the reaction was purged and back filled with N_2 (× 3) before being sealed under an atmosphere of H_2. The reaction was stirred for 48 h. The reaction was filtered through Celite® and concentrated *in vacuo*. Purification via reverse phase chromatography (eluent H_2O) gave **163** as a colourless glass (58 mg, 48% over 3 steps); mp 182 °C (dec.); $[\alpha]_D^{25}$ +26.6 (*c* 1.0 in H_2O); ν_{max} (film) 3268 (O−H), 2923 (C−H), 2851 (C−H), 1611 (COO^-) 1363 (S=O), 1224 (S=O)1612 (S=O), ; δ_H (400 MHz, D_2O) 1.38 – 1.56 (m, 2H, C(9)H_2), 1.58 – 1.78 (m, 4H, C(8)H_2, C(10)H_2), 3.01 (dd, J = 8.1, 6.9 Hz, 2H, C(11)H_2), 3.27 (dd, J = 10.0, 3.6 Hz, 1H, C(2)*H*), 3.55 (dt, J = 9.9, 5.9 Hz, 1H, C(7)H_A), 3.67 – 3.80 (m, 3H, C(3)*H*, C(4)*H*, C(7)H_B), 3.93 – 3.97 (m, 1H, C(4')*H*), 3.98

(t, J = 4.3 Hz, 1H, C(3')*H*), 4.05 (dt, J = 10.1, 3.3 Hz, 1H, C(5)*H*), 4.20 – 4.28 (m, 1H, C(2')*H*), 4.47 (d, J = 3.3 Hz, 2H, C(6)H_2), 5.13 (d, J = 3.7 Hz, 1H, C(1)*H*), 5.15 (d, J = 2.7 Hz, 1H, C(1')*H*); δ_C (151 MHz, D_2O) 22.5 (*C*(9)), 26.5 (*C*(10)), 28.0 (*C*(8)), 39.5 (*C*(11)), 57.8 (*C*(2)), 68.2 (*C*(7)), 68.3 (*C*(5)), 68.5 (*C*(6)), 69.3 (*C*(4')), 69.4 (*C*(5')), 69.6 (*C*(3')), 69.8 (*C*(4)), 75.1 (*C*(2')), 77.9 (*C*(4)), 97.2 (*C*(1)), 99.2 (*C*(1')); *m/z* (ESI^+) 724 ($[M-Na+H]^+$, 100%); HRMS (ESI^+) $C_{17}H_{32}N_3Na_2O_{19}S_3^+$ ($[M-Na+H]^+$) requires 724.0582; found 724.0581.

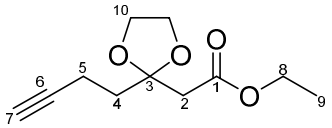
Ethyl 3-oxohept-6-ynoate (165)



n-BuLi (2.5 M in hexane, 101 mL, 254 mmol) was added dropwise to a solution of diisopropylamine (35.5 mL, 254 mmol) in THF (200 mL) at – 78 °C under N_2 and the reaction was stirred for 15 min. Ethyl acetoacetate (14.6 mL, 115 mmol) was added and the reaction was stirred at – 78 °C for 30 min. Propargyl bromide (80% w/w in $PhCH_3$, 18.9 g, 127 mmol) was added and the reaction was stirred at – 78 °C for 15 min, then warmed to rt over 16 h. The reaction was quenched by addition of satd aq NH_4Cl (5 mL) and extracted with Et_2O (3 × 40 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentane, 5:95), followed by Kugelrohr distillation to remove ethyl acetoacetate, gave **165** as a yellow oil (7.10 g, 37%); ν_{max} (film) 3287 (C≡C–H), 2986 (C–H), 2935 (C–H), 1739 (C=O), 1715 (C=O); δ_H (400 MHz, $CDCl_3$) 1.28 (t, *J* = 7.14 Hz, 3H, C(9)H_3), 1.96 (t, *J* = 2.65 Hz, 1H, C(7)*H*), 2.48 (ddd, *J* = 8.74, 6.73, 2.69 Hz, 2H, C(5)H_2),

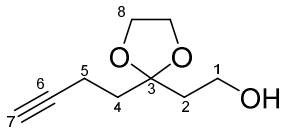
2.81 (dd, *J* = 7.77, 6.70 Hz, 2H, C(4)H_2), 3.46 (s, 2H, C(2)H_2), 4.20 (q, *J* = 7.14 Hz, 2H, C(8)H_2); δ_C (126 MHz, $CDCl_3$) 13.0 (*C*(5)), 15.4 (*C*(9)), 41.8 (*C*(4)), 49.4 (*C*(2)), 61.7 (*C*(8)), 69.1 (*C*(7)), 82.7 (*C*(6)), 167.1 (*C*(3)), 200.7 (*C*(1)); *m/z* (ESI^+) 191 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_9H_{12}NaO_3^+$ ($[M+Na]^+$) requires 191.0679, found 191.0680.

Ethyl 2-(2-(but-3-yn-1-yl)-1,3-dioxolan-2-yl)acetate (166)



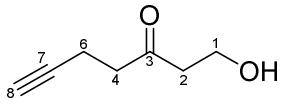
$BF_3{\cdot}OEt_2$ (3.31 mL, 26.8 mmol) was added dropwise to **164** (3.00 g, 17.9 mmol) and ethylene glycol (4.00 mL, 71.7 mmol) in CH_2Cl_2 (36 mL) at 0 °C under N_2. The reaction was stirred at rt for 16 h. The mixture was cooled to 0 °C and quenched by addition of H_2O (4 mL). The reaction was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo.* Purification via column chromatography (eluent EtOAc/pentane, 3:7) gave **166** as a yellow oil (3.34 g, 87%); ν_{max} (film) 3290 (C≡C–H), 2986 (C–H), 2896 (C–H), 1733 (C=O); δ_H (400 MHz, $CDCl_3$) 1.27 (t, *J* = 7.13 Hz, 3H, C(9)H_3), 1.93 (t, *J* = 2.67 Hz, 1H, C(7)*H*), 2.08 – 2.17 (m, 2H, C(4)H_2), 2.27 – 2.36 (m, 2H, C(5)H_2), 2.65 (s, 2H, C(2)H_2), 3.93 – 4.06 (m, 4H, 2 × C(10)H_2), 4.16 (q, *J* = 7.15 Hz, 2H, C(8)H_2); δ_C (126 MHz, $CDCl_3$) 12.9 (*C*(5)), 14.3 (*C*(9)), 36.5 (*C*(4)), 42.9 (*C*(2)), 60.8 (*C*(8)), 65.4 (2 × *C*(10)), 68.2 (*C*(7)), 84.1 (*C*(6)), 108.4 (*C*(3)), 169.3 (*C*(1)); *m/z* (ESI^+) 235 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{11}H_{16}NaO_4^+$ ($[M+Na]^+$) requires 235.0941; found 235.0942.

2-(2-(But-3-yn-1-yl)-1,3-dioxolan-2-yl)ethan-1-ol (167)



A solution of **166** (3.24 g, 15.3 mmol) in Et_2O (76 mL) was added dropwise to a solution of $LiAlH_4$ (1 M in THF, 15.3 mL, 15.3 mmol) in Et_2O (96 mL) at 0 °C under N_2 and the reaction was stirred at rt for 2 h. The reaction was quenched by sequential addition of H_2O (0.58 mL), 15 w/v% NaOH (0.58 mL) and H_2O (1.7 mL) at 0 °C and stirred at rt for 15 min. The reaction was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give **167** as a yellow oil that was used without further purification (2.55 g, 98%); ν_{max} (film) 3367 (O–H), 3289 (C≡C–H), 2958 (C–H), 2889 (C–H); δ_H (400 MHz, $CDCl_3$) 1.88 – 1.99 (m, 5H, C(2)H_2, C(4)H_2, C(7)*H*), 2.27 (ddd, J = 8.37, 7.01, 2.74 Hz, 2H, C(5)H_2), 3.71 – 3.79 (m, 2H, C(1)H_2), 3.93 – 4.06 (m, 4H, 2 × C(8)H_2); δ_C (126 MHz, $CDCl_3$) 13.3 (*C*(5)), 36.0 (*C*(2)), 38.4 (*C*(4)), 58.8 (*C*(1)), 65.1 (*C*(8)), 68.4 (*C*(7)), 84.1 (*C*(6)), 111.2 (*C*(3)); ***m/z*** (ESI$^+$) 193 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_9H_{14}NaO_3^+$ ([M+Na]$^+$) requires 193.0835; found 193.0836.

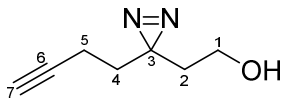
1-Hydroxyhept-6-yn-3-one (168)



5 M aq HCl (12.0 mL) was added to a solution of **167** (2.55 g, 15.0 mmol) in THF (40 mL) at 0 °C and the reaction was stirred at rt for 6 h. The reaction was diluted with H_2O (20 mL) and neutralised by addition of satd aq $NaHCO_3$, before being extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give **168** as a yellow oil that was used without further purification (1.61 g, 85%); ν_{max} (film) 3417 (O–H), 3290 (C≡C–H), 2922 (C–H), 2903 (C–H), 1711 (C=O); δ_H (400 MHz, Chloroform-*d*) 1.96 (t, *J* = 2.68 Hz, 1H, C(8)*H*), 2.47 (ddd, *J* = 7.92, 6.64, 2.71 Hz, 2H, C(6)H_2), 2.66 – 2.75 (m, 4H, C(2)H_2, C(4)H_2), 3.87 (t, *J* = 5.38 Hz, 2H, C(1)H_2); δ_C (126 MHz, Chloroform-*d*) 12.9 (*C*(6)), 41.9 (*C*(2)), 44.7 (*C*(4)), 57.9 (*C*(1)),

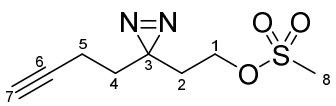
69.1 (*C*(8)), 82.9 (*C*(7)), 209.2 (*C*(3)); *m/z* (ESI$^+$) 149 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_7H_{10}NaO_2^+$ ([M+Na]$^+$) requires 149.0573; found 149.0574.

2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethan-1-ol (170)



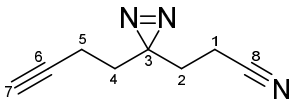
Anhydrous NH_3 was condensed into a round-bottomed flask containing **168** (1.00 g, 800 μmol) at –78 °C and the reaction was stirred at –78 °C for 1 h, then for 5 h at room temperature. A solution of hydroxylamine-*O*-sulfonic acid (1.03 g, 910 μmol) in CH_3OH (7 mL) was then added dropwise at 0 °C and stirred at rt for 16 h. Excess NH_3 was removed by degassing with N_2 for 1 h, and then the reaction was filtered and the solid washed with CH_3OH. The filtrate was concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2 (7 mL). Et_3N (1.4 mL, 10.0 mmol) was added and the reaction was cooled to 0 °C. I_2 (2.89 g, 11.4 mmol) was added portionwise, until the appearance of a persistent orange-brown coloration occurred, and the mixture was stirred at 0 °C for 1 h. The crude product was extracted with Et_2O (3 × 50 mL), washed with brine and concentrated *in vacuo*. Purification via column chromatography (eluent Et_2O/pentane, 1:4) gave **170** as a colourless oil (441 mg, 40%); ν_{max} (film) 3376 (broad, O–H), 3295 (C≡C–H), 2922 (C–H), 1586 (N=N); δ_H (400 MHz, $CDCl_3$) 1.64 – 1.77 (m, 4H, C(2)H_2, C(4)H_2), 1.97 – 2.08 (m, 3H, C(5)H_2, C(7)*H*), 3.50 (t, *J* = 6.15 Hz, 2H, C(1)H_2); δ_C (101 MHz, $CDCl_3$) 13.4 (*C*(5)), 26.7 (*C*(3)), 32.8 (*C*(4)), 35.7 (*C*(2)), 57.6 (*C*(1)), 69.4 (*C*(7)), 83.0 (*C*(6)); HRMS *m/z* not found.

2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethyl methanesulfonate (171)



Methanesulfonyl chloride (86.0 µL, 1.11 mmol) was added dropwise to a solution of **168** (154 mg, 1.11 mmol) and Et_3N (186 µL, 1.34 mmol) in THF (5.5 mL) at 0 °C and the reaction was stirred at rt for 2 h. The reaction was diluted with CH_2Cl_2 (5 mL) and quenched by addition of H_2O (5 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give **169** as a yellow oil that was used without further purification (200 mg, 84%); ν_{max} (film) 3290 (C≡C–H), 2960 (C–H), 2927 (C–H), 1351 (S=O), 1172 (S=O); δ_H (400 MHz, Chloroform-*d*) 1.70 (t, *J* = 7.21 Hz, 2H, C(4)H_2), 1.90 (t, *J* = 6.25 Hz, 2H, C(2)H_2), 1.99 – 2.09 (m, 3H, C(5)H_2, C(7)*H*), 3.06 (s, 3H, C(8)H_3), 4.09 (t, *J* = 6.27 Hz, 2H, C(1)H_2); δ_C (101 MHz, Chloroform-*d*) 13.4 (*C*(5)), 26.0 (*C*(3)), 32.4 (*C*(4)), 33.0 (*C*(2)), 37.8 (*C*(8)), 64.1 (*C*(1)), 69.7 (*C*(7)), 82.5 (*C*(6)); HRMS (ESI^+) $C_8H_{12}N_2NaO_3S^+$ ($[M+Na]^+$) requires 239.0461; found 239.0465.

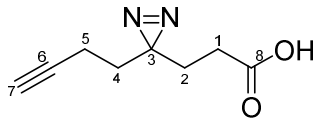
3-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)propanenitrile (172)



KCN (83.0 mg, 130 µmol) was added to **171** (200 mg, 870 µmol) in DMF (2 mL) and the reaction was heated at 70 °C for 16 h. The reaction was diluted with H_2O (10 mL) and extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with ½ sat brine (5 × 5 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentane, 3:17) gave **172** as a colourless oil (110 mg, 86%); ν_{max} (film) 3292 (C≡C–H), 2955 (C–H), 2850 (C–H), 2360 (C≡N), 1589 (N=N); δ_H (400 MHz, $CDCl_3$) 1.66 – 1.74 (m, 2H, C(4)H_2), 1.87 (dd, J = 8.0, 7.2 Hz, 2H, C(2)H_2), 2.01 – 2.09 (m, 3H, C(5)H_2, C(7)*H*), 2.18 (dd, J = 8.0, 7.2 Hz, 2H, C(1)H_2); δ_C (101 MHz,

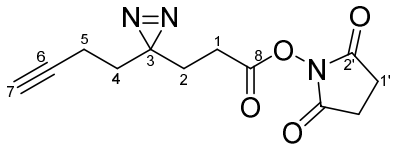
$CDCl_3$) 12.2 (*C*(4)), 13.3 (*C*(2)), 27.1 (*C*(3)), 29.7 (*C*(5)), 32.0 (*C*(1)), 69.9 (*C*(6)), 82.4 (*C*(7)), 118.5 (*C*(8)); HRMS *m/z* not found.

3-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)propanoic acid (173)



NaOH (10% aq, 1.25 mL) was added to **172** (110 mg, 750 μmol) and the reaction was heated at 70 °C for 5 h. The reaction was cooled to rt, and extracted with Et_2O (3 mL), this layer was discarded. The aqueous layer was acidified to pH~1 by addition of 1 M HCl, and extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give **173** as a colourless oil (25 mg, 20%); ν_{max} (film) 3299 (C≡C–H), ~3000 (br, O–H) 2918 (C–H), 2849 (C–H), 1712 (C=O), 1588 (N=N); δ_H (400 MHz, $CDCl_3$) 1.60 (t, J = 7.3 Hz, 2H, C(4)H_2), 1.76 (dd, J = 8.1, 7.2 Hz, 2H, C(2)H_2), 1.89 – 2.00 (m, 3H, C(5)H_2, C(7)*H*), 2.12 (t, J = 7.7 Hz, 2H, C(1)H_2); δ_C (151 MHz, $CDCl_3$) 13.4 (*C*(5)), 27.8 (*C*(1)), 28.0 (*C*(2)), 32.4 (*C*(4)), 69.4 (*C*(7)), 82.7 (*C*(6)), 175.6 (*C*=O); HRMS (ESI^-) $C_8H_9N_2O_2^-$ ([M−H$^-$]) requires 165.0670; found 165.0660.

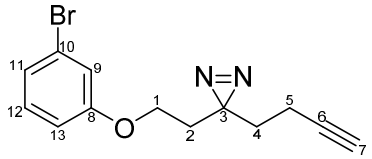
2,5-Dioxopyrrolidin-1-yl 3-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)propanoate (174)



EDC.HCl (36.0 mg, 190 μmol) and *N*-Hydroxysuccinimide (22.0 mg, 190 μmol) were added in quick succession to a solution of **173** (25.0 mg, 150 μmol) in CH_3CN (0.75 mL) under N_2 and the reaction was stirred for 16 h in the dark. The reaction was concentrated *in vacuo*, and the residue was partitioned between EtOAc (3 mL) and H_2O (3 mL). The aqueous layer was extracted with EtOAc (2 × 3 mL), and the combined organic layers were

washed with satd aq NH_4Cl (3 mL), satd aq $NaHCO_3$ (3 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo* to give **174** as a colourless oil that was used without further purification (30 mg, 76%); ν_{max} (film) 3290 (C≡C–H), 2918 (C–H), 2850 (C–H), 1737 (C=O); δ_H (400 MHz, $CDCl_3$)1.62 – 1.71 (m, 2H, C(4)H_2), 1.85 – 1.92 (m, 2H, C(2)H_2), 1.98 – 2.05 (m, 3H, C(5)H_2, C(7)*H*), 2.46 (dd, J = 8.5, 7.2 Hz, 2H, C(1)H_2), 2.82 (s, 4H, 2 × C(1')H_2); δ_C (101 MHz, $CDCl_3$) 13.3 (*C*(5)), 25.6 (*C*(1)), 25.7 (*C*(1')), 27.3 (*C*(3)), 28.1 (*C*(2)), 32.1 (*C*(4), 69.6 (*C*(7)), 82.5 (*C*(6)), 167.6 (*C*(8)), 169.0 (*C*(2')); *m/z* (ESI^+) 286 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{12}H_{13}N_3NaO_4^+$ ($[M+Na]^+$) requires 286.0798; found 286.0797.

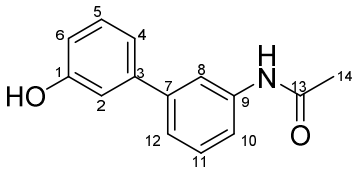
3-(2-(3-Bromophenoxy)ethyl)-3-(but-3-yn-1-yl)-3*H*-diazirine (178)



3-Bromophenol (55.0 mg, 320 μmol) was added to a suspension of **171** (84.0 mg, 390 μmol) and Cs_2CO_3 (127 mg, 390 μmol) in MeCN (1.6 mL) and the reaction was heated at 85 °C for 4 h. The reaction was concentrated *in vacuo* and the residue was partitioned between CH_2Cl_2 (5 mL) and satd aq NH_4Cl (5 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→5% Et_2O in pentane) gave **178** as a free-flowing, colourless oil (94 mg, 99%); ν_{max} (film) 3298 (C≡C–H), 2925 (C–H), 2881 (C–H), 1590 (N=N); δ_H (400 MHz,$CDCl_3$) 1.73 (t, J = 7.45 Hz, 2H, C(4)H_2), 1.89 (t, J = 6.17 Hz, 2H, C(2)H_2), 1.99 (t, J = 2.66 Hz, 1H, C(7)*H*), 2.07 (td, J = 7.45, 2.67 Hz, 2H, C(5)H_2), 3.80 (t, J = 6.17 Hz, 2H, C(1)H_2), 6.82 (ddd, J = 8.04, 2.49, 1.26 Hz, 1H, C(13)*H*), 7.05 (dd, J = 2.46, 1.77 Hz, 1H, C(9)*H*), 7.07 – 7.11 (m, 1H, C(11)*H*), 7.14 (t, J = 7.94 Hz, 1H, C(12)*H*);

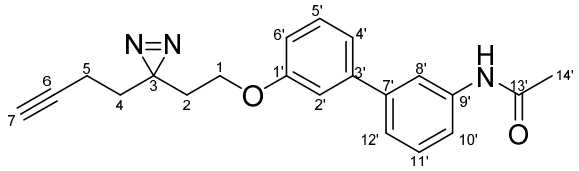
δ_C (101 MHz, $CDCl_3$) 13.4 (*C*(5)), 26.5 (*C*(3)), 32.8 (*C*(4)), 33.0 (*C*(2)), 63.0 (*C*(1)), 69.4 (*C*(7)), 77.4 (*C*(6)), 113.7 (*C*(13)), 118.0 (*C*(9)), 123.0 (*C*(10)), 124.4 (*C*(11)), 130.7 (*C*(12)), 159.4 (*C*(8)); HRMS *m/z* not found.

_N_-(3'-hydroxy-[1,1'-biphenyl]-3-yl)acetamide (180)



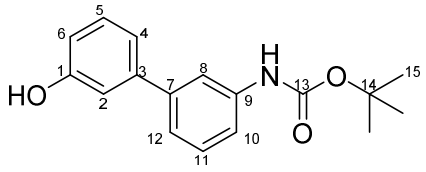
3-Bromophenol (865 mg, 5.00 mmol) and 3-acetamidophenylboronic acid (895 mg, 5.00 mmol) and Na_2CO_3 (1.06 g, 10.0 mmol) were suspended in DME (36 mL) and H_2O (5 mL) and the reaction was degassed with N_2 for 5 min. $Pd(dppf)Cl_2$ (185 mg, 0.25 mmol) was added and the reaction was degassed with N_2 for a further 5 min and the reaction was heated at 80 °C for 30 min. The reaction was concentrated *in vacuo* and the residue was partitioned between CH_2Cl_2 (50 mL) and H_2O (50 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→75% EtOAc in pentane) gave **180** as an orange oil (773 mg, 68%); ν_{max} (film) 3306 (br. O−H), 3055 (C−H), 2981 (C−H), 1743 (C=O); δ_H (400 MHz, $CDCl_3$) 2.20 (s, 3H, C(14)*H*$_3$), 5.94 (s, 1H, O*H*), 6.83 (dd, J = 7.9, 2.1 Hz, 1H, C(6)*H*), 7.03 (t, J = 2.1 Hz, 1H, C(2)*H*), 7.10 (dt, J = 7.7, 1.3 Hz, 1H, C(4)*H*), 7.23 – 7.37 (m, 3H, C(5)*H*, C(11)*H*, C(12)*H*), 7.39 – 7.49 (m, 2H, C(10)*H*, N*H*), 7.71 (t, J = 1.9 Hz, 1H, C(8)*H*); δ_C (101 MHz, MeOH-d_4) 23.9 (*C*(14)), 114.8 (*C*(2)), 115.4 (*C*(6)), 119.3 (*C*(8)), 119.7 (*C*(4)), 120.0 (*C*(10)), 123.7 (*C*(12)), 130.1 (*C*(11)), 130.8 (*C*(5)), 140.2 (*C*(9)), 143.2 (*C*(7)), 143.6 (*C*(3)), 158.8 (*C*(1)), 171.7 (*C*=O); *m/z* (ESI^+) 228 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{14}H_{14}NO_2^+$ ($[M+H]^+$) requires 228.1019; found 228.1020.

***N*-(3'-(2-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)ethoxy)-[1,1'-biphenyl]-3-yl)acetamide (181)**



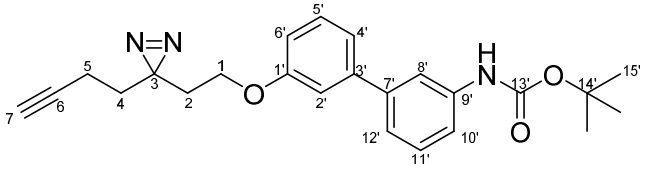
180 (89.0 mg, 390 μmol) was added to a suspension of **171** (100 mg, 390 μmol) and Cs_2CO_3 (127 mg, 390 μmol) in MeCN (1.6 mL) and the reaction was heated at 85 °C for 4 h. The reaction was concentrated *in vacuo* and the residue was partitioned between CH_2Cl_2 (5 mL) and satd aq NH_4Cl (5 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→50% EtOAc in pentane) gave **181** as yellow oil (67 mg, 50%); ν_{max} (film) 3294 (C≡C–H), 2926 (C–H), 1664 (C=O), 1598 (N=N); δ_H (400 MHz, $CDCl_3$) 1.69 (t, J = 7.5 Hz, 2H, C(4)H_2), 1.84 (t, J = 6.2 Hz, 2H, C(2)H_2), 1.93 (q, J = 2.6 Hz, 1H, C(7)*H*), 2.01 (td, J = 7.5, 2.6 Hz, 2H, C(5)H_2), 2.14 (s, 3H, C(14')H_3), 3.82 (t, J = 6.2 Hz, 2H, C(1)H_2), 6.81 (ddd, J = 8.2, 2.5, 1.0 Hz, 1H, C(6')*H*), 7.03 (dd, J = 2.5, 1.7 Hz, 1H, C(2')*H*), 7.11 (ddd, J = 7.7, 1.7, 1.0 Hz, 1H, C(4')*H*), 7.17 (s, 1H, N*H*), 7.23 – 7.40 (m, 3H, C(5')*H*, C(11')*H*, C(12')*H*), 7.45 (d, J = 7.7 Hz, 1H, C(10')*H*), 7.7 (t, J = 1.92 Hz, 1H, C(8')*H*); δ_C (101 MHz, $CDCl_3$) 13.4 (*C*(5)), 24.7 (*C*(14'), 26.8 (*C*(3)), 32.8 (*C*(4)), 33.1 (*C*(2)), 62.7 (*C*(1)), 69.4 (*C*(6)), 82.9 (*C*(7)), 113.6 (*C*(2'), *C*(6')), 118.8 (*C*(8')), 119.2 (*C*(10')), 120.2 (*C*(4')), 123.3 (*C*(12')), 129.5 (*C*(11')), 129.9 (*C*(5')), 138.4 (*C*(9')), 141.9 (*C*(7')), 142.4 (*C*(3')), 158.9 (*C*(1')), 168.8 (*C*(13')); *m/z* (ESI^+) 348 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{21}H_{22}N_3O_2^+$ ($[M+H]^+$) requires 348.1707; found 348.1707.

***tert*-Butyl (3'-hydroxy-[1,1'-biphenyl]-3-yl)carbamate (182)**



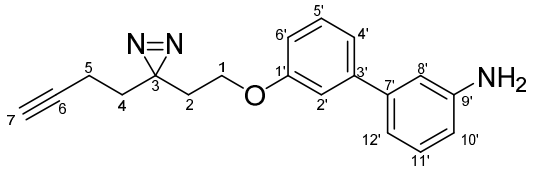
3-Bromophenol (342 mg, 2.00 mmol) and 3-acetamidophenylboronic acid (474 mg, 2.00 mmol) and Na_2CO_3 (424 mg, 4.00 mmol) were suspended in DME (14 mL) and H_2O (2 mL) and the reaction was degassed with N_2 for 5 min. Pd(dppf)Cl_2 (74.0 mg, 100 μmol) was added and the reaction was degassed with N_2 for a further 5 min and the reaction was heated at 80 °C for 30 min. The reaction was concentrated *in vacuo* and the residue was partitioned between CH_2Cl_2 (50 mL) and H_2O (50 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→75% EtOAc in pentane) gave **182** as an orange oil (423 mg, 82%); ν_{max} (film) 3305 (br. O−H), 2981 (C−H), 1695 (C=O); δ_H (400 MHz, $CDCl_3$) 1.46 (s, 9H, 3 × C(15)*H*$_3$), 5.75 (s, 1H, O*H*), 6.57 (s, 1H, N*H*), 6.73 (ddd, J = 8.1, 2.5, 1.0 Hz, 1H, C(6)*H*), 6.94 (td, J = 2.7, 1.6 Hz, 1H, C(2)*H*), 7.02 (ddd, J = 7.7, 1.6, 1.0 Hz, 1H, C(4)*H*), 7.10 – 7.25 (m, 4H, C(5)*H*, C(10)*H*, C(11)*H*, C(12)*H*), 7.47 – 7.55 (m, 1H, C(8)*H*); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × *C*(15)), 81.1 (*C*(14)), 114.3 (*C*(2)), 114.6 (*C*(6)), 117.7 (*C*(8)), 118.0 (*C*(10)), 119.6 (*C*(4)), 122.2 (*C*(12)), 129.4 (*C*(11)), 130.0 (*C*(5)), 138.6, 141.9, 142.6 (*C*(3), *C*(7) and *C*(9)), 153.3 (*C*=O), 156.2 (*C*(1)); *m/z* (ESI^+) 286 ($[M+H]^+$, 100%).

tert-Butyl (3'-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethoxy)-[1,1'-biphenyl]-3-yl)carbamate (183)



Cs_2CO_3 (285 mg, 880 μmol) was added to a solution of **182** (250 mg, 877 μmol) and **171** (160 mg, 730 μmol) in CH_3CN (5 mL) and reaction was heated at 85 °C for 6 h. The reaction was filtered, concentrated *in vacuo* and the residue was partitioned between CH_2Cl_2 (10 mL) and sat aq NH_4Cl (10 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent, $PhCH_3$) gave **183** as a yellow oil (183 mg, 62%); ν_{max} (film) 3299 (C≡C–H), 2975 (C–H), 2929 (C–H), 1725 (C=O), 1600 (C=O), 1578 (N=N); δ_H (400 MHz, $CDCl_3$) 1.53 (s, 9H, 3 × C(15')H_3), 1.76 (t, J = 7.5 Hz, 2H, C(4)H_2), 1.91 (t, J = 6.2 Hz, 2H, C(2)H_2), 2.00 (t, J = 2.6 Hz, 1H, C(7)*H*), 2.08 (td, J = 7.5, 2.6 Hz, 2H, C95)H_2), 3.89 (t, J = 6.2 Hz, 2H, C(1)H_2), 6.87 (ddd, J = 8.2, 2.6, 1.0 Hz, 1H, N*H*), 7.10 (dd, J = 2.5, 1.6 Hz, 1H, C(6')*H*), 7.18 (ddd, J = 7.7, 1.7, 1.0 Hz, 1H, C(2')*H*), 7.24 (dd, J = 4.5, 1.8 Hz, 1H, C(10')*H*), 7.29 – 7.37 (m, 3H, C(4')*H*, C(5')*H*, C(11')*H*), 7.55 – 7.61 (m, 1H, C(8')*H*); δ_C (101 MHz, $CDCl_3$) 13.5 (*C*(5)), 26.9 (*C*(3)), 28.5 (3 × *C*(15')), 32.8 (*C*(4)), 33.2 (*C*(2)), 62.8 (*C*(1)), 69.4 (*C*(7)), 80.7 (*C*(14')), 82.9 (*C*(6)), 113.6 (*C*(6')), 113.7 (*C*(8')), 114.0 (*C*(8')), 117.5 (*C*(4')), 117.7 (*C*(2')), 120.3 (*C*(10')), 122.1 (*C*(12')), 129.5, 129.9 (*C*(5') and C(8')), 138.9, 142.1, 142.7 (*C*(3'), *C*(7') and *C*(9')), 152.9 (*C*(1')), 158.9 (*C*(13')); ***m/z*** (ESI^+) 286 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{24}H_{28}N_3O_3^+$ ($[M+H]^+$) requires 406.2125; found 406.2126.

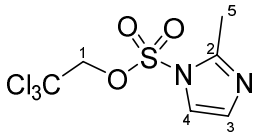
3'-(2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)ethoxy)-[1,1'-biphenyl]-3-amine (179)



TFA (345 μL, 4.50 mmol) was added to a solution of **183** (183 mg, 450 μmol) in CH_2Cl_2 (2.25 mL) and the reaction was stirred at rt for 6 h. The reaction was basified by addition

of 1M NaOH and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent Et_2O/pentane, 4:6) gave **179** as a colourless oil (130 mg, 95%); ν_{max} (film) 3373 (N−H), 3290 (C≡C–H), 2917 (C–H), 2849 (C–H), 1576 (N=N); δ_H (400 MHz, CD_2Cl_2) 1.67 (t, J = 7.5 Hz, 2H, C(4)H_2), 1.82 (t, J = 6.2 Hz, 2H, C(2)H_2), 1.94 (t, J = 2.6 Hz, 1H, C(7)*H*), 2.00 (td, J = 7.5, 2.5 Hz, 2H, C(5)H_2), 3.72 (s, 2H, NH_2), 3.81 (t, J = 6.2 Hz, 2H, C(1)H_2), 6.58 (ddd, J = 7.9, 2.3, 1.0 Hz, 1H, C(10')*H*), 6.78 (ddd, J = 8.2, 2.6, 1.0 Hz, 1H, C(6')*H*), 6.81 (t, J = 2.1 Hz, 1H, C(8')*H*), 6.87 (ddd, J = 7.7, 1.7, 1.0 Hz, 1H, C(12')*H*), 7.01 (dd, J = 2.6, 1.7 Hz, 1H, C(2')*H*), 7.05 – 7.16 (m, 2H, C(4')*H*, C(11')*H*), 7.25 (t, J = 7.9 Hz, 1H, C(5')*H*); δ_C (126 MHz, CD_2Cl_2) 13.6 (*C*(5)), 27.2 (*C*(3)), 33.0 (*C*(4)), 33.3 (*C*(6)), 63.1 (*C*(1)), 69.2 (*C*(6)), 83.3 (*C*(7)), 113.6 (*C*(2')), 113.7 (*C*(8')), 113.9 (*C*(6')), 114.5 (*C*(10')), 117.6 (*C*(12')), 120.2 (*C*(4')), 130.0 (*C*(11')), 130.1 (*C*(5')), 142.3 (*C*(3')), 143.3 (*C*(7')), 147.5 (*C*(9')), 159.3 (*C*(1')); *m/z* (ESI$^+$) 306 ([M+H]$^+$, 100%); HRMS (ESI$^+$) $C_{19}H_{20}N_3O^+$ ([M+H]$^+$) requires 306.1601; found 306.1599.

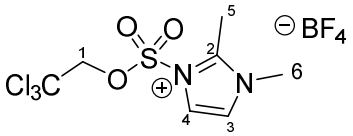
2,2,2-Trichloroethyl 2-methyl-1*H*-imidazole-1-sulfonate (185)



2,2,2-Trichloroethanol (1.00 mL, 10.4 mmol) and pyridine (850 μL, 10.4 mmol) in Et_2O (5 mL) was added dropwise to SO_2Cl_2 (1M in CH_2Cl_2, 10.4 mL, 10.4 mmol) at −78 °C under N_2. The reaction was stirred at −78 °C, then at rt for 2 h. The reaction filtered and the filtrate was concentrated *in vacuo*, and dissolved in THF (8 mL). This solution was added to a suspension of 2-methylimidazole (2.0 g, 26 mmol) in THF (12 mL) at 0 °C and the reaction

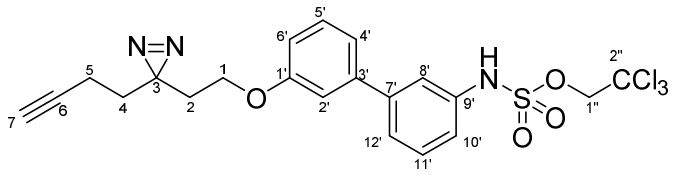
was stirred at rt for 16 h. The reaction was filtered, concentrated *in vacuo* and the residue was partitioned between EtOAc and H_2O (1:1, 40 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→30% EtOAc in penatne) gave **185** as a colourless, free-flowing oil (2.10 g, 69%); ν_{max} (film) 3162 (C–H), 3128 (C–H), 3005 (C–H), 2954 (C–H), 1422 (S=O), 1206 (S=O); δ_H (400 MHz, $CDCl_3$) 2.70 (s, 3H, C(5)H_3), 4.66 (s, 2H, C(1)H_2), 6.97 (d, J = 1.8 Hz, 1H, C(4)*H*), 7.33 (d, J = 1.8 Hz, 1H, C(3)*H*); δ_C (101 MHz, $CDCl_3$) 15.0 (*C*(5)), 80.2 (*C*(1)), 91.8 (*C*Cl_3), 120.3 (*C*(4)), 128.0 (*C*(3)), 146.7 (*C*(2)); *m/z* (ESI^+) 314 ($[M+Na]^+$, 100%). Characterisation consistent with literature.[86]

2,3-Dimethyl-1-((2,2,2-trichloroethoxy)sulfonyl)-1*H*-imidazol-3-ium tetrafluoroborate (186)



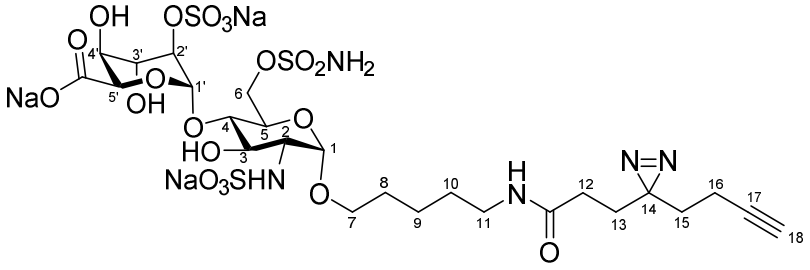
Trimethyloxonium tetrafluoroborate (100 mg, 687 μmol) was added to a solution of **185** (200 mg, 687 μmol) in CH_2Cl_2 (2.0 mL) under N_2 at 0 °C and the reaction was stirred at rt for 16 h. The precipitate was isolated via vacuum filtration and washed with ice-cold pentane to give **186** as a white solid that was used without further purification (245 mg, 91%); mp 113-116 °C; {lit.[86] mp 118.5-120.5 °C}; ν_{max} (film) 3151 (C–H), 3035 (C–H), 1454 (S=O), 1277 (S=O); δ_H (400 MHz, MeOH-*d*$_4$) 2.93 (s, 3H, C(5)H_3), 3.94 (s, 3H, C(6)H_2), 5.34 (s, 2H, C(1)H_2), 7.73 (d, J = 2.5 Hz, 1H, C(3)*H*), 8.08 (d, J = 2.5 Hz, 1H, C(4)*H*); δ_C (101 MHz, MeOH-*d*$_4$) 11.8 (*C*(5)), 36.7 (*C*(6)), 83.4 (*C*(1)), 93.0 (*C*Cl_3), 122.1 (*C*(3)), 124.9 (*C*(4)), 150.0 (*C*(2)); δ_F (376 MHz, MeOH-*d*$_4$) -154.4 (s, 4F, BF_4). Characterisation data are consistent with the literature.[86]

2,2,2-trichloroethyl (3'-(2-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)ethoxy)-[1,1'-biphenyl]-3-yl)sulfamate (176)



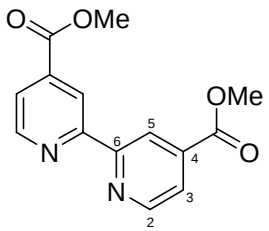
186 (85.0 mg, 216 μmol) was added to a solution of **179** (22.0 mg, 72.0 μmol) in THF (720 μL) and the reaction was heated at 67 °C for 30 h in the dark. The reaction was concentrated *in vacuo* and purification via column chromatography (eluent EtOAc/pentane, 1:4) gave **176** as a colourless oil (35 mg, 94%); ν_{max} (film) 3298 (C≡C–H), 2952 (C–H), 2921 (C–H), 2851 (C–H), 1435 (S=O), 1225 (S=O); δ_H (600 MHz, CD_2Cl_2) 1.75 (t, J = 7.5 Hz, 2H, C(4)H_2), 1.92 (t, J = 6.2 Hz, 2H, C(2)H_2), 2.03 (t, J = 2.6 Hz, 1H, C(7)*H*), 2.09 (td, J = 7.5, 2.7 Hz, 2H, C(5)H_2), 3.89 (t, J = 6.1 Hz, 2H, C(1)H_2), 4.71 (s, 2H, C(1'')H_2), 6.91 (ddd, J = 8.3, 2.6, 0.9 Hz, 1H, C(6')*H*), 6.95 (s, 1H, N*H*), 7.08 – 7.11 (m, 1H, C(2')*H*), 7.18 (ddd, J = 7.7, 1.7, 0.9 Hz, 1H, C(4')*H*), 7.27 (dt, J = 7.1, 2.0 Hz, 1H, C(8')*H*), 7.37 (t, J = 8.0 Hz, 1H, C(5')*H*), 7.44 – 7.50 (m, 3H, C(10')*H*, C(11')*H*, C(12')*H*); δ_C (151 MHz, CD_2Cl_2) 13.6 (*C*(5)), 27.1 (*C*(3)), 33.0 (*C*(4)), 33.3 (*C*(2)), 63.2 (*C*(1)), 69.3 (*C*(7)), 79.4 (*C*(1'')), 83.3 (*C*(6)), 93.5 (*C*(2'')), 113.9 (*C*(2')), 114.2 (*C*(6')), 120.2 (*C*(4')), 120.3 (*C*(8')), 120.5 (*C*(10')), 125.2 (*C*(12')), 130.4 (*C*(5'), *C*(11')), 136.2 (*C*(7')), 141.8 (*C*(9')), 143.0 (*C*(3')), 159.4 (*C*(1')); HRMS $C_{21}H_{20}(^{35}Cl)_3N_3NaO_4S^+$ ([M($^{35}Cl)_3$+Na]$^+$) requires 538.0132; found 538.0135.

Sodium (2*R*,3*S*,4*S*,5*R*,6*R*)-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-6-((5-(3-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)propanamido)pentyl)oxy)-4-hydroxy-2-((sulfamoyloxy)methyl)-5-(sulfonatoamino)tetrahydro-2*H*-pyran-3-yl)oxy)-3,4-dihydroxy-5-(sulfonatooxy)tetrahydro-2*H*-pyran-2-carboxylate (187)



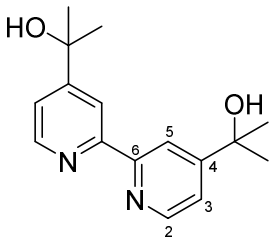
163 (30.0 mg, 40.0 μmol), **174** (21.0 mg, 80.0 μmol) and Na_2CO_3 (8.00 mg, 80.0 μmol) were suspended in DMF (200 μL) under N_2 in the dark and the reaction was stirred at rt for 16 h. Purification via reversed phase chromatography (eluent, H_2O), followed by elution through a DOWEX 50WX4 Na^+-form column (eluent, H_2O) gave **187** as a brown glass (21 mg, 59%); $[\alpha]_D^{25}$ +33.0 (*c* 0.1 in CH_3OH); ν_{max} 3379 (br., O–H), 2948 (C–H), 2898 (C–H), 2849 (C–H), 1726 (C=O), 1431 (S=O), 1228 (S=O); δ_H (400 MHz, D_2O) 1.36 (p, J = 7.3 Hz, 2H, C(9)H_2), 1.48 (p, J = 7.2 Hz, 2H, C(10)H_2), 1.53 – 1.65 (m, 4H, C(8)H_2, C(15)H_2), 1.70 (t, J = 7.3 Hz, 2H, C(13)H_2), 1.96 (td, J = 7.2, 2.7 Hz, 2H, C(16)H_2), 2.04 (t, J = 7.3 Hz, 2H, C(12)H_2), 2.31 (t, J = 2.7 Hz, 1H, C(18)*H*), 3.13 (t, J = 6.7 Hz, 2H, C(11)H_2), 3.20 (dd, J = 10.0, 3.7 Hz, 1H, C(2)*H*), 3.48 (dt, J = 9.9, 6.2 Hz, 1H, C(7)H_A), 3.58 – 3.73 (m, 3H, C(3)*H*, C(4)*H*, C(7)H_B), 3.90 (d, J = 4.7 Hz, 2H, C(3')*H*, C(4')*H*), 3.98 (d, J = 9.5 Hz, 1H, C(5)*H*), 4.11 – 4.19 (m, 1H, C(2')*H*), 4.37 – 4.47 (m, 2H, C(6)H_2), 4.64 (d, J = 2.7 Hz, 1H, C(5')*H*), 5.03 (d, J = 3.2 Hz, 1H, C(1')*H*), 5.07 (d, J = 3.7 Hz, 1H, C(1)*H*); δ_C (151 MHz, D_2O) 12.5 (*C*(16)), 22.9 (*C*(9)), 28.0 (*C*(10)), 28.2 (*C*(8)), 28.4 (*C*(10)), 28.7 (*C*(13)), 30.0 (*C*(16)), 30.9 (*C*(15)), 39.3 (*C*(11)), 57.8 (*C*(2)), 68.3 (*C*(5)), 68.5 (*C*(6)), 68.6 (*C*(7)), 69.5 (*C*(18)), 69.6 (*C*(5')), 69.8 (*C*(3') or *C*(4')), 69.9 (*C*(3') or *C*(4')), 69.9 (*C*(3)), 75.6 (*C*(2')), 77.8 (*C*(4)), 84.4 (*C*(17)), 97.2 (*C*(1)), 99.2 (*C*(1')), 174.9 (C_{Acid}=O), 176.1 (C_{Amide}=O); *m/z* (ESI^+) 893 ([M+H]+, 100%); HRMS (ESI^-) $C_{25}H_{40}N_5O_{20}S_3^-$ ($[M-3Na+2H]^-$) requires 826.1434; found 826.1426.

Dimethyl 2,2′-bipyridine-4,4′-dicarboxylate (189)



H_2SO_4 (10 mL) was added to a suspension of 2,2′-bipyridine-4,4′-dicarboxylic acid (4.25 g, 17.4 mmol) in CH_3OH (100 mL) and the reaction was heated at 80 °C for 16 h. The reaction was cooled to rt, then poured into H_2O (250 mL) and neutralised to pH 7-8 by addition of 1M NaOH. The aqueous layer was extracted with $CHCl_3$ (5 × 50 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give **189** as a pale yellow crystalline solid (4.28 g, 90%); mp 210-212 °C; {lit.[162] mp 221.1-221.5 °C (chloroform, acetone)}; ν_{max} (film) 2925 (C–H), 2850 (C–H), 1731 (C=O); δ_H (400 MHz, $CDCl_3$) 4.00 (s, 6H, 2 × *Me*), 7.91 (dd, J = 5.0, 1.6 Hz, 2H, 2 × C(3)*H*), 8.87 (dd, J = 5.0, 0.9 Hz, 2H, 2 × C(5)*H*), 8.97 (dd, J = 1.6, 0.9 Hz, 2H, 2 × C(2)*H*); δ_C (101 MHz, $CDCl_3$) 52.9 (2 × *C*H_3), 120.9 (2 × *C*(5)), 123.5 (2 × *C*(3)), 139.0 (2 × *C*(4)), 150.2 (2 × *C*(2)), 156.3 (2 × *C*(6)), 165.7 (2 × *C*=O); *m/z* (ESI^+) 273 ($[M+H]^+$, 89%), 567 ($[2M+Na]^+$, 100%); HRMS (ESI+) $C_{14}H_{13}N_2O_4^+$ ($[M+H]^+$) requires 273.0870; found 273.0869. Characterisation data are consistent with the literature.[162]

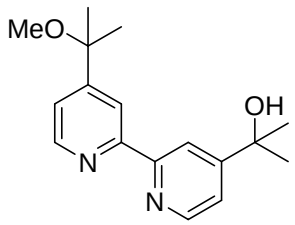
2,2'-([2,2'-Bipyridine]-4,4'-diyl)bis(propan-2-ol) (190)



MeMgBr (1M in THF, 97.0 mL, 97.0 mmol) was added rapidly via cannula to a vigorously stirred solution of **189** (4.28 g, 17.4 mmol) in THF (175 mL) at −78 °C under N_2. The reaction was stirred at −78 °C for 30 min, then at rt for 2 h. The reaction was quenched by slow addition of satd aq NH_4Cl (25 mL) and concentrated *in vacuo*. The residue was partitioned between EtOAc and H_2O (1:1, 500 mL) and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via recrystallization

(EtOAc/pentane, 3:17, 200 mL) gave **190** as a white solid (3.81 g, 89%); mp 146-151 °C; {lit.[163] mp 149.6 - 152.8 °C}; ν_{max} (film) 3355 (O–H), 2975 (C–H), 2927 (C–H); δ_H (400 MHz, $CDCl_3$) 1.64 (s, 12H, 4 × C*H*$_3$), 7.48 (dd, J = 5.2, 1.9 Hz, 2H, 2 C(3)*H*), 8.51 (d, J = 1.8 Hz, 2H, 2 × C(5)*H*), 8.64 (d, J = 5.1 Hz, 2H, 2 × C(2)*H*); δ (101 MHz, CDCl3) 31.5 (4 × *C*H_3), 72.4 (2 × *C*$(CH_3)_2$OH), 117.7 (2 × *C*(5)), 120.1 (2 × *C*(3)), 149.1 (2 × *C*(2)), 155.8 (2 × *C*(6)), 159.7 (2 × *C*(4)); *m/z* (ESI^+) 273 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{16}H_{21}N_2O_2^+$ ($[M+H]^+$) requires 273.1598; found 273.1595. Characterisation data are consistent with the literature.[163]

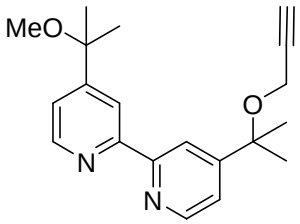
2-(4'-(2-Methoxypropan-2-yl)-[2,2'-bipyridin]-4-yl)propan-2-ol (191)



NaH (60% in mineral oil, 504 mg, 12.6 mmol) was added to a solution of **190** (3.83 g, 14.1 mmol) in THF (14 mL) at 0 °C under N_2. The reaction was stirred at rt for 1 h, over which time the reaction formed a saturated suspension. Iodomethane (785 μL, 12.6 mmol) was added, the reaction vessel was sealed and then heated at 80 °C for 5 h. The reaction was quenched by addition of sat aq NH_4Cl, and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification via reversed phase chromatography (gradient elution 5%→50% CH_3CN in H_2O) gave **191** as a yellow crystalline solid (1.41 g, 35%); mp 105-109 °C; ν_{max} (film) 3367 (O–H), 2987 (C–H), 2975 (C–H), 2934 (C–H), 2827 (C–H); δ_H (400 MHz, $CDCl_3$) 1.59 (s, 6H, 2 × C*H*$_3$), 1.64 (s, 6H, 2 × C*H*$_3$), 3.15 (s, 3H, OC*H*$_3$), 7.42 (dd, J = 5.2, 1.9 Hz, 1H, *Ar*), 7.47 (dd, J = 5.2, 1.9 Hz, 1H, *Ar*), 8.42 (dd, J = 1.9, 0.8 Hz, 1H, *Ar*), 8.51 (dd, J = 1.9, 0.8 Hz, 1H, *Ar*), 8.65 (ddd, J = 5.2, 2.1, 0.8 Hz, 2H, *Ar*); δ_C (151 MHz, $CDCl_3$)

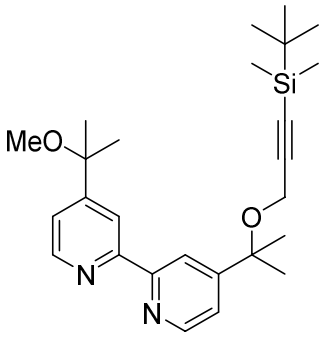
27.5, 31.5, 51.1, 72.4, 76.7, 117.5, 118.7, 120.0, 121.2, 149.3, 149.3, 156.1, 156.4, 156.9, 159.4; *m/z* (ESI^+) 287 ($[M+H]^+$, 100%); HRMS (ESI^+): $C_{17}H_{23}N_2O_2^+$ ($[M+H]^+$) requires 287.1754; found 287.1751. Characterisation data are consistent with the literature.[129]

4-(2-Methoxypropan-2-yl)-4'-(2-(prop-2-yn-1-yloxy)propan-2-yl)-2,2'-bipyridine (192)



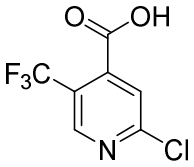
NaH (60% in mineral oil, 190 mg, 4.73 mmol) was added to a solution of **191** (920 mg, 3.15 mmol) in DMF (16 mL) at 0 °C under N_2 and the reaction was stirred at rt for 1 h. Propargyl bromide (80 w/v% in PhMe, 440 μL, 3.94 mmol) was added and the reaction was heated at 80 °C for 16 h. The reaction was quenched by addition of ½ sat brine, and extracted with CH_2Cl_2 (2 × 25 mL). The combined organic layers were washed with ½ sat brine (5 × 15 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 10%→80% EtOAc in pentanes) gave **192** as a brown oil (385 mg, 38%); ν_{max} (film) 3298 (C≡C−H), 2979 (C–H), 2933 (C–H); δ_H (400 MHz, $CDCl_3$) 1.59 (s, 6H), 1.65 (s, 6H), 2.41 (t, J = 2.4 Hz, 1H), 3.15 (s, 3H), 3.93 (d, J = 2.4 Hz, 2H), 7.42 (dd, J = 5.2, 1.8 Hz, 1H), 7.47 (dd, J = 5.2, 1.8 Hz, 1H), 8.42 (d, J = 1.8 Hz, 2H), 8.68 (ddd, J = 9.4, 5.2, 0.8 Hz, 2H); δ_C (151 MHz, $CDCl_3$) 27.5, 27.9, 51.1, 52.1, 73.9, 76.7, 78.1, 80.8, 118.6, 118.8, 121.1, 121.3, 149.3, 149.6, 156.0, 156.2, 156.5, 157.0; *m/z* (ESI^+) 325 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{20}H_{25}N_2O_2^+$ ($[M+H]^+$) requires 325.1911; found 325.1912. Characterisation data are consistent with the literature.[129]

4-(2-((3-(*tert*-Butyldimethylsilyl)prop-2-yn-1-yl)oxy)propan-2-yl)-4'-(2-methoxypropan-2-yl)-2,2'-bipyridine (193)



n-BuLi (2.5M in hexane, 1.0 mL, 2.44 mmol) was added dropwise to a solution of **192** (500 mg, 1.54 mmol) in THF (10.3 mL) at – 78 °C under N_2. The reaction was stirred for 15 min at – 78 °C, before TBSOTf (530 μL, 2.31 mmol) was added and the reaction was allowed to warm to rt over 1 h. The reaction was quenched by addition of CH_3OH (1 mL) and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 20%→60% EtOAc in pentane) gave **193** as a brown oil (469 mg, 70%); ν_{max} (film) 2953 (C–H), 2931 (C–H), 2895 (C–H), 2827 (C–H), 2179 (C≡C); δ_H (400 MHz, $CDCl_3$) 0.08 (s, 6H), 0.91 (s, 9H), 1.58 (s, 6H), 1.63 (s, 6H), 3.14 (s, 3H), 3.96 (s, 2H), 7.39 (dd, J = 5.1, 1.8 Hz, 1H), 7.46 (dd, J = 5.1, 1.8 Hz, 1H), 8.39 (dd, J = 1.9, 0.8 Hz, 2H), 8.66 (td, J = 5.3, 0.8 Hz, 2H); δ_C (101 MHz, $CDCl_3$) -4.6, 16.6, 26.2, 27.5, 28.0, 51.1, 53.0, 76.6, 78.1, 88.8, 103.5, 118.5, 118.6, 121.1, 121.2, 149.4, 149.6, 156.2, 156.5, 156.7*m/z* (ESI^+) 439 ($[M+H]^+$, 100%), 899 ($[2M+Na]^+$, 17%); HRMS (ESI^+) $C_{26}H_{39}N_2O_2Si^+$ ($[M+H]^+$) requires 439.2775; found 439.2782. Characterisation data are consistent with the literature.[129]

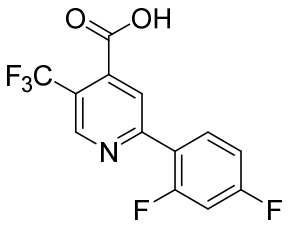
2-Chloro-5-(trifluoromethyl)isonicotinic acid (195)



n-BuLi (2.5 M in hexanes, 2.60 mL, 6.50 mmol) was added dropwise to a solution of 2-chloro-4-iodo-5-(trifluoromethyl)pyridine (1.00 g, 3.26 mmol) in THF (13 mL) at −78 °C

under N_2. The reaction was stirred at −78 °C for 15 min then poured over freshly crushed solid CO_2 and the reaction was stirred at rt for 30 min. The reaction was concentrated *in vacuo* and the residue was dissolved in H_2O (15 mL) and extracted with Et_2O (2 × 15 mL), these organic layers were discarded. The aqueous layer was then acidifed to pH 1-2 by addition of 1M HCl, and was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent CH_3OH/CH_2Cl_2, 1:4) gave **195** as a yellow solid (450 mg, 63%); mp 187-189 °C; {lit.[164] mp 185-186 °C (ethyl acetate, hexane)}; ν_{max} (film) 3648 (O–H), 2973 (C–H), 2923 (C–H), 1732 (C=O); δ_H (400 MHz, MeOH-*d*$_4$) 7.83 (s, 1H), 8.80 (s, 1H); δ_C (101 MHz, MeOH-*d*$_4$) 122.1 (q, J = 33.3 Hz), 123.9, 122.7 (q, J = 273.0 Hz), 142.8, 147.9 (q, J = 5.9 Hz), 155.6, 164.9; δ_F (376 MHz, MeOH-*d*$_4$) -60.9; *m/z* (ESI^-) 223 ($[M(^{35}Cl)-H]^-$); HRMS (ESI^-) $C_7H_2ClF_3NO_2^-$ ($[M(^{35}Cl)-H]^-$) requires 223.9721; found 223.9724. Characterisation data are consistent with the literature.[164]

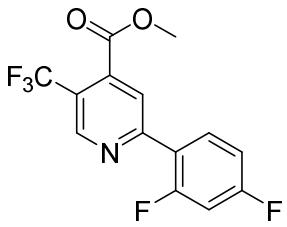
2-(2,4-difluorophenyl)-5-(trifluoromethyl)isonicotinic acid (196)



195 (500 mg, 2.22 mmol), (2,4-difluorophenyl)boronic acid (421 mg, 2.66 mmol) and Cs_2CO_3 (2.53 g, 7.77 mmol) were suspended in dioxane (6.7 mL) and H_2O (2.2 mL) under N_2 and the reaction was degassed for 10 min. $Pd(dppf)Cl_2.CH_2Cl_2$ (36 mg, 0.044 mmol) was added and the reaction was degassed for a further 10 min, before being heated at 100 °C for 4 h. The reaction was cooled to rt and concentrated *in vacuo*. The residue was partitioned between EtOAc and H_2O (1:1, 50 mL). The aqueous layer was acidified to pH 1-2 by addition of 1M HCl and it was extracted with EtOAc (2 × 20 mL). The combined

organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentane/CH_3OH, 50:50:1) gave **196** as a pale yellow solid (513 mg, 68%); mp 195-200 °C; ν_{max} (film) 3034 (O–H), 2974 (C–H), 2923 (C–H), 1731 (C=O); δ_H (400 MHz, MeOH-d_4) 7.09 – 7.21 (m, 2H), 8.00 – 8.21 (m, 2H), 9.07 (d, J = 1.1 Hz, 1H); δ_C (101 MHz, MeOH-d_4) 104.7 – 106.1 (t, J = 26.6 Hz), 113.3 (dd, J = 21.7, 3.6 Hz), 122.7 (q, J = 33.0 Hz), 123.0 (dd, J = 11.4, 3.9 Hz), 124.2 (d, J = 10.8 Hz), 124.4 (q, J = 272.6 Hz), 133.8 (dd, J = 10.1, 3.9 Hz), 142.2, 148.7 (q, J = 5.8 Hz), 158.0, 162.4 (dd, J = 253.3, 12.2 Hz), 165.7 (dd, J = 252.2, 12.4 Hz), 167.8; δ_F (377 MHz, MeOH-d_4) −113.4 (q, *J* = 10.2 Hz, 1F), −108.5 - −108.4 (m, 1F), −60.8 (s, 3F); *m/z* (ESI^-) 302 ($[M-H]^-$, 100%); HRMS (ESI^-) $C_{13}H_5F_5NO_2^-$ ($[M-H]^-$) requires 302.0235; found 302.0240. Characterisation data are consistent with the literature.[129]

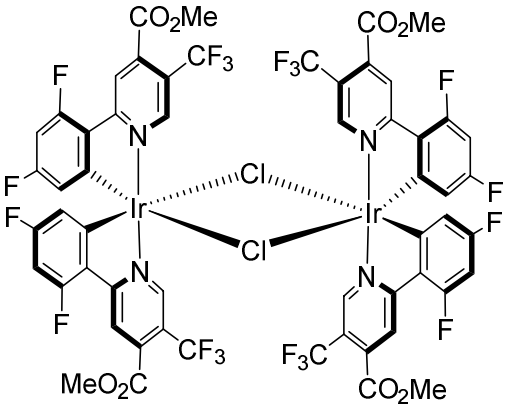
2-(2,4-Difluorophenyl)-5-(trifluoromethyl)isonicotinic acid (197)



Oxalyl chloride (688 μL, 8.25 mmol) was added to a suspension of **196** (500 mg, 1.65 mmol) and DMF (1 drop) in CH_2Cl_2 (8.25 mL) under N_2 and the reaction was stirred at rt for 1 h. CH_3OH (5 mL) was added and the reaction was stirred for an additional 1 h. The reaction was concentrated *in vacuo* and the residue was portioned between sat aq $NaHCO_3$ (10 mL) and EtOAc (10 mL). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent Et_2O/pentane, 1:19) gave **197** as a white crystalline solid (395 mg, 76%); mp 61-64 °C; ν_{max} (film) 2961 (C−H), 2900 (C−H), 1749 (C=O); δ_H (400 MHz, $CDCl_3$) 4.00 (s, 3H), 6.97 (ddd, J = 11.3, 8.6, 2.5 Hz, 1H), 7.06 (dddd, J = 8.7, 7.7, 2.5, 1.0 Hz, 1H), 8.02 – 8.20 (m, 2H), 9.06 (t, J

= 0.8 Hz, 1H); δ (151 MHz, $CDCl_3$) 53.6, 104.9 (t, J = 26.5 Hz), 112.6 (dd, J = 21.4, 3.5 Hz), 121.9 (q, *J* = 33.0 Hz), 121.8 (dd, *J* = 10.9, 3.8 Hz), 123.0 (q, *J* = 273.6 Hz), 123.4 (d, *J* = 11.5 Hz), 132.6 (dd, J = 10.0, 3.9 Hz), 139.3 (d, J = 2.3 Hz), 148.0 (q, J = 5.9 Hz), 156.8, 161.3 (dd, J = 254.6, 12.2 Hz), 164.4 (dd, J = 253.9, 12.4 Hz), 165.5; δ_F (376 MHz, $CDCl_3$) -111.3 (q, J = 9.8 Hz), -106.0 (ddd, J = 14.4, 9.2, 7.2 Hz), -59.6; *m/z* (ESI^+) 318 ($[M+H]^+$, 100%); HRMS (ESI^+) $C_{14}H_9F_5NO_2^+$ ($[M+H]^+$) requires 318.0548; found 318.0548.

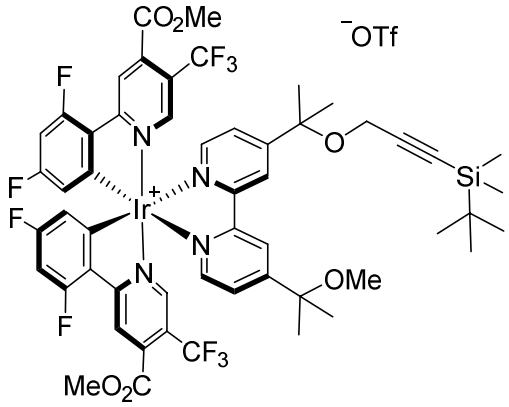
'Gen 1' dimethyl-ester Ir-dimer (198)



197 (444 mg, 1.40 mmol) and $IrCl_3.H_2O$ (316 mg, 600 μmol) were suspended in H_2O/2-ethoxyethanol (2:1, 12 mL, thoroughly degassed) under N_2 in a sealed microwave vial, and the reaction was heated at 135 °C for 16 h. The reaction was cooled to rt and the precipitate was isolated via vacuum filtration, washed with H_2O and dried under vacuum, to give **198** as a bright orange solid (420 mg, 82%); δ_H (400 MHz, $CDCl_3$) 4.11 (s, 6H), 5.03 (dd, J = 8.6, 2.3 Hz, 2H), 6.48 (ddd, J = 12.3, 8.8, 2.3 Hz, 2H), 8.73 (d, J = 3.6 Hz, 2H), 9.67 (s,

2H); δ_C (151 MHz, $CDCl_3$) 54.0, 99.7 (t, J = 26.7 Hz), 112.4 – 113.0 (m), 120.8, 122.0 (d, J = 34.9 Hz), 122.6, 123.4 (d, J = 20.4 Hz), 124.4, 126.1, 140.4, 148.1 (d, J = 7.7 Hz), 148.8, 161.1 (d, J = 13.6 Hz), 162.9 (d, J = 13.1 Hz), 163.4 (d, J = 13.3 Hz), 163.6, 165.1 (d, J = 13.2 Hz), 169.7 (d, J = 6.2 Hz); δ_F (377 MHz, $CDCl_3$) -106.4 (td, J = 12.4, 4.0 Hz, 4F), -101.9 – -100.9 (m, 4F), -59.5 (s, 12F); *m/z* (ESI^+) 825 ($[M/2\text{-}Cl]^+$, 100%); HRMS (ESI^+) $C_{56}H_{28}Cl_2F_{20}Ir_2N_4NaO_8^+$ ($[M+Na]^+$) requires 1743.0099; found 1743.0071.

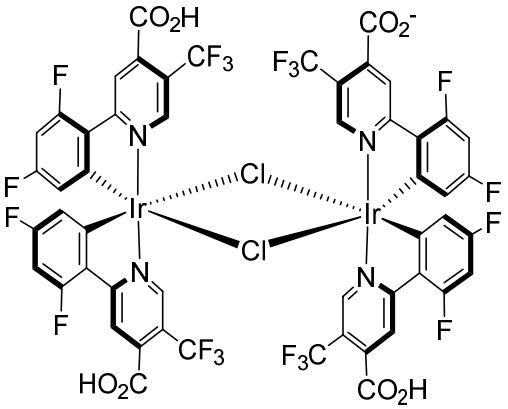
TBS-protected 'Gen 1' dimethyl-ester Ir-photocatalyst (199)



198 (130 mg, 76.0 μmol) and **193** (68.0 mg, 154 μmol) were dissolved in CH_3OH/CH_2Cl_2 (1:1, 7.6 mL, thoroughly degassed) under N_2 and the reaction was heated at 75 °C for 16 h. The reaction was cooled to rt and AgOTf (130 mg) was added and the reaction was stirred at rt for 30 min. The precipitate (AgCl) was removed via vacuum filtration and the filtrate was concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→5% CH_3OH in CH_2Cl_2) gave **199** as a bright yellow solid (155 mg, 81%); δ_H

(400 MHz, MeOH-d_4) 0.00 (d, J = 1.0 Hz, 9H), 0.87 (s, 6H), 1.57 – 1.74 (m, 12H), 3.24 (s, 3H), 4.00 (s, 6H), 4.15 (d, J = 1.3 Hz, 2H), 5.88 (ddd, J = 8.2, 5.0, 2.3 Hz, 2H), 6.85 (ddt, J = 12.7, 9.1, 2.3 Hz, 2H), 7.59 – 7.70 (m, 2H), 7.81 (ddd, J = 6.8, 4.5, 1.8 Hz, 1H), 7.88 (dd, J = 5.9, 1.8 Hz, 1H), 8.07 (dd, J = 5.8, 4.6 Hz, 2H), 8.70 – 8.76 (m, 2H), 8.77 – 8.87 (m, 2H); δ_F (377 MHz, MeOH-d_4) -107.4 (dtd, J = 21.3, 12.8, 3.7 Hz), -102.8 (ddt, J = 53.8, 13.1, 8.7 Hz), -80.0, -61.6 (d, J = 2.5 Hz); *m/z* (ESI^+) 1263 ($[M-H]^+$, 100%); HRMS (ESI^+) $C_{54}H_{52}F_{10}IrN_4O_6Si^+$ ($[M-OTf]^+$) requires 1263.3123; found 1263.3065.

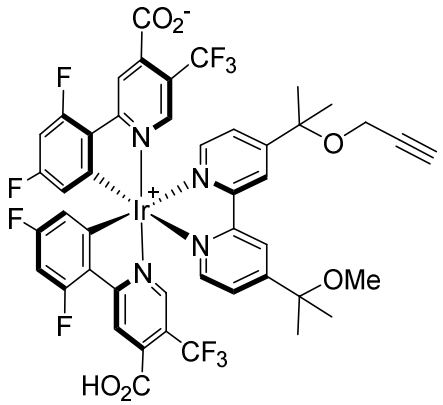
'Gen 1' Ir-dimer (201)



196 (424 mg, 1.40 mmol) and $IrCl_3.H_2O$ (190 mg, 600 μmol) were suspended in H_2O/2-ethoxyethanol (2:1, 12 mL, thoroughly degassed) under N_2 in a sealed microwave vial, and the reaction was heated t 135 °C for 16 h. The reaction was cooled to rt, and diluted with satd aq NH_4Cl (12 mL). The reaction was extracted with EtOAc (3 × 15 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in Et_2O (10 mL) and cooled to −20 °C for 2 h. The

precipitate was isolated via vacuum filtration, washed with ice-cold Et_2O and dried under vacuum, to give **201** as a bright orange solid (226 mg, 45%); δ_H (400 MHz, MeOH-*d*$_4$) 5.54 (d, *J* = 8.6 Hz, 2H), 5.71 (d, *J* = 8.3 Hz, 2H), 6.45 – 6.76 (m, 4H), 8.62 (d, *J* = 2.7 Hz, 4H), 9.20 (s, 2H), 10.31 (s, 2H); δ_F (377 MHz, MeOH-*d*$_4$) -110.3 (2F), -109.2 (2F), -105.9 (2F), -105.1 (2F), -60.9 (6F), -60.9 (6F); *m/z* (ESI^+) 797 ($[M/2\text{-}Cl]^+$, 100%). Characterisation data are consistent with the literature.[129]

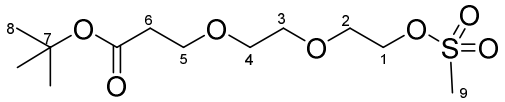
'Gen 1' Ir-photocatalyst (200)



(1) 201 (83.0 mg, 50.0 μmol) and **193** (44.0 mg, 100 μmol) were dissolved in degassed CH_3OH/CH_2Cl_2 (1:1, 5 mL) under N_2 and the reaction was heated at 75 °C for 16 h. The reaction was cooled to rt and AgOTf (100 mg) was added and the reaction was stirred at rt for 15 min. The precipitate was removed via vacuum filtration and the filtrate was concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→30% CH_3OH in CH_2Cl_2) gave **202** as a bright yellow solid (123 mg, 89%). **(2) Silyl-deprotection.** TBAF (1M in THF, 178 μL, 0.178 mmol) was added to a suspension of **202** (123 mg, 0.089 mmol) in MeCN (9 mL) under N_2 at rt. The reaction was stirred at rt for 16 h, after which time the reaction had become a homogenous dark brown solution, and the reaction was quenched by addition of $CaCO_3$ (100 mg) and filtered through an Isolute® SCX-2 cartridge (strong cation exchange column) and concentrated *in vacuo*. Purification

via successive column chromatography (normal phase, gradient elution, 20%→80% CH_3OH in CH_2Cl_2); then (reversed phase, gradient elution, 0%→30% MeCN in H_2O) gave **200** as a bright yellow solid (40 mg, 71%); δ_H (400 MHz, MeOH-*d*$_4$) 1.63 (d, *J* = 2.5 Hz, 6H), 1.68 (s, 6H), 2.84 (t, *J* = 2.5 Hz, 1H), 3.23 (s, 3H), 4.10 (d, *J* = 2.5 Hz, 2H), 5.85 (dd, *J* = 6.5, 4.2 Hz, 2H), 6.71 – 6.85 (m, 2H), 7.51 (d, *J* = 12.7 Hz, 2H), 7.75 – 7.84 (m, 2H), 8.03 – 8.12 (m, 2H), 8.39 (d, *J* = 2.7 Hz, 2H), 8.75 – 8.88 (m, 2H); δ_F (377 MHz, MeOH-*d*$_4$) -108.44 – -108.30 (m, 2F), -104.82 – -104.63 (m, 2F), -62.10 (3F), -62.07 (3F); *m/z* (ESI$^+$) 1121 ([M+H]$^+$, 100%); HRMS $C_{46}H_{34}F_{10}IrN_4O_6^+$ ([M+H]$^+$) requires 1121.1946; found 1121.192. Characterisation data are consistent with the literature.[129]

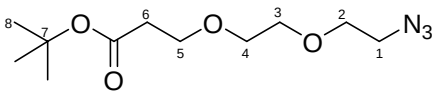
***tert*-Butyl 3-(2-(2-((methylsulfonyl)oxy)ethoxy)ethoxy)propanoate (204)**



Methanesulfonyl chloride (140 µL, 1.81 mmol) was added to a solution of *tert*-Butyl 3-(2-(2-hydroxyethoxy)ethoxy)propanoate (350 mg, 1.50 mmol) and Et_3N (630 µL, 4.52 mmol) in anhydrous CH_2Cl_2 (15 mL) at 0 °C. The reaction was allowed to warm to rt over 16 h. The reaction was quenched by addition of sadt aq $NaHCO_3$ (15 mL) and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give **204** as a pale yellow oil that was used without further purification (455 mg, 97%); ν_{max} (film) 2932 (C−H), 2874 (C−H), 1727 (C=O), 1352 (S=O), 1173 (S=O); δ_H (400 MHz, $CDCl_3$) 1.45 (s, 9H, 3 × C(8)*H*$_3$), 2.48 (t, J = 6.5 Hz, 2H, C(6)*H*$_2$), 3.07 (s, 3H, C(9)*H*$_3$), 3.58 – 3.63 (m, 2H, C(4)*H*$_2$), 3.63 – 3.67 (m, 2H, C(3)*H*$_2$), 3.70 (t, J = 6.4 Hz, 2H, C(5)*H*$_2$), 3.73 – 3.79 (m, 2H, C(2)*H*$_2$), 4.32 – 4.41 (m, 2H, C(1)*H*$_2$); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × *C*(8)), 36.4 (*C*(6)), 37.9 (*C*(9)), 67.1 (*C*(5)), 69.2 (*C*(1)), 69.4 (*C*(2)), 70.5 (*C*(4)), 70.8

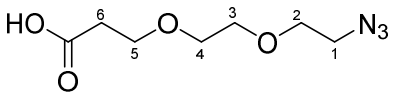
(*C*(3)), 80.8 (*C*(7)), 171.0 (*C*=O); HMRS (ESI$^+$) $C_{12}H_{24}NaO_7S^+$ ([M+Na]$^+$) requires 335.1135; found 335.1134.

***tert*-Butyl 3-(2-(2-azidoethoxy)ethoxy)propanoate (205)**



NaN_3 (105 mg, 1.60 mmol) and **204** (455 mg, 1.46 mmol) were suspended in DMA (5.7 mL) and the reaction was heated at 80 °C for 4 h. The reaction was cooled to rt and diluted with H_2O (15 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with ½ sat brine (5 × 5 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo* to give **205** as a colourless oil (370 mg, 98%); ν_{max} (film) 2925 (C−H), 2870 (C−H), 2105 (N=N=N), 1729 (C=O); δ_H (400 MHz, $CDCl_3$) 1.38 (s, 9H, 3 × C(8)H_3), 2.44 (t, J = 6.5 Hz, 2H, C(6)H_2), 3.32 (t, J = 5.1 Hz, 2H, C(1)H_2), 3.54 – 3.59 (m, 4H, C(3)H_2, C(4)H_2), 3.60 (dd, J = 5.7, 4.6 Hz, 2H, C(2)H_2), 3.66 (t, J = 6.5 Hz, 2H, C(5)H_2); δ_C (101 MHz, $CDCl_3$) 28.2 (3 × *C*(8)), 36.4 (*C*(6)), 50.8 (*C*(1)), 67.1 (*C*(5)), 70.2 (*C*(3)), 70.6 (*C*(4)), 70.8 (*C*(2)), 80.7 (*C*(7)), 171.0 (*C*=O); HRMS (ESI$^+$) $C_{11}H_{21}N_3NaO_4^+$ ([M+Na]$^+$) requires 282.1424; found 282.1422.

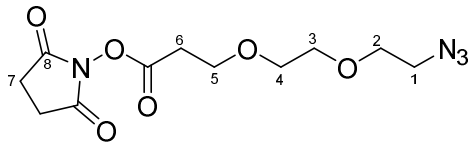
3-(2-(2-Azidoethoxy)ethoxy)propanoic acid (206)



F_3CCO_2H (2.80 mL) was added dropwise to a solution of **205** (370 mg, 1.43 mmol) in CH_2Cl_2 (26 mL). The reaction was stirred at rt for 2 h and then concentrated *in vacuo* and the residue was azeotroped with PhMe (2 × 15 mL). Purification via column chromatography (gradient elution, 0%→5% CH_3OH in CH_2Cl_2) gave **206** as a colourless oil (241 mg, 83%); ν_{max} (film) 3085 (O−H), 2923 (C−H), 2884 (C−H), 2105 (N=N=N),

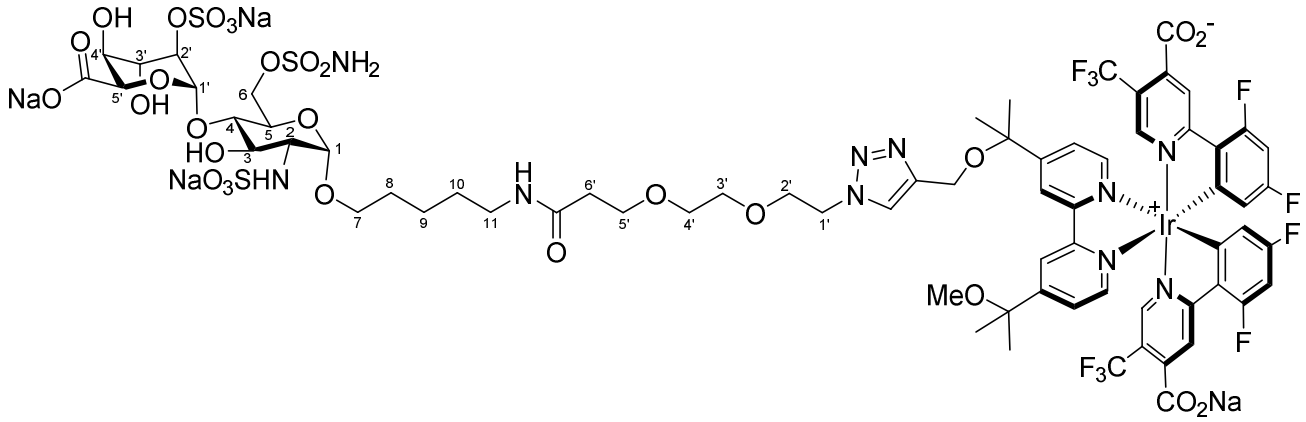
1727 (C=O); δ_H (400 MHz, $CDCl_3$) 2.70 (t, *J* = 6.1 Hz, 2H, C(6)H_2), 3.41 (t, *J* = 5.0 Hz, 2H, C(1)H_2), 3.64 – 3.71 (m, 6H, C(3)H_2, C(4)H_2, C(5)H_2), 3.82 (t, *J* = 6.1 Hz, 2H, C(2)H_2); δ_C (101 MHz, $CDCl_3$) 34.7 (*C*(6)), 50.8 (*C*(1)), 66.4 (*C*(5)), 70.2 (*C*(3)), 70.5 (*C*(4)), 70.6 (*C*(5)), 176.9 (*C*=O); *m/z* (ESI^-) 202 ($[M-H]^-$, 100%); HRMS (ESI^+) $C_7H_{13}N_3NaO_4^+$ ($[M+Na]^+$) requires 226.0798; found 226.0798.

3-(2-(2-Azidoethoxy)ethoxy)propanoic acid (207)



N-Hydroxysuccinimide (163 mg, 1.42 mmol) and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC.HCl) (273 mg, 1.42 mmol) were added to a solution of **206** (240 mg, 1.18 mmol) in CH_2Cl_2 (6 mL) under N_2. The reaction was stirred at rt for 16 h. The reaction was washed with satd aq $NaHCO_3$ (15 mL), satd aq NH_4Cl (15 mL), brine dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→100% EtOAc in pentane) gave **207** as a colourless oil (120 mg, 32%); ν_{max} (film) 2921 (C−H), 2874 (C−H), 2108 (N=N=N), 1815 (C=O), 1783 (C=O), 1737 (C=O); δ_H (400 MHz, $CDCl_3$) 2.8 (s, 4H, 2 × C(7)H_2), 2.8 (t, *J* = 6.3 Hz, 2H, C(6)H_2), 3.3 (t, *J* = 5.1 Hz, 2H, C(1)H_2), 3.6 – 3.6 (m, 6H, C(2)H_2, C(3)H_2, C(4)H_2), 3.8 (t, *J* = 6.3 Hz, 2H, C(5)H_2); δ_C (101 MHz, $CDCl_3$) 25.7 (*C*(7)), 32.4 (*C*(6)), 50.9 (*C*(1)), 65.9 (*C*(5)), 70.2 (*C*(3)), 70.7 (*C*(4)), 71.0 (*C*(2)), 166.9 (*C*=O), 169.1 (*C*(8)); *m/z* (ESI^+) 323 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{11}H_{16}N_4NaO_6^+$ ($[M+Na]^+$) requires 323.0962; found 323.0961.

IdoA(2S)-GlcNS(6Sulfamate) Gen1-Ir PC catalyst (208)

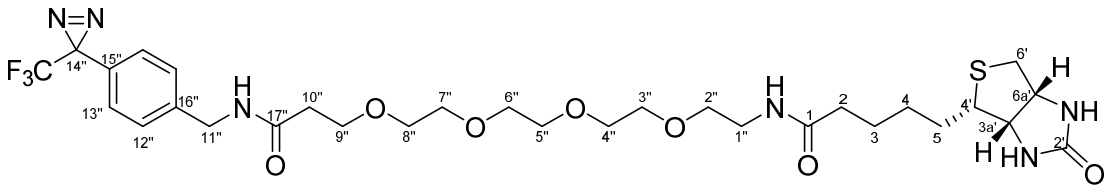


(1) Amide coupling. 163 (10.0 mg, 27.0 μmol), **207** (33.0 mg, 109 μmol) and Na_2CO_3 (19.0 mg, 181 μmol) were suspended in DMF (500 μL) and the reaction was heated at 50 °C for 1 h under microwave irradiation. Purification via reversed phase column chromatography (gradient elution, 0%→20% MeCN in H_2O) gave intermediate azide as a colourless solid (28 mg, 80%). **(2) Click reaction.** Intermediate azide (9.00 mg, 10.0 μmol), **200** (10.0 mg, 10.0 μmol) and DIPEA (16.0 μL, 100 μmol) were suspended in MeCN (0.2 mL). A freshly prepared solution of $CuSO_4$ (1.40 mg, 5.00 μmol) and sodium ascorbate (3.30 mg, 20.0 μmol) in H_2O (0.3 mL) was added to the reaction. This reaction mixture was stirred at room temperature for 16 hours. Purification via reversed phase chromatography (eluent, H_2O), followed by elution through a DOWEX 50WX4 Na^+-form column, gave **208** as a bright yellow solid as a mixture of diastereomers (7 mg, 34%, purity 93%[xiii]); δ_H (400 MHz, D_2O) 1.19 – 1.32 (m, 2H), 1.32 – 1.42 (m, 2H), 1.45 – 1.56 (m, 2H), 1.63 (dd, J = 11.4, 3.6 Hz, 8H), 1.72 (d, J = 2.8 Hz, 5H), 2.41 (t, J = 6.0 Hz, 2H), 3.04 (t, J = 6.9 Hz, 2H), 3.17 (s, 3H), 3.26 (dd, J = 9.7, 3.7 Hz, 1H), 3.40 – 3.78 (m, 10H), 3.89 (q, J = 5.4 Hz, 2H), 3.94 – 4.04 (m, 3H), 4.23 (s, 1H), 4.42 (s, 2H), 4.48 (s, 2H), 4.56 (t, J = 5.0 Hz, 2H), 4.71 (d, J = 2.8 Hz, 1H), 5.07 – 5.16 (m, 2H), 5.93 (td, J = 8.4, 2.3 Hz, 2H),

[xiii] Data for purity analysis in appendix

6.68 – 6.82 (m, 2H), 7.58 (d, J = 9.5 Hz, 2H), 7.61 – 7.72 (m, 2H), 8.01 (s, 1H), 8.13 (t, J = 6.5 Hz, 2H), 8.35 (dd, J = 6.4, 3.1 Hz, 2H), 8.59 (s, 1H), 8.73 (s, 1H); δ_C (151 MHz, D_2O) 22.7, 26.2, 26.2, 26.6, 26.6, 26.9, 26.9, 28.0, 28.2, 29.5, 29.6, 29.9, 30.0, 30.1, 30.1, 30.3, 36.1, 39.2, 50.0, 50.8, 56.9, 57.8, 66.8, 68.4, 68.4, 68.5, 68.5, 68.6, 68.8, 69.3, 69.4, 69.7, 69.7, 69.9, 72.1, 75.2, 75.3, 77.5, 78.0, 78.2, 97.2, 99.1, 99.3, 99.3, 99.5, 99.7, 114.1, 114.1, 114.1, 114.1, 114.2, 114.2, 114.2, 114.2, 114.2, 114.3, 114.3, 119.0, 120.1, 120.2, 120.3, 120.3, 120.6, 120.8, 120.8, 122.1, 122.7, 125.1, 125.4, 125.8, 125.9, 125.9, 126.0, 126.7, 127.7, 128.6, 129.2, 144.1, 146.2, 146.3, 146.3, 146.3, 146.4, 148.9, 148.9, 148.9, 148.9, 149.0, 149.0, 151.5, 151.6, 154.4, 154.4, 154.5, 154.6, 155.7, 155.8, 159.6, 159.7, 159.8, 160.3, 161.4, 161.5, 163.2, 163.3, 163.7, 163.7, 163.7, 163.8, 165.4, 165.4, 165.5, 167.7, 167.7, 171.5, 171.5, 173.5, 173.5, 176.1; δ_F (565 MHz, D_2O) -106.88 (dq, J = 25.3, 12.8 Hz, 2 × 3F), -104.04 (dq, J = 51.3, 10.6 Hz, 2 × 1F), -61.99 – -60.05 (m, 2 × 1F); HRMS (ESI^-) $C_{70}H_{76}F_{10}IrN_{10}O_{28}S_3^-$ ($[M-4Na+3H]^-$) requires 1983.3470; found 1983.3494.

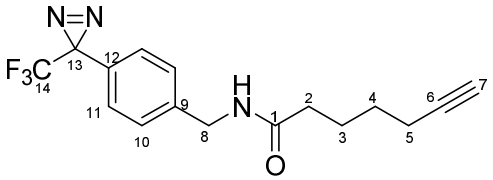
Diazirine-PEG4-Biotin (211)



DIPEA (32.0 μL, 180 μmol) was added to a solution of 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzenemethanamine hydrochloride (35.0 mg, 140 μmol) and Biotin-PEG4-NHS ester (50.0 mg, 85.0 μmol) in CH_2Cl_2 (500 μL) and the reaction was stirred at rt for 16 h in the dark. The reaction was concentrated *in vacuo*. Purification via column chromatography (eluent, EtOAc/(2M NH_3 in CH_3OH) 85:15, then EtOAc/CH_3OH 2:3) gave **211** as a viscous white oil (45 mg, 77%); ν_{max} (film) 3275 (N−H), 2980 (C−H), 2971 (C−H), 2917 (C−H), 2881 (C−H), 2872 (C−H), 1696 (C=O), 1650 (C=O), 1550 (N=N); δ_H (400 MHz, MeOH-

d$_4$) 1.40 –1.77 (m, 6H, C(3)H_2, C(4)H_2, C(5)H_2), 2.21 (t, J = 7.3 Hz, 2H, C(2)H_2), 2.51 (t, J = 6.0 Hz, 2H, C(10'')H_2), 2.70 (d, J = 12.8 Hz, 1H, C(6')H_A), 2.92 (dd, J = 12.7, 5.0 Hz, 1H, C(6')H_B), 3.20 (ddd, J = 8.9, 5.9, 4.5 Hz, 1H, C(4')*H*), 3.32 – 3.39 (m, 2H, C(1'')H_2), 3.53 (t, J = 5.5 Hz, 2H, C(2'')H_2), 3.61 (t, J = 2.5 Hz, 12H, C(3'')H_2, C(4'')H_2, C(5'')H_2, C(6'')H_2, C(7'')H_2, C(8'')H_2), 3.76 (t, J = 6.0 Hz, 2H, C(9'')H_2), 4.30 (dd, J = 7.9, 4.4 Hz, 1H, C(3a')*H*), 4.40 – 4.45 (m, 2H, C(11'')H_2), 4.49 (ddd, J = 7.9, 5.0, 0.9 Hz, 1H, C(6a')*H*), 7.15 – 7.25 (m, 2H, C(12'')H_2), 7.36 – 7.43 (m, 2H, C(13'')H_2), 7.96 – 8.07 (m, 1H, N*H*), 8.40 – 8.53 (m, 1H, N*H*); δ_C (151 MHz, MeOH-*d*$_4$) 26.8 (*C*(3)), 29.4 (q, J = 40.3 Hz, *C*(14)), 29.5 (*C*(4)), 29.8 (*C*(5)), 36.7 (*C*(2)), 37.7 (*C*(10'')), 40.3 (*C*(6')), 41.0 (*C*(1'')), 43.5 (*C*(11'')), 57.0 (*C*(4')), 61.6 (*C*(6a')), 63.4 (*C*(3a')), 68.3 (*C*(9'')), 70.6, 71.2, 71.3, 71.5, 71.5, 71.5, 71.6 (*C*(2''), *C*(3''), *C*(4''), *C*(5''), *C*(6''), *C*(7''), *C*(8'')), 123.6 (q, J = 273.9 Hz, *C*F_3), 127.7 (*C*(12'')), 128.6 (*C*(15'')), 129.2 (*C*(13'')), 142.6 (*C*(16'')), 166.1 (*C*(2')), 174.1 (*C*(17'')), 176.1 (*C*(1)); δ_F (377 MHz, MeOH-*d*$_4$) -67.10 (3F); *m/z* (ESI$^+$) 689 ([M+H]$^+$, 29%), 711 ([M+Na]$^+$, 100%); HRMS (ESI$^+$) $C_{30}H_{44}F_3N_6O_7S^+$ ([M+H]$^+$) requires 689.2939; found 689.2939.

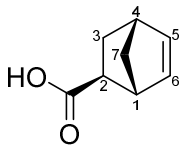
_N_-(4-(3-(trifluoromethyl)-3_H_-diazirin-3-yl)benzyl)hept-6-ynamide (213)



HATU (160 mg, 420 μmol) was added to a solution of 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzenemethanamine hydrochloride (75.0 mg, 300 μmol), 6-heptynoic acid (40.0 μL, 300 μmol) and DIPEA (150 μL, 900 μmol) in CH_2Cl_2 (1.5 mL) and the reaction was stirred at rt for 16 h in the dark. The reaction was diluted with CH_2Cl_2 (5 mL) and quenched by addition of satd aq $NaHCO_3$ (5 mL). The organic layer was separated, washed with satd aq

NH_4Cl (5 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentane, 3:7) gave **213** as a white solid (74 mg, 78%); ν_{max} (film) 3307 (C≡C–H), 2944 (C–H), 2866 (C–H), 1684 (C=O), 1457 (N=N); mp 63-65 °C; δ_H (400 MHz, $CDCl_3$) δ 1.53 – 1.61 (m, 2H, C(3)H_2), 1.73 – 1.84 (m, 2H, C(4)H_2), 1.94 (t, J = 2.6 Hz, 1H, C(7)*H*), 2.17 – 2.28 (m, 4H, C(2)H_2, C(5)H_2), 4.45 (d, J = 5.9 Hz, 2H, C(8)H_2), 5.76 (s, 1H, N*H*), 7.12 – 7.20 (m, 2H, 2 × C(10)*H*), 7.28 – 7.35 (m, 2H, 2 × C(11)*H*); δ_C (151 MHz, $CDCl_3$) 18.3 (*C*(5)), 24.8 (*C*(3)), 28.1 (*C*(4)), 28.5 (q, J = 40.2 Hz, *C*(13)), 36.2 (*C*(2)), 43.1 (*C*(8)), 68.8 (*C*(7)), 84.1 (*C*(6)), 122.2 (q, J = 274.8 Hz, *C*(14)), 127.0 (2 × *C*(10)), 128.3 (2 × *C*(11)), 128.5 (*C*(12)), 140.4 (*C*(9)), 172.7 (*C*(1)); δ_F (376 MHz, $CDCl_3$) -65.30 (3F); *m/z* (ESI^+) 346 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{16}H_{16}F_3N_3NaO^+$ ($[M+H]^+$) requires 346.1318; found 346.1319.

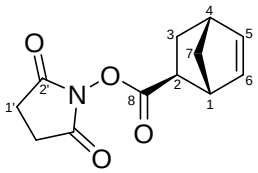
(±)-*Exo*-norbornene carboxylic acid (216)



A solution of KI (8.90 g, 53.4 mmol) and I_2 (4.50 g, 17.8 mmol) in H_2O (25 mL) was added dropwise to a solution of norbornene carboxylic acid (2.50 mL, 20.4 mmol, commercially available racemic exo/endo, ~1:4) in aq $NaHCO_3$ (0.75 M, 30 mL). The reaction was extracted with Et_2O (3 × 10 mL) and these organic layers were discarded. The aqueous layer was quenched by addition of aq $Na_2S_2O_3$ (10 w/v%, 10 mL) and acidified to pH 2 by addition pf 1N H_2SO_4, then extracted with Et_2O (5 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give racemic **216** as a pale yellow solid (520 mg, 18%, exo/endo 92:8); mp 40-42 °C; ν_{max} (film) 3062 (C=H), 2945 (C–H), 2874 (C–H), 1700 (C=O); δ_H (400 MHz, $CDCl_3$) 1.36 – 1.42 (m, 2H, C(5)H_A, C(7)H_A), 1.51 – 1.57 (m, 1H, C(7)H_B), 1.96 (ddd, J = 11.9, 4.6, 3.5 Hz, 1H, C(5)H_B), 2.24

– 2.30 (m, 1H, C(2)*H*), 2.95 (br s, 1H, C(4)*H*), 3.11 (s, 1H, C(1)*H*), 6.12 (dd, J = 5.7, 3.0 Hz, 1H, C(6)*H*), 6.16 (dd, J = 5.7, 2.9 Hz, 1H, C(5)*H*); δ_C (101 MHz, $CDCl_3$) 30.5 (*C*(5)), 41.8 (*C*(4)), 43.1 (*C*(2)), 46.6 (*C*(7)), 46.8 (*C*(1)), 135.9 (*C*(6)), 138.3 (*C*(5)), 181.7 (*C*OOH); *m/z* (ESI^-) 137 ($[M-H]^-$, 100%); HRMS (ESI^-) $C_8H_9O_2^-$ ($[M-H]^-$) requires 137.0608; found 137.0596. Analytical data were consistent with data reported in the literature.[165]

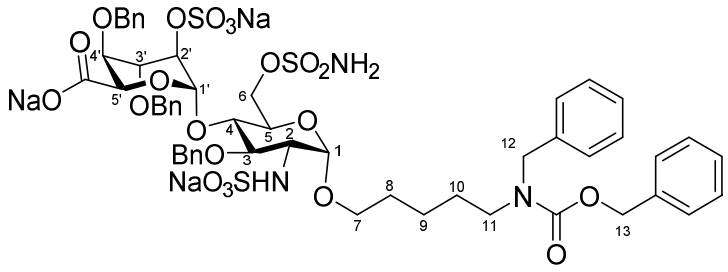
(±)-*Exo N*-Hydroxysuccinimide 5-norbornene-2-carboxylate (217)



N-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC.HCl) (904 mg, 4.71 mmol) and *N*-hydroxysuccinimide (542 mg, 4.71 mmol) were added in quick succession to a solution of **216** (520 mg, 3.77 mmol) in MeCN (12.5 mL) at rt and the reaction was stirred for 16 h. The reaction was concentrated *in vacuo* and the residue was partitioned between EtOAc and H_2O (1:1, 25 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with satd aq NH_4Cl (2 × 10 mL), satd aq $NaHCO_3$ (2 × 10 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentane, 1:4) gave racemic **217** as a crystalline solid (753 mg, 85%); mp 93-95 °C; ν_{max} (film) 3064 (C=C), 2947 (C–H), 2875 (C–H), 1811 (C=O), 1780 (C=O), 1736 (C=O); δ_H (400 MHz, $CDCl_3$) 1.42 – 1.49 (m, 1H, C(7)H_A), 1.49 – 1.55 (m, 2H, C(3)H_A, C(7)H_B), 2.05 (ddd, J = 11.9, 4.6, 3.6 Hz, 1H, C(3)H_B), 2.51 (ddd, J = 9.0, 4.5, 1.5 Hz, 1H, C(2)*H*), 2.83 (s, 4H, 2 × C(1')H_2), 3.00 (br s, 1H, C(4)*H*), 3.28 (br s, 1H, C(1)*H*), 6.15 (dd, J = 5.7, 3.0 Hz, 1H, C(6)*H*), 6.21 (dd, J = 5.7, 3.0 Hz, 1H, C(5)*H*); δ_C (101 MHz, $CDCl_3$) 25.8 (*C*(1')), 31.1 (*C*(3)), 40.5 (*C*(2)), 42.0 (*C*(4)), 46.6 (*C*(7)), 47.3 (*C*(1)), 135.4 (*C*(6)), 138.7

(*C*(7)), 169.4 (*C*(2')), 171.8 (*C*(8)); *m/z* (ESI$^+$) 258 ([M+Na]$^+$, 43%), 493 ([2M+Na]$^+$). Analytical data were consistent with data reported in the literature.[165]

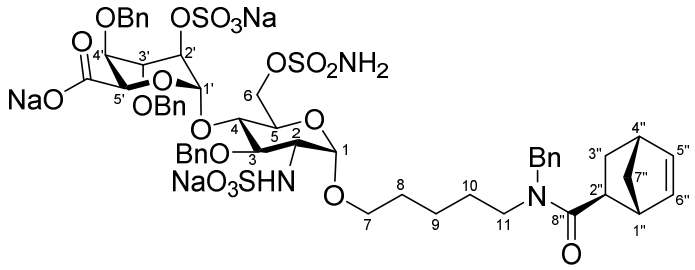
(2*R*,3*S*,4*S*,5*R*,6*R*)-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-6-((5-(benzyl((benzyloxy)carbonyl)amino)pentyl)oxy)-4-(benzyloxy)-2-((sulfamoyloxy)methyl)-5-(sulfoamino)tetrahydro-2*H*-pyran-3-yl)oxy)-3,4-bis(benzyloxy)-5-(sulfooxy)tetrahydro-2*H*-pyran-2-carboxylic acid (220)



(1) Staudinger reduction. PMe_3 (1M in THF, 1.53 mL, 1.53 mmol) was added to a solution of **160** (810 mg, 764 μmol) in THF (64 mL) and 0.1 M NaOH (6.4 mL) under N_2. The reaction was stirred at rt for 16 h. The reaction was neutralised by addition of CH_3COOH and concentrated *in vacuo*. Purification via column chromatography (eluent, EtOAc/IPA/H_2O, 82:16:2) gave the intermediate amine as a colourless oil. **(2) *O,N*-disulfation.** SO_3.Py (787 mg, 4.35 mmol) and Et_3N (2.25 mL) were added to a solution of the above intermediate (450 mg, 435 μmol) in pyridine (11.25 mL) and the reaction was stirred at rt until the reagents were fully dissolved (ca. 5 min). The reaction was then heated at 100 °C under microwave irradiation for 15 min. The reaction was cooled to rt, quenched by addition of CH_3OH (5 mL) and stirred at rt for 15 min, then concentrated *in vacuo*. Purification via reversed phase column chromatography (gradient elution, 0%→40% CH_3CN in H_2O), followed by elution through a DOWEX 50WX4 Na^+-form column, gave **220** as a pale yellow solid (480 mg, 67% over 2 steps); mp 169-177 °C (dec.); $[\alpha]_D^{25}$ –2.4

(*c* 1.0 in CH_3OH); ν_{max} (film) 3471 (O−H), 3063 (C−H), 3033 (C−H), 2942 (C−H), 2874 (C−H), 1612 (C=O), 1366 (S=O), 1228 (S=O); δ_H (600 MHz, MeOH-*d*$_4$) 1.29 – 1.44 (m, 2H, C(9)H_2), 1.44 – 1.73 (m, 4H, C(8)H_2, C(10)H_2), 3.21 – 3.29 (m, 2H, C(11)H_2), 3.36 – 3.54 (m, 3H, C(2)*H*, C(7)H_A, C(4)*H*), 3.59 – 3.76 (m, 1H, C(7)H_B), 3.77 – 3.83 (m, 2H, C(3)*H*, C(4')*H*), 3.85 – 3.97 (m, 1H, C(5)*H*), 4.19 (t, J = 2.5 Hz, 1H, C(3')*H*), 4.35 (d appt, J = 11.3 Hz, 2H, C(6)H_A, C(4')OCH_APh), 4.42 – 4.50 (m, 1H, C(6)H_B), 4.50 – 4.56 (m, 3H, C(12)H_2, C(3)OCH_APh), 4.59 (d, J = 11.4 Hz, 1H, C(3')OCH_APh), 4.60 – 4.66 (m, 2H, C(2')*H*, C(4')OCH_BPh), 4.79 (d, J = 11.3 Hz, 2H, C(3)OCH_BPh, C(3')OCH_BPh), 4.92 (s, 1H, C(5')*H*), 5.10 – 5.20 (m, 3H, C(1)*H*, C(13)H_2), 5.25 (s, 1H, C(1')*H*), 7.10 – 7.44 (m, 25H, 5 × *Ph*); δ_C (151 MHz, MeOH-*d*$_4$) 24.5 (*C*(9)), 24.6 (*C*(9)), 28.6 (*C*(10)), 29.1 (*C*(10)), 30.2 (*C*(8)), 30.3 (*C*(8)), 47.7 (*C*(11)), 51.4 (*C*(12)), 51.6 (*C*(12)), 59.8 (*C*(2)), 68.4 (*C*(13)), 68.5 (*C*(13)), 68.8 (*C*(5')), 69.3 (*C*(7)), 69.6 (*C*(6)), 71.0 (*C*(5)), 71.3 (*C*(2')), 72.2 (*C*(3')), 72.9, 73.0 (*C*(3')OCH_2Ph and *C*(4')OCH_2Ph), 75.0 (*C*(4')), 76.0 (*C*(3)), 76.4 (*C*(3)OCH_2Ph), 79.3 (*C*(4)), 99.0 (*C*(1)), 99.9 (*C*(1')), 128.3 (*Ar*), 128.4 (*Ar*), 128.7 (*Ar*), 128.8 (*Ar*), 129.0 (*Ar*), 129.2 (*Ar*), 129.4 (*Ar*), 129.5 (*Ar*), 129.6 (*Ar*), 130.0 (*Ar*), 138.1 (*i-Ph*), 138.9 (*i-Ph*), 139.3 (*i-Ph*), 139.4 (*i-Ph*), 158.0 (C_{Cbz}=O), 158.5 (C_{Cbz}=O), 173.3 (C_{Acid}=O); *m/z* (ESI$^+$) 1174 (48%, $[M+H]^+$), 1196 (41%, $[M+Na]^+$), 1218 (99%, $[M-H+2Na]^+$), 1240 (10%, $[M-2H+3Na]^+$), 1256 (12%, $[M-3H+4Na]^+$); HRMS (ESI$^-$) $C_{53}H_{62}N_3O_{21}S_3^-$ ($[M-3Na+2H]^-$) requires 1172.3043; 1172.3041.

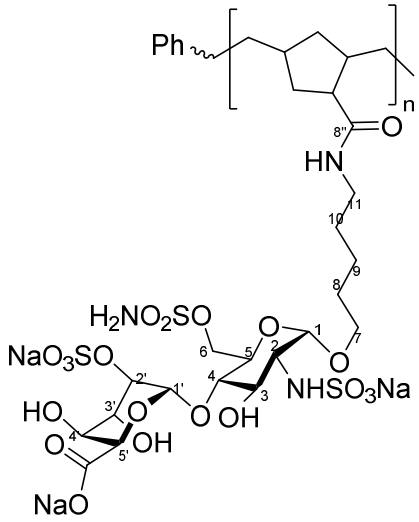
Sodium (2*R*,3*S*,4*S*,5*R*,6*R*)-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-6-((5-((1*R*,2*S*,4*R*)-N-benzylbicyclo[2.2.1]hept-5-ene-2-carboxamido)pentyl)oxy)-4-(benzyloxy)-2-((sulfamoyloxy)methyl)-5-(sulfonatoamino)tetrahydro-2*H*-pyran-3-yl)oxy)-3,4-bis(benzyloxy)-5-(sulfonatooxy)tetrahydro-2*H*-pyran-2-carboxylate (221)



(1) *N*-Cbz deprotection. Pd/C (10 wt. % loading, 6 mg) was added to a solution of **220** (62 mg, 0.048 mmol) in CH_3OH (1 mL) under N_2 and the reaction was degassed with N_2 for 5 min before being placed under an atmosphere of H_2. The reaction was stirred at rt for 45 min, then filtered through Celite® and concentrated *in vacuo*. **(2) Amide coupling.** *N*-methylmorpholine (12.0 μL, 109 μmol) was added to a solution of the above amine intermediate and **217** (12 mg, 79.0 μmol) in DMF (1.0 mL) under N_2 and the reaction was heated at 50 °C under microwave irradiation for 1 h. Purification via reversed phase column chromatography (gradient elution, 0%→40% CH_3CN in H_2O) gave **221** as a white gum as a mixture of two diastereomers (32 mg, 55%, over two steps); ν_{max} (film) 3463 (O−H), 3061 (C−H), 3031 (C−H), 2940 (C−H), 2871 (C−H), 1735 (C=O), 1635 (C=O), 1372 (S=O), 1231 (S=O); δ_H (400 MHz, MeOH-*d*$_4$) 1.11 – 1.41 (m, 4H, C(9)*H*$_2$, C(3’’)*H*$_A$, C(7’’)*H*$_A$), 1.42 – 1.77 (m, 5H, C(8)*H*$_2$, C(10)*H*$_2$, C(7’’)*H*$_B$), 1.66 – 1.76 (m, 1H, C(3’’)*H*$_B$), 2.19 – 2.44 (m, 1H, C(2’’)*H*), 2.71 – 2.85 (m, 2H, C(1’’)*H*, C(4’’)*H*), 3.13 – 3.30 (m, 1H, C(11)*H*$_A$), 3.31 – 3.52 (m, 4H, C(2)*H*, C(3)*H*, C(8)*H*$_A$, C(11)*H*$_B$), 3.61 (q, J = 10.1 Hz, 2H, C(7)*H*$_B$, C(4)*H*), 3.84 (s, 2H, C(4’)*H*, C(5)*H*), 4.11 (s, 1H, C(3’)*H*), 4.19 (td, J = 11.5, 5.5 Hz, 1H, C(6)*H*$_A$), 4.31 (dd, J = 11.2, 2.0 Hz, 1H, Bn), 4.39 (d, J = 9.8 Hz, 1H, C(6)*H*$_B$, C(12)*H*$_A$), 4.52 (t, J = 11.7 Hz, 1H, C(5)*H*), 4.82 – 4.88 (m, 2H, Bn, C(2’)*H*), 5.06 (t, J = 3.6 Hz, 1H, C(1)*H*, Bn), 5.21 (s, 1H, C(1’)*H*), 5.87 – 6.03 (m, 1H, C(5’’)*H*), 6.09 (q, J = 4.3 Hz, 1H, C(6’’)*H*), 7.01 – 7.42 (m, 20H, 4 × *Ph*); δ_C (151 MHz, MeOH-*d*$_4$) 24.8 (*C*(9)), 24.8 (*C*(9)), 29.6 (*C*(10)), 29.6 (*C*(10)), 30.3 (*C*(8)), 30.3 (*C*(8)), 32.3 (*C*(3’’)), 32.3 (*C*(3’’)), 32.4 (*C*(3’’)), 32.5 (*C*(3’’)), 41.9 (*C*(2’’)), 42.3 (*C*(2’’)), 42.8 (*C*(1’’)), 42.9

(*C*(1’’)), 47.6, 47.7, 47.7, 47.8, 47.8, 47.9 (*C*(4’’) and (*C*(7’’)), 49.6 (*C*(11)), 49.6 (*C*(11)), 59.9 (*C*(2)), 68.5 (N*C*H_2Ph), 68.7 (N*C*H_2Ph), 69.5 (*C*(6)), 69.5 (*C*(6)), 69.7 (*C*(7)), 69.7 (*C*(7)), 70.4 (*C*(2’)), 71.2 (*C*(5)), 71.4 (C(5)), 71.5 (*C*(5’)), 71.5 (*C*(5’)), 72.0 (*C*(3’)), 72.2 (*C*(3’)), 72.8 (*C*H_2Ph), 72.8 (*C*H_2Ph), 73.4 (*C*H_2Ph), 73.4 (*C*H_2Ph), 75.8 (*C*(4’)), 75.9 (*C*(4’)), 77.0 (*C*H_2Ph), 77.0 (*C*H_2Ph), 78.6 (*C*(4)), 78.7 (*C*(4)), 79.0 (*C*(3)), 79.0 (*C*(3)), 98.9 (*C*(1)), 101.1 (*C*(1’)), 126.3 (*Ar*), 127.6 (*Ar*), 128.2 (*Ar*), 128.5 (*Ar*), 128.7 (*Ar*), 128.7 (*Ar*), 128.9 (*Ar*), 129.2 (*Ar*), 129.3 (*Ar*), 129.4 (*Ar*), 129.5 (*Ar*), 129.6 (*Ar*), 129.8 (*Ar*), 129.9 (*Ar*), 129.9 (*Ar*), 130.7 (*Ar*), 137.2 (*C*(6’’)), 137.2 (*C*(6’’)), 137.3 (*C*(6’’)), 137.3 (*C*(6’’)), 138.5 (*i-Ph*), 138.7 (*i-Ph*), 138.8 (*i-Ph*), 139.1 (*i-Ph*), 139.2 (*C*(5’’)), 139.3 (*C*(5’’)), 139.3 (*C*(5’’)), 139.3 (*C*(5’’)), 178.1 (C_{Acid}=O), 178.2 (C_{Acid}=O)., 178.5 (C_{Amide}=O), 178.5 (C_{Amide}=O); *m/z* (ESI$^+$) 1182 ([M-2Na+3H]$^+$, 10%), 1204 ([M-Na+2H]$^+$, 20%), 1226 ([M+Na]$^+$, 13%); HRMS (ESI$^-$) $C_{53}H_{64}N_3O_{20}S_3^-$ ([M−3Na+2H]$^-$) requires 1158.3251; found 1158.3268.

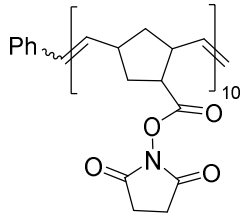
IdoA(2S)-GlcNS(6sulfamate) containing polymer (222)



(1) ROMP. Grubbs Catalyst® M300 (0.35 mg, 0.39 μmol) in $ClCH_2CH_2Cl$ (190 μL) was added rapidly to a solution of monomer **221** (9.5 mg, 7.76 μmol) in thoroughly degassed $CH_3OH/ClCH_2CH_2Cl$ (1:1, 600 μL) under N_2 at 55 °C and the reaction was stirred at stirred

at 55 °C for 2 h before being quenched by addition of ethyl vinyl ether (100 µL). The reaction was concentrated *in vacuo* and the residue was dissolved in the minimal amount of CH_2Cl_2/CH_3OH (9:1) then poured into Et_2O (10 mL) and the precipitate was collected to give the intermediate as an off-white solid (7.4 mg, 78%). **(2) Hydrogenation.** $Pd(OH)_2/C$ (10 mg) was added to a solution of the above intermediate in CH_3OH/PBS (9:1, 1 mL) under N_2 and the reaction was placed under an atmosphere of H_2. The reaction was stirred at rt for 48 h. The reaction was filtered and concentrated *in vacuo* to give **222** as a white solid.

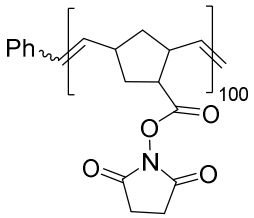
NHS-activated ester containing polymer (223)



Grubbs Catalyst® M300 (18.8 mg, 22 µmol) in CH_2Cl_2 (190 µL) was added rapidly to a solution of monomer **217** (50 mg, 0.22 mmol) in thoroughly degassed CH_2Cl_2 (2.13 mL) under N_2 and the reaction was stirred at rt for 1 h before being quenched by addition of ethyl vinyl ether (100 µL). The crude reaction was poured into Et_2O/PhH (v/v 9:1, 30 mL) and the precipitate was collected via vacuum filtration to give **223** as an off-white solid (91%); δ_H (400 MHz, $CDCl_3$) 1.23 (d, J = 14.2 Hz, 1H), 1.78 (s, 1H), 2.02 (s, 1H), 2.25 (s, 1H), 2.61 – 2.92 (m, 6H), 3.10 (s, 1H), 3.32 (s, 1H), 5.13 – 5.67 (m, 2H), 7.09 – 7.40 (m, 0.5H); Mn_{GPC} = 3069, PD_{GPC} = 1.28.[xiv]

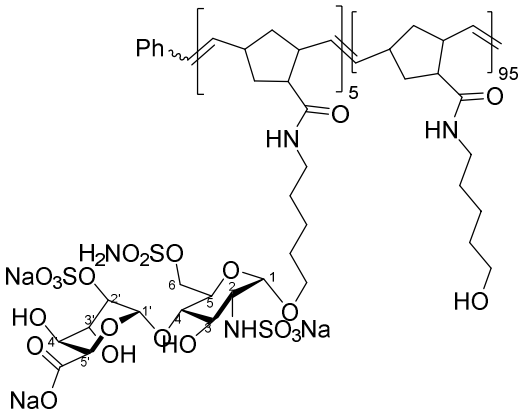
NHS-activated ester containing polymer (224)

[xiv] For GPC data see appendix



Grubbs Catalyst® M300 (18.8 mg, 22 μmol) in CH_2Cl_2 (190 μL) was added rapidly to a solution of monomer **217** (50 mg, 0.22 mmol) in thoroughly degassed CH_2Cl_2 (2.13 mL) under N_2 and the reaction was stirred at rt for 1 h before being quenched by addition of ethyl vinyl ether (100 μL). The crude reaction was poured into Et_2O/PhH (v/v 9:1, 30 mL) and the precipitate was collected via vacuum filtration to give **224** as an off-white solid (89%); δ_H (400 MHz, $CDCl_3$) 1.03 – 1.42 (m, 2H), 1.78 (s, 1H), 2.03 (s, 1H), 2.25 (s, 1H), 2.79 (s, 5H), 2.97 – 3.48 (m, 1H), 5.08 – 5.67 (m, 2H); Mn_{GPC} = 20522, PD_{GPC} = 1.23.

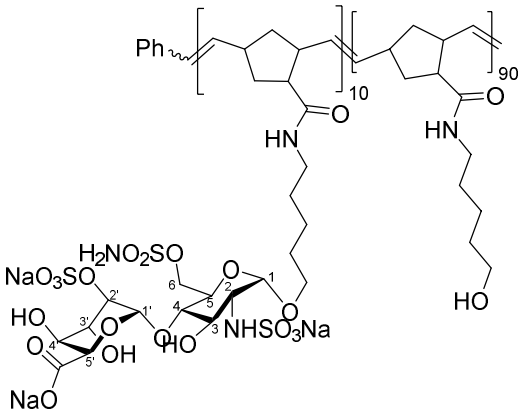
Polymer with 5% IdoA(2S)-GlcNS(6Sulfamate) (226)



163 (1.35 mg), **224** (8.88 mg) and Et_3N (2 μL) in DMF (1 mL) and the reaction was stirred at rt for 24 h, after which an excess of 5-aminopentan-1-ol (18 mg) was added and the reaction was stirred for an additional 24 h. The reaction was precipitated into Et_2O (5 mL) and the oily residue was isolated to give **226** as a sticky solid; δ_H (400 MHz, MeOH-*d*$_4$) 1.15 (s, 15H), 1.27 (t, J = 7.2 Hz, 9H), 1.32 – 1.73 (m, 295H), 2.00 (s, 38H), 2.27 – 2.42 (m, 37H), 2.42 – 2.53 (m, 39H), 2.68 (s, 16H), 2.74 – 2.83 (m, 25H), 2.94 – 3.29 (m, 94H),

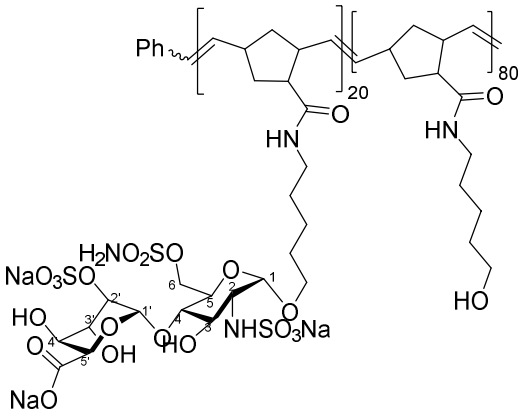
3.55 (dq, J = 6.8, 5.4 Hz, 87H), 3.73 (q, J = 6.7 Hz, 3H), 3.88 (s, 3H), 4.20 (s, 1H), 4.42 (s, 2H), 4.58 (s, 1H), 5.07 – 5.53 (m, 36H).

Polymer with 10% IdoA(2S)-GlcNS(6Sulfamate) (227)



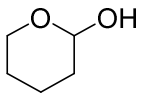
163 (1.09 mg), **224** (3.57 mg) and Et_3N (2 μL) in DMF (1 mL) and the reaction was stirred at rt for 24 h, after which an excess of 5-aminopentan-1-ol (18 mg) was added and the reaction was stirred for an additional 24 h. The reaction was precipitated into Et_2O (5 mL) and the oily residue was isolated to give **227** as a sticky solid; δ_H (400 MHz, MeOH-*d*$_4$) 1.15 (s, 8H), 1.27 (t, J = 7.2 Hz, 8H), 1.33 – 1.75 (m, 172H), 2.00 (s, 22H), 2.30 – 2.52 (m, 35H), 2.53 – 2.88 (m, 31H), 2.92 – 3.27 (m, 50H), 3.56 (dt, J = 8.6, 6.5 Hz, 50H), 3.62 – 3.79 (m, 3H), 3.79 – 4.02 (m, 3H), 4.20 (s, 1H), 4.43 (s, 2H), 4.58 (s, 1H), 5.08 – 5.54 (m, 20H).

Polymer with 20% IdoA(2S)-GlcNS(6Sulfamate) (228)



163 (1.05 mg), **224** (1.85 mg) and Et_3N (2 μL) in DMF (1 mL) and the reaction was stirred at rt for 24 h, after which an excess of 5-aminopentan-1-ol (18 mg) was added and the reaction was stirred for an additional 24 h. The reaction was precipitated into Et_2O (5 mL) and the oily residue was isolated to give **228** as a sticky solid; δ_H (400 MHz, MeOH-*d*$_4$) 1.23 – 1.72 (m, 141H), 2.02 (s, 11H), 2.29 – 2.51 (m, 21H), 2.54 – 2.84 (m, 26H), 3.16 (t, J = 7.0 Hz, 23H), 3.22 (td, J = 7.1, 4.2 Hz, 4H), 3.50 – 3.62 (m, 41H), 3.73 (d, J = 7.0 Hz, 3H), 3.90 (s, 3H), 4.21 (s, 1H), 4.42 (s, 2H), 4.59 (s, 1H), 5.05 – 5.50 (m, 10H).

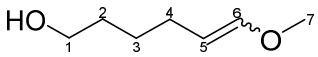
Tetrahydro-2*H*-pyran-2-ol (230)



Sodium metabisulfite (1.05 g, 5.23 mmol) in H_2O (3 mL) was added to 3,4-dihydro-2*H*-pyran (1.00 mL, 11.0 mmol) and the reaction was stirred at rt for 16 h. The reaction was concentrated in vacuo and the residue was dissolved in 10% w/v NaOH and stirred at rt for 30 min. The reaction was extracted with Et_2O (3 × 10 mL), and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give the **230** that was used without further purification (622 mg, 55%); δ_H (400 MHz, $CDCl_3$) 1.43 – 1.61 (m, 4H), 1.73 – 1.94 (m, 2H), 3.54 (dt, J = 11.6, 5.7 Hz, 1H), 4.01 (ddt, J = 9.5, 4.2, 2.3 Hz,

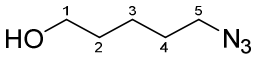
1H), 4.89 (dd, J = 5.9, 2.7 Hz, 1H). Characterisation data are consistent with the literature.[166]

(E,Z)-6-Methoxy-5-hexen-1-ol (231)



n-BuLi (1.6 M in hexane, 5.6 mL, 9.0 mmol) was added dropwise to a suspension of (methoxymethyl)triphenylphosphonium chloride (2.46 g, 7.2 mmol) in THF (15 mL) under N_2 at −78 °C. The reaction was stirred at −78 °C for 30 min and then a solution of 5-hydroxypentanal (290 µL, 3.0 mmol) in THF (5 mL) was added dropwise to the reaction. The reaction was allowed to warm to rt over 16 h. The reaction was quenched by addition of satd aq NH_4Cl (20 mL) and extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→50% EtOAc in pentane) gave **231** as a colourless oil (55 mg, 14%, E/Z, 8:2); δ_H (400 MHz, $CDCl_3$) 1.27 – 1.41 (m, 2H, C(2)H_2), 1.45 – 1.56 (m, 2H, C(3)H_2), 1.89 (qd, J = 7.3, 1.3 Hz, 1.6H, C(4*E*)H_2), 2.03 (qd, J = 7.4, 1.5 Hz, 0.4H, C(4*Z*)H_2), 3.43 (s, 2.4H, C(7*E*)H_3), 3.51 (s, 0.6H, C(7*Z*)H_3), 3.58 (td, J = 6.6, 1.2 Hz, 2H, C(1)H_2), 4.27 (td, J = 7.3, 6.2 Hz, 0.2H, C(5*Z*)*H*), 4.65 (dt, J = 12.6, 7.3 Hz, 0.8H, C(4*E*)*H*), 5.82 (dt, J = 6.2, 1.5 Hz, 0.3H, C(6*Z*)*H*), 6.22 (dt, J = 12.6, 1.3 Hz, 0.8H, C(6*E*)*H*). Characterisation data are consistent with the literature.[146]

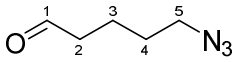
5-Azido-pentan-1-ol (234)



Sodium azide (234 mg, 3.6 mmol) was added to a solution of 5-bromo-pentan-1-ol (360 µL, 3.0 mmol) in DMF (6.8 mL) and the reaction was heated at 90 °C for 4 h. The reaction was cooled to rt and diluted with EtOAc (15 ml). The reaction was washed with ½ sat brine (5 × 5 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column

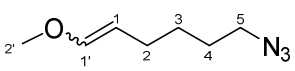
chromatography (eluent EtOAc/pentane, 1:3) gave **234** as a colourless oil (225 mg, 58%); δ_H (400 MHz, $CDCl_3$) 1.40 – 1.54 (m, 2H, C(3)H_2), 1.54 – 1.70 (m, 4H, C(2)H_2, C(4)H_2), 3.28 (td, J = 6.8, 2.5 Hz, 2H, C(5)H_2), 3.65 (t, J = 6.4 Hz, 2H, C(1)H_2); δ_C (101 MHz, $CDCl_3$) 23.1 (*C*(3)), 28.8 (*C*(4)), 32.3 (*C*(2)), 51.5 (*C*(5)), 62.7 (*C*(1)); *m/z* (ESI^+) 152 ($[M+Na]^+$, 100%). Characterisation data are consistent with the literature.[167]

5-Azido-pentan-1-al (235)



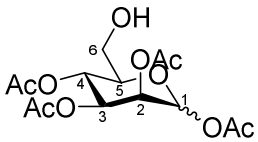
DMSO (271 µL, 3.83 mmol) was added to a solution of oxalyl chloride (164 µL, 1.914 mmol) in CH_2Cl_2 (3.2 mL) at −78 °C under N_2. The reaction was stirred at −78 °C for 20 min. A solution of **234** (225 mg, 1.74 mmol) in CH_2Cl_2 (3.9 mL) was added and the reaction was stirred at −78 °C for an additional 30 min. Et_3N (605 µL, 4.35 mmol) was added and the reaction was warmed to rt for 5 min. The reaction was quenched by addition of satd aq $NaHCO_3$ (15 mL) and the organic layer was separated. The aqueous was extracted with CH_2Cl_2 (2 × 15 mL) and the combined organics were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→10% EtOAc in pentane) gave **235** as a colourless oil (150 mg, 68%); δ_H (400 MHz, $CDCl_3$) 1.56 – 1.68 (m, 2H, C(4)H_2), 1.68 – 1.78 (m, 2H, C(3)H_2), 2.50 (td, J = 7.0, 1.5 Hz, 2H, C(2)H_2), 3.31 (t, J = 6.6 Hz, 2H, C(5)H_2), 9.78 (t, J = 1.5 Hz, 1H, C(1)*H*); δ_C (101 MHz, $CDCl_3$) 19.4 (*C*(3)), 28.4 (*C*(4)), 43.4 (*C*(2)), 51.3 (*C*(5)), 201.8 (*C*(1)). Characterisation data are consistent with the literature.[168]

(E,Z)-6-Azido-1-methoxyhex-1-ene (232)



n-BuLi (1.6 M in hexanes, 885 µL, 1.417 mmol) was added to a suspension of (methoxymethyl)triphenylphosphonium chloride (485 mg, 1.417 mmol) in THF (5 mL) under N_2 at -78 °C. The reaction was stirred at –78 °C for 30 min and then a solution of **235** (150 mg, 1.18 mmol) in THF (1 mL) was added dropwise to the reaction. The reaction was allowed to warm to rt over 16 h and then was quenched by addition of H_2O (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→20% EtOAc in pentane) gave **232** as a colourless oil (75 mg, 41%); δ_H (400 MHz, $CDCl_3$) 1.37 – 1.52 (m, 2H, C(3)H_2), 1.55 – 1.68 (m, 2H, C(4)H_2), 1.96 (qd, J = 7.3, 1.2 Hz, 1.5H, C(2)*H* trans), 2.04 – 2.14 (m, 0.5H, C(2)*H* cis), 3.26 (t, J = 6.9 Hz, 2H, C(5)H_2), 3.50 (s, 2.25H, C(2')H_3 trans), 3.58 (s, 0.75H, C(2')H_3 cis), 4.31 (td, J = 7.4, 6.2 Hz, 0.25H, C(1)*H* cis), 4.70 (dt, J = 12.6, 7.3 Hz, 0.75H, C(1)*H* trans), 5.89 (dt, J = 6.2, 1.5 Hz, 0.25H, C(1')*H* cis), 6.29 (dt, J = 12.6, 1.3 Hz, 0.75H, C(1')*H* trans). Characterisation data are consistent with the literature.[146]

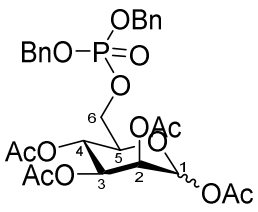
(3*S*,4*S*,5*R*,6*R*)-6-(Hydroxymethyl)tetrahydro-2*H*-pyran-2,3,4,5-tetrayl tetraacetate (237)



(1) 6*O*-Trityl protection. Trityl chloride (8.2 g, 29 mmol) was added to a solution of mannose (5.0 g, 28 mmol) in pyridine (50 mL) and the reaction was heated at 50 °C for 4 h. **(2) Peracetylation.** Without workup, acetic anhydride (25 mL, 270 mmol) was added and the reaction was stirred at rt for 16 h. The reaction was concentrated *in vacuo* and the residue was partitioned between H_2O and EtOAc (1:1, 100 mL) and the organic layer was washed with satd aq NH_4Cl (2 × 50 mL), brine, dried over Na_2SO_4 and concentrated *in*

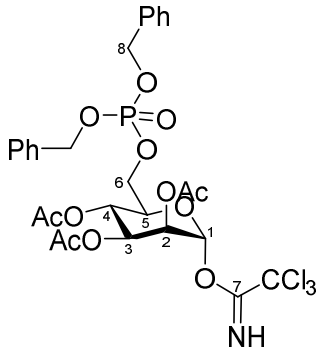
vacuo. **(3) 6*O*-Trityl deprotection.** TMSCl (6.06 mL, 48.1 mmol) was added dropwise to a solution of the above intermediate (9.15 g, 15.5 mmol) and NaI (7.16 g, 48.1 mmol) in CH_3CN (75 mL) at 0 °C under N_2. The reaction was stirred at 0 °C for 20 min, then quenched by addition of ice-cold 10 w/v% $Na_2S_2O_3$ and concentrated *in vacuo* to remove the CH_3CN. The residue was partitioned between CH_2Cl_2 (50 mL) and H_2O (50 mL) and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→60% EtOAc in penatnes) gave **237** as a colourless oil (4.35 g, 67% over 3 steps, α/β, 0.4:0.6); δ_H (400 MHz, $CDCl_3$) 1.93 – 2.18 (m, 12H, 4 × *Ac*), 3.54 – 3.75 (m, 2.4H, C(6α)*H*$_2$, C(6β)*H*$_2$, C(5α)*H*), 3.79 – 3.85 (m, 0.6H, C(5β)*H*), 5.14 (dd, J = 10.0, 3.2 Hz, 0.4H, C(3α)*H*), 5.21 (d, J = 9.6 Hz, 0.4H, C(4α)*H*), 5.22 – 5.26 (m, 0.6H, C(2β)*H*), 5.28 (d, J = 10.0 Hz, 0.6H, C(4β)*H*), 5.35 (dd, J = 10.1, 3.5 Hz, 0.6H, C(3β)*H*,), 5.46 (dd, J = 3.2, 1.2 Hz, 0.4H, C(2α)*H*), 5.84 (d, J = 1.2 Hz, 0.4H, C(1α)*H*), 6.05 (d, J = 1.9 Hz, 0.6H, C(1β)*H*); δ_C (101 MHz, $CDCl_3$) 20.6 (*Ac*), 20.7 (*Ac*), 20.7 (*Ac*), 20.8 (*Ac*), 20.8 (*Ac*), 20.9 (*Ac*), 61.2 (*C*(6)), 61.2 (*C*(6)), 65.8 (*C*(4α)), 65.9 (*C*(4β)), 68.4 (*C*(2α)), 68.5 (*C*(2β)), 68.7 (*C*(3β)), 70.7 (*C*(3α)), 73.0 (*C*(5β)), 75.5 (*C*(5α)), 90.4 (*C*(1α)), 90.7 (*C*(1β)), 168.4 (*C*=O), 168.6 (*C*=O), 169.9 (*C*=O), 169.9 (*C*=O), 170.1 (*C*=O), 170.3 (*C*=O), 170.4 (*C*=O), 170.5 (*C*=O); *m/z* (ESI^+) 371 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{14}H_{20}NaO_{10}^+$ ($[M+Na]^+$) requires 371.0949; found 371.0946. Characterisation data are consistent with the literature.[169]

(3*S*,4*S*,5*R*,6*R*)-6-(Hydroxymethyl)tetrahydro-2*H*-pyran-2,3,4,5-tetrayl tetraacetate (238)



Dibenzyl *N*,*N*-diisopropylphosphoramidite (4.30 mL, 12.9 mmol) was added dropwise (over 5 min) to a solution of **237** (3.0 g, 8.62 mmol) and tetrazole (0.45 M in CH_3CN, 38 mL, 17.1 mmol) in CH_2Cl_2 (172 mL) at 0 °C, under N_2. The reaction was stirred at 0 °C for 2 h, then at rt for 16 h. The reaction was cooled to – 78 °C and *m*-CPBA (7.70 g, 34.4 mmol) was added as a solid in one portion. The reaction was stirred at – 78 °C for 1 h, then allowed to warm to rt over 3 h. The reaction was quenched with satd aq $Na_2S_2O_3$ (150 mL) and the organic layer was separated, washed with satd aq $NaHCO_3$ (2 × 150 mL), brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 10%→90% EtOAc in pentane) gave **238** as a colourless oil (4.29 g, 82%, α/β, 0.45/0.55); δ_H (400 MHz, $CDCl_3$) 1.95 – 2.19 (m, 16H, 4 × *Ac*), 3.73 (dtd, J = 9.5, 4.1, 0.9 Hz, 0.4H, C(5α)*H*), 4.02 – 4.17 (m, 2.6H, C(5α)*H*, C(6)H_2), 5.03 (ddd, J = 8.1, 4.1, 2.5 Hz, 4H, 2 × CH_2Ph), 5.09 (dd, J = 9.9, 3.3 Hz, 0.4H, C(4α)*H*), 5.20 – 5.35 (m, 2.2H, C(2β)*H*, C(3α)*H*, C(3β)*H*, C(4β)*H*), 5.45 (dd, J = 3.2, 1.3 Hz, 0.4H, C(2α)*H*), 5.83 (d, J = 1.2 Hz, 0.4H, C(1α)*H*), 6.06 (d, J = 2.0 Hz, 0.6H, C(1β)*H*), 7.29 – 7.40 (m, 10H, 2 × *Ph*); δ_C (101 MHz, $CDCl_3$) 20.6, 20.6, 20.7, 20.7, 20.7, 20.7, 20.7, 20.8 (8 × *C*H_3, 65.5, 65.6, 65.7, 65.8, 65.8 (*C*(6)) and *C*(3)), 68.2 (*C*(2α)), 68.4 , 68.8 (*C*(2β) and *C*(4β)), 69.5 (d, J = 5.5 Hz, *C*H_2Ph), 69.5 (d, J = 5.6 Hz, *C*H_2Ph), 70.6 (*C*(4α)), 71.3 (d, J = 7.5 Hz, C(5β)*H*), 73.8 (d, J = 7.6 Hz, *C*(5α)), 90.3 (*C*(1α)), 90.5 (*C*(1β)), 127.9 (*Ph*), 128.0 (*Ph*), 128.1 (*Ph*), 128.6 (*Ph*), 128.6 (*Ph*), 128.6 (*Ph*), 135.8 (d, J = 7.4 Hz, *i-Ph*) 135.9 (d, J = 7.2 Hz, *i-Ph*), 168.1 (*C*=O), 168.3 (*C*=O), 169.5 (*C*=O), 169.6 (*C*=O), 169.7 (*C*=O), 169.8 (*C*=O), 170.0 (*C*=O), 170.2 (*C*=O); $\delta_P\{^1H\}$ (162 MHz, $CDCl_3$) -1.4, -1.3; *m/z* (ESI^+) 549 ($[M-H_2O+H]^+$, 93%), 609 ($[M+H]^+$, 100%), 631 ([M+Na]+, 87%), 1217 (($[2M+H]^+$, 100%), 1239 (($[2M+Na]^+$, 92%), 1825 (($[3M+H]^+$, 33%), 1847 (($[3M+Na]^+$, 100%); HRMS (ESI^+) $C_{28}H_{34}O_{13}P^+$ ($[M+H]^+$) requires 609.1732; found 609.1733. Characterisation data are consistent with the literature. [170]

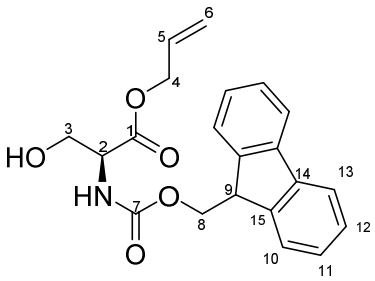
(3*S*,4*S*,5*R*,6*R*)-6-(Hydroxymethyl)tetrahydro-2*H*-pyran-2,3,4,5-tetrayl tetraacetate (239)



(1) 1*O*-deacetylation. 3-(Dimethylamino)-1-propylamine (415 μL, 3.30 mmol) was added to a solution of **238** (800 mg, 1.32 mmol) in THF (6.6 mL) at 0 °C under N_2. The reaction was stirred at rt for 2 h and then quenched by addition of 1M HCl (10 mL). The reaction was extracted with CH_2Cl_2 (2 × 15 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. **(2) 1*O*-trichloroacetimidate formation.** DBU (20 μL, 0.133 mmol) was added to a solution of the above intermediate and trichloroacetonitrile (660 μL, 6.60 mmol) in CH_2Cl_2 (25 mL) under N_2. The reaction was stirred at rt for 1 h, the concentrated *in vacuo*. Purification via column chromatography (gradient elution, 20%→60% EtOAc in pentane) gave **239** as a yellow oil (490 mg, 53% over two steps); δ_H (400 MHz, $CDCl_3$) 2.00 (s, 3H, *Ac*), 2.02 (s, 3H, *Ac*), 2.11 (s, 3H, *Ac*), 4.06 – 4.24 (m, 3H, C(5)*H*, C(6)H_2), 5.04 (dd, J = 7.6, 6.0 Hz, 4H, 2 × CH_2Ph), 5.33 – 5.49 (m, 3H, C(2)*H*, C(3)*H*, C(4)*H*), 6.27 (d, J = 1.9 Hz, 1H, C(1)*H*), 7.27 – 7.39 (m, 10H, 2 × *Ph*), 8.72 (s, 1H, N*H*); δ_C (101 MHz, $CDCl_3$) 20.7 (*Ac*), 20.7 (*Ac*), 20.8 (*Ac*), 65.4 (*C*(4)), 65.7 (d, J = 5.1 Hz, C(6)), 67.9, 68.9 (*C*(3) and *C*(4)), 69.5 (d, J = 2.2 Hz, *C*(8)), 69.5 (d, J = 2.1 Hz, *C*(8)), 72.0 (d, J = 7.8 Hz, *C*(5))), 90.6 (*C*Cl_3), 94.5 (*C*(1)), 128.0 (*Ar*), 128.0 (*Ar*), 128.6 (*Ar*), 128.7 (*Ar*), 135.8 (d, J = 4.2 Hz, *i-Ph*), 135.9 (d, J = 4.2 Hz, *i-Ph*), 159.8 (*C*(7)), 169.6 (*C*=O), 169.8 (*C*=O), 169.9 (*C*=O); $\delta_P\{^1H\}$ (162 MHz, $CDCl_3$) −1.3; *m/z* (ESI^+) 549 ($[M-(OCNHCCl_3)]^+$, 94%), 581 ($[M-(NHCCl_3)+H_3]^+$, 100%), 732 ($[M(^{35}Cl)_3+Na]^+$,

69%), 734 ([M(^{35}Cl)$_2$(^{37}Cl)+Na]$^+$, 66%), 736 ([M(^{35}Cl)(^{37}Cl)$_2$+Na]$^+$, 32%), 738 ([M(^{37}Cl)$_3$+Na]$^+$, 6%). Characterisation data are consistent with the literature.[170]

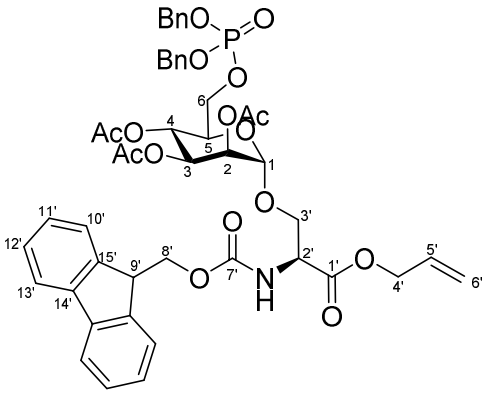
Allyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-serinate (241)



(1) *N*-Fmoc protection. *N*-(9-Fluorenylmethoxycarbonyloxy)succinimide (1.68 g, 5.0 mmol) was added to a solution of L-serine (500 mg, 4.76 mmol) in dioxane (10 mL) and 10 w/v% $NaHCO_3$ (8.75 mL) and the reaction was stirred at rt for 16 h. The reaction was concentrated *in vacuo* to remove the 1,4-dioxane, and the resulting aqueous layer was washed with EtOAc (this organic layer was discarded). The aqueous layer was acidified to pH 1 by addition of 1M HCl and then extracted with EtOAc (3 × 15 mL), these combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. **(2) O-Allyl protection.** Allyl bromide (820 μL, 9.52 mmol) was added to a solution of the above intermediate and DIPEA (1.66 mL, 9.52 mmol) in DMF (30 mL) and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of ½ sat brine (50 mL) and extracted with EtOAc (50 mL). The organic layer was washed with ½ sat brine (5 × 20 mL), brine, dried over Na_2SO_4 and concentrated in vacuo. Purification via column chromatography (gradient elution, 0%→20% EtOAc in pentanes) gave **241** as a white crystalline solid (1.65 g, 90% over 2 steps); mp 82-84 °C; δ_H (400 MHz, $CDCl_3$) 3.87 – 4.08 (m, 2H, C(3)H_2), 4.23 (t, J = 6.9 Hz, 1H, C(9)*H*), 4.35 – 4.56 (m, 3H, C(2)*H*, C(8)H_2), 4.69 (d, J = 5.7 Hz, 2H, C(4)H_2), 5.27 (dd, J = 10.4, 1.2 Hz, 1H, C(6)H_A), 5.35 (d, J = 17.4 Hz, 1H, C(6)H_B), 5.74 (br s, 1H, O*H*), 5.91 (ddt, J = 16.5, 11.0, 5.7 Hz, 1H, C(5)*H*), 7.32

(tt, J = 7.5, 1.2 Hz, 2H, 2 × C(12)*H*), 7.40 (t, J = 7.5 Hz, 2H, 2 × C(11)*H*), 7.53 – 7.66 (m, 2H, 2 × C(10)*H*), 7.77 (d, J = 7.5 Hz, 2H, 2 × C(13)*H*); δ_C (101 MHz, $CDCl_3$) 47.3 (*C*(9)), 56.3 (*C*(2)), 63.5 (*C*(3)), 66.5 (*C*(4)), 67.4 (*C*(8)), 119.2 (*C*(6)), 120.1, 120.2 (2 × *C*(13)), 125.2 (2 × *C*(10)) 127.2, 127.2 (2 × *C*(12)), 127.9 (2 × *C*(11)), 131.4 (*C*(5)), 141.4, 141.5 (2 × *C*(14)), 143.8, 143.9 (2 × *C*(15)), 156.4 (*C*(7)), 170.3 (*C*(1)); *m/z* (ESI^+) 368 ($[M+H]^+$, 100%). Characterisation data are consistent with the literature.[171]

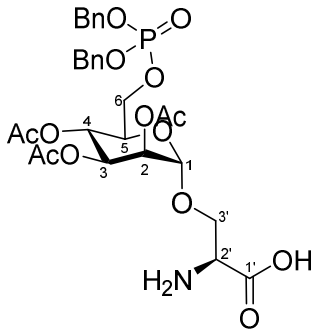
(2*S*,3*S*,4*S*,5*R*,6*R*)-2-((*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(allyloxy)-3-oxopropoxy)-6-(((bis(benzyloxy)phosphoryl)oxy)methyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (242)



4Å molecular sieves (freshly activated, 6 g) were added to a solution of **239** (2.12 g, 2.99 mmol) and **241** (1.30 g, 3.59 mmol) under N_2 and the reaction was stirred at rt for 30 min. The reaction was cooled to 0 °C and TMSOTf (150 μL, 830 μmol) was added. The reaction was allowed to warm to rt over 6 h and the reaction was quenched by addition of Et_3N (0.5 mL), and the reaction was filtered through celite and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 10%→80% EtOAc in pentane) gave **242** as a pale yellow oil (810 mg, 30%); δ_H (400 MHz, $CDCl_3$) 1.97 (s, 3H, *Ac*), 1.99 (s, 3H, *Ac*), 2.06 (s, 3H, *Ac*), 3.86 – 4.02 (m, 3H), 4.23 (t, J = 7.1 Hz, 1H), 4.31 – 4.44 (m, 2H), 4.50 – 4.59 (m, 1H), 4.70 (t, J = 5.7 Hz, 2H), 4.73 – 4.80 (m, 1H), 4.97 – 5.09 (m, 5H), 5.17 (s, 1H), 5.20 – 5.38 (m, 4H), 5.79 (d, J = 8.2 Hz, 1H), 5.92 (ddt, J = 16.6, 11.0, 5.9 Hz, 1H), 7.27 – 7.35 (m, 12H), 7.35 – 7.41 (m, 3H), 7.61 (d, J = 7.6 Hz, 2H), 7.76 (dt, J = 7.6, 1.0

Hz, 2H); δ_C (151 MHz, $CDCl_3$) 20.8, 20.9, 47.2, 66.0, 66.7, 67.6, 69.0, 69.1, 69.4, 69.5 (d, J = 5.5 Hz), 69.8 (d, J = 7.7 Hz), 98.3, 119.7, 120.1, 125.4 (d, J = 3.8 Hz), 127.3, 127.9, 128.0, 128.1, 128.7, 128.7, 131.4, 135.9 (dd, J = 7.3, 3.0 Hz), 141.4, 143.9 (d, J = 4.4 Hz); *m/z* (ESI^+) 916 ($[M+H]^+$, 85%), 938 ($[M+Na]^+$, 100%); HRMS (ESI^+) $C_{47}H_{51}NO_{16}P^+$ ($[M+H]^+$) requires 916.2940; found 916.2933. Characterisation data are consistent with the literature.[170]

_O_-((2_S_,3_S_,4_S_,5_R_,6_R_)-3,4,5-Triacetoxy-6-(((bis(benzyloxy)phosphoryl)oxy)methyl)tetrahydro-2_H_-pyran-2-yl)-L-serine (243)



(1) _O_-Allyl deprotection. $Pd(PPh_3)_4$ (73 mg, 0.063 mmol) was added to a degassed solution of **242** (830 mg, 0.91 mmol) and *N*-methylaniline (980 μL, 9.1 mmol) in THF (45 mL) under N_2 at rt . The reaction was stirred at rt for 2 h and was concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→100% EtOAc in pentane) gave intermediate acid which was used directly in the next step. **(2) _N_-Fmoc deproteciton.** The above intermediate was dissolved in DMF (5 mL) and morpholine (1 mL) was added. The reaction was stirred at rt for 1 h and then the reaction was concentrated *in vacuo.* Purification via column chromatography (eluent gradient, 100%→33% CH_3OH in EtOAc, followed by 0%→17% H_2O in CH_3OH and EtOAc, 1:2) gave **243** as a brown solid (190 mg, 32%); δ_H (400 MHz, MeOH-d_4) 1.92 – 2.05 (m, 9H), 3.71 – 3.85 (m, 2H), 4.06 – 4.16 (m, 2H), 4.16 – 4.24 (m, 2H), 5.06 – 5.11 (m, 3H), 5.32 – 5.35 (m, 1H), 5.35 – 5.44 (m,

1H), 7.30 – 7.45 (m, 10H); δ_P (162 MHz, MeOH-d_4) -1.83; *m/z* (ESI^+) 654 ($[M+H]^+$, 100%), 676 ($[M+Na]^+$, 40%), 1307 ($[2M+H]^+$, 87%), 1329 ($[2M+Na]^+$, 13%). Characterisation data are consistent with the literature.[170]

9. Biology experimental methods

9.1 Materials

HSulf-2 protein was kindly provided by Prof. Romain Vivès (CNRS). MSulf-2 and MSulf-2ΔCC overexpressing RAW 264.7 cells were kindly provided by Dr. Maarten Swart (KIR, University of Oxford). The following materials were obtained from commercial sources: TAMRA-biotin-azide (DC Biosciences #CCR-1048), TBTA (Cayman Chemical #18816), TCEP (Sigma #C4706), $CuSO_4$ (Sigma #451657), 4-Methylumbelliferyl sulfate potassium salt (Sigma #M7133).

9.2 Cell culture

MCF-7 cells were maintained in DMEM (Life Technologies) supplemented with 10% Fetal Bovine Serum (Life Technologies), 1 mM Sodium Pyruvate (ThermoFisher), 1 mM MEM Non-Essential Amino Acids Solution (ThermoFisher) and 1% Penicillin Streptomycin (Life Technologies). Cells were maintained at 5% CO_2 at 37 °C. RAW 264.7 cells were maintained in RPMI-1640 (ThermoFisher) supplemented with 10% Fetal Bovine Serum (Life Technologies) and 1% Penicillin Streptomycin (Life Technologies). Cells were maintained at 5% CO_2 at 37 °C.

9.3 HSulf-2 activity assay (4-MUS assay)

Arylsulfatase activity was monitored by the hydrolysis of 4-MUS to fluorescent methylumbelliferone (4MU, λ_{ex} 360 nm, λ_{em} 460 nm).[172] Assays were performed in white 96-well plates (Corning). HSulf (160 ng μL^{-1}) activity was measured by using 4-MUS (10 mM) in assay buffer (50 mM Tris base, 10 mM $MgCl_2$, (pH 7.5)) in the absence of presence of inhibitors. Experiments were incubated at 37 °C for 4 h, then quenched by addition of an equal volume of 1M Tris base (pH 11.5) and analysed on a fluorescence plate reader

(Fusion, Perkin–Elmer). Inhibition is reported as a percentage of the uninhibited control: % activity=[rate with inhibitor/rate control×100]; and % Inhibition=[100−% activity]. All values represent an average of two or more independent measurements. Half-maximal biochemical activity values, or IC_{50} values, were determined by using a dose–response curve.

9.4 MSulf-2 isolation

Recovering Sulf DNA from bacterial culture: LB agar plates with were ampicillin prepared 24 hours in advance and stored at 4 °C. A sterile inoculation loop was used to spread a Sulf stab culture (pcDNA3.1/Myc-His(-)-MSulf-2, addgene) over a section of a LB agar plate (Sigma Aldrich). Using a fresh loop the bacteria was spread further over the surface of the agar plate. The plates were incubated at 37 °C for 18 h. A single colony was inoculated into 5 ml of LB broth containing ampicillin and grown overnight at 37 °C at 320 rpm. 1 mL of bacteria was pelleted for 10 min at maximum speed at room temperature. The Sulf DNA was isolated and purified using a QIAprep® Spin Miniprep Kit. The supernatant was removed and the pellet was resuspended in 250 µL Buffer P1 and transferred to a microcentrifuge tube and 250 µL Buffer P2 was added, the tubes were inverted to mix and initiate the lysis reaction. The tubes were incubated at room temperature for 5 min and was quenched by addition of 350 µL Buffer N3. The tubes were inverted to mix. The tubes were centrifuged for 10 min at 13,000 rpm at rt. The supernatant was decanted by pipetting and transferred to the miniprep spin column. The columns were centrifuged for 1 min and the flow through was discarded. The spin column was washed with 0.5 mL Buffer PB followed by 0.75 mL Buffer PE, centrifuging for 1 min at 13,000 rpm (~17,900 x g) and discarding the flow through each time. The spin column was then centrifuged for 1 min at 13,000 rpm (~17,900 x g) to remove residual buffer and the column was placed in a clean

microcentrifuge tube. The DNA was eluted from the spin column by addition of 50 µL water, standing at rt for 1 min followed by 1 min centrifuge. The concentration of DNA was quantified using NanoDrop One Microvolume UV-Vis Spectrophotometer by placing 1 µL of DNA solution.

Characterisation of DNA: Restriction digestion of the DNA plasmid was performed to quickly confirm the identity of a plasmid by diagnostic digest. Samples were prepared containing 1 µg DNA solution, 2 µL Buffer R (10x), 1 µL HindIII restriction enzyme, 1 µL NheI restriction enzyme and made up to 20 µL with nuclease free water. The sample was mixed by pipetting and incubated at 37 °C for 45 min. The digest was confirmed by agorose gel electrophoresis. A standard 1% agarose gel was prepared by suspending 1 g agarose in 100 mL TAE buffer and microwaving until boiling. The agarose solution was cooled before the addition of Sybr Safe (10 µL). 2-Log DNA ladder (1 µg) was placed in the first well and 4 µL Gel Loading Dye, Purple (6X) was added to the digested sample before loading into the agarose gel. The gel electrophoresis was run at 100 V for 1 h using TAE buffer. The gel was visualised and imaged using UV excitation and indicated two bands at approx. 2600 bp and 5500 bp, corresponding to the Sulf1/2 insert and the remaining plasmid, respectively. The plasmid identity was further confirmed by sequencing analysis from the T7 promotor.

Transient trasfrection of Sulf DNA into HEK293 cells: The sulf plasmids were transfected into HEK 293 cells using jetPRIME® transfection reagent according to the manufactures protocol (Polyplus Transfection®). Briefly, HEK 293 cells were seeded for 24 h in TC treated 6-well plate until approx. 80% confluent. The transfection reagent was prepared by

adding 4 µg of DNA plasmid, 400 µL jetPRIME® buffer and 8 µL jetPRIME® reagent. The solution was vortexed for 10 s and spun down using a bench top centrifuge, before a 10 min incubation at room temperature. 200 µL per well was added dropwise to the cells in 10% serum containing medium. The cells were incubated at 37 °C for 18 h, after which the medium was changed for 0.5% fetal bovine serum (FBS) containing medium, and incubated at 37 °C for 18 h. The cells were lysed by addition of 160 µL/well of SDS-buffer with β-mercaptoethanol and gel loading dye.

Analysis of column fractions by western blot: 5 µL of ibright protein ladder and 5 µL of protein sample was loaded on to a 4-12% gel, run at 170 V for 55 min. Gel was wet transferred onto the membrane run at 30 V for 1 h. blocking 45 min at rt, primary incubation 2.5 h at rt, followed by 3 × PBST washes, secondary incubation for 1.5 h, 3 × PBST washes. Membrane imaged using fluorescence at 800 nm.

9.5 Target engagement study

MCF-7 cells were grown to 60% confluency in 6 well plates, the medium was removed and replaced with 3 mL of fresh medium (0% FBS, 1% Penicillin Streptomycin Solution) and incubated at 37 °C for 16 h. target engagement probes (stock solution 5 mM) was added into the medium and the cells were incubated at 37 °C for 1 h. Cells treated with **176** and **187** were irradiated with a high intensity UV lamp (354 nm) for 20 min on ice. And the cells treated with **200** and **208**, were supplemented with **213** (250 µM) and irradiated with high intensity blue light (440 nm) for 20 min. The medium was collected and the proteins in the supernatant were concentrated by TCA precipitation. The precipitate was dissolved in 'lysis buffer' and the protein concentration was determined by BSA assay using albumin as a standard. Lysis buffer contained 0.1% (wt/vol) SDS and 1% (vol/vol) Triton X-100 in

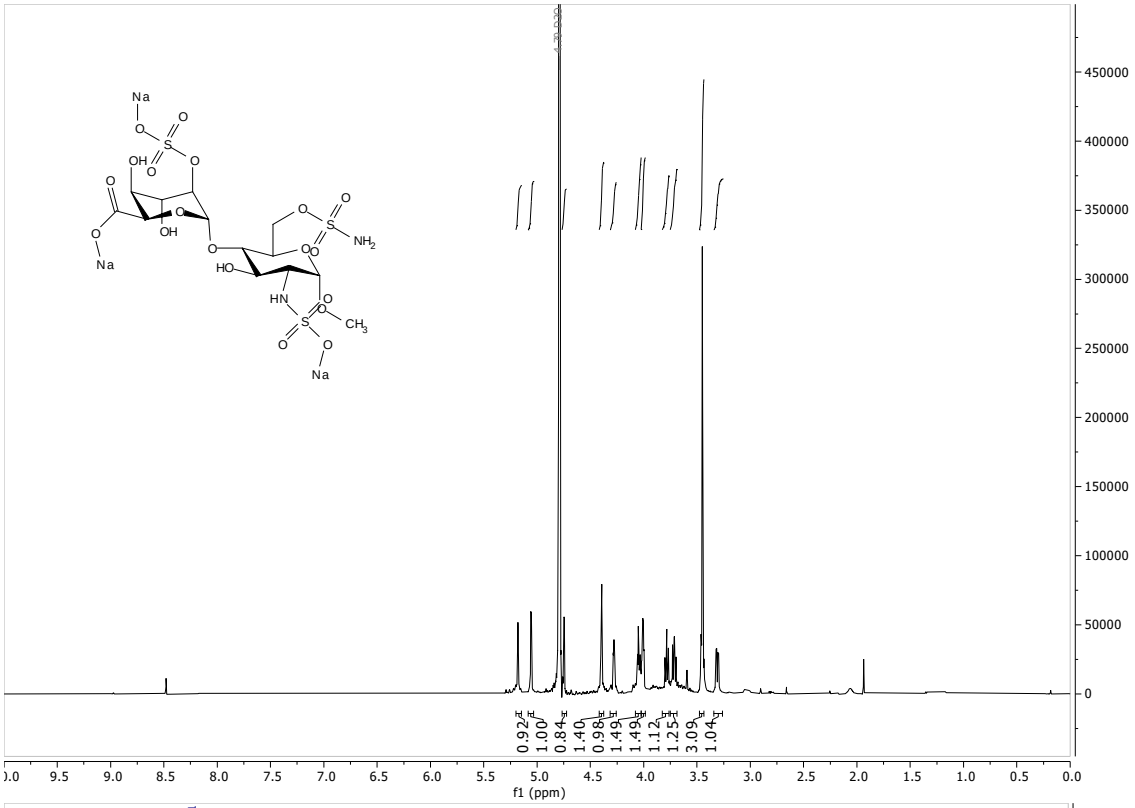
PBS, pH 7.4, with cOmplete EDTA-free protease inhibitor cocktail (Sigma-Aldrich). The samples were diluted to 1 mg/mL concentration for the CuAAC reaction. The 'click mix' was prepared by adding 1 volume 1 volume of 10 mM TAMRA-biotin-azide (AzTB, clickchemistrytools, catalogue number 1048), 2 volumes of 50 mM $CuSO_4$ solution, 2 volumes of 50 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP) solution and 1 volume of 10 mM tris(benzyltriazolylmethyl)amine (TBTA) solution (final concentrations of 100 μM, 1 mM, 1 mM and 100 μM, respectively). Then 6 μL of 'click mix' was added per 100 μL of sample and the samples were incubated it for 1 h at rt while shaking vigorously. The CuAAC reaction was quenched by addition of 1M ethylenediaminetetraacetic acid (EDTA) (final concentration 5 mM). The samples were precipitated by adding 4 sample volumes of methanol, 1 volume of chloroform and 2 volumes of water and vortex vigorously at 3,000 rpm for 10 s. The samples were centrifuged at 5,000g for 2 min at rt. The top layer was discarded and methanol (500 μL) was added. The samples were centrifuged at 17,000g for 4 min at rt. The supernatant was discarded, and the pellet was washed with methanol (2 × 500 μL). The pellets were dried, leaving the tube open, upside down on tissue paper. The pellet was dissolved in 10 μL of (resuspension buffer), centrifuged at 17,000g for 2 min at rt. The supernatant was transferred into a clean Eppendorf and HEPES solution (90 μL) was added. Dynabeads™ MyOne™ Streptavidin T1 (30 μL/100 μL protein sample) were washed with HEPES buffer (3 × 50 μL). The protein samples were centrifuged at 17,000 g for 5 min at rt, and the supernatant was transferred into a new Protein LoBind® tube (Eppendorf, catalogue number 0030108094) with the Dynabeads™ MyOne™ Streptavidin T1 (Invitrogen, catalogue number. 65601). The samples were incubated at 4 °C for 16 h with shaking. The beads were washed with HEPES buffer (3 × 1 mL) and then HEPES buffer 2 (3 × 1 mL). The samples were denatured in 30 μL of NuPAGE™ LDS Sample Buffer with NuPAGE™

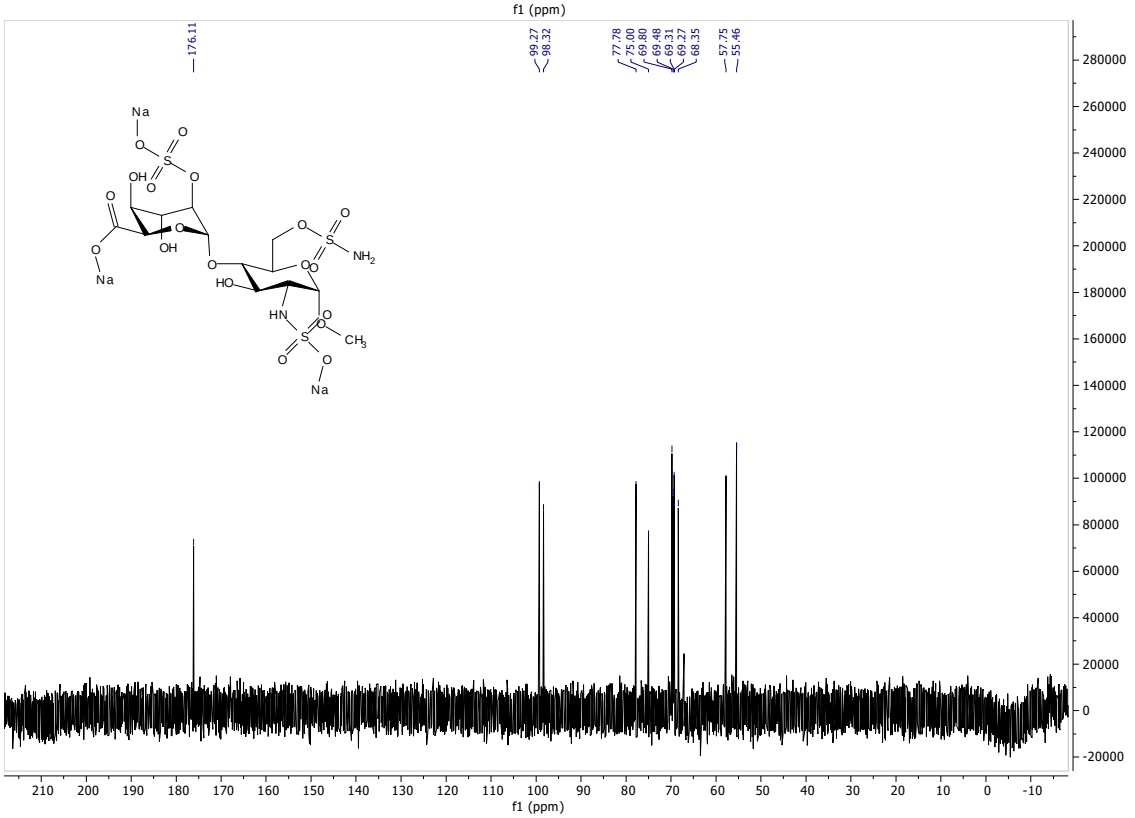
Sample Reducing Agent. Samples were heated at 90 °C for 5 min, and 12.5 µL was added loaded on SDS-PAGE gel (4–12% Bis-Tris Midi Gel, NuPAGE® MES SDS Running Buffer and NuPAGE® Antioxidant. 170V for 1 h). Gels visualised using silver staining protocol (Pierce™ Silver Stain for Mass Spectrometry).

Gel bands were excised and destained. Gel pieces were reduced by adding 30 µl of 10 mM DTT in 100 mM NH_4HCO_3 and heated at 56 °C for 45 min with agitation. The excess liquid was removed and the samples were alkylated by reaction with 30 µl of 55 mM of iodoacetamide in 100 mM NH_4HCO_3 for 30 min at rt. The liquid was removed and the samples were washed with 100 µl of 100mM NH_4HCO_3 (5 min), 100 µl of MeCN (15 min) and air dried for 15 min. The samples were digested with 50µL of 0.01mg/mL trypsin solution at 4°C for 30 min, then at 37°C for 16 h. the enzymatic digested was quenched by addition of formic acid (final concentration 5%). The peptides were washed out of the gel pieces by washing with 1% formic acid in H2O (100 µl, 15 min), MeCN (100 µl, 15 min), MeCN/H_2O (1:1, 100 µl, 5 min) and 1% formic acid in MeCN (100 µl, 5 min). the combined wash layers were concentrated using a SpeedVac. Samples were dissolved in 10 µl 0.1% formic acid in H_2O and cleaned up using ZipTip. Samples were submitted for analysis by LC-MS/MS.

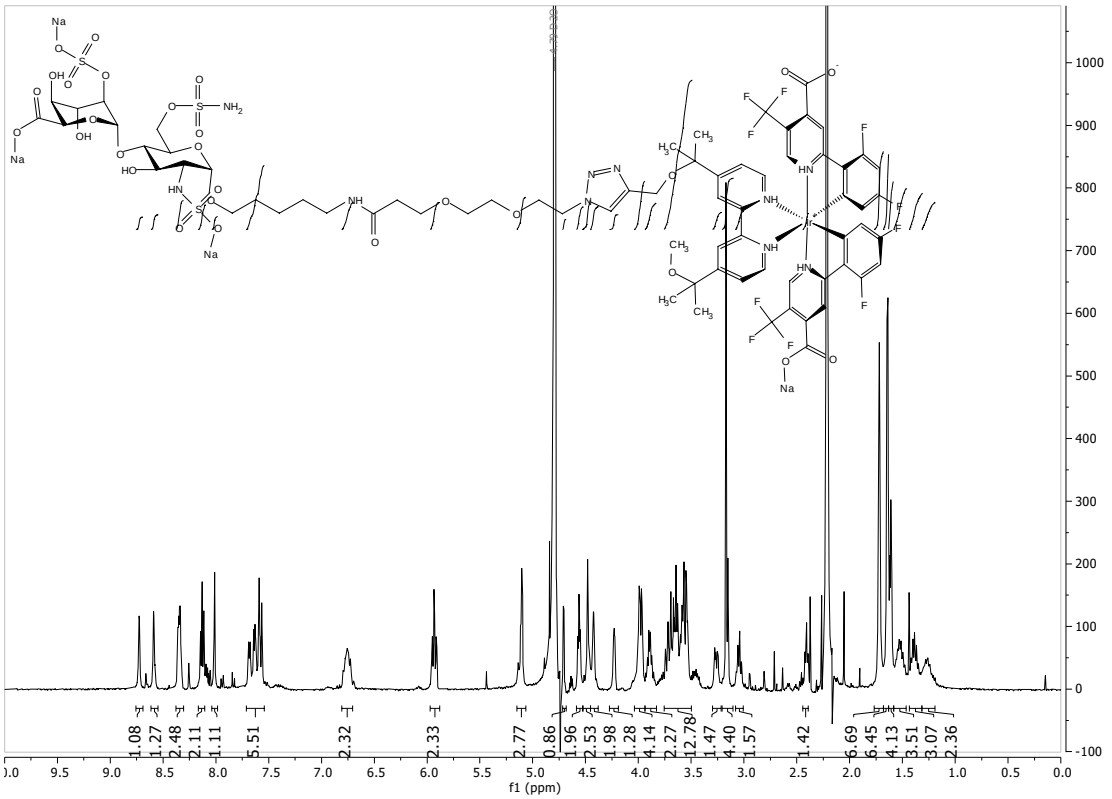
10. Appendix

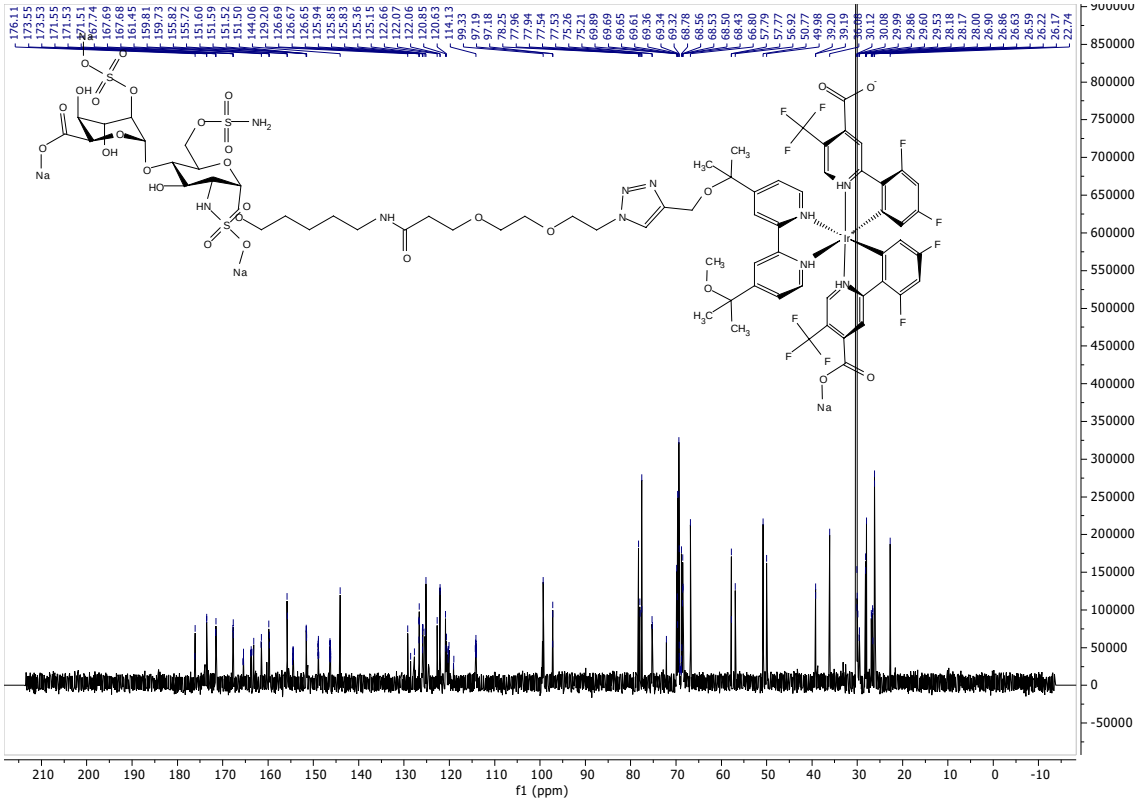
10.1 NMR data for Compound 89





10.2 NMR and HPLC data for Compound 208





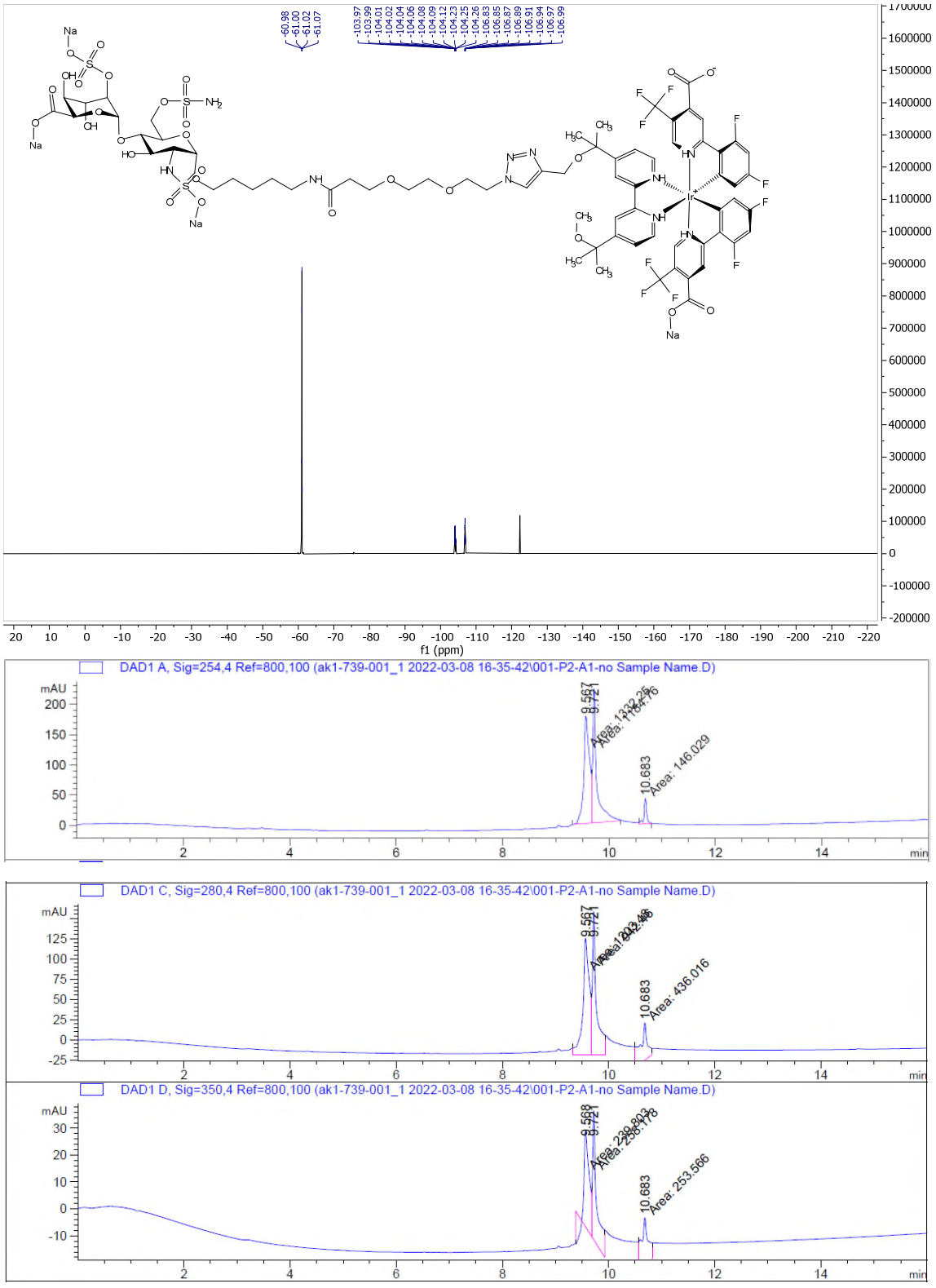
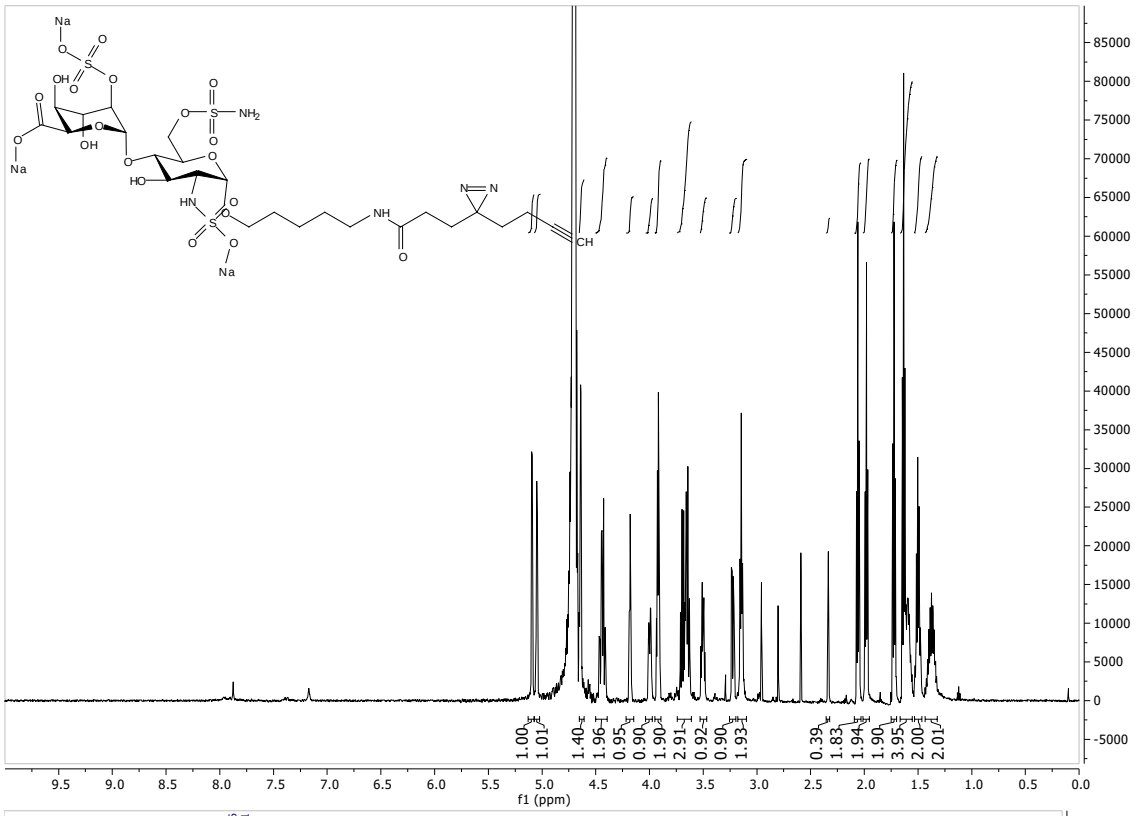
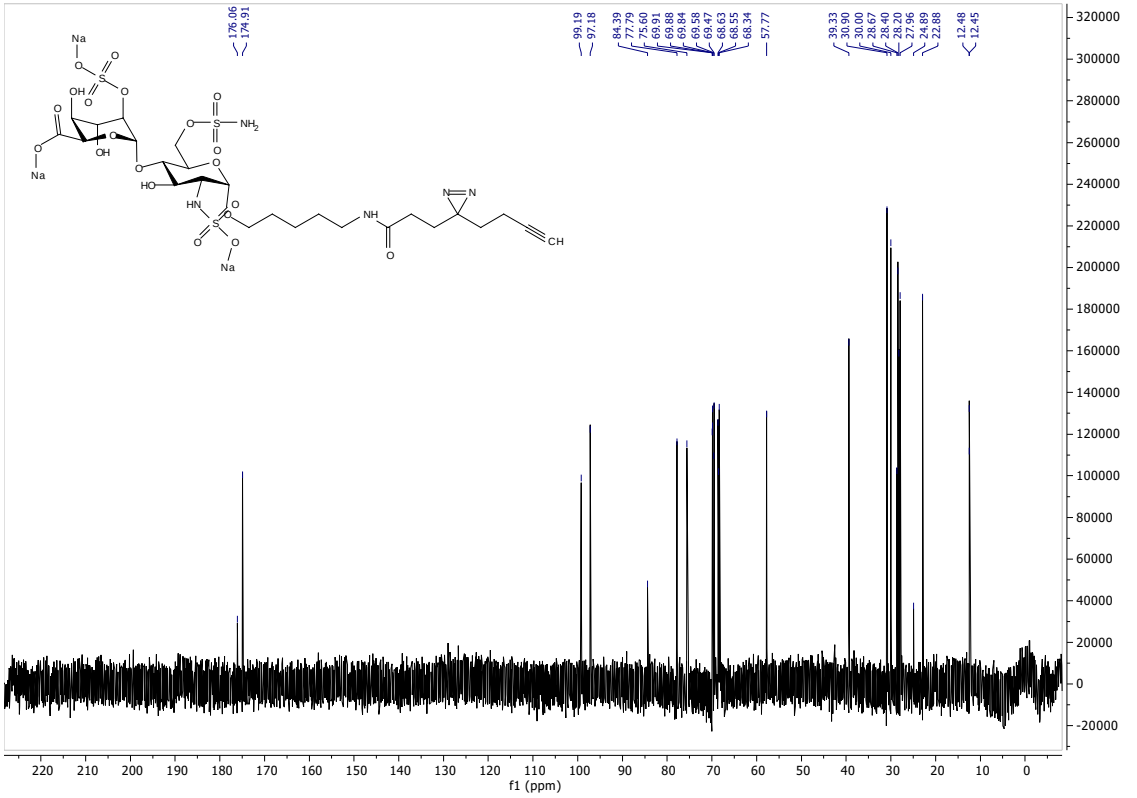


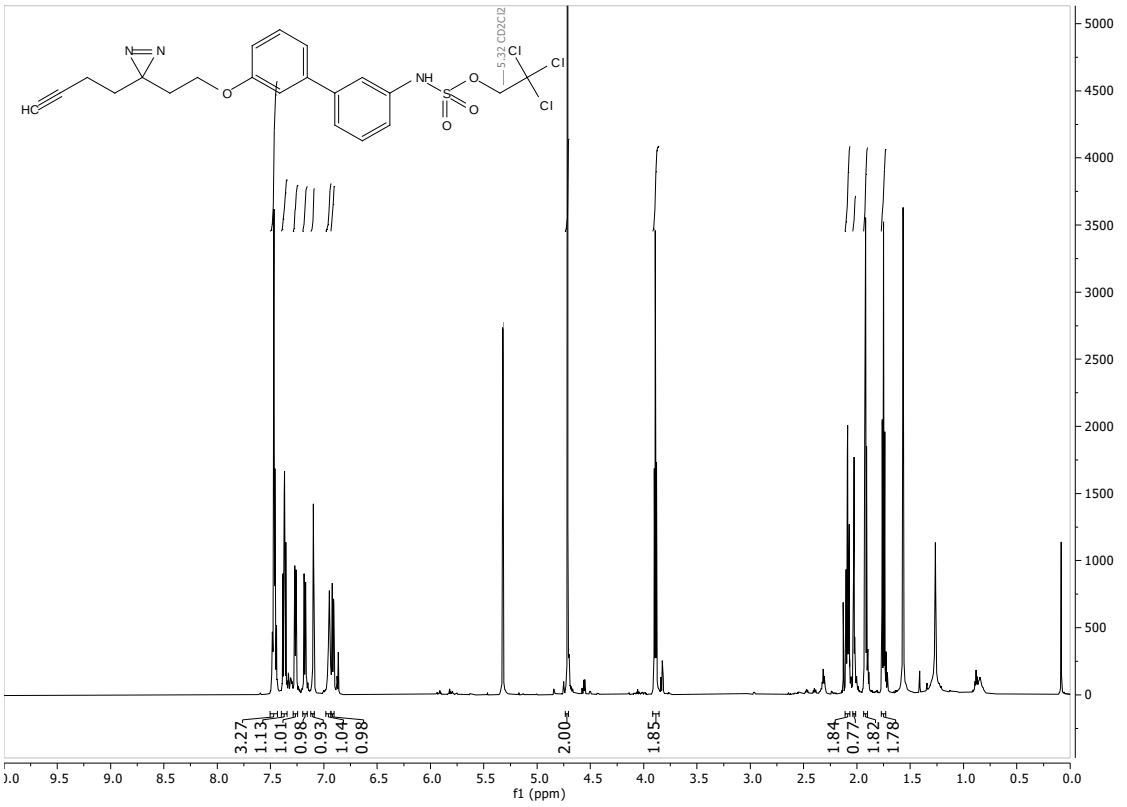
Figure S1: HPLC data recorded for compound **208**. (0%→100% MeCN in H2O with 0.1% formic acid), DAD1 A, Sig=254,4 Ref=800,100, purity = 94.5%.

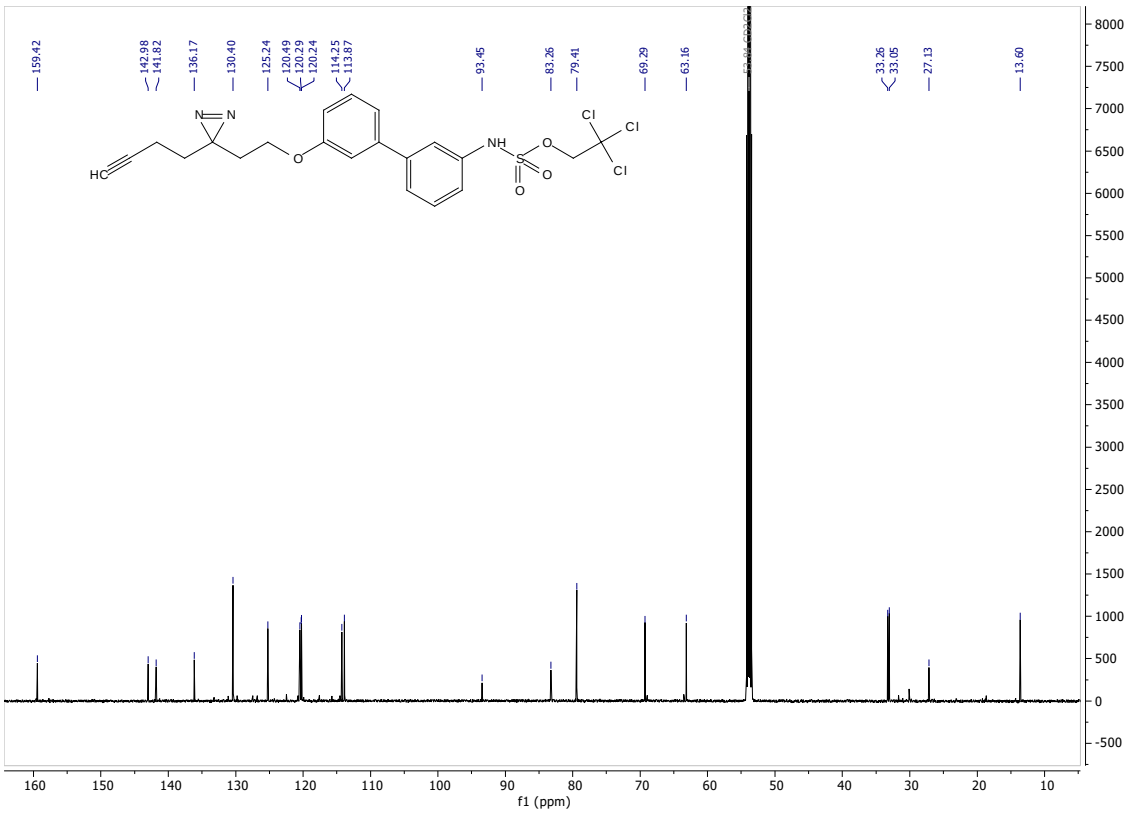
10.3 NMR data for compound 187





10.4 NMR data for 175





10.5 Diazirine activation via Dexter energy transfer NMR experiment data

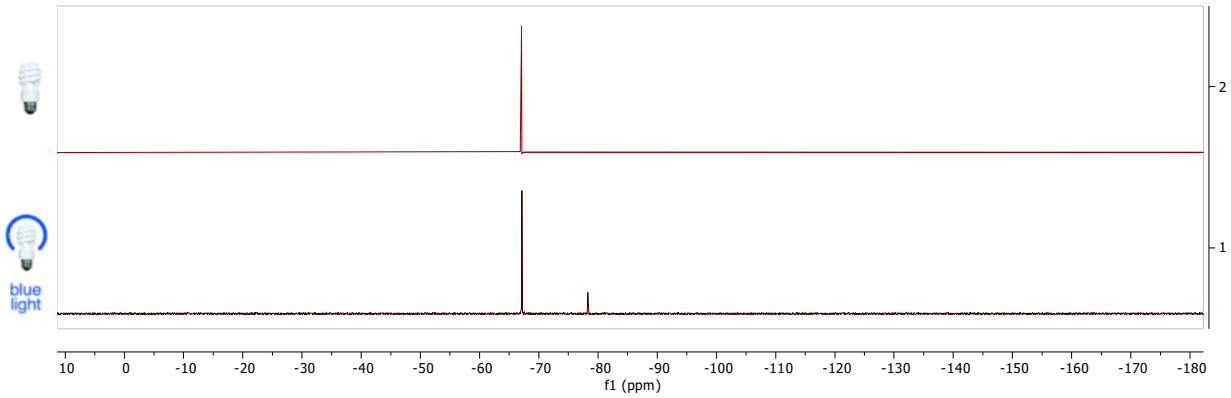


Figure S2: ^{19}F NMR spectra of a mixture of **208** (0.1 mg) and **211** (5 mg) in 500 µL CD_3OD. Top: before blue light irradiation. Bottom: with high intensity 440 nm irradiation for 20 min. Experiments performed in a Quartz NMR tube using Kessil 440 nm lamp (PR160L-440) positioned 20 cm from NMR tube.

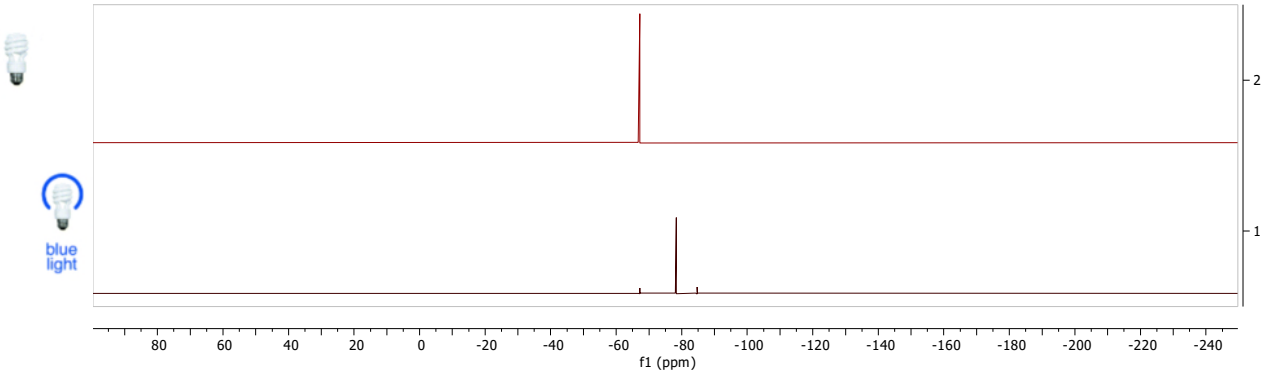


Figure S3: ^{19}F NMR spectra of a mixture of **208** (0.1 mg) and **213** (5 mg) in 500 µL CD_3OD. Top: before blue light irradiation. Bottom: with high intensity 440 nm irradiation for 20 min. Experiments performed in a Quartz NMR tube using Kessil 440 nm lamp (PR160L-440) positioned 20 cm from NMR tube.

10.6 Polymer chacterisation

Table 16 from chapter 6

Entry		*mol% Grubbs cat*	M_n	PD	*n*	yield
1	**223**	10	3069	1.28	12	91
2	**224**	1	20522	1.23	87	89

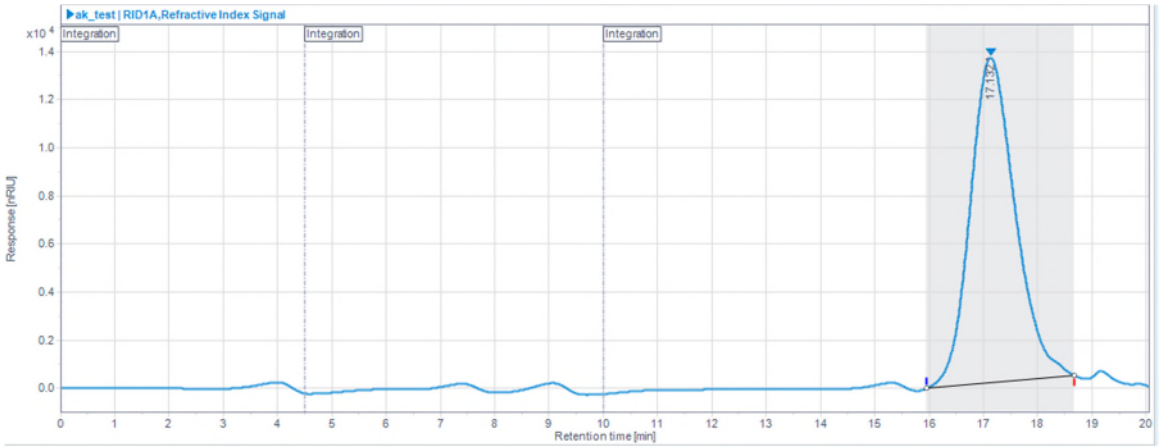


Figure S4: GPC data for polymer **223**

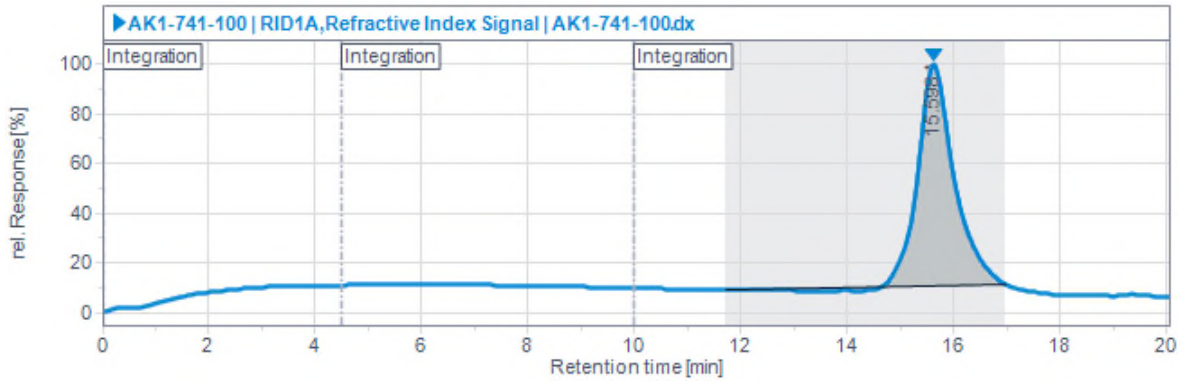
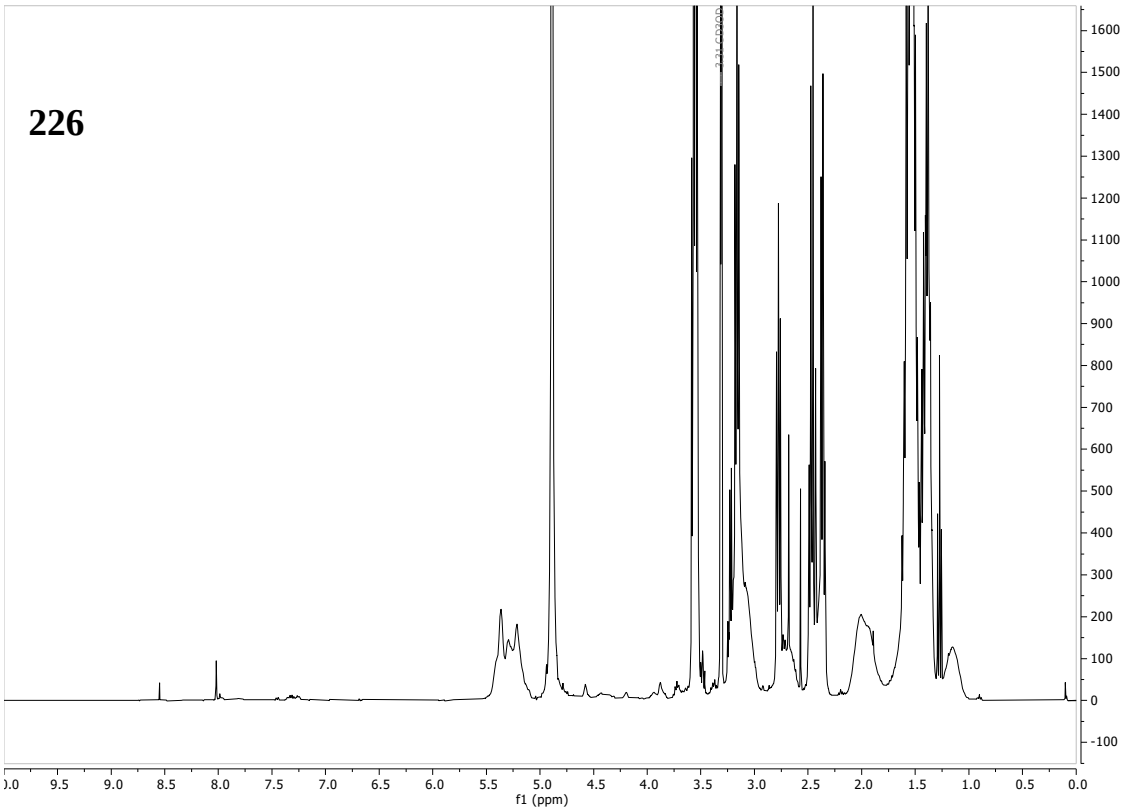


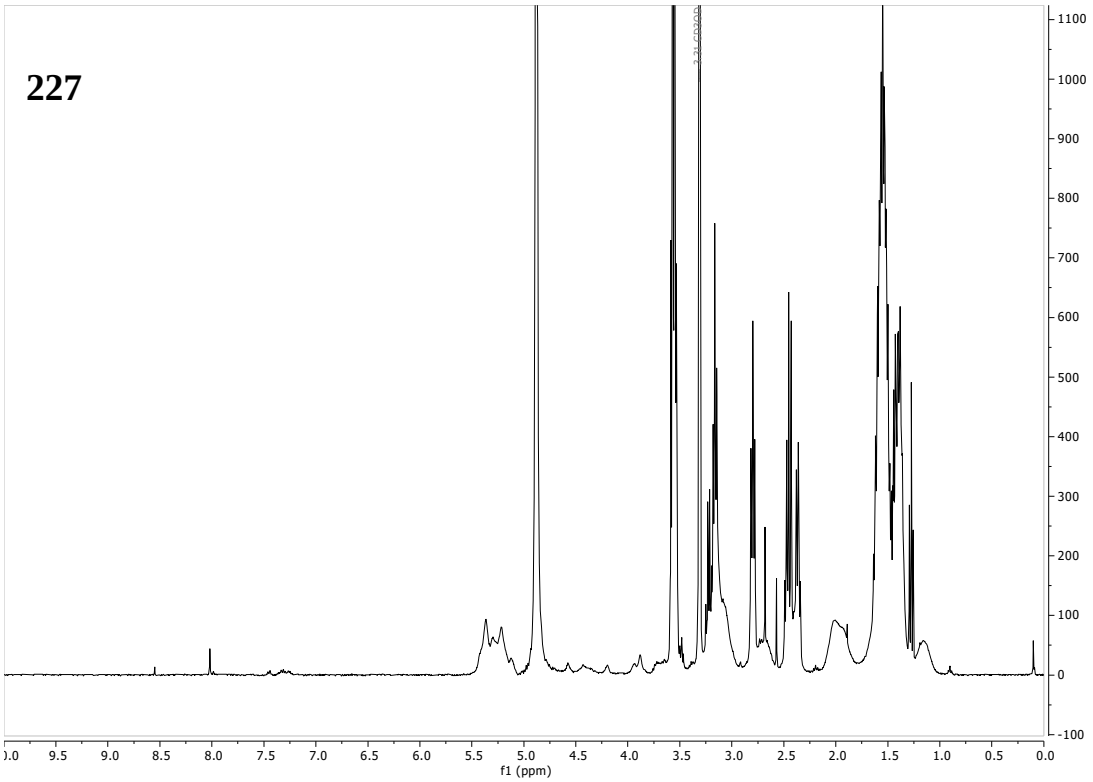
Figure S5: GPC data for polymer **224**.

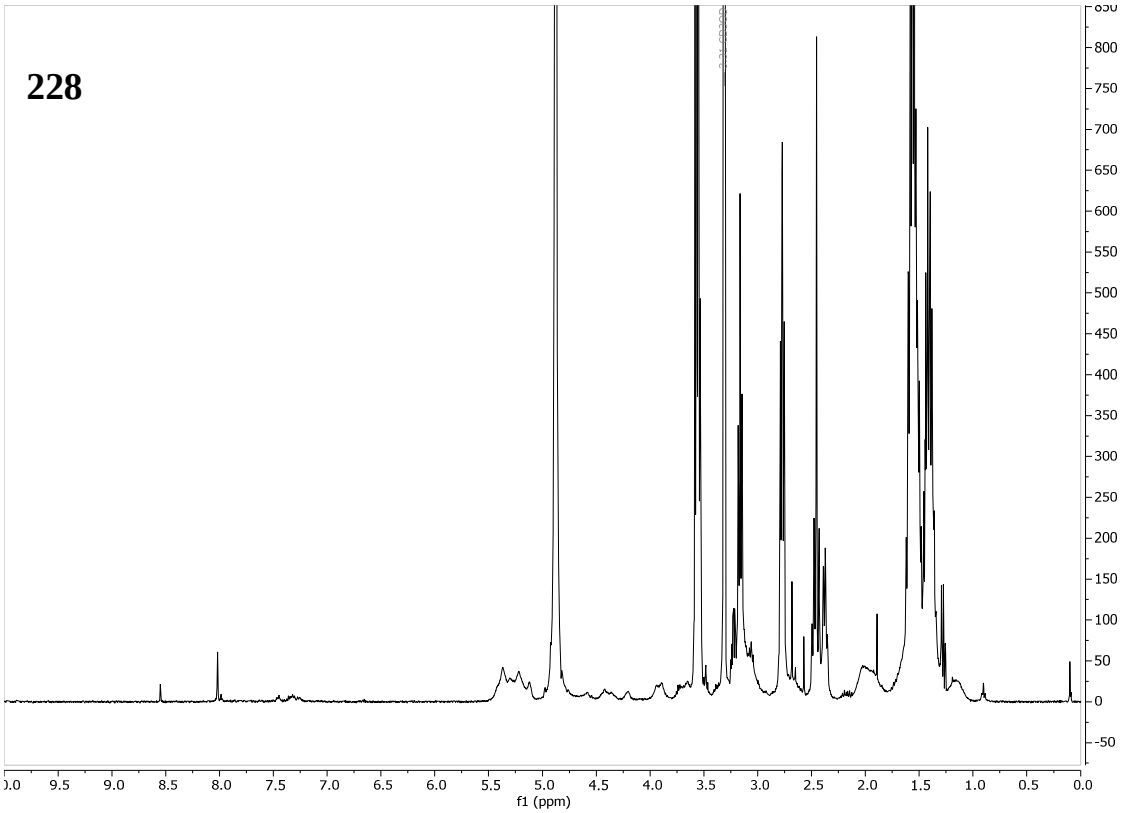
10.7 ^{1}H NMR data for glycopolymers 226, 227 and 228

226



227




228
f1 (ppm)

11. Bibliography

(1) Hanson, S. R.; Best, M. D.; Wong, C. H. Sulfatases: Structure, mechanism, biological activity, inhibition, and synthetic utility. *Angewandte Chemie-International Edition* **2004**, *43* (43), 5736-5763. DOI: 10.1002/anie.200300632.

(2) Schelwies, M.; Brinson, D.; Otsuki, S.; Hong, Y. H.; Lotz, M. K.; Wong, C. H.; Hanson, S. R. Glucosamine-6-sulfamate Analogues of Heparan Sulfate as Inhibitors of Endosulfatases. *Chembiochem* **2010**, *11* (17), 2393-2397. DOI: 10.1002/cbic.201000401.

(3) Coughtrie, M. W. H.; Sharp, S.; Maxwell, K.; Innes, N. P. Biology and function of the reversible sulfation pathway catalysed by human sulfotransferases and sulfatases. *Chemico-Biological Interactions* **1998**, *109* (1-3), 3-27. DOI: 10.1016/s0009-2797(97)00117-8.

(4) Bojarova, P.; Williams, S. J. Sulfotransferases, sulfatases and formylglycine-generating enzymes: a sulfation fascination. *Current Opinion in Chemical Biology* **2008**, *12* (5), 573-581. DOI: 10.1016/j.cbpa.2008.06.018.

(5) Barbeyron, T.; Brillet-Gueguen, L.; Carre, W.; Carriere, C.; Caron, C.; Czjzek, M.; Hoebeke, M.; Michel, G. Matching the Diversity of Sulfated Biomolecules: Creation of a Classification Database for Sulfatases Reflecting Their Substrate Specificity. *Plos One* **2016**, *11* (10). DOI: 10.1371/journal.pone.0164846.

(6) Boltes, I.; Czapinska, H.; Kahnert, A.; von Bulow, R.; Dierks, T.; Schmidt, B.; von Figura, K.; Kertesz, M. A.; Uson, I. 1.3 angstrom structure of arylsulfatase from Pseudomonas aeruginosa establishes the catalytic mechanism of sulfate ester cleavage in the sulfatase family. *Structure* **2001**, *9* (6), 483-491. DOI: 10.1016/s0969-2126(01)00609-8.

(7) Mueller, I.; Kahnert, A.; Pape, T.; Sheldrick, G. M.; Meyer-Klaucke, W.; Dierks, T.; Kertesz, M.; Uson, I. Crystal structure of the alkylsulfatase AtsK: Insights into the catalytic mechanism of the Fe(II) alpha-ketoglutarate-dependent dioxygenase superfamily. *Biochemistry* **2004**, *43* (11), 3075-3088, Article.

(8) Hagelueken, G.; Adams, T. M.; Wiehlmann, L.; Widow, L.; Kolmar, H.; Tummler, B.; Heinz, D. W.; Schubert, W. D. The crystal structure of SdsA1, an alkylsulfatase from Pseudomonas aeruginosa, defines a third class of sulfatases. *Proceedings of the National Academy of Sciences of the United States of America* **2006**, *103* (20), 7631-7636, Article. DOI: 10.1073/pnas.0510501103.

(9) Melino, S.; Capo, C.; Dragani, B.; Aceto, A.; Petruzzelli, R. A zinc-binding motif conserved in glyoxalase II, beta-lactamase and arylsulfatases. *Trends in Biochemical Sciences* **1998**, *23* (10), 381-382, Article. DOI: 10.1016/s0968-0004(98)01264-x.

(10) Barbeyron, T.; Brillet-Gueguen, L.; Carre, W.; Carriere, C.; Caron, C.; Czjzek, M.; Hoebeke, M.; Michel, G. Matching the Diversity of Sulfated Biomolecules: Creation of a Classification Database for Sulfatases Reflecting Their Substrate Specificity. *Plos One* **2016**, *11* (10), 33, Article. DOI: 10.1371/journal.pone.0164846.

(11) Appel, M. J.; Bertozzi, C. R. Formylglycine, a Post-Translationally Generated Residue with Unique Catalytic Capabilities and Biotechnology Applications. *Acs Chemical Biology* **2015**, *10* (1), 72-84, Review. DOI: 10.1021/cb500897w.

(12) Dierks, T.; Schmidt, B.; Borissenko, L. V.; Peng, J. H.; Preusser, A.; Mariappan, M.; von Figura, K. Multiple sulfatase deficiency is caused by mutations in the gene encoding the human C-alpha-formylglycine generating enzyme. *Cell* **2003**, *113* (4), 435-444. DOI: 10.1016/s0092-8674(03)00347-7.

(13) Schlotawa, L.; Ennemann, E. C.; Radhakrishnan, K.; Schmidt, B.; Chakrapani, A.; Christen, H. J.; Moser, H.; Steinmann, B.; Dierks, T.; Gartner, J. SUMF1 mutations affecting stability and activity of formylglycine generating enzyme predict clinical outcome in multiple sulfatase deficiency. *European Journal of Human Genetics* **2011**, *19* (3), 253-261. DOI: 10.1038/ejhg.2010.219.

(14) Lukatela, G.; Krauss, N.; Theis, K.; Selmer, T.; Gieselmann, V.; von Figura, K.; Saenger, W. Crystal structure of human arylsulfatase A: The aldehyde function and the metal ion at the active site suggest a novel mechanism for sulfate ester hydrolysis. *Biochemistry* **1998**, *37* (11), 3654-3664. DOI: 10.1021/bi9714924.

(15) Recksiek, R.; Selmer, T.; Dierks, T.; Schmidt, B.; von Figura, K. Sulfatases, trapping of the sulfated enzyme intermediate by substituting the active site formylglycine. *Journal of Biological Chemistry* **1998**, *273* (11), 6096-6103, Article. DOI: 10.1074/jbc.273.11.6096.

(16) Chai, C. L. L.; Loughlin, W. A.; Lowe, G. The stereochemical course of sulphuryl transfer catalysed by arylsulphatase II from Aspergillus oryzae. *Biochemical Journal* **1992**, *287*, 805-812, Article. DOI: 10.1042/bj2870805.

(17) Marino, T.; Russo, N.; Toscano, M. Catalytic Mechanism of the Arylsulfatase Promiscuous Enzyme from Pseudomonas Aeruginosa. *Chemistry-a European Journal* **2013**, *19* (6), 2185-2192. DOI: 10.1002/chem.201201943.

(18) Morimoto-Tomita, M.; Uchimura, K.; Werb, Z.; Hemmerich, S.; Rosen, S. D. Cloning and characterization of two extracellular heparin-degrading endosulfatases in mice and humans. *Journal of Biological Chemistry* **2002**, *277* (51), 49175-49185. DOI: 10.1074/jbc.M205131200.

(19) Pempe, E. H.; Burch, T. C.; Law, C. J.; Liu, J. Substrate specificity of 6-O-endosulfatase (Sulf-2) and its implications in synthesizing anticoagulant heparan sulfate. *Glycobiology* **2012**, *22* (10), 1353-1362. DOI: 10.1093/glycob/cws092.

(20) Seffouh, A.; El Masri, R.; Makshakova, O.; Gout, E.; Hassoun, Z. E.; Andrieu, J. P.; Lortat-Jacob, H.; Vives, R. R. Expression and purification of recombinant extracellular sulfatase HSulf-2 allows deciphering of enzyme sub-domain coordinated role for the binding and 6-O-desulfation of heparan sulfate. *Cellular and Molecular Life Sciences* **2019**, *76* (9), 1807-1819. DOI: 10.1007/s00018-019-03027-2.

(21) Rosen, S. D.; Lemjabbar-Alaoui, H. Sulf-2: an extracellular modulator of cell signaling and a cancer target candidate. *Expert Opinion on Therapeutic Targets* **2010**, *14* (9), 935-949. DOI: 10.1517/14728222.2010.504718.

(22) Sahota, A. P.; Dhoot, G. K. A novel SULF1 splice variant inhibits Wnt signalling but enhances angiogenesis by opposing SULF1 activity. *Experimental Cell Research* **2009**, *315* (16), 2752-2764. DOI: 10.1016/j.yexcr.2009.06.029.

(23) Milz, F.; Harder, A.; Neuhaus, P.; Breitkreuz-Korff, O.; Walhom, V.; Lubke, T.; Anselmetti, D.; Dierks, T. Cooperation of binding sites at the hydrophilic domain of cell-surface sulfatase Sulf1 allows for dynamic interaction of the enzyme with its substrate heparan sulfate. *Biochimica Et Biophysica Acta-General Subjects* **2013**, *1830* (11), 5287-5298. DOI: 10.1016/j.bbagen.2013.07.014.

(24) Staples, G. O.; Shi, X. F.; Zaia, J. Glycomics Analysis of Mammalian Heparan Sulfates Modified by the Human Extracellular Sulfatase HSulf2. *Plos One* **2011**, *6* (2). DOI: 10.1371/journal.pone.0016689.

(25) Nagamine, S.; Tamba, M.; Ishimine, H.; Araki, K.; Shiomi, K.; Okada, T.; Ohto, T.; Kunita, S.; Takahashi, S.; Wismans, R. G. P.; et al. Organ-specific Sulfation Patterns of Heparan Sulfate Generated by Extracellular Sulfatases Sulf1 and Sulf2 in Mice. *Journal of Biological Chemistry* **2012**, *287* (12), 9579-9590. DOI: 10.1074/jbc.M111.290262.

(26) Saad, O. M.; Ebel, H.; Uchimura, K.; Rosen, S. D.; Bertozzi, C. R.; Leary, J. A. Compositional profiling of heparin/heparan sulfate using mass spectrometry: assay for specificity of a novel extracellular human endosulfatase. *Glycobiology* **2005**, *15* (8), 818-826. DOI: 10.1093/glycob/cwi064.

(27) Chiu, L. T.; Sabbavarapu, N. M.; Lin, W. C.; Fan, C. Y.; Wu, C. C.; Cheng, T. J. R.; Wong, C. H.; Hung, S. C. Trisaccharide Sulfate and Its Sulfonamide as an Effective Substrate and Inhibitor of Human Endo-O-sulfatase-1. *Journal of the American Chemical Society* **2020**, *142* (11), 5282-5292. DOI: 10.1021/jacs.0c00005.

(28) Pomin, V. H.; Mulloy, B. Glycosaminoglycans and Proteoglycans. *Pharmaceuticals* **2018**, *11* (1). DOI: 10.3390/ph11010027.

(29) Kreuger, J.; Spillmann, D.; Li, J. P.; Lindahl, U. Interactions between heparan sulfate and proteins: the concept of specificity. *Journal of Cell Biology* **2006**, *174* (3), 323-327. DOI: 10.1083/jcb.200604035.

(30) Nadanaka, S.; Kitagawa, H. Heparan sulphate biosynthesis and disease. *Journal of Biochemistry* **2008**, *144* (1), 7-14. DOI: 10.1093/jb/mvn040.

(31) Malavaki, C. J.; Theocharis, A. D.; Lamari, F. N.; Kanakis, I.; Tsegenidis, T.; Tzanakakis, G. N.; Karamanos, N. K. Heparan sulfate: biological significance, tools for biochemical analysis and structural characterization. *Biomedical Chromatography* **2011**, *25* (1-2), 11-20. DOI: 10.1002/bmc.1536.

(32) Shriver, Z.; Capila, I.; Venkataraman, G.; Sasisekharan, R. Heparin and heparan sulfate: analyzing structure and microheterogeneity. *Handbook of experimental pharmacology* **2012**, (207), 159-176. DOI: 10.1007/978-3-642-23056-1_8.

(33) Kreuger, J.; Kjellen, L. Heparan Sulfate Biosynthesis: Regulation and Variability. *Journal of Histochemistry & Cytochemistry* **2012**, *60* (12), 898-907. DOI: 10.1369/0022155412464972.

(34) Uchimura, K.; Morimoto-Tomita, M.; Bistrup, A.; Li, J.; Lyon, M.; Gallagher, J.; Werb, Z.; Rosen, S. D. HSulf-2, an extracellular endoglucosamine-6-sulfatase, selectively mobilizes heparin- bound growth factors and chemokines: effects on VEGF, FGF-1, and SDF-1. *BMC Biochemistry* **2006**, *7*. DOI: 10.1186/1471-2091-7-2.

(35) Billings, P. C.; Pacifici, M. Interactions of signaling proteins, growth factors and other proteins with heparan sulfate: mechanisms and mysteries. *Connective Tissue Research* **2015**, *56* (4), 272-280. DOI: 10.3109/03008207.2015.1045066.

(36) Schlessinger, J.; Plotnikov, A. N.; Ibrahimi, O. A.; Eliseenkova, A. V.; Yeh, B. K.; Yayon, A.; Linhardt, R. J.; Mohammadi, M. Crystal structure of a ternary FGF-FGFR-heparin complex reveals a dual role for heparin in FGFR binding and dimerization. *Molecular Cell* **2000**, *6* (3), 743-750. DOI: 10.1016/s1097-2765(00)00073-3.

(37) Lai, J. P.; Sandhu, D. S.; Yu, C. R.; Han, T.; Moser, C. D.; Jackson, K. K.; Guerrero, R. B.; Aderca, I.; Isomoto, H.; Garrity-Park, M. M.; et al. Sulfatase 2 up-regulates glypican 3, promotes fibroblast growth factor signaling, and decreases survival in hepatocellular carcinoma. *Hepatology* **2008**, *47* (4), 1211-1222. DOI: 10.1002/hep.22202.

(38) Ai, X. B.; Do, A. T.; Lozynska, O.; Kusche-Gullberg, M.; Lindahl, U.; Emerson, C. P. QSulf1 remodels the 6-O sulfation states of cell surface heparan sulfate proteoglycans to promote Wnt signaling. *Journal of Cell Biology* **2003**, *162* (2), 341-351. DOI: 10.1083/jcb.200212083.

(39) Kleinschmit, A.; Koyama, T.; Dejima, K.; Hayashi, Y.; Kamimura, K.; Nakato, H. Drosophila heparan sulfate 6-O endosulfatase regulates Wingless morphogen gradient formation. *Developmental Biology* **2010**, *345* (2), 204-214. DOI: 10.1016/j.ydbio.2010.07.006.

(40) Hassing, H. C.; Surendran, R. P.; Derudas, B.; Verrijken, A.; Francque, S. M.; Mooij, H. L.; Moens, S. J. B.; t Hart, L. M.; Nijpels, G.; Dekker, J. M.; et al. SULF2 Strongly Prediposes to Fasting and Postprandial Triglycerides in Patients with Obesity and Type 2 Diabetes Mellitus. *Obesity* **2014**, *22* (5), 1309-1316. DOI: 10.1002/oby.20682.

(41) Alhasan, A. A.; Spielhofer, J.; Kusche-Gullberg, M.; Kirby, J. A.; Ali, S. Role of 6-O-Sulfated Heparan Sulfate in Chronic Renal Fibrosis. *Journal of Biological Chemistry* **2014**, *289* (29), 20295-20306. DOI: 10.1074/jbc.M114.554691.

(42) Kim, H.-J.; Kim, H.-S.; Hong, Y.-H. Sulfatase 1 and sulfatase 2 as novel regulators of macrophage antigen presentation and phagocytosis. *Yeungnam University journal of medicine* **2021**, *38* (4), 326-336. DOI: 10.12701/yujm.2021.01025.

(43) Maltseva, I.; Chan, M.; Kalus, I.; Dierks, T.; Rosen, S. D. The SULFs, Extracellular Sulfatases for Heparan Sulfate, Promote the Migration of Corneal Epithelial Cells during Wound Repair. *Plos One* **2013**, *8* (8). DOI: 10.1371/journal.pone.0069642.

(44) Singer, M. S.; Phillips, J. J.; Lemjabbar-Alaoui, H.; Wang, Y. Q.; Wu, J.; Goldman, R.; Rosen, S. D. SULF2, a heparan sulfate endosulfatase, is present in the blood of healthy individuals and increases in cirrhosis. *Clinica Chimica Acta* **2015**, *440*, 72-78. DOI: 10.1016/j.cca.2014.10.038.

(45) Phillips, J. J.; Huillard, E.; Robinson, A. E.; Ward, A.; Lum, D. H.; Polley, M. Y.; Rosen, S. D.; Rowitch, D. H.; Werb, Z. Heparan sulfate sulfatase SULF2 regulates PDGFR alpha signaling and growth in human and mouse malignant glioma. *Journal of Clinical Investigation* **2012**, *122* (3), 911-922. DOI: 10.1172/jci58215.

(46) Zhu, C. F.; He, L.; Zhou, X.; Nie, X.; Gu, Y. Sulfatase 2 promotes breast cancer progression through regulating some tumor-related factors. *Oncology Reports* **2016**, *35* (3), 1318-1328. DOI: 10.3892/or.2015.4525.

(47) Khurana, A.; Beleford, D.; He, X. P.; Chien, J.; Shridhar, V. Role of heparan sulfatases in ovarian and breast cancer. *American Journal of Cancer Research* **2013**, *3* (1), 34-45.

(48) Yang, J. D.; Sun, Z. F.; Hu, C. L.; Lai, J. P.; Dove, R.; Nakamura, I.; Lee, J. S.; Thorgeirsson, S. S.; Kang, K. J.; Chu, I. S.; et al. Sulfatase 1 and Sulfatase 2 in Hepatocellular Carcinoma: Associated Signaling Pathways, Tumor Phenotypes, and Survival. *Gene Chromosomes Cancer* **2011**, *50* (2), 122-135. DOI: 10.1002/gcc.20838.

(49) Li, J. S.; Kleeff, J.; Abiatari, I.; Kayed, H.; Giese, N. A.; Felix, K.; Giese, T.; Buchler, M. W.; Friess, H. Enhanced levels of Hsulf-1 interfere with heparin-binding growth factor signaling in pancreatic cancer. *Molecular Cancer* **2005**, *4*. DOI: 10.1186/1476-4598-4-14.

(50) Abiatari, I.; Kleeff, J.; Li, J.; Felix, K.; Buchler, M. W.; Friess, H. Hsulf-1 regulates growth and invasion of pancreatic cancer cells. *Journal of Clinical Pathology* **2006**, *59* (10), 1052-1058. DOI: 10.1136/jcp.2005.031716.

(51) Hur, K.; Han, T. S.; Jung, E. J.; Yu, J.; Lee, H. J.; Kim, W. H.; Goel, A.; Yang, H. K. Up-regulated expression of sulfatases (SULF1 and SULF2) as prognostic and metastasis predictive markers in human gastric cancer. *Journal of Pathology* **2012**, *228* (1), 88-98. DOI: 10.1002/path.4055.

(52) Lee, H. Y.; Yeh, B. W.; Chan, T. C.; Yang, K. F.; Li, W. M.; Huang, C. N.; Ke, H. L.; Li, C. C.; Yeh, H. C.; Liang, P. I.; et al. Sulfatase-1 overexpression indicates poor prognosis in urothelial carcinoma of the urinary bladder and upper tract. *Oncotarget* **2017**, *8* (29), 47216-47229. DOI: 10.18632/oncotarget.17590.

(53) Guedes, N.; Kopitzki, S.; Echeverria, B.; Pazos, R.; Elosegui, E.; Calvo, J.; Reichardt, N. C. Solid-phase assembly of glycosaminoglycan oligosaccharide precursors. *Rsc Advances* **2015**, *5* (13), 9325-9327. DOI: 10.1039/c4ra09854d.

(54) Hahm, H. S.; Schlegel, M. K.; Hurevich, M.; Eller, S.; Schuhmacher, F.; Hofmann, J.; Pagel, K.; Seeberger, P. H. Automated glycan assembly using the Glyconeer 2.1 synthesizer. *Proceedings of the National Academy of Sciences of the United States of America* **2017**, *114* (17), E3385-E3389. DOI: 10.1073/pnas.1700141114.

(55) Budhadev, D.; Saxby, K.; Walton, J.; Davies, G.; Tyler, P. C.; Schworer, R.; Fascione, M. A. Using automated glycan assembly (AGA) for the practical synthesis of heparan sulfate oligosaccharide precursors. *Organic & Biomolecular Chemistry* **2019**, *17* (7), 1817-1821. DOI: 10.1039/c8ob02756k.

(56) Kuberan, B.; Lech, M. Z.; Beeler, D. L.; Wu, Z. L. L.; Rosenberg, R. D. Enzymatic synthesis of antithrombin III-binding heparan sulfate pentasaccharide. *Nature Biotechnology* **2003**, *21* (11), 1343-1346. DOI: 10.1038/nbt885.

(57) Xu, Y.; Liu, J. Chemoenzymatic synthesis of structurally homogeneous ultra-low molecular weight heparins. US 11203772, 2021.

(58) Pawar, N. J.; Wang, L.; Higo, T.; Bhattacharya, C.; Kancharla, P. K.; Zhang, F. M.; Baryal, K.; Huo, C. X.; Liu, J.; Linhardt, R. J.; et al. Expedient Synthesis of Core Disaccharide Building Blocks from Natural Polysaccharides for Heparan Sulfate Oligosaccharide Assembly. *Angewandte Chemie-International Edition* **2019**, *58* (51), 18577-18583. DOI: 10.1002/anie.201908805.

(59) Pater, R. H.; Coelho, R. A.; Mowery, D. F. Chromatographic Adsorption. VI. Isomer Distribution and Mechanism of Formation of the Methyl Glycosides of d-Glucose and D-Galactose by the Fischer Method. *Journal of Organic Chemistry* **1973**, *38* (19), 3272-3277. DOI: 10.1021/jo00959a007.

(60) Edward, J. T. STABILITY OF GLYCOSIDES TO ACID HYDROLYSIS - A CONFORMATIONAL ANALYSIS. *Chemistry & Industry* **1955**, (36), 1102-1104.

(61) Lemieux, R. U.; Kullnig, R. K.; Bernstein, H. J.; Schneider, W. G. Configurational Effects on the Proton Magnetic Resonance Spectra of Six-membered Ring Compounds. *Journal of the American Chemical Society* **1958**, *80* (22), 6098-6105. DOI: 10.1021/ja01555a051.

(62) Roy, B.; Mukhopadhyay, B. Sulfuric acid immobilized on silica: an excellent catalyst for Fischer type glycosylation. *Tetrahedron Letters* **2007**, *48* (22), 3783-3787. DOI: 10.1016/j.tetlet.2007.03.165.

(63) Bornaghi, L. F.; Poulsen, S. A. Microwave-accelerated Fischer glycosylation. *Tetrahedron Letters* **2005**, *46* (20), 3485-3488. DOI: 10.1016/j.tetlet.2005.03.126.

(64) Shaikh, N.; Russo, L.; Cipolla, L.; Nicotra, F. Ultrasonic assisted Fischer glycosylation: generating diversity for glycochemistry. *Molecular Diversity* **2011**, *15* (2), 341-345. DOI: 10.1007/s11030-010-9281-2.

(65) Aronow, J.; Stanetty, C.; Baxendale, I. R.; Mihovilovic, M. D. Methyl glycosides via Fischer glycosylation: translation from batch microwave to continuous flow processing. *Monatshefte Fur Chemie* **2019**, *150* (1), 11-19. DOI: 10.1007/s00706-018-2306-8.

(66) Schmidt, R. R.; Michel, J. Facile Synthesis of α- and β-O-Glycosyl Imidates; Preparation of Glycosides and Disaccharides. *Angewandte Chemie-International Edition in English* **1980**, *19* (9), 731-732. DOI: 10.1002/anie.198007311.

(67) Schmidt, R. R. New Methods for the Synthesis of Glycosides and Oligosaccharides—Are There Alternatives to the Koenigs-Knorr Method? *Angewandte Chemie-International Edition in English* **1986**, *25* (3), 212-235. DOI: 10.1002/anie.198602121.

(68) Mukaiyama, T.; Jona, H. Glycosyl fluoride - A superb glycosyl donor in glycosylation. *Proceedings of the Japan Academy Series B-Physical and Biological Sciences* **2002**, *78* (4), 73-83. DOI: 10.2183/pjab.78.73.

(69) Zeng, J.; Liu, Y.; Chen, W.; Zhao, X.; Meng, L. K.; Wan, Q. Glycosyl Sulfoxides in Glycosylation Reactions. *Topics in Current Chemistry* **2018**, *376* (4). DOI: 10.1007/s41061-018-0205-4.

(70) Zu, Y. J.; Cai, C. L.; Sheng, J. Y.; Cheng, L. L.; Feng, Y. L.; Zhang, S.; Zhang, Q.; Chai, Y. H. n-Pentenyl-Type Glycosides for Catalytic Glycosylation and Their Application in Single-Catalyst One-Pot Oligosaccharide Assemblies. *Organic Letters* **2019**, *21* (20), 8270-8274. DOI: 10.1021/acs.orglett.9b03038.

(71) Codee, J. D. C.; Litjens, R.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. Thioglycosides in sequential glycosylation strategies. *Chemical Society Reviews* **2005**, *34* (9), 769-782. DOI: 10.1039/b417138c.

(72) Lian, G. Y.; Zhang, X. H.; Yu, B. Thioglycosides in Carbohydrate Research. *Carbohydrate Research* **2015**, *403*, 13-22. DOI: 10.1016/j.carres.2014.06.009.

(73) Veeneman, G. H.; Vanleeuwen, S. H.; Vanboom, J. H. Iodonium ion promoted reactions at the anomeric centre. II An efficient thioglycoside mediated approach toward the formation of 1,2-trans linked glycosides and glycosidic esters. *Tetrahedron Letters* **1990**, *31* (9), 1331-1334. DOI: 10.1016/s0040-4039(00)88799-7.

(74) Das, R.; Mukhopadhyay, B. Chemical O-Glycosylations: An Overview. *Chemistryopen* **2016**, *5* (5), 401-433. DOI: 10.1002/open.201600043.

(75) Cai, L.; Meng, L. K.; Zeng, J.; Wan, Q. Sequential activation of thioglycosides enables one-pot glycosylation. *Organic Chemistry Frontiers* **2021**, *8* (12), 3150-3165. DOI: 10.1039/d0qo01414a.

(76) Zhu, X. M.; Schmidt, R. R. New Principles for Glycoside-Bond Formation. *Angewandte Chemie-International Edition* **2009**, *48* (11), 1900-1934. DOI: 10.1002/anie.200802036.

(77) Mootoo, D. R.; Date, V.; Fraserreid, B. n-Pentenyl glycosides permit the chemospecific liberation of the anomeric center. *Journal of the American Chemical Society* **1988**, *110* (8), 2662-2663. DOI: 10.1021/ja00216a057.

(78) Fraserreid, B.; Wu, Z. F.; Udodong, U. E.; Ottosson, H. Armed/disarmed effects in glycosyl donors: rationalization and sidetracking. *Journal of Organic Chemistry* **1990**, *55* (25), 6068-6070. DOI: 10.1021/jo00312a004.

(79) Chang, C. W.; Wu, C. H.; Lin, M. H.; Liao, P. H.; Chang, C. C.; Chuang, H. H.; Lin, S. C.; Lam, S.; Verma, V. P.; Hsu, C. P.; et al. Establishment of Guidelines for the Control of Glycosylation Reactions and Intermediates by Quantitative Assessment of Reactivity. *Angewandte Chemie-International Edition* **2019**, *58* (47), 16775-16779. DOI: 10.1002/anie.201906297.

(80) Chang, C. W.; Lin, M. H.; Chan, C. K.; Su, K. Y.; Wu, C. H.; Lo, W. C.; Lam, S.; Cheng, Y. T.; Liao, P. H.; Wong, C. H.; et al. Automated Quantification of Hydroxyl Reactivities: Prediction of Glycosylation Reactions. *Angewandte Chemie-International Edition* **2021**, *60* (22), 12413-12423. DOI: 10.1002/anie.202013909.

(81) Zhang, Z. Y.; Ollmann, I. R.; Ye, X. S.; Wischnat, R.; Baasov, T.; Wong, C. H. Programmable one-pot oligosaccharide synthesis. *Journal of the American Chemical Society* **1999**, *121* (4), 734-753. DOI: 10.1021/ja982232s.

(82) Hossain, M. M.; Hosono-Fukao, T.; Tang, R.; Sugaya, N.; van Kuppevelt, T. H.; Jenniskens, G. J.; Kimata, K.; Rosen, S. D.; Uchimura, K. Direct detection of HSulf-1 and HSulf-2 activities on extracellular heparan sulfate and their inhibition by PI-88. *Glycobiology* **2010**, *20* (2), 175-186. DOI: 10.1093/glycob/cwp159.

(83) Zheng, X.; Gai, X. H.; Han, S. S.; Moser, C. D.; Hu, C. L.; Shire, A. M.; Floyd, R. A.; Roberts, L. R. The human sulfatase 2 inhibitor 2,4-disulfonylphenyl-tert-butylnitrone (OKN-007) has an antitumor effect in hepatocellular carcinoma mediated via suppression of TGFB1/SMAD2 and Hedgehog/GLI1 signaling. *Gene Chromosomes Cancer* **2013**, *52* (3), 225-236. DOI: 10.1002/gcc.22022.

(84) Hanson, S. R.; Whalen, L. J.; Wong, C. H. Synthesis and evaluation of general mechanism-based inhibitors of sulfatases based on (difluoro)methyl phenyl sulfate and cyclic phenyl sulfamate motifs. *Bioorganic & Medicinal Chemistry* **2006**, *14* (24), 8386-8395. DOI: 10.1016/j.bmc.2006.09.002.

(85) Miller, D. C.; Carbain, B.; Beale, G. S.; Alhasan, S. F.; Reeves, H. L.; Baisch, U.; Newell, D. R.; Golding, B. T.; Griffin, R. J. Regioselective sulfamoylation at low temperature enables concise syntheses of putative small molecule inhibitors of sulfatases. *Organic & Biomolecular Chemistry* **2015**, *13* (18), 5279-5284, Article. DOI: 10.1039/c5ob00211g.

(86) Reuillon, T.; Alhasan, S. F.; Beale, G. S.; Bertoli, A.; Brennan, A.; Cano, C.; Reeves, H. L.; Newell, D. R.; Golding, B. T.; Miller, D. C.; et al. Design and synthesis of biphenyl and biphenyl ether inhibitors of sulfatases. *Chemical Science* **2016**, *7* (4), 2821-2826. DOI: 10.1039/c5sc03612g.

(87) Bojarova, P.; Williams, S. J. Aryl sulfamates are broad spectrum inactivators of sulfatases: Effects on sulfatases from various sources. *Bioorganic & Medicinal Chemistry Letters* **2009**, *19* (2), 477-480, Article. DOI: 10.1016/j.bmcl.2008.11.059.

(88) Liu, Y.; Ahmed, V.; Hill, B.; Taylor, S. D. Synthesis of a non-hydrolyzable estrone sulfate analogue bearing the difluoromethanesulfonamide group and its evaluation as a steroid sulfatase inhibitor. *Organic & Biomolecular Chemistry* **2005**, *3* (18), 3329-3335, Article. DOI: 10.1039/b508852f.

(89) Bojarova, P.; Denehy, E.; Walker, I.; Loft, K.; De Souza, D. P.; Woo, L. W. L.; Potter, B. V. L.; McConville, M. J.; Williams, S. J. Direct evidence for ArO-S bond cleavage upon inactivation of Pseudomonas aeruginosa arylsulfamates by aryl sulfatase. *Chembiochem* **2008**, *9* (4), 613-623, Article. DOI: 10.1002/cbic.200700579.

(90) Wiliams, S. J.; Denehy, E.; Krenske, E. H. Experimental and Theoretical Insights into the Mechanisms of Sulfate and Sulfamate Ester Hydrolysis and the End Products of Type I Sulfatase Inactivation by Aryl Sulfamates. *Journal of Organic Chemistry* **2014**, *79* (5), 1995-2005, Article. DOI: 10.1021/jo4026513.

(91) Potter, B. V. L. Steroid sulphatase inhibition via aryl sulphamates: clinical progress, mechanism and future prospects. *Journal of Molecular Endocrinology* **2018**, *61* (2), T233-T252, Review. DOI: 10.1530/jme-18-0045.

(92) Ahmed, S.; James, K.; Owen, C. P.; Patel, C. K.; Sampson, L. The mechanism of the irreversible inhibition of estrone sulfatase (ES) through the consideration of a range of methane- and amino-sulfonate-based compounds. *Bioorganic & Medicinal Chemistry Letters* **2002**, *12* (9), 1279-1282, Article. DOI: 10.1016/s0960-894x(02)00137-3.

(93) Myette, J. R.; Soundararajan, V.; Shriver, Z.; Raman, R.; Sasisekharan, R. Heparin/Heparan Sulfate 6-O-Sulfatase from Flavobacterium heparinum. *Journal of Biological Chemistry* **2009**, *284* (50), 35177-35188. DOI: 10.1074/jbc.M109.053801.

(94) Woo, L. W. L.; Purohit, A.; Reed, M. J.; Potter, B. V. L. Oestrone 3-O-(N-acetyl)sulphamate, a potential molecular probe of the active site of oestrone sulphatase. *Bioorganic & Medicinal Chemistry Letters* **1997**, *7* (24), 3075-3080, Article; Proceedings Paper. DOI: 10.1016/s0960-894x(97)10167-6.

(95) Simpson, L. S.; Widlanski, T. S. A comprehensive approach to the synthesis of sulfate esters. *Journal of the American Chemical Society* **2006**, *128* (5), 1605-1610. DOI: 10.1021/ja056086j.

(96) Blackburn, J. M.; Short, M. A.; Castanheiro, T.; Ayer, S. K.; Muellers, T. D.; Roizen, J. L. Synthesis of N-Substituted Sulfamate Esters from Sulfamic Acid Salts by Activation with Triphenylphosphine Ditriflate. *Organic Letters* **2017**, *19* (21), 6012-6015, Article. DOI: 10.1021/acs.orglett.7b03059.

(97) Fu, S. M.; Yang, H. H.; Li, G. Q.; Deng, Y. F.; Jiang, H. F.; Zeng, W. Copper(II)-Catalyzed Enantioselective Intramolecular Cyclization of N-Alkenylureas. *Organic Letters* **2015**, *17* (4), 1018-1021, Article. DOI: 10.1021/acs.orglett.5b00131.

(98) Navidpour, L.; Lu, W.; Taylor, S. D. Synthesis of alpha-fluorosulfonate and alpha-fluorosulfonamide analogues of a sulfated carbohydrate. *Organic Letters* **2006**, *8* (24), 5617-5620. DOI: 10.1021/ol062357z.

(99) Pabis, A.; Duarte, F.; Kamerlin, S. C. L. Promiscuity in the Enzymatic Catalysis of Phosphate and Sulfate Transfer. *Biochemistry* **2016**, *55* (22), 3061-3081. DOI: 10.1021/acs.biochem.6b00297.

(100) Sunden, F.; AlSadhan, I.; Lyubimov, A.; Doukov, T.; Swan, J.; Herschlag, D. Differential catalytic promiscuity of the alkaline phosphatase superfamily bimetallo core reveals mechanistic features underlying

enzyme evolution. *Journal of Biological Chemistry* **2017**, *292* (51), 20960-20974. DOI: 10.1074/jbc.M117.788240.

(101) Safaiyan, F.; Kolset, S. O.; Prydz, K.; Gottfridsson, E.; Lindahl, U.; Salmivirta, M. Selective effects of sodium chlorate treatment on the sulfation of heparan sulfate. *Journal of Biological Chemistry* **1999**, *274* (51), 36267-36273. DOI: 10.1074/jbc.274.51.36267.

(102) Kirsch, P.; Hartman, A. M.; Hirsch, A. K. H.; Empting, M. Concepts and Core Principles of Fragment-Based Drug Design. *Molecules* **2019**, *24* (23). DOI: 10.3390/molecules24234309.

(103) Hu, Y. P.; Zhong, Y. Q.; Chen, Z. G.; Chen, C. Y.; Shi, Z. H.; Zulueta, M. M. L.; Ku, C. C.; Lee, P. Y.; Wang, C. C.; Hung, S. C. Divergent Synthesis of 48 Heparan Sulfate-Based Disaccharides and Probing the Specific Sugar-Fibroblast Growth Factor-1 Interaction. *Journal of the American Chemical Society* **2012**, *134* (51), 20722-20727. DOI: 10.1021/ja3090065.

(104) Yeh, B. K.; Eliseenkova, A. V.; Plotnikov, A. N.; Green, D.; Pinnell, J.; Polat, T.; Gritli-Linde, A.; Linhardt, R. J.; Mohammadi, M. Structural basis for activation of fibroblast growth factor signaling by sucrose octasulfate. *Molecular and Cellular Biology* **2002**, *22* (20), 7184-7192. DOI: 10.1128/mcb.22.20.7184-7192.2002.

(105) Lee, J. C.; Lu, X. A.; Kulkarni, S. S.; Wen, Y. S.; Hung, S. C. Synthesis of heparin oligosaccharides. *Journal of the American Chemical Society* **2004**, *126* (2), 476-477. DOI: 10.1021/ja038244h.

(106) Hung, S. C.; Lu, X. A.; Lee, J. C.; Chang, M. D. T.; Fang, S. L.; Fan, T. C.; Zulueta, M. M. L.; Zhong, Y. Q. Synthesis of heparin oligosaccharides and their interaction with eosinophil-derived neurotoxin. *Organic & Biomolecular Chemistry* **2012**, *10* (4), 760-772. DOI: 10.1039/c1ob06415k.

(107) van den Bos, L. J.; Codee, J. D. C.; van der Toorn, J. C.; Boltje, T. J.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. Thioglycuronides: Synthesis and application in the assembly of acidic oligosaccharides. *Organic Letters* **2004**, *6* (13), 2165-2168. DOI: 10.1021/ol049380+.

(108) Scheidt, K. A.; Chen, H.; Follows, B. C.; Chemler, S. R.; Coffey, D. S.; Roush, W. R. Tris(dimethylamino)sulfonium difluorotrimethylsilicate, a mild reagent for the removal of silicon protecting groups. *Journal of Organic Chemistry* **1998**, *63* (19), 6436-6437. DOI: 10.1021/jo981215i.

(109) Bernotas, R. C.; Cube, R. V. THE USE OF PEARLMAN CATALYST FOR SELECTIVE N-DEBENZYLATION IN THE PRESENCE OF BENZYL ETHERS. *Synth. Commun.* **1990**, *20* (8), 1209-1212, Article. DOI: 10.1080/00397919008052829.

(110) Crawford, C. J.; Qiao, Y.; Liu, Y. Q.; Huang, D. M.; Yan, W. J.; Seeberger, P. H.; Oscarson, S.; Chen, S. Defining the Qualities of High-Quality Palladium on Carbon Catalysts for Hydrogenolysis. *Organic Process Research & Development* **2021**, *25* (7), 1573-1578. DOI: 10.1021/acs.oprd.0c00536.

(111) Hanessian, S.; Liak, T. J.; Vanasse, B. Facile Cleavage of Benzyl Ethers by Catalytic Transfer Hydrogenation. *Synthesis-Stuttgart* **1981**, (5), 396-397.

(112) Sakagami, M.; Hamana, H. A selective ring opening reaction of 4,6-O-benzylidene acetals in carbohydrates using trialkylsilane derivatives. *Tetrahedron Letters* **2000**, *41* (29), 5547-5551. DOI: 10.1016/s0040-4039(00)00877-7.

(113) Goddard-Borger, E. D.; Stick, R. V. An efficient, inexpensive, and shelf-stable diazotransfer reagent: Imidazole-1-sulfonyl azide hydrochloride. *Organic Letters* **2007**, *9* (19), 3797-3800. DOI: 10.1021/ol701581g.

(114) Ye, H.; Liu, R. H.; Li, D. M.; Liu, Y. H.; Yuan, H. X.; Guo, W. K.; Zhou, L. F.; Cao, X. F.; Tian, H. Q.; Shen, J.; et al. A Safe and Facile Route to Imidazole-1-sulfonyl Azide as a Diazotransfer Reagent. *Organic Letters* **2013**, *15* (1), 18-21. DOI: 10.1021/ol3028708.

(115) Titz, A.; Radic, Z.; Schwardt, O.; Ernst, B. A safe and convenient method for the preparation of triflyl azide, and its use in diazo transfer reactions to primary amines. *Tetrahedron Letters* **2006**, *47* (14), 2383-2385. DOI: 10.1016/j.tetlet.2006.01.157.

(116) Jensen, H. H.; Nordstrom, L. U.; Bols, M. The disarming effect of the 4,6-acetal group on glycoside reactivity: Torsional or electronic? *Journal of the American Chemical Society* **2004**, *126* (30), 9205-9213. DOI: 10.1021/ja047578j. Fraserreid, B.; Wu, Z. F.; Andrews, C. W.; Skowronski, E.; Bowen, J. P. Torsional effects in glycoside reactivity: saccharide couplings mediated by acetal protecting groups. *Journal of the American Chemical Society* **1991**, *113* (4), 1434-1435. DOI: 10.1021/ja00004a066.

(117) Pedersen, C. M.; Marinescu, L. G.; Bols, M. Conformationally armed glycosyl donors: reactivity quantification, new donors and one pot reactions. *Chemical Communications* **2008**, (21), 2465-2467. DOI: 10.1039/b801305e.

(118) Park, S. S.; Hsieh, H. W.; Gervay-Hague, J. Anomeric O-Functionalization of Carbohydrates for Chemical Conjugation to Vaccine Constructs. *Molecules* **2018**, *23* (7). DOI: 10.3390/molecules23071742.

(119) Frem, D.; Urban, D.; Norsikian, S.; Beau, J. M. From Chitin to alpha-Glycosides of N-Acetylglucosamine Using Catalytic Copper Triflate in a Heated Sealed-Vessel Reactor. *European Journal of Organic Chemistry* **2017**, *2017* (34), 5094-5101. DOI: 10.1002/ejoc.201700933.

(120) Wei, G. H.; Lv, X.; Du, Y. U. FeCl3-catalyzed alpha-glycosidation of glycosamine pentaacetates. *Carbohydrate Research* **2008**, *343* (18), 3096-3099. DOI: 10.1016/j.carres.2008.09.003.

(121) Nielsen, M. M.; Pedersen, C. M. Catalytic Glycosylations in Oligosaccharide Synthesis. *Chemical Reviews* **2018**, *118* (17), 8285-8358. DOI: 10.1021/acs.chemrev.8b00144.

(122) Hock, S.; Marti, R.; Riedl, R.; Simeunovic, M. Thermal Cleavage of the Fmoc Protection Group. *Chimia* **2010**, *64* (3), 200-202. DOI: 10.2533/chimia.2010.200.

(123) Ngoje, G.; Li, Z. T. Study of the stereoselectivity of 2-azido-2-deoxyglucosyl donors: protecting group effects. *Organic & Biomolecular Chemistry* **2013**, *11* (11), 1879-1886. DOI: 10.1039/c3ob26994a.

(124) Ma, Y. Y.; Lian, G. Y.; Li, Y.; Yu, B. A. Identification of 3,6-di-O-acetyl-1,2,4-O-orthoacetyl-alpha-D-glucopyranose as a direct evidence for the 4-O-acyl group participation in glycosylation. *Chemical Communications* **2011**, *47* (26), 7515-7517. DOI: 10.1039/c1cc11680k.

(125) Schurmann, M.; Janning, P.; Ziegler, S.; Waldmann, H. Small-Molecule Target Engagement in Cells. *Cell Chemical Biology* **2016**, *23* (4), 435-441. DOI: 10.1016/j.chembiol.2016.03.008.

(126) Seath, C. P.; Trowbridge, A. D.; Muir, T. W.; MacMillan, D. W. C. Reactive intermediates for interactome mapping. *Chemical Society Reviews* **2021**, *50* (5), 2911-2926. DOI: 10.1039/d0cs01366h.

(127) Li, Z. Q.; Hao, P. L.; Li, L.; Tan, C. Y. J.; Cheng, X. M.; Chen, G. Y. J.; Sze, S. K.; Shen, H. M.; Yao, S. Q. Design and Synthesis of Minimalist Terminal Alkyne-Containing Diazirine Photo-Crosslinkers and Their Incorporation into Kinase Inhibitors for Cell- and Tissue-Based Proteome Profiling. *Angewandte Chemie-International Edition* **2013**, *52* (33), 8551-8556. DOI: 10.1002/anie.201300683.

(128) Ichiishi, N.; Moore, K. P.; Wassermann, A. M.; Wolkenberg, S. E.; Krska, S. W. Reducing Limitation in Probe Design: The Development of a Diazirine-Compatible Suzuki-Miyaura Cross Coupling Reaction. *Acs Medicinal Chemistry Letters* **2019**, *10* (1), 56-61. DOI: 10.1021/acsmedchemlett.8b00403.

(129) Geri, J. B.; Oakley, J. V.; Reyes-Robles, T.; Wang, T.; McCarver, S. J.; White, C. H.; Rodriguez-Rivera, F. P.; Parker, D. L.; Hett, E. C.; Fadeyi, O. O.; et al. Microenvironment mapping via Dexter energy transfer on immune cells. *Science* **2020**, *367* (6482), 1091-+. DOI: 10.1126/science.aay4106.

(130) Turro, N. J.; Cha, Y.; Gould, I. R.; Moss, R. A. Direct photochemical population of triplet diazirines: comparison of triplet energies by direct excitation and by energy transfer. *Journal of Photochemistry* **1987**, *37* (1), 81-86. DOI: 10.1016/0047-2670(87)85029-3.

(131) Sauvageot, E.; Lafite, P.; Duverger, E.; Marion, R.; Hamel, M.; Gaillard, S.; Renaud, J. L.; Daniellou, R. Iridium complexes inhibit tumor necrosis factor-alpha by utilizing light and mixed ligands. *Journal of Organometallic Chemistry* **2016**, *808*, 122-127. DOI: 10.1016/j.jorganchem.2016.02.001.

(132) Fan, K.; Nie, W. X.; Wang, L. P.; Liao, C. H.; Bao, S. S.; Zheng, L. M. Defective Metal-Organic Frameworks Incorporating Iridium-Based Metalloligands: Sorption and Dye Degradation Properties. *Chemistry-a European Journal* **2017**, *23* (27), 6615-6624. DOI: 10.1002/chem.201700365.

(133) Huang, S. L.; Liu, N. F.; Ling, Y.; Luo, H. K. Ir-III-based Octahedral Metalloligands Derived Primitive Cubic Frameworks for Enhanced CO2/N-2 Separation. *Chemistry-an Asian Journal* **2017**, *12* (24), 3110-3113. DOI: 10.1002/asia.201701339.

(134) Morimoto-Tomita, M.; Uchimura, K.; Bistrup, A.; Lum, D. H.; Egeblad, M.; Boudreau, N.; Werb, Z.; Rosen, S. D. Sulf-2, a proangiogenic heparan sulfate endosulfatase, is upregulated in breast cancer. *Neoplasia* **2005**, *7* (11), 1001-1010. DOI: 10.1593/neo.05496.

(135) Nie, L. J.; Zhang, H.; Wu, Z. K.; Wang, Y. B.; Liu, H.; Wu, X. L.; Wang, W. Modified TCA/acetone precipitation of plant proteins for proteomic analysis. *Plos One* **2018**, *13* (12). DOI: 10.1371/journal.pone.0202238.

(136) Arsiwala, A.; Castro, A.; Frey, S.; Stathos, M.; Kane, R. S. Designing Multivalent Ligands to Control Biological Interactions: From Vaccines and Cellular Effectors to Targeted Drug Delivery. *Chemistry-an Asian Journal* **2019**, *14* (2), 244-255. DOI: 10.1002/asia.201801677.

(137) Erlendsson, S.; Teilum, K. Binding Revisited-Avidity in Cellular Function and Signaling. *Frontiers in Molecular Biosciences* **2021**, *7*. DOI: 10.3389/fmolb.2020.615565.

(138) Oh, Y. I.; Sheng, G. J.; Chang, S. K.; Hsieh-Wilson, L. C. Tailored Glycopolymers as Anticoagulant Heparin Mimetics. *Angewandte Chemie-International Edition* **2013**, *52* (45), 11796-11799. DOI: 10.1002/anie.201306968.

(139) Xu, P.; Laval, S.; Guo, Z.; Yu, B. Microwave-assisted simultaneous O,N-sulfonation in the synthesis of heparin-like oligosaccharides. *Organic Chemistry Frontiers* **2016**, *3* (1), 103-109. DOI: 10.1039/c5qo00320b.

(140) Sultane, P. R.; Mete, T. B.; Bhat, R. G. A convenient protocol for the deprotection of N-benzyloxycarbonyl (Cbz) and benzyl ester groups. *Tetrahedron Letters* **2015**, *56* (16), 2067-2070. DOI: 10.1016/j.tetlet.2015.02.131.

(141) Gestwicki, J. E.; Cairo, C. W.; Mann, D. A.; Owen, R. M.; Kiessling, L. L. Selective immobilization of multivalent ligands for surface plasmon resonance and fluorescence microscopy. *Analytical Biochemistry* **2002**, *305* (2), 149-155. DOI: 10.1006/abio.2002.5652.

(142) Gordon, E. J.; Gestwicki, J. E.; Strong, L. E.; Kiessling, L. L. Synthesis of end-labeled multivalent ligands for exploring cell-surface-receptor-ligand interactions. *Chemistry & Biology* **2000**, *7* (1), 9-16. DOI: 10.1016/s1074-5521(00)00060-0.

(143) Nagarkar, A. A.; Kilbinger, A. F. M. End functional ROMP polymers via degradation of a ruthenium Fischer type carbene. *Chemical Science* **2014**, *5* (12), 4687-4692. DOI: 10.1039/c4sc02242d.

(144) Pontrello, J. K.; Allen, M. J.; Underbakke, E. S.; Kiessling, L. L. Solid-phase synthesis of polymers using the ring-opening metathesis polymerization. *Journal of the American Chemical Society* **2005**, *127* (42), 14536-14537. DOI: 10.1021/ja053931p.

(145) Nagarkar, A. A.; Crochet, A.; Fromm, K. M.; Kilbinger, A. F. M. Efficient Amine End-Functionalization of Living Ring-Opening Metathesis Polymers. *Macromolecules* **2012**, *45* (11), 4447-4453. DOI: 10.1021/ma300602p.

(146) Mangold, S. L.; Carpenter, R. T.; Kiessling, L. L. Synthesis of fluorogenic polymers for visualizing cellular internalization. *Organic Letters* **2008**, *10* (14), 2997-3000. DOI: 10.1021/ol800932w.

(147) Madkour, A. E.; Koch, A. H. R.; Lienkamp, K.; Tew, G. N. End-Functionalized ROMP Polymers for Biomedical Applications. *Macromolecules* **2010**, *43* (10), 4557-4561. DOI: 10.1021/ma100330u.

(148) Vinczer, P.; Juvancz, Z.; Novak, L.; Szantay, C. An Improved Synthesis of 5-hydroxypentanal. *Organic Preparations and Procedures International* **1989**, *21* (3), 344-346. DOI: 10.1080/00304948909356391.

(149) Zhu, Y. X.; Li, X. M.; Kyazike, J.; Zhou, Q.; Thurberg, B. L.; Raben, N.; Mattaliano, R. J.; Cheng, S. H. Conjugation of mannose 6-phosphate-containing oligosaccharides to acid alpha-glucosidase improves the clearance of glycogen in Pompe mice. *Journal of Biological Chemistry* **2004**, *279* (48), 50336-50341. DOI: 10.1074/jbc.M409676200.

(150) Gary-Bobo, M.; Nirde, P.; Jeanjean, A.; Morere, A.; Garcia, M. Mannose 6-phosphate receptor targeting and its applications in human diseases. *Current Medicinal Chemistry* **2007**, *14* (28), 2945-2953. DOI: 10.2174/092986707782794005.

(151) Li, L.; Cen, J.; Pan, W. H.; Zhang, Y. B.; Leng, X. X.; Tan, Z. Q.; Yin, H.; Liu, S. Y. Synthesis of Polypeptides with High-Fidelity Terminal Functionalities under NCA Monomer-Starved Conditions. *Research* **2021**, *2021*. DOI: 10.34133/2021/9826046.

(152) Hyun, J. Y.; Kim, S.; Lee, H. S.; Shin, I. A Glycoengineered Enzyme with Multiple Mannose-6-Phosphates Is Internalized into Diseased Cells to Restore Its Activity in Lysosomes. *Cell Chemical Biology* **2018**, *25* (10), 1255-+. DOI: 10.1016/j.chembiol.2018.07.011.

(153) Saito, H.; Nishimura, Y.; Kondo, S.; Takeuchi, T. Synthesis of Carbocyclic Lignan Variants Related to Podophyllotoxin. *Chemistry Letters* **1988**, (7), 1235-1238.

(154) Jacquinet, J. C.; Petitou, M.; Duchaussoy, P.; Lederman, I.; Choay, J.; Torri, G.; Sinay, P. Synthesis of heparin fragments. A chemical synthesis of the trisaccharide O-(2-deoxy-2-sulfamido-3,6-di-O-sulfo-α-d-glucopyranosyl)-(1→4)-O-(2-O-sulfo-α-l-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamido-6-O-sulfo-d-glucopyranose heptasodium salt. *Carbohydrate Research* **1984**, *130* (JUL), 221-241. DOI: 10.1016/0008-6215(84)85281-7.

(155) Soderberg, E.; Westman, J.; Oscarson, S. Rapid carbohydrate protecting group manipulations assisted by microwave dielectric heating. *Journal of Carbohydrate Chemistry* **2001**, *20* (5), 397-410. DOI: 10.1081/car-100105712.

(156) Goud, P. M.; Venkatachalam, T. K.; Uckun, F. M. Introduction of a carbohydrate moiety into the structure of thiourea compounds targeting HIV-1 reverse transcriptase. *Synthetic Communications* **2003**, *33* (7), 1185-1193. DOI: 10.1081/scc-120017195.

(157) Li, T. H.; Ye, H.; Cao, X. F.; Wang, J. J.; Liu, Y. H.; Zhou, L. F.; Liu, Q.; Wang, W. J.; Shen, J.; Zhao, W.; et al. Total Synthesis of Anticoagulant Pentasaccharide Fondaparinux. *Chemmedchem* **2014**, *9* (5), 1071-1080. DOI: 10.1002/cmdc.201400019.

(158) Polat, T.; Wong, C. H. Anomeric reactivity-based one-pot synthesis of heparin-like oligosaccharides. *Journal of the American Chemical Society* **2007**, *129* (42), 12795-12800. DOI: 10.1021/ja073098r.

(159) Yoza, K.; Amanokura, N.; Ono, Y.; Akao, T.; Shinmori, H.; Takeuchi, M.; Shinkai, S.; Reinhoudt, D. N. Sugar-integrated gelators of organic solvents - Their remarkable diversity in gelation ability and aggregate structure. *Chemistry-a European Journal* **1999**, *5* (9), 2722-2729. DOI: 10.1002/(sici)1521-3765(19990903)5:9<2722::Aid-chem2722>3.0.Co;2-n.

(160) Tatina, M.; Yousuf, S. K.; Mukherjee, D. 2,4,6-Trichloro-1,3,5-triazine (TCT) mediated one-pot sequential functionalisation of glycosides for the generation of orthogonally protected monosaccharide building blocks. *Organic & Biomolecular Chemistry* **2012**, *10* (28), 5357-5360. DOI: 10.1039/c2ob25452b.

(161) Lv, Y. M.; Laborda, P.; Huang, K.; Cai, Z. P.; Wang, M.; Lu, A. M.; Doherty, C.; Liu, L.; Flitsch, S. L.; Voglmeir, J. Highly efficient and selective biocatalytic production of glucosamine from chitin. *Green Chemistry* **2017**, *19* (2), 527-535. DOI: 10.1039/c6gc02910h.

(162) Muldoon, J.; Ashcroft, A. E.; Wilson, A. J. Selective Protein-Surface Sensing Using Ruthenium(II) Tris(bipyridine) Complexes. *Chemistry-a European Journal* **2010**, *16* (1), 100-103. DOI: 10.1002/chem.200902368.

(163) Yu, H. H.; Xiao, L.; Yang, X. C.; Shao, L. M. Controllable access to multi-substituted imidazoles via palladium(II)-catalyzed C-C coupling and C-N condensation cascade reactions. *Chemical Communications* **2017**, *53* (70), 9745-9748. DOI: 10.1039/c7cc05315k.

(164) Cottet, F.; Marull, M.; Lefebvre, O.; Schlosser, M. Recommendable routes to trifluoromethyl-substituted pyridine- and quinolinecarboxylic acids. *European Journal of Organic Chemistry* **2003**, *2003* (8), 1559-1568.

(165) Yerushalmi, S. M.; Buck, M. E.; Lynn, D. M.; Lemcoff, N. G.; Meijler, M. M. Multivalent alteration of quorum sensing in Staphylococcus aureus. *Chemical Communications* **2013**, *49* (45), 5177-5179. DOI: 10.1039/c3cc41645c.

(166) Murphy, P. J.; Williams, H. L.; Hibbs, D. E.; Hursthouse, M. B.; Malik, K. M. A. Biomimetic model studies towards ptilomycalin A. *Tetrahedron* **1996**, *52* (24), 8315-8332. DOI: 10.1016/0040-4020(96)00385-7.

(167) Magoulas, G. E.; Afroudakis, P.; Georgikopoulou, K.; Roussaki, M.; Borsari, C.; Fotopoulou, T.; Santarem, N.; Barrias, E.; Nevado, P. T.; Hachenberg, J.; et al. Design, Synthesis and Antiparasitic Evaluation of Click Phospholipids. *Molecules* **2021**, *26* (14). DOI: 10.3390/molecules26144204.

(168) Rubinshtein, M.; James, C. R.; Young, J. L.; Ma, Y. J.; Kobayashi, Y.; Gianneschi, N. C.; Yang, J. Facile Procedure for Generating Side Chain Functionalized Poly(alpha-hydroxy acid) Copolymers from Aldehydes via a Versatile Passerini-Type Condensation. *Organic Letters* **2010**, *12* (15), 3560-3563. DOI: 10.1021/ol101433v.

(169) Miyagawa, A.; Tomita, R.; Kurimoto, K.; Yamamura, H. Selective deprotection of trityl group on carbohydrate by microflow reaction inhibiting migration of acetyl group. *Synthetic Communications* **2016**, *46* (6), 556-562. DOI: 10.1080/00397911.2016.1156703.

(170) Banik, S. M.; Pedram, K.; Wisnovsky, S.; Ahn, G.; Riley, N. M.; Bertozzi, C. R. Lysosome-targeting chimaeras for degradation of extracellular proteins. *Nature* **2020**, *584* (7820), 291-+. DOI: 10.1038/s41586-020-2545-9.

(171) Ficht, S.; Payne, R. J.; Guy, R. T.; Wong, C. H. Solid-phase synthesis of peptide and glycopeptide thioesters through side-chain-anchoring strategies. *Chemistry-a European Journal* **2008**, *14* (12), 3620-3629. DOI: 10.1002/chem.200701978.

(172) Zhi, H. H.; Wang, J. D.; Wang, S. J.; Wei, Y. J. Fluorescent Properties of Hymecromone and Fluorimetric Analysis of Hymecromone in Compound Dantong Capsule. *Journal of Spectroscopy* **2013**, *2013*. DOI: 10.1155/2013/147128.