UNIVERSITY OF BIRMINGHAM

Thesis submitted to

The University of Birmingham

for the degree of

Doctor of Philosophy

The Synthesis of Galectin-3-Targeted Cancer Imaging Agents

Glenn Lees

July 2014

Supervisor: Dr J Snaith
School of Chemistry
University of Birmingham
Birmingham
B15 2TT

for Dad

Acknowledgements

First and foremost, thanks to John for your excellent supervision, support, encouragement and pragmatism – the coffees and occasional pastries were much appreciated too! Thanks also to Liam for helpful tips and to Richard for providing advice and re-homing me after my lab eviction.

Pete, Neil, Jon & Chi have my gratitude for providing excellent analytical facilities as do Katie & Rachel for ensuring that the undergraduate labs ran smoothly.

Thanks in especial to Gail for convincing me to begin my PhD studies – who would have thought that an afternoon coffee could have led to so much?

Mom – I cannot thank you enough for all the emotional, financial and domestic support you have given me; for patiently listening to my rants, putting up with my snarky moods and still making my dinner!

Thanks to Dad for all your encouragement, support and for countless chats and putting the world to right over a game of Scrabble; though you're not here at the finish, you're always in my thoughts. This work is for you.

Thanks also to past and present members of the Snaith, Davies, Cox, Grainger and Tucker groups; to Saddlebags, Teebs, Renate, Justyna, Holster, Gillie, Pam, Horniblow, Little Haydn, Squeaky, Kraken, Gem, Rosie, Kedge, Huy, James, Dan, Shoes & Fish.

Last, but by no means least, thanks to Persil for all the banter, beer and burgers; my time here would have been far less enjoyable without you!

Abstract

Galectin-3 is an animal lectin that recognises and binds to β-galactosides in glycoconjugates. It performs numerous functions within the body including mediating cell adhesion, apoptosis and the cell cycle. It is frequently over-expressed in cancerous states, potentially allowing inhibitors to serve as vector tags for targeted image contrast agents to give enhanced MRI and fluorescence images. Derivatives of lactosamine with 3'-arylamides have been shown to be excellent inhibitors of galectin-3, exploiting a π-cation interaction between the aryl ring and ARG144 of galectin-3. 3'-Naphthamide derivatives of lactosamine have previously been shown to bind to galectin-3 with $k_d < 1$ μM, and form the basis of the target imaging agent.

This project is focussed on the synthesis of 3'-naphthamide derivatives of lactosamine, the incorporation of a spacer unit and attachment of a probe molecule. Initially FITC has been used as probe, though the design of the target allows for incorporation of other probe molecules, such as paramagnetic species, with minimal changes to the synthetic pathway.

Problems with the reduction/acylation of an azide in a key disaccharide intermediate could not be resolved by alterations in the reaction conditions; incorporation of the required amide earlier in the synthesis failed to lead to disaccharide products.

Employing a phthalimide as nitrogen protecting group has successfully produced a glycosyl donor precursor that will only require ring opening rather than the troublesome reduction/acylation.

Using a propargyl group to protect the anomeric position in the glycosyl acceptor has allowed for the attachment of the linker *via* a click reaction and subsequent deprotection reactions and FITC installation has produced one of the compounds required for comparison studies.

Abbreviations

Abbreviation	Meaning
Ac	Acetyl
ADMB	4-Ally-1,2-Dimethoxybenzene
An	p-Anisyl / 4-Methoxylphenyl
Bn	Benzyl
Boc	^{t}Butyloxycarbonyl
Bu	Butyl
CRD	Carbohydrate Recognition Domain
CuAAC	Copper Catalysed Alkyne Azide Click Reaction
DCC	Dicyclohexyl Carbodiimide
DCM	Dichloromethane
DMAP	4-(Dimethylamino)pyridine
DMDS	Dimethyl Disulfide
DMF	N,N-Dimethylformamide
DOWEX	DOWEX 50WX8-200 Ion Exchange Resin (Acid Form)
DTBMP	2,6-Di-t-Butyl-4-Methylpyridine
ECM	Extra-cellular Matrix
Et	Ethyl
FDG	2-Deoxy-2-(^{18}F)-fluoro-D-glucose
FeNP	Iron Nanoparticle
FITC	Fluorescein Isothiocyanate
Gal	Galactose / Galactoside
Gal-3	Galectin-3
GFP	Green Fluorescent Protein
Glc	Glucose / Glucoside
HMBC	Heteronuclear Multiple Bond Correlation
Im	Imidazole
Lac	Lactose / Lactose Derivative
LacNAc	N-Acetyllactosamine
mCPBA	Meta-Chloroperbenzoic Acid

Abbreviation	Meaning
Me	Methyl
Ms	Mesyl / Methanesulfonyl
NADH	Nicotinamide Adenine Dinucleotide Reduced Form
NBS	N-Bromosuccinimide
NDL	Methyl *N*-Acetyl-3'-(2-naphthamido)-3'-deoxy-β-D-lactosamine
NHS	*N*-hydroxy Succinimide
NIS	N-Iodosuccinimide
NMSC	Non-Melanoma Skin Cancer
PDC	Pyridinium Dichromate
PEG	Poly(ethylene glycol)
Ph	Phenyl
Py	Pyridine
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium Hexafluorophosphate
ROS	Reactive Oxygen Species
TBAF	Tetra-^{n}butylammonium Iodide
TCAN	Trichloro Acetonitrole
TCP	Tetrachlorophthalimide
TCPA	Tetrachlorophthalic Anhydride
TES	Triethylsilane
Tf	Triflyl / Trifluoromethanesulfonyl
TFA	Trifluoroacetic Acid
TFMBL	Methyl *N*-Acetyl-3'-(2,3,5,6-tetrafluoro-4-methoxybenzamido)-3'-deoxy-β-D-lactosamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl
TNFR	Tumour Necrosis Factor Receptor
Troc	2,2,2-trichloroethoxycarbonyl
Ts	Tosyl / 4-methylbenzenesulfonyl
VEGF	Vascular Endothelial Growth Fator

1 Introduction

The primary aim of this project is to develop a new diagnostic tool to aid in the earlier detection of cancer. Cancer is a catch-all term for over 200 different types of malignant neoplastic disease, which becomes progressively more difficult to treat as the disease advances – thus there is considerable benefit from tools that allow early detection. The design of such a tool requires the understanding of healthy cell function (discussed in Section 1.2) and how processes become altered in malignant neoplasms (discussed in Section 1.3). The mode of action that the tool employs requires an understanding of current diagnostic tools and treatments (discussed in Section 1.4).

Galectin-3 is involved with many of the processes of healthy cells that become perturbed in malignant states (discussed in Section 1.5) and compounds which exhibit good binding characteristics (discussed in Section 1.5.3) have formed that basis of the synthetic target, shown in Figure 1-1.

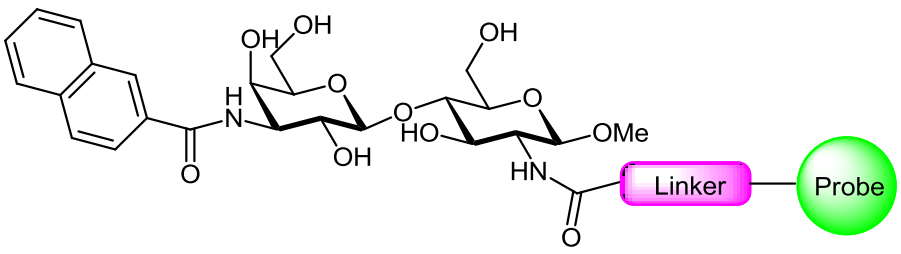


Figure 1-1: Synthetic Target

1.1 Cancer

In 2011, over 330,000 cases of cancer were diagnosed in the UK*, with over half of these being diagnoses for breast, lung, colorectal or prostate cancers (see Figure 1-2).[1]

* Excluding Non-Melanoma Skin Cancer (NMSC)

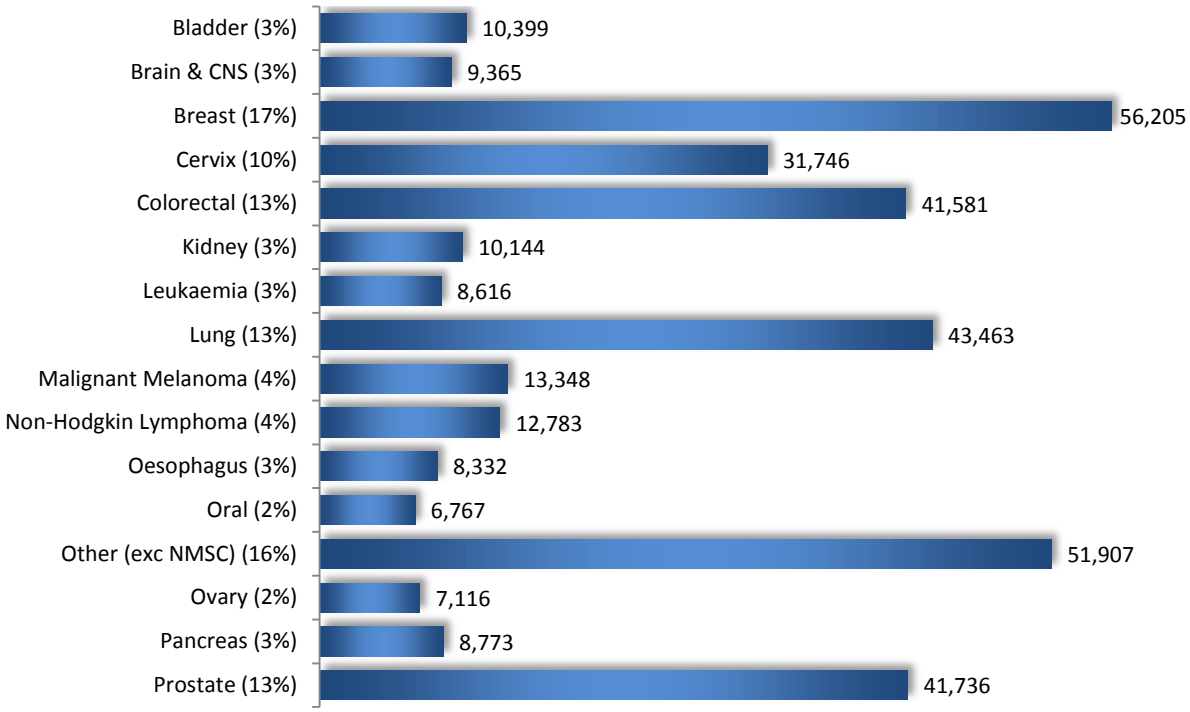


Figure 1-2: UK incidence, 2011 - Commonly Diagnosed Cancers

Cancer is predominantly an age-related disease with more than 75% of diagnosed cases in 2009-2011 being in people aged 60 and over.[2]

In 2011, cancer was attributable to the deaths of over 158 000 people, accounting for nearly a quarter of all deaths in the UK, with breast, lung, colorectal and prostate cancers causing nearly one-half of all cancer deaths (see Figure 1-3).[1]

Although incidence of cancer has increased in the period 1977-2006, mortality actually decreased by almost 20%.[3]

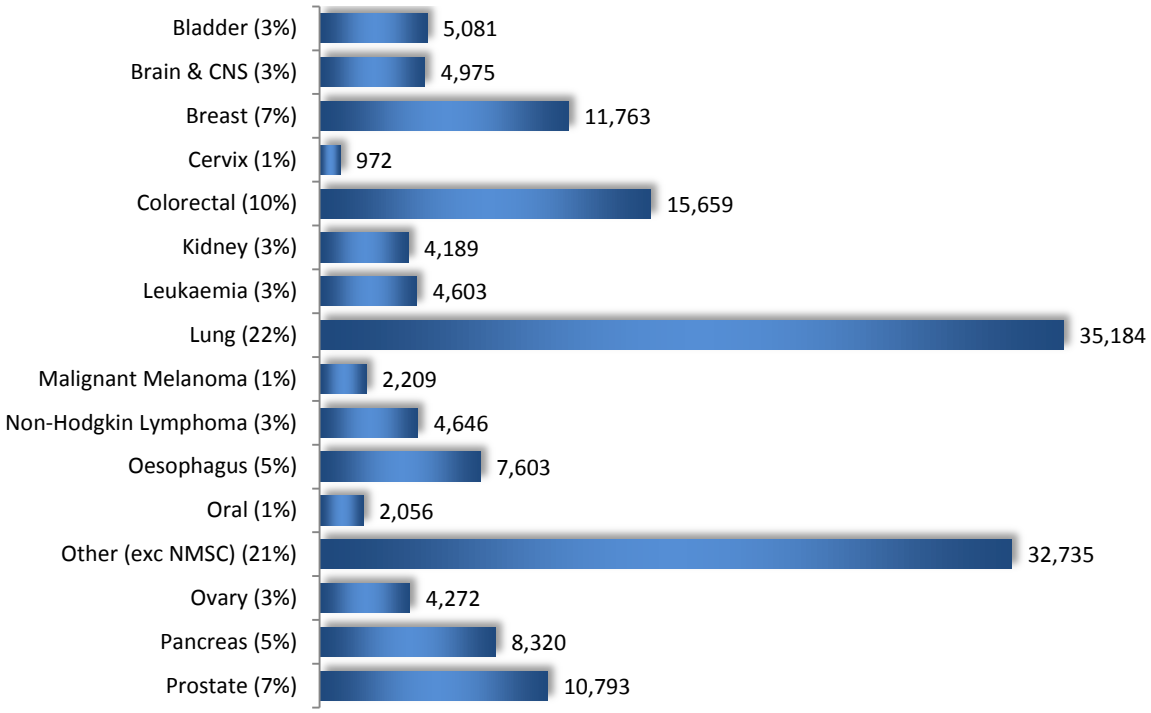


Figure 1-3: UK Mortality, 2011 - Common Deaths Caused by Cancer

1.2 Normal Cell Life

1.2.1 The Cell Cycle

The adult human body is made up of 100 million million cells,[4] only dividing when required – some dividing frequently e.g. stomach epithelia and bone marrow and some not at all e.g. neurons and skeletal muscle.

The cell cycle (see Figure 1-4) lists the stages involved in cell division. Most cells in an adult human spend most of their time in the G_0 phase – that is, not undergoing nor preparing to undergo division, performing their normal bodily functions instead.

When a growth signal is received e.g. from cell damage, positive and negative regulation factors are produced and the cell moves into the G_1 phase[5]. During this phase the equipment needed for DNA replication (such as DNA polymerase III) is synthesised. If the cell's DNA is undamaged, it will move into the S phase, where DNA synthesis occurs, otherwise the cell cycle is halted whilst repair takes

place – should the repair fail, then apoptosis will be induced. In the G_2 phase the remaining cell components are duplicated in preparation for mitosis, which occurs in the M phase. The two daughter cells may then enter a second G_0 phase or pass directly on to a second G_1 phase.

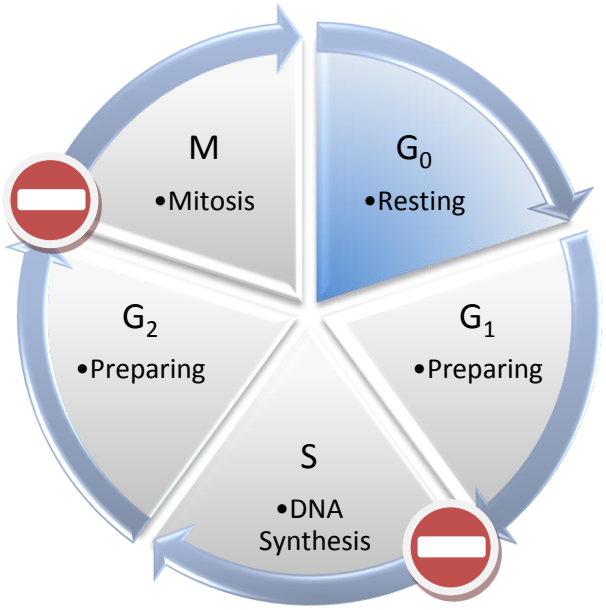


Figure 1-4: The Cell Cycle

There is a second restriction point between the G_2 and M phases – though the mechanisms operating at the point are less well-known.[5]

Extracellular actions also need to be performed during the cell cycle: the extracellular matrix (ECM) needs to be sculpted to allow room for the new cells, a process mediated by metalloproteinases, and angiogenesis need to occur in order that the new cells receive adequate blood supply.

1.2.2 Apoptosis

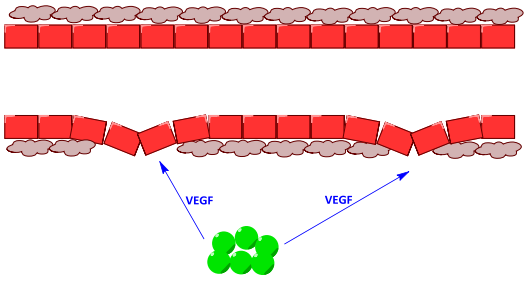
Death is essential to life. The human brain would not develop properly unless millions of neurons die during infancy.[6] The mechanism for programmed and controlled cell death is called apoptosis, where 2 different pathways operate.

Cells need constant signals to not commit suicide. These signals are local to the cells, such that if the cell migrates to a region that it is not supposed to be in, the survival signals cease and the cell undergoes apoptosis. These signals stimulate the production of anti-apoptotic Bcl-2 proteins (B-cell lymphoma type 2), thus withdrawal of the signals leads to a shift in the balance of pro- and anti apoptotic proteins which begins a cascade of reactions culminating in death. DNA damage also results in death by this pathway, as protein P53, which prevents the cell from entering the S phase of the cell cycle, also affects the pro-anti-apoptotic balance.

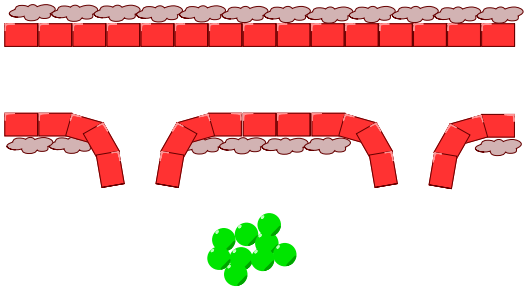
The second pathway, known as the death receptor pathway, involves receptors of the TNFR (tumour necrosis factor receptor) in the cell membrane. When the cell receives a death signal, the receptors trimerise and initiate another cascade resulting in cell death.

1.2.3 Angiogenesis

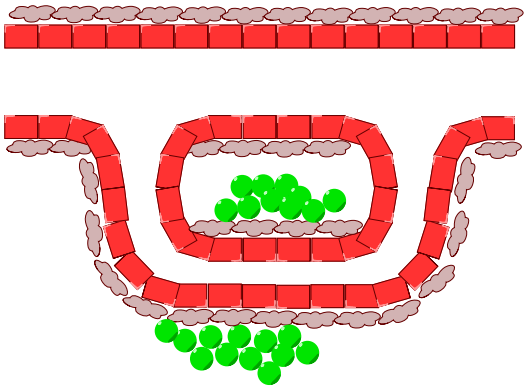
Diffusion limits the distance away from blood vessels that cells can survive to around 2 mm. Thus as tissues grow, vascularisation must occur such that the daughter cells receive adequate blood supply. The important stages of angiogenesis are:



A. VEGF (vascular endothelial growth factor) released from the dividing cells diffuses to the endothelial cells of a nearby blood vessel, causing them to migrate toward the source. Metalloproteinases are also secreted to breakdown to break down the ECM, allowing room for the new vessel.



B. Cells in the vascular endothelium divide and a lumen begins to form. Fibroblasts are stimulated to create new matrix around the growing vessel.



C. Nascent tubules form into loops and the new vessel is stabilised by cell-to-cell and cell-to-matrix binding.

1.3 Carcinogenesis

The processes of cell division, apoptosis and angiogenesis discussed above become altered in cancerous states and these changes can reasonably be ascribed to the failure of regulatory processes.

The cell cycle contains numerous checks and feedback mechanisms to ensure that the cell does not turn cancerous: genes that promote growth often trigger apoptosis should they become over-active.

Genes can be classified into two families: proto-oncogenes, which stimulate growth and tumour suppressing genes, which retard it.

In order for a cell to develop into a carcinoma, several mutations or malfunctions must occur: proto-oncogenes must become fully-fledged oncogenes, jamming the cell's growth switches into the 'on' position; tumour suppressing genes must become broken, jamming the cell's regulation and repair switches into the 'off' position; mutations must occur to render the cell independent of the survival signals needed to suppress apoptosis. All-in-all, the chances of this happening are minute – if it weren't for the mind-bogglingly enormous number of cells in the body and the fact that only one of them needs to turn cancerous, then it would be all but impossible.

These mutations confer 3 characteristics on cancer cells:

1. Uncontrolled proliferation: this says nothing about the speed of proliferation, merely that the cell's progress around the cell cycle is unrestricted.
2. Invasiveness: as the cells no longer require the local survival signals to prevent apoptosis, they can spread into areas where they would not normally be found.
3. Loss of function: as the cell contains many mutations and is constantly dividing, the biochemistry of the cell is fundamentally altered, leading to the cell ceasing to perform its normal function.

The 4th characteristic of metastasis is unique to cancer cells and infectious disease. Here, the cancer cells may leave their original location and move anywhere in the body, forming secondary tumours; the location of the secondary tumour usually has some similarity to the location of the primary tumour e.g. breast cancer cells often metastasise to the bones, as calcium is readily available in both locations.

1.4 Imaging and Treatment

The changes that occur in carcinogenesis such as altered metabolic demand and the formation of a solid mass tumour have been used to develop imaging techniques to aid in diagnosis and in treatment regimes.

1.4.1 Imaging

Accurate imaging is essential to effective treatment of cancer: the size and characteristics of a tumour may dictate which drugs should be used for treatment and the precise location of a tumour is needed for radiotherapy and surgery, such that damage to healthy tissue is minimised.

The main imaging techniques used are:

- CT Scanning – an anatomical technique using x-rays
- MR Imaging – an anatomical technique using magnetic resonance
- PET Scanning – a functional technique using radio-emissions
- Fluorescence Spectroscopy – a functional technique using fluorescence.

1.4.1.1 CT Scanning

A CT scan is essentially an advanced x-ray. In a conventional x-ray, the rays pass through the body in a single direction toward a detector. Dense tissues such as bone absorb more x-rays than diffuse tissues such as blood vessels and therefore bone appears light on the developed image. In a CT scan, several x-ray beams are used, angled at different directions in a single plane. This allows an image of a slice through the body to be taken rather than a composite image of all tissues between the x-ray source and the detector.

A contrast agent (commonly iodinated compounds) may be used to attenuate the signal from certain tissues such as blood vessels, giving greater contrast between them and the surrounding tissue.

Figure 1-5: CT Image[7]

1.4.1.2 MR Imaging

MR imaging is similar to CT scanning except that the permanent magnetic moment of the protons in hydrogen is exploited rather than the absorption of x-rays. Water is the commonest constituent of the body, and the intensity of the magnetic resonance of the protons can be used to calculate densities. Gradient magnetic fields are used to localise the source of the resonance.

MR images are often weighted with respect to longitudinal (spin-lattice) and transverse (spin-spin) relaxation times (T_1 and T_2 respectively).

When molecular motion occurs with a similar frequency to the proton resonance frequency, the protons may relax by interaction with the lattice. This reduces T_1, leading to more protons being relaxed, and therefore excitable by the subsequent magnetic pulse: resulting in increased signal in the MR image. Very small molecules such as water and very large molecules such as proteins tumble with a frequency that is very different to the resonance frequency – consequently T_1 is long, and the MRI signal weak (bulk water and proteins appear dark and fat, light.)

Motion of the protons within the molecules causes local magnetic fields and therefore field inhomogeneities. These inhomogeneities result in loss of phase coherence and thus a decay in the M_z magnetisation vector. Flexible molecules generate stronger local magnetic fields and therefore have a shorter T_2 than rigid molecules (both bulk water and fat appear light.)

MRI can be used to detect pathological conditions, for example, cerebro-spinal fluid (CSF) contains a high proportion of bulk water and therefore appears dark on T_1-weighted images and light on T_2-weighted images. If CSF leaks into the brain, it will form hydration sheaths around myelin, reducing molecular tumbling: T_1 is decreased and the MRI signal, increased.

Contrast agents are often used in MR imaging. Gadolinium (III) is paramagnetic and shortens both T_1 and T_2, the effect on image brightness depends on the composition of the tissue, the proton density and the concentration of the contrast agent; T_1 images often show brightening and T_2 images, darkening in the locality of the contrast agent.

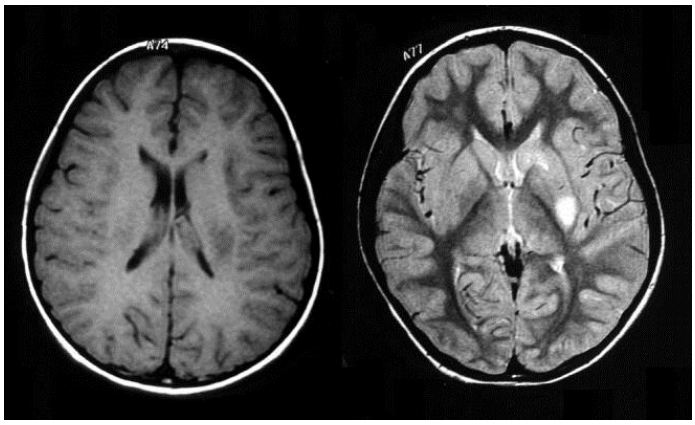


Figure 1-6: T_1 (L) and T_2 (R) Images[8]

The different magnetic properties of iron in oxyhaemoglobin (oHb) and deoxyhaemoglobin (dHb) can also be exploited. On binding to oxygen, a water molecule is displaced by an oxygen molecule. Oxygen is higher up in the spectrochemical series than water and thus produces a larger splitting (Δ_O) and results in a change from high-spin Fe^{2+} in dHb to low-spin Fe^{2+} in oHb, see Figure 1-7. As high-spin

Fe^{2+} is paramagnetic, poorly oxygenated areas will have greater contrast and stronger T_1 signals than well oxygenated areas; this forms the basis of functional MR imaging (fMRI), which is used to image brain function.

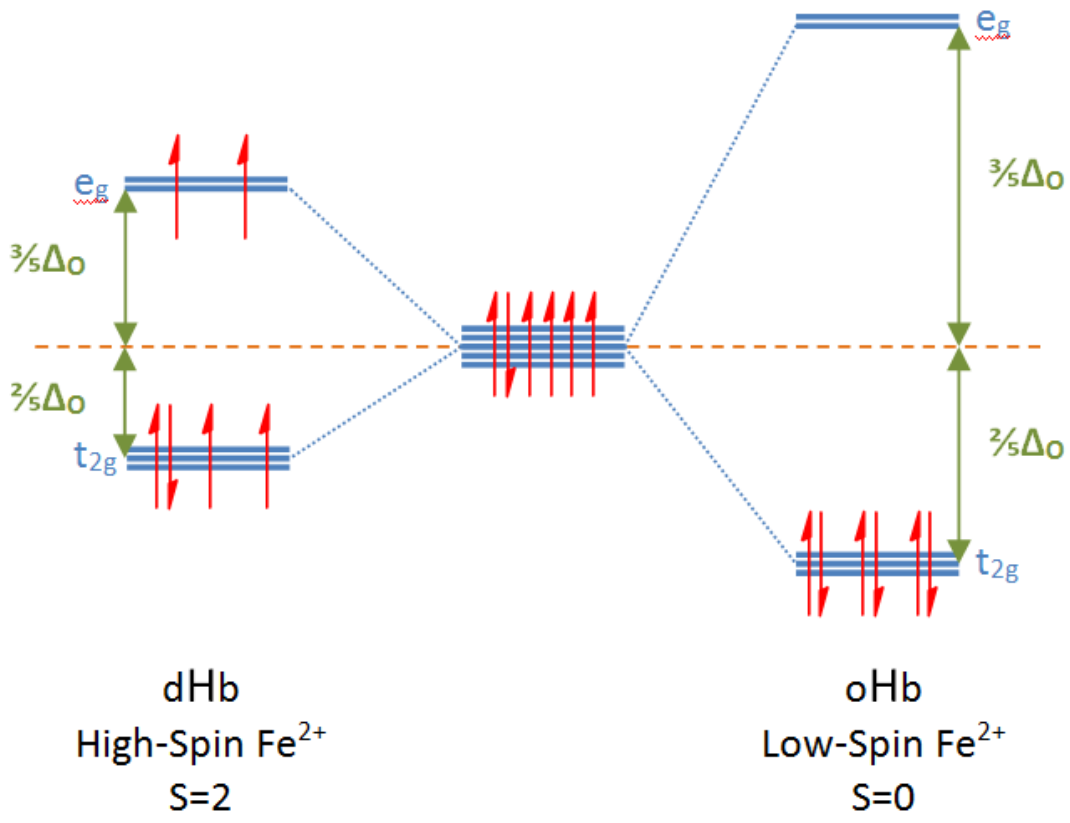


Figure 1-7: Spin States of Fe^{2+} in Deoxyhaemoglobin (dHb) and Oxyhaemoglobin (oHb)

Elemental iron is ferromagnetic and iron nanoparticles (FeNPs) have excellent potential for use as MRI contrast agents.

1.4.1.3 PET Scanning

PET scanning is a functional rather than an anatomical technique which can be used to distinguish between harmless scar tissue and cancerous tissue, because of their different metabolic demands. The technique uses positron-emitting nuclides: the positron annihilates an electron, producing 2 gamma-rays at almost 180° apart, coincident arrival of these γ-rays at the detectors allow the

location of the emission to be calculated, and an image constructed in a similar way to CT scans. ^{11}C, ^{13}N and ^{15}O can be used as the radio-nuclide but logistical problems are apparent as they have half-lives of under 20 min. ^{18}F has a half-life of 110 min and is often used in 2-deoxy-2-(^{18}F)-fluoro-D-glucose (FDG, see Figure 1-8)

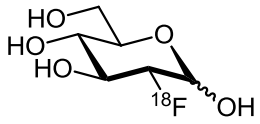


Figure 1-8: FDG

The advantages of FDG are:

1. Being a glucose derivative, it is actively absorbed by cells with high metabolic demands – such as cancer cells.
2. The emissive half-life of 110 min is long enough for the images to be acquired, but short enough for the radiation to diminish to negligible levels within 24h.
3. After entering a cell, hexokinase phosphorylates the 6-hydroxyl of FDG, preventing it from leaving the cell before emission occurs.
4. The decay product is 2-(^{18}O)-D-glucose, which is harmless.

It is often advantageous to combine PET scanning with CT scanning or MR imaging, as this combines the functional aspect of PET with the anatomical aspects of CT/MRI, examples are shown in Figure 1-9.

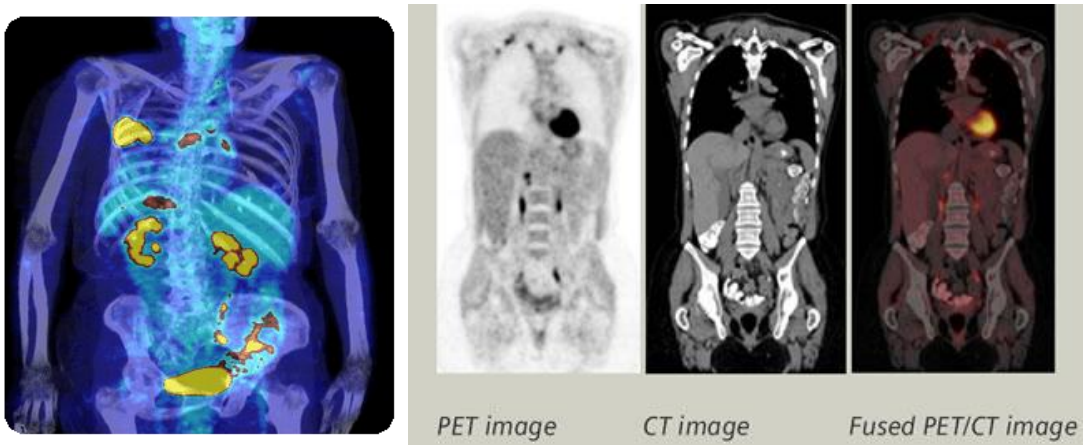


Figure 1-9: PET/CT Images[9, 10]

1.4.1.4 *Fluorescence spectroscopy*

Fluorescence spectroscopy is another important technique that uses the fluorescence of endogenous fluorophores such as porphyrins and NADH or exogenous fluorophores such as Green Fluorescent Protein (GFP), scorpion toxin and lanthanide complexes. Incident radiation is used from the UV, VIS or NIR regions and the technique can be used both *in vitro* and *in vivo*. The fluorescence of endogenous fluorophores often show a change in their emission spectrum on going from a healthy to a diseased state[11] and this can be used for diagnosis or during surgery. Exogenous fluorophores may be combined with a targeting vector, such as an antibody, so that the fluorescence only occurs at the site of interest. Forward scattering by cells limits fluorescence to being a surface technique; UV light only penetrates to a few hundred microns, whereas NIR may penetrate to several centimetres.

1.4.2 Treatment

Once diagnosed, one of the greatest difficulties in treating cancer is the difficulty in discriminating between cancerous and non-cancerous cells. Penicillins work by interfering with the mechanism that bacteria use to make cell walls; as eukaryotes do not have cell walls, it is not possible for penicillins to disrupt human cell wall production. In cancer, all of the proteins and pathways are human proteins and pathways, and it is very difficult to target the cancer cells without interfering with healthy cells.

Differences in protein expression and metabolism can be used for the discrimination and forms the basis of cancer chemotherapy.

Cancer chemotherapeutic drugs offer little more than blanket cytotoxicity and kill any cell that they accumulate in. As cancer cells have increased metabolic demand, cytotoxic drugs accumulate in them, killing the tumour cells. These drugs have significant and serious side-effects as they exert a toxic effect on any cell-line that divides frequently: on stomach epithelia, producing nausea and vomiting; on bone marrow, producing anaemia; on hair follicles, producing alopecia. Targeting these drugs such that they only show a cytotoxic effect on cancer cells is highly desirable.

High doses of ionising radiation are also cytotoxic and can be used for treatment. In order that only the tumour is targeted, several radiation beams are used, with them converging on the treatment site. The main mechanism of action of radiation damage is through the production of free radicals, notably reactive oxygen species (ROS), including the ferociously reactive hydroxyl radical. One of the main drawbacks of radiotherapy is that the tumour may outgrow its blood supply resulting in local hypoxia and limiting the amount of ROS that may be formed. This can lead to malignant cells being 2-3 times more resistant to radiation than healthy cells.

The most conceptually simple treatment for cancer is surgery to remove the tumour. Surgery is not always possible as the tumour may be in an inaccessible site, such as the brain stem or there may be no tumour, such as in leukaemia. Further complications arise from the fact that every single malignant cell must be removed, otherwise the tumour may re-grow. Thus the margin of the tumour must be known precisely and healthy cells are often removed to ensure that the entire tumour is excised.

1.4.2.1 Targeted Drug Delivery

It is highly desirable that cytotoxic drugs are only active in the target cells/tissues. In passive drug targeting, the drug or inactive drug precursor (pro-drug) is administered and preferentially accumulates in the target zone. This could be effected by specific uptake in certain cell lines or by conversion of the pro-drug in the active form by enzymes present in the target zone that are not found elsewhere. In active drug targeting, the drug actively seeks out the target, often by use of a vector tag such as an antibody. The antibody binds to specific surface proteins expressed in the target and may then be transported into the cell or simply increase the local concentration of the drug.

1.5 Galectins

1.5.1 Role of Galectins

Galectins are a family of 15[†] animal lectins which bind to β-D-galactoside residues in glyco-conjugates. The region of the protein where the carbohydrate binds is known as the carbohydrate recognition domain (CRD), some galectins contain a single CRD and form dimers or aggregates and others contain two CRDs (see Table 1-1.)[12]

[†] So far discovered

Table 1-1: Galectin Types

Type	Structure	Galectins
One CRD (Dimer)		1, 2, 5, 6, 8, 10, 11, 13, 14, 15
One CRD (Aggregates)		3
Two CRDs		4, 6, 8, 9, 12

The role of galectins in the body is varied: they act both intra- and extracellularly. Extracellularly they may form cross-links between membrane-bound glyco-conjugates and the ECM, increasing cell adhesion or they may form lattices between different membrane-bound conjugates, thereby reducing cell adhesion. Galectin-3 (Gal-3) also has a role in angiogenesis: breast cancer tissues that over-express Gal-3 have a higher density of capillaries. Intracellularly, galectins can modify signal transduction by binding to membrane-bound, cytosolic or nuclear glyco-conjugates;[12, 13] they have a role in regulating the cell cycle and can act in an anti-apoptotic manner. The exact mechanisms of these complicated and diverse actions have not been fully elucidated.

1.5.2 Galectins and Carcinogenesis

Acquisition of a cancerous phenotype is frequently coincident with a change in galectin expression. An increase in galectin production is frequently seen in cell lines that do not express a high level of galectin.[12] The actions of galectins can clearly be seen to contribute to the characteristics of cancer: altered cell adhesion contributes to invasiveness and metastasis; uncontrolled proliferation is contributed to by galectins' role in regulating the cell cycle and their anti-apoptotic effects.

1.5.3 Substrate Binding

The CRD of galectins is a saddle-shaped groove as opposed to the classical pocket of the lock-and-key analogy. Whist galectins are selective for β-D-galactose, there is sufficient room for other saccharides present to interact with the CRD, increasing binding and specificity; many substrates contain polylactosamines chains. Figure 1-10 shows N-acetyllactosamine (LacNAc) bound to Gal-3.

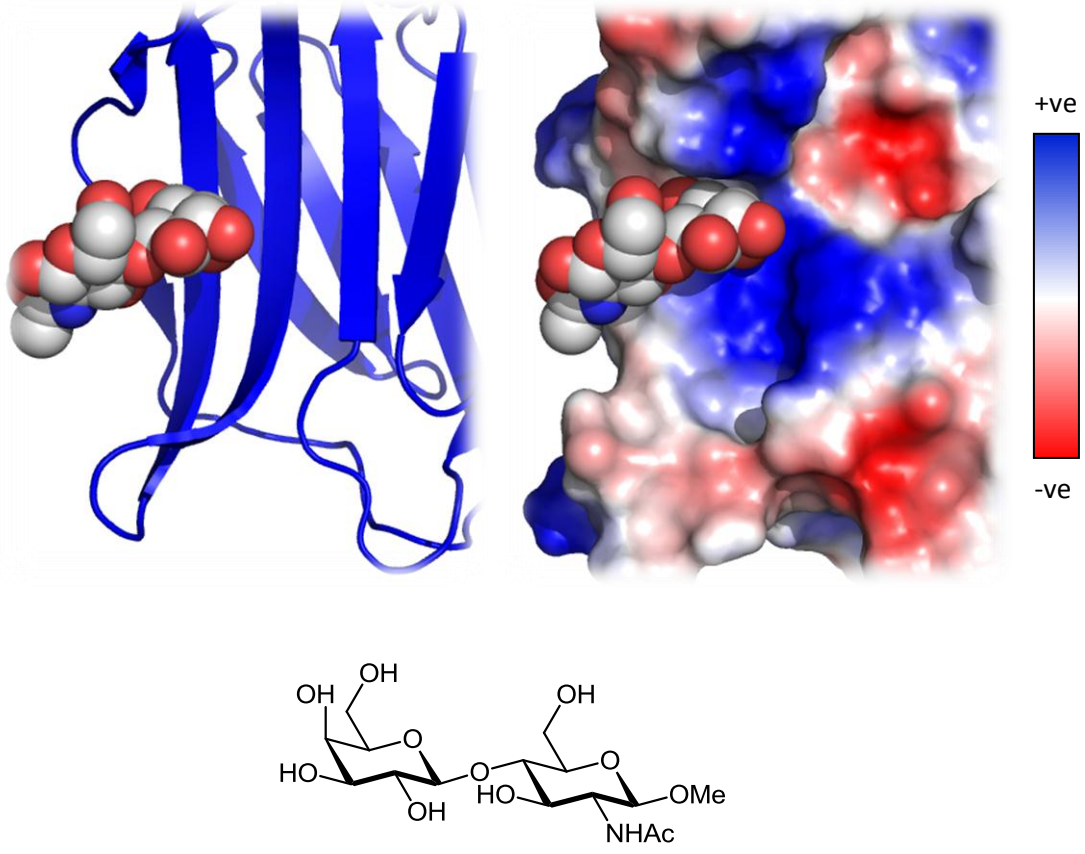


Figure 1-10: LacNAc bound to Gal-3 – created from PDB: 1KJL[14] (L: Ribbon, R: Electrostatic)

Whilst the binding to LacNAc is selective, it is also relatively weak (K_d = 67 µM.) It has been shown that 3'-arene derivatisation of LacNAc can decrease the dissociation constant by 2 orders of magnitude: methyl *N*-acetyl-3'-(2,3,5,6-tetrafluoro-4-methoxybenzamido)-3'-deoxy-lactosamine (TFMBL, Figure 1-11) binds with K_d = 880 nM.[14]

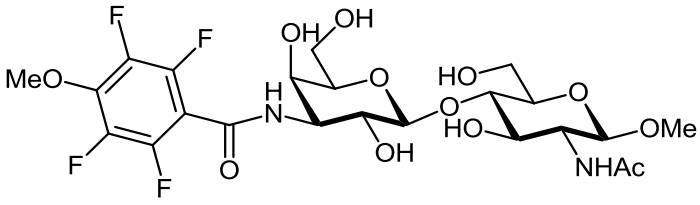


Figure 1-11: TFMBL

The increase in binding is thought to be caused by π-interactions between the 3'-arene group and ARG144 of Gal-3, there are also increased direct and indirect hydrogen bonding interactions. Figure 1-12 shows TFMBL bound to Gal-3.

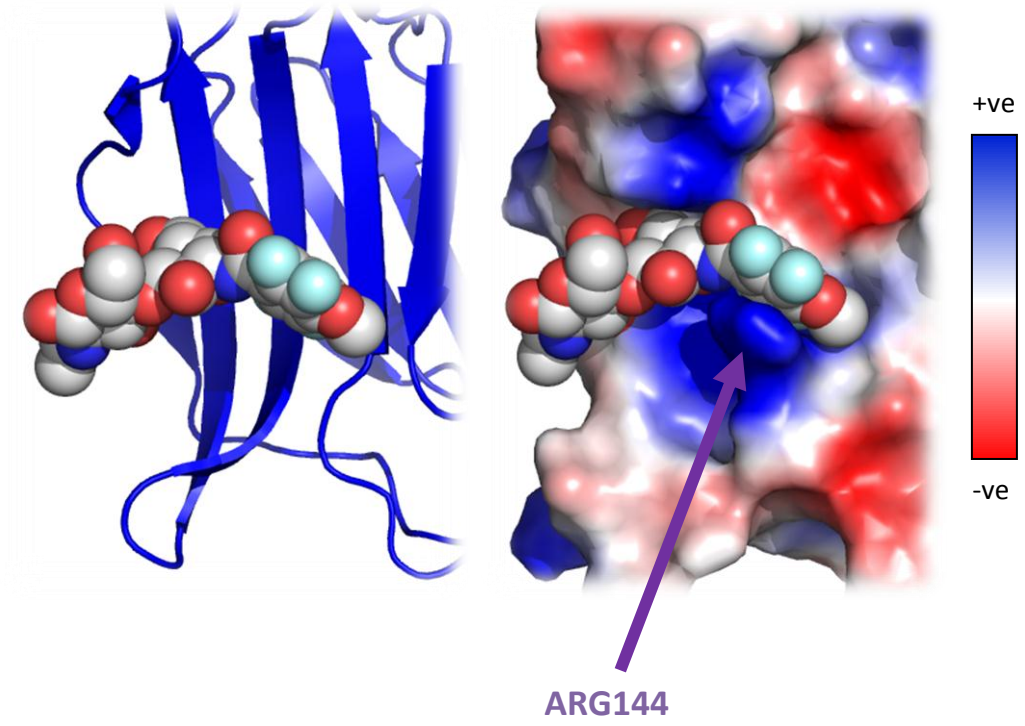


Figure 1-12: TFMBL bound to Gal-3 – created from PDB: 1KJR[14] (L: Ribbon, R: Electrostatic)

The amide group of LacNAc points away from the CRD of Gal-3, and can thus serve as a point of attachment for drug targeting.

When the substrate binds to Gal-3, the binding groove becomes more tightly curved. This change increases the distance between the side chains on the opposite face and is accompanied by an

increase in the conformational entropy of these groups. This increase helps to offset the reduction in entropy that occurs on binding.[15, 16] The entropy of binding lactose to Gal-3 has been calculated by ITC at ΔS = -15.6 J K^{-1} mol^{-1}.

1.6 Active Gal-3-Targeted Imaging and Contrast Agents

The low K_d and accessible nitrogen of 3'-arene-lactosamines allow for the synthesis of Gal-3-targeted imaging and contrast agents. Methyl *N*-acetyl-3'-(2-naphthamido)-3'-deoxy-lactosamine (NDL) exhibits stronger binding to Gal-3 (K_d = 480 nM) than either LacNAc (K_d = 67 µM) or TFMBL (K_d = 880 nM), and will form the basis of the indicator shown in Figure 1-13. Also shown are compounds required to perform comparison studies.

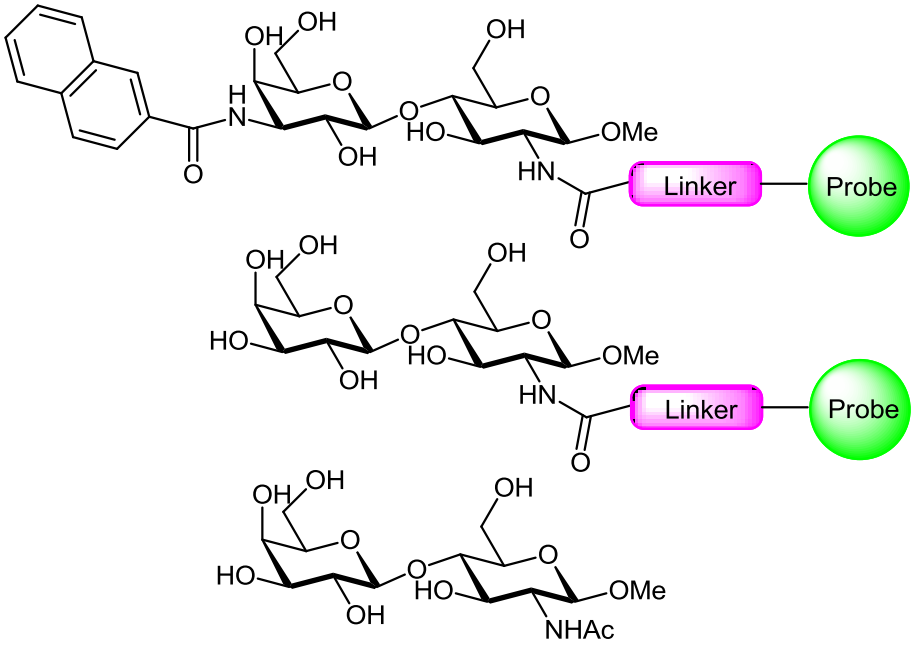


Figure 1-13: NDL-Tagged Imaging/Contrast Agent

The combination of NDL with MRI contrast agents or a suitable fluorophore would allow for enhanced images of cancerous growths; the combination with chemotherapeutic drugs would allow for treatment with fewer side-effects.

Iron nanoparticles offer an enticing combination of being ferromagnetic, and thus excellent for MRI contrast, and generating heat in an oscillating magnetic field;[17] the local hyperthermia could potentially be used to kill cancer cells – possibly in the same machine as that used for the imaging.

2 First Generation

2.1 Design & Retrosynthesis

The proposed first-generation target **1**, shown in Figure 2-1 contains the required aromatic amide, lactosamine, linker and probe molecule. Figure 2-1 also shows compounds required to allow for comparison studies (**2** & **3**).

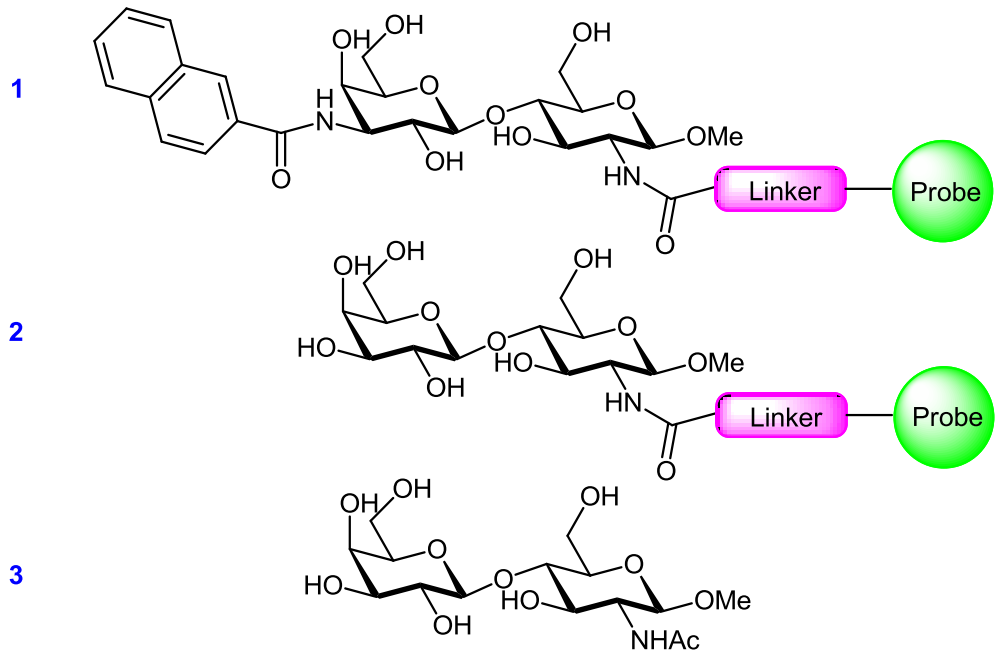


Figure 2-1: Proposed First-Generation Target and Comparison Compounds

Retrosynthetic analysis of target **1**, shown in Figure 2-2, leads to the use of 2-naphthoyl chloride, 2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-β-D-galactopyranoside **5** and methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside **4** as starting materials.

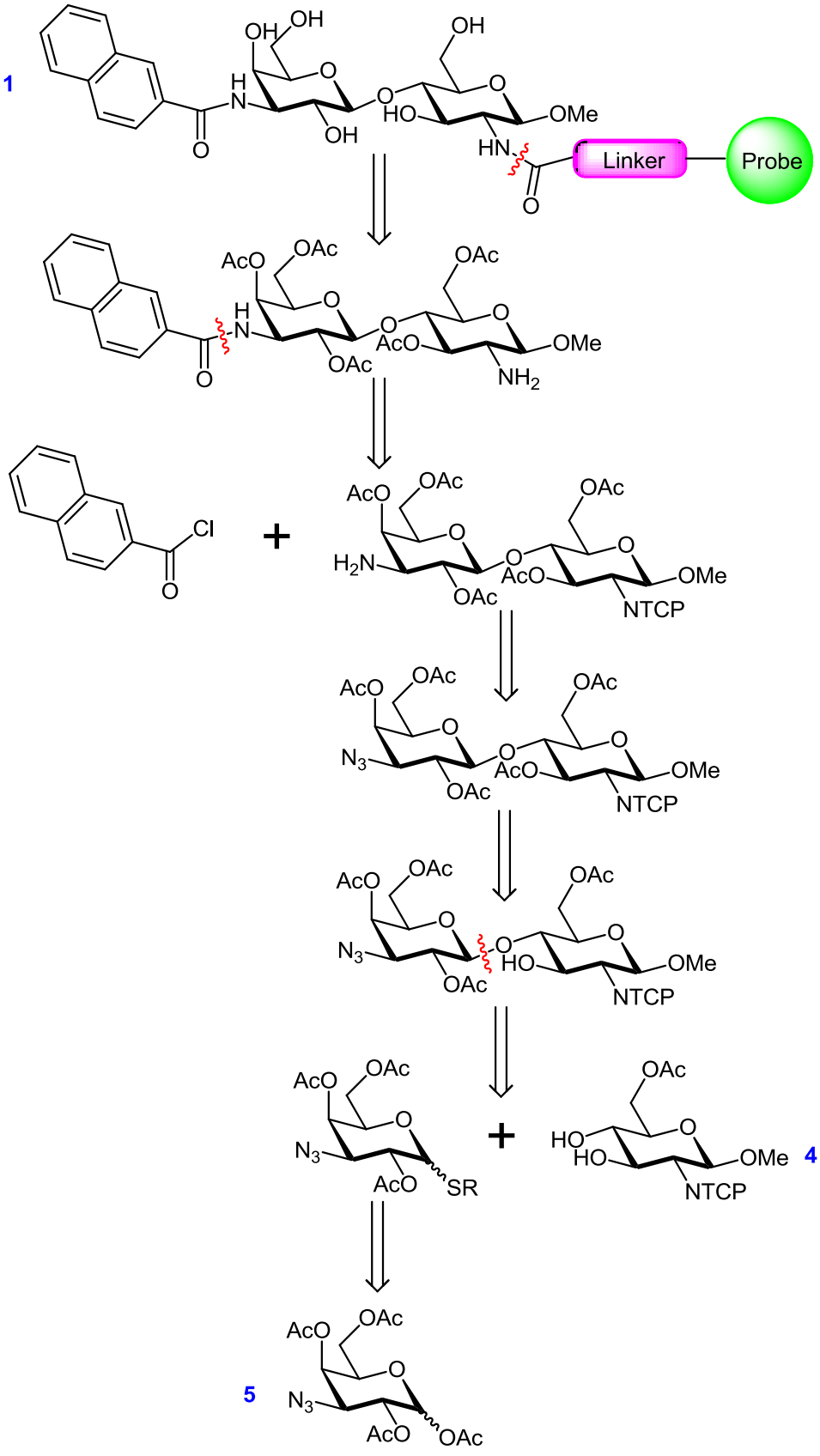


Figure 2-2: Retrosynthetic Analysis of Target 1

Similar retrosynthetic analyses on the comparison compounds leads to the use of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose **6** and glucosamine derivative **4** as starting materials. The required monosaccharides are summarised in Figure 2-3.

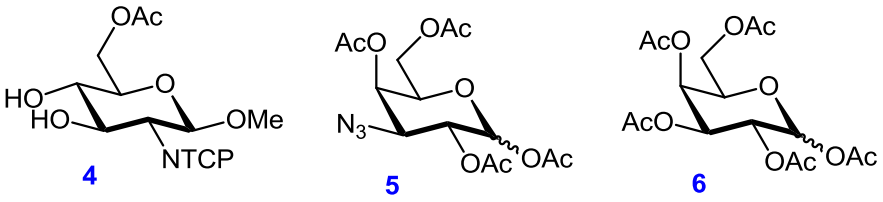


Figure 2-3: Required Monosaccharides

2-Naphthoyl chloride is commercially available. Azido-galactosides derivatives have been synthesised by Lowary[18] and Sörme[19] from diacetone glucose using zu Reckendorf's synthesis of gulose[20] in a total of 8 steps, and from galactose by Oberg[21] in a total of 6 steps. The 1,2,6-tri-protected glucosamine **4** has been synthesised from glucosamine hydrochloride in 7 steps by Main.[22]

2.2 Glycosyl Donor

One of the required monosaccharides is shown in Figure 2-4. Acetyl groups were chosen to protect the hydroxyl groups as this renders the latter non-nucleophilic, and the groups may be attached and removed with ease. The 1-*O*-acetyl group allows for flexibility as it is simple to convert **5** into glycosyl halides or thioglycosides for use in coupling reactions.

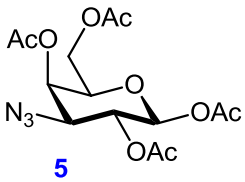


Figure 2-4: Galactose Derivative

Two routes to the required galactose derivative have been investigated: one route starting from diacetone glucose and the other from galactose penta-acetate.

2.2.1 Diacetone Glucose Route

The transformation of glucose into azido-galactose **5** requires epimerisation at C4 (shown in red in Figure 2-5) and retention at C3 i.e. a double inversion at C3, with the azide group installed on the second inversion (shown in green).

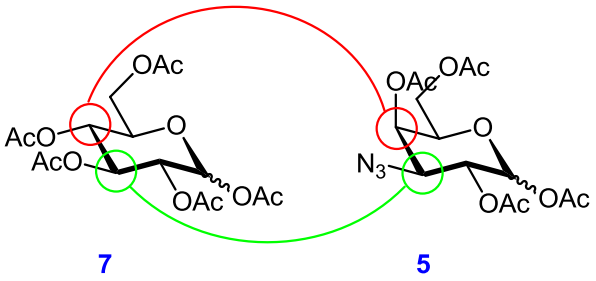
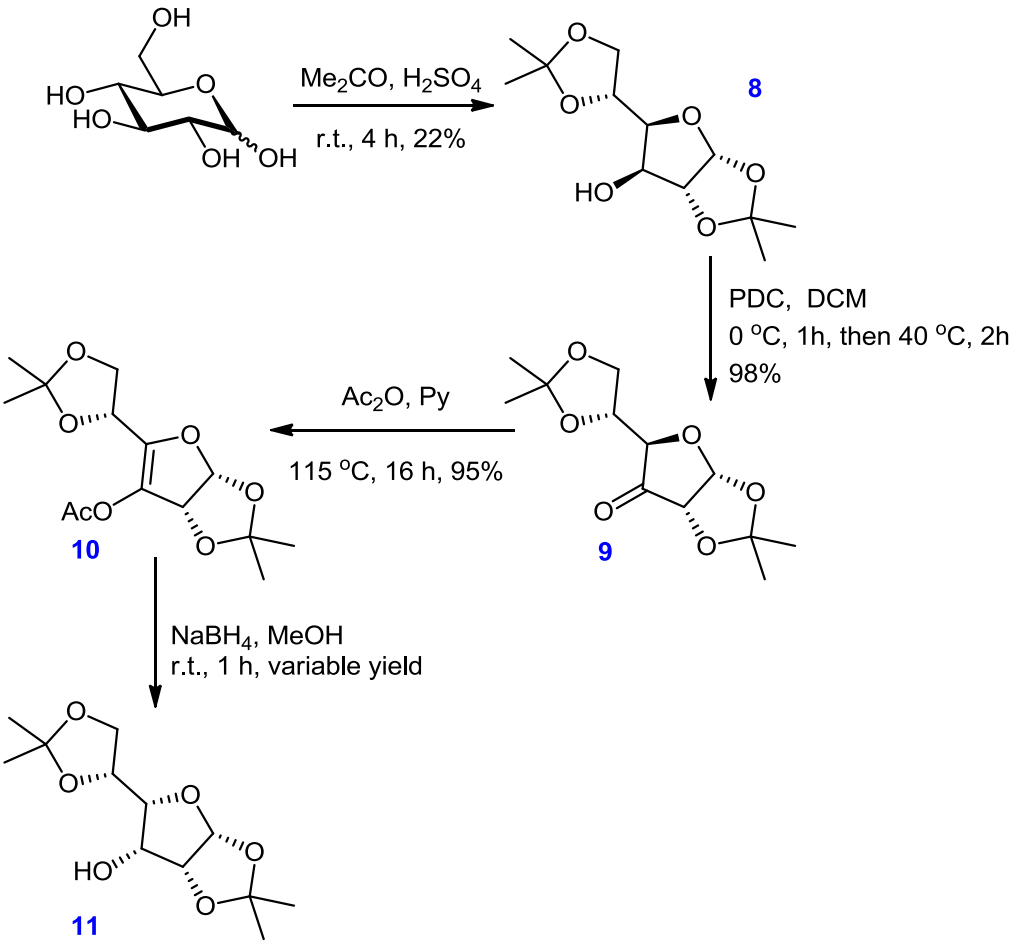


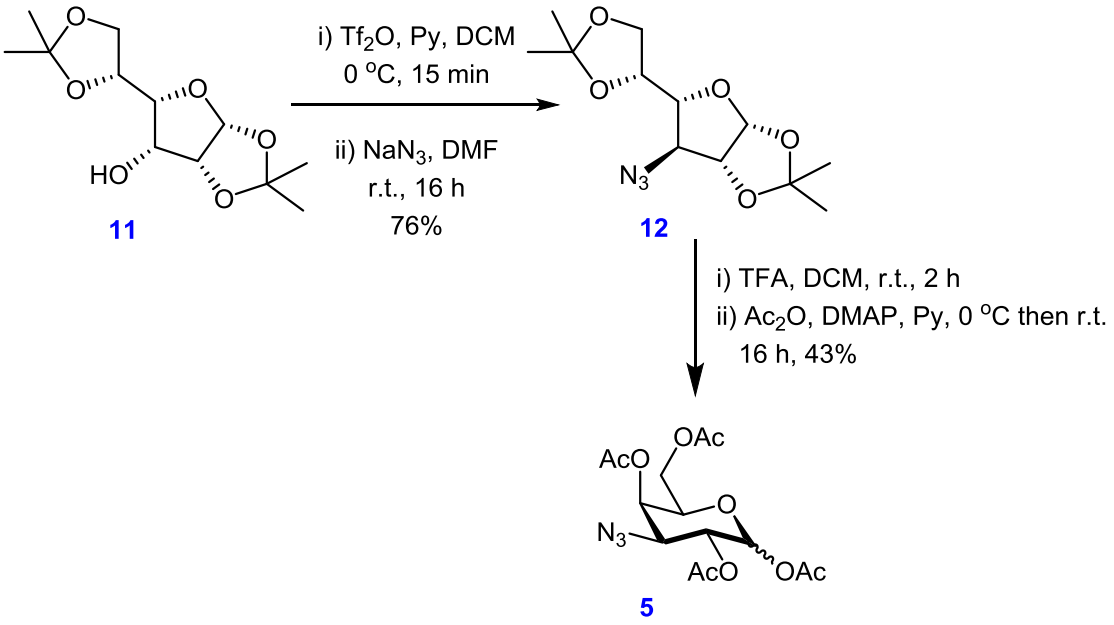
Figure 2-5: The required stereochemical changes at C3 (Green) and C4 (Red)

Diacetone gulose **11**, is a key intermediate on this route as C3 remains as an unprotected hydroxyl and available for activation and azide substitution; the epimerisation at C4 has been effected. The synthesis of diacetone gulose is outlined in Scheme 2-1.



Scheme 2-1: Synthesis of Gulose

The remainder of the synthesis of azido-galactose **5** requires azide substitution with inversion at C3 and transprotection of the acetonides to acetates, as outlined in Scheme 2-2.



Scheme 2-2: Synthesis of Azido-Galactose Derivative

2.2.1.1 *Synthesis of Diacetone Gulose*

The first step to the synthesis of diacetone gulose is the acetonide protection of glucose. Acetonide protection favours *cis* 1,2 diols and drives the formation of the less-favoured furanose form, which allows 2 acetonide groups to be installed. This leaves the C3 hydroxyl free for further transformations. Diacetone glucose **8** was only obtained in 22% yield (compared to 70% reported in the literature[23]), attributable to the poor solubility of glucose in acetone. Fortunately diacetone glucose is cheap and readily available, thus no further work on this acetonide protection was required.

Oxidation of diacetone glucose to hexos-3-ulose **9** was achieved in 98% yield by employing PDC.[24] The carbonyl group is readily hydrated and the compound can be precipitated as the hydrate from a toluene solution by addition of water. The IR spectrum of the hydrate showed no carbonyl stretching band. The ketone can easily be regenerated by dissolving the hydrate in $CHCl_3$ and drying over magnesium sulfate.

Formation of enol acetate **10** was accomplished in 95% yield by heating **9** in pyridine and acetic anhydride,[25] proceeding *via* the enolate. Only one of the possible two enol acetates is formed, see Figure 2-6. Enol acetate **A** is thought to be the thermodynamic product as the geometry of enol acetate **B** imposes a twist on the double bond, thus destabilising the molecule compared to enol acetate **A**.

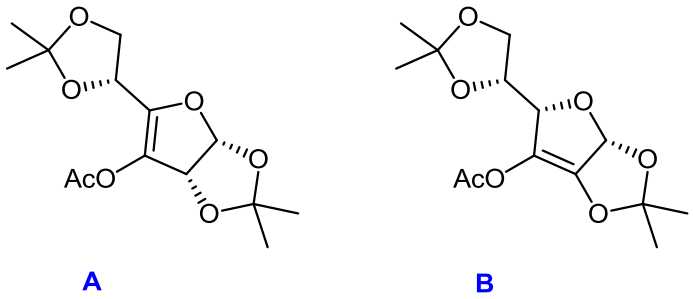
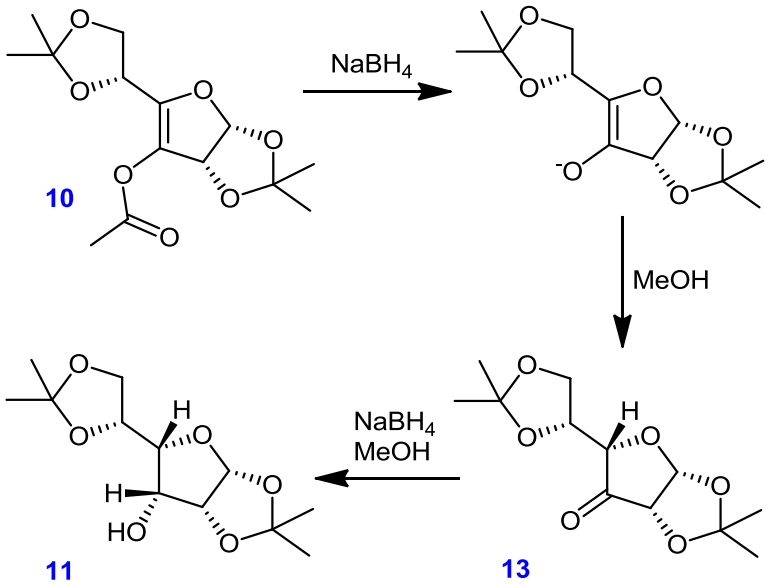


Figure 2-6: Possible Enol Acetates

The planarity of the enol acetate is key to the required stereochemical changes. The original method for the conversion of the enol acetate to diacetone gulose was reduction with $NaBH_4$.[20]



Scheme 2-3: Reduction of Enol Acetate 10

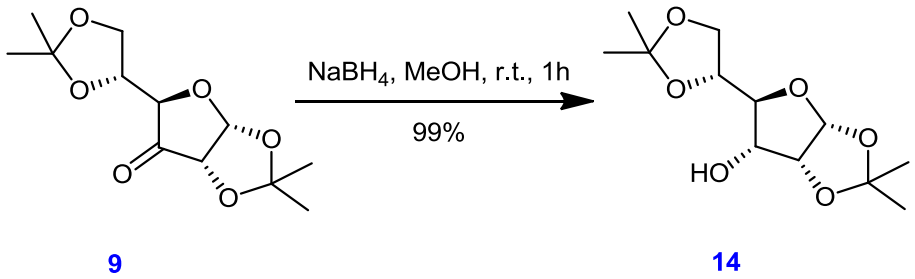
This reaction is hypothesised to proceed by reduction of the acetate, exposing the enolate, which is re-protonated on the more-available top face followed by reduction of the carbonyl, again from the top face, as shown in Scheme 2-3. Yields were modest and frustratingly variable; ranging from 13% to 84%, see Table 2-1. The reduction of the enol acetate also produced unidentified, partially deprotected side-products. ^{1}H NMR suggested that these may be dimers, due to the number of proton signals; the mass spectra were complicated and did not help in the identification of these by-products.

Table 2-1: Results from a series of enol acetate reduction experiments†

Entry	Reductant	Solvent	Time	Yield	Comment
1	$NaBH_4$	1 mL MeOH	5 min	57%	Crude yield
2		2 mL MeOH	2 h	76%	Crude yield
3		5 mL MeOH	3.5 h	37%	Isolated yield
4		5 mL MeOH	40 min	42%	Crude yield
5		5 mL MeOH	20 min	84%	Crude yield
6		2.5 mL MeOH	20 min	51%	Crude yield
7		7.5 mL MeOH	20 min	47%	Crude yield
8		20 mL MeOH	40 min	56%	Crude yield
9		20 mL MeOH	5 min	68%	Isolated yield
10*		50 mL MeOH	2 h	13%	Isolated Yield
11		THF		0%	
12	$LiBH_4$	5 mL MeOH	25 min	<50%	Determined by crude NMR

† Reactions performed with 100 mg (0.33 mmol) of starting material and 1.2 eq. of reductant.
*** Reaction performed with 5.8 g (19.3 mmol) of starting material and 1.2 eq. of reductant.**

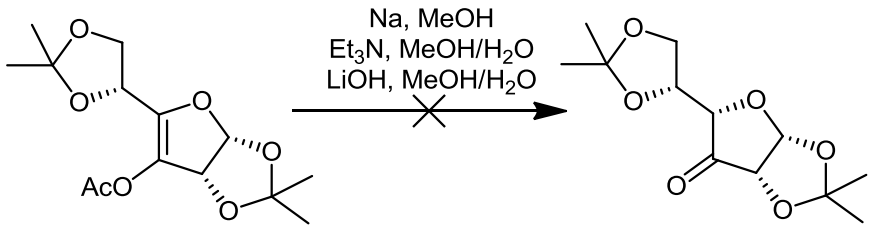
The issues with this reduction could stem from the reactivity of the deprotected enolate or the reduction of the subsequently produced *xylo*-hexos-3-ulose **13**, and both possibilities were investigated. Reduction of *ribo*-hexos-3-ulose **9** with $NaBH_4$ produced diacetone allose **14** cleanly and in high yield, see Scheme 2-4. This suggests that the reduction of the ketone intermediate of the enol acetate reduction should proceed smoothly.



9 **14**

Scheme 2-4: Reduction of Hexos-3-ulose 9

Attempts to remove the acetate group of **10** with NaOMe/MeOH, Et_3N or LiOH all failed to produce *xylo*-hexos-3-ulose **13** (Scheme 2-5), again producing unidentified products with partial deprotection. Therefore it was posited that the exposure of the enolate was causing the reduced and variable yields, and an alternative method was sought.

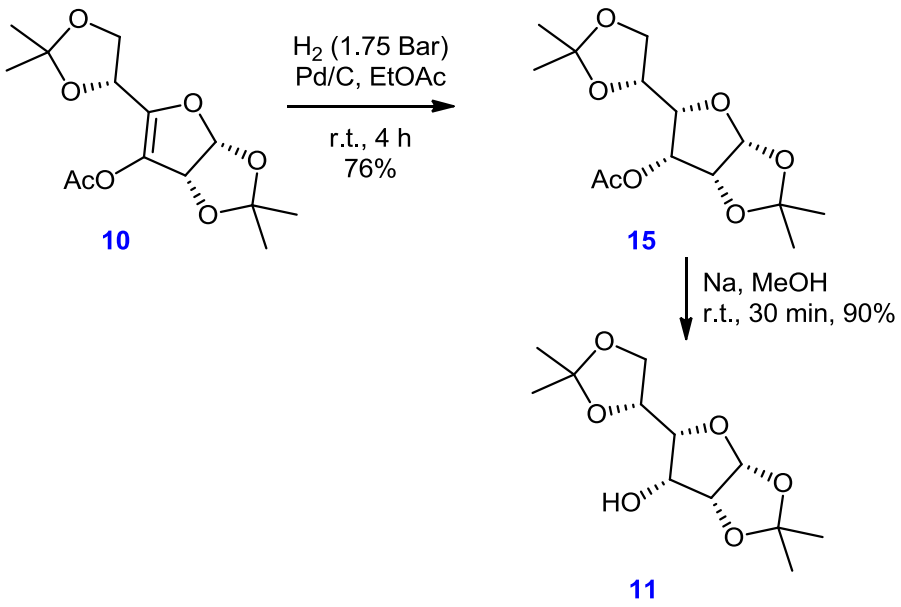


10 **13**

Scheme 2-5: Deacetylation of Enol Acetate 10

Catalytic hydrogenation of **10** followed by methanolysis of the acetate has been used for the reduction of the enol acetate,[25-27] and was investigated. Hydrogenation at atmospheric pressure with catalytic Pd/C produced modest yields of acetate **15** after 3 days in both Et_2O and EtOAc, with an

unknown, partially-deprotected side-product formed. It was later discovered that increasing the quantity of catalyst from 5 mol% to 10 mol% increased the yield to 88% and reduced the reaction time to 2.5 days. Using DMF as reaction solvent further decreased the reaction time to 2 days, although with a small reduction in isolated yield to 74%. Finally, increasing the pressure of H_2 to 1.75 Bar dramatically reduced the reaction time to 4 hours whilst preserving a high yield of 76%, see Scheme 2-6.



Scheme 2-6: Hydrogenation and Hydrolysis of 10

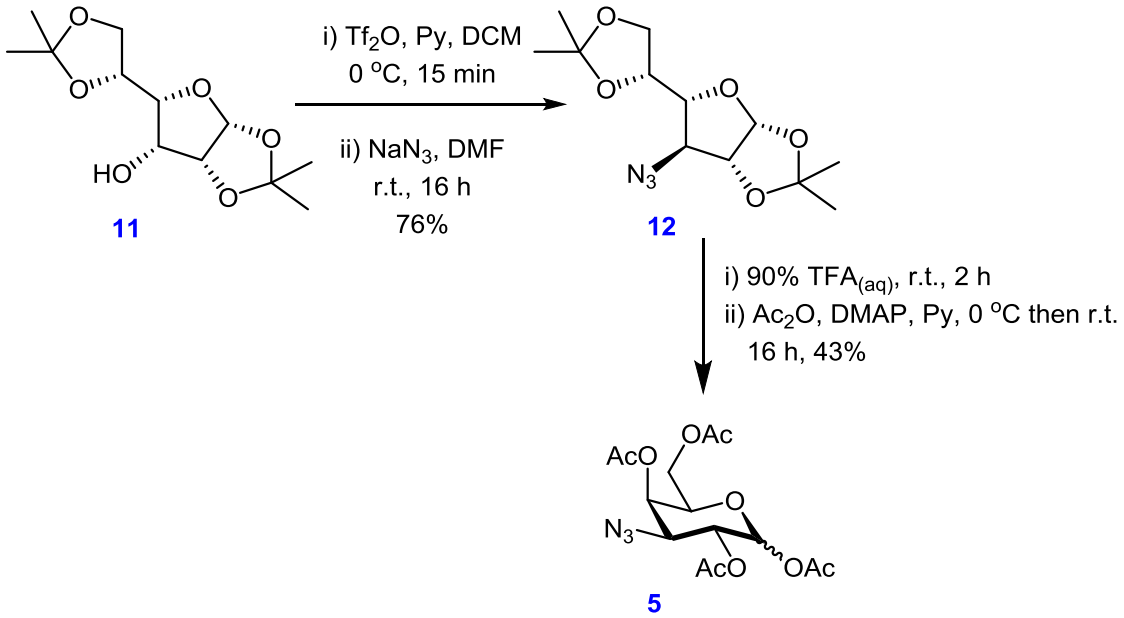
Methanolysis of acetate **15** by Na/MeOH resulted in high-yielding, clean production of diacetone gulose **11** when the reaction mixture was neutralised with dry ice, which presumably introduces a small amount of carbonic acid to the reaction liquor. It was found that neutralisation with DOWEX 50WX8-200 resulted in decomposition of the product with, curiously, some apparent re-formation of the starting material. This is possibly due to the low local pH in the pores of the DOWEX. TLC of the reaction liquor prior to neutralisation showed complete consumption of the starting material. It was discovered that methanolysis by K_2CO_3/MeOH allowed for a faster reaction and a simpler work-up –

only filtering and partitioning between water and $CHCl_3$ was required, rather than a tedious neutralisation with dry ice.

Overall, diacetone gulose was produced from diacetone glucose in 48% yield over 4 steps.

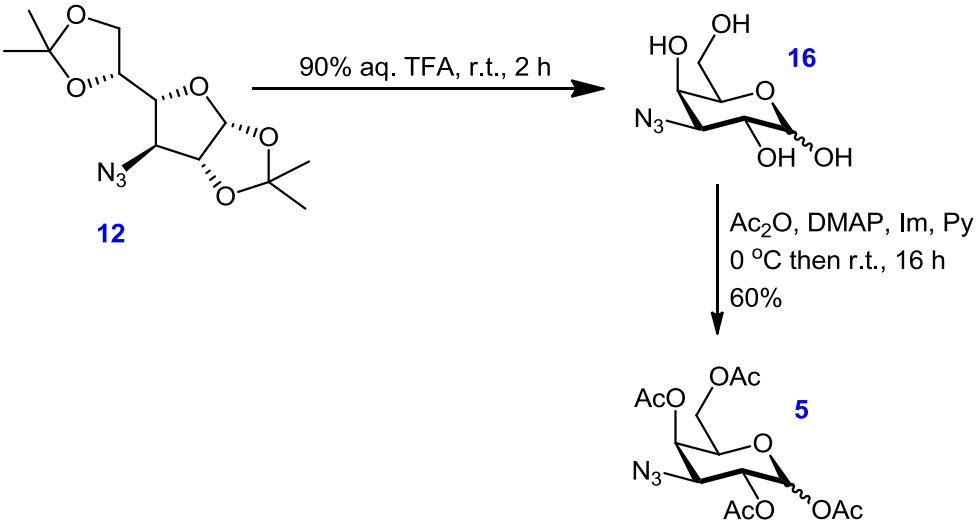
2.2.1.2 *Diacetone Gulose to Azido-Galactose*

Conversion of diacetone gulose to azide **12** was easily accomplished by formation of the triflate with Tf_2O in DCM followed by substitution with NaN_3 in DMF,[18] in 80% yield, see Scheme 2-7.



Scheme 2-7: Azide Substitution with Inversion

The crude product was sufficiently pure to be transprotected to azido-galactopyranose **5** using 90% aq. TFA followed by Ac_2O/DMAP/Py, with Ac_2O in 5-fold excess. It was found that addition of imidazole reduced the reaction time from 3 days to 16 hours and increased the yield from 43% to 60%, see Scheme 2-8.



Scheme 2-8: Transprotection of Azide 12

The anomeric mixture (α:β 1:1) was not considered problematic as further reactions at the anomeric centre are controlled by either anchimeric assistance in the formation of thioglycosides, or by the anomeric effect in the formation of glycosyl halides. The main difference between the further reactions of the anomers involves rate: the α anomer is expected to be slower to activate than the β anomer due to steric effects, but once activated the α leaving group is quicker to leave, due to the anomeric effect.

Overall, galactose derivative **5** was produced in 29% yield over 6 steps.

2.2.2 Galactose Route

Due to the difficulties in reducing the enol acetate in the diacetone glucose route, a second route was investigated in parallel. Azido-galactose derivative **17** has been prepared by Oberg[21], giving a thiogalactoside derivative that may be used as a glycosyl donor, see Figure 2-7.

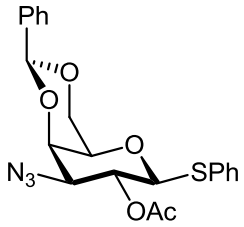
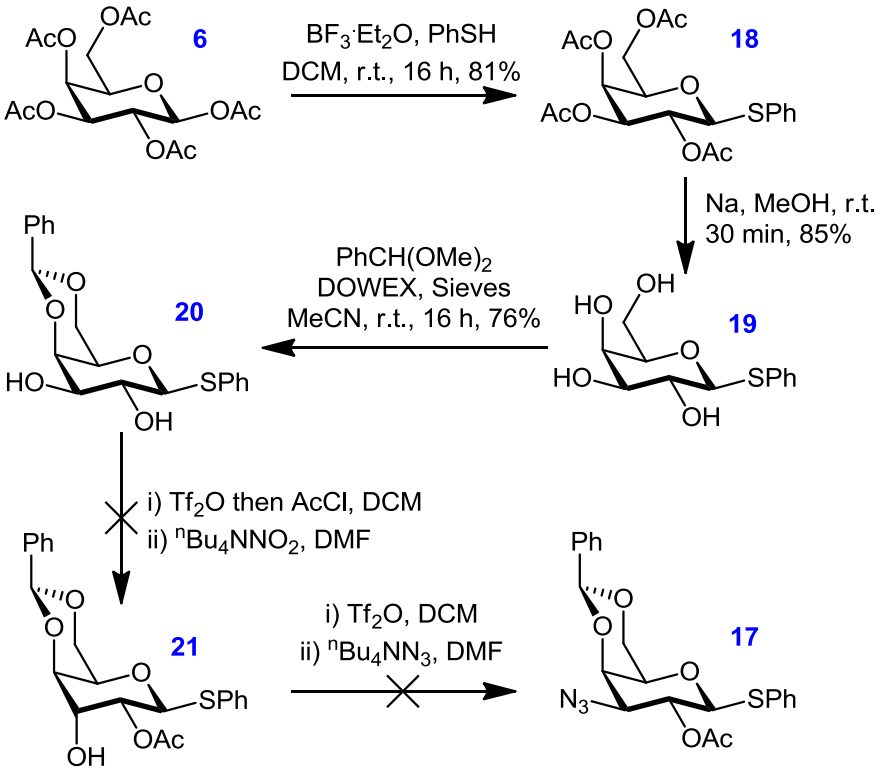


Figure 2-7: Oberg's Galactose Derivative

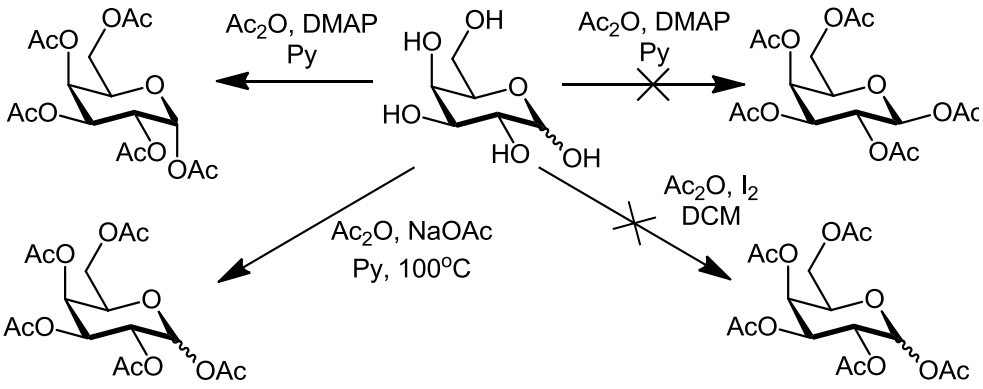
The synthesis of **17** is outlined in Scheme 2-9, below. A key step in this synthesis is the conversion of galactose-derivative **20** into gulose-derivative **21**. Inductive effects deactivate the C2 hydroxyl, due to its proximity to the hemithioacetal, resulting in preferential reaction with triflic anhydride at C3.



Scheme 2-9: Synthesis of 17

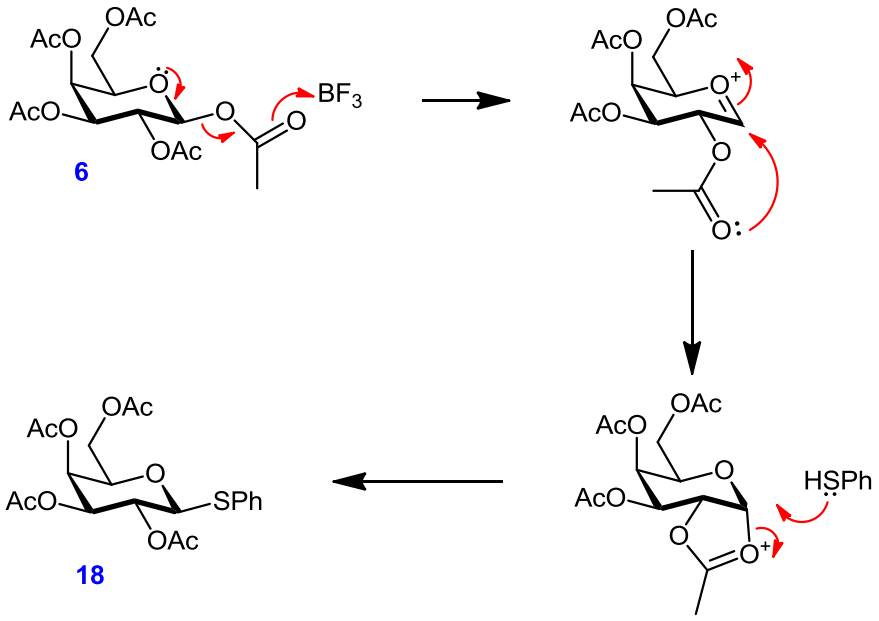
The preparation of galactose penta-acetate was first attempted using Ac_2O/I_2[28] but no product was formed. Galactose was fully acetylated with Ac_2O/DMAP/Py but only the α anomer of **6** was produced, this anomer is slower to react in subsequent reactions than the desired β anomer of **6**.

Heating galactose, Ac_2O and NaOAc in pyridine[29] at reflux resulted in a 1:1 mixture of anomers, as shown in Scheme 2-10. Thus, attempts to produce the β anomer in a cheap synthesis were unsuccessful; fortunately this anomer is commercially available.



Scheme 2-10: Peracetylation of Galactose

The conversion of pentaacetate **6** into thiogalactoside **18** was easily accomplished using $BF_3{\cdot}Et_2O$/PhSH,[30, 31] proceeding *via* a glycosyl cation as per Scheme 2-11.



Scheme 2-11: Formation of Thiogalactoside 18

Participation of the C2 acetyl group blocks the bottom face of the molecule, resulting in exclusive production of the β anomer. It was discovered that modifying the literature procedure to washing with sat. Na_2CO_3, instead of sat. $NaHCO_3$, removed the need to purify the product by chromatography: the main impurity is unreacted thiophenol (pK_a = 6.6), which is deprotonated by Na_2CO_3 (pK_a = 10.4) but not by $NaHCO_3$ (pK_a = 6.3).

To allow for the deacetylation to produce **19**, thiogalactoside **18** was dissolved in MeOH and reacted with a sub-stoichiometric amount of Na, giving the product in 85% yield. The remaining thiogalactoside was left as a methanolic solution for 1 week, after which it was serendipitously discovered that complete transesterification had occurred in 93% yield. As a single run of each deacetylation reaction produced a combined total of 5 g of product, neither reaction needed to be repeated.

Benzylidene protection of thiogalactoside **19** preferentially protects the C4 and C6 hydroxyls as this results in a *cis*-decalin structure, allowing the bulky phenyl group to occupy an equatorial position. 1,2-Benzylidene protection is not favoured as the available pseudo-equatorial position on the formed 5-membered ring does not confer the same steric advantage.

4,6-Benzylidenes have previously been synthesised using α,α-dimethoxytoluene in acetonitrile with catalytic tosic acid,[32] which can present purification difficulties. The literature protocol was modified to use DOWEX 50WX8-200 as an acid catalyst and 4 Å molecular sieves. Benzylidene **19** is poorly soluble in MeCN, and precipitated on to the DOWEX/sieves; the use of DCM as co-solvent prevented this from occurring and gave the product in 76% yield.

The next step was the conversion of galactose derivative **20** into gulose derivative **21**. Unfortunately all attempts at this conversion failed to produce the desired product. The reaction was difficult to

monitor due severe streaking in the TLC which did not improve by adding NEt_3 to the eluent. Crude NMR spectra did not appear to show product peaks, though these may have been swamped by the tetrabutylammonium peaks. Column chromatography failed to produce identifiable saccharides though the majority of the crude material failed to pass through the column despite increasing the polarity of the eluent to 1:4 hexane/EtOAc and flushing with 100% EtOAc. These difficulties may have been introduced by insufficient differentiation between the hydroxyl groups at C2 and C3 in the reaction with Tf_2O. At this point, the diacetone glucose route – which was run in parallel to this route – had successfully produced the desired galactose derivative **5**.

2.3 Glycosyl Acceptor

The second monosaccharide required is shown in Figure 2-8. Tetrachlorophthalimide was chosen for N-protection as the nucleophilicity of the nitrogen is completely suppressed and the group may be removed under milder conditions than the popular phthalimide group.[33] The TCP group also participates in glycosyl coupling reactions, giving the required β anomer and significantly reduces the nucleopilicity of the C3 hydroxyl, through inductive and possibly steric effects. This deactivation of the C3 hydroxyl significantly simplifies the synthesis as it is only necessary to protect the C6 hydroxyl, which may be achieved easily as it is the only primary centre in **4**.

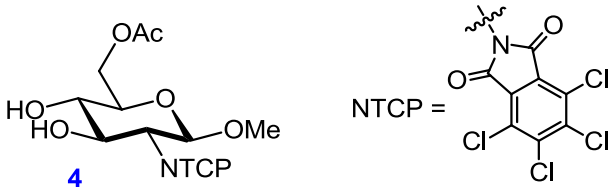
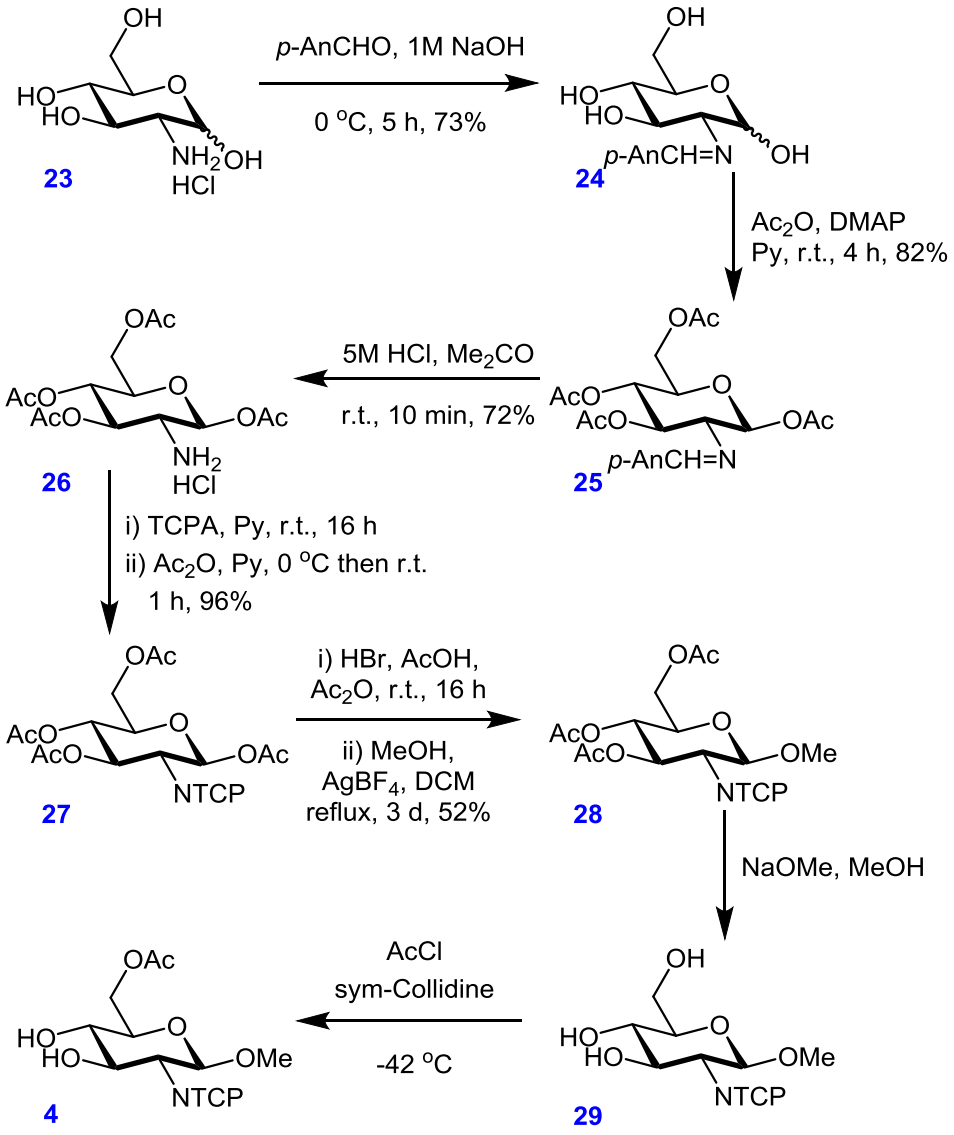


Figure 2-8: Glucosamine Derivative

Main's[22] synthesis of **4** is outlined in Scheme 2-12, the majority of which concerns protecting group transformations. The hydroxyl groups cannot be protected with acetyl groups in the presence of the

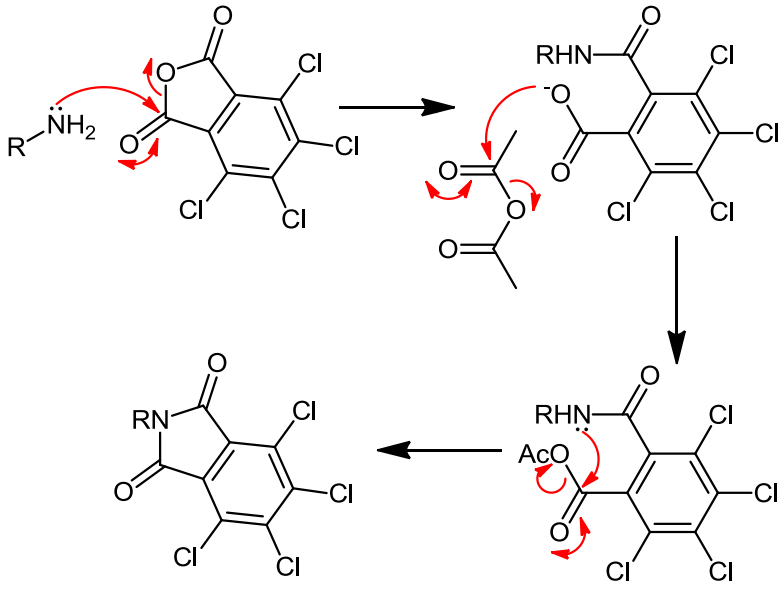
more nucleophilic amine; the amine cannot be protected with a TCP group in the presence of free hydroxyls. Thus the amine was temporarily protected as imine **24**, allowing the acetyl groups to be installed. Acid hydrolysis of the peracetylated imine was accomplished with 5M HCl in Me_2CO; the amount of Me_2CO was doubled to prevent the formation of a semi-solid swollen gel and produced hydrochloride **26** in 72% yield.



Scheme 2-12: Synthesis of 4

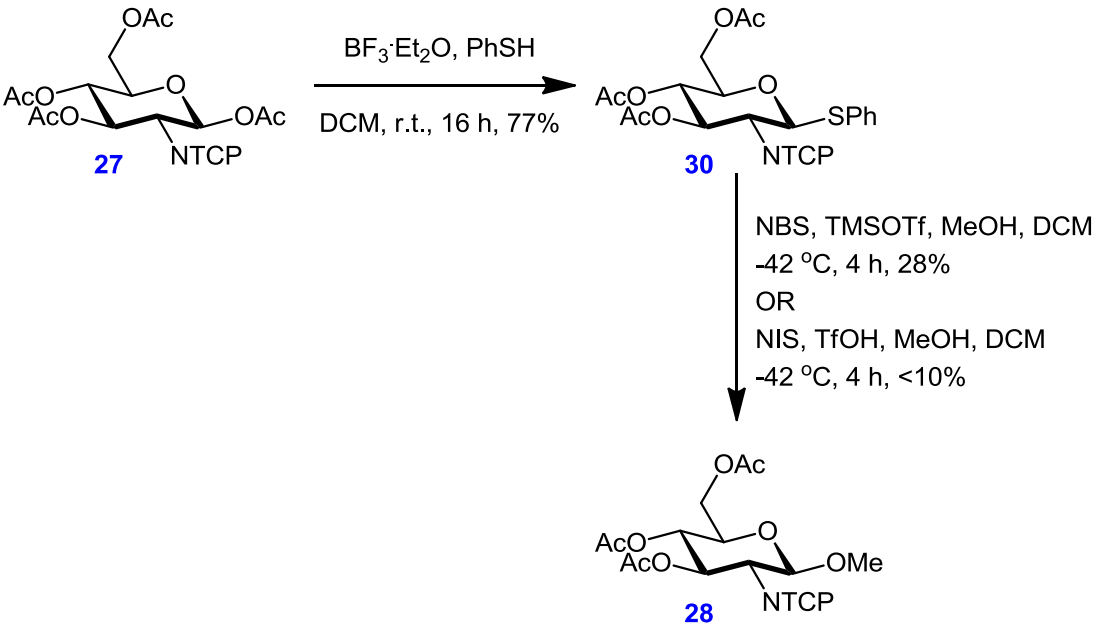
Hydrochloride **26** was poorly soluble in pyridine and produced a thick gel which prevented TCPA from dissolving, seriously affecting the TCP protection step. It was found that partially dissolving the TCPA

in a small amount of DCM allowed the reaction to begin, accompanied by a swift reduction in viscosity. The first step in the protection is the formation of a phthalamic acid; subsequent addition of Ac_2O produces a phthalamic anhydride which immediately ring-closes to the phthalimide, as shown in Scheme 2-13.



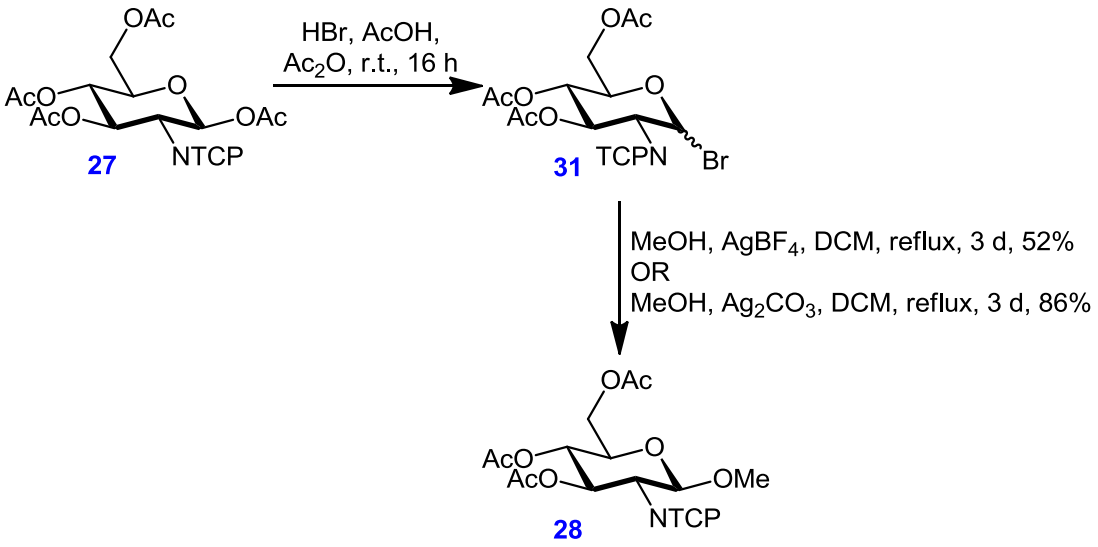
Scheme 2-13: Mechanism of TCP Protection

The next step to produce methyl glycoside **28** was attempted using phenyl thioglycoside **30** (Scheme 2-14) and glycosyl bromide **31** (Scheme 2-15). Formation of the thioglycoside proceeded smoothly and in 77% yield. Unfortunately conversion of the thioglycoside to the methyl glycoside using NBS/TMSOTf gave the product in only 28% yield while NIS/TfOH did not give any appreciable conversion. This was probably due to the strongly disarming nature of the TCP group inductively suppressing the nucleophilicity of the sulfur.



Scheme 2-14: Methyl Glycoside Formation *via* Thioglycoside

The glycosyl bromide was formed in quantitative yield with HBr/AcOH/Ac_2O. At lower concentrations only the α-anomer is produced, but as the concentration increases so too does the proportion of the β-anomer.

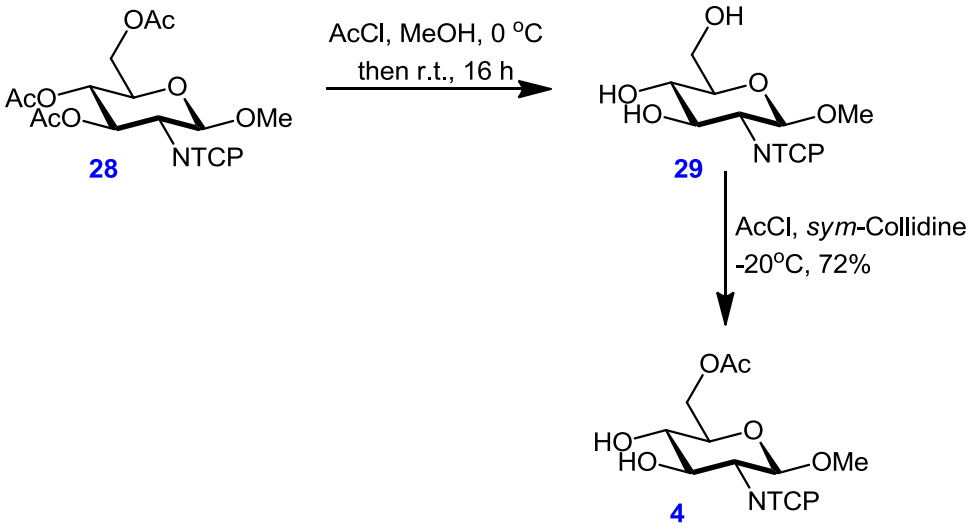


Scheme 2-15: Methyl Glycoside Formation *via* Glycosyl Bromide

Conversion of the glycosyl bromide to the methyl glycoside was first attempted using $AgBF_4$, but this was found to cause a variable amount of deacetylation, possibly due to traces of HBF_4. The use of

AgOTs caused complete deacetylation. Changing the silver salt to Ag_2CO_3 (i.e. to mildly basic conditions) produced the methyl glycoside in 86% yield, and in sufficient purity to carry the crude forward.

Removal of the acetate groups to give triol **29** using catalytic NaOMe in MeOH failed, producing a residue that was insoluble in $CHCl_3$ or MeOH, probably due to the opening of the phthalimide ring. As reported by Ellervik,[32] the reaction is concentration dependent, due to the base-sensitive nature of the TCP group. Performing the reaction in 18 mM NaOMe in MeOH produced a crude product which was soluble in $CHCl_3$, but was difficult to fully characterise because of severe line-broadening in the ^{1}H NMR spectra. At this point, acid-catalysed deacetylation was attempted using AcCl/MeOH (generating a methanolic solution of HCl). A ^{1}H NMR spectrum was obtained confirming the deacetylation had completed. In order to avoid possible complications arising from trace residues of HCl, the triol was immediately taken through to the next step. The literature procedure for the mono-acetylation[19] involves using 1.1 equivalents of AcCl at -42 °C followed by a further addition of 0.25 equivalents of AcCl at -20 °C. It was found that no reaction occurred at -42 °C and that the reaction can be conducted at -20 °C with an excess of AcCl. Interestingly, the starting material is not soluble in DCM whereas the product is – thus the reaction can be monitored visually, indeed the reaction was quenched when the suspension had cleared.



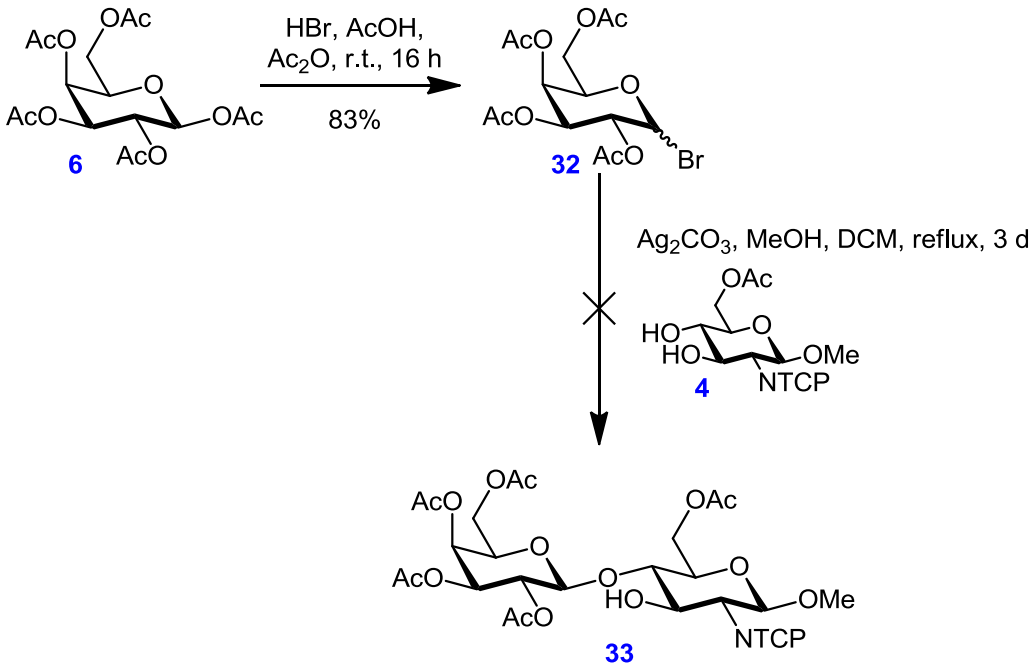
Scheme 2-16: Formation of Glycosyl Acceptor

2.4 Disaccharides

The next stage was to couple the galactose and glucosamine monosaccharides together and attention was turned to selecting a suitable glycosyl donor. Glycosyl bromides and thioglycosides were investigated in parallel.

2.4.1 Glycosyl Bromide

A glycosyl bromide had successfully been employed to perform the aglycosyl coupling producing methyl glycoside **28**. This protocol was employed to form lactosamine **33** as shown in Scheme 2-17.



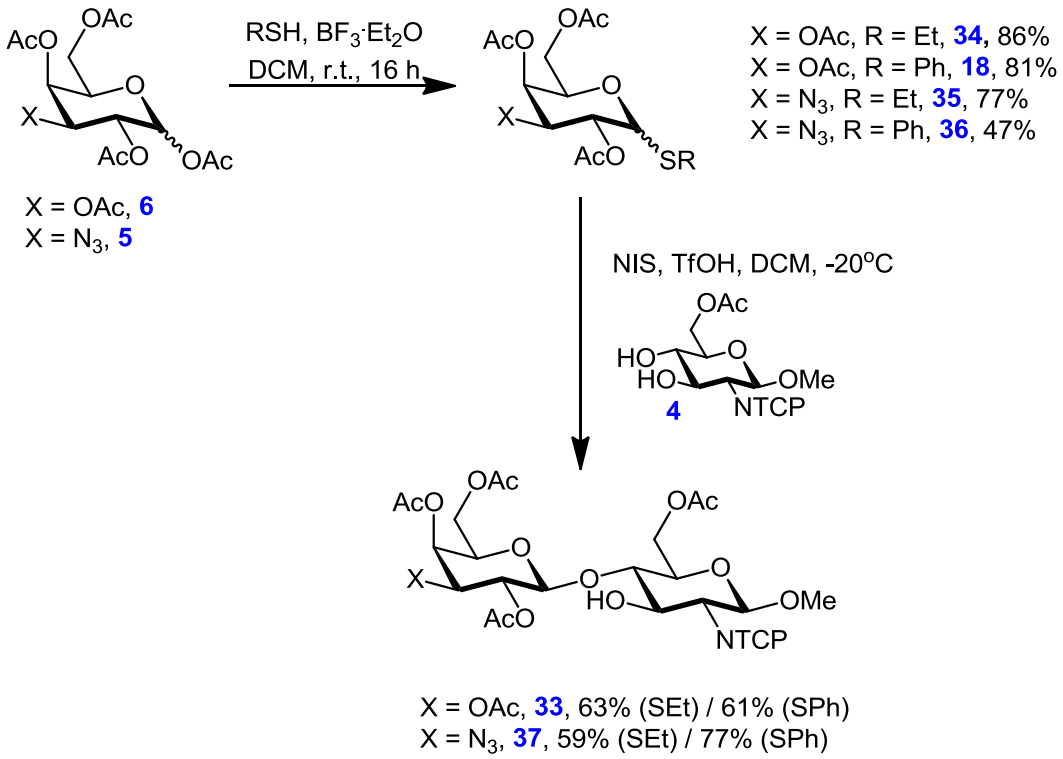
Scheme 2-17: Glycosyl Coupling *via* Glycosyl Bromide

The glycosyl bromide was successfully formed, as determined by NMR spectroscopy, but no disaccharide was formed. This may have been due to difficulties in maintaining the rigorously anhydrous conditions required for glycosyl couplings for 3 days. Fortunately, couplings *via* thioglycosides were successful, and are discussed below.

2.4.2 Thioglycosides

Methyl and phenyl thioglycosides have precedent for their use in glycosyl coupling reactions. Whilst methyl thioglycosides are the more reactive of the two, they are also the more difficult to synthesise, requiring either trimethyl(methylthio)silane or the formation of *S*-glycosyl isothioureas from glycosyl halides followed by treatment with MeI and hydrolysis. This complication arises from the simple fact that MeSH is a gas at room temperature. Phenyl glycosides have reduced reactivity due to overlap between the sulfur lone pair and the phenyl π-system reducing the nucleophilicity of the sulfur. As well as phenyl thioglycosides, ethyl thioglycosides were investigated as they should have similar

reactivity to methyl thioglycosides, but are simpler to synthesise as EtSH is a liquid at room temperature.



Scheme 2-18: Thioglycoside Couplings

The yields for the formation of the various thioglycosides and their glycosyl coupling reactions are shown in Table 2-2. Ethyl thioglycoside was chosen for galactose **6** due to the higher overall yield and phenyl thioglycoside was chosen for azido-galactose **5** – although the overall yield is lower the handling and purification of the phenyl thiogalactoside is much simpler.

Table 2-2: Thioglycoside Formation and Glycosyl Coupling Results

Entry	X	R	% Yield Thioglycoside Formation	% Yield Glycosyl Coupling	% Yield Overall
1	OAc	Et	86	63	54
2	OAc	Ph	81	61	49
3	N_3	Et	77	59	45
4	N_3	Ph	47*	77	36

* Reaction not fully optimised

Figure 2-9 shows the spectra from HMBC NMR experiments that confirm the formation of β(1-4) glycoside linkages, the peaks showing the $^{3}J_{H\text{-}C}$ correlations are ringed in blue.

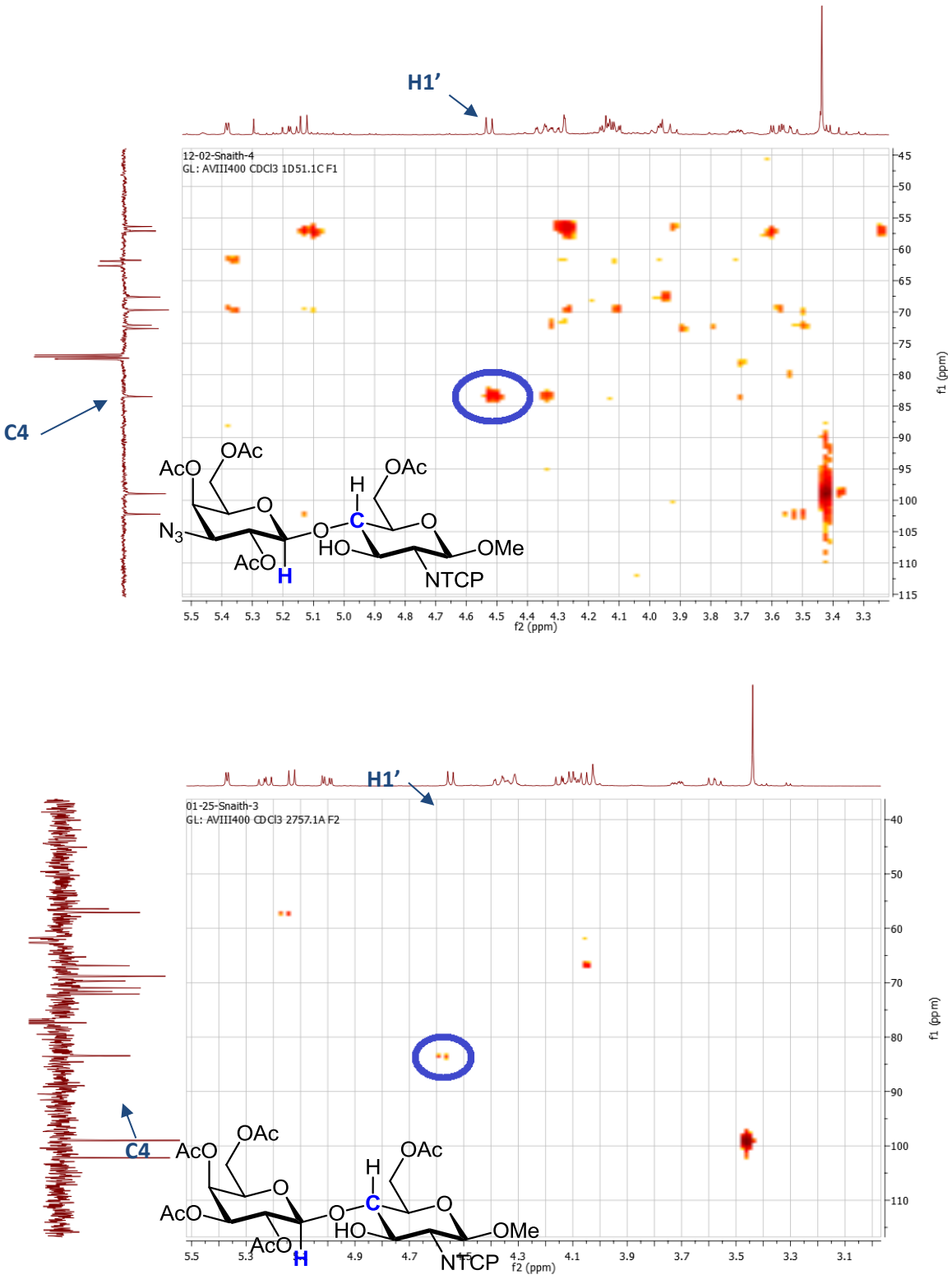
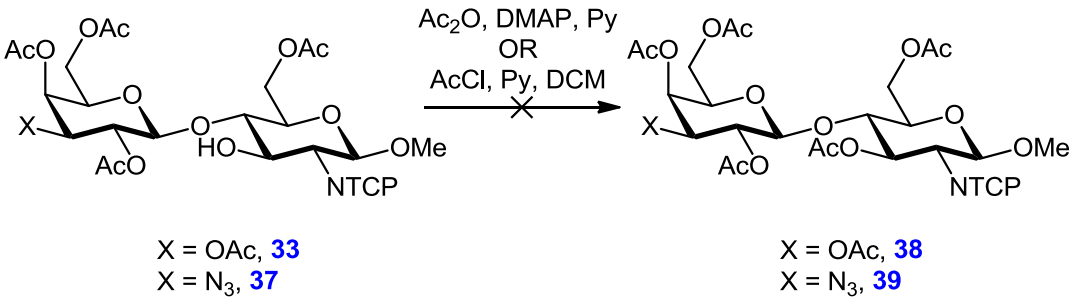


Figure 2-9: HMBC Spectra Confirming Regiochemistry of Glycosyl Couplings.

2.4.3 Alcohol Protection

In order to prevent complications in subsequent reactions, acetyl protection of the free alcohol was investigated, see Scheme 2-19.

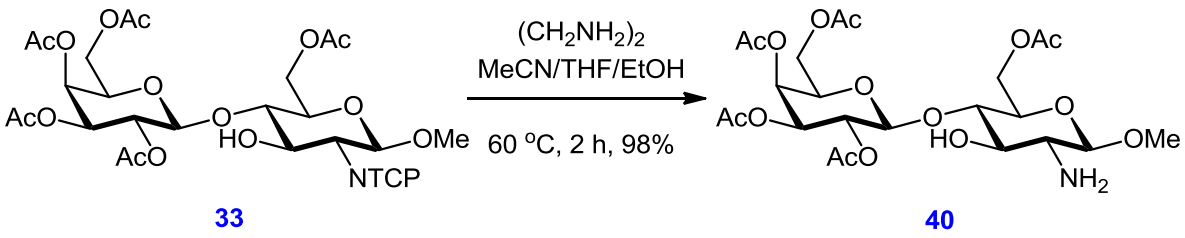


Scheme 2-19: Acetylation of Free Alcohol

Neither acetic anhydride nor acetyl chloride successfully acetylated the free alcohol, suggesting that the alcohol is sufficiently unreactive to be left unprotected.

2.4.4 TCP Removal

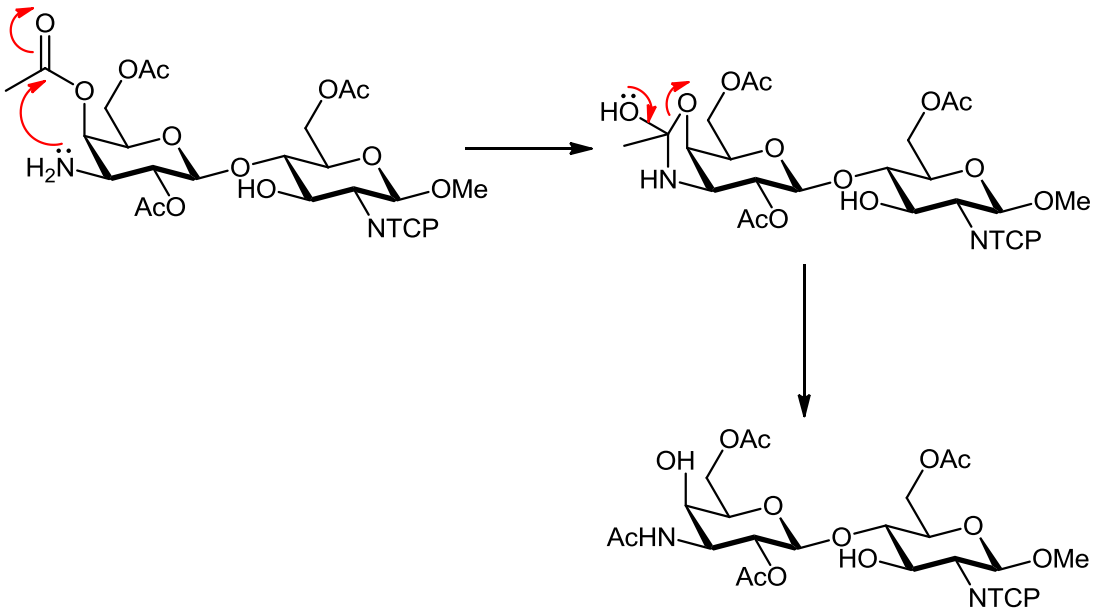
Removal of the TCP group was attempted using Debenham's[33] method and was found to proceed in high yield, see Scheme 2-20. This method employs ethylene diamine in MeCN/THF/EtOH to remove the phthalimide. It was discovered that filtering and a water wash was sufficient to remove the impurities.



Scheme 2-20: TCP Removal

2.4.5 Azide Reduction & Naphthamide Installation

The azide group of lactosamine **37** is acting as a nitrogen protecting group and thus reduction of the azide prior to installation of the naphthamide was required. It was envisioned that care would be needed to avoid a possible acetyl migration, show in Scheme 2-21.



Scheme 2-21: Acetyl Migration

Aplander[34] used catalytic hydrogenation to produce the amine that was then converted to the amide. Initial reactions were run in EtOH but this was modified to 30% EtOH in Et_2O as this facilitated easier removal of the solvent without heating – to reduce the possibility of an acetyl migration. The intermediate amine was immediately taken through to the acylation with 2-naphthoyl chloride. Only a small amount of naphthamide was produced, as determined by crude NMR spectra, even when the reaction time was increased to 1 week.

The reduction was attempted with both Pd/C and $Pd(OH)_2/C$; TLC analysis showed complete consumption of the starting material, though IR analysis on the intermediate did not show the

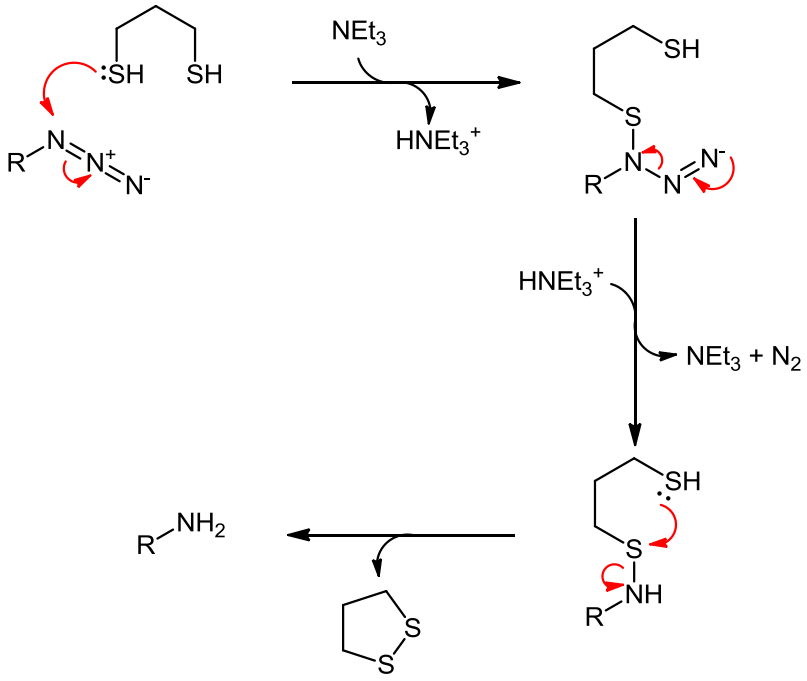
characteristic symmetric and asymmetric stretches of a primary amine. Despite over 20 repeats of the reduction/acylation involving variations in stoichiometry, Pd catalyst and reaction time, no optimal conditions were found, and other methods were sought.

Burés[35] showed that it was possibly to modify the Staudinger reduction to directly produce amides without releasing the intermediate amine. As this procedure requires 20 mol% 2,2'-dipyridyl diselenide (which is not commercially available) and Me_3P, it was not attempted. As expected, conducting the reaction with Ph_3P instead of Me_3P and without PySeSePy failed to produce any amide.

An unmodified Staudinger reaction successfully produced the phosphazene intermediate, as shown by MS, but hydrolysis did not occur at room temperature. Heating the hydrolysis reaction was not attempted due to the possibility of acetyl migration.

A sulfur equivalent of the Staudinger reaction[36] (Scheme 2-22) which utilises propane-1,3-dithiol/NEt_3 in place of Ph_3P/H_2O was attempted as heating is not required. Once again, TLC showed the complete consumption of starting material but NMR and IR spectra showed no evidence of the amine.

Due to these difficulties, further reactions were not pursued and the glycosyl donor was redesigned, as discussed in section 2.7.



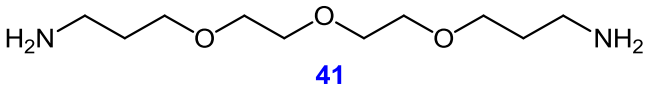
Scheme 2-22: Mechanism of Dithiol Reduction of an Azide

2.5 Linkers

Investigations into the synthesis of the 3'-amide-derivatised lactosamine derivative were run in parallel with the synthesis of the non-derivatised lactosamine. Although none of the amide-derivatised lactosamine was available, the non-derivatised lactosamine was, and methods for the attachment of linkers were investigated, such that these protocols could be applied to the amide derivative when it became available.

Previous work within the group used linkers derived from caproic acid. This, however, has the possibility of introducing solubility problems. Linkers based on poly(ethylene glycol) are topologically similar to caproic acid, and have the advantage of being water soluble and biocompatible. It is also desirable to be able to produce both an amine-terminated linker for conjugation to FITC and a carboxyl-terminated linker for conjugation with FeNPs, as used by Hadjipanayis.[17] Finally, inclusion of amide bonds within the chain is thought to stabilise the coating shell of FeNPs.

It was found that diamine **41** was commercially available and cheap. This diamine could easily be converted to being carboxyl-terminated by reaction with succinic anhydride producing ω-amino acids; this would also introduce amide bonds within the chain.

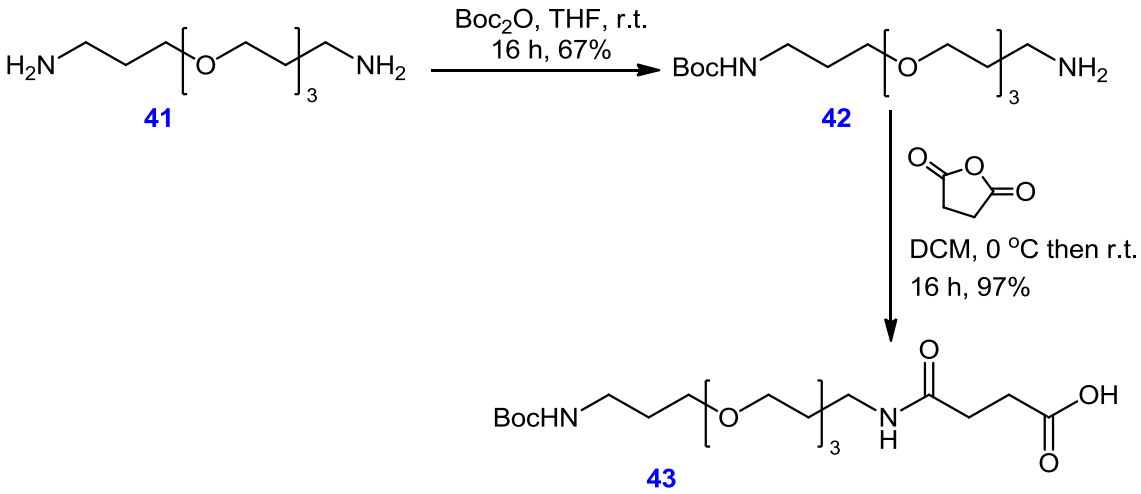


41

Figure 2-10: Diamine Building Block Used for Linkers

2.5.1 ω-Amino Acid Synthesis

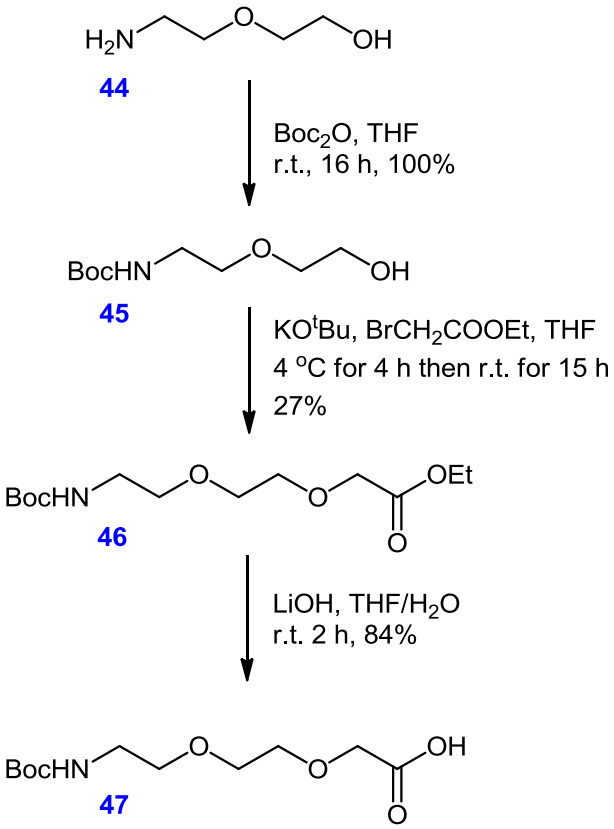
The first probe molecule to be used was FITC, which thus requires a carboxyl terminus for conjugation to the disaccharide, and an amine terminus for conjugation to FITC. The synthesis of this linker is shown in Scheme 2-23.



Scheme 2-23: Linker Synthesis

The mono-Boc protection of **41** was straightforward, employing 0.1 equivalents of Boc_2O to prevent bis-protection. The yield was only 67%, probably due to the fact that the starting material and product are soluble in both EtOAc and water, reducing the efficiency of the partitioning. The acylation reaction was also straightforward and proceeded in 97% yield.

An alternative linker based on aminoethoxy ethanol, without the amide in the chain was also synthesised, as shown in Scheme 2-24. *N*-protection proceeded smoothly and in 96% yield and the alkylation was attempted using KO^{t}Bu/ethyl bromoacetate.[37] Unfortunately, this procedure only produced the desired product once, and in a low yield of 27%. This may possibly be due the similarity in the predicted pK_as of the alcohol and BocNH leading to over-alkylation or exclusive *N*-alkylation. Attempts to avoid this complication by varying the amount of butoxide, the reaction temperature and reaction time failed and thus only 350 mg of **46** was available to take forward.

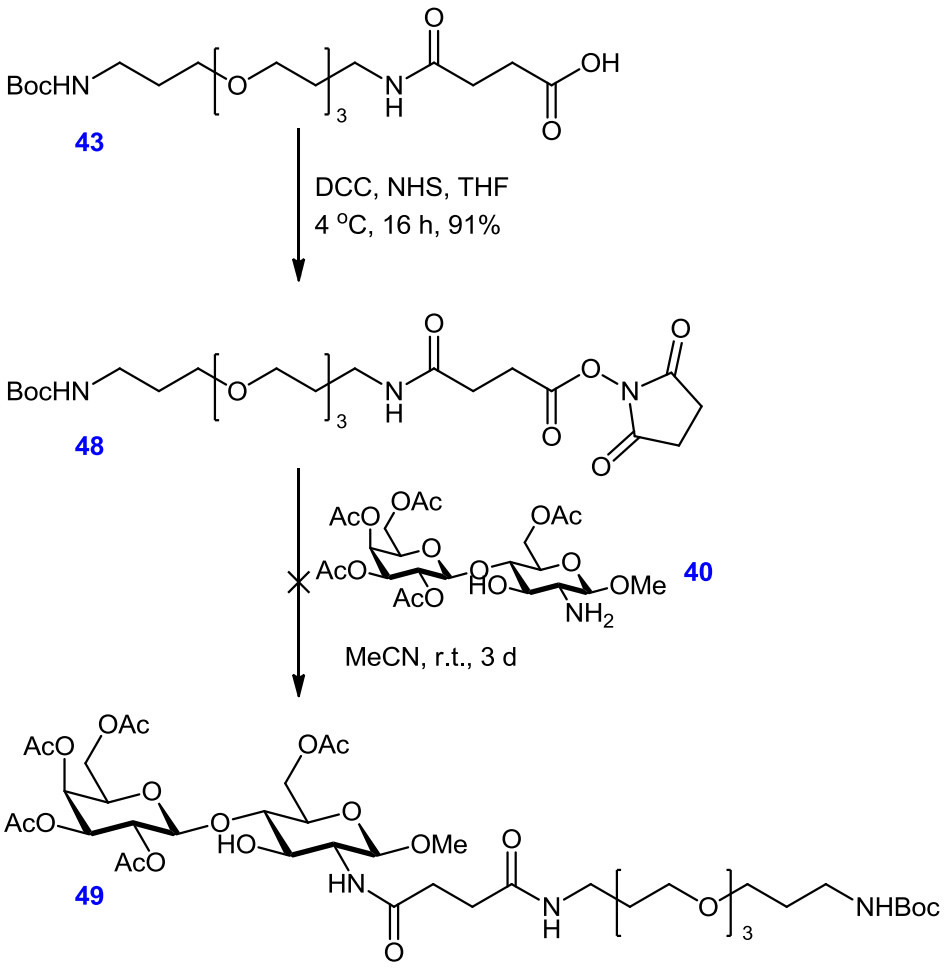


Scheme 2-24: Synthesis of Alternative Linker

The literature method for hydrolysing the ester involved the use of LiOH in TFH/H_2O followed by acidification to pH 1 with HCl. It was felt that the use of HCl would promote removal of the Boc group, and 1 M citric acid was used instead. This modification improved the yield from 75% to 84%.

2.6 Coupling to Disaccharide

Only lactosamine **40** was available for coupling, due to problems with the azide reduction, and a suitable coupling method was sought. Initially, coupling *via* an *N*-hydroxysuccinimide ester was attempted, as shown in Scheme 2-25.

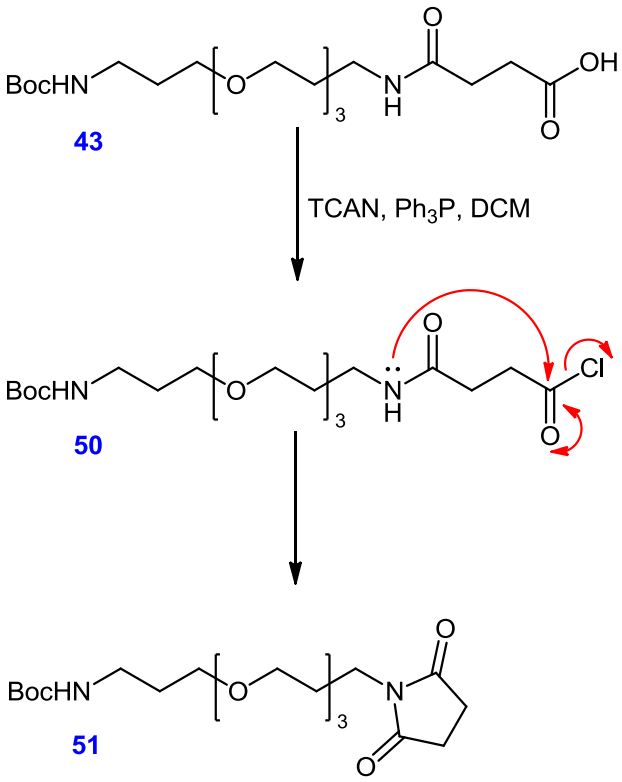


Scheme 2-25: Coupling of Disaccharide to Diamine-Derived Linker

Once production of the NHS-ester had been confirmed by isolation and characterisation, the coupling was run as domino reactions. After stirring with the lactosamine for 3 days, no product had been

produced – this suggests the NHS ester is not sufficiently reactive to promote amide formation at room temperature.

The acid chloride **50** suffers the opposite problem and is too reactive. Even when the acid chloride is produced under mild conditions using TCAN/Ph_3P,[38] a succinimide was produced (as determined by mass spectrometry), see Scheme 2-26.



Scheme 2-26: Succinimide Formation

Finally a PyBOP-mediated coupling was attempted and successfully produced the linker-derivatised lactosamine **49** in yields ranging from <5% - 54%. As this protocol requires heating at reflux for 20 h, it was anticipated that linker cyclization would occur – this may account for the variable yield as succinimide **51** would be unreactive under these conditions.

Characterisation by ^{1}H NMR spectroscopy (see Figure 2-11) showed that the coupling was successful, due the presence of 2 amide proton signals at δ = 6.78 and 6.66 ppm. HRMS also confirmed the formation of the product.

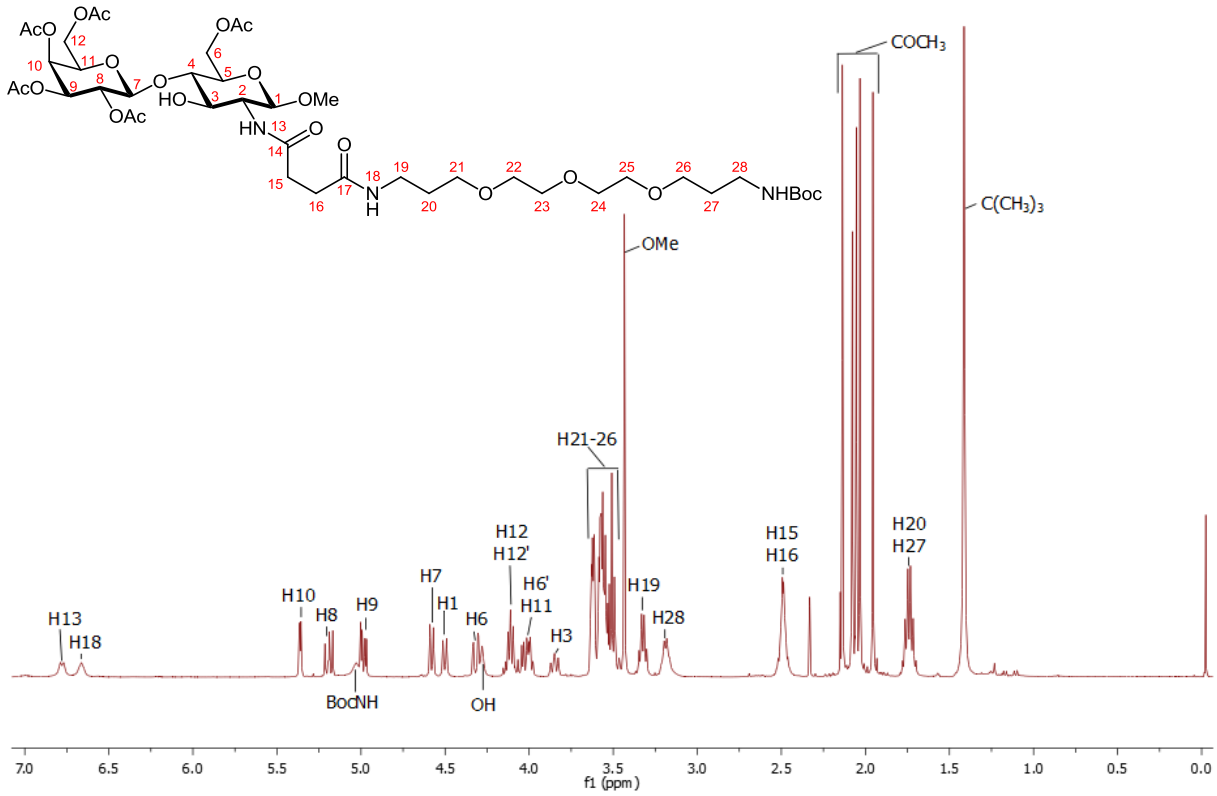
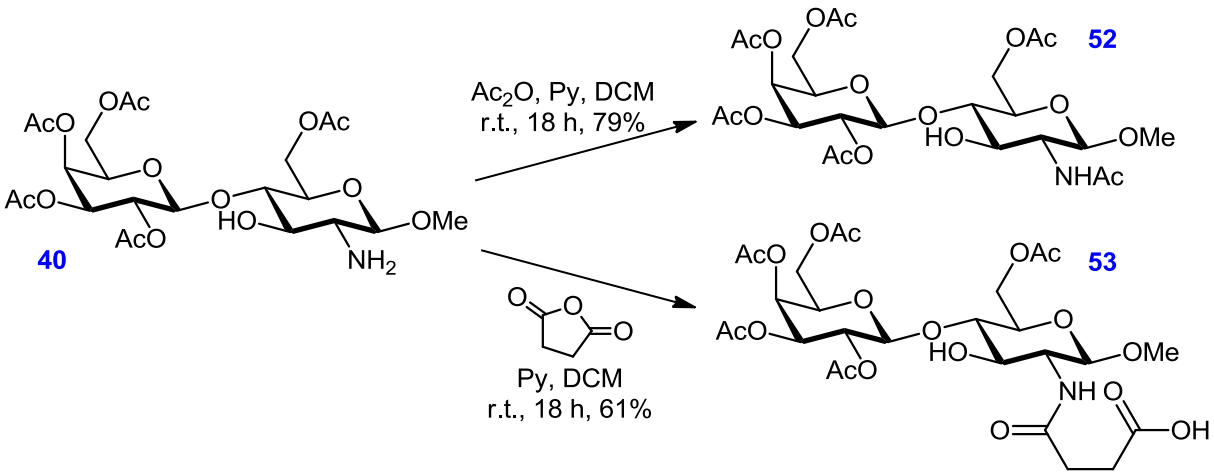


Figure 2-11: ^{1}H NMR Spectrum of Compound 49

Although the coupling was successful, the variability in yield meant that this protocol was not suitable for the naphthamide-derivatised lactosamine.

In order to confirm that the problems in the coupling were due to the linker cyclising, amine **40** was reacted with acetic and succinic anhydrides successfully, as shown in Scheme 2-27.



Scheme 2-27: Acylations at Lactosamine Nitrogen

The success of these acylations was confirmed by the presence of amide proton signals in the ^{1}H NMR spectra and by MS.

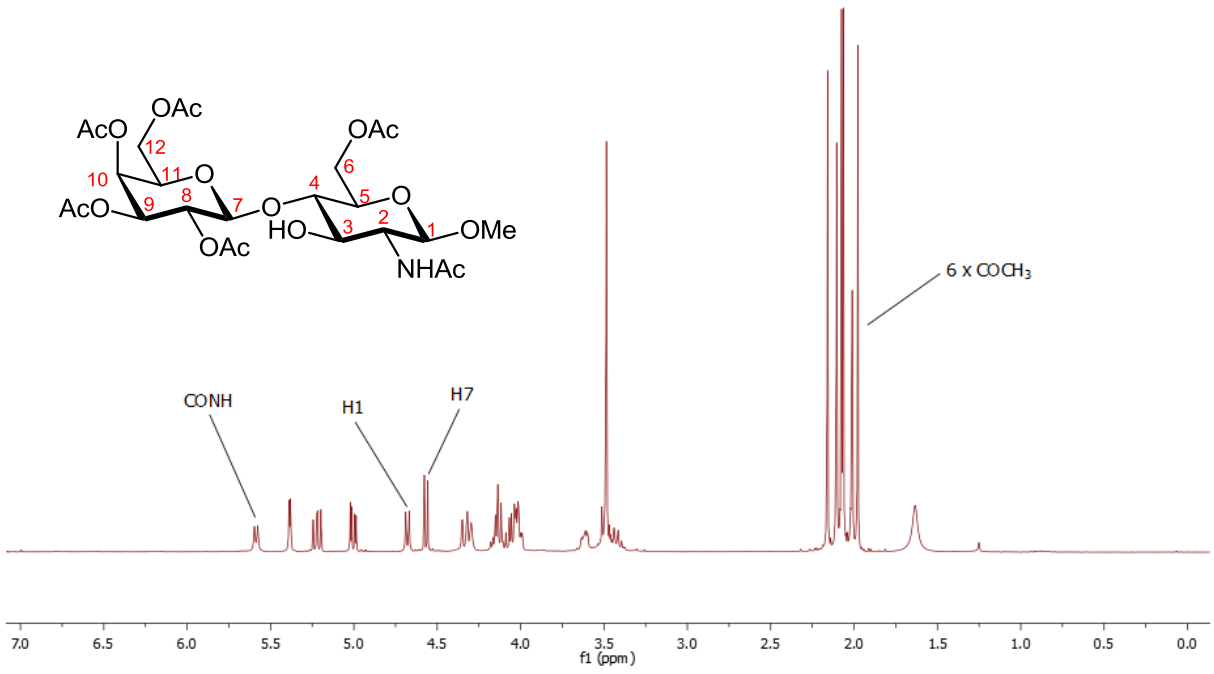


Figure 2-12: ^{1}H NMR Spectrum of Compound 52

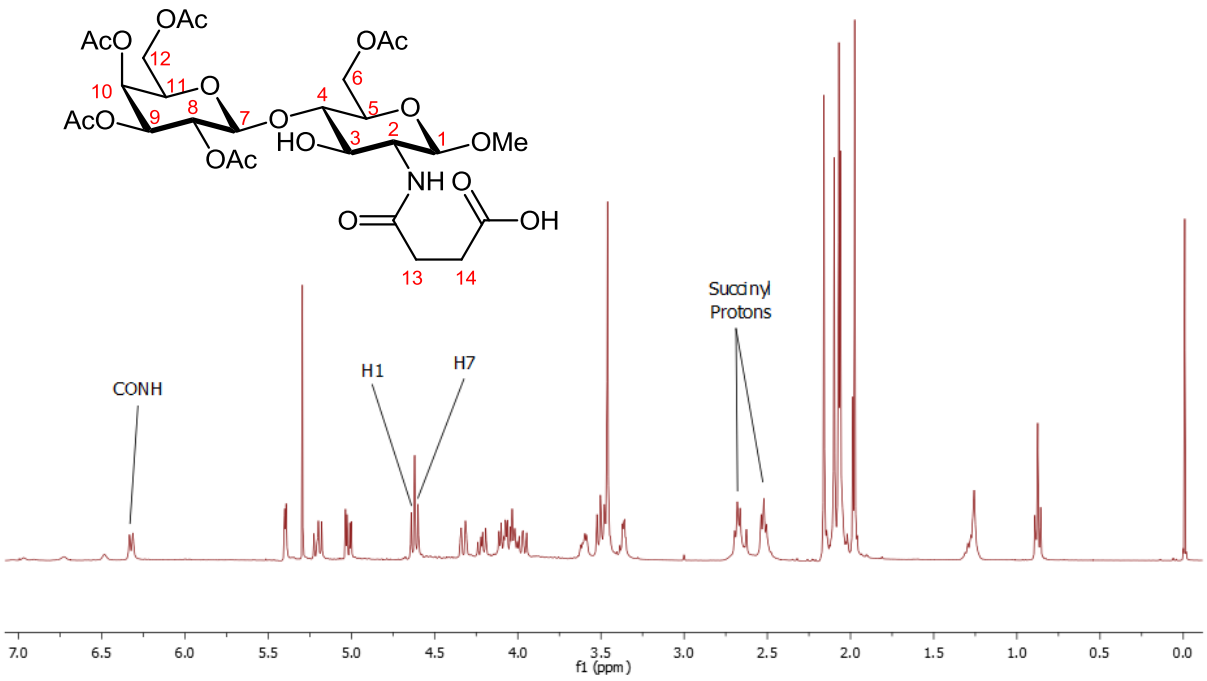
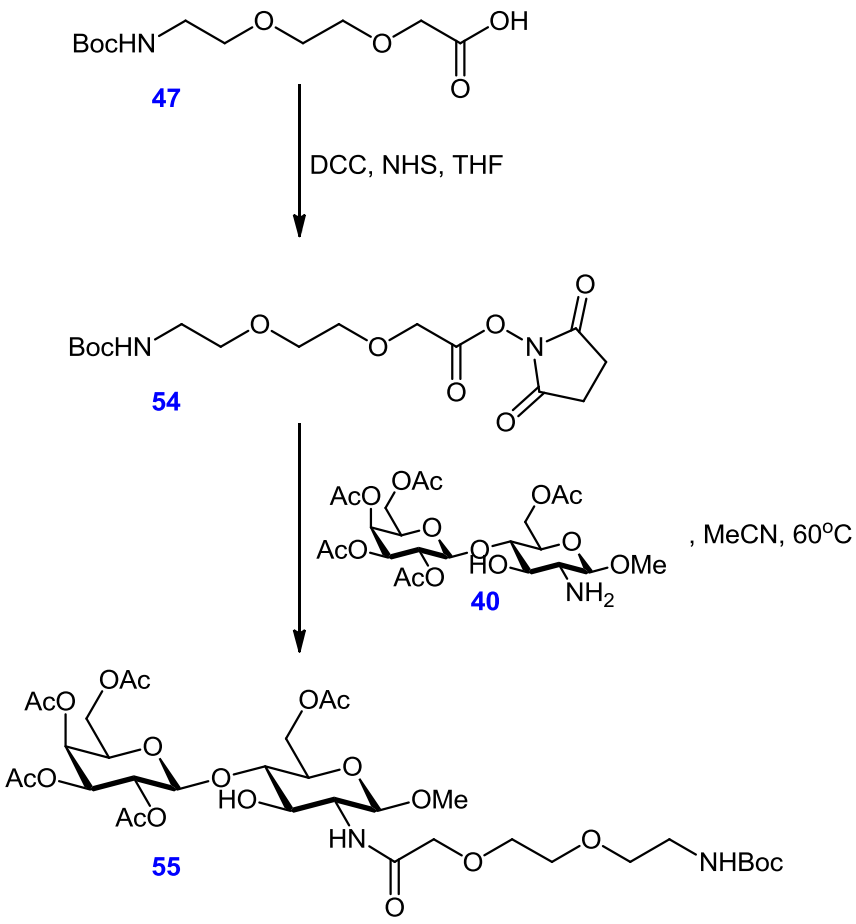


Figure 2-13: ^{1}H NMR Spectrum of Compound 53

The results of these acylations suggest that the difficulties in attaching the linker stem from the ability of the linker to cyclise to form a succinimide.

In order to avoid these difficulties lactosamine **40** was coupled to the diethylene glycol-derived linker **47**. Whilst this linker does not contain the desired amide bonds within the chain, there is no possibility of it cyclising. An NHS ester was again chosen, as in this case, heating the reaction is possible should the coupling fail at room temperature (see Scheme 2-28).

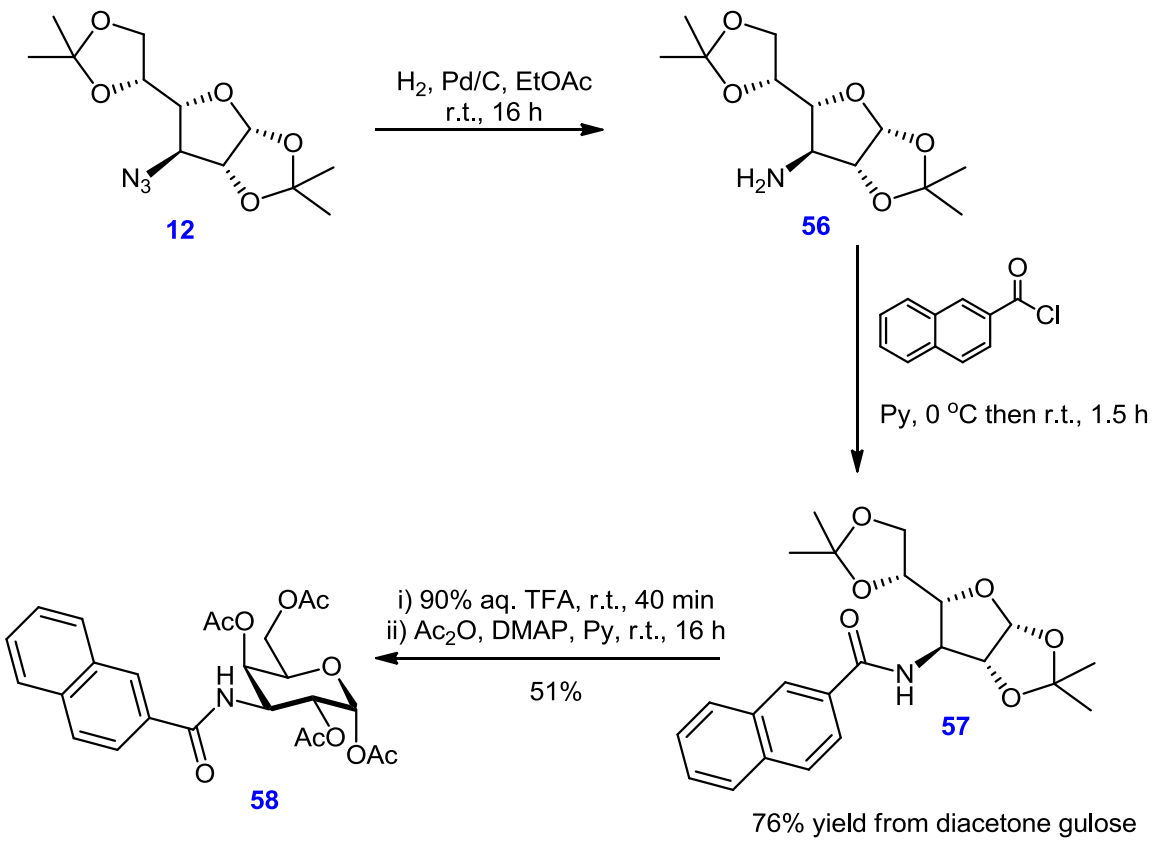


Scheme 2-28: Coupling of Disaccharide to Amino-Alcohol-Derived Linker

Whilst the coupling was successful, producing **55** in a modest yield of 41%, difficulties in the production of ω-amino acid **47** mentioned in section 2.5.1, meant that this route was unsuitable.

2.7 Redesigned Glycosyl Donor

When the difficulties in the reduction and acylation of azide **37** became apparent, an alternative glycosyl donor was designed. It was envisioned that earlier incorporation of the amide may lead to the successful production of the required disaccharide. Incorporation of the amide at the galactofuranose stage eliminates the possibility of an acetyl migration, leading to the revised synthesis shown in Scheme 2-29.



Scheme 2-29: Synthesis of Revised Glycosyl Donor Precursor

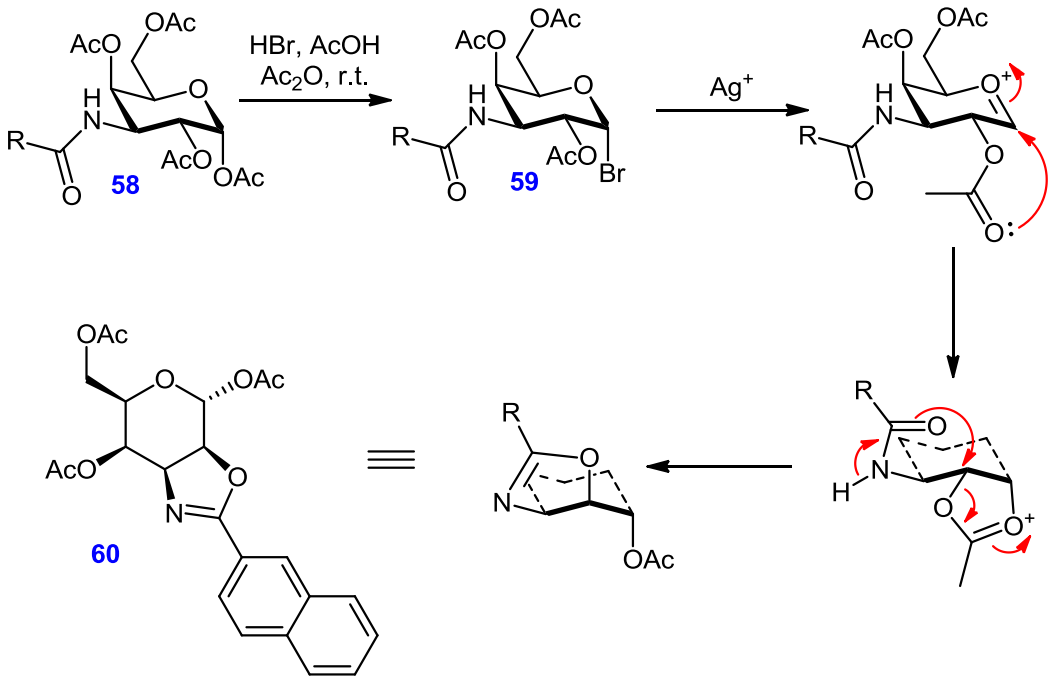
Catalytic hydrogenation of the azide using Pd/C smoothly produced the amine, which was acylated with 2-naphthoyl chloride producing **57** in 76% overall yield from diacetone gulose.

Furanose **57** was transprotected to tetra-acetate **58** with TFA followed by Ac_2O/DMAP/Py in 76% yield. Unlike the acetylation to produce the azide derivative **5** on page 32, this reaction only produced the α anomer. This is possibly caused by the amide being more electron withdrawing than the azide, withdrawing electron density from the ring and increasing the strength of the anomeric effect – favouring the production of the α-anomer.

Attempts to convert tetra-acetate **58** to a thioglycoside using $BF_3{\cdot}Et_2O$/PhSH failed and it was not possible to identify the products of these reactions. In light of the results from the reactions of the

glycosyl bromide, below, it seems likely that the PhSH was reacting with the intermediate oxazoline, and further reactions were not pursued.

The glycosyl bromide **59** could successfully be produced using HBr/AcOH/Ac_2O, but it was found that on exposure to silver salts, the glycosyl bromide cyclised to oxazoline **60** rather than producing disaccharides, see Scheme 2-30. Similar oxazolines have previously been reported by Paulsen[39] on exposure of 3-acetamido glucose derivatives on exposure to SbF_5 or $AgBF_4$.



Scheme 2-30: Silver Promoted Cyclisation

Whilst this reaction failed to produce the desired product it provides an interesting pathway to 3-amino-3-deoxy-D-talose derivatives through acid hydrolysis of the isoamide. No literature reference has been found for 3-amino-3-deoxy-D-talose derivatives.

2.8 Conclusions

A route to diacetone gulose has been established and although there were initial difficulties with the reduction of enol acetate **10**, these difficulties were successfully bypassed. Diacetone gulose can now be produced on the multi-gramme scale within 1 week in greater than 40% overall yield.

Transformation of diacetone gulose into azido-galactose **5** has also been optimised, giving the product in 60% yield over 4 steps. The only disadvantage is that the product forms a sticky semi-solid that is rather inconvenient to work with. Thioglycoside donors have also been successfully produced: whilst the ethyl thioglycosides are produced in higher overall yield, the phenyl thioglycosides are easier to handle and there is still room for optimisation to improve yields.

The synthesis of the glycosyl acceptor, devised by previous members of the group, has been further optimised with improvements in yield and purity gained from the substitution of $AgBF_4$ for Ag_2CO_3 in the production of the methyl glycoside **28**, the use of an acid catalysed transesterification to produce triol **29**, and a simplification of the monoacetylation procedure for the synthesis of **4**.

Disaccharides have been produced using standard NIS/TfOH-mediated glycosyl couplings in excellent yield. Unfortunately further transformations have all proved problematic; reduction of the azide **37** and acylation failed to produce the desired amide and attachment of linkers to the lactosamine nitrogen failed to produce a satisfactory pathway.

Attempts to solve the azide reduction problem by introducing the amide earlier in the synthesis resulted in the formation of oxazoline derivatives rather than disaccharides; although this has produced a previously unreported D-talose derivative.

In light of these problems, both the donor and acceptor were re-designed. The donor was modified to use a nitrogen protecting group that would be simpler to transform to the relevant amide. The acceptor was modified to provide alternative linker attachment sites. The modifications are discussed in the next chapter.

3 Second Generation

3.1 Design & Retrosynthesis

Sörme has show that disaccharide **61** binds to galectin-3 with K_d = 320 nM,[14] and is thus a suitable motif for our target. This naphthalamide could be generated from a naphthalimide, which should serve as a nitrogen protecting group through the synthesis, see Figure 3-1.

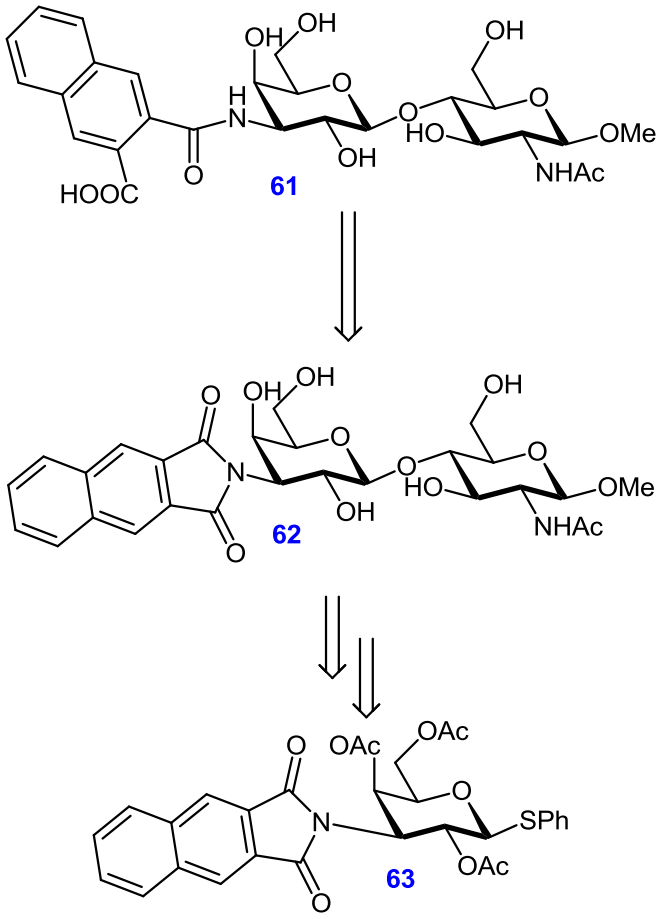


Figure 3-1:Retrosynthesis of Naphthalamic Saccharide Derivative

Due to the expense of 2,3-naphthalimide, a phthalimide group would serve as a model, allowing the reactions to be optimised whilst minimising expense. Phthalimide **64** can be synthesised from

diacetone gulose using a protocol similar to the production of the azide derivative discussed earlier, see Figure 3-2.

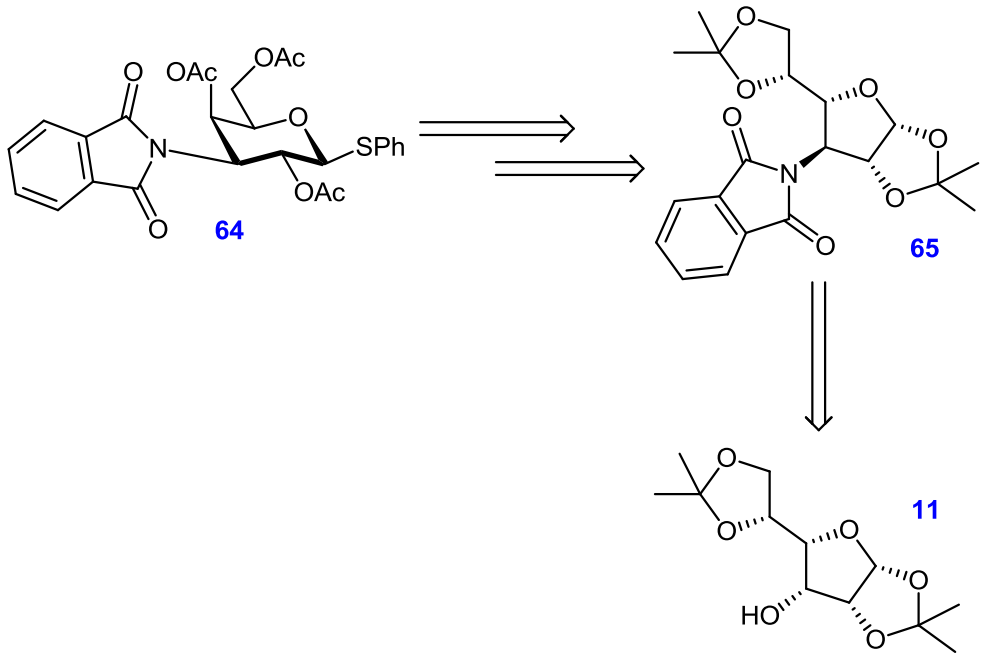


Figure 3-2: Retrosynthesis of Phthalimide

For the acceptor, a literature search revealed that **66** has been synthesised by Izumi[40] in 2 steps from Troc-protected glucosamine. This allows for flexibility as the linker could be attached either through the nitrogen or through the alkyne.

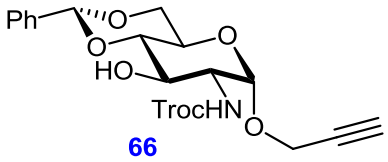


Figure 3-3: Izumi's Protected Glucosamine

The literature method produces only the α-anomer. Examination of the crystal structure for LacNAc bound to Gal-3 suggests that there should be sufficient room to accommodate α-substituents, see Figure 3-4. Thus it is viewed that the exact anomer used is unimportant, although anomerically pure materials are desirable to simplify characterisation.

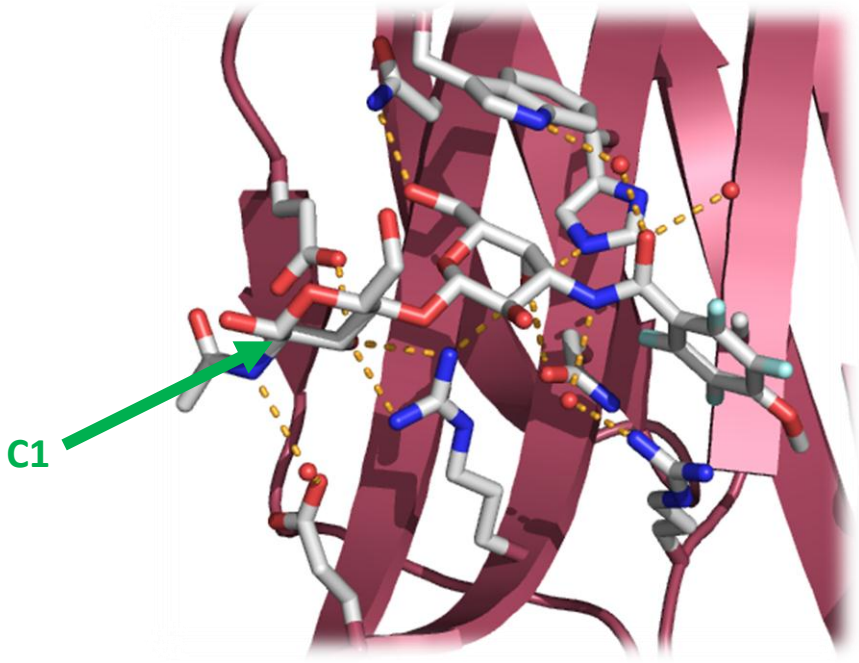
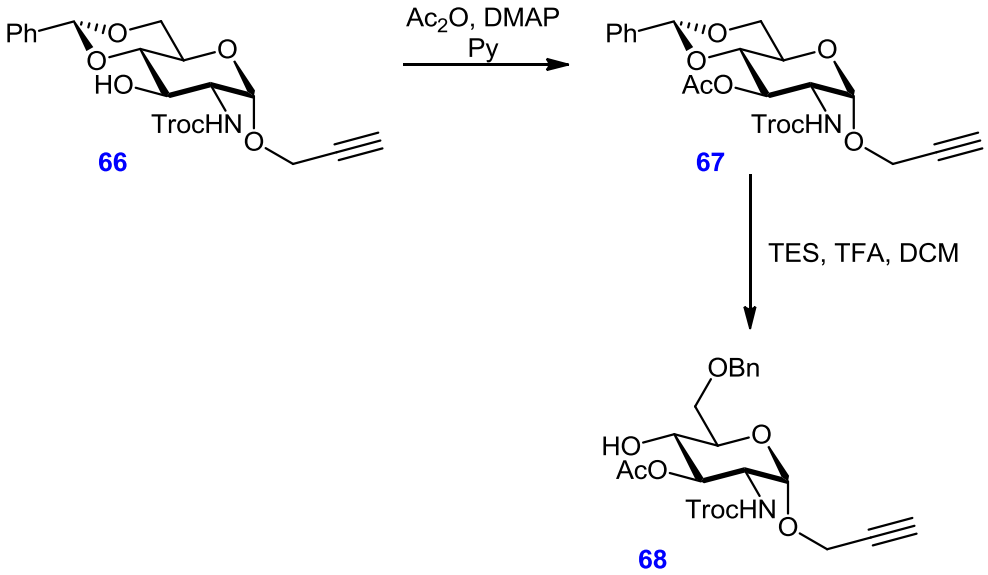


Figure 3-4: Gal-3 Bound LacNAc Showing Room for α-Substituents at C1 (Marked in Green) – created from PDB: 1KJL[14]

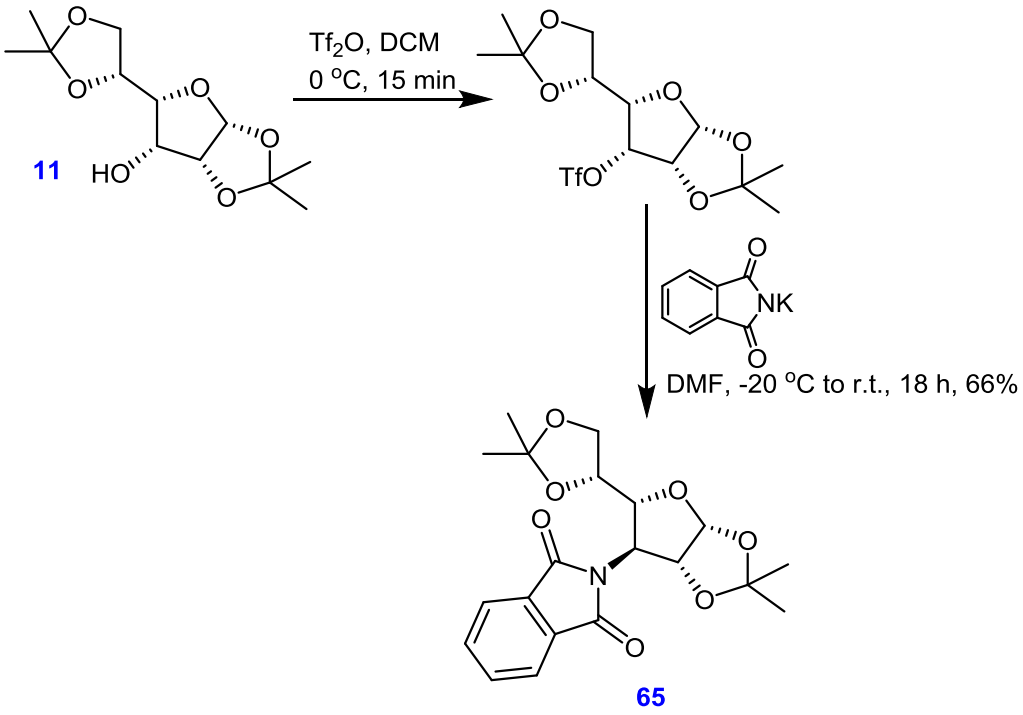
In the first generation synthesis, the inductively withdrawing TCP group was used to differentiate between OH3 and OH4 and thus allow the exclusive formation of β(1-4) glycosyl bonds. As the second generation acceptor does not contain such a group, another strategy to differentiate between OH3 and OH4 would be needed – this can be achieved by acetyl protection prior to the reductive ring opening of the benzylidene acetal, as shown in Scheme 3-1.



Scheme 3-1: Synthesis of Second-Generation Acceptor 68

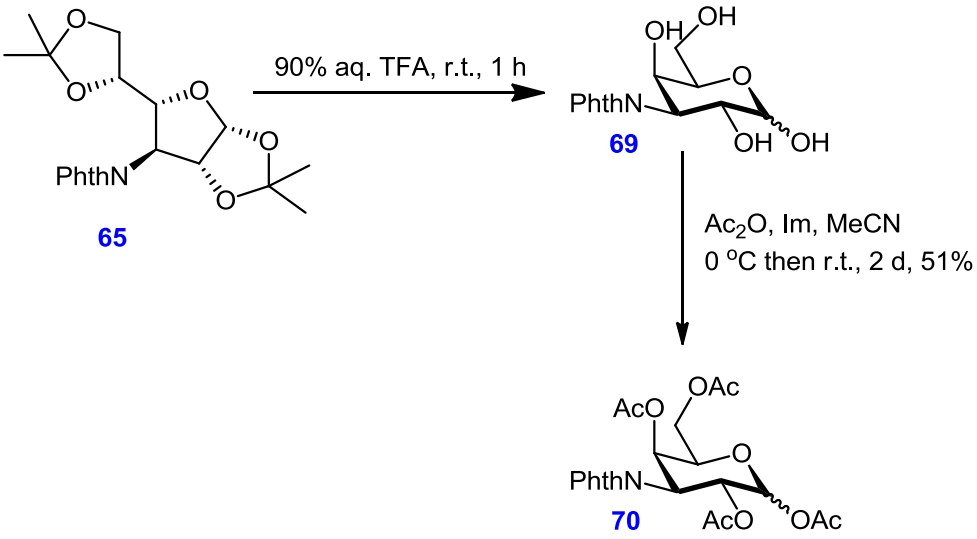
3.2 Glycosyl Donor

The first step in the preparation of the second generation glycosyl donor is the activation of diacetone gulose with triflic anhydride followed by substitution with phthalimide. This is similar to the process for preparing the azide derivative **12**, although the temperature for the reaction was reduced to -20 °C to reduce the possibility of elimination reactions. The phthalimide was produced in 66% yield.



Scheme 3-2: Synthesis of Phthalimide 65

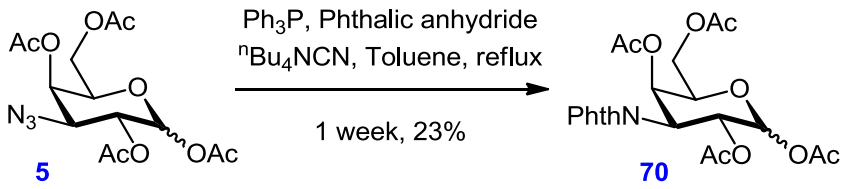
The acetonide protecting groups were easily removed with TFA/H_2O, though the acetylation proved troublesome – probably due to the electron withdrawing nature of the phthalimide group, combined with the successive additional deactivation caused by the addition of each acetyl group. Several acetylation conditions were attempted and the best was found to be Ac_2O/Im/MeCN, which produced the product in 51% yield in 2 days.



Scheme 3-3: Transprotection of Phthalimide 70

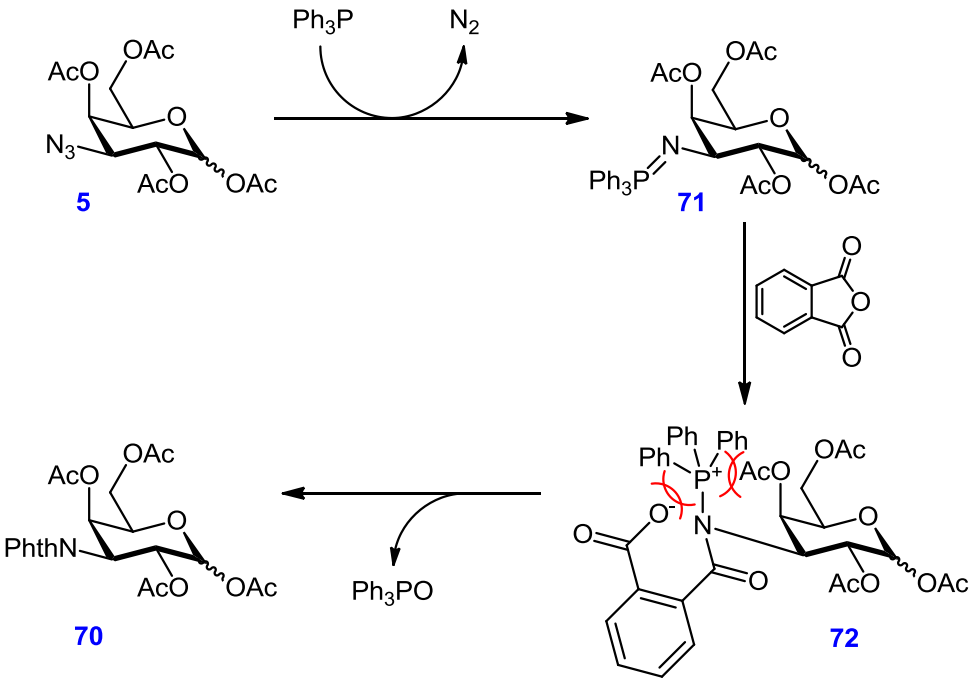
As **70** is novel, no spectra were available for comparison and it was unclear that the reaction had produced the desired product. This uncertainty was compounded by the appearance of a $[2M + Na]^+$ peak in the mass spectrum, which suggests the possibility of dimerization, and an alternative synthesis was sought.

Garcia[41] has demonstrated a method of converting azides directly to phthalimides by using Ph_3P and phthalic anhydride, see Scheme 3-4.



Scheme 3-4: Alternative Synthesis of Phthalimide

The reaction was conducted in toluene under reflux for 1 week, yet only a yield of 23% was achieved with the phosphazene intermediate **71** still present in the crude. This can be accounted for by the high steric demand of arranging Ph_3P, phthalic anhydride and galactose around the nitrogen, see intermediate **72** in Scheme 3-5.

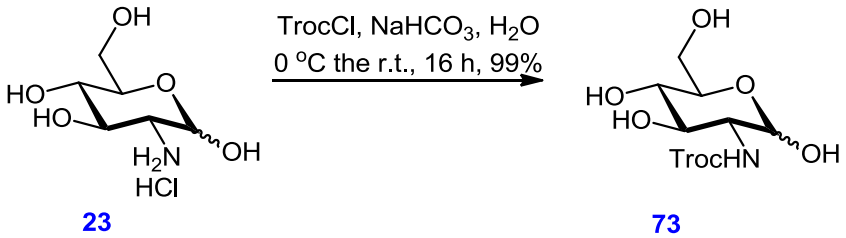


Scheme 3-5: Formation of Phthalimide from Azide

Although the yield was low, a sample of phthalimide **70** was synthesised providing reference spectra and thus confirming that the method in Scheme 3-3 was successful. Unfortunately, due to time constraints, it was not possible to investigate further reactions in this sequence.

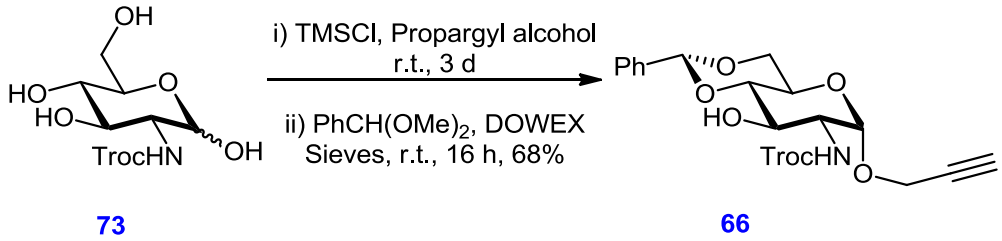
3.3 Glycosyl Acceptor

The first stage of the synthesis of the glycosyl acceptor is the Troc protection of glucosamine. This was easily achieved in 99% yield, the only quirk was that the reaction generates a foam that floats on top of the reaction solvent, thus a large reaction flask is needed.



Scheme 3-6: Troc Protection of Glucosamine

The next step is a Fischer glycosidation with propargyl alcohol and TMSCl as an acid catalyst,[40] immediately followed by a benzylidene acetal formation, see Scheme 3-7.

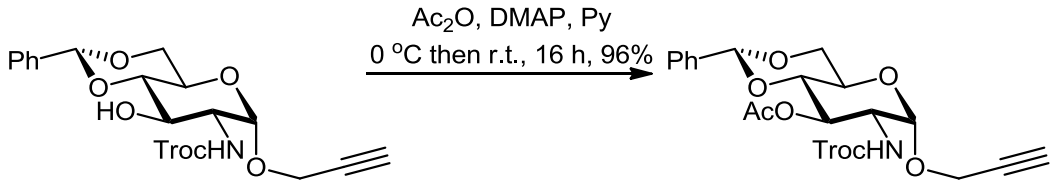


73 **66**

Scheme 3-7: Fischer Glycosidation & Benzylidene Formation

The Fischer glycosidation was straightforward, although the removal of the propargyl alcohol solvent was laborious, involving extensive co-distillation with toluene. The procedure for the formation of the benzylidene acetal was modified (as discussed in section 2.2.2) allowing for a simple work-up and giving the product in 68% yield.

The acetylation of the unprotected hydroxyl group presented no difficulties and was easily accomplished with Ac_2O/DMAP/Py in 96% yield.

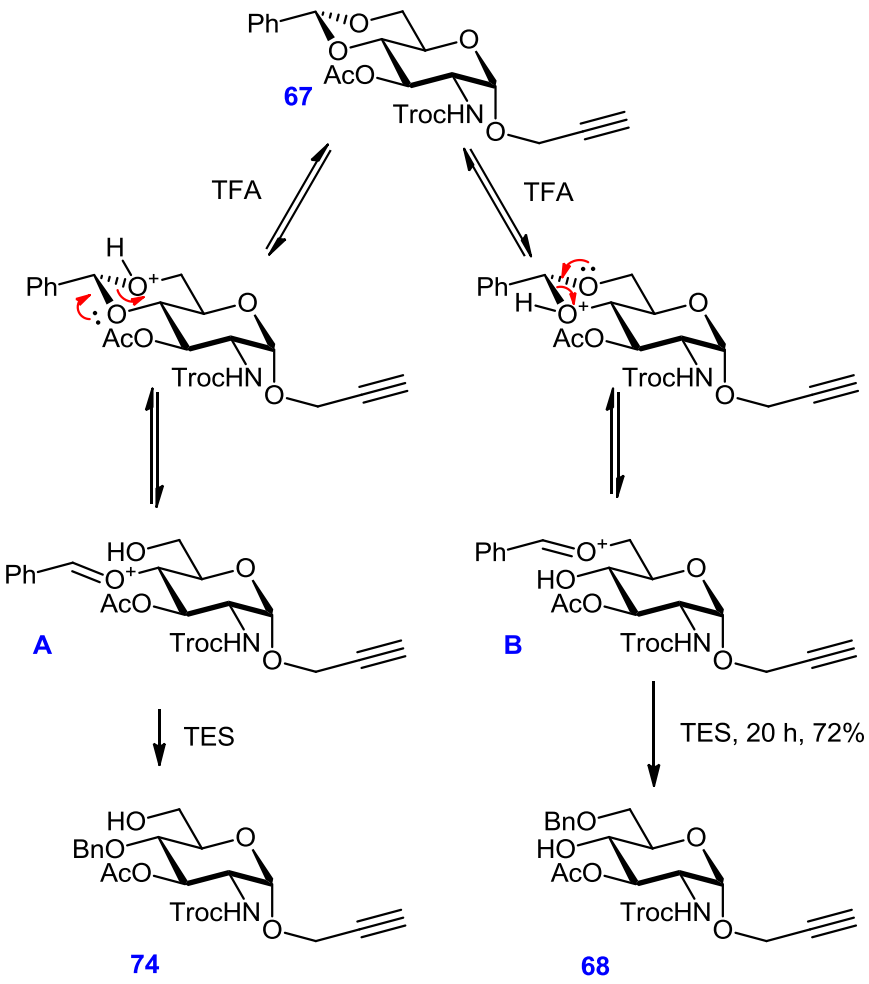


66 **67**

Scheme 3-8: Acetylation of Open Hydroxyl Group

The final step to produce the acceptor is reductive ring opening of the benzylidene acetal to produce a 6-*O*-benzyl derivative **68**. DeNinno[42] has shown that this may be achieved selectively by the use of TES/TFA.

It is hypothesised that the origin of the selectivity is a fast equilibration between ring-opened oxocarbenium cations followed by rate limiting reduction by the silane, as shown in Scheme 3-9. Of the two oxocarbenium cations, **B** is anticipated to be the more stable, on steric grounds, and thus be the dominant oxocarbenium cation. **B** would also be expected to be reduced faster than **A**, again on steric grounds, thus allowing time for **A** to be converted to **B**.



Scheme 3-9: Selectivity in Reductive Ring Opening

Figure 3-5 shows a reaction coordinate diagram that shows the anticipated relative energies of the species involved in this reaction. We suppose that protonation at O4 is less favourable than protonation at O6, but the O4-protonated species opens to form the oxocarbenium ion that is more

easily attacked by TES – the transition state is predicted to be lower in energy that that of the alternative oxocarbenium ion, and therefore lead to the dominant product.

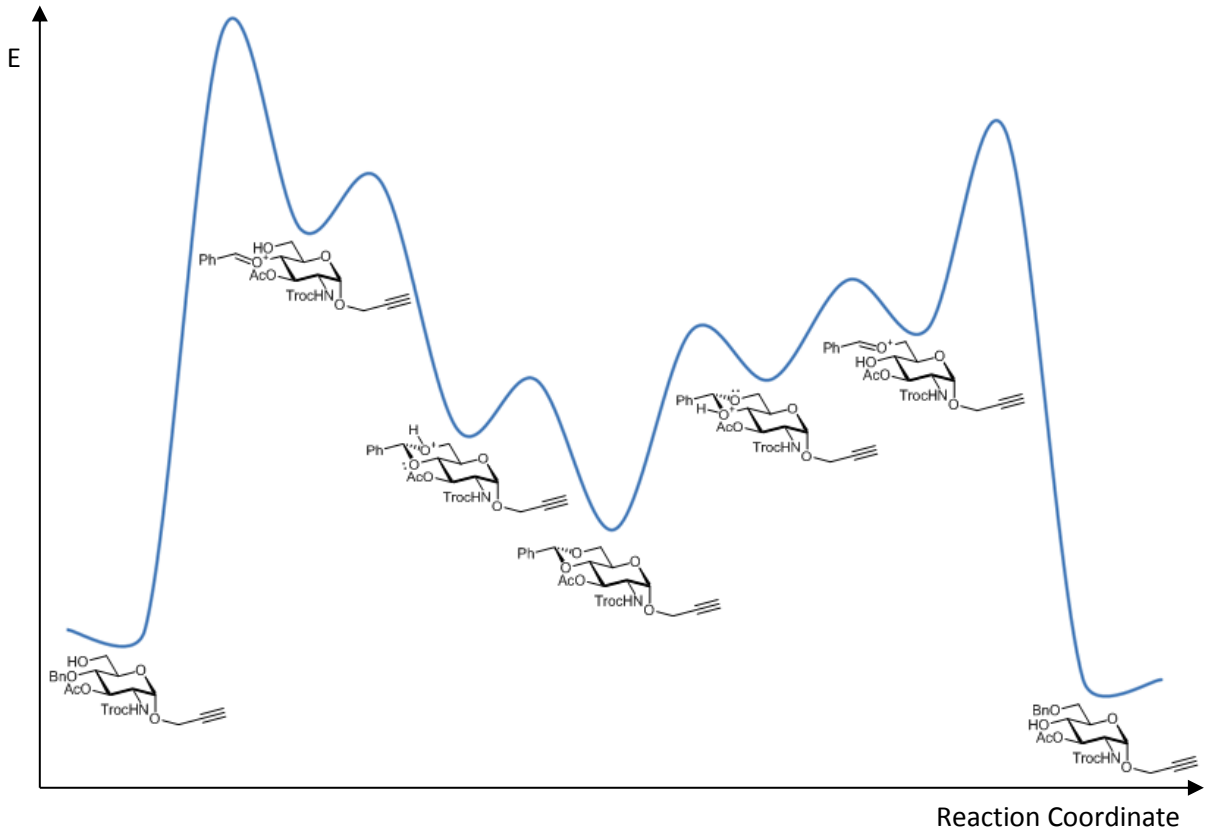
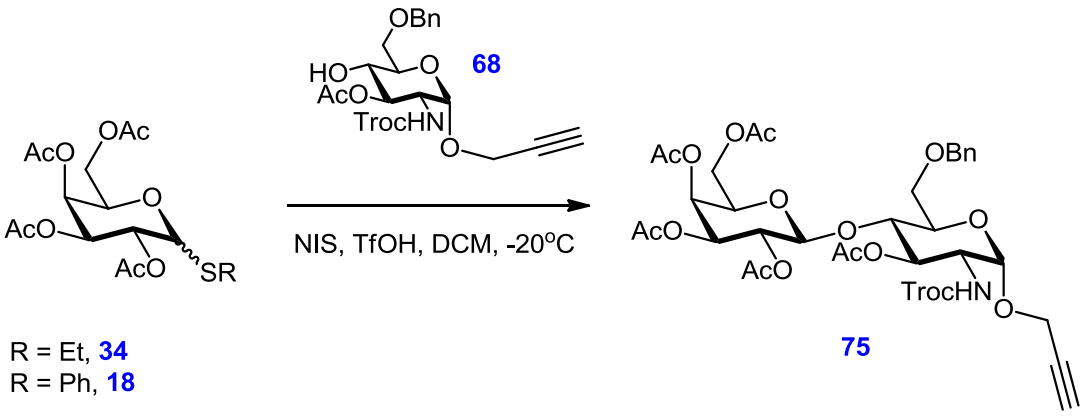


Figure 3-5: Reaction Coordinate Diagram for Reductive Ring Opening

The glycosyl acceptor **68** has been synthesised in 46% yield over 4 steps, as compared to 26% yield over 5 steps for the first generation acceptor **4**.

3.4 Disaccharides

Thiogalactosides **18** and **34** were coupled to acceptor **68** in a NIS/TfOH-mediated glycosyl coupling, as shown in Scheme 3-10. The yields for ethyl and phenyl thiogalactosides couplings are very similar and are shown in Table 3-1. As the yields are almost identical phenyl thiogalactoside was chosen due to handling considerations.

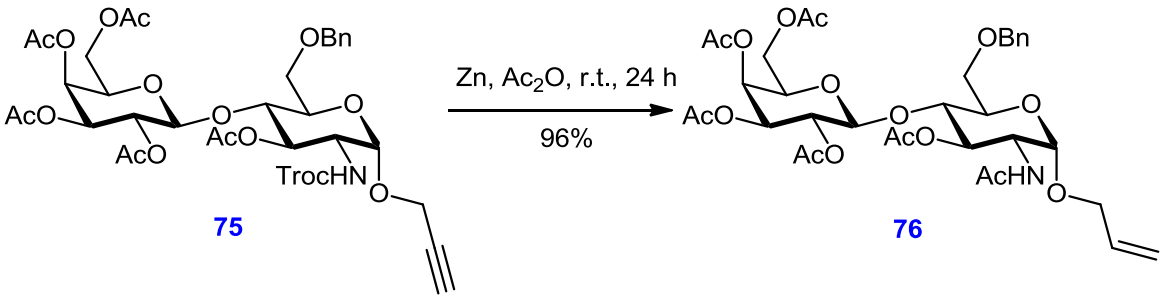


Scheme 3-10: Glycosyl Couplings

Table 3-1: Thioglycoside Formation and Glycosyl Coupling Results

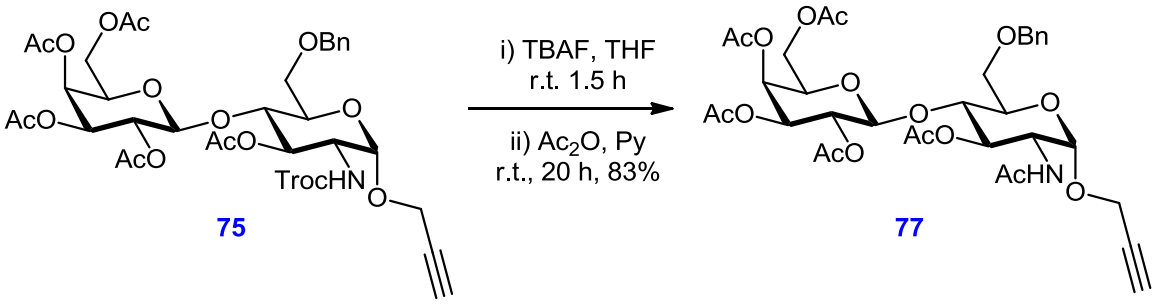
Entry	R	% Yield Thioglycoside Formation	% Yield Glycosyl Coupling	% Yield Overall
1	Et	86	65	56
2	Ph	81	66	53

The final transformation before the linker attachment was the transprotection of the Troc group to an acetate. A typical method for the removal of Troc groups is with Zn/Ac_2O, it was found that this method also performed a dissolving metal reduction on the alkyne, see Scheme 3-11.



Scheme 3-11: Transprotection with Concomitant Alkyne Reduction

Huang[43] has shown that it also possible to remove a Troc group with TBAF – a method that is compatible with an alkyne group, see Scheme 3-12.



Scheme 3-12: Troc Removal

This method produced *N*-acetyl lactosamine derivative **77** in 83% yield.

3.5 Linkers

The presence of an alkyne group in disaccharide **77** allows for the use of alkyne-azide click reactions for the attachment of the linker. As before, an FITC probe was investigated first and thus the synthesis of PEG-based amino-azides was required. The linkers show in Figure 3-6 could be based on those used in the first generation synthesis discussed in Section 2.5.

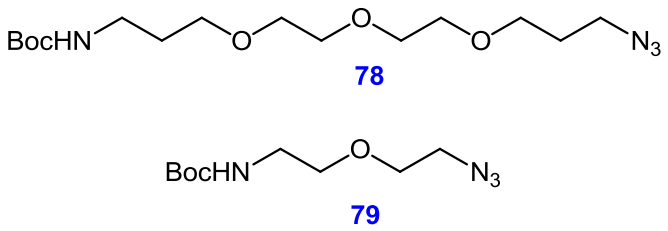
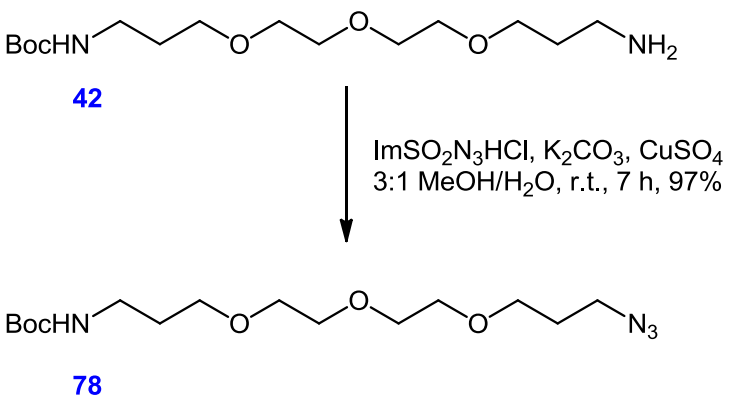


Figure 3-6: Amino-Azide Linkers

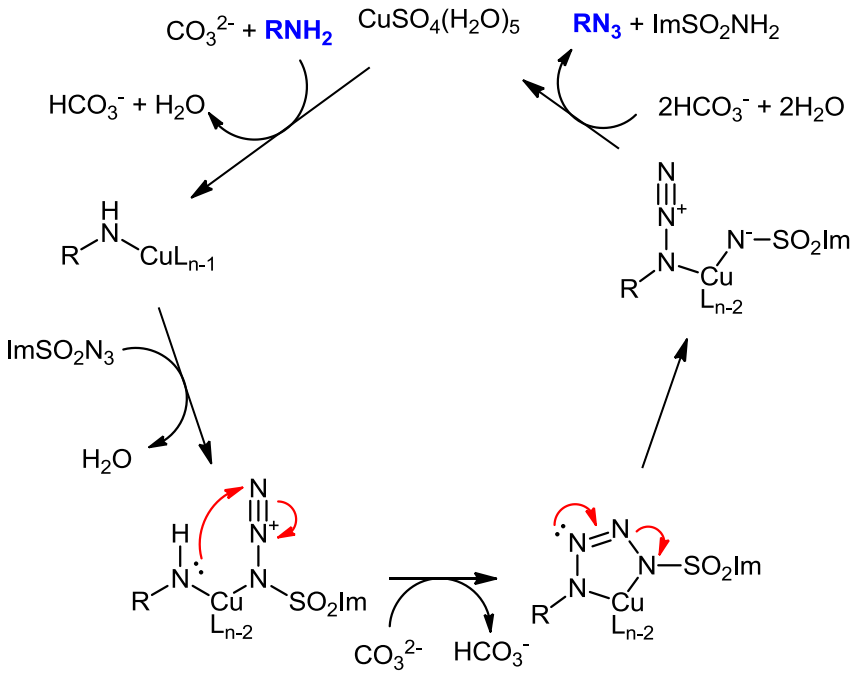
3.5.1 Long Linker

The long linker **78** was synthesised in a single step from the Boc-protected diamine by employing a diazo-transfer reaction in 97% yield, see Scheme 3-13. Imidazole-1-sulfonyl azide hydrochloride was chosen as the diazo-transfer reagent as it is simple to prepare and handle, and is shelf-stable.[44]



Scheme 3-13: Diazo-Transfer Reaction

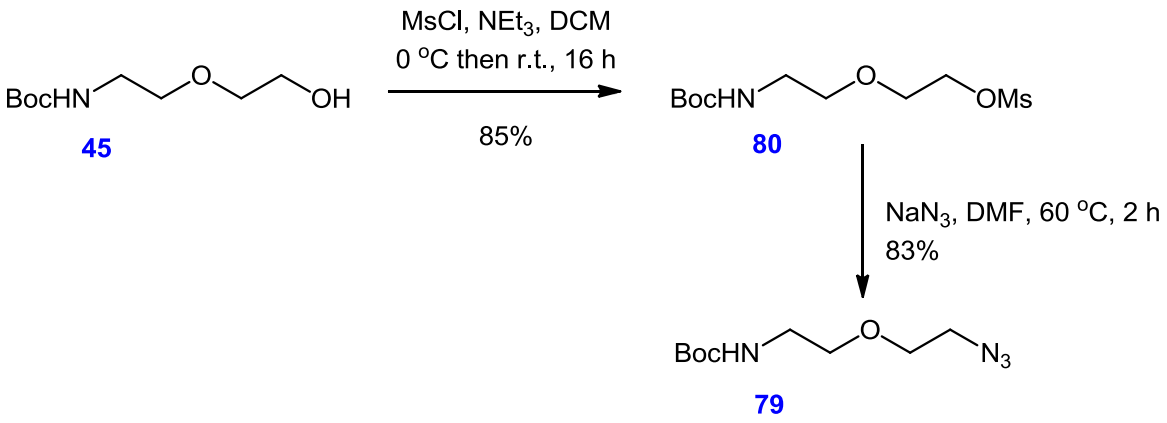
A possible mechanism for this reaction involves the co-ordination of the alkyl amine and sulfonyl azide at copper followed by cyclization to form a tetra-azo-cupracycle which collapses to form the alkyl azide and sulfonamide, as shown in Scheme 3-14.



Scheme 3-14: Possible Mechanism of Diazo Transfer

3.5.2 Short Linker

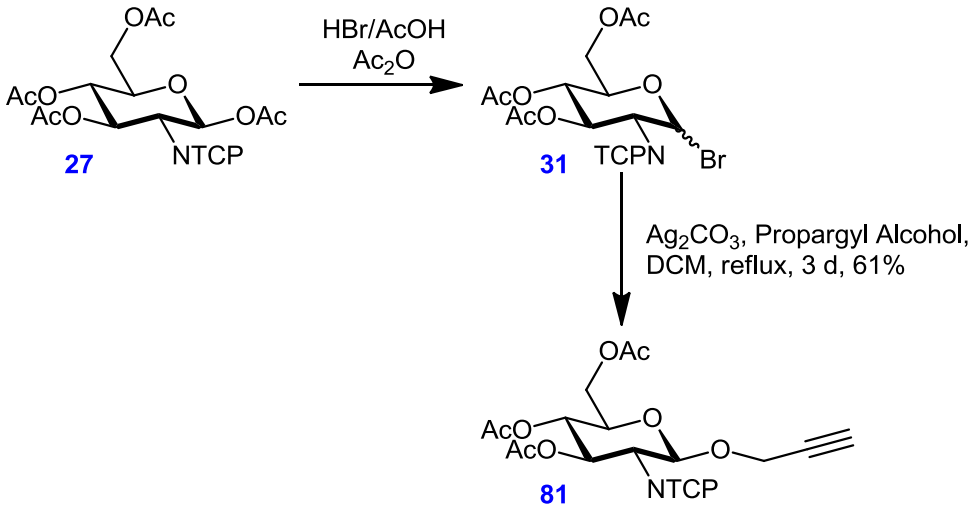
The short linker **79** was synthesised from the Boc-protected alcohol by activation with MsCl followed by substitution with NaN_3 in 71% yield over 2 steps, see Scheme 3-15.



Scheme 3-15: Alcohol Activation and Azide Substitution

3.6 Click Reactions & Conjugation to FITC

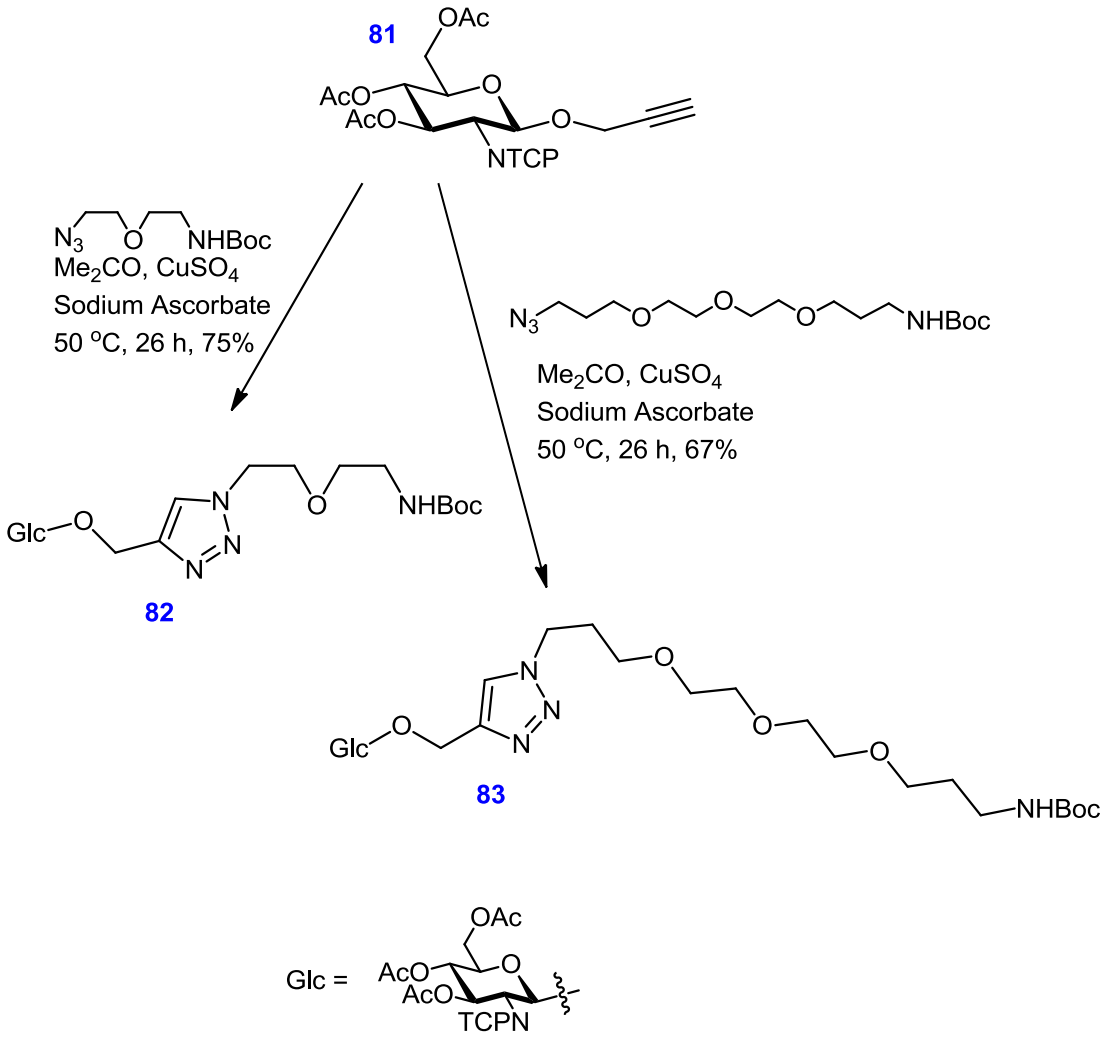
In order to avoid potentially wasting disaccharide, conditions for the attachment of the linker by CuAAC were optimised using a glucosamine-derived propargyl glycoside **81**. This substrate was prepared from **27** *via* a glycosyl bromide in 61% yield, see Scheme 3-16.



Scheme 3-16: Synthesis of Model Substrate 81

3.6.1 Optimisation on Model System

CuAAC reactions require Cu(I), which was generated in situ by reduction of Cu(II) with sodium ascorbate. $^{t}BuOH/H_2O$ is commonly employed as solvent,[45, 46] but solubility problems were encountered; it was found that switching to acetone solved these problems.



Scheme 3-17: CuAAC Reactions on Model Substrate

The cycloaddition reactions shown in Scheme 3-17 were complete within 26 hours at 50 °C, producing the short- and long-linker triazoles in 75% & 67% yield respectively. ^{1}H NMR studies (see Figure 3-7) showed the appearance of a triazole signal at ca. δ = 7.6 ppm and the disappearance of the alkyne signal at δ = 2.43 ppm, confirming the successful attachment of the linker.

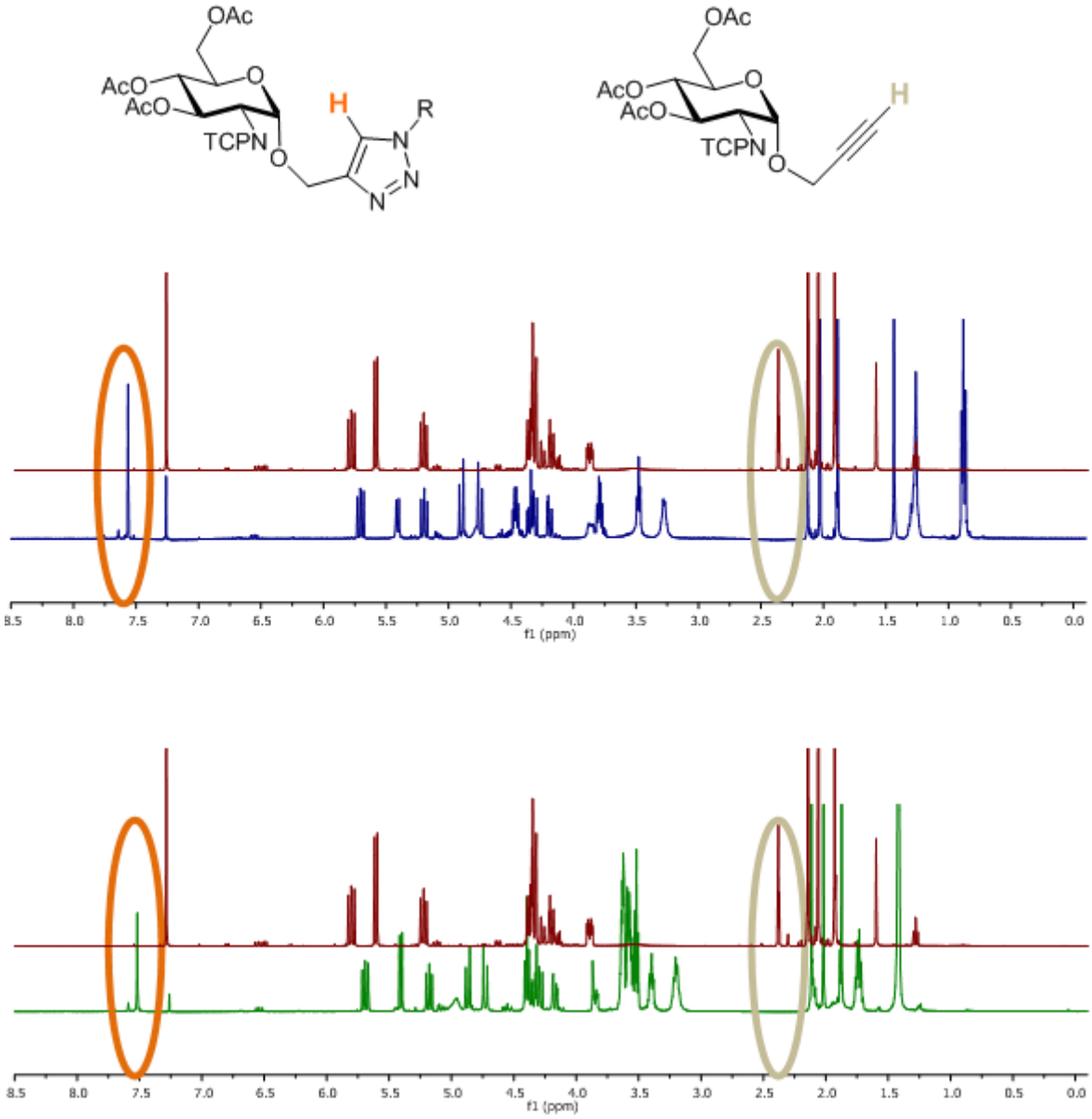
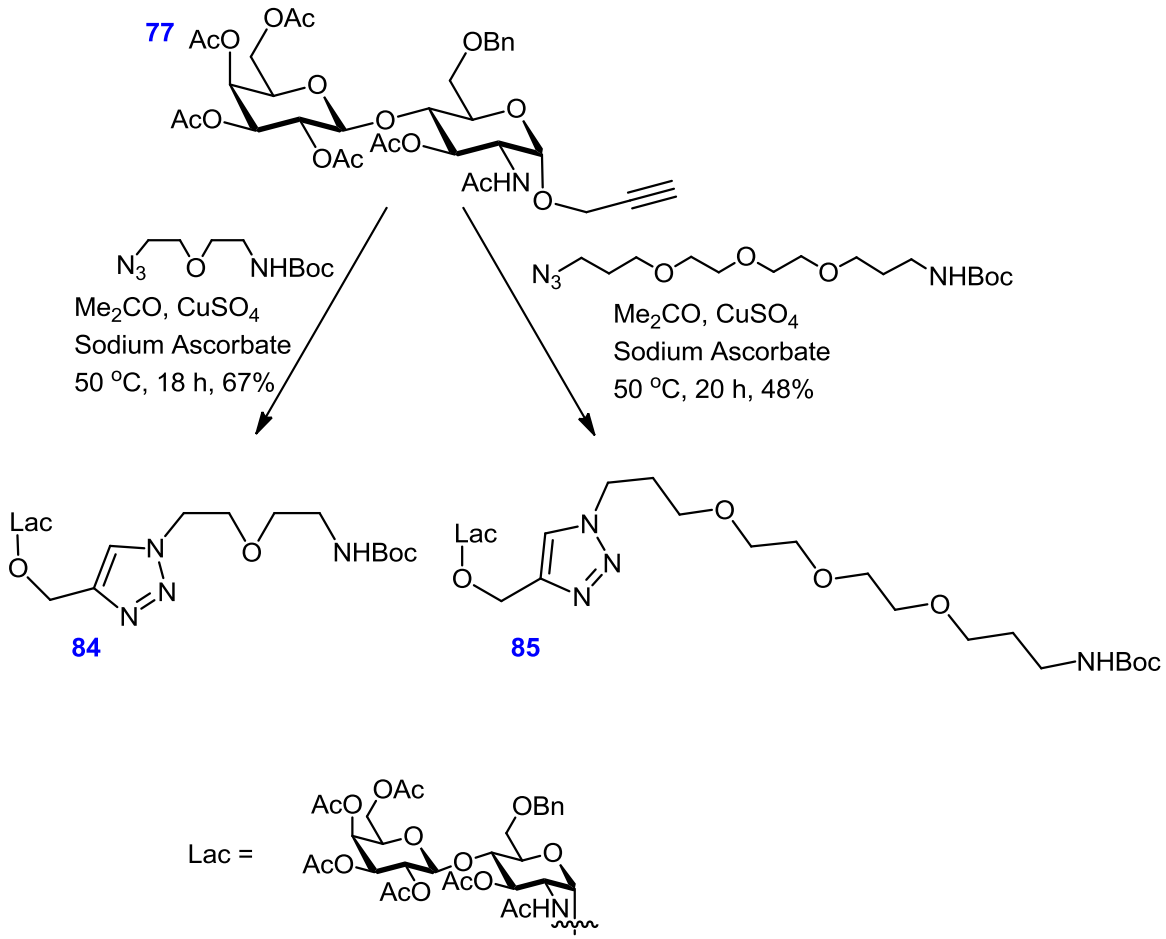


Figure 3-7: ^{1}H NMR Showing the Appearance of a Triazole Signal (Orange) and the Disappearance of the Alkyne Signal (Grey) for Short (Blue) and Long (Green) Linker Click Reactions on Model Alkyne (Red)

3.6.2 Linker Attachment to Disaccharides

The modified conditions for the CuAAC reactions above were then applied to disaccharide **77**, producing the short- and long-linker triazoles in 67% & 48% yield respectively (Scheme 3-18). These yields are similar to those obtained for the first generation linker attachment reactions, but without the issues surrounding linker cyclisation and synthesis (as discussed in sections 2.5.1 and 2.6)



Scheme 3-18: CuAAC Reactions on Disaccharides

Once again, the success of the reactions was confirmed by ^{1}H NMR spectroscopy (see Figure 3-8), which showed the appearance of the triazole signal at ca. δ = 7.6 ppm and the disappearance of the alkyne signal at δ = 2.43 ppm.

Click reactions on 1-*O*-propargyl lactosamine derivatives have not been reported, and thus this method provides an excellent alternative to attaching linkers through the amine.

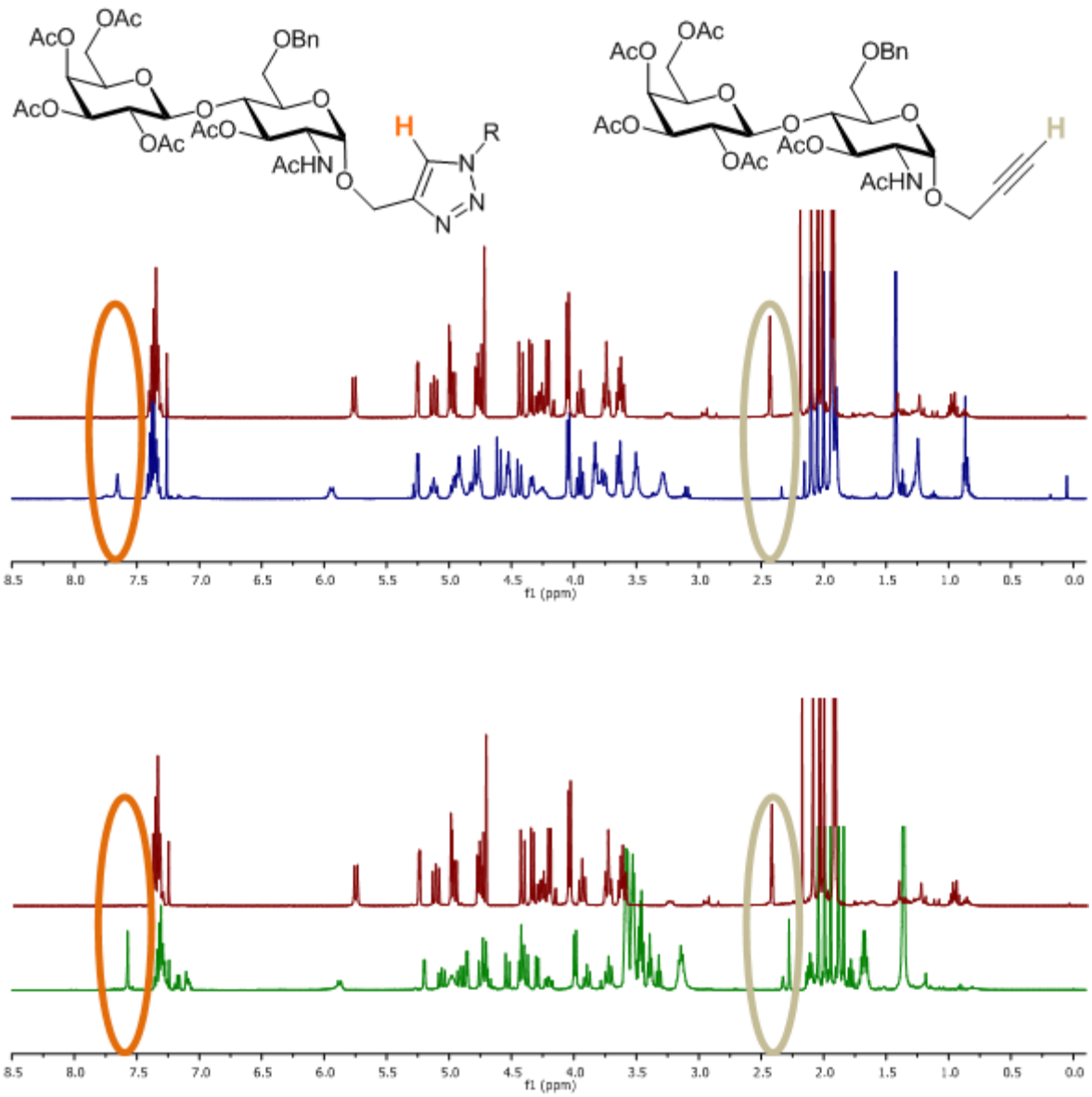


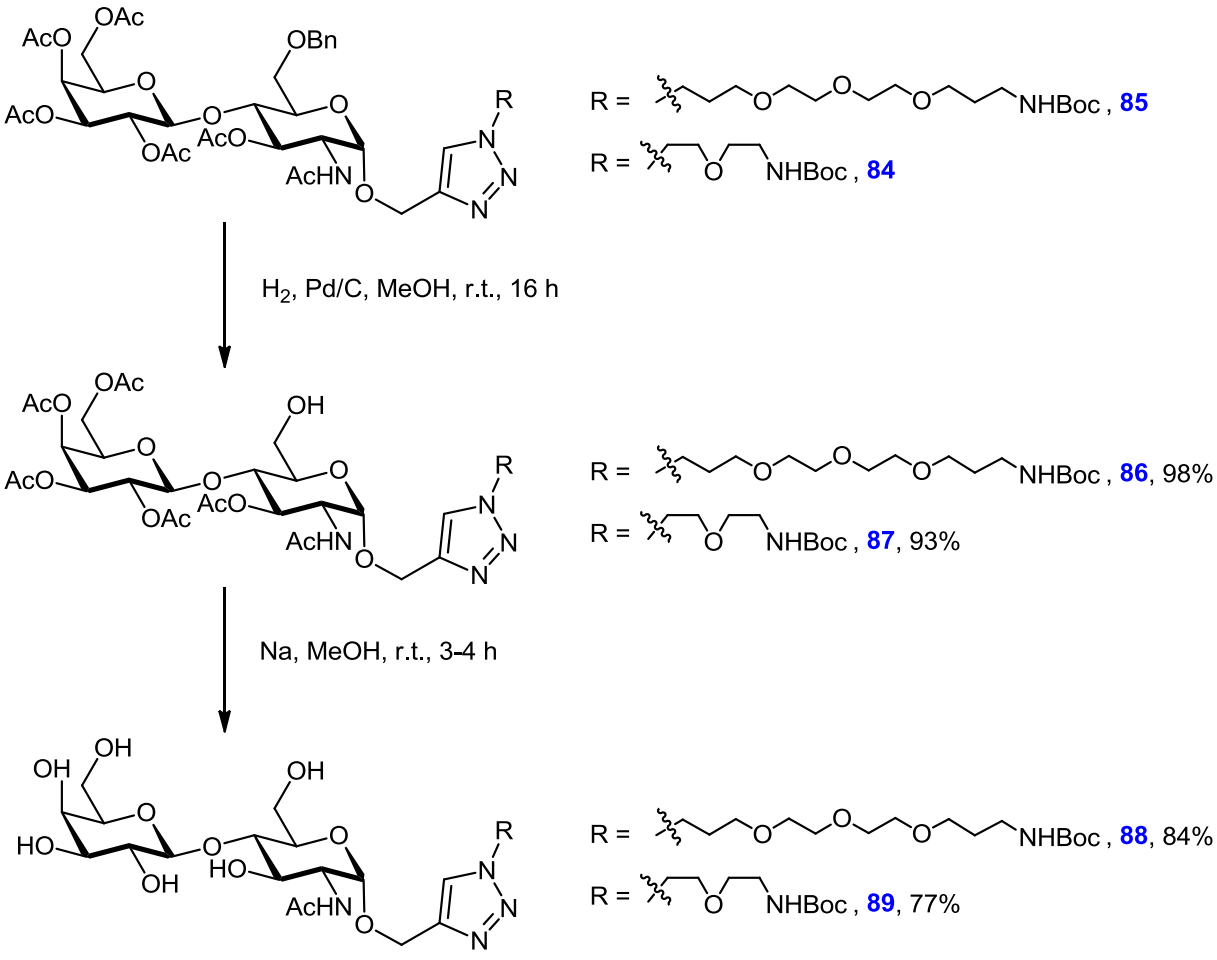
Figure 3-8: ^{1}H NMR Showing the Appearance of a Triazole Signal (Orange) and the Disappearance of the Alkyne Signal (Grey) for Short (Blue) and Long (Green) Linker Click Reactions on Disaccharide 77 (Red)

3.6.3 Deprotection and FITC Installation

For the final set of transformations there was a choice between installing the FITC before or after the deprotection. It was uncertain whether the deprotection conditions would interfere with the FTIC group, therefore the deprotection was done first. The deprotection steps are shown in Scheme 3-19. In order to maximise solubility in organic solvents, the benzyl group was removed first. Benzyl ethers can be removed by hydrogenation,[47, 48] strong acids,[49] oxidation to benzoyl esters followed by

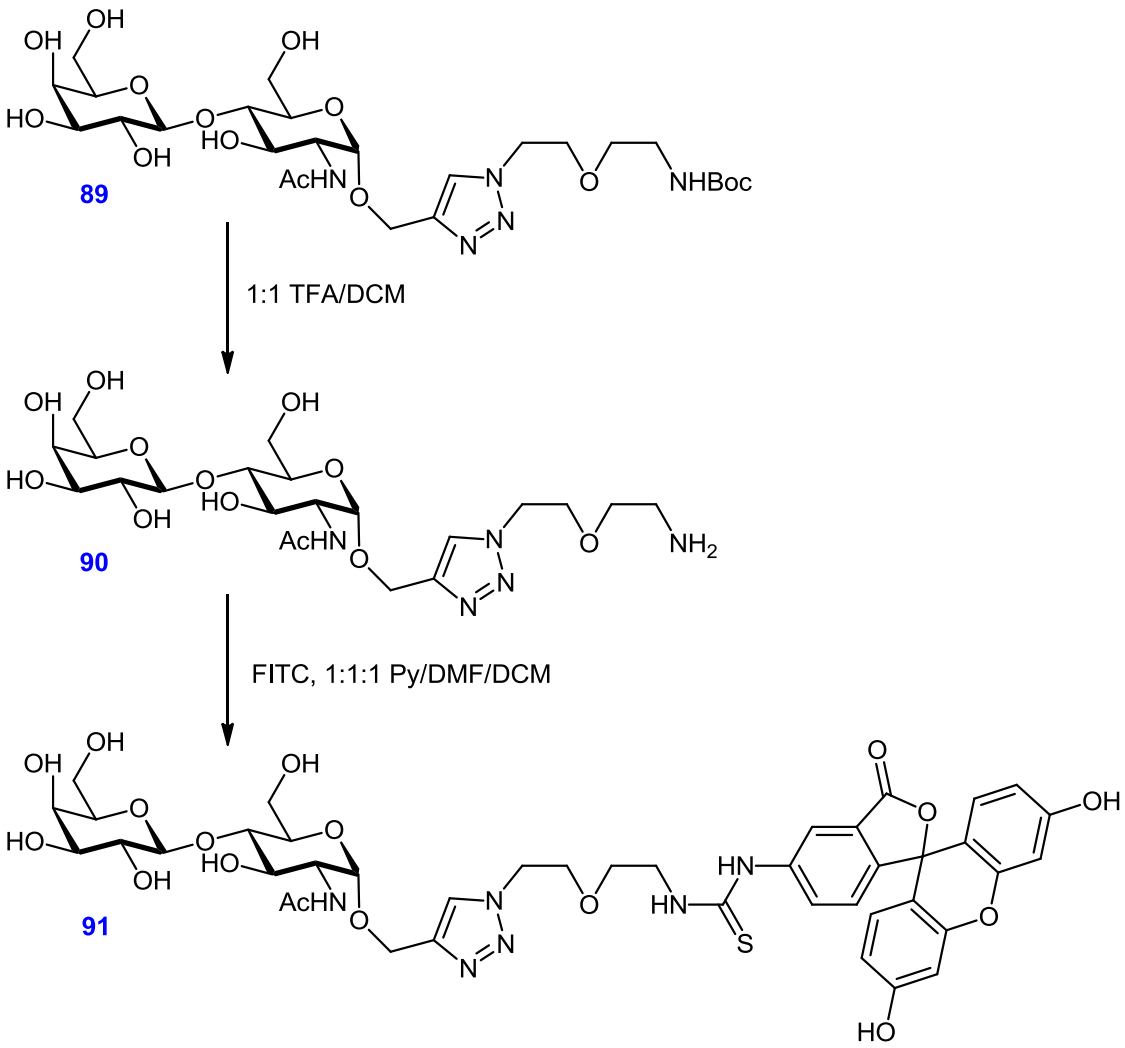
hydrolysis[50, 51] or dissolving metal reduction.[52] Wilkinson[53] has shown that H_2, Pd/C is compatible with glycosyl triazoles; this method successfully produced the de-benzylated product in 98% (**86**) and 93% (**87**) yield.

Deacetylation was achieved using Na/MeOH in 84% (**88**) and 77% (**89**) yield. Whilst the reaction appeared clean by ^{1}H NMR, some impurities were evident in the analytical HPLC trace. These impurities were also evident in the HPLC traces of subsequent reactions with the same retention time, suggesting that they do not participate in the reactions. These impurities were carried though in order to avoid loss of material during purification and would be removed by purification after the final step.



Scheme 3-19: Deprotection Steps

Boc removal and FITC installation were run as domino reactions. Boc removal was achieved with 1:1 TFA/DCM and the crude was taken up in 1:1:1 Py/DMF/DCM to ensure maximal solubility of materials, see Scheme 3-20. The reaction was stirred for 1 week to ensure maximal conversion.



Scheme 3-20: Boc Removal and FITC Installation

Analytical HPLC of **90** showed complete removal of the Boc group and HPLC analysis of **91** showed that no amine **90** was present in the reaction crude after FITC addition; the relevant HPLC traces are shown in Figure 3-9. A molecular ion of **91** was not evident in the mass spectrum, though amine **90** and aminofluoroscein were. This, in combination with the HPLC analysis strongly suggests that product had formed but fragmented at the thiourea during acquisition of the mass spectrum.

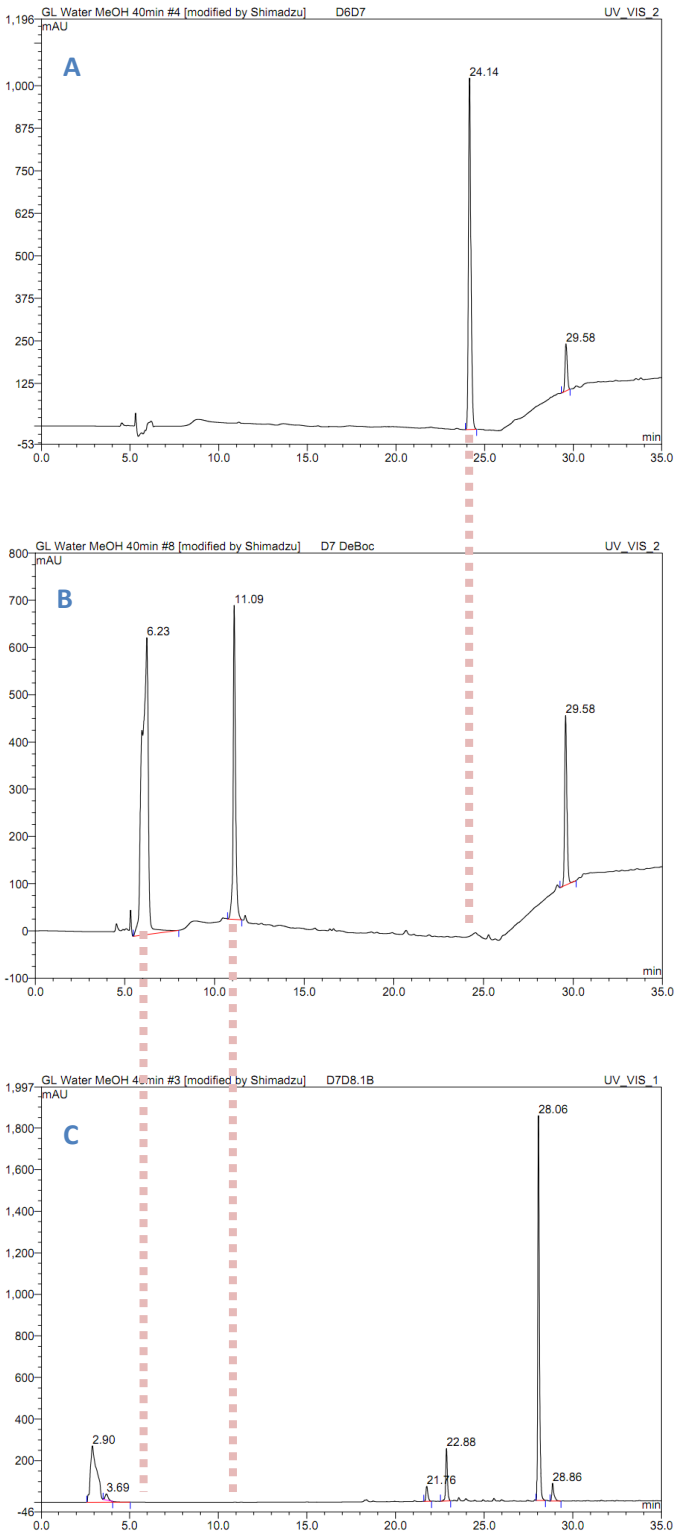


Figure 3-9: HPLC Traces; A) Boc-Protected Amine 98, B) Deprotected Amine 90, C) FITC Derivative 91

Semi-preparative HPLC did not yield enough material for NMR analysis and the mass spectra of the isolated fractions were complicated by the presence of $[TFA]_nNa^+$ clusters, which swamped the peaks

associated with the product. Work on the conjugation of FITC to the long linker was not attempted in order to avoid potential loss of material due to the issues mentioned above. Unfortunately, time constraints precluded further work in the synthesis and characterisation of **91**.

3.7 Conclusion

Encouraging work has been done on the redesign and synthesis of the glycosyl acceptor **68**, producing an acceptor in higher yield and fewer steps than the first generation synthesis.

The redesigned glycosyl donor **65** has proved more challenging, partly due to the replacement of the electron-donating azide group with an electron-withdrawing phthalimide group and partly due to there being no literature sources available to provide reference data for the product.

Synthesis of the donor from azide **5** by using a modified Staudinger reaction did not provide a useful alternative synthesis of **70** but has provided a sample allowing for confirmation of the product from the original route. Phthalimide **70** has been synthesised from diacetone glucose in 21% yield over 6 steps.

The second generation disaccharide **75** has been synthesised *via* thioglycoside **18** and transprotected to *N*-acetyl lactosamine derivative **77** ready for attachment of the linker.

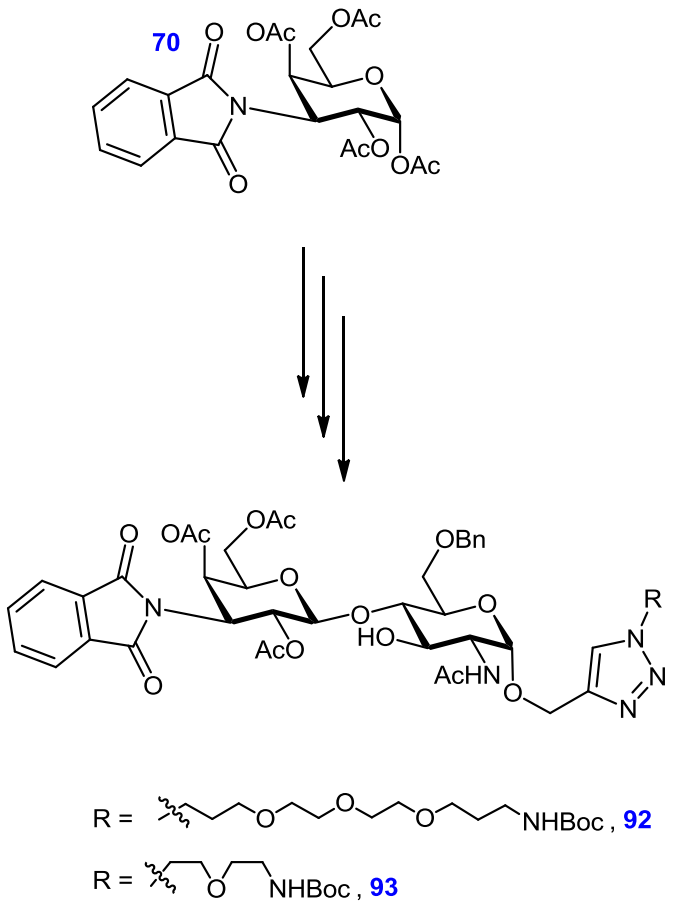
The attachment of 2 different linkers *via* CuAAC reactions was first investigated on a monosaccharide and the modified procedure successfully applied to the disaccharide, giving linker-attached products in greater than 48% yield.

Deprotection reactions proceeded smoothly and without problem and initial experiments into conjugation to FITC are promising.

4 Future Work

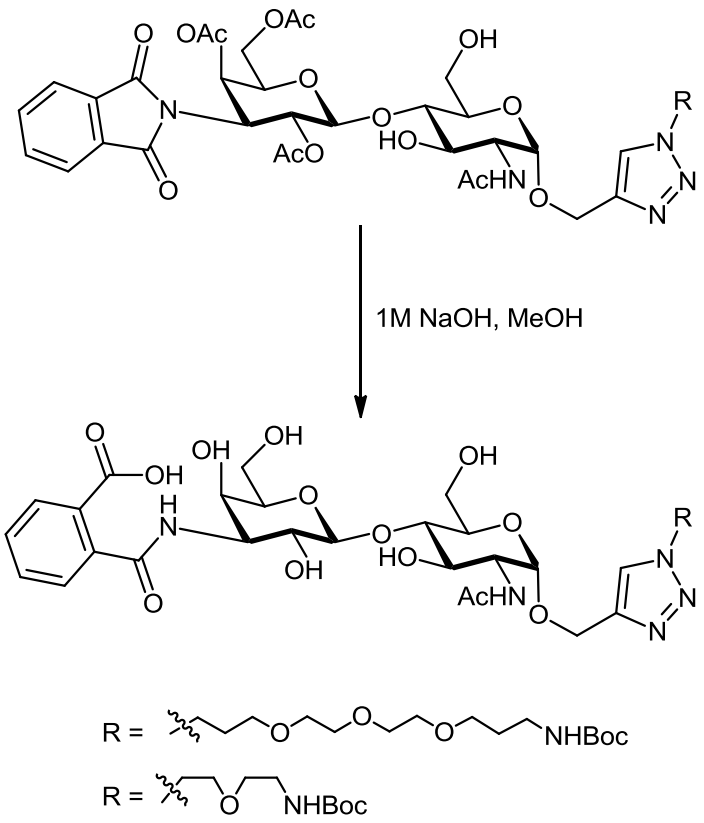
The next steps in the immediate future are to scale up the synthesis of the FITC conjugation to the short-linker disaccharide **89** to allow for further characterisation to be performed and to create a stock of material for comparisons during binding studies, and to create a similar stock of long-linker disaccharide **88**.

In concert with this, further work on phthalimide **70** will create the precursors to 2 more indicators, see Scheme 4-1.



Scheme 4-1: Phthalimide Indicators

Experiments will need to be conducted to ascertain the optimal time to open the phthalimide ring. The most opportune time may be to perform this ring opening in tandem with the deacetylations by using 1M NaOH in MeOH, as employed by Hada,[54] see Scheme 4-2.



Scheme 4-2: Phthalimide Ring Opening

Conjugation of these phthalamides to FITC will provide 2 indicators which, whilst not hypothesised to bind to Gal-3 with a sufficiently low K_d, may still prove useful.

Once the synthesis of the phthalamide indicators has been achieved, work on producing the second generation target should be relatively straightforward, producing the naphthalamide indicators shown in Figure 4-1.

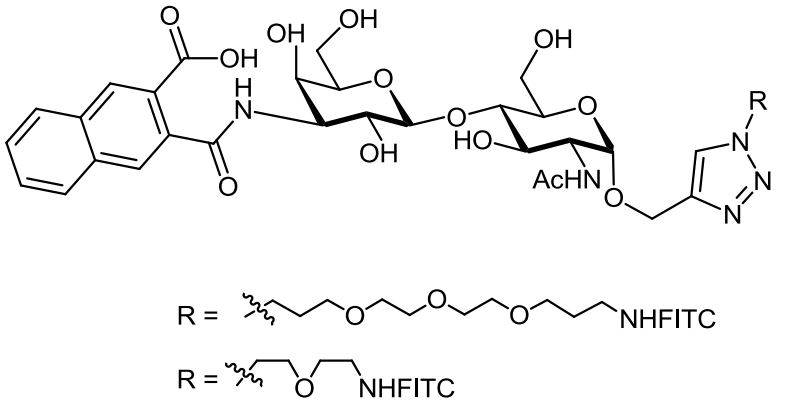
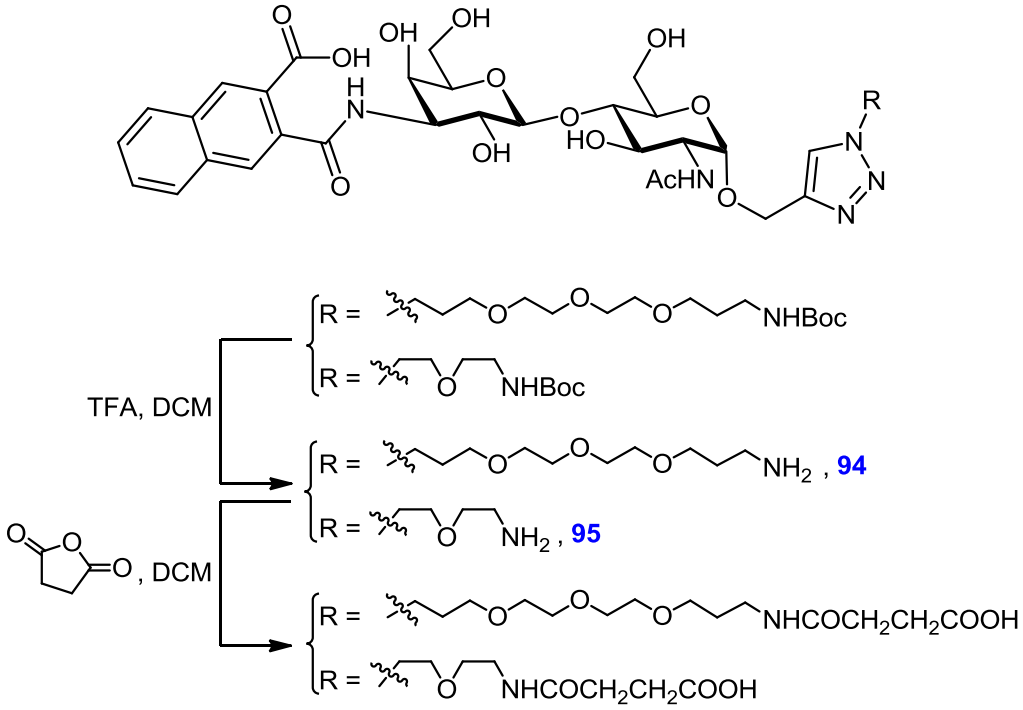


Figure 4-1: Naphthalamide Indicators

Finally, MRI contrast agents could be produced by reacting amines **94** and **95** with succinic anhydride for the creation of functionalised FeNPs, see Scheme 4-3.



Scheme 4-3: Disaccharides Required for Functionalised FeNPs

5 Experimental

5.1 Index of Compounds

5.2 General Methods

Solution phase reactions were carried out, when appropriate, in flame or oven-dried (175 ˚C) glassware. Anhydrous solvents were either purchased or dried according to standard methods[55]. Unless otherwise stated, reagents were purchased and used without further purification.

DOWEX refers to DOWEX 50WX8-200 ion-exchange resin pre-washed with 5M HCl and MeOH.

Thin layer chromatography (TLC) was performed on pre-coated glass- or aluminium-backed silica-gel plates: (silica gel 60, F_{254} s, 0.25mm thickness, supplied by Merck). Visualisation was achieved using a variety of methods including UV light (254 nm lamp), basic $KMnO_4$ solution and ceric ammonium molybdate solution.

Flash column chromatography was performed using laboratory-grade solvents on silica gel 40-63µ 60A, supplied by Fluorochem, UK.

^{1}H, ^{13}C and 2D- NMR spectra were recorded on Bruker AVIII300, AVIII400 or DRX500 spectrometers. Coupling constants (J) are given in Hertz (Hz) and the multiplicities of spectral signals as follows: ‘s’ singlet; ‘d’ doublet; ‘t’ triplet; ‘q’ quartet; ‘p’ quintet; ‘m’ multiplet; ‘br’ broad; ‘ps’ *pseudo*. Chemical shifts (δ) are expressed in parts per million (ppm) downfield from tetramethyl silane (TMS): individual spectra are referenced relative to the residual solvent signal. Off-line processing was accomplished using MNova software.

Positive and negative ion electrospray (ES+ & ES-) mass spectra were measured on a Micromass LCT spectrometer using a methanol mobile phase. High resolution mass spectra (HRMS) were achieved using an appropriate lockmass.

Infra-red spectra were obtained on a Perkin Elmer Spectrum 100 FTIR spectrophotometer. Wavenumbers (ν) are reported in cm^{-1} and spectra obtained from neat samples.

Melting points were determined in open ended glass capillaries using Stuart scientific SMP1 apparatus and are uncorrected.

High performance liquid chromatography (HPLC) was performed on Dionex summit HPLC systems with Chromeleon 6.11 software. Analytical and semi-preparative separation and purifications were acquired with the aid of a Summit p580 quaternary low pressure gradient pump with built in vacuum degasser. Helium-degassed HPLC-grade solvents were used throughout.

The following columns were used:

Analytical – Phenomenex Kinetex 5 µm C18 100Å, 250 x 4.6 mm.

Semi-preperative – Phenomenex Luna 10 µm C18 100Å, 250 x 10mm.

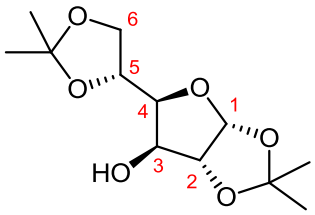
The HPLC solvent programme outlined below was employed to maximise separation and streamline purification.

Solvent A	Solvent B	Run Details
H_2O + 0.05% TFA	MeOH + 0.05% TFA	0% → 100% Solvent B over 40 min, then 100% Solvent B for 20 min.

5.3 Glycosyl Donors

5.3.1 First Generation

5.3.1.1 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (8)[23, 26, 56, 57]



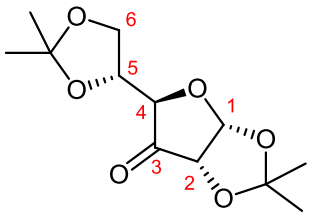
Chemical Formula: $C_{12}H_{20}O_6$

D-Glucose (10 g, 56 mmol) and H_2SO_4 (98%, 8.0 mL) was stirred in dry acetone (200 mL) at room temperature for 4 h. The reaction mixture was bought to pH 8 by addition of sat. Na_2CO_3 solution and the solvents evaporated. Dry acetone (200 mL) was added with shaking, the mixture filtered and the solvents evaporated to afford a pale yellow solid. Precipitation from $CHCl_3$ with hexane, followed by filtering and drying resulted in a fine white solid (3.2 g, 22%).

δ_H(400 MHz, $CDCl_3$)	5.92 (d, *J*=3.6, 1H, H1), 4.51 (d, *J*=3.6, 1H, H2), 4.35 – 4.28 (m, 2H, H3 & H5), 4.15 (dd, *J*=8.7, 6.2, 1H, H6), 4.04 (dd, *J*=7.8, 2.8, 1H, H4), 3.98 (dd, *J*=8.7, 5.3, 1H, H6'), 2.80 (d, *J*=3.9, 1H, exchangeable, OH), 1.48 (s, 3H, Me), 1.43 (s, 3H, Me), 1.35 (s, 3H, Me), 1.30 (s, 3H, Me). Data match literature values.[56, 57]
δ_C(101 MHz, $CDCl_3$)	111.9 ($(CH_3)_2\underline{C}$), 109.7 ($(CH_3)_2\underline{C}$), 105.4 (C1), 85.2 (C2), 81.3 (C4), 75.2 (C3), 73.4 (C5), 67.7 (C6), 26.9 (Me), 26.9 (Me), 26.3 (Me), 25.3 (Me). Data match literature values.[57]
R_f	0.5 (2:3 hexane/EtOAc)
m.p.	112-113 °C (Lit. [26] 110 °C)

ν (cm^{-1})	3424 (OH), 2984, 2950, 2903, 2873 (CH)
m/z (ES+ TOF)	283.1146 $[M+Na]^+$ ($C_{12}H_{20}O_6Na$), calc. 283.1158 – 100%

5.3.1.2 1,2:5,6-Di-O-isopropylidene-ribo-α-D-hexofuran-3-ulose (9)[24, 25, 58]



Chemical Formula: $C_{12}H_{18}O_6$

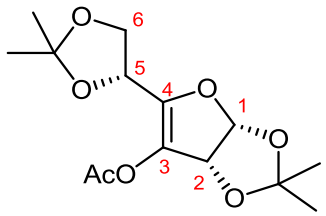
1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**8**, 10 g, 38 mmol) in DCM (50 mL) was added, dropwise over 1 h to a stirred suspension of PDC (8.7 g, 23 mmol) and Ac_2O (11 mL, 120 mmol) in DCM (150 mL) at 0 °C. The mixture was heated under reflux for 2 h and then cooled to room temperature. EtOAc (100 mL) was added and the DCM removed by evaporation. The residue was filtered through a plug of silica and eluted with EtOAc. Removal of the solvents by co-evaporation with toluene afforded the product as a golden syrup (9.7 g, 98%).

δ_H(400 MHz, $CDCl_3$)	6.09 (d, *J*=4.5, 1H, H1), 4.36 – 4.28 (m, 3H, H2, H4 & H5), 4.00 – 3.95 (m, 2H, H6 & H6'), 1.40 (s, 3H, Me), 1.38 (s, 3H, Me), 1.28 (s, 6H, 2 x Me). Data match literature values, although assignments differ.[25]
δ_C(101 MHz, $CDCl_3$)	208.9 (C3), 114.3 ($(CH_3)_2\underline{C}$), 110.4 ($(CH_3)_2\underline{C}$), 103.1 (C1), 79.0 (C4), 77.3 (C2), 76.4 (C5), 64.3 (C6), 27.6 (Me), 27.2 (Me), 26.0 (Me), 25.3 (Me). Data match literature values.[25]
R_f	0.3 (2:3 hexane/EtOAc)
ν (cm^{-1})	Ketone: 2988 (CH), 1772 (C=O) Hydrate: 3403 (OH), 2982, 2865 (CH)

m/z (ES+ TOF) 299.1100 $[M+H_2O+Na]^+$ ($C_{12}H_{20}O_7Na$), calc.299.1107 – 70%

313.2 $[M+MeOH+Na]^+$ ($C_{13}H_{22}O_7Na$) – 100%

5.3.1.3 3-O-acetyl-1,2:5,6-di-O-isopropylidene-erythro-α-D-hexofuran-3-enose (10)[20, 25]



Chemical Formula: $C_{14}H_{20}O_7$

1,2:5,6-Di-*O*-isopropylidene-*ribo*-α-D-hexofuran-3-ulose (**9**, 3.0 g, 12 mmol) and Ac_2O (30 mL) were heated in pyridine at reflux overnight. The solvents were removed by co-evaporation with toluene, producing a dark brown syrup. Purification by dry flash chromatography (0% → 50% EtOAc in hexane, in 5% increments) and removal of the solvents afforded the product as a pale yellow solid. (3.3 g, 95%).

δ_H(400 MHz, $CDCl_3$) 6.03 (d, *J*=5.5, 1H, H1), 5.39 (d, *J*=5.5, 1H, H2), 4.70 (ps t, *J*=6.4, 1H, H5), 4.11 – 4.03 (m, 2H, H6 & H6'), 2.21 (s, 3H, $COCH_3$), 1.54 (s, 3H, Me), 1.47 (s, 3H, Me), 1.45 (s, 3H, Me), 1.38 (s, 3H, Me).

Data match literature values.[20, 25]

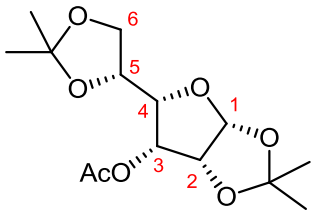
δ_C(101 MHz, $CDCl_3$) 169.1 ($\underline{C}OCH_3$), 145.4 (C4), 129.2 (C3), 113.6 ($(CH_3)_2\underline{C}$), 110.6 ($(CH_3)_2\underline{C}$), 104.2 (C1), 81.0 (C2), 68.8 (C5), 66.1 (C6), 28.1 (Me), 28.0 (Me), 25.9 (Me), 25.8 (Me), 20.7 ($COCH_3$). Data match literature values.[25]

R_f 0.8 (2:3 hexane/EtOAc)

m.p. 49-51 °C, recrystallised from hexane (Lit. [20] 62-63 °C, recrystallisation details not specified)

ν (cm^{-1})	2992, 2940, 2905 (CH), 1762 (C=O)
	C=C not visible
m/z (ES+ TOF)	323.1092 $[M+Na]^+$ ($C_{14}H_{20}O_7Na$), calc. 323.1107 – 100%

5.3.1.4 3-O-acetyl-1,2:5,6-di-O-isopropylidene-α-D-gulofuranose (15)[25, 26]



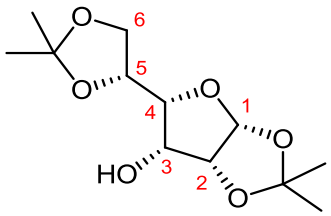
Chemical Formula: $C_{14}H_{22}O_7$

3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene-*erythro*-α-D-hexofuran-3-enose (**10**, 2.00 g, 6.62 mmol) was stirred in EtOAc (40 mL) with Pd/C (ca. 0.2 g) under hydrogen at 1.75 Bar for 4 h. Filtering through Celite and concentration afforded a pale yellow solid. Purification by column chromatography (4:1 hexane/EtOAc) afforded the product as a colourless syrup which slowly solidified to a white solid on standing (1.52 g, 76%).

δ_H(400 MHz, $CDCl_3$)	5.71 (d, *J*=4.1, 1H, H1), 4.98 (dd, *J*=6.5, 5.7, 1H, H3), 4.72 (dd, *J*=5.7, 4.1, 1H, H2), 4.51 (d ps t, *J*=9.2, 7.2, 1H, H5), 4.03 – 3.95 (m, 2H, H4 & H6), 3.44 (dd, *J*=8.3, 7.2, 1H, H6'), 2.03 (s, 3H, $COCH_3$), 1.48 (s, 3H, Me), 1.33 (s, 3H, Me), 1.28 (s, 3H, Me), 1.25 (s, 3H, Me). Data match literature values.[25]
δ_C(101 MHz, $CDCl_3$)	169.4 ($\underline{C}OCH_3$), 114.3 ($(CH_3)_2\underline{C}$), 109.0 ($(CH_3)_2\underline{C}$), 104.9 (C1), 81.1 (C4), 78.4 (C2), 75.0 (C5), 71.6 (C3), 66.2 (C6), 26.7 (Me), 26.6 (Me), 26.5 (Me), 25.1 (Me), 20.4 ($CO\underline{C}H_3$). Data match literature values.[25]
R_f	0.6 (2:3 hexane/EtOAc)
m.p.	96-98 °C, not recrystallised (Lit.[26] 73-74 °C, recrystallised from EtOH)

v (cm^{-1})	2989, 2939, 2870 (CH), 1742 (C=O)
m/z (ES+ TOF)	325.1253 $[M+Na]^+$ ($C_{14}H_{22}O_7Na$), calc. 325.1263 – 100%

5.3.1.5 1,2:5,6-Di-O-isopropylidene-α-D-gulofuranose (11)[20, 25, 26]



Chemical Formula: $C_{12}H_{20}O_6$

From 3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene-*erythro*-α-D-hexofuran-3-enose (10):

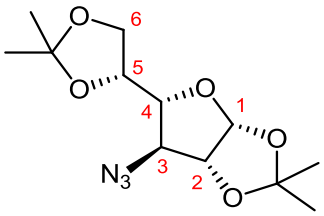
3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene-*erythro*-α-D-hexofuran-3-enose (**10**, 1.00 g, 3.33 mmol) was dissolved in MeOH (20 mL) at room temperature. $NaBH_4$ (151 mg, 4.00 mmol) was added and the mixture stirred for 1 h. Water (20 mL) was added and the product extracted into $CHCl_3$. Purification by column chromatography (2:3 hexane/EtOAc) afforded the product as a white solid (687 mg, 68%).

From 3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene-α-D-gulofuranose (15):

3-*O*-acetyl-1,2:5,6-Di-*O*-isopropylidene-α-D-gulofuranose (**15**, 24.2 g, 80.0 mmol) was dissolved in MeOH (200 mL) at room temperature. K_2CO_3 (1.00 g) was added and the mixture stirred for 30 min. The solvent was swapped to $CHCl_3$ (100 mL), washed with water, dried over $MgSO_4$, filtered and ca. 80 mL of $CHCl_3$ removed by evaporation. Precipitation with hexane, filtering and drying afforded the product as a white solid (18.9 g, 90%).

δ_H(400 MHz, $CDCl_3$)	5.76 (d, *J*=4.1, 1H, H1), 4.64 (dd, *J*=6.2, 4.1, 1H, H2), 4.46 (d ps t, *J*=8.7, 7.2, 1H, H5), 4.25 – 4.17 (m, 2H, H3 & H6), 3.88 (dd, *J*=8.7, 5.8, 1H, H4), 3.69 (dd, *J*=8.6, 7.2, 1H, H6'), 2.69 (d, *J*=6.0, 1H, exchangeable, OH), 1.61 (s, 3H, Me), 1.43 (s, 3H, Me), 1.40 (s, 3H, Me), 1.36 (s, 3H, Me). Data match literature values.[25]
δ_C(101 MHz, $CDCl_3$)	115.1 ($(CH_3)_2\underline{C}$), 109.3 ($(CH_3)_2\underline{C}$), 105.4 (C1), 84.4 (C4), 80.0 (C2), 75.6 (C5), 69.8 (C3), 66.5 (C6), 27.2 (Me), 27.2 (Me), 26.8 (Me), 25.3 (Me). Data match literature values.[25]
R_f	0.5 (2:3 hexane/EtOAc)
m.p.	103-104 °C (Lit.[26] 105-106 °C)
$[\alpha]_D$	+1.6° (c = 1, $CHCl_3$) (Lit.[20] +7.5°, c=1, $CHCl_3$)
v (cm^{-1})	3486 (OH), 2989, 2938 (CH)
m/z (ES+ TOF)	283.1162 $[M+Na]^+$ ($C_{12}H_{20}O_6Na$), calc. 283.1158 – 100%

5.3.1.6 3-Azido-3-deoxy-1,2:5,6-di-O-ispropylidene-α-D-galactofuranose (12)[18]



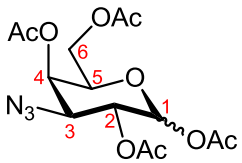
Chemical Formula: $C_{12}H_{19}N_3O_5$

1,2:5,6-Di-*O*-isopropylidene-α-D-gulofuranose (**11**, 200 mg, 0.76 mmol) was dissolved in DCM (5 mL) and pyridine (0.5 mL) at 0 °C. Tf_2O (0.26 mL, 1.5 mmol) was added dropwise and the mixture stirred for 15 min, when TLC revealed the complete consumption of starting material. The mixture was diluted with DCM, washed with ice-cold 1M HCl then water and the solvent removed by evaporation

to give an orange oil. The crude triflate was immediately taken up in DMF (10 mL), NaN_3 (249 mg, 3.83 mmol) added and the mixture stirred for 2 h. The mixture was diluted with DCM and washed 6 times with water, twice with brine and dried over $MgSO_4$. Removal of the solvent gave the azide as a pale yellow oil (165 mg, 76%) which analysis revealed to be sufficiently pure to take forward without further purification.

δ_H(400 MHz, $CDCl_3$)	5.76 (d, *J*=3.9, 1H, H1), 4.57 (dd, *J*=3.9, 1.8, 1H, H2), 4.32 (d ps t, *J*=6.7, 5.7, 1H, H5), 4.04 (dd, *J*=8.4, 6.7, 1H, H6), 3.91 (dd, *J*=5.7, 1.8, 1H, H3), 3.84 (dd, *J*=8.4, 6.7, 1H, H6'), 3.79 (ps t, *J*=5.7, 1H, H4), 1.54 (s, 3H, Me), 1.42 (s, 3H, Me), 1.35 (s, 3H, Me), 1.34 (s, 3H, Me). Data match literature values.[18]
δ_C(101 MHz, $CDCl_3$)	114.4 ($(CH_3)_2\underline{C}$), 110.1 ($(CH_3)_2\underline{C}$), 104.9 (C1), 85.8 (C2), 83.1 (C4), 74.6 (C5), 65.6 (C6), 65.5 (C3), 27.5 (Me), 26.9 (Me), 26.4 (Me), 25.2 (Me). Data match literature values.[18]
R_f	0.6 (3:1 hexane/EtOAc)
v (cm^{-1})	2988, 2939 (CH), 2104 (N_3)
m/z (ES+ TOF)	308.1235 $[M+Na]^+$ ($C_{12}H_{19}N_3O_5Na$), calc. 308.1222 – 100%

5.3.1.7 1,2,4,6-Tetra-O-acetyl-3-azido-3-deoxy-D-galactopyranose (5)[18, 59]



Chemical Formula: $C_{14}H_{19}N_3O_9$

1,2:5,6-Di-*O*-isopropylidene-α-D-gulofuranose (**11**, 4.00 g, 15.4 mmol) was converted to 3-azido-3-deoxy-1,2:5,6-di-*O*-ispropylidene-α-D-galactofuranose using the procedure in 5.3.1.6. The azido-

galactofuranose was dissolved in 80% aq. TFA (20 mL) and stirred at room temperature for 2 h, when TLC (2:3 hexane/EtOAc) revealed only a baseline spot. The solvent was removed by co-evaporation with toluene. The residue was taken up in pyridine (100 mL) and cooled to 0 °C. DMAP (ca. 0.5 g), imidazole (5.00 g, 73.4 mmol) and Ac_2O (20.0 mL, 212 mmol) were added and the mixture stirred for 16 h whilst warming to room temperature. The mixture was poured into ice-cold sat. $NaHCO_3$ solution (500 mL) and the product extracted into DCM, washed with sat. $NaHCO_3$ solution and dried over $MgSO_4$. Removal of the solvents by evaporation gave crude product as a sticky brown solid. Purification by stepped gradient column chromatography (20% → 30% → 40% EtOAc in hexane) yielded the product as a colourless syrup, which turned to a semi-solid on standing (3.43 g, 60%, α:β 1:1).

δ_H(400 MHz, $CDCl_3$) 6.34 (d, *J*=3.6, 0.5H, $H1_\alpha$), 5.66 (d, *J*=8.2, 0.5H, $H1_\beta$), 5.48 (dd, *J*=3.2, 1.2, 0.5H, $H4_\alpha$), 5.44 (d, *J*=3.2, 0.5H, $H4_\beta$), 5.26 – 5.21 (m, 1H, H2), 4.27 (d ps t, *J*=6.5, 1.2, 0.5H, $H5_\alpha$), 4.16 – 3.97 (m, 3H, $H3_\alpha$, $H5_\beta$,H6 & H6'), 3.67 (dd, *J*=10.6, 3.2, 0.5H, $H3_\beta$), 2.19 – 2.01 (m, 12H, 4 x COC*H*$_3$)

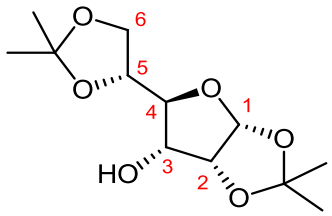
Anomeric proton signals match those found by Lowary[18] (other peaks are not reported.)

NMR data reported by Hainrichson[59] are inconsistent with either anomerically pure products nor an anomeric mixture and are disregarded.

δ_C(101 MHz, $CDCl_3$) 170.5 (C$\underline{O}$$CH_3$), 169.9 (C$\underline{O}$$CH_3$), 169.7 (C$\underline{O}$$CH_3$), 169.3 (C$\underline{O}$$CH_3$), 169.1 (C$\underline{O}$$CH_3$), 168.8 (C$\underline{O}$$CH_3$), 92.3 ($C1_\beta$), 89.3 ($C1_\alpha$), 72.7 ($C5_\beta$), 69.1 ($C5_\alpha$), 68.8 (C2), 68.1 (C2), 67.7 ($C4_\alpha$), 67.4 ($C4_\beta$), 61.7 ($C3_\beta$), 61.5 (C6), 61.3 (C6), 57.7 ($C3_\alpha$), 21.1 (CO$\underline{C}$$H_3$), 20.9 (CO$\underline{C}$$H_3$), 20.8 (CO$\underline{C}$$H_3$), 20.7 (CO$\underline{C}$$H_3$), 20.6 (CO$\underline{C}$$H_3$), 20.6 (CO$\underline{C}$$H_3$). No literature values found.

R_f	0.6 (2:3 hexane/EtOAc)
v (cm^{-1})	2977 (CH), 2110 (N_3), 1746 (C=O)
m/z (ES+ TOF)	396.1023 $[M+Na]^+$ ($C_{14}H_{19}N_3O_9Na$), calc. 396.1019 – 100%

5.3.1.8 1,2:5,6-Di-O-isopropylidene-α-D-allofuranose (14)[26, 58, 60]



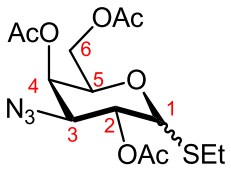
Chemical Formula: $C_{12}H_{20}O_6$

1,2:5,6-Di-*O*-isopropylidene-*ribo*-α-D-hexofuran-3-ulose (**9**, 100 mg, 0.33 mmol) was dissolved in MeOH (5 mL) at room temperature. $NaBH_4$ (17 mg, 0.46 mmol) was added and the mixture stirred for 1 h. Water (10 mL) was added and the product extracted into $CHCl_3$. Removal of the solvent by evaporation afforded the product as a white solid (100 mg, 99%).

δ_H(400 MHz, $CDCl_3$)	5.79 (d, *J*=3.9, 1H, H1), 4.59 (dd, *J*=5.1, 3.9, 1H, H2), 4.29 (d ps t, *J*=6.6, 4.8, 1H, H5), 4.08 – 3.97 (m, 3H, H3, H6 & H6'), 3.80 (dd, *J*=8.5, 4.8, 1H, H4), 2.58 (d, *J*=8.3, 1H, OH), 1.56 (s, 3H, Me), 1.44 (s, 3H, Me), 1.36 (s, 3H, Me), 1.35 (s, 3H, Me). Data match literature values.[25]
δ_C(101 MHz, $CDCl_3$)	112.9 ($(CH_3)_2\underline{C}$), 109.9 ($(CH_3)_2\underline{C}$), 104.0 (C1), 79.8 (C4), 79.0 (C2), 75.68 (C5), 72.5 (C3), 65.9 (C6), 26.6 (Me), 26.6 (Me), 26.4 (Me), 25.3 (Me). Data match literature values.[25]
R_f	0.5 (2:3 hexane/EtOAc)
m.p.	73-75 °C, not recrystallised (Lit.[26] 73-74 °C, recrystallised from cyclohexane)
$[\alpha]_D$	+22.4° (c=1, $CHCl_3$) (Lit.[26] +38°, c=1, $CHCl_3$)

v (cm^{-1})	3471 (OH), 2993, 2949, 2919, 2891, 2874 (CH)
m/z (ES+ TOF)	283.1163 $C_{12}H_{20}O_6Na$ (calc. 283.1158) – 100%

5.3.1.9 Ethyl 1,2,4,6-tetra-O-acetyl-3-azido-3-deoxy-D-thiogalactopyranoside (35)



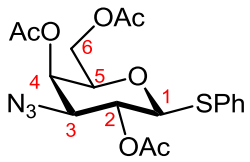
Chemical Formula: $C_{14}H_{21}N_3O_7S$

No literature reference found

1,2,4,6-Tetra-O-acetyl-3-azido-3-deoxy-D-galactopyranose (**5**, 300 mg, 0.80 mmol), was dissolved in DCM (10 mL) at 0 °C. EtSH (0.09 mL, 1.1 mmol) and $BF_3{\cdot}Et_2O$ (0.30 mL, 2.4 mmol) were added and stirred overnight at reflux. The mixture was quenched with sat. $NaHCO_3$, extracted into DCM, dried with $MgSO_4$ and the solvent removed by evaporation producing a dark brown syrup. Purification by flash column chromatography (5:1 hexane/EtOAc) gave the product as an orange syrup (232 mg, 77%, α:β 1:1). A small amount of pure α and pure β products were obtained which allowed for NMR characterisation of each anomer.

δ_H(400 MHz, $CDCl_3$)	**α-anomer:**
	5.71 (d, *J*=5.6, 1H, H1), 5.40 (dd, *J*=3.4, 1.1, 1H, H4), 5.18 (dd, *J*=10.9, 5.6, 1H, H2), 4.51 (ddd, *J*=8.7, 5.7, 1.1, 1H, H5), 4.11 (dd, *J*=11.5, 5.7, 1H, H6), 4.02 (dd, *J*=11.5, 7.1, 1H, H6'), 3.89 (dd, *J*=10.9, 3.4, 1H, H3), 2.61 – 2.48 (m, 2H, $CH_3C\underline{H}_2$), 2.13 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.31 – 1.18 (m, 3H, $C\underline{H}_3CH_2$)
	β-anomer:
	5.45 (dd, *J*=3.4, 1.1, 1H, H4), 5.19 (ps t, *J*=10.0, 1H, H2), 4.46 (d, *J*=10.0, 1H, H1), 4.10 (dd, *J*=6.6, 0.9, 2H, H6 & H6'), 3.88 (d ps t, *J*=6.6, 1.1, 1H, H5), 3.64 (dd, *J*=10.0, 3.4, 1H, H3), 2.79 – 2.05 (m, 2H, $CH_3C\underline{H}_2$), 2.16 (s, 3H, $COCH_3$), 2.13 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 1.30 – 1.24 (m, 3H, $C\underline{H}_3CH_2$)
δ_C(101 MHz, $CDCl_3$)	**α-anomer:**
	170.4 ($\underline{C}OCH_3$), 170.0 ($\underline{C}OCH_3$), 169.9 ($\underline{C}OCH_3$), 81.7 (C1), 69.8 (C2), 68.3 (C4), 66.9 (C5), 62.0 (C6), 58.7 (C3), 24.1 ($CH_3\underline{C}H_2$), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 14.8 ($\underline{C}H_3CH_2$)
	β-anomer:
	170.5 ($\underline{C}OCH_3$), 170.4 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCH_3$), 84.3 (C1), 75.3 (C5), 68.4 (C2), 67.9 (C4), 63.0 (C3), 61.8 (C6), 24.4 ($CH_3\underline{C}H_2$), 20.9 ($\underline{C}OCH_3$), 20.8 ($\underline{C}OCH_3$), 20.7 ($\underline{C}OCH_3$), 14.9 ($\underline{C}H_3CH_2$)
R_f	0.6 (2:3 hexane/EtOAc)
v (cm^{-1})	2967, 2930 (CH), 2109 (N_3), 1744 (C=O)
m/z (ES+ TOF)	398.0990 $[M+Na]^+$ ($C_{14}H_{21}N_3O_7SNa$), calc. 398.0998 – 100%

5.3.1.10 Phenyl 1,2,4,6-tetra-O-acetyl-3-azido-3-deoxy-β-D-thiogalactopyranoside (36)[61]



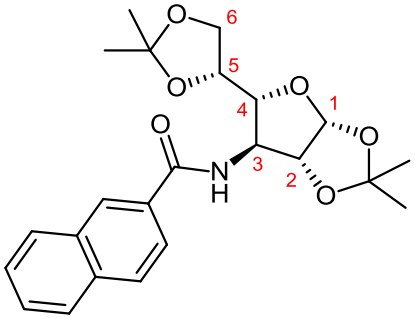
Chemical Formula: $C_{18}H_{21}N_3O_7S$

1,2,4,6-Tetra-O-acetyl-3-azido-3-deoxy-D-galactopyranose (**5**, 2.28 g, 6.11 mmol), was dissolved in DCM (20 mL) at 0 °C. PhSH (1.00 mL, 9.74 mmol) and $BF_3{\cdot}Et_2O$ (2.5 mL, 20 mmol) were added and stirred overnight at room temperature followed by 3 h heated at reflux. The mixture was quenched with sat. Na_2CO_3, extracted into DCM, dried with $MgSO_4$ and the solvent removed by evaporation, producing a pale pink syrup which was solidified by the addition of a few drops of EtOAc. Purification by trituration under hexane gave the product as an off-white solid (1.22 g, 47%, β only).

δ_H(400 MHz, $CDCl_3$)	7.57 – 7.46 (m, 2H, ArH), 7.37 – 7.29 (m, 3H, ArH), 5.44 (dd, *J*=3.3, 0.8, 1H, H4), 5.21 (ps t, *J*=10.0, 1H, H2), 4.70 (d, *J*=10.0, 1H, H1), 4.12 (d, *J*=6.5, 2H, H6 & H6'), 3.89 (d ps t, *J*=6.5, 0.8, 1H, H5), 3.63 (dd, *J*=10.0, 3.3, 1H, H3), 2.18 (s, 3H, $COCH_3$), 2.15 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$). Data match literature values.[61]
δ_C(101 MHz, $CDCl_3$)	173.1 ($\underline{C}OCH_3$), 171.4 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCH_3$), 132.6 (Ar), 130.0 (Ar_q), 129.0 (Ar), 128.3 (Ar), 87.1 (C1), 75.4 (C5), 68.4 (C2), 67.9 (C4), 63.0 (C3), 62.0 (C6), 21.0 ($CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$). No literature values found.
R_f	0.8 (2:3 hexane/EtOAc)
m.p.	104-106 °C. No literature value found.
$[\alpha]_D$	+14.4° (c=0.5, $CHCl_3$). No literature value found.
v (cm^{-1})	3069, 3033, 2989, 2969, 2948, 2879 (CH), 2094 (N_3), 1748, 1737 (C=O)

m/z (ES+ TOF) 446.0987 $[M+Na]^+$ ($C_{18}H_{21}N_3O_7SNa$), calc. 446.0998 – 100%

5.3.1.11 3-Deoxy-1,2:5,6-di-O-isopropylidene-3-(2-naphthamido)-α-D-galactofuranose (57)



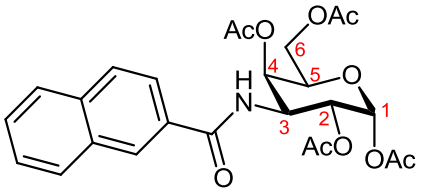
Chemical Formula: $C_{23}H_{27}NO_6$

No literature reference found

1,2:5,6-Di-*O*-isopropylidene-α-D-gulofuranose (**11**, 2.00 g, 7.68 mmol) was converted to the *galacto*-azide using the method detailed in 5.3.1.6. The azide was dissolved in EtOAc (40 mL), Pd/C (ca. 0.2 g) was added and the mixture stirred under hydrogen at atmospheric pressure overnight. The mixture was filtered through Celite and the solvent swapped to pyridine (40 mL). 2-Naphthoyl chloride (1.46 g, 7.68 mmol) was added at 0 °C and the liquor stirred at room temperature for 1.5 h. The reaction mixture was poured over ice, extracted into DCM and washed with sat. $NaHCO_3$, water, dried over $MgSO_4$ and the solvents removed by evaporation. Precipitation from $CHCl_3$ with hexane, followed by filtering and drying resulted in an off-white solid (2.45 g, 76%).

δ_H(400 MHz, $CDCl_3$)	8.28 (d, *J*=1.3, 1H, ArH), 7.94 – 7.87 (m, 3H, ArH), 7.81 (dd, *J*=8.6, 1.8, 1H, ArH), 7.63 – 7.53 (m, 2H, ArH), 6.50 (d, *J*=6.5, 1H, NH), 6.05 (d, *J*=3.8, 1H, H1), 4.84 (dd, *J*=3.8, 1.1, 1H, H2), 4.52 (ps q, *J*=7.0, 1H, H5), 4.31 (ddd, *J*=6.5, 4.3, 1.1, 1H, H3), 4.20 (dd, *J*=7.0, 4.3, 1H, H4), 4.15 (dd, *J*=8.6, 7.0, 1H, H6), 3.93 (dd, *J*=8.6, 7.0, 1H, H6'), 1.63 (s, 3H, Me), 1.46 (s, 3H, Me), 1.39 (s, 3H, Me), 1.36 (s, 3H, Me)
δ_C(101 MHz, $CDCl_3$)	167.4 (CONH), 135.1 (Ar_q), 129.1 (Ar), 128.9 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.1 (Ar), 123.4 (Ar), 113.7 ($C(CH_3)_2$), 110.1 ($C(CH_3)_2$), 105.7 (C1), 85.9 (C2), 85.1 (C4), 75.7 (C5), 66.0 (C6), 57.6 (C3), 27.3 (Me), 26.8 (Me), 26.6 (Me), 25.4 (Me) 2 x Ar_q not seen
R_f	0.5 (2:3 Hexane/EtOAc)
m.p.	208-210 °C
$[\alpha]_D$	+13.6° (c = 1, $CHCl_3$)
v (cm^{-1})	3343 (NH), 2989, 2887 (CH), 1639 (C=O)
m/z (ES+ TOF)	436.1729 $[M+Na]^+$ ($C_{23}H_{27}NO_6Na$), calc. 436.1736 – 100%

5.3.1.12 1,2,4,6-Tetra-O-acetyl-3-deoxy-3-(2-naphthamido)-α-D-galactopyranose (58)



Chemical Formula: $C_{25}H_{27}NO_{10}$

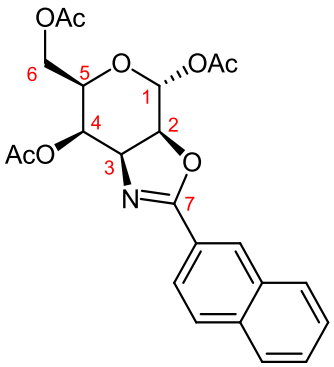
No literature reference found

3-Deoxy-1,2:5,6-di-*O*-isopropylidene-3-(2-naphthamido)-α-D-galactofuranose (**57**, 1.00 g, 2.41 mmol) was dissolved in 90% aq. TFA (20 mL) and stirred at room temperature for 40 min, when TLC (2:3 Hexane/EtOAc) revealed only a baseline spot. The solvent was removed by co-evaporation with toluene and the crude intermediate was taken up in pyridine (40 mL) and cooled to 0 °C. DMAP (ca. 0.2 g) and distilled Ac_2O (2.3 mL, 24 mmol) were added and the mixture stirred overnight at room temperature. The product was extracted into DCM and washed with water then sat. $NaHCO_3$ solution and dried over $MgSO_4$. Removal of the solvent by evaporation gave crude product as a pale orange solid. Purification by stepped gradient column chromatography (0% → 0.5% → 1% MeOH/DCM) yielded the naphthamide as a cream solid (925 mg, 76%, α only).

δ_H(400 MHz, $CDCl_3$)	8.20 (d, *J*=1.4, 1H, ArH), 7.95 – 7.85 (m, 3H, ArH), 7.71 (dd, *J*=8.6, 1.8, 1H, ArH), 7.60 – 7.52 (m, 2H, ArH), 6.55 (d, *J*=7.9, 1H, NH), 6.38 (d, *J*=3.6, 1H, H1), 5.68 (dd, *J*=3.1, 1.1, 1H, H4), 5.44 (dd, *J*=11.5, 3.6, 1H, H2), 4.91 (ddd, *J*=11.5, 7.9, 3.1, 1H, H3), 4.45 (ddd, *J*=7.1, 6.5, 1.1, 1H, H5), 4.14 (dd, *J*=11.5, 6.5, 1H, H6), 4.05 (dd, *J*=11.5, 7.1, 1H, H6'), 2.22 (s, 3H, $COCH_3$), 2.15 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$)
δ_C(101 MHz, $CDCl_3$)	172.1 ($\underline{C}OCH_3$), 170.5 ($\underline{C}OCH_3$), 169.8 ($\underline{C}OCH_3$), 169.1 ($\underline{C}OCH_3$), 167.5 ($\underline{C}OCH_3$), 135.0 (Ar_q), 132.7 (Ar_q), 130.6 (Ar_q), 129.2 (Ar), 128.8 (Ar), 128.1 (Ar), 127.8 (Ar), 127.8 (Ar), 127.0 (Ar), 123.2 (Ar), 89.6 (C1), 69.4 (C5), 68.9 (C4), 67.0 (C2), 61.7 (C6), 49.2 (C3), 21.1 ($CO\underline{C}H_3$), 21.0 ($CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$)
R_f	0.5 (2:3 hexane/EtOAc)
m.p.	212-214 °C
$[\alpha]_D$	+133.6° (c = 1, $CHCl_3$)

v (cm^{-1})	3371 (NH), 3058, 2943 (CH), 1738, 1646 (C=O)
m/z (ES+ TOF)	524.1525 $[M+Na]^+$ ($C_{25}H_{27}NO_{10}Na$), calc. 524.1533 – 100%

5.3.1.13 1,4,6-Tri-O-acetyl-3-deoxy-2-O,3-N-(2-naphthimidato)-α-D-talopyranose (60)



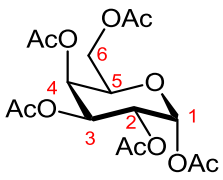
Chemical Formula: $C_{23}H_{23}NO_8$

No literature reference found

1,2,4,6-Tetra-*O*-acetyl-3-Deoxy-3-(2-naphthamido)-α-D-galactopyranose (**58**, 100 mg, 0.20 mmol) was dissolved in HBr (32% in AcOH, 1.0 mL, 4.0 mmol), Ac_2O (47 µL, 0.5 mmol) added and the mixture stirred overnight in darkness. The liquor was diluted with DCM, poured over ice, washed with water and sat. $NaHCO_3$, dried over $MgSO_4$ and the solvents removed by evaporation. The crude glycosyl bromide was dissolved in DCM (10 mL), methyl 6-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (110 mg. 0.22 mmol) and 3Å molecular sieves added and the mixture stirred at room temperature for 30 min. Ag_2CO_3 (55 mg, 0.20 mmol) was added and the mixture heated at reflux in darkness for 3 days. Cooling, filtering through Celite and purification by flash column chromatography gave the oxazoline as a white solid (67 mg, 35%). None of the expected disaccharide was obtained.

δ_H(400 MHz, $CDCl_3$)	8.49 (s, 1H, ArH), 8.06 (dd, *J*=8.7, 1.7, 1H, ArH), 7.95 – 7.90 (m, 1H, ArH), 7.89 – 7.83 (m, 2H, ArH), 7.59 – 7.48 (m, 2H, ArH), 5.75 (ps t, *J*=2.2, 1H, H1), 5.63 (dd, *J*=7.3, 4.8, 1H, H4), 5.16 (dd, *J*=4.8, 2.2, 1H, H2), 4.55 (d ps t, *J*=7.3, 4.8, 1H, H5), 4.34 (d ps t, *J*=4.8, 2.2, 1H, H3), 4.26 (dd, *J*=12.0, 4.8, 1H, H6), 4.18 (dd, *J*=12.0, 7.3, 1H, H6'), 2.20 (s, 3H, $COCH_3$), 2.16 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$)
δ_C(101 MHz, $CDCl_3$)	170.4 ($\underline{C}OCH_3$), 170.3 ($\underline{C}OCH_3$), 169.8 ($\underline{C}OCH_3$), 156.0 (C7), 135.0 (Ar_q), 132.7 (Ar_q), 129.2 (Ar), 128.7 (Ar_q), 128.6 (Ar), 128.2 (Ar), 127.8 (Ar), 126.6 (Ar), 124.4 (Ar), 89.8 (C1), 70.4 (C5), 68.0 (C4), 66.2 (C2), 63.51 (C6), 48.3 (C3), 21.0 ($CO\underline{C}H_3$), 21.0 ($CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$)
R_f	0.3 (2:3 hexane/EtOAc)
m.p.	214-216 °C
$[\alpha]_D$	+72.0 (c = 1, $CHCl_3$)
v (cm^{-1})	3059, 2946, 2902, 2852 (CH), 1742, 1714 (C=O), 1641 (C=N)
m/z (ES+ TOF)	464.1303 $[M+Na]^+$ ($C_{23}H_{23}NO_8Na$) calc. 464.1320 – 100%

5.3.1.14 Penta-O-acetyl-α-D-galactopyranose (6)[62]



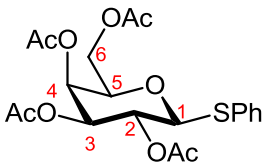
Chemical Formula: $C_{16}H_{22}O_{11}$

D-galactose (5.00 g, 27.8 mmol) and DMAP (ca. 0.5 g) were dissolved in pyridine (20 mL) at 0 °C. Ac_2O (20 mL) was added slowly and the reaction stirred for 4 h. The mixture was poured over ice, allowed

to warm to room temperature and the product extracted into DCM. Solvents were removed by evaporation and the crude product dissolved in a minimum of ethyl acetate and precipitated with hexane, affording an off-white solid (8.07 g, 75%, α only).

δ_H(400 MHz, $CDCl_3$)	6.36 (d, *J*=1.7, 1H, H1), 5.49 – 5.46 (m, 1H, H4), 5.36 – 5.31 (m, 2H, H2 & H3), 4.32 (d ps t, *J*=6.7, 1.0, 1H, H5), 4.08 (dd, *J*=6.7, 4.9, 2H, H6 & H6'), 2.14 (s, 6H, 2 x $COCH_3$), 2.02 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$). Data match literature values.[62]
δ_C(101 MHz, $CDCl_3$)	170.4 ($\underline{C}OCH_3$), 170.2 ($\underline{C}OCH_3$), 169.9 ($\underline{C}OCH_3$), 169.0 ($\underline{C}OCH_3$), 89.8 (C1), 68.8 (C5), 67.5 (C4), 67.4 (C2/3), 66.5 (C3/2), 61.3 (C6), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$). Data match literature values.[62]
R_f	0.5 (2:3 hexane/EtOAc)
v (cm^{-1})	2960 (CH), 1760, 1738 (C=O)
m/z (ES+ TOF)	413.1065 $[M+Na]^+$ ($C_{16}H_{22}O_{11}Na$), calc. 413.1060 – 100%

5.3.1.15 Phenyl 2,3,4,6-tetra-O-acetyl-β-D-thiogalactopyranoside (18)[30, 63, 64]



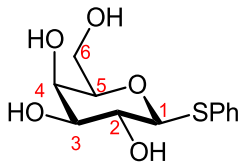
Chemical Formula: $C_{20}H_{24}O_9S$

Penta-*O*-acetyl-β-D-galactopyranose (10.0 g, 25.7 mmol), was dissolved in DCM (150 mL) at 0 °C. PhSH (3.5 mL, 34 mmol) and $BF_3{\cdot}Et_2O$ (9.8 mL, 79 mmol) were added and stirred overnight at room temperature. The mixture was quenched with sat. Na_2CO_3, extracted into DCM, dried with $MgSO_4$ and the solvent removed by evaporation producing a soft white foam which was solidified by

freezing (9.11 g, 81%, β only).

δ_H(400 MHz, $CDCl_3$)	7.49 – 7.43 (m, 2H, ArH), 7.27 – 7.23 (m, 3H, ArH), 5.34 (dd, *J*=3.4, 0.8, 1H, H4), 5.18 (ps t, *J*=10.0, 1H, H2), 5.01 (dd, *J*=10.0, 3.4, 1H, H3), 4.68 (d, *J*=10.0, 1H, H1), 4.11 (dd, *J*=11.3, 6.7, 1H, H6), 4.04 (dd, *J*=11.3, 6.7, 1H, H6'), 3.88 (d ps t, *J*= 6.7, 0.8, 1H, H5), 2.05 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$), 1.91 (s, 3H, $COCH_3$). Data match literature values.[30]
δ_C(101 MHz, $CDCl_3$)	170.2 ($\underline{C}OCH_3$), 170.0 ($\underline{C}OCH_3$), 169.8 ($\underline{C}OCH_3$), 169.2 ($\underline{C}OCH_3$), 132.4 (2 x Ar), 128.8 (Ar), 128.0 (Ar), 86.3 (C1), 74.3 (C5), 71.8 (C3), 67.1 (C2 & C4), 61.5 (C6), 20.7 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$), 20.4 ($CO\underline{C}H_3$). Data match literature values.[63]
R_f	0.7 (2:3 hexane/EtOAc)
m.p.	74-76 °C not recrystallised (Lit.[64] 80-81 °C, recrystallised from Et_2O/hexane)
v (cm^{-1})	3064, 2981 (CH), 1743 (C=O)
m/z (ES+ TOF)	463.1040 $[M+Na]^+$ ($C_{20}H_{24}O_9SNa$), calc. 463.1039 – 100%

5.3.1.16 Phenyl β-D-thiogalactopyranoside (19) [31, 65]

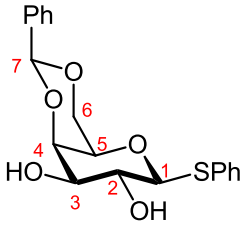


Chemical Formula: $C_{12}H_{16}O_5S$

Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-thiogalactopyranoside (**18**, 7.28 g, 16.5 mmol) was dissolved in 73 mL of MeOH and stood for 1 week at room temperature. The liquor was concentrated by removing roughly 65 mL of MeOH by evaporation and the product precipitated with $CHCl_3$, to afford a white solid (4.19 g, 93%).

δ_H(400 MHz, DMSO-d^6)	7.48 – 7.42 (m, 2H, ArH), 7.33 – 7.26 (m, 2H, ArH), 7.23 – 7.17 (m, 1H, ArH), 5.13 (d, *J*=5.1, 1H, OH2), 4.88 (d, *J*=4.2, 1H, OH4), 4.63 (t, *J*=4.7, 1H, OH6), 4.58 (d, *J*=9.5, 1H, H1), 4.48 (d, *J*=4.2, 1H, OH3), 3.71 (ps t, *J*=4.2, 1H, H3), 3.56 – 3.39 (m, 4H, H2, H5, H6 & H6'), 3.37 (ps t, *J*=4.2, 1H, H4). Literature values not found for DMSO-d^6.[31]
δ_C(101 MHz, DMSO-d^6)	135.5 (Ar), 129.2 (Ar_q), 128.7 (Ar), 126.0 (Ar), 87.7 (C1), 79.1 (C2/5), 74.69 (C4), 69.2 (C5/2), 68.3 (C3), 60.5 (C6). Literature values not found for DMSO-d^6.[31]
m.p.	98-100 °C (Lit.[65] 98-100 °C)
v (cm^{-1})	3276 (br, OH), 2896 (CH)
m/z	295.0629 $[M+Na]^+$ ($C_{12}H_{16}O_5SNa$), calc. 295.0616 – 100%

5.3.1.17 Phenyl 4,6-O-benzylidene-β-D-thiogalactopyranoside (20)[32]

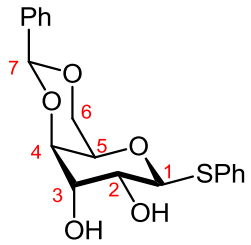


Chemical Formula: $C_{19}H_{20}O_5S$

Phenyl β-D-thiogalactopyranoside (**19**, 4.00 g, 14.4 mmol) was dissolved in MeCN (40 mL) and DCM (5 mL), with 4 Å sieves and DOWEX and stirred at room temperature overnight. The solvent was removed by evaporation after filtering, and recrystallisation from EtOH afforded the product as a white solid (4.05 g, 76%).

δ_H(400 MHz, $CDCl_3$)	7.73 – 7.66 (m, 2H, ArH), 7.44 – 7.27 (m, 8H, ArH), 5.52 (s, 1H, H7), 4.54 (d, *J*=9.2, 1H, H1), 4.39 (dd, *J*=12.5, 1.5, 1H, H6), 4.23 (dd, *J*=2.1, 1.5, 1H, H4), 4.05 (dd, *J*=12.5, 1.5, 1H, H6'), 3.76 – 3.65 (m, 2H, H2 & H3), 3.57 (ps q, *J*=1.5, 1H, H5), 2.52 (d, *J*=1.3, 1H, OH3), 2.51 – 2.47 (m, 1H, OH2). Data match literature values.[32]
δ_C(101 MHz, $CDCl_3$)	137.7 (Ar_q), 133.9 (Ar), 130.8 (Ar_q), 129.5 (Ar), 129.1 (Ar), 128.3 (2 x Ar), 126.6 (Ar), 101.5 (C7), 87.1 (C1), 75.4 (C4), 73.9 (C2), 70.2 (C5), 69.4 (C6), 68.9 (C3). Data match literature values.[32]
m.p.	151-153 °C (Lit.[32] 164-165 °C, recrystallisation details not specified)
ν (cm^{-1})	3534 (OH) ,3393 (br, OH), 3061, 2977, 2910, 2869 (CH)
m/z	383.0934 $[M+Na]^+$ ($C_{19}H_{20}O_5SNa$), calc. 383.0929 – 100%

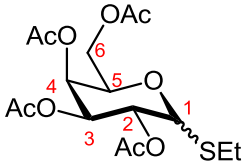
5.3.1.18 Phenyl 4,6-O-benzylidene-β-D-thiogulopyranoside (21)[21]



Chemical Formula: $C_{19}H_{20}O_5S$

Phenyl 4,6-*O*-benzylidene-β-D-thiogalactopyranoside (**20**, 500 mg, 1.39 mmol) and pyridine (0.3 mL) were dissolved in DCM (5 mL) at -20 °C. Triflic anhydride (270 µL, 1.60 mmol) was added dropwise over 5 mins and the reaction stirred for 1.5 h. AcCl (151 µL, 1.60 mmol) was added and the reaction allowed to warm to room temperature over 1 h. Further AcCl (132 µL, 1.40 mmol) and pyridine (0.1 mL) were added and the reaction stirred at room temperature for 1 h. The mixture was diluted with DCM, washed with 0.5 M HCl, sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and the solvents removed by evaporation. The residue was taken up in MeCN (5 mL), $^{n}Bu_4NNO_2$ (1.21 g, 4.16 mmol) added and the reaction stirred at 50 °C for 14 h. The mixture was cooled, diluted with DCM and washed with 0.5 M HCl, sat. $NaHCO_3$ and brine. Drying over $MgSO_4$ and removal of the solvent by evaporation produced a dark brown syrup (1.15 g, 206%). Analysis showed that no product had been formed, and the by-products could not be identified.

5.3.1.19 Ethyl 2,3,4,6-tetra-O-acetyl-D-thiogalactopyranoside (34)[66, 67]



Chemical Formula: $C_{16}H_{24}O_9S$

Penta-*O*-acetyl-D-galactopyranose (10.0 g, 25.7 mmol), was dissolved in DCM (150 mL) at 0 °C. EtSH (2.5 mL, 34 mmol) and $BF_3{\cdot}Et_2O$ (9.8 mL, 79 mmol) were added and stirred overnight at room temperature. The mixture was quenched with sat. $NaHCO_3$, extracted into DCM, dried with $MgSO_4$ and the solvent removed by evaporation, producing a pale yellow syrup. Purification by trituration with 5% Et_2O in hexane followed by filtering and drying gave the product as an off-white solid (8.68 g, 86%, α:β 3:2).

δ_H(400 MHz, $CDCl_3$) 5.29 – 5.17 (m, 3H, $H2_\alpha$, $H2_\beta$ & $H3_\alpha$)

α-anomer:

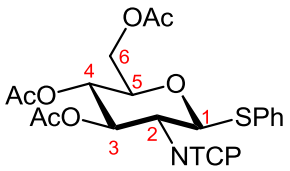
5.74 (d, *J*=5.4, 1H, H1), 5.44 (dd, *J*=3.1, 1.0, 1H, H4), 4.58 (d ps t, *J*=6.6, 1.0, 1H, H5), 4.10 (d, *J*=6.6, 2H, H6 & H6'), 2.64 – 2.46 (m, 2H, $C\underline{H}_2CH_3$), 2.13 (s, 3H, $COCH_3$), 2.06(s, 3H, $COCH_3$), 2.03(s, 3H, $COCH_3$), 1.98(s, 3H, $COCH_3$), 1.26 (t, *J*=7.4, 3H, $CH_2C\underline{H}_3$). No literature values found.

β-anomer:

5.42 (dd, *J*=3.4, 0.9, 1H, H4), 5.04 (dd, *J*=10.0, 3.4, 1H, H3), 4.48 (d, *J*=9.9, 1H, H1), 4.16 (dd, *J*=11.4, 6.6, 1H, H6), 4.09 (dd, *J*=11.4, 6.6, 3H, H6'), 3.92 (d ps t, *J*=6.6, 0.9, 1H, H5), 2.80 – 2.64 (m, 2H, $C\underline{H}_2CH_3$), 2.14(s, 3H, $COCH_3$), 2.06(s, 3H, $COCH_3$), 2.03(s, 3H, $COCH_3$), 1.97(s, 3H, $COCH_3$), 1.27 (t, *J*=7.4, 3H, $CH_2C\underline{H}_3$). Data match literature values.[66]

δ_C(101 MHz, $CDCl_3$)	170.5 ($\underline{C}OCH_3$), 170.3 ($\underline{C}OCH_3$), 170.2 ($\underline{C}OCH_3$), 170.2 ($\underline{C}OCH_3$), 169.9 ($\underline{C}OCH_3$), 169.7 ($\underline{C}OCH_3$), 84.2 ($C1_\beta$), 82.0 ($C1_\alpha$), 74.5 ($C5_\beta$), 72.0 ($C3_\beta$), 68.3 & 68.0 ($C2_\alpha$, $C2_\beta$ & $C3_\alpha$), 67.3 ($C4_{\alpha/\beta}$), 67.3 ($C4_{\beta/\alpha}$), 66.5 ($C5_\alpha$), 61.9 ($C6_\alpha$), 61.6 ($C6_\beta$), 24.5 ($\underline{C}H_2CH_3$), 24.1 ($\underline{C}H_2CH_3$), 20.9, 20.7 & 20.7 (5 x $CO\underline{C}H_3$), 14.9 ($CH_2\underline{C}H_3$), 14.8 ($CH_2\underline{C}H_3$). Data match literature values for β-anomer[66]. Only partial data available for α-anomer[67], data match although assignments differ.
R_f	0.7 (2:3 hexane/EtOAc)
v (cm^{-1})	2985, 2966, 2943, 2916, 2872 (CH), 1750, 1738, 1729 (C=O)
m/z (ES+ TOF)	415.1043 $[M+Na]^+$ ($C_{16}H_{24}O_9SNa$), calc. 415.1039 – 100%

5.3.1.20 *Phenyl 1,3,4,6-tetra-O-acetyl-2-tetrachlorophthalimido-2-deoxy-β-D-thioglucopyranoside (30)*[68, 69]



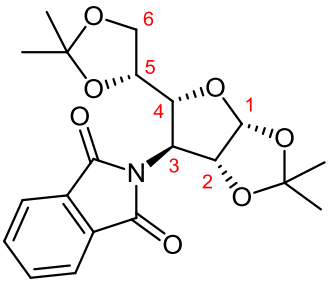
Chemical Formula: $C_{26}H_{21}Cl_4NO_9S$

1,3,4,6-Tetra-O-acetyl-2-tetrachlorophthalimido-2-deoxy-β-D-glucopyranoside (**27**, 1.00 g, 1.65 mmol), was dissolved in DCM (25 mL) at 0 °C. PhSH (0.23 mL, 2.2 mmol) and $BF_3{\cdot}Et_2O$ (0.60 mL, 4.9 mmol) were added and the mixture stirred overnight at room temperature. The mixture was quenched with sat. Na_2CO_3, extracted into DCM, dried with $MgSO_4$ and the solvent removed. The crude was dissolved in a minimum of $CHCl_3$ and precipitated with hexane affording the product as a white solid (0.83 g, 77%, β only).

δ_H(400 MHz, $CDCl_3$)	7.44 – 7.38 (m, 2H, ArH), 7.32 – 7.27 (m, 3H, ArH), 5.72 (dd, *J*=10.4, 9.2, 1H, H3), 5.67 (d, *J*=10.4, 1H, H1), 5.14 (dd, *J*=10.2, 9.2, 1H, H4), 4.33 (ps t, *J*=10.4, 1H, H2), 4.28 (dd, *J*=12.3, 5.2, 1H, H6), 4.20 (dd, *J*=12.3, 2.4, 1H, H6'), 3.86 (ddd, *J*=10.2, 5.2, 2.4, 1H, H5), 2.10 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.87 (s, 3H, $COCH_3$). Data match literature values.[68, 69]
δ_C(101 MHz, $CDCl_3$)	170.7 ($\underline{C}OCH_3$), 169.4 ($\underline{C}OCH_3$), 163.4 ($\underline{C}OCH_3$), 145.6 ($\underline{C}ON\underline{C}O$), 133.4 (Ar), 130.6 (Ar), 130.2 (Ar), 129.1 (Ar), 128.7 (Ar), 127.2 (Ar), 126.9 (Ar), 82.5 (C1), 76.1 (C5), 71.8 (C3), 68.5 (C4), 62.2 (C6), 54.5 (C2), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$). Peaks at δ < 135 match literature values;[68] too many peaks at δ > 135 are reported in the literature to be consisted with **30**.
m.p.	180-182 °C (Lit.[69] 181-183 °C)
$[\alpha]_D$	+70.8° (c=1, $CHCl_3$) (Lit.[69] +55.6°, c=1, $CHCl_3$)
ν (cm^{-1})	2970, 2876 (CH), 1744, 1725 (C=O)
m/z (ES+ TOF)	685.9594 $[M+Na]^+$ ($C_{26}H_{21}{}^{35}Cl_4NO_9SNa$), calc. 685.9589 – 70% 688.0 $[M+Na]^+$ ($C_{26}H_{21}{}^{35}Cl_3{}^{37}Cl_1NO_9SNa$) – 100% 690.0 $[M+Na]^+$ ($C_{26}H_{21}{}^{35}Cl_2{}^{37}Cl_2NO_9SNa$) – 10%

5.3.2 Second Generation

5.3.2.1 3-Deoxy-1,2:5,6-di-O-ispropylidene-3-phthalimido-α-D-galactofuranose (65)



Chemical Formula: $C_{20}H_{23}NO_7$

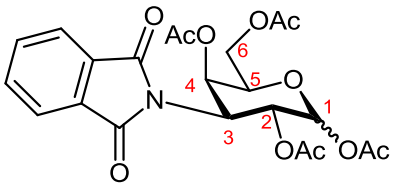
No literature reference found

1,2:5,6-Di-*O*-isopropylidene-α-D-gulofuranose (**11**, 2.00 g, 7.68 mmol) was converted to the triflate using the procedure described in 5.3.1.6. The crude triflate was taken up in DMF (60 mL) and cooled to -20 °C, potassium phthalimide (7.10 g, 38.3 mmol) added and the mixture stirred for 18 h whilst warming to room temperature. The mixture was diluted with DCM and washed 6 times with water, twice with brine and dried over $MgSO_4$. Removal of the solvent and purification by flash column chromatography (30% EtOAc in hexane) afforded the phthalimide as a white solid (1.95 g, 66%).

δ_H(400 MHz, $CDCl_3$)	7.91 – 7.81 (m, 2H, ArH), 7.81 – 7.71 (m, 2H, ArH), 6.18 (d, *J*=3.5, 1H, H1), 4.89 (dd, *J*=3.5, 1.4, 1H, H2), 4.67 (dd, *J*=6.6, 1.4, 1H, H3), 4.32 (ps q, *J*=6.6, 1H, H5), 4.22 (ps t, *J*=6.6, 1H, H4), 4.07 (dd, *J*=8.5, 6.6, 1H, H6), 3.76 (dd, *J*=8.5, 6.6, 1H, H6'), 1.63 (s, 3H, Me), 1.42 (s, 3H, Me), 1.35 (s, 6H, 2 x Me)
δ_C(101 MHz, $CDCl_3$)	167.4 (CONCO), 134.6 (Ar), 131.6 (Ar_q), 123.7 (Ar), 114.6 ($C(CH_3)_2$), 110.1 ($C(CH_3)_2$), 106.0 (C1), 85.2 (C2), 81.8 (C4), 76.1 (C5), 65.5 (C6), 54.7 (C3), 28.0 (Me), 27.1 (Me), 26.4 (Me), 25.2 (Me)
R_f	0.4 (2:3 hexane/EtOAc)
m.p.	96-98 °C

$[\alpha]_D$	56.8° (c=1, $CHCl_3$)
v (cm^{-1})	3063, 2992, 2946, 2893 (CH), 1703 (C=O)
m/z (ES+ TOF)	412.1373 $[M+Na]^+$ ($C_{20}H_{23}NO_7Na$), calc. 631.9661 – 100%

5.3.2.2 3-Deoxy-1,2,5,6-tetra-O-acetyl-3-phthalimido-D-galactofuranose (70)



Chemical Formula: $C_{22}H_{23}NO_{11}$

No literature reference found

From 3-deoxy-1,2:5,6-di-*O*-ispropylidene-3-phthalimido-α-D-galactofuranose (65):

3-Deoxy-1,2:5,6-di-*O*-ispropylidene-3-phthalimido-α-D-galactofuranose (**65**, 1.26 g, 3.23 mmol) was dissolved in 80% aq. TFA (30 mL) and stirred at room temperature for 1 h, when TLC (2:3 hexane/EtOAc) revealed only a baseline spot. The solvent was removed by co-evaporation with toluene and the residue was taken up in MeCN (15 mL), cooled to 0 °C and imidazole (132 mg, 1.94 mmol) and Ac_2O (1.47 mL, 15.5 mmol) were added and the mixture stirred for 2 days at room temperature. The mixture was poured into ice-cold sat. $NaHCO_3$ solution (50 mL) and the product extracted into DCM, washed with sat. $NaHCO_3$ solution and dried over $MgSO_4$. Removal of the solvents by evaporation gave crude product as a cream foam. Purification by stepped gradient column chromatography (20% → 30% → 40% EtOAc in hexane) yielded the product as a white solid (785 mg, 51%, α:β 2:1).

From 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-D-galactopyranose (4):

1,2,4,6-Tetra-*O*-acetyl-3-azido-3-deoxy-D-galactopyranose (**4**, 264 mg, 0.71 mmol) was suspended in toluene (20 mL), triphenylphosphine (204 mg, 0.78 mmol) was added and the suspension stirred at room temperature until a solution formed. Phthalic anhydride (115 mg, 0.78 mmol) and $^{n}Bu_4NCN$ (20 mg, 0.10 mmol) were added and the mixture heated at reflux for 1 week. Removal of the solvents and purification by stepped gradient column chromatography (20% → 30% → 40% → 50% EtOAc in hexane) afforded the product as a white solid (77 mg, 23%, α:β 2:1).

δ_H(400 MHz, $CDCl_3$)	7.92 – 7.81 (m, 2H, ArH), 7.81 – 7.69 (m, 2H, ArH), 6.45 (d, *J*=4.5, 0.7H, $H1_\alpha$), 6.35 (dd, *J*=11.6, 8.1, 0.3H, $H2_\beta$), 5.97 (dd, *J*=9.4, 4.5, 0.7H, $H2_\alpha$), 5.75 (d, *J*=8.1, 0.3H, $H1_\beta$), 5.37 (d, *J*=2.5, 0.3H, $H4_\beta$), 5.20 (d ps t, *J*=6.2, 3.3, 0.7H, $H5_\alpha$), 5.15 (d ps t, *J*=7.0, 3.7, 0.3H, $H5_\beta$), 4.96 (dd, *J*=9.4, 8.7, 0.7H, $H3_\alpha$), 4.82 (dd, *J*=8.7, 6.2, 0.7H, $H4_\alpha$), 4.65 (dd, *J*=11.6, 2.5, 0.3H, $H3_\beta$), 4.38 (dd, *J*=12.1, 3.7, 0.3H, $H6_\beta$), 4.34 (dd, *J*=12.4, 3.3, 0.7H, $H6_\alpha$), 4.20 – 4.04 (m, 1H, $H6_\beta$' & $H6_\alpha$'), 2.22 – 1.78 (stack, 12H, 4 x $COCH_3$)
δ_C(101 MHz, $CDCl_3$)	170.0 ($\underline{C}OCH_3$), 169.4 ($\underline{C}OCH_3$), 168.7 ($\underline{C}OCH_3$), 167.6 ($\underline{C}OCH_3$), 166.6 ($\underline{C}OCH_3$), 134.7 (Ar), 131.5 (Ar_q), 123.9 (Ar), 94.0 ($C1_\beta$), 93.0 ($C1_\alpha$), 75.1 ($C4_\alpha$), 71.8 ($C2_\alpha$), 71.0 ($C5_\alpha$), 68.5 ($C4_\beta$), 67.9 ($C5_\beta$), 65.5 ($C2_\beta$), 62.3 ($C6_{\alpha/\beta}$), 61.3 ($C6_{\beta/\alpha}$), 53.6 ($C3_\beta$), 52.1 ($C3_\alpha$), 21.2 ($CO\underline{C}H_3$), 21.0 ($CO\underline{C}H_3$), 20.9 ($CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$), 20.4 ($CO\underline{C}H_3$) $\underline{C}ON\underline{C}O$ not visible.
R_f	0.4 (2:3 hexane/EtOAc)
ν (cm^{-1})	2960, 2939 (CH), 1742, 1713 (C=O)

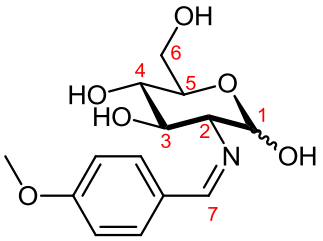
m/z (ES+ TOF) 500.1192 $[M+Na]^+$ ($C_{22}H_{23}NO_{11}Na$), calc. 500.1169 – 100%

977.2 $[2M+Na]^+$ ($C_{44}H_{46}N_2O_{22}Na$) – 75%

5.4 Glycosyl Acceptors

5.4.1 First Generation

5.4.1.1 2-Deoxy-2-(4-methoxybenzimino)-D-glucopyranose (24)[22, 70, 71]



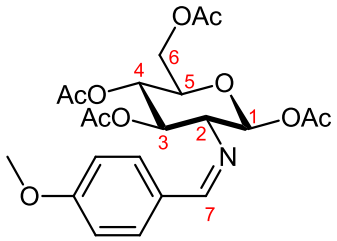
Chemical Formula: $C_{14}H_{19}NO_6$

D-glucosamine hydrochloride (20.0 g, 92.8 mmol) was dissolved in 1M NaOH (100 mL) at room temperature. p-Anisaldehyde (12.7 mL, 104 mmol) was added and the mixture stirred until cloudy, then stirred at 0 °C for a further 5 h. The precipitated product was filtered and washed with water (2 x 100 mL) and 1:1 MeOH/Et_2O (2 x 100 mL) and dried to afford a white solid (20.1 g, 73%).

δ_H(400 MHz, DMSO) 8.11 (s, 1H, H7), 7.72 – 7.63 (m, 2H, Ar), 7.03 – 6.96 (m, 2H, Ar), 6.52 (d, *J*=6.8, 1H, OH1), 4.91 (d, *J*=5.3, 1H, OH4), 4.80 (d, *J*=5.7, 1H, OH3), 4.70 (dd, *J*=7.9, 6.8 1H, H1), 4.54 (ps t, *J*=5.9, 1H, OH6), 3.80 (s, 3H, OMe), 3.72 (ddd, *J*=11.9, 5.9, 1.9, 1H, H6), 3.48 (d ps t, *J*=11.9, 5.9, 1H, H6'), 3.42 (d ps t, *J*=9.1, 5.7, 1H, H3), 3.24 (ddd, *J*=9.1, 5.9, 1.9, 1H, H5), 3.15 (d ps t, *J*=9.1, 5.7, 1H, H4), 2.79 (dd, *J*=9.1, 7.9, 1H, H2). Data match literature values, except for the multiplicity of H3 – reported as dd, found to be d ps t.[70]

δ_C(101 MHz, DMSO)	161.1 (C7), 161.0 (Ar), 129.6 (Ar), 129.1 (Ar), 113.8 (Ar), 95.6 (C1), 78.1 (C2), 76.8 (C5), 74.5 (C3), 70.3 (C4), 61.2 (C6), 55.2 (OMe). Data match literature values.[70]
m.p.	153-154 °C (d) (Lit.[71] 154-155 °C (d))
v (cm^{-1})	3487 (OH), 3319, 3205 (br, OH), 3027, 2970, 2932, 2895,2843 (CH), 1637 (C=N)
m/z (ES+ TOF)	320.1095 $[M+Na]^+$ ($C_{14}H_{19}NO_6Na$), calc. 320.1110 – 100%

5.4.1.2 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(4-methoxybenzimino)- β-D-glucopyranose (25)[22, 72]

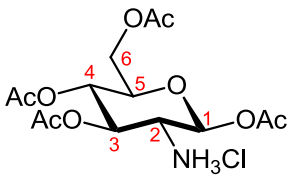


Chemical Formula: $C_{22}H_{27}NO_{10}$

2-Deoxy-2-(4-methoxybenzimino)-D-glucopyranose (**24**, 12.5 g, 42.1 mmol) and DMAP (ca. 1 g) were dissolved in pyridine (70 mL) at room temperature. Ac_2O (40 mL) was added slowly and the mixture stirred for 4 h, then poured over ice (200 mL) and allowed to warm to room temperature. The precipitated product was filtered and washed with H_2O (2 x 100 mL) and Et_2O (2 x 100 mL) then dried to afford the product as a white solid (16.1 g, 82%).

δ_H(400 MHz, DMSO-d^6)	8.28 (s, 1H, H7), 7.68 – 7.63 (m, 2H, Ar), 7.02 – 6.96 (m, 2H, Ar), 6.07 (d, *J*=8.3, 1H, H1), 5.44 (ps t, *J*=9.7, 1H, H3), 4.97 (ps t, *J*=9.7, 1H, H4), 4.26 (ddd, *J*=9.7, 4.6, 1.8, 1H, H5), 4.22 (dd, *J*=12.1, 4.6, 1H, H6), 4.01 (dd, *J*=12.1, 1.8, 1H, H6'), 3.79 (s, 3H, OMe), 3.44 (dd, *J*=9.7, 8.3, 1H, H2), 2.02 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.82 (s, 3H, $COCH_3$). No literature reference found for spectrum in DMSO-d^6, data is consistent with spectrum reported in $CDCl_3$ except for a downfield shift in the signal for H5 ($\Delta\delta$ = 0.3).
δ_C(101 MHz, DMSO-d^6)	170.0 ($\underline{C}OCH_3$), 169.4 ($\underline{C}OCH_3$), 168.9 ($\underline{C}OCH_3$), 168.5 ($\underline{C}OCH_3$), 164.4 (C7), 161.8 (Ar), 129.9 (Ar), 128.2 (Ar), 114.1 (Ar), 92.5 (C1), 72.3 (C2/3), 72.2 (C2/3), 71.5 (C5), 67.8 (C4), 61.6 (C6), 55.3 (OMe), 20.5 ($CO\underline{C}H_3$), 20.4 ($CO\underline{C}H_3$), 20.1 ($CO\underline{C}H_3$). No literature reference found for spectrum in DMSO-d^6, data is consistent with spectrum reported in $CDCl_3$.[72]
m.p.	181-183 °C, not recrystallised (Lit. 175-176 °C, recrystallisation details not specified)
v (cm^{-1})	2970 , 2921 (CH), 1748, 1737 (C=O), 1647 (C=N)
m/z (ES+ TOF)	488.1529 $[M+Na]^+$ ($C_{22}H_{27}NO_{10}Na$), calc. 488.1533 – 100%

5.4.1.3 1,3,4,6-Tetra-O-acetyl-β-D-glucosamine hydrochloride (26)[22, 72]

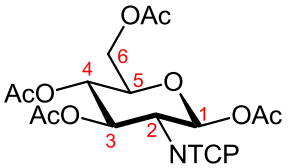


Chemical Formula: $C_{14}H_{22}ClNO_9$

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(4-methoxybenzimino)-β-D-glucopyranose (**25**, 16.1 g, 34.7 mmol) was dissolved in Me_2CO (180 mL) and 5M HCl (6 mL) added dropwise over 10 min with stirring. The precipitated product was filtered, washed with Me_2CO (2 x 100 mL), Et_2O (2 x 100 mL) and dried to afford the product as a white solid (9.62 g, 72%).

δ_H(400 MHz, DMSO)	8.79 (s, 3H, NH_3), 5.91 (d, *J*=8.7, 1H, H1), 5.35 (dd, *J*=10.3, 9.3, 1H, H3), 4.93 (dd, *J*=10.0, 9.3 1H, H4), 4.19 (dd, *J*=12.5, 4.4, 1H, H6), 4.05 (ddd, *J*=10.0, 4.4, 2.2, 1H, H5), 3.99 (dd, *J*=12.5, 2.2, 1H, H6'), 3.56 (dd, *J*=10.3, 8.7, 1H, H2), 2.17 (s, 3H, COC*H*$_3$), 2.03 (s, 3H, COC*H*$_3$), 1.99 (s, 3H, COC*H*$_3$), 1.97 (s, 3H, COC*H*$_3$). Data are consistent with literature values, [72] greater resolution of H5 and H6' has been achieved.
δ_C(101 MHz, DMSO)	169.9 (C$\underline{O}$CH$_3$), 169.7 (C$\underline{O}$CH$_3$), 169.2 (C$\underline{O}$CH$_3$), 168.6 (C$\underline{O}$CH$_3$), 90.0 (C1), 71.5 (C5), 70.3 (C3), 67.7 (C4), 61.2 (C6), 52.1 (C2), 20.9 (CO$\underline{C}$H$_3$), 20.8 (CO$\underline{C}$H$_3$), 20.4 (CO$\underline{C}$H$_3$), 20.3 (CO$\underline{C}$H$_3$). Data match literature values.[72]
m.p.	219-222 °C (d) (Lit.[72] 220-222 °C)
v (cm^{-1})	2948, 2842, 2829 (br, NH), 2750, 2687 (CH), 1766, 1744 (C=O)
m/z (ES+ TOF)	370.1110 $[M\text{-}HCl+Na]^+$ ($C_{14}H_{21}NO_9Na$), calc. 370.1114 – 100%

5.4.1.4 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranose (27)[22, 32]

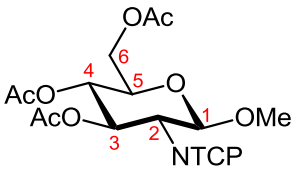


Chemical Formula: $C_{22}H_{19}Cl_4NO_{11}$

1,3,4,6-Tetra-*O*-acetyl-β-D-glucosamine hydrochloride (**26**, 5.00 g, 13.0 mmol) was dissolved in pyridine (50 mL) and TCPA (4.43 g, 15.5 mmol) partially dissolved in DCM (5 mL) was added and the mixture stirred overnight at room temperature. The mixture was cooled to 0 °C and Ac_2O (15 mL) added and then allowed to warm to room temperature and stirred for a further 1 h. The solvents were removed by co-evaporation with toluene. Purification by flash column chromatography (1:1 hexane/EtOAc) afforded the product as a white solid (7.71 g, 96%).

δ_H(400 MHz, $CDCl_3$)	6.47 (d, *J*=8.8, 1H, H1), 5.79 (dd, *J*=10.4, 9.1, 1H, H3), 5.23 (dd, *J*=10.2, 9.1, 1H, H4), 4.45 (dd, *J*=10.4, 8.8, 1H, H2), 4.36 (dd, J=12.5, 4.5, 1H, H6), 4.14 (dd, *J*=12.5, 2.2, 1H, H6'), 3.98 (ddd, *J*=10.2, 4.5, 2.2, 1H, H5), 2.11 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.90 (s, 3H, $COCH_3$). Data match literature values.[32]
δ_C(101 MHz, $CDCl_3$)	170.7 ($\underline{C}OCH_3$), 170.6 ($\underline{C}OCH_3$), 169.4 ($\underline{C}OCH_3$), 168.7 ($\underline{C}OCH_3$), 149.3 ($\underline{C}ON\underline{C}O$), 140.9 (Ar), 130.3 (Ar), 126.9 (Ar), 89.7 (C1), 72.7 (C5), 70.8 (C3), 68.0 (C4), 61.5 (C6), 54.4 (C2), 20.9 ($CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$). Data match literature values.[32]
R_f	0.7 (hexane/EtOAc 2:3)
m.p.	163-165 °C, not recrystallised (Lit.[32] 169-171 °C, recrystallisation details not specified)
v (cm^{-1})	2967 (CH), 1749, 1721 (C=O)
m/z (ES+ TOF)	635.9595 $[M+Na]^+$ ($C_{22}H_{19}{}^{35}Cl_4NO_{11}Na$), calc. 635.9610 – 70% 638.0 $[M+Na]^+$ ($C_{22}H_{19}{}^{35}Cl_3{}^{37}Cl_1NO_{11}Na$) – 100% 640.0 $[M+Na]^+$ ($C_{22}H_{19}{}^{35}Cl_2{}^{37}Cl_2NO_{11}Na$) – 10%

5.4.1.5 Methyl 3,4,6-tri-O-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (28)[32]



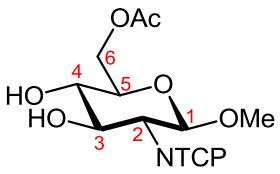
Chemical Formula: $C_{21}H_{19}Cl_4NO_{10}$

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranose (**27**, 8.00 g, 13.0 mmol) was dissolved in 32% HBr in AcOH (22 mL, 86 mmol) with Ac_2O (4 mL) and stirred, in darkness, overnight at room temperature. 200 mL of DCM was added and the mixture poured over ice (200 mL). Washing with water then sat. $NaHCO_3$, drying over $MgSO_4$ and removal of the solvent afforded the crude bromide as a cream solid, which was immediately dissolved in DCM (200 mL), MeOH (8.0 mL, 200 mmol) and Ag_2CO_3 (3.60 g, 13.1 mmol) were added and the mixture refluxed in darkness for 3 d. Filtering through Celite and removal of the solvent afforded the product as a cream solid (7.14 g, 86%). Analysis showed that the crude was sufficiently pure to be taken through without further purification.

δ_H(400 MHz, $CDCl_3$)	5.68 (dd, *J*=10.6, 9.1, 1H, H3), 5.25 (d, *J*=8.5, 1H, H1), 5.18 (dd, *J*=10.1, 9.1, 1H, H4), 4.33 (dd, *J*=12.3, 4.6, 1H, H6), 4.27 (dd, *J*=10.6, 8.5, 1H, H2), 4.17 (dd, *J*=12.3, 2.3, 1H, H6'), 3.82 (ddd, *J*=10.1, 4.6, 2.3, 1H, H5), 3.44 (s, 3H, OMe), 2.10 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 1.88 (s, 3H, $COCH_3$). Data match literature values.[32]
δ_C(101 MHz, $CDCl_3$)	170.7 ($\underline{C}OCH_3$), 170.6 ($\underline{C}OCH_3$), 169.4 ($\underline{C}OCH_3$), 140.6 ($\underline{C}ON\underline{C}O$), 130.1 (Ar), 127.1 (Ar), 98.7 (C1), 72.0 (C5), 71.0 (C3), 68.7 (C4), 61.9 (C6), 57.1 (OMe), 55.4 (C2), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$). No literature reference found, data match values previously found in the group.
R_f	0.7 (2:3 hexane/EtOAc)

m.p.	90-92 °C. No literature reference found.
v (cm^{-1})	2958 (CH), 1745, 1722 (C=O)
m/z (ES+ TOF)	607.9668 $[M+Na]^+$ ($C_{21}H_{19}{}^{35}Cl_4NO_{10}Na$), calc. 607.9661 – 80%
	609.8 $[M+Na]^+$ ($C_{21}H_{19}{}^{35}Cl_3{}^{37}Cl_1NO_{10}Na$) – 100%
	611.8 $[M+Na]^+$ ($C_{21}H_{19}{}^{35}Cl_2{}^{37}Cl_2NO_{10}Na$) – 40%

5.4.1.6 Methyl 6-O-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (4)[19]



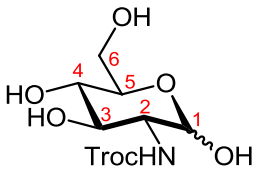
Chemical Formula: $C_{17}H_{15}Cl_4NO_8$

Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (**28**, 6.18 g, 10.5 mmol) was dissolved in MeOH (200 mL) and cooled to 0 °C. AcCl (5.0 mL) was added and the mixture stirred overnight at room temperature. The solvents were removed by evaporation and the intermediate triol suspended in DCM (100 mL) at -20 °C. AcCl (2.0 mL, 28 mmol) and *sym*-collidine (7.0 mL, 53 mmol) were added and the mixture stirred at -20 °C until the suspension cleared, when the reaction was quenched with MeOH (10 mL) warmed to room temperature and washed with 0.5 M HCl, sat. $NaHCO_3$ and water, dried over $MgSO_4$ and the solvents removed by evaporation. Purification by stepped gradient column chromatography (2:1 → 3:2 hexane/EtOAc) gave the glycosyl acceptor as an off-white solid (3.82 g, 72%).

δ_H(400 MHz, MeOD)	5.10 (d, *J*=8.5, 1H, H1), 4.50 (dd, *J*=12.0, 2.2, 1H, H6), 4.33 (dd, *J*=12.0, 5.6, 1H, H6'), 4.24 (dd, *J*=10.7, 8.7, 1H, H3), 3.99 (dd, *J*=10.7, 8.5, 1H, H2), 3.67 (ddd, *J*=9.8, 5.6, 2.2, 1H, H5), 3.50 – 3.43 (m, 4H, H4 & OMe), 2.14 (s, 3H, $COCH_3$). No literature reference found for spectrum in MeOD, data is consistent with spectrum reported in $CDCl_3$.[19]
δ_C(101 MHz, MeOD)	172.7 ($\underline{C}OCH_3$), 100.2 (C1), 75.5 (C5), 72.3 (C4), 72.1 (C3), 64.5 (C6), 58.9 (C2), 56.9 (OMe), 20.7 ($CO\underline{C}H_3$). Ar_qs not seen. No literature reference found for spectrum in MeOD, data is consistent with spectrum reported in $CDCl_3$.[19]
R_f	0.3 (2:3 hexane/EtOAc)
m.p.	129-131 °C. No literature reference found.
$[\alpha]_D$	+4.8° (c = 0.5, MeOH) (Lit. -18°, c=1, $CHCl_3$)
v (cm^{-1})	3385 (br, OH), 2974, 2895 (CH), 1719 (C=O)
m/z (ES+ TOF)	523.9446 $[M+Na]^+$ ($C_{17}H_{17}{}^{35}Cl_4NO_8Na$), calc. 532.9449 – 60% 525.8 $[M+Na]^+$ ($C_{17}H_{17}{}^{35}Cl_3{}^{37}ClNO_8Na$) – 100% 527.8 $[M+Na]^+$ ($C_{17}H_{17}{}^{35}Cl_2{}^{37}Cl_2NO_8Na$) – 30%

5.4.2 Second Generation

5.4.2.1 2-Deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (73)[73, 74]

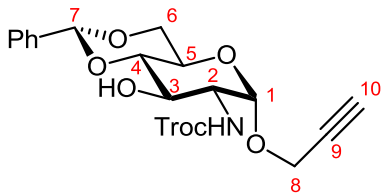


Chemical Formula: $C_9H_{14}Cl_3NO_7$

D-glucosamine hydrochloride (6.00 g, 27.8 mmol) was dissolved in water (60 mL), TrocCl (5.1 mL, 37 mmol) was added at 0 °C and the mixture stirred at room temperature overnight. Filtering, washing with Et_2O and drying gave the product as a fine white solid (9.80 g, 99%). Analysis showed that the crude was sufficiently pure to be taken through without further purification.

δ_H(400 MHz, DMSO)	7.41 (d, *J*=8.1, 1H, NH), 6.45 (d, *J*=4.5, 1H, OH1), 4.98 (ps t, *J*=4.0, 1H, H1), 4.91 (d, *J*=5.5, 1H, OH4), 4.81 (d, *J*=12.3, 1H, C<u>H</u>HCl$_3$), 4.75 (d, *J*=12.3, 1H, CH<u>H</u>Cl$_3$), 4.69 (d, *J*=5.7, 1H, OH3), 4.42 (ps t, *J*=5.8, 1H, OH6), 3.61 – 3.44 (m, 4H, H3, H4, H6 & H6'), 3.30 (d, *J*=1.2, 1H, H2), 3.12 (d ps t, *J*=9.3, 5.5, 1H, H5). No literature reference found.
δ_C(101 MHz, DMSO)	154.4 (N<u>C</u>OOCH$_2$), 96.2 (<u>C</u>Cl$_3$), 90.4 (C1), 73.5 (<u>C</u>H$_2$CCl$_3$), 72.0 (C4), 71.0 (C5), 70.0 (C3), 61.0 (C6), 56.7 (C2). No literature reference found.
m.p.	184-186 °C (d) (Lit.[74] 183-184 °C (d))
v (cm^{-1})	3320 (br, OH), 2941, 2919, 2878 (CH), 1698 (C=O), 1539 (NH bend)
m/z (ES+ TOF)	375.9739 [M+Na]$^+$ ($C_9H_{14}{}^{35}Cl_3NO_7Na$) ,calc. 375.9734 – 100% 378.0 [M+Na]$^+$ ($C_9H_{14}{}^{35}Cl_2{}^{37}ClNO_7Na$) – 90% 380.0 [M+Na]$^+$ ($C_9H_{14}{}^{35}Cl^{37}Cl_2NO_7Na$) – 30%

5.4.2.2 *Propargyl 4,6-O-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (66)*[75]



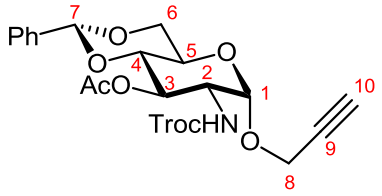
Chemical Formula: $C_{19}H_{20}Cl_3NO_7$

2-Deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (**73**, 3.55 g, 10.0 mmol) was dissolved in propargyl alcohol (30 mL), TMSCl (15 mL, 120 mmol) was added and the reaction stirred at room temperature for 3 days. The solvent was removed by co-evaporation with toluene and the brown residue taken up in MeCN (25 mL). 4 Å molecular sieves, DOWEX and benzaldehyde dimethyl acetal (2.3 mL, 15 mmol) were added and the mixture stirred at room temperature overnight. The mixture was filtered and the product precipitated as a beige solid by addition to a large excess of sat. $NaHCO_3$ solution followed by filtering and drying (3.28 g, 68%). Analysis showed that the crude was sufficiently pure to be taken through without further purification.

δ_H(400 MHz, $CDCl_3$)	7.53 – 7.45 (m, 2H, ArH), 7.42 – 7.34 (m, 3H, ArH), 5.55 (s, 1H, H7), 5.34 (d, *J*=9.6, 1H, TrocNH), 5.08 (d, *J*=3.7, 1H, H1), 4.82 (d, *J*=12.0, 1H, C<u>H</u>HCCl_3), 4.69 (d, *J*=12.0, 1H, CH<u>H</u>CCl_3), 4.31 – 4.26 (m, 3H, H6 & 2 x H8), 4.00 (d ps t, *J*=9.6, 3.7, 1H, H2), 3.92 (d ps t, *J*=9.6, 3.0, 1H, H3), 3.87 (d ps t, *J*=9.6, 4.6, 1H, H5), 3.76 (t, *J*=9.6, 1H, H6'), 3.59 (ps t, *J*=9.6, 1H, H4), 2.69 (d, *J*=3.0, 1H, OH, exchangeable), 2.48 (t, *J*=2.3, 1H, H10). Data consistent with literature values, except that the literature reports an O<u>CH_2</u>CH=CH_2 peak, when no such functionality is present.[75]
δ_C(101 MHz, $CDCl_3$)	155.0 (O<u>C</u>OCH_2), 137.0 (Ar_q), 129.5 (Ar), 128.5 (Ar), 126.4 (Ar), 102.1 (C7), 96.9 (C1), 95.5 (*C*Cl_3), 81.8 (C4), 78.3 (C9), 75.6 (C10), 74.9 (<u>C</u>H_2CCl_3), 70.1 (C3), 68.7 (C6), 63.1 (C5), 55.6 (C2), 55.3 (C8). No literature reference found.
R_f	0.6 (2:3 hexane/EtOAc)
m.p.	163-166 °C. No literature reference found.

v (cm^{-1})	3425 (br, OH), 3331 (NH), 3302, 3066, 2956, 2917, 2870 (CH), 1709 (C=O), 1531 (NH bend)
m/z (ES+ TOF)	375.9739 $[M+Na]^+$ ($C_{19}H_{20}{}^{35}Cl_3NO_7Na$), calc. 375.9734 – 80% 378.0 $[M+Na]^+$ ($C_{19}H_{20}{}^{35}Cl_2{}^{37}ClNO_7Na$)– 100% 380.0 $[M+Na]^+$ ($C_{19}H_{20}{}^{35}Cl^{37}Cl_2NO_7Na$) – 35%

5.4.2.3 *Propargyl 3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (67)*



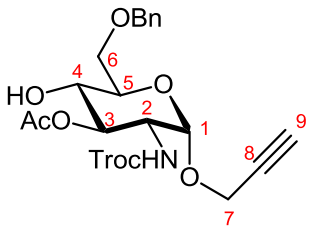
Chemical Formula: $C_{21}H_{22}Cl_3NO_8$

No literature reference found

Propargyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (**66**, 3.16 g, 6.57 mmol) and DMAP (ca. 0.2 g) were dissolved in pyridine (40 mL). The mixture was cooled to 0 °C and Ac_2O (1.2 mL, 13 mmol) was added and the mixture stirred overnight at room temperature. The mixture was poured over ice and extracted with DCM, washed with sat. $NaHCO_3$, dried over $MgSO_4$. Removal of the solvents by evaporation afforded the product as a light brown solid (3.31 g, 96%) which analysis showed to be sufficiently pure to be taken through without further purification.

δ_H(400 MHz, $CDCl_3$)	7.50 – 7.43 (m, 2H, ArH), 7.42 – 7.34 (m, 3H, ArH), 5.55 (s, 1H, H7), 5.43 – 5.35 (m, 2H, H3 & AcNH), 5.12 (d, *J*=3.7, 1H, H1), 4.81 (d, *J*=12.0, 1H, C<u>H</u>HCCl$_3$), 4.71 (d, *J*=12.0, 1H, CH<u>H</u>CCl$_3$), 4.35 – 4.30 (m, 3H, H6 & 2 x H8), 4.13 (d ps t, *J*=10.3, 3.7, 1H, H2), 3.98 (d ps t, *J*=9.6, 4.8, 1H, H5), 3.81 (ps t, *J*=9.6, 1H, H6'), 3.75 (ps t, *J*=9.6, 1H, H4), 2.50 (ps t, *J*=2.4, 1H, H10), 2.07 (s, 3H, COCH$_3$)
δ_C(101 MHz, $CDCl_3$)	170.9 (<u>C</u>OCH$_3$), 154.5 (N<u>C</u>OOCH$_2$), 137.0 (Ar$_q$), 129.2 (Ar), 128.3 (Ar), 126.3 (Ar), 101.7 (C7), 96.8 (C1), 95.5 (<u>C</u>Cl$_3$), 79.1 (C4), 78.1 (C9), 75.7 (C10), 74.7 (<u>C</u>H$_2$CCl$_3$), 69.9 (C3), 68.7 (C6), 63.5 (C5), 55.3 (C8), 54.6 (C2), 20.9 (CO<u>C</u>H$_3$)
R_f	0.7 (2:3 hexane/EtOAc)
m.p.	70-72 °C
$[\alpha]_D$	+4.0 (c=1.5, $CHCl_3$)
v (cm^{-1})	3308 (br, NH), 3075, 2920, 2868 (CH), 2106 (C≡C), 1744, 1714 (C=O), 1539 (NH bend)

5.4.2.4 *Propargyl 3-O-acetyl-6-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (68)*



Chemical Formula: $C_{21}H_{24}Cl_3NO_8$

No literature reference found

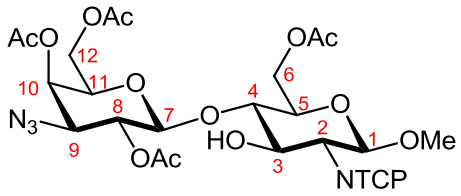
Propargyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (**67**, 6.05 g, 11.6 mmol) and TES (9.2 mL, 58 mmol) were dissolved in DCM at 0 °C and TFA (4.3 mL, 58 mmol) added. The mixture was stirred for 20 h at room temperature before the solvents were removed by co-evaporation with toluene. Purification by stepped gradient column chromatography (20% → 30% →40% EtOAc in hexane) afforded the product as a pale yellow foam which collapsed to an extremely viscous syrup (4.38 g, 72%).

δ_H(400 MHz, $CDCl_3$)	7.40 – 7.24 (m, 5H, ArH), 5.41 (d, *J*=10.3, 1H, TrocNH), 5.12 (dd, *J*=10.3, 8.8, 1H, H3), 5.06 (d, *J*=3.7, 1H, H1), 4.79 (d, *J*=12.0, 1H, C<u>H</u>HCCl$_3$), 4.63 (d, *J*=12.0, 1H, CH<u>H</u>CCl$_3$), 4.62 (d, *J*=12.0, 1H, PhC<u>H</u>H), 4.56 (d, *J*=12.0, 1H, PhCH<u>H</u>), 4.30 (dd, *J*=15.9, 2.4, 1H, H7), 4.24 (dd, *J*=15.8, 2.4, 1H, H7'), 4.01 (td, *J*=10.3, 3.7, 1H, H2), 3.88 – 3.70 (m, 4H, H4, H5, H6 &H6'), 2.90 (d, *J*=4.0, 1H, OH, exchangeable), 2.43 (t, *J*=2.4, 1H, H9), 2.07 (s, 3H, COCH$_3$)
δ_C(101 MHz, $CDCl_3$)	172.1 (<u>C</u>OCH$_3$), 154.4 (N<u>C</u>OOCH$_2$), 137.7 (Ar$_q$), 128.5 (Ar), 127.9 (Ar), 127.7 (Ar), 96.1 (C1), 95.5 (<u>C</u>Cl$_3$), 78.4 (C8), 75.5 (C9), 74.6 (<u>C</u>H$_2$CCl$_3$), 73.8 (Ph<u>C</u>H$_2$), 73.8 (C3), 70.9 (C4/5), 70.1 (C5/4), 69.5 (C6), 55.0 (C7), 53.6 (C2), 21.0 (CO<u>C</u>H$_3$)
R_f	0.5 (2:3 hexane/EtOAc)
$[\alpha]_D$	+64.5° (c=0.75, $CHCl_3$)
ν (cm^{-1})	3428 (br, OH), 3301 (br, NH), 3069, 3032, 2959, 2922, 2871 (CH), 2124 (C≡C), 1722 (C=O), 1518 (NH bend)
m/z (ES+ TOF)	546.0452 $[M+Na]^+$ ($C_{21}H_{24}{}^{35}Cl_3NO_8Na$), calc. 546.0465 – 100% 548.1 $[M+Na]^+$ ($C_{21}H_{24}{}^{35}Cl_2{}^{37}ClNO_8Na$)– 80% 550.0 $[M+Na]^+$ ($C_{21}H_{24}{}^{35}Cl{}^{37}Cl_2NO_8Na$) – 20%

5.5 Disaccharides

5.5.1 First Generation

5.5.1.1 Methyl 2,4,6-tri-O-acetyl-3-azido-3-deoxy-β(1-4)-D-galactopyranosyl-6-O-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (37)[19]



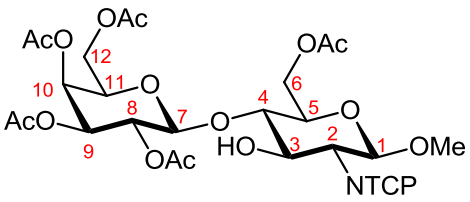
Chemical Formula: $C_{29}H_{30}Cl_4N_4O_{15}$

Phenyl 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-β-D-thiogalactopyranoside (**36**, 400 mg, 0.95 mmol) and methyl 6-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (**4**, 317 mg, 0.63 mmol) were dissolved in DCM (20 ml) and cooled to -20 °C. NIS (319 mg, 1.42 mmol) and TfOH (3 drops) were added and the mixture stirred for 2 h. The mixture was warmed to room, temperature and washed with 5% aq. $Na_2S_2O_3$, dried over $MgSO_4$ and the solvents removed by evaporation. Purification by flash column chromatography (2:1 hexane/EtOAC) yielded product as an off white solid (395 mg, 77%).

δ_H(400 MHz, $CDCl_3$) 5.36 (d, *J*=3.3, 1H, H10), 5.15 (dd, *J*=10.6, 7.9, 1H, H8), 5.11 (d, *J*=8.4, 1H, H1), 4.52 (d, *J*=7.9, 1H, H7), 4.36 – 4.24 (m, 3H, H3, H6 & OH), 4.16 – 4.03 (m, 3H, H2, H6' & H12), 4.01 – 3.85 (m, 2H, H11 &H12'), 3.70 (ddd, *J*=9.7, 4.7, 1.7, 1H, H5), 3.59 (dd, *J*=10.6, 3.3, 1H, H9), 3.52 (dd, *J*=9.7, 7.8, 1H, H4), 3.41 (s, 3H, OMe), 2.15 (s, 3H, $COCH_3$), 2.13 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 1.90 (s, 3H, $COCH_3$). Data match literature values.[19]

δ_C(101 MHz, $CDCl_3$)	170.8 ($\underline{C}OCH_3$), 170.6 ($\underline{C}OCH_3$), 169.9 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCH_3$), 140.4 ($\underline{C}ON\underline{C}O$), 127.4 ($Ar_q$), 126.8 ($Ar_q$), 126.5 ($Ar_q$), 102.2 (C7), 98.9 (C1), 83.4 (C4), 72.6 (C11), 72.1 (C5), 69.6 (C3 & C8), 67.6 (C10), 62.7 (C6), 61.8 (C12), 61.7 (C9), 57.1 (OMe), 56.3 (C2), 21.0 ($CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$). No literature reference found
R_f	0.6 (2:3 hexane/EtOAc)
m.p.	140-142 °C. No literature reference found.
$[\alpha]_D$	-40.0° (c=1, $CHCl_3$). No literature reference found.
v (cm^{-1})	3473 (OH), 2954, 2899 (CH), 2108 (N_3), 1744, 1719 (C=O)
m/z (ES+ TOF)	837.0380 $[M+Na]^+$ ($C_{29}H_{30}{}^{35}Cl_4N_4O_{15}$), calc. 837.0359 – 60% 839.0 $[M+Na]^+$ ($C_{29}H_{30}{}^{35}Cl_3{}^{37}ClN_4O_{15}$) – 100% 841.1 $[M+Na]^+$ ($C_{29}H_{30}{}^{35}Cl_2{}^{37}Cl_2N_4O_{15}$) – 30%

5.5.1.2 Methyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-6-O-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (33)



Chemical Formula: $C_{31}H_{33}Cl_4NO_{17}$

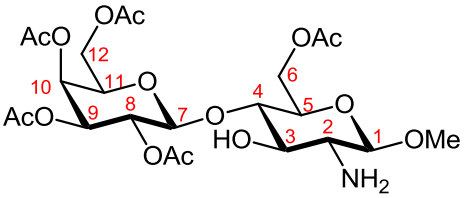
No literature reference found

Ethyl 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-D-thiogalactopyranoside (**35**, 1.17 g, 2.98 mmol) and methyl 6-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-Glucopyranoside (**4**, 1.00 g, 1.99 mmol) were dissolved in DCM (50 ml) and cooled to -20 °C. NIS (671 mg, 2.98 mmol) and TfOH (3 drops)

were added and the mixture stirred overnight whilst warming to room temperature. The mixture was washed with 5% aq. $Na_2S_2O_3$, dried over $MgSO_4$ and the solvents removed by evaporation. Purification by dissolution in Et_2O, filtration and removal of the solvents gave product as an off white solid (1.05 g, 63%).

δ_H(400 MHz, $CDCl_3$)	5.37 (d, *J*=3.4, 1H, H10), 5.23 (dd, *J*=10.4, 8.0, 1H, H8), 5.13 (d, *J*=8.5, 1H, H1), 5.00 (dd, *J*=10.4, 3.4, 1H, H9), 4.55 (d, *J*=8.0, 1H, H7), 4.40 – 4.29 (m, 3H, H3, H12 & H12'), 4.17 – 4.01 (m, 5H, H2, H6, H6', H11 & OH), 3.72 (ddd, *J*=9.8, 4.7, 1.8, 1H, H5), 3.58 (dd, *J*=9.8, 8.1, 1H, H4), 3.44 (s, 3H, OMe), 2.15 (s, 3H, $COCH_3$), 2.14 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.94 (s, 3H, $COCH_3$)
δ_C(101 MHz, $CDCl_3$)	170.1 ($\underline{C}OCH_3$), 170.0 ($\underline{C}OCH_3$), 169.6 ($\underline{C}OCH_3$), 140.4 ($\underline{C}ON\underline{C}O$), 102.1 (C7), 98.9 (C1), 83.4 (C4), 72.1 (C5), 71.6 (C11), 70.9 (C9), 69.6 (C3), 68.8 (C8), 66.8 (C10), 62.6 (C12), 61.7 (C6), 57.0 (OMe), 56.4 (C2), 21.0 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$) Ar_qs not visible.
R_f	0.5 (2:3 hexane/EtOAc)
m.p.	182-184 °C
$[\alpha]_D$	+9.4° (c=1, $CHCl_3$)
v (cm^{-1})	3570 (OH), 2914, 2849 (CH), 1743, 1712 (C=O)
m/z (ES+ TOF)	854.0423 $[M+Na]^+$ ($C_{31}H_{33}{}^{35}Cl_4NO_{17}Na$), calc. 854.0400 – 60% 856.0 $[M+Na]^+$ ($C_{31}H_{33}{}^{35}Cl_3{}^{37}Cl_1NO_{17}Na$) – 100% 856.0 $[M+Na]^+$ ($C_{31}H_{33}{}^{35}Cl_2{}^{37}Cl_2NO_{17}Na$) – 30%

5.5.1.3 *Methyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-6-O-acetyl-2-amino-2-deoxy-β-D-glucopyranoside (40)*



Chemical Formula: $C_{23}H_{35}NO_{15}$

No literature reference found

Methyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-6-*O*-acetyl-2-deoxy-2-tetrachloro-phthalimido-β-D-glucopyranoside (**34**, 100 mg, 0.12 mmol) was dissolved in 2:1:1 MeCN/THF/EtOH (8 mL), ethylene diamine (16 µL, 0.24 mmol) was added and the reaction stirred at 60 °C for 2 h when TLC (2:3 Hexane/EtOAc) revealed only a baseline spot. The reaction mixture was cooled, filtered through Celite and the solvents removed. The residue was dissolved in DCM, washed with water and dried over $MgSO_4$. Removal of the solvents afforded the product as a pale yellow solid (67 mg, 98%).

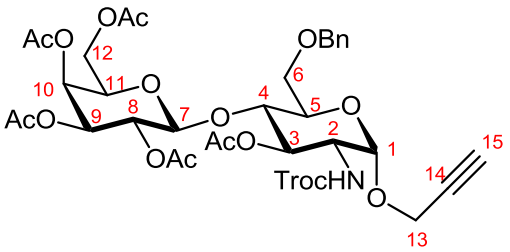
δ_H(400 MHz, $CDCl_3$) 5.39 (dd, *J*=3.3, 0.6, 1H, H10), 5.23 (dd, *J*=10.5, 8.0, 1H, H8), 5.00 (dd, *J*=10.5, 3.3, 1H, H9), 4.54 (d, *J*=8.0, 1H, H7), 4.30 (dd, *J*=11.8, 1.9, 1H, H6), 4.19 (dd, *J*=11.4, 4.6, 1H, H12), 4.14 – 4.00 (m, 4H, H1, H6', H11 & H12'), 3.59 – 3.41 (m, 7H, H3, H4, H5, OH & OMe), 2.75 (dd, *J*=9.2, 8.1, 1H, H2), 2.16 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$), 1.28 (br s, 2H, NH_2)

δ_C(101 MHz, $CDCl_3$) 170.8 ($\underline{C}OCH_3$), 170.5 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 170.0 ($\underline{C}OCH_3$), 169.6 ($\underline{C}OCH_3$), 104.8 (C1), 102.2 (C7), 82.9 (C4), 74.9 (C3), 71.9 (C5), 71.5 (C11), 71.0 (C9), 68.8 (C8), 67.0 (C10), 63.1 (C6), 61.9 (C12), 57.4 (OMe), 56.7 (C2), 21.0 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$)

$[\alpha]_D$	+8.6° (c=0.5, $CHCl_3$)
v (cm^{-1})	3505 (br, OH, NH), 2999, 2966, 2939, 2917, 2889, 2852 (CH), 1745, 1733 (C=O)
m/z (ES+ TOF)	588.1925 $[M+Na]^+$ ($C_{23}H_{35}NO_{15}Na$), calc. 588.1904 – 100%

5.5.2 Second Generation

5.5.2.1 Propargyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-3-O-acetyl-6-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (75)



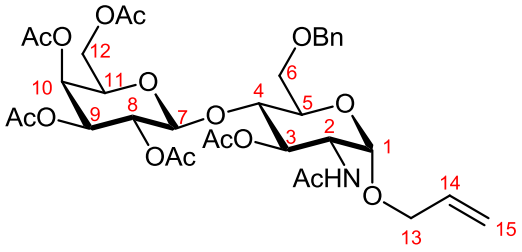
Chemical Formula: $C_{35}H_{42}Cl_3NO_{17}$

No literature reference found

Phenyl penta-*O*-acetyl-β-D-thiogalactopyranoside (**18**, 672 mg, 1.52 mmol) and propargyl 3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (**68**, 533 mg, 1.02 mmol) were dissolved in DCM (20 ml) and cooled to -20 °C. NIS (343 mg, 1.52 mmol) and TfOH (3 drops) were added and the mixture stirred for 2 h. The mixture was warmed to room, temperature and washed with 5% aq. $Na_2S_2O_3$, dried over $MgSO_4$ and the solvents removed by evaporation. Purification by stepped gradient flash column chromatography (3:1 → 2:1 → 1:1 Hexane/EtOAc) yielded product as a white solid (577 mg, 66%).

δ_H(400 MHz, $CDCl_3$)	7.41 – 7.28 (m, 5H, ArH), 5.31 (d, *J*=9.9, 1H, AcNH), 5.24 (d, *J*=3.5, 1H, H10), 5.14 (dd, *J*=10.7, 9.5, 1H, H3), 5.05 (d, *J*=3.7, 1H, H1), 4.95 (dd, *J*=10.6, 8.0, 1H, H8), 4.75 (d, *J*=12.0, 1H, PhC<u>H</u>H), 4.74 (dd, *J*=10.6, 3.5, 1H, H9), 4.73 (d, *J*=12.0, 1H, C<u>H</u>HCCl_3), 4.63 (d, *J*=12.0, 1H, CH<u>H</u>CCl_3), 4.41 (d, *J*=12.0, 1H, PhCH<u>H</u>), 4.31 (d, *J*=8.0, 1H, H7), 4.24 (dd, *J*=15.8, 2.4, 1H, H13), 4.19 (dd, *J*=15.8, 2.4, 1H, H13'), 4.08 – 3.85 (m, 5H, H2, H4, H12 & H12'), 3.77 – 3.70 (m, 2H, H5 & H6), 3.65 – 3.56 (m, 2H, H11 & H6'), 2.43 (t, *J*=2.4, 1H, H15), 2.08 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.92 (s, 3H, $COCH_3$), 1.90 (s, 3H, $COCH_3$)
δ_C(101 MHz, $CDCl_3$)	170.6 (<u>C</u>OCH_3), 170.3 (<u>C</u>OCH_3), 170.2 (<u>C</u>OCH_3), 170.0 (<u>C</u>OCH_3), 168.8 (<u>C</u>OCH_3), 154.3 (<u>C</u>OCH_2), 137.6 (Ar_q), 128.7 (Ar), 128.2 (Ar), 128.2 (Ar), 100.3 (C7), 96.1 (C1), 95.4 (<u>C</u>Cl_3), 78.2 (C14), 75.5 (C15), 74.6 (C4), 74.5 (<u>C</u>H_2CCl_3), 73.7 ($PhCH_2$), 71.0 (C9), 70.8 (C5), 70.4 (C3), 69.1 (C11), 67.0 (C8), 66.8 (C6), 60.9 (C10), 55.1 (C12), 53.9 (C13), 20.8 (CO<u>C</u>H_3), 20.7 (CO<u>C</u>H_3), 20.7 (CO<u>C</u>H_3), 20.6 (CO<u>C</u>H_3), 20.5 (CO<u>C</u>H_3)
R_f	0.6 (2:3 hexane/EtOAc)
m.p.	72-74 °C
$[\alpha]_D$	+52.8° (c=0.5, $CHCl_3$)
ν (cm^{-1})	3285 (NH), 3068, 3032, 2939, 2914, 2866 (CH), 2122 (C≡C), 1741 (C=O), 1526 (NH bend)
m/z (ES+ TOF)	876.1411 $[M+Na]^+$ ($C_{35}H_{42}{}^{35}Cl_3NO_{17}Na$), calc. 876.1416 – 60% 878.2 $[M+Na]^+$ ($C_{35}H_{42}{}^{35}Cl_2{}^{37}ClNO_{17}Na$) – 100% 880.2 $[M+Na]^+$ ($C_{35}H_{42}{}^{35}Cl^{37}Cl_2NO_{17}Na$) – 30%

5.5.2.2 *Allyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-3-O-acetyl-6-O-benzyl-2-deoxy-(2,2,2-trichloroethoxycarbamido)-β-D-glucopyranoside (76)*



Chemical Formula: $C_{34}H_{45}NO_{16}$

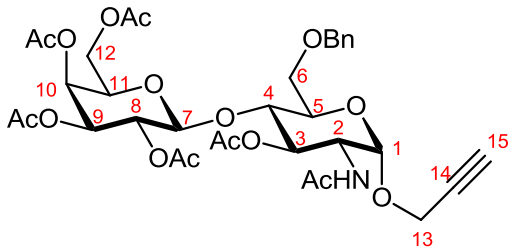
No literature reference found

Propargyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbamido)-α-D-glucopyranoside (**75**, 577 mg, 0.67 mmol) was dissolved in Ac_2O (40 mL) and Zn (activated with 1M HCl followed by filtering and washing with MeOH then Et_2O, 2.20 g, 33.6 mmol) was stirred at room temperature for 24 h. The mixture was filtered and the solvent removed by repeated co-evaporation with toluene. The residue was taken up in DCM, washed with water, sat. $NaHCO_3$ and dried over $MgSO_4$. Removal of the solvents afforded the product as a white solid (468 mg, 96%).

δ_H(400 MHz, $CDCl_3$) 7.43 – 7.30 (m, 5H, ArH), 6.19 (d, *J*=9.5, 1H, AcNH), 5.90 – 5.71 (m, 1H, H14), 5.29 – 5.20 (m, 3H, H10 & 2x H15), 5.16 (dd, *J*=10.8, 9.3, 1H, H3), 5.00 (dd, *J*=10.4, 8.0, 1H, H8), 4.88 (d, *J*=3.8, 1H, H1), 4.79 (dd, *J*=10.4, 3.5, 1H, H9), 4.78 (d, *J*=12.1, 1H, PhC<u>H</u>H), 4.45 (d, *J*=12.1, 1H, PhCH<u>H</u>), 4.37 (d, *J*=8.0, 1H, H7), 4.26 (ddd, *J*=10.8, 9.5, 3.8, 1H, H2), 4.16 (dd, *J*=12.8, 5.3, 1H, H13), 4.07 (d, *J*=6.8, 2H, H12 & H12'), 4.01 – 3.92 (m, 2H, H4 & H13'), 3.80 – 3.70 (m, 2H, H5 & H6), 3.69 – 3.60 (m, 2H, H6' & H11), 2.12 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 1.94 (s, 3H, $COCH_3$)

δ_C(101 MHz, $CDCl_3$)	172.2 ($\underline{C}OCH_3$), 171.4 ($\underline{C}OCH_3$), 170.4 ($\underline{C}OCH_3$), 170.2 ($\underline{C}OCH_3$), 170.0 ($\underline{C}OCH_3$), 169.0 ($\underline{C}OCH_3$), 137.6 (Ar_q), 133.2 (C14), 128.6 (Ar), 128.1 (Ar), 118.4 (C15), 100.4 (C7), 96.1 (C1), 74.7 (C4), 73.7 ($Ph\underline{C}H_2$), 71.3 (C3), 70.9 (C9), 70.4 (C5/11), 70.2 (C11/5), 69.1 (C8), 68.7 (C13), 67.1 (C10), 66.8 (C6), 60.9 (C12), 52.4 (C2), 23.2 ($CO\underline{C}H_3$), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$)
R_f	0.2 (2:3 hexane/EtOAc)
m.p.	82-84 °C
$[\alpha]_D$	+28.0° (c=1, $CHCl_3$)
v (cm^{-1})	3330 (NH), 2962, 2931, 2847 (CH), 1745, 1609 (C=O), 1554 (NH bend)
m/z (ES+ TOF)	746.2603 $[M+Na]^+$ ($C_{34}H_{45}NO_{16}Na$), calc. 746.2636 – 100%

5.5.2.3 *Propargyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (77)*



Chemical Formula: $C_{34}H_{43}NO_{16}$

No literature reference found

TBAF (1M in THF, 3.0 mL, 3.0 mmol) was added to a solution of propargyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (**75**, 506 mg, 0.59 mmol) in THF (5 mL) and the mixture stirred at room temperature

for 1.5 h. Pyridine (1 mL) and Ac_2O (1.0 mL, 11 mmol) were added and the reaction stirred for a further 20 h. The mixture was diluted with DCM, washed with sat. $NaHCO_3$ and dried over $MgSO_4$. Removal of the solvents afforded the product as a light beige solid (354 mg, 83%).

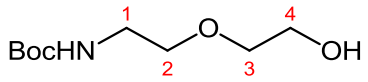
δ_H(400 MHz, $CDCl_3$)	7.42 – 7.29 (m, 5H, ArH), 5.76 (d, *J*=9.6, 1H, AcNH), 5.25 (dd, *J*=3.5, 0.8, 1H, H10), 5.12 (dd, *J*=10.9, 9.5, 1H, H3), 4.99 (d, *J*=3.6, 1H, H1), 4.98 (dd, *J*=10.5, 8.1, 2H, H8), 4.77 (dd, *J*=10.5, 3.5, 1H, H9), 4.75 (d, *J*=12.1, 1H, PhC<u>H</u>H), 4.43 (d, *J*=12.1, 1H, PhCH<u>H</u>), 4.35 (d, *J*=8.1, 1H, H7), 4.28 (ddd, *J*=10.9, 9.6, 3.6, 1H, H2), 4.24 (dd, *J*=15.9, 2.4, 1H, H13), 4.18 (dd, *J*=15.9, 2.4, 1H, H13'), 4.05 (d, *J*=6.8, 2H, H12 & H12'), 3.95 (ps t, *J*=9.5, 1H, H4), 3.78 – 3.70 (m, 2H, H5 & H6), 3.67 – 3.58 (m, 2H, H6' & H11), 2.43 (t, *J*=2.4, 1H, H15), 2.10 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 1.94 (s, 3H, $COCH_3$), 1.93 (s, 3H, $COCH_3$), 1.92 (s, 3H, $COCH_3$)
δ_C(101 MHz, $CDCl_3$)	171.3 (<u>C</u>OCH_3), 170.5 (<u>C</u>OCH_3), 170.3 (<u>C</u>OCH_3), 170.2 (<u>C</u>OCH_3), 170.1 (<u>C</u>OCH_3), 169.0 (<u>C</u>OCH_3), 137.8 (Ar_q), 128.8 (Ar), 128.3 (Ar), 128.2 (Ar), 100.5 (C1), 96.3 (C7), 78.5 (C14), 75.3 (C15), 74.8 (C4), 73.8 (Ph<u>C</u>H_2), 71.3 (C3), 71.1 (C9), 70.9 (C5), 70.5 (C11), 69.3 (C8), 67.2 (C6), 66.9 (C10), 61.1 (C12), 55.2 (C13), 51.9 (C2), 23.3 (CO<u>C</u>H_3), 21.0 (CO<u>C</u>H_3), 20.8 (CO<u>C</u>H_3), 20.8 (CO<u>C</u>H_3), 20.7 (CO<u>C</u>H_3), 20.6 (CO<u>C</u>H_3)
R_f	0.6 (2:1 toluene/Me_2CO)
m.p.	55-57 °C
$[\alpha]_D$	+27.7° (c=1.5, $CHCl_3$)
v (cm^{-1})	3373, 3277 (NH), 2936, 2872 (CH), 2117 (C≡C), 1742, 1674(C=O), 1533 (NH bend)

m/z (ES+ TOF) 744.2480 $[M+Na]^+$ ($C_{34}H_{43}NO_{16}Na$), calc. 744.2480 – 100%

5.6 Linkers

5.6.1 Short Linkers

5.6.1.1 N-Boc-2-(2-aminoethoxy)ethanol (45)[76]



Chemical Formula: $C_9H_{19}NO_4$

2-(2-Aminoethoxy)ethanol (5.0 mL, 50 mmol) and $NaHCO_3$ (10.5 g, 125 mmol) were dissolved in 1:1 THF/H_2O (100 mL) at room temperature and Boc_2O (10.9 g, 49.9 mmol) in THF (50 mL) was added dropwise over 2 h and the mixture stirred overnight. The THF was removed by evaporation and the product was extracted into EtOAc and washed with 1 M citric acid solution and dried over $MgSO_4$. Removal of the solvents by evaporation afforded the product as a colourless syrup (10.2 g, 100%).

δ_H(300 MHz, $CDCl_3$) 5.61 (s, 0.25H, BocNH), 5.01 (s, 0.75H, BocNH), 3.83 – 3.68 (m, 2H, 2 x H4), 3.67 – 3.44 (m, 4H, 4 x H2-3), 3.32 (br ps q, *J*=4.9, 2H, 2 x H1), 1.44 (s, 9H, $C(CH_3)_3$). Data match literature values.[76]

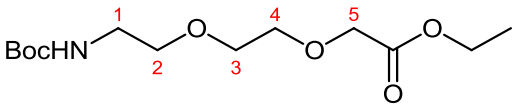
δ_C(101 MHz, $CDCl_3$) 156.3 (COO^tBu), 79.5 ($\underline{C}(CH_3)_3$), 72.2 (C2), 70.3 (C3), 61.7 (C4), 40.4 (C1), 28.48 ($C(\underline{C}H_3)_3$). Data match literature values.[76]

R_f 0.3 (2:3 hexane/EtOAc)

v (cm^{-1}) 3347 (br, OH), 2976, 2932, 2872 (CH), 1687 (C=O), 1532 (NH bend)

m/z (ES+ TOF) 228.1216 $[M+Na]^+$ ($C_9H_{19}NO_4Na$), calc. 228.1212 – 100%

5.6.1.2 Ethyl 3,6-dioxa-8-^{t}butyloxycarbonylaminooctanoate (46)[37]

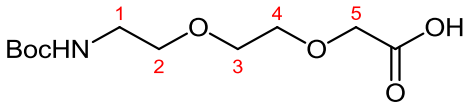


Chemical Formula: $C_{13}H_{25}NO_6$

N-Boc-2-(2-aminoethoxy)ethanol (**45**, 959 mg, 4.67 mmol) and KO^{t}Bu (524 mg, 5.07 mmol) were stirred in THF (20 mL) at 0 °C for 30 min. Ethyl bromoacetate (0.56 mL, 5.1 mmol) was added dropwise over 10 min and the reaction stirred at 4 °C for 4 h and then a further 15 h at room temperature. Water (50 mL) was added, the THF removed by evaporation and the product extracted into EtOAc. Drying over $MgSO_4$ and removal of the solvents by evaporation gave a colourless syrup that was purified by flash column chromatography (1:1 hexane/EtOAc) to afford the product as a colourless syrup (346 mg, 27%).

δ_H(300 MHz, $CDCl_3$)	5.02 (br s, 1H, BocNH), 4.20 (q, *J*=7.1, 2H, C<u>H</u>$_2$CH$_3$), 4.12 (s, 2H, 2 x H5), 3.75 – 3.66 (m, 2H, 2 x H2), 3.66 – 3.59 (m, 2H, 2 x H3), 3.53 (t, *J*=5.1, 2H, 2 x H4), 3.30 (br ps q, *J*=5.2, 2H, 2 x H1), 1.42 (s, 9H, C(CH$_3$)$_3$), 1.27 (t, *J*=7.1, 3H, CH$_2$C<u>H</u>$_3$). Data match literature values.[37]
δ_C(101 MHz, $CDCl_3$)	170.4 (COOEt), 156.1 (COO^{t}Bu), 79.2 (<u>C</u>(CH$_3$)$_3$), 71.0 (C5), 70.5 (C2), 70.1 (C4), 68.8 (C3), 60.9 (<u>C</u>H$_2$CH$_3$), 40.5 (C1), 28.5 (C(<u>C</u>H$_3$)$_3$), 14.3 (CH$_2$<u>C</u>H$_3$)
R_f	0.3 (2:3 hexane/EtOAc). Data match literature values.[37]
v (cm^{-1})	3347 (br, OH), 2976, 2932, 2872 (CH), 1687 (C=O), 1523 (NH bend)

5.6.1.3 3,6-Dioxa-8-^{t}butyloxycarbonylaminooctanoic Acid (47)[37]



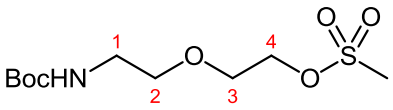
Chemical Formula: $C_{11}H_{21}NO_6$

Ethyl 3,6-dioxa-8-^{t}butyloxycarbonylaminooctanoate (**46**, 355 mg, 1.22 mmol) was dissolved in THF (5 mL). LiOH (112 mg, 2.68 mmol) was added followed by enough water to form a clear solution. The reaction was stirred at room temperature for 2 h. The THF was removed by evaporation and the mixture diluted with water, washed with EtOAc, acidified with 1 M citric acid, extracted into EtOAc and dried over $MgSO_4$. Removal of the solvents by evaporation afforded the product as a colourless syrup (270 mg, 84%).

δ_H(300 MHz, $CDCl_3$) 10.67 (s, 1H, COOH), 6.11 (s, 0.25H, BocNH), 5.06 (s, 0.75H, BocNH), 4.16 (s, 2H, 2 x H5), 3.79 – 3.69 (m, 2H, 2 x H2), 3.69 – 3.59 (m, 2H, 2 x H3), 3.54 (t, *J*=4.8, 2H, 2 x H4), 3.31 (br d, *J*=4.3, 2H, 2 x H1), 1.42 (s, 9H, $C(CH_3)_3$). Data match literature values.[37]

δ_C(101 MHz, $CDCl_3$) 173.4 (COOH), 156.2 ($COO^{t}Bu$), 79.6 ($\underline{C}(CH_3)_3$), 71.1 (C5), 70.5 (C4), 70.2 (C3), 68.6 (C2) 40.4 (C1), 28.4 ($C(\underline{C}H_3)_3$). Data match literature values.[37]

5.6.1.4 N-Boc-O-methanesulfonyl-2-(2-aminoethoxy)ethanol (80)[77]

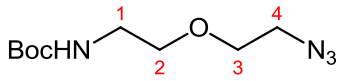


Chemical Formula: $C_{10}H_{21}NO_6S$

N-Boc-2-(2-aminoethoxy)ethanol (**45**, 3.00 g, 14.6 mmol) and NEt_3 (4.1 mL, 29 mmol) were dissolved in DCM (30 mL) at 0 °C. Methanesulfonyl chloride (1.24 mL, 16.1 mmol) was added dropwise over 30 min and the reaction stirred overnight at room temperature. The solvents were removed by evaporation and the residue taken up in EtOAc, washed with water, then brine and dried over $MgSO_4$. Removal of the solvents by evaporation afforded the product as a pale yellow syrup (3.51 g, 85%).

δ_H(300 MHz, $CDCl_3$)	4.90 (br s, 1H, BocNH), 4.38 – 4.32 (m, 2H, 2 x H4), 3.74 – 3.68 (m, 2H, 2 x H3), 3.54 (t, *J*=5.2, 2H, 2 x H2), 3.31 (br ps q, *J*=5.9, 2H, 2 x H1), 3.05 (s, 3H, CH_3S), 1.42 (s, 9H, $C(CH_3)_3$). Data match literature values.[77]
δ_C(101 MHz, $CDCl_3$)	156.0 (COO^tBu), 79.5 ($\underline{C}(CH_3)_3$), 70.5 (C4), 68.8 (C3), 68.8 (C2), 40.3 (C1), 37.8 (CH_3S), 28.5 ($C(\underline{C}H_3)_3$). No literature reference found.
R_f	0.4 (2:3 hexane/EtOAc)
v (cm^{-1})	3397 (br NH), 2977, 2963, 2880 (CH), 1700 (C=O), 1514 (NH bend)
m/z (ES+ TOF)	306.0981 $[M+Na]^+$ ($C_{10}H_{21}NO_6SNa$), calc. 306.0987 – 100%

5.6.1.5 N-Boc-2-(2-azidoethoxy)ethylamine (79)[78]



Chemical Formula: $C_9H_{18}N_4O_3$

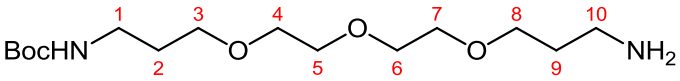
N-Boc-*O*-methanesulfonyl-2-(2-aminoethoxy)ethanol (**80**, 3.51 g, 12.4 mmol) was dissolved in DMF (30 mL) and NaN_3 (4.03 g, 62.0 mmol) added. The reaction was heated at 60 °C for 2 h, cooled, diluted with EtOAc and washed with water and then brine. Drying over $MgSO_4$ and removal of the solvents by evaporation afforded the product as a pale yellow syrup (2.37 g, 83%).

δ_H(300 MHz, $CDCl_3$)	4.97 (br s, 1H, BocNH), 3.68 – 3.57 (m, 2H, 2 x H2), 3.52 (t, *J*=5.1, 2H, 2 x H3), 3.39 – 3.25 (m, 4H, 2 x H1 & 2 x H4), 1.42 (s, 9H, $C(CH_3)_3$). Data match literature values.[78]
δ_C(101 MHz, $CDCl_3$)	156.0 (COO^tBu), 79.3 ($\underline{C}CH_3)_3$), 70.4 (C2), 69.9 (C3), 50.7 (C4), 40.4 (C1), 28.4 ($C(\underline{C}H_3)_3$). Data match literature values.[78]
R_f	0.6 (2:3 hexane/EtOAc)

v (cm^{-1})	3355 (br, NH), 2977, 2932, 2869 (CH), 2101 (N_3), 1694 (C=O), 1511 (NH bend)
m/z (ES+ TOF)	253.1243 $[M+Na]^+$ ($C_9H_{18}N_4O_3Na$), calc. 253.1277 – 100%

5.6.2 Long Linkers

5.6.2.1 Mono-N-Boc-4,7,10-trioxa-trideca-1,13-diamine (42)[79, 80]



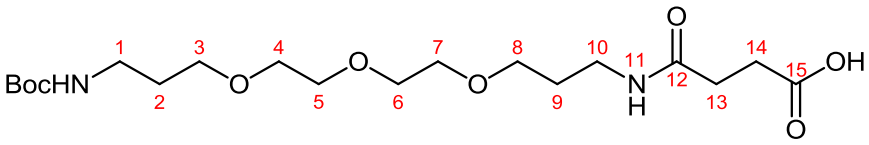
Chemical Formula: $C_{15}H_{32}N_2O_5$

4,7,10-Trioxa-trideca-1,13-diamine (60 mL, 270 mmol) was dissolved in THF (400 mL), Boc_2O (10.0 g, 45.8 mmol) dissolved in THF (100 mL) was added dropwise over 1 h and the mixture stirred overnight at room temperature. The solvents were removed by evaporation and the residue taken up in water (200 mL) and washed with EtOAc (3 x 100 mL). The organic portions were combined and washed with brine (3 x 50 mL). Removal of the solvents by evaporation gave product as a colourless liquid (9.78 g, 67%) which analysis revealed to be sufficiently pure to proceed without further purification.

δ_H(300 MHz, $CDCl_3$)	5.17 (br t, *J*=5.8 1H, BocNH), 3.63 – 3.44 (m, 12H, 12 x H3-8), 3.15 (dt, *J*=12.3, 5.8, 2H, 2 x H1), 2.73 (t, *J*=6.7, 2H, 2 x H10), 1.75 – 1.60 (m, 4H, 2 x H2 & 2 x H9), 1.37 (s, 9H, $C(CH_3)_3$), 1.22 (s, 2H, NH_2). Data match literature values.[79]
δ_C(101 MHz, $CDCl_3$)	156.0 (COO^tBu), 78.7 ($\underline{C}(CH_3)_3$), 70.6 (CH_2O), 70.6 (CH_2O), 70.2 (CH_2O), 70.2 (CH_2O), 69.5 (CH_2O), 69.4 (CH_2O), 39.6 (C1), 38.5 (C10), 33.4 (C2), 29.6 (C9), 28.4 ($C(\underline{C}H_3)_3$). Data match incomplete set of peaks given in literature.[80]
v (cm^{-1})	3357 (br, NH), 2977, 2929, 2866 (CH), 1706 (C=O), 1517 (NH bend)

m/z (ES+ TOF) 321.2391 $[M+H]^+$ ($C_{15}H_{33}N_2O_5$), calc. 321.2389 – 100%

5.6.2.2 N-*Boc*, N'-*succinyl-4,7,10-trioxa-trideca-1,13-diamine (43)*[79]



Chemical Formula: $C_{19}H_{36}N_2O_8$

Succinic anhydride (1.42 g, 14.2 mmol) and pyridine (5 mL) were added to mono-*N*-Boc-4,7,10-trioxa-trideca-1,13-diamine (**42**, 3.80 g, 11.9 mmol) in DCM (20 mL) at 0 °C, and the mixture stirred overnight at room temperature. Removal of the solvents by co-evaporation with toluene gave an orange syrup. Purification by flash column chromatography (10% MeOH, 1% AcOH in DCM) afforded the product as a pale yellow syrup (4.82 g, 97%).

δ_H(400 MHz, MeOD) 10.29 (br s, 1H, COOH), 6.98 (s, 0.4H, H11), 6.90 (s, 0.6H, H11), 6.30 (s, 0.3H, BocNH), 5.07 (s, 0.7H, BocNH), 3.69 – 3.48 (m, 12H, 12 x H3-8), 3.36 (ps q, *J*=5.8, 2H, 2 x H10), 3.20 (br ps q, *J*=6.0 2H, 2 x H1), 2.66 (t, *J*=5.6, 2H, 2 x H13), 2.49 (t, *J*=5.6, 2H, 2 x H14), 1.84 – 1.67 (m, 4H, 2 x H2 & 2 x H9), 1.42 (s, 9H, $C(CH_3)_3$). Data match literature values.[79]

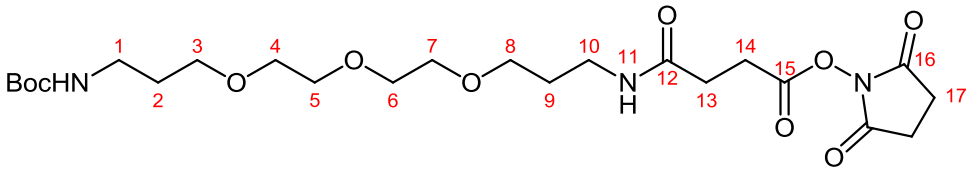
δ_C(101 MHz, MeOD) 175.1, 172.5 (C12 & C15), 156.2 (COO^tBu), 79.2 ($\underline{C}(CH_3)_3$), 70.5 (CH_2O), 70.3 (CH_2O), 70.1 (CH_2O), 69.6 (CH_2O), 69.4 (CH_2O), 68.4 (CH_2O), 38.6 (C1/10), 38.4 (C10/1), 30.9, 30.2, 29.7, 28.7 (C2, C9, C13 & C14), 28.5 ($C(\underline{C}H_3)_3$). Data match literature values.[79]

R_f 0.6 (10% MeOH, 1% AcOH in DCM)

v (cm^{-1}) 3502 (OH), 3348 (NH), 2978, 2927, 2871 (CH), 1694 (C=O), 1525 (NH bend)

m/z (ES+ TOF) 443.2381 $[M+Na]^+$ ($C_{19}H_{36}N_2O_8Na$), calc. 443.2369 – 100%

5.6.2.3 N-Boc, N'-succinyl-4,7,10-trioxa-trideca-1,13-diamine NHS Ester (48)[81]

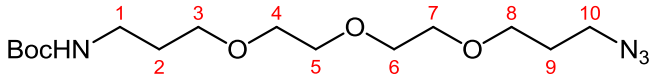


Chemical Formula: $C_{23}H_{39}N_3O_{10}$

DCC (413 mg, 2.00 mmol) and NHS (230 mg, 2.00 mmol) were added to *N*-Boc, *N'*-succinyl-4,7,10-trioxa-trideca-1,13-diamine (**43**, 0.1 M solution in THF, 20 mL, 2.0 mmol) and the mixture left to stand overnight at 4 °C. Filtration through Celite and removal of the solvents by evaporation gave the product as a thick golden syrup (937 mg, 91%) which analysis revealed to sufficiently pure to proceed without further purification.

δ_H(400 MHz, $CDCl_3$) 6.53 (br s, 1H, H11), 4.97 (br s, 1H, BocNH), 3.67 – 3.48 (m, 12H, CH_2O), 3.37 (ps q, *J*=5.9, 2H, 2 x H10), 3.19 (ps q, *J*=5.9, 2H, 2 x H1), 2.97 (t, *J*=7.6, 2H, 2 x H14), 2.82 (br s, 4H, 4 x H17), 2.56 (t, *J*=7.6, 2H, 2 x H13), 1.80 – 1.69 (m, 4H, 2 x H2 & 2 x H9), 1.42 (s, 9H, $C(CH_3)_3$). Data match literature values.[81]

5.6.2.4 N-Boc-13-azido-4,7,10-trioxa-tridecylamine (78)[82]



Chemical Formula: $C_{15}H_{30}N_4O_5$

Mono-*N*-Boc-4,7,10-trioxa-trideca-1,13-diamine (**42**, 2.02 g, 6.25 mmol), $CuSO_4 \cdot 5H_2O$ (156 mg, 0.63 mmol) and K_2CO_3 (1.72 g, 12.5 mmol) were stirred in 3:1 MeOH/H_2O (60 mL) for 15 min at room temperature. $ImSO_2N_3 \cdot HCl$[44] (2.36 g, 11.2 mol) was added and the mixture stirred for 7 h at room temperature. The MeOH was removed by evaporation and the residue extracted into EtOAc, washed

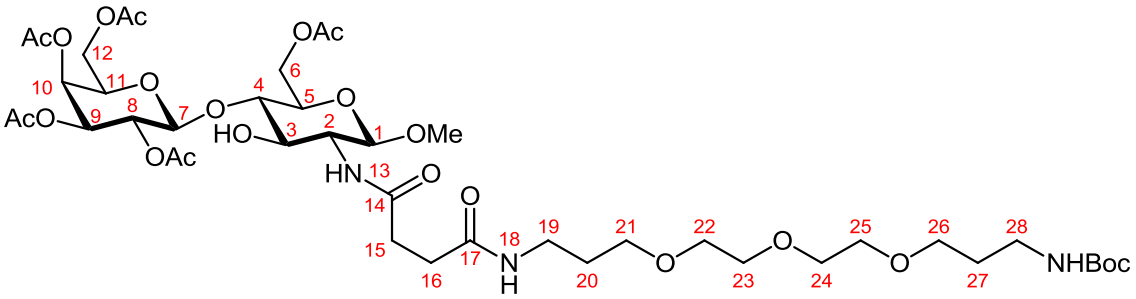
with 1 M citric acid solution and dried over $MgSO_4$. Removal of the solvent by evaporation afforded the product as a pale yellow syrup (2.09 g, 97%).

δ_H(300 MHz, $CDCl_3$)	4.98 (s, 1H, BocNH), 3.71 – 3.49 (m, 12H, 12 x H3-8), 3.38 (t, *J*=6.6, 2H, 2 x H10), 3.22 (ps q, *J*=6.1, 2H, 2 x H1), 1.85 (p, *J*=6.6, 2H, 2 x H9), 1.75 (p, *J*=6.1, 2H, 2 x H2), 1.43 (s, 9H, $C(CH_3)_3$). Data match literature values.[82]
δ_C(101 MHz, $CDCl_3$)	156.1 (COO^tBu), 79.0 ($\underline{C}(CH_3)_3$), 70.7 (CH_2O), 70.5 (CH_2O), 70.3 (CH_2O), 69.7 (CH_2O), 68.0 (CH_2O), 48.6 (C10), 38.7 (C1), 29.7 (C2), 29.2 (C9), 28.5 ($C(\underline{C}H_3)_3$). Data match literature values.[82]
R_f	0.6 (2:3 hexane/EtOAc)
v (cm^{-1})	3351 (br, NH), 2977, 2930, 2870 (CH),2096 (N_3), 1710 (C=O), 1517 (NH bend)
m/z (ES+ TOF)	369.2124 $[M+Na]^+$ ($C_{15}H_{30}N_4O_5Na$), calc. 369.2114 – 100%

5.7 Linker Attached Disaccharides

5.7.1 First Generation

5.7.1.1 Methyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-6-O-acetyl-2-deoxy-2-[N'-(13-^{t}butyloxycarbonylamino-4,7,10-trioxa-tridecyl)succinamido]-β-D-glucopyranoside (49)



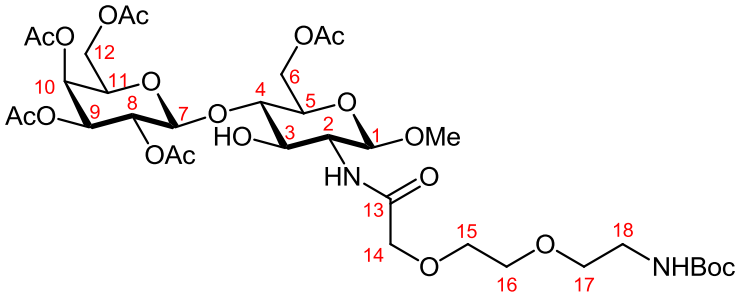
Chemical Formula: $C_{42}H_{69}N_3O_{22}$

No literature reference found

N-Boc, *N'*-succinyl-4,7,10-trioxa-trideca-1,13-diamine (**43**, 0.1 M in THF, 2.7 mL, 0.27 mmol) was diluted with MeCN (2.5 mL), DIPEA (0.1 mL, 0.6 mmol) and PyBOP (142 mg, 0.27 mmol) were added and the mixture stirred at room temperature for 30 min. Methyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-6-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside (**40**, 77 mg, 0.14 mmol) in MeCN (2.5 mL) was added and the reaction heated at reflux for 20 h. Removal of the solvents by evaporation and purification by flash column chromatography (60% Me_2CO in toluene) afforded the product as a colourless glass (71 mg, 54%).

δ_H(400 MHz, $CDCl_3$)	6.78 (d, *J*=7.7, 1H, H13), 6.66 (br s, 1H, H18), 5.36 (dd, *J*=3.4, 0.7, 1H, H10), 5.19 (dd, *J*=10.4, 8.0, 1H, H8), 5.03 (s, *J*=13.8, 2H, BocNH), 4.98 (dd, *J*=10.4, 3.4, 1H, H9), 4.58 (d, *J*=8.0, 1H, H7), 4.50 (d, *J*=8.3, 1H, H1), 4.32 (dd, *J*=11.8, 1.7, 1H, H6), 4.28 (s, 1H, OH), 4.16 – 4.05 (m, 2H, H12 & H12'), 4.05 – 3.96 (m, 2H, H6' & H11), 3.89 – 3.81 (m, 1H, H3), 3.65 – 3.47 (m, 15H, H2, H4, H5 & 12 x H21-26), 3.43 (s, 3H, OMe), 3.32 (ps q, *J*=6.0, 2H, 2 x H19), 3.19 (br ps q, *J*=6.0, 2H, 2 x H28), 2.54 – 2.44 (m, 4H, 2 x H15 & 2 x H16), 2.14 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 1.80 – 1.68 (m, 4H, 2 x H20 & 2 x H27), 1.41 (s, 9H, $C(CH_3)_3$).
δ_C(101 MHz, $CDCl_3$)	173.1 ($\underline{C}OCH_3$), 172.3 ($\underline{C}OCH_3$), 170.7 ($\underline{C}OCH_3$), 170.5 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 170.0 ($CH_2\underline{C}ONH$), 169.6 ($CH_2\underline{C}ONH$), 156.1 (COO^tBu), 101.8 (C7), 101.5 (C1), 82.5 (C4), 79.0 ($\underline{C}(CH_3)_3$), 72.5 (C3), 71.8 (C5), 71.3 (C11), 71.0 (C9), 70.6 (CH_2O), 70.2 (CH_2O), 70.2 (CH_2O), 69.5 (CH_2O), 68.9 (C8), 66.9 (C10), 62.9 (C6), 61.5 (C12), 56.8 (OMe), 56.0 (C2), 38.5 (C28), 38.2 (C19), 32.2 (C15/16), 31.9 (C16/15), 29.8 (C20/27), 28.8 (C27/20), 28.5 ($C(\underline{C}H_3)_3$), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$).
R_f	0.4 (10% MeOH in DCM)
m/z (ES+ TOF)	990.4296 $[M+Na]^+$ ($C_{42}H_{69}N_3O_{22}Na$), calc. 990.4270 – 100%

5.7.1.2 Methyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-6-O-acetyl-2-(3,6-dioxa-8-^{t}butyloxycarbonylaminooctanamido)-2-deoxy-β-D-glucopyranoside (55)



Chemical Formula: $C_{34}H_{54}N_2O_{20}$

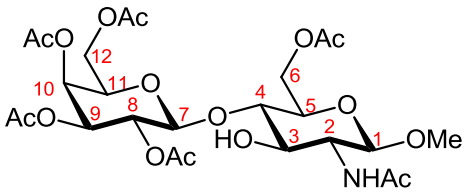
No literature reference found

3,6-Dioxa-8-^{t}butyloxycarbonylaminooctanoic Acid (**47**, 270 mg, 1.03 mmol), DCC (212 mg, 1.03 mmol) and NHS (118 mg, 1.03 mmol) were dissolved in THF (10 mL), stood overnight at 4 °C and filtered. The filtrate was added to methyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-6-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside (**40**, 130 mg, 0.23 mmol) in MeCN (10 mL) and the mixture stirred at 60 °C for 18 h. Removal of the solvents by evaporation and purification by flash column chromatography (100% EtOAc) afforded the product as a off-white foam (77 mg, 41%).

δ_H(400 MHz, $CDCl_3$) 7.01 (d, *J*=6.0, 1H, CH_2CONH), 5.36 (d, *J*=3.4, 1H, H10), 5.19 (dd, *J*=10.4, 8.3, 1H, H8), 5.08 (s, 1H, BocNH), 4.98 (dd, *J*=10.4, 3.4, 1H, H9), 4.70 – 4.61 (m, 1H, H1), 4.56 (d, *J*=8.3, 1H, H7), 4.32 (dd, *J*=11.8, 1.7, 1H, H6), 4.24 (s, 1H, OH), 4.16 – 4.07 (m, 2H, H12 & H12'), 4.07 – 3.98 (m, 3H, H3, H6' & H11), 3.96 (s, 2H, 2 x H14), 3.69 – 3.63 (m, 2H, 2 x H15), 3.63 – 3.57 (m, 3H, H5 & 2x H16), 3.56 – 3.47 (m, 4H, H2, H4 & 2 x H17), 3.46 (s, 3H, OMe), 3.34 – 3.25 (m, 2H, 2 x H18), 2.13 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.42 (s, 9H, $C(CH_3)_3$)

δ_C(101 MHz, $CDCl_3$)	170.7 ($\underline{C}OCH_3$), 170.4 ($\underline{C}OCH_3$), 170.4 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 170.0 ($\underline{C}OCH_3$), 169.5 (C13), 156.0 ($\underline{C}OO^tBu$), 101.9 (C7), 101.0 (C1), 83.0 (C4), 79.5 ($\underline{C}(CH_3)_3$), 72.0 (C11), 71.8 (C5), 71.4 (C3), 71.0 (C9), 70.9 (C15), 70.5 (C14), 70.5 (C17), 70.1 (C16), 68.9 (C10), 66.9 (C8), 62.8 (C6), 61.6 (C12), 56.8 (OMe), 56.1 (C2), 40.5 (C18), 28.5 ($C(\underline{C}H_3)_3$), 20.9 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$)
R_f	0.3 (100% EtOAc)

5.7.1.3 Methyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-6-O-acetyl-2-deoxy-β-D-glucopyranoside (52)



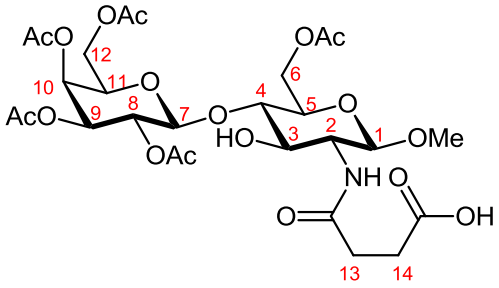
Chemical Formula: $C_{25}H_{37}NO_{16}$

No literature reference found

Methyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-6-*O*-acetyl-2-deoxy-2-tetrachloro-phthalimido-β-D-glucopyranoside (**33**, 400 mg, 0.48 mmol) was converted to the amine using the method in 5.5.1.3. The crude amine was dissolved in DCM (20 mL), pyridine (5 mL) and Ac_2O (45 µL, 1.0 mmol) were added and the mixture stirred at room temperature for 18 h. The mixture was washed with water, sat. $NaHCO_3$, dried over $MgSO_4$ and the solvents removed by evaporation. Purification by trituration with Et_2O afforded the product as a pale yellow solid (231 mg, 79%).

δ_H(400 MHz, $CDCl_3$)	5.59 (d, *J*=7.7, 1H, NH), 5.38 (d, *J*=3.4, 1H, H10), 5.22 (dd, *J*=10.5, 8.0, 1H, H8), 5.00 (dd, *J*=10.5, 3.4, 1H, H9), 4.68 (d, *J*=8.2, 1H, H1), 4.57 (d, *J*=8.0, 1H, H7), 4.34 (dd, *J*=11.8, 1.9, 1H, H6), 4.30 (s, 1H, OH), 4.19 – 3.97 (m, 5H, H3, H6', H11, H12 & H12'), 3.65 – 3.58 (m, 1H, H5), 3.53 – 3.42 (s, 5H, H2, H4 & OMe), 2.16 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$)
δ_C(101 MHz, $CDCl_3$)	170.8 ($\underline{C}OCH_3$), 170.3 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 170.0 ($\underline{C}OCH_3$), 169.8 ($\underline{C}OCH_3$), 169.6 ($\underline{C}OCH_3$), 101.9 (C7), 101.1 (C1), 82.8 (C4), 71.9 (C5 & C11), 71.5 (C3), 71.0 (C9), 68.9 (C8), 66.9 (C10), 62.9 (C6), 61.7 (C12), 56.9 (OMe), 56.8 (C2), 21.0 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$)
R_f	0.4 (60% Me_2CO in toluene)
m.p.	193-194 °C
$[\alpha]_D$	+21.1° (c=1, $CHCl_3$)
v (cm^{-1})	3482 (br, OH), 3267 (br, NH), 3096, 2939 (CH), 1743, 1652 (C=O), 1555 (NH bend)
m/z (ES+ TOF)	608.2202 $[M+H]^+$ ($C_{25}H_{38}NO_{16}$), calc. 608.2191 – 100% 630.2 $[M+Na]^+$ ($C_{25}H_{37}NO_{16}Na$) – 50%

5.7.1.4 *Methyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-6-O-acetyl-2-amino-2-deoxy-β-D-glucopyranoside-N-succinate (53)*



Chemical Formula: $C_{27}H_{39}NO_{18}$

No literature reference found

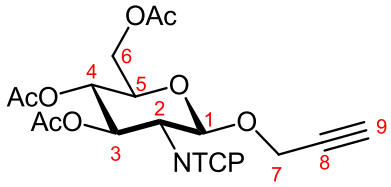
Methyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-6-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside (**40**, 77 mg, 0.14 mmol), was dissolved in 1:1 DCM/pyridine (4 mL), succinic anhydride (15 mg, 1.5 mmol) was added and the reaction stirred at room temperature for 18 h. Removal of the solvents by evaporation and purification by flash column chromatography (60% Me_2CO, 1% AcOH in toluene) afforded the product as a colourless glass (56 mg, 61%).

δ_H(400 MHz, $CDCl_3$) 6.32 (d, *J*=8.0, 1H, AcNH), 5.40 (d, *J*=3.4, 1H, H10), 5.20 (dd, *J*=10.5, 8.1, 1H, H8), 5.02 (dd, *J*=10.5, 3.4, 1H, H9), 4.63 (d, *J*=8.3, 1H, H1), 4.61 (d, *J*=8.1, 1H, H7), 4.33 (dd, *J*=11.8, 1.7, 1H, H6), 4.22 (dd, *J*=11.3, 7.7, 1H, H12), 4.12 – 4.00 (m, 3H, H6', H11 & H12'), 3.97 (dd, *J*=10.0, 8.1, 1H, H3), 3.63 – 3.57 (m, 1H, H5), 3.54 – 3.44 (m, 6H, H2, H4, OH & OMe), 2.68 (t, *J*=6.4, 2H, 2 x H14), 2.52 (t, *J*=6.4, 2H, 2 x H13), 2.16 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$)

δ_C(101 MHz, $CDCl_3$)	175.5 ($\underline{C}OCH_3$), 173.1 ($\underline{C}OCH_3$), 171.1 ($\underline{C}OCH_3$), 170.8 ($\underline{C}OCH_3$), 170.2 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 169.7 ($\underline{C}OCH_3$), 101.8 (C7), 101.1 (C1), 82.3 (C4), 71.9 (C3 &C5), 71.4 (C11), 71.0 (C9), 68.9 (C8), 67.0 (C10), 62.9 (C6), 61.6 (C12), 56.9 (OMe), 56.7 (C2), 31.1 (C14), 29.8 (C13), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$)
m/z (ES+ TOF)	688.2085 $[M+Na]^+$ ($C_{27}H_{39}NO_{18}Na$), calc. 688.2065 – 100%

5.7.2 Model Reactions

5.7.2.1 Propargyl 3,4,6-tri-O-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (81)



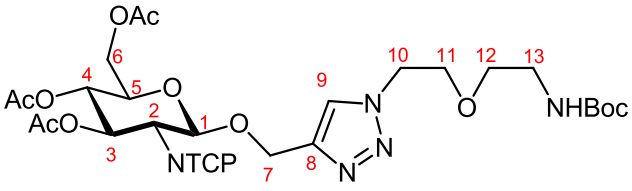
Chemical Formula: $C_{23}H_{19}Cl_4NO_{10}$

No literature reference found

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranose (**27**, 4.00 g, 6.53 mmol) was dissolved in 32% HBr in AcOH (11 mL) with Ac_2O (2.0 mL, 21 mmol) and stirred, in darkness, overnight at room temperature. 100 mL of DCM was added and the mixture poured over ice. Washing with water then sat. $NaHCO_3$, drying over $MgSO_4$ and removal of the solvent afforded the crude bromide as a cream solid, which was immediately dissolved in DCM (50 mL), propargyl alcohol (6.0 mL, 100 mmol) and Ag_2CO_3 (1.82 g, 6.53 mmol) were added and the mixture refluxed in darkness for 3 days. Filtering through Celite and removal of the solvent produced an orange solid which was purified by stepped gradient column chromatography (15% → 20% → 25% EtOAc in hexane) to give the product as an off-white solid (2.43 g, 61%).

δ_H(400 MHz, $CDCl_3$)	5.78 (dd, *J*=10.5, 9.0, 1H, H3), 5.58 (d, *J*=8.5, 1H, H1), 5.20 (dd, *J*=10.1, 9.1, 1H, H4), 4.38 – 4.28 (m, 4H, H2, H6, H7 & H7'), 4.17 (dd, *J*=12.4, 2.3, 1H, H6'), 3.87 (ddd, *J*=10.2, 4.5, 2.3, 1H, H5), 2.36 (t, *J*=2.4, 1H, H9), 2.12 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.91 (s, 3H, $COCH_3$)
δ_C(101 MHz, $CDCl_3$)	170.8 ($\underline{C}OCH_3$), 170.6 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCH_3$), 140.7 ($\underline{C}ON\underline{C}O$), 135.9 ($Ar_q$), 130.1 ($Ar_q$), 127.1 ($Ar_q$), 95.6 (C1), 78.1 (C9), 75.9 (C8), 72.1 (C5), 70.8 (C3), 68.6 (C4), 61.8 (C6), 56.3 (C7), 55.2 (C2), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$)
R_f	0.7 (2:3 hexane/EtOAc)
m.p.	88-90 °C
$[\alpha]_D$	+25.3 (c=0.6, $CHCl_3$)
v (cm^{-1})	3261, 2950, 2898 (CH), 2122 (C≡C), 1724 (C=O)
m/z (ES+ TOF)	631.9673 $[M+Na]^+$ ($C_{23}H_{19}{}^{35}Cl_4NO_{10}Na$), calc. 631.9661 – 80% 634.0 $[M+Na]^+$ ($C_{23}H_{19}{}^{35}Cl_3{}^{37}ClNO_{10}Na$) – 100% 636.0 $[M+Na]^+$ ($C_{23}H_{19}{}^{35}Cl_2{}^{37}Cl_2NO_{10}Na$) – 60%

5.7.2.2 1-(3-Oxa-5-^{t}butyloxycarbonylaminopent-1-yl)-1,2,3-triazol-4-ylmethyl 3,4,6-tri-O-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (82)



Chemical Formula: $C_{32}H_{37}Cl_4N_5O_{13}$

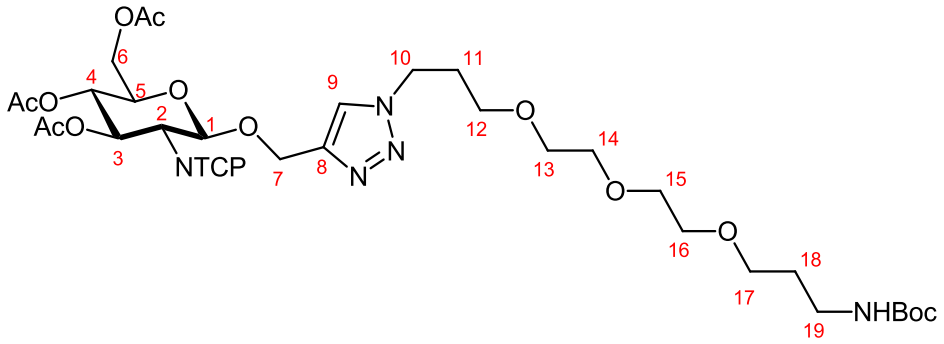
No literature reference found

Propargyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (**81**, 200 mg, 0.33 mmol) and *N*-Boc-2-(2-azidoethoxy)ethylamine (**79**, 113 mg, 0.49 mmol) were dissolved in Me_2CO (5 mL), sodium ascorbate (26 mg, 0.13 mmol) and $CuSO_4$ (0.1 M in H_2O, 163 µL, 16.3 µmol) were added and the mixture stirred at 50 °C for 26 h. The mixture was cooled, extracted into DCM, washed with brine and dried over $MgSO_4$. Removal of the solvents gave a pale yellow syrup that was purified by flash column chromatography (3:1 Hexane/EtOAc) to afford the product triazole as a colourless syrup (206 mg, 75%).

δ_H(400 MHz, $CDCl_3$)	7.56 (s, *J*=10.5, 1H, H9), 5.70 (dd, *J*=10.5, 9.1, 1H, H3), 5.41 (d, *J*=8.5, 1H, H1, H1), 5.19 (dd, *J*=10.0, 9.2, 1H, H4), 4.90 (d, *J*=12.6, 1H, H7), 4.78 (br s, 1H, BocNH), 4.75 (d, *J*=12.6, 1H, H7'), 4.47 (t, *J*=6.1, 1H, H10), 4.46 (t, *J*=6.1, 1H, H10'), 4.35 (dd, *J*=12.1, 4.7, 1H, H6), 4.32 (dd, *J*=10.4, 8.4, 1H, H2), 4.19 (dd, *J*=12.3, 2.3, 1H, H6'), 3.91 – 3.83 (m, 1H, H5), 3.80 (t, *J*=6.3, 1H, H11), 3.79 (t, *J*=6.3, 1H, H11'), 3.48 (t, *J*=5.1, 2H, 2 x H12), 3.28 (br ps q, *J*=5.1, 2H, 2 x H13), 2.13 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.89 (s, 3H, $COCH_3$), 1.44 (s, 9H, $C(CH_3)_3$)
δ_C(101 MHz, $CDCl_3$)	170.8 ($\underline{C}OCH_3$), 170.6 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCH_3$), 161.7 ($\underline{C}ON\underline{C}O$), 140.5 ($\underline{C}OO^tBu$), 130.0 (C8), 129.1 ($Ar_q$), 128.3 ($Ar_q$), 125.4 ($Ar_q$), 124.1 (C9), 96.9 (C1), 73.9 ($\underline{C}(CH_3)_3$), 72.0 (C5), 70.8 (C3), 70.5 (C12), 69.1 (C11), 68.7 (C4), 62.8 (C7), 61.9 (C6), 55.4 (C2), 50.4 (C10), 40.3 (C13), 28.5 ($C(\underline{C}H_3)_3$), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$)
R_f	0.6 (2:3 hexane/EtOAc)
$[\alpha]_D$	+25.5° (c=1, $CHCl_3$)

v (cm^{-1})	3413 (NH), 3152, 2961, 2904, 2853 (CH), 1747, 1723 (C=O), 1512 (NH bend)
m/z (ES+ TOF)	840.1187 $[M+H]^+$ ($C_{32}H_{38}{}^{35}Cl_4N_5O_{13}$), calc. 840.1220 – 80% 842.2 $[M+H]^+$ ($C_{32}H_{38}{}^{35}Cl_3{}^{37}ClN_5O_{13}$) – 100% 844.2 $[M+H]^+$ ($C_{32}H_{38}{}^{35}Cl_2{}^{37}Cl_2N_5O_{13}$)– 60%

5.7.2.3 1-(4,7,10-Trioxa-13-^{t}butyloxycarbonylaminotridec-1-yl)-1,2,3-triazol-4-ylmethyl 3,4,6-tri-O-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (83)



Chemical Formula: $C_{38}H_{49}Cl_4N_5O_{15}$

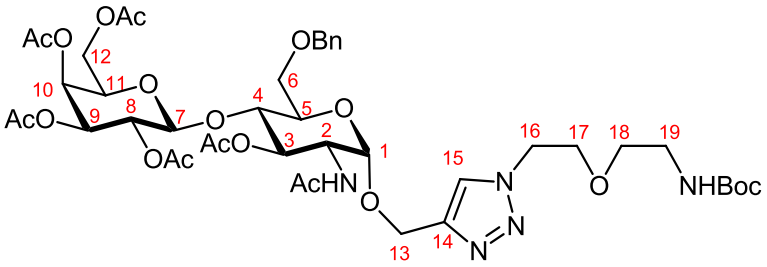
No literature reference found

Propargyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranoside (**81**, 200 mg, 0.33 mmol) and *N*-Boc-13-azido-4,7,10-trioxa-tridecylamine (**78**, 170 mg, 0.49 mmol) were dissolved in Me_2CO (5mL), sodium ascorbate (26 mg, 0.13 mmol) and $CuSO_4$ (0.1 M in H_2O, 163 µL, 16.3 µmol) were added and the mixture stirred at 50 °C for 26 h. The mixture was cooled, extracted into DCM, washed with brine and dried over $MgSO_4$. Removal of the solvents gave a pale yellow syrup that was purified by stepped gradient column chromatography (10% → 20% → 30% → 40% → 50% Me_2CO in toluene) to afford the product triazole as a colourless syrup (208 mg, 67%).

δ_H(400 MHz, $CDCl_3$)	7.52 (s, 1H, H9), 5.69 (dd, *J*=10.5, 9.0, 1H, H3), 5.41 (d, *J*=8.5, 1H, H1), 5.18 (dd, *J*=10.1, 9.1, 1H, H4), 4.96 (br s, 1H, BocNH), 4.87 (d, *J*=12.6, 1H, H7), 4.73 (d, *J*=12.6, 1H, H7'), 4.40 (t, *J*=6.9, 2H, 2 x H10), 4.34 (dd, *J*=12.2, 4.3, 1H, H6), 4.29 (dd, *J*=10.5, 8.4, 1H, H2), 4.17 (dd, *J*=12.2, 2.3, 1H, H6'), 3.85 (ddd, *J*=6.9, 4.3, 2.2, 1H, H5), 3.66 – 3.49 (m, 10H, 10 x H13-17), 3.40 (t, *J*=6.2, 2H, 2 x H12), 3.24 – 3.15 (m, 4H, 2 x H19), 2.14 – 2.08 (m, 5H, 2 x H11 & $COCH_3$), 2.02 (s, 3H, $COCH_3$), 1.87 (s, 3H, $COCH_3$), 1.78 – 1.69 (m, 2H, 2 x H18), 1.42 (s, 9H, $C(CH_3)_3$)
δ_C(101 MHz, $CDCl_3$)	170.8 ($\underline{C}OCH_3$), 170.6 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCH_3$), 156.1 ($\underline{C}ON\underline{C}O$), 143.2 (C8), 140.4 ($\underline{C}OO^tBu$), 130.0 ($Ar_q$), 127.2 ($Ar_q$), 127.0 ($Ar_q$), 123.7 (C9), 96.9 (C1), 79.0 ($\underline{C}(CH_3)_3$), 72.0 (C5), 70.7 (C3), 70.6 (OCH_2), 70.6 (OCH_2), 70.5 (OCH_2), 70.4 (OCH_2), 70.3 (OCH_2), 68.7 (C4), 67.1 (C12), 62.7 (C7), 61.9 (C6), 55.3 (C2), 47.2 (C10), 38.6 (C19), 30.2 (C11), 29.7 (C18), 28.5 ($\underline{C}(CH_3)_3$), 20.9 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$)
R_f	0.1 (2:3 hexane/EtOAc)
$[\alpha]_d$	+23.9° (c=1, $CHCl_3$)
v (cm^{-1})	3880 (NH), 3140, 2980, 2935, 2874 (CH), 1749, 1723 (C=O), 1515 (NH bend)
m/z (ES+ TOF)	956.2030 $[M+H]^+$ ($C_{38}H_{50}{}^{35}Cl_4N_5O_{15}$), calc. 956.2058 – 80% 958.2 $[M+H]^+$ ($C_{38}H_{50}{}^{35}Cl_3{}^{37}ClN_5O_{15}$)– 100% 960.2 $[M+H]^+$ ($C_{38}H_{50}{}^{35}Cl_2{}^{37}Cl_2N_5O_{15}$)– 60%

5.7.3 Second Generation

5.7.3.1 1-(3-Oxa-5-^{t}butyloxycarbonylaminopent-1-yl)-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (84)



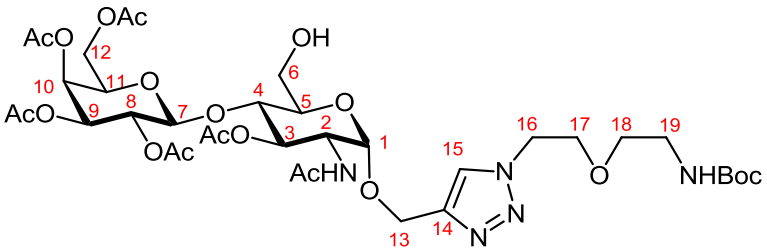
Chemical Formula: $C_{43}H_{61}N_5O_{19}$

No literature reference found

Propargyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**77**, 344 mg, 0.48 mmol) and *N*-Boc-2-(2-azidoethoxy)ethylamine (**79**, 165 mg, 0.72 mmol) were dissolved in Me_2CO (10 mL), sodium ascorbate (38 mg, 0.19 mmol) and $CuSO_4$ (0.1 M in H_2O, 238 µL, 23.8 µmol) were added and the mixture stirred at 50 °C for 18 h. The mixture was cooled, extracted into DCM, washed with brine and dried over $MgSO_4$. Removal of the solvents gave a beige solid that was purified by flash column chromatography (50% Me_2CO, 2% NEt_3 in toluene) to afford the product triazole as a white solid (305 mg, 67%).

δ_H(400 MHz, $CDCl_3$)	7.65 (s, 1H, H15), 7.44 – 7.29 (m, 5H, ArH), 5.94 (d, *J*=9.0, 1H, AcNH), 5.25 (d, *J*=3.0, 1H, H10), 5.12 (ps t, *J*=9.8, 1H, H3), 4.96 (dd, *J*=10.6, 7.9, 1H, H8), 4.94 (br s, 1H, BocNH), 4.92 (d, *J*=3.1, 1H, H1), 4.86 – 4.72 (m, 3H, H9, H13 & PhC<u>H</u>H), 4.60 (d, *J*=12.3, 1H, H13'), 4.52 (br t, *J*=4.9, 2H, 2 x H16), 4.44 (d, *J*=12.1, 1H, PhCH<u>H</u>), 4.34 (d, *J*=7.9, 1H, H7), 4.30 – 4.19 (m, 1H, H2), 4.04 (d, *J*=6.8, 2H, H12 & H12'), 3.95 (ps t, *J*=9.8, 1H, H4), 3.83 (t, *J*=4.9, 2H, 2 x H17), 3.81 – 3.72 (m, 2H, H5 & H6), 3.68 – 3.60 (m, 2H, H6' & H11), 3.50 (t, *J*=5.2, 2H, 2 x H18), 3.33 – 3.24 (m, 2H, 2 x H19), 2.10 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.94 (s, 3H, $COCH_3$), 1.93 (s, 3H, $COCH_3$), 1.90 (s, 3H, $COCH_3$), 1.42 (s, 9H, $C(CH_3)_3$)
δ_C(101 MHz, $CDCl_3$)	171.3 ($\underline{C}OCH_3$), 170.5 ($\underline{C}OCH_3$), 170.3 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 169.1 ($\underline{C}OCH_3$), 156.1 ($\underline{C}OCH_3$), 143.5 (C14), 137.8 ($\underline{C}OO^tBu$), 128.8 (Ar), 128.3 (Ar), 128.3 (Ar), 124.0 (C14), 100.5 (C7), 96.8 (C1), 79.6 ($\underline{C}(CH_3)_3$), 74.9 (C4), 73.8 (Ph$\underline{C}H_2$), 71.5 (C3), 71.1 (C9), 70.7 (C5), 70.5 (C11), 70.4 (C18), 69.3 (C8), 69.1 (C17), 67.3 (C10), 66.9 (C6), 61.1 (C13), 61.0 (C12), 52.0 (C2), 50.4 (C16), 40.3 (C19), 28.5 ($C(\underline{C}H_3)_3$), 23.2 ($CO\underline{C}H_3$), 21.0 ($CO\underline{C}H_3$), 20.9 ($CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$) Ar_q not seen.
R_f	0.3 (50% Me_2CO, 2% NEt_3 in toluene)
m.p.	63-65 °C
$[\alpha]_D$	+27.2° (c=1.5, $CHCl_3$)
v (cm^{-1})	3350 (br, NH), 2942, 2937, 2873 (CH), 1745, 1708, 1673, 1522 (C=O)
m/z (ES+ TOF)	952.4019 $[M+H]^+$ ($C_{43}H_{62}N_5O_{19}$), calc. 952.4039 – 100%

5.7.3.2 1-(3-Oxa-5-^{t}butyloxycarbonylaminopent-1-yl)-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-3-O-acetyl-2-deoxy-α-D-glucopyranoside (87)



Chemical Formula: $C_{36}H_{55}N_5O_{19}$

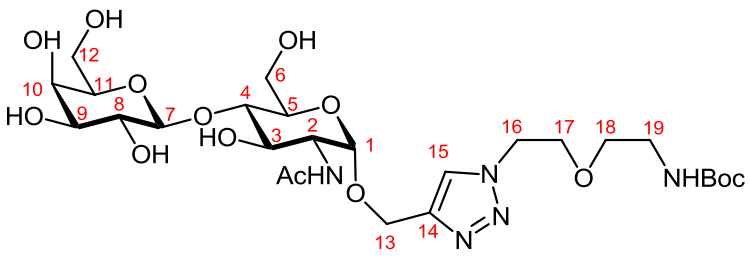
No literature reference found

1-(3-Oxa-5-^{t}butyloxycarbonylaminopent-1-yl)-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**84**, 50 mg, 0.05 mmol) was dissolved in MeOH (10 mL), Pd/C (10%, ca. 10 mg) was added and the mixture stirred under hydrogen at 1 bar for 16 h. Filtration through Celite and removal of the solvents afforded the product alcohol as a white solid (45 mg, 98%).

δ_H(400 MHz, $CDCl_3$) 7.69 (s, 1H, H15), 5.98 (d, *J*=8.4, 1H, AcNH), 5.32 (d, *J*=3.3, 1H, H10), 5.20 (ps t, *J*=10.0, 1H, H3), 5.09 (dd, *J*=10.4, 7.9, 1H, H8), 4.98 (dd, *J*=10.4, 3.3, 1H, H9), 4.89 (br s, 1H, BocNH), 4.89 (d, *J*=3.3, 1H, H1), 4.78 (d, *J*=13.3, 1H, H13), 4.65 – 4.60 (m, 2H, H7 & H13'), 4.57 – 4.50 (m, 2H, 2 x H16), 4.29 – 4.15 (m, 1H, H2), 4.07 (dd, *J*=6.6, 5.1, 2H, H12 & H12'), 3.95 – 3.87 (m, 2H, H4 & H11), 3.83 (t, *J*=4.8, 2H, 2 x H17), 3.80 – 3.76 (m, 1H, H6) 3.75 – 3.67 (m, 2H, H5 & H6'), 3.51 (t, *J*=5.2, 2H, 2 x H18), 3.30 (br s, 2H, 2 x H19), 2.12 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.03 (s, 6H, 2 x $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.90 (s, 3H, $COCH_3$), 1.42 (s, 9H, $C(CH_3)_3$)

δ_C(101 MHz, $CDCl_3$)	171.1 ($\underline{C}OCH_3$), 170.5 ($\underline{C}OCH_3$), 170.3 ($\underline{C}OCH_3$), 170.2 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCH_3$), 156.1 ($\underline{C}OCH_3$), 143.4 (C14), 124.2 (C15), 101.2 (C1), 96.7 (C7), 79.6 ($\underline{C}(CH_3)_3$), 75.4 (C4/11), 71.8 (C3), 71.2 (C5), 71.1 (C9), 70.6 (C11/4), 70.4 (C18), 69.4 (C8), 69.1 (C17), 66.9 (C10), 70.0 (C12), 70.0 (C13), 60.5 (C6), 52.2 (C2), 50.4 (C16), 40.3 (C19), 28.5 ($C(\underline{C}H_3)_3$), 23.2 ($CO\underline{C}H_3$), 21.0 ($CO\underline{C}H_3$), 20.9 ($CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$) $\underline{C}OO^tBu$ not seen.
R_f	0.3 (50% Me_2CO in toluene)
m.p.	84-87 °C
$[\alpha]_D$	-4.6° (c=1, $CHCl_3$)
v (cm^{-1})	3361 (br, OH), 2958, 2935, 2901 (CH), 1744, 1642, 1723, 1522 (C=O)
m/z (ES+ TOF)	862.3561 $[M+H]^+$ ($C_{36}H_{56}N_5O_{19}$), calc. 862.3569 – 100%

5.7.3.3 1-(3-Oxa-5-^{t}butyloxycarbonylaminopent-1-yl)-1,2,3-triazol-4-ylmethyl β(1-4)-D-galactopyranosyl-2-acetamido-2-deoxy-α-D-glucopyranoside (89)



Chemical Formula: $C_{26}H_{45}N_5O_{14}$

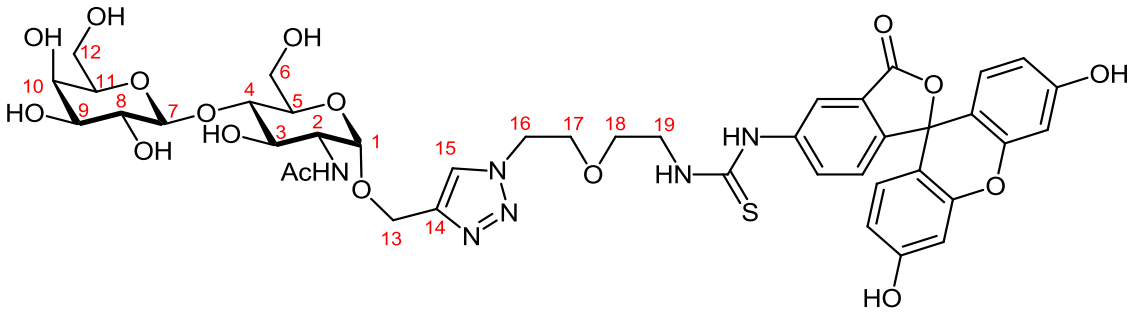
No literature reference found

1-(3-Oxa-5-^{t}butyloxycarbonylaminopent-1-yl)-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**87**, 46 mg, 0.05 mmol) was

dissolved in MeOH (4mL), Na (ca. 5mg) was added and the reaction stirred at room temperature for 4h, when TLC (50% Me_2CO in toluene) showed only a baseline spot. The mixture was neutralised by addition of DOWEX and filtered. Removal of the solvents by evaporation afforded the product as a colourless film (29 mg, 84%).

δ_H(500 MHz, MeOD)	8.04 (s, 1H, H15), 4.90 (d, *J*=3.6, 1H, H1), 4.79 (d, *J*=12.5, 1H, H13), 4.63 (d, *J*=12.5, 1H, H13'), 4.59 (t, *J*=5.0, 2H, H16), 4.37 (d, *J*=7.5, 1H, H7), 3.97 (dd, *J*=10.8, 3.6, 1H, H2), 3.89 – 3.83 (m, 4H, H6, H6' & 2 X H17), 3.81 (br s, 1H, H10), 3.81 (dd, *J*=10.8, 9.5, 1H, H3), 3.75 (dd, *J*=11.5, 7.4, 1H, H12), 3.74 (dd, *J*=7.1, 3.0, 1H, H5), 3.68 (dd, *J*=11.5, 4.6, 1H, H12'), 3.63 (dd, *J*=9.5, 8.6, 1H, H4), 3.58 (dd, *J*=7.4, 4.6, 1H, H11), 3.53 (dd, *J*=9.7, 7.5, 1H, H8), 3.49 (dd, *J*=9.7, 3.3, 1H, H9), 3.48 (t, *J*=5.7, 2H, H18), 3.19 (t, *J*=5.7, 2H, H19), 1.95 (s, 3H, $COCH_3$), 1.43 (s, 9H, $C(CH_3)_3$)
δ_C(126 MHz, MeOD)	173.5 ($\underline{C}OCH_3$), 158.4 ($\underline{C}OO^tBu$), 145.0 (C14), 126.0 (C15), 105.1 (C7), 97.9 (C1), 81.2 (C4), 80.1 ($\underline{C}(CH_3)_3$), 77.1 (C11), 74.8 (C9), 72.6 (C8), 72.5 (C5), 71.0 (C18), 71.0 (C3), 70.3 (C10), 70.0 (C19), 62.4 (C12), 61.8 (C6), 61.4 (C13), 54.8 (C2), 51.5 (C16), 41.1 (C19), 28.7 ($C(\underline{C}H_3)_3$), 22.5 ($CO\underline{C}H_3$)
t_R	24.1 min
m.p.	111-114 °C
$[\alpha]_D$	+42.5° (c=0.7, MeOH)
v (cm^{-1})	3345 (br, OH), 2922 (CH), 1636, 1540 (C=O)
m/z (ES+ TOF)	652.3025 $[M+H]^+$ ($C_{26}H_{46}N_5O_{14}$), calc.652.3041 – 100%

5.7.3.4 1-(3-Oxa-5-(fluorescein-5-thioureyl)pent-1-yl)-1,2,3-triazol-4-ylmethyl β(1-4)-D-galactopyranosyl-2-acetamido-2-deoxy-α-D-glucopyranoside (91)



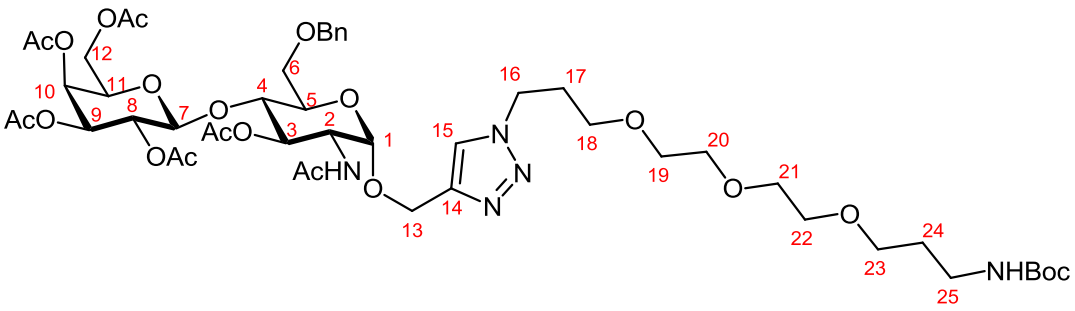
Chemical Formula: $C_{42}H_{48}N_6O_{17}S$

No literature reference found

1-(3-Oxa-5-^{t}butyloxycarbonylaminopent-1-yl)-1,2,3-triazol-4-ylmethyl β(1-4)-D-galactopyranosyl-2-acetamido-2-deoxy-α-D-glucopyranoside (**89**, 2 mg, 3 µmol) was dissolved in 1:1 TFA/DCM (2 mL) and stirred for 4 h when HPLC showed complete consumption of the starting material (intermediate amine t_R = 11.1 min). The solvents were removed and the residue dissolved in 1:1:1 DCM/DMF/pyridine (0.5 mL), FITC (3 mg, 6 µmol) was added and the mixture stirred at room temperature for 1 week. Removal of the solvents and purification by HPLC and lyophilisation afforded the product as a yellow/orange solid (2 mg, 77%).

t_R	28.1 min
m/z (ES+ TOF)	552.3 $C_{21}H_{38}N_5O_{12}$ – 100%
	348.1 $C_{20}H_{14}NO_5$ – 85%
Fluorescence	Absorption: 446 nm
	Emission: 518 nm

5.7.3.5 1-(4,7,10-Trioxa-13-^{t}butyloxycarbonylaminotridec-1-yl)-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (85)



Chemical Formula: $C_{49}H_{73}N_5O_{21}$

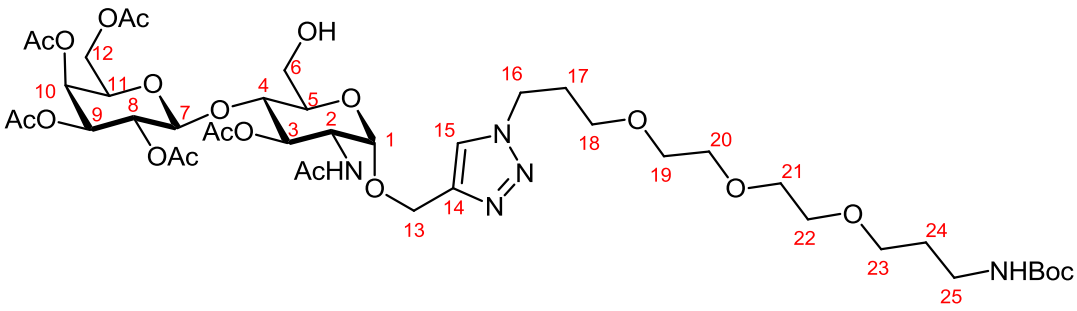
No literature reference found

Propargyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**77**, 184 mg, 0.26 mmol) and *N*-Boc-13-azido-4,7,10-trioxa-tridecylamine (**78**, 133 mg, 0.38 mmol) were dissolved in Me_2CO (7 mL), sodium ascorbate (20 mg, 0.10 mmol) and $CuSO_4$ (0.1 M in H_2O, 128 µL, 12.8 µmol) were added and the mixture stirred at 50 °C for 20 h. The mixture was cooled, extracted into DCM, washed with brine and dried over $MgSO_4$. Removal of the solvents gave a light brown syrup that was purified by stepped gradient column chromatography (30% → 40% → 50% Me_2CO, 2% NEt_3 in toluene) to afford the product triazole as a colourless film (130 mg, 48%).

δ_H(400 MHz, $CDCl_3$)	7.56 (s, 1H, H15), 7.40 – 7.25 (m, 5H, ArH), 5.92 (d, *J*=9.5, 1H, AcNH), 5.20 (d, *J*=2.8, 1H, H10), 5.06 (dd, *J*=10.8, 9.5, 1H, H3), 4.97 (br s, 1H, BocNH), 4.91 (dd, *J*=10.4, 8.0, 1H, H8), 4.86 (d, *J*=3.6, 1H, H1), 4.78 – 4.67 (m, 3H, H9, H13 & PhC<u>H</u>H), 4.53 (d, *J*=12.3, 1H, H13'), 4.46 – 4.35 (m, 3H, 2 x H16 & PhCH<u>H</u>), 4.28 (d, *J*=8.0, 1H, H7), 4.21 (ddd, *J*=10.8, 9.5, 3.6, 1H, H2), 3.99 (d, *J*=6.8, 2H, H12), 3.90 (ps t, *J*=9.5, 1H, H4), 3.77 – 3.71 (m, 1H, H5), 3.62 – 3.50 (m, 11H, H6, H6', H11 & 8 x H19-22), 3.46 (t, *J*=6.0, 2H, 2 x H23), 3.39 (t, *J*=5.7, 2H, 2 x H18), 3.14 (br ps q, *J*=6.0, 2H, H25), 2.17 – 2.08 (m, 2H, 2 x H17), 2.05 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.89 (s, 3H, $COCH_3$), 1.88 (s, 3H, $COCH_3$), 1.84 (s, 3H, $COCH_3$), 1.67 (p, *J*=6.0, 2H, 2 x H24), 1.36 (s, 9H, $C(CH_3)_3$)
δ_C(101 MHz, $CDCl_3$)	171.1 ($\underline{C}OCH_3$), 170.3 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 170.1 ($\underline{C}OCH_3$), 168.9 ($\underline{C}OCH_3$), 156.0 ($\underline{C}OO^tBu$), 143.1 (C14), 128.6 (Ar), 128.1 (Ar), 128.1 (Ar), 123.5 (C15), 100.4 (C7), 96.8 (C1), 78.8 ($\underline{C}(CH_3)_3$), 74.8 (C4), 73.7 ($PhCH_2$), 71.4 (C3), 71.0 (C9), 70.5 (OCH_2), 70.5 (C5), 70.5 (OCH_2), 70.3 (C11), 70.3 (OCH_2),70.2 (OCH_2), 69.1 (C8), 67.2 (OCH_2), 67.0 (OCH_2), 66.7 (C10), 61.0 (C13), 60.9 (C12), 51.8 (C2), 47.2 (C16), 38.5 (C25), 30.2 (C17), 29.6 (C24), 28.4 ($C(\underline{C}H_3)_3$), 23.1 ($CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$), 20.5 ($CO\underline{C}H_3$) Ar_q not seen.
R_f	0.3 (50% Me_2CO, 2% NEt_3 in toluene)
$[\alpha]_D$	-9.4° (c=2, $CHCl_3$)
ν (cm^{-1})	3356 (br, NH), 2981, 2938, 2873 (CH), 1745, 1707, 1675, 1522 (C=O)

m/z (ES+ TOF) 1090.4653 $[M+Na]^+$ ($C_{49}H_{73}N_5O_{21}Na$), calc. 1090.4696 – 100%

5.7.3.6 1-(4,7,10-Trioxa-13-^{t}butyloxycarbonylaminotridec-1-yl)-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-O-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-3-O-acetyl-2-deoxy-α-D-glucopyranoside (86)



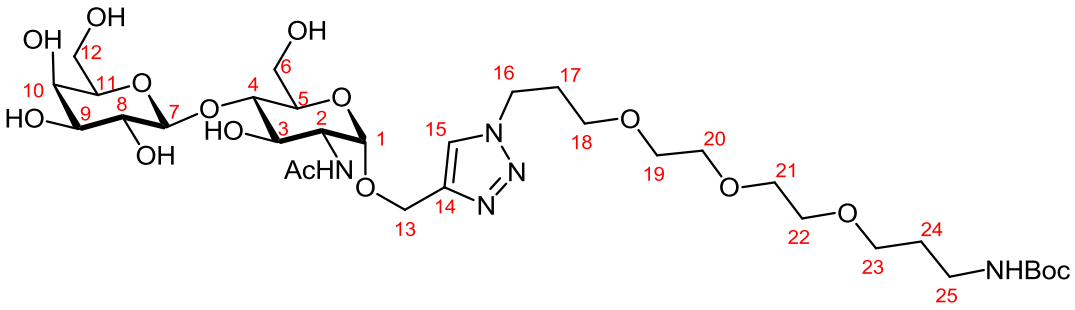
Chemical Formula: $C_{42}H_{67}N_5O_{21}$

No literature reference found

1-(4,7,10-Trioxa-13-^{t}butyloxycarbonylaminotridec-1-yl)-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**85**, 86 mg, 0.08 mmol) was dissolved in MeOH (10 mL), Pd/C (10%, ca. 10 mg) was added and the mixture stirred under hydrogen at 1 bar for 16 h. Filtration through Celite and removal of the solvents afforded the product alcohol as a white foam which slowly collapsed to a colourless, viscous syrup (73 mg, 93%).

δ_H(400 MHz, $CDCl_3$)	7.67 (s, 1H, H15), 5.97 (d, *J*=9.1, 1H, AcNH), 5.32 (d, *J*=2.8, 1H, H10), 5.19 (dd, *J*=10.6, 9.3, 1H, H3), 5.09 (dd, *J*=10.4, 7.9, 1H, H8), 5.00 (br s, 1H, BocNH), 4.98 (dd, *J*=10.4, 3.4, 1H, H9), 4.88 (d, *J*=3.6, 1H, H1), 4.78 (d, *J*=12.4, 1H, H13), 4.63 (d, *J*=7.8, 1H, H7), 4.61 (d, *J*=12.4, 1H, H13'), 4.50 (t, *J*=6.8, 2H, 2 x H16), 4.20 (ddd, *J*=10.6, 9.6, 3.5, 1H, H2), 4.13 – 4.02 (m, 2H, H12 & H12'), 3.95 – 3.86 (m, 2H, H4 & H11), 3.84 – 3.78 (m, 1H, H6), 3.76 – 3.70 (m, 2H, H5 & H6'), 3.67 – 3.56 (m, 8H, 8 x H19-H22), 3.52 (t, *J*=6.1, 2H, 2 x H23), 3.44 (t, *J*=5.9, 2H, 2 x H18), 3.20 (br ps q, *J*=6.1, 2H, 2 x H25), 2.17 (tt, *J*=6.8, 5.9, 2H, 2 x H17), 2.12 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.90 (s, 3H, $COCH_3$), 1.73 (p, *J*=6.1, 2H, 2 x H24), 1.41 (s, 9H, $C(CH_3)_3$)
δ_C(101 MHz, $CDCl_3$)	171.1 ($\underline{C}OCH_3$), 170.5 ($\underline{C}OCH_3$), 170.2 ($\underline{C}OCH_3$), 170.2 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCH_3$), 156.1 (COO^tBu), 143.0 (C14), 123.9 (C15), 101.2 (C7), 96.7 (C1), 79.1 ($\underline{C}(CH_3)_3$), 75.3 (C4), 71.8 (C3), 71.2 (C5), 71.1 (C9), 70.6 (C11 & OCH_2), 70.4 (OCH_2), 70.30 (OCH_2), 69.6 (C23), 69.4 (C8), 67.0 (C18), 66.8 (C10), 60.9 (C13), 60.9 (C12), 60.4 (C6), 52.1 (C2), 47.3 (C16), 38.6 (C25), 30.2 (C17), 29.7 (C24), 28.5 ($C(\underline{C}H_3)_3$), 23.2 ($CO\underline{C}H_3$), 21.0 ($CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$), 20.7 ($CO\underline{C}H_3$), 20.6 ($CO\underline{C}H_3$)
R_f	0.1 (50% Me_2CO in toluene)
$[\alpha]_D$	-2.5° (c=2, $CHCl_3$)
v (cm^{-1})	3362 (br, OH), 2980, 2933, 2871 (CH), 1746, 1706, 1675, 1525 (C=O)
m/z (ES+ TOF)	1000.4244 $[M+Na]^+$ ($C_{42}H_{67}N_5O_{21}Na$), calc. 1000.4226 – 100%

5.7.3.7 1-(4,7,10-Trioxa-13-^{t}butyloxycarbonylaminotridec-1-yl)-1,2,3-triazol-4-ylmethyl β(1-4)-D-galactopyranosyl-2-acetamido-2-deoxy-α-D-glucopyranoside (88)



Chemical Formula: $C_{32}H_{57}N_5O_{16}$

No literature reference found

1-(4,7,10-Trioxa-13-^{t}butyloxycarbonylaminotridec-1-yl)-1,2,3-triazol-4-ylmethyl 2,3,4,6-tetra-*O*-acetyl-β(1-4)-D-galactopyranosyl-2-acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**86**, 50 mg, 0.05 mmol) was dissolved in MeOH (4mL), Na (ca. 5mg) was added and the reaction stirred at room temperature for 3h, when TLC (80% Me_2CO in toluene) showed only a baseline spot . The mixture was neutralised by addition of DOWEX and filtered. Removal of the solvents by evaporation afforded the product as a sticky white film (31 mg, 77%).

δ_H(500 MHz, MeOD) 8.11 (s, 1H, H15), 4.92 (d, *J*=3.7, 1H, H1), 4.81 (d, *J*=12.5, 1H, H13), 4.66 (d, *J*=12.5, 2H, H13), 4.57 (t, *J*=6.8, 3H, 2 x H16), 4.39 (d, *J*=7.5, 1H, H7), 3.99 (dd, *J*=10.8, 3.7, 1H, H2), 3.89 – 3.80 (m, 4H, H3, H6, H6' & H10), 3.80 – 3.69 (m, 3H, H5, H12 & H12'), 3.69 – 3.58 (m, 10H, H4, H11 & 8 x H19-22), 3.56 – 3.46 (m, 6H, H8, H9, 2 x H18 & 2 x H23), 3.13 (t, *J*=6.8, 2H, 2 x H25), 2.18 (p, *J*=6.5, 3H, 2 x H17), 1.97 (s, 3H, $COCH_3$), 1.73 (p, *J*=6.5, 2H, 2 x H24), 1.45 (s, 9H, $C(CH_3)_3$)

δ_C(126 MHz, MeOD)	173.5 ($\underline{C}OCH_3$), 158.4 ($\underline{C}OO^tBu$), 145.0 (C14), 125.7 (C15), 105.1 (C7), 97.8 (C1), 81.2 (C4), 79.9 ($\underline{C}(CH_3)_3$), 77.1 (C11), 74.8 (C9), 72.6 (C8), 72.6 (C5), 71.5 (OCH_2), 71.3 (OCH_2), 71.3 (OCH_2), 71.0 (C3), 70.3 (C10), 69.9 (C23), 68.3 (C18), 62.5 (C12), 61.8 (C6), 61.4 (C13), 54.8 (C2), 38.7 (C25), 31.4 (C17), 30.9 (C24), 28.8 ($C(\underline{C}H_3)_3$), 22.6 ($CO\underline{C}H_3$)
t_R	23.4 min
$[\alpha]_D$	+58.0° (c=0.6, MeOH)
m/z (ES+ TOF)	768.3904 $[M+H]^+$ ($C_{32}H_{58}N_5O_{16}$) calc. 768.3879 – 100%

6 References

1. *Cancer Cases and Deaths in the UK*, http://publications.cancerresearchuk.org/downloads/Product/CS_DT_CASESDEATHS.xlsx, Accessed 07/07/2014.
2. *Cancer Incidence by Age*, http://www.cancerresearchuk.org/prod_consump/idcplg?IdcService=GET_FILE&dDocName=CASES_CRUDE_ALL_XLS&RevisionSelectionMethod=LatestReleased&allowInterrupt=1, Accessed 07/07/2014.
3. *Mortality - UK, 2007*, http://publications.cancerresearchuk.org/epages/crukstore.sf/en_GB/?ObjectPath=/Shops/crukstore/Products/CSMORT06, Accessed 23/12/2009.
4. N. Lane, *Oxygen: The Molecule That Made The World*, 1st edn., OUP, 2002.
5. H. Rang, M. Dale, J. Ritter and P. Moore, *Pharmacology*, 5th edn., Churchill Livingstone, 2003.
6. M. Ridley, *Genome*, 1st edn., Fourth Estate, 1999.
7. *CT*, http://www.med.harvard.edu/JPNM/TF00_01/Oct3/CT.gif, Accessed 19/5/2014.
8. *MRI*, http://www.hawaii.edu/medicine/pediatrics/pemxray/v3c09.html, Accessed 19/5/2014.
9. *3D-PET/CT*, http://www.texascancerclinic.com/equipment/sami_petct.cfm, Accessed 28/6/2010.
10. *PET/CT*, http://www.njironline.org/pet-ct-radiology-imaging-center-carlstadt-new-jersey.htm, Accessed 28/6/2010.
11. N. Ramanujam, *Enc. Anal. Chem.*, 2000, **1**, 20-56.

12. F. T. Liu and G. A. Rabinovich, *Nat. Rev. Cancer*, 2005, **5**, 29-41.

13. S. H. Barondes, D. N. W. Cooper, M. A. Gitt and H. Leffler, *J. Biol. Chem.*, 1994, **269**, 20807-20810.

14. P. Sorme, P. Arnoux, B. Kahl-Knutsson, H. Leffler, J. M. Rini and U. J. Nilsson, *J. Am. Chem. Soc.*, 2005, **127**, 1737-1743.

15. C. Diehl, S. Genheden, K. Modig, U. Ryde and M. Akke, *J. Biomol. NMR*, 2009, **45**, 157-169.

16. C. Diehl, O. Engström, T. Delaine, M. Håkansson, S. Genheden, K. Modig, H. Leffler, U. Ryde, U. J. Nilsson and M. Akke, *J. Am. Chem. Soc.*, 2010, **132**, 14577-14589.

17. C. G. Hadjipanayis, M. J. Bonder, S. Balakrishnan, X. Wang, H. Mao and G. C. Hadjipanayis, *Small*, 2008, **4**, 1925-1929.

18. T. L. Lowary and O. Hindsgaul, *Carbohydr. Res.*, 1994, **251**, 33-67.

19. P. Sorme, Y. N. Qian, P. G. Nyholm, H. Leffler and U. J. Nilsson, *ChemBioChem*, 2002, **3**, 183-189.

20. W. zu Reckendorf, *Angew. Chem., Int. Ed.*, 1967, **6**, 177.

21. C. Oberg, A. L. Noresson, T. Delaine, A. Larumbe, J. Tejler, H. von Wachenfeldt and U. J. Nilsson, *Carbohydr. Res.*, 2009, **344**, 1282-1284.

22. M. Main, PhD Thesis, University of Birmingham, 2010.

23. D. J. Bell, *J. Chem. Soc.*, 1935, 1874-1875.

24. F. Andersson and B. Samuelsson, *Carbohydr. Res.*, 1984, **129**, C1-C3.

25. J. Elhalabi and K. G. Rice, *Nucleosides Nucleotides Nucleic Acids*, 2004, **23**, 195-205.

26. K. N. Slessor and A. S. Tracey, *Can. J. Chem.*, 1969, **47**, 3989-3995.

27. M. P. Watterson, L. Pickering, M. D. Smith, S. J. Hudson, P. R. Marsh, J. E. Mordaunt, D. J. Watkin, C. J. Newman and G. W. J. Fleet, *Tetrahedron: Asymmetry*, 1999, **10**, 1855-1859.

28. P. Phukan, *Tetrahedron Lett.*, 2004, **45**, 4785-4787.

29. Z. Wang, M. Matin and S. Sheikh, *Molecules*, 2005, **10**, 1325-1334.

30. S. Deng, U. Gangadharmath and C.-W. T. Chang, *J. Org. Chem.*, 2006, **71**, 5179-5185.

31. A. J. Janczuk, W. Zhang, P. R. Andreana, J. Warrick and P. G. Wang, *Carbohydr. Res.*, 2002, **337**, 1247-1259.

32. U. Ellervik and G. Magnusson, *J. Org. Chem.*, 1998, **63**, 9314-9322.

33. J. S. Debenham, S. D. Debenham and B. Fraser-Reid, *Bioorg. Med. Chem.*, 1996, **4**, 1909-1918.

34. K. Aplander, J. Tejler, J. Toftered, S. Carlsson, B. Kahl-Knutsson, A. Sundin, H. Leffler and U. J. Nilsson, *Carbohydr. Res.*, 2006, **341**, 1363-1369.

35. J. Burés, M. Martín, F. Urpí and J. Vilarrasa, *J. Org. Chem.*, 2009, **74**, 2203-2206.

36. I. L. Cartwright, D. W. Hutchinson and V. W. Armstrong, *Nucleic Acids Res.*, 1976, **3**, 2331-2340.

37. M. Adamczyk, J. R. Fishpaugh and M. Thiruvazhi, *Org. Prep. Proced. Int.*, 2002, **34**, 326-331.

38. D. O. Jang, D. J. Park and K. Joonggon, *Tetrahedron Lett.*, 1999, **40**, 5323-5326.

39. H. Paulsen and C.-p. Herold, *Chem. Ber.*, 1971, **104**, 1311-1321.

40. M. Izumi, K. Fukase and S. Kusumoto, *Biosci. Biotechnol. Biochem.*, 2002, **66**, 211-214.

41. J. Garcia, J. Vilarrasa, X. Bordas and A. Banaszek, *Tetrahedron Lett.*, 1986, **27**, 639-640.

42. M. P. DeNinno, J. B. Etienne and K. C. Duplantier, *Tetrahedron Lett.*, 1995, **36**, 669-672.

43. C. Huang, N. Wang, K. Fujiki, Y. Otsuka, M. Akamatsu, Y. Fujimoto and K. Fukase, *J. Carbohydr. Chem.*, 2010, **29**, 289-298.

44. E. D. Goddard-Borger and R. V. Stick, *Org. Lett.*, 2007, **9**, 3797-3800.

45. F. Himo, T. Lovell, R. Hilgraf, V. V. Rostovtsev, L. Noodleman, K. B. Sharpless and V. V. Fokin, *J. Am. Chem. Soc.*, 2004, **127**, 210-216.

46. V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596-2599.

47. H. J. Cristau, A. Hervé, F. Loiseau and D. Virieux, *Synthesis*, 2003, **2003**, 2216-2220.

48. P. K. Mandal and J. S. McMurray, *J. Org. Chem.*, 2007, **72**, 6599-6601.

49. S. K. Boovanahalli, D. W. Kim and D. Y. Chi, *J. Org. Chem.*, 2004, **69**, 3340-3344.

50. P. Angibeaud, J. Defaye, A. Gadelle and J.-P. Utille, *Synthesis*, 1985, **1985**, 1123-1125.

51. M. A. Rahim, S. Matsumura and K. Toshima, *Tetrahedron Lett.*, 2005, **46**, 7307-7309.

52. E. Alonso, D. J. Ramón and M. Yus, *Tetrahedron*, 1997, **53**, 14355-14368.

53. B. L. Wilkinson, L. F. Bornaghi, S.-A. Poulsen and T. A. Houston, *Tetrahedron*, 2006, **62**, 8115-8125.

54. N. Hada, T. Takeda and Y. Ogihara, *Carbohydr. Res.*, 1994, **258**, 93-104.

55. W. L. F. Armarego, Perrin, D. D., *Purification of laboratory chemicals*, Butterworth Heinemann, Oxford ; Boston :, 1996.

56. L. Hall, S. Black, K. Slessor and A. Tracey, *Can. J. Chem.*, 1972, **50**, 1912-1924.

57. M. R. Maddani and K. R. Prabhu, *Synlett*, 2011, **2011**, 821-825.

58. M. Ellermann, R. Paulini, R. Jakob-Roetne, C. Lerner, E. Borroni, D. Roth, A. Ehler, W. B. Schweizer, D. Schlatter, M. G. Rudolph and F. Diederich, *Chem. Eur. J.*, 2011, **17**, 6369-6381.

59. M. Hainrichson, V. Pokrovskaya, D. Shallom-Shezifi, M. Fridman, V. Belakhov, D. Shachar, S. Yaron and T. Baasov, *Bioorg. Med. Chem.*, 2005, **13**, 5797-5807.

60. S. M. Christensen, H. F. Hansen and T. Koch, *Org. Process Res. Dev.*, 2004, **8**, 777-780.

61. *US Pat.*, US20110130553A1, 2011.

62. P. Tiwari and A. K. Misra, *J. Org. Chem.*, 2006, **71**, 2911-2913.

63. J. M. Jacobson, P. I. Kitov and D. R. Bundle, *Carbohydr. Res.*, 2013, **378**, 4-14.

64. G. Agnihotri, P. Tiwari and A. K. Misra, *Carbohydr. Res.*, 2005, **340**, 1393-1396.

65. A. K. Sarkar and K. L. Matta, *Carbohydr. Res.*, 1992, **233**, 245-250.

66. S. Valerio, A. Iadonisi, M. Adinolfi and A. Ravidà, *J. Org. Chem.*, 2007, **72**, 6097-6106.

67. M. Contour, J. Defaye, M. Little and E. Wong, *Carbohydr. Res.*, 1989, **193**, 283-287.

68. C. Xu, H. Liu and X. Li, *Carbohydr. Res.*, 2011, **346**, 1149-1153.

69. L. Lay, L. Manzoni and R. R. Schmidt, *Carbohydr. Res.*, 1998, **310**, 157-171.

70. A. Kyas and M. Feigel, *Helv. Chim. Acta*, 2005, **88**, 2375-2396.

71. G. Sosnovsky and C. T. Gnewuch, *J. Pharm. Sci.*, 1994, **83**, 989-998.

72. C. Dang, C. Nguyen, T. Nguyen and C. Im, *RSC Advances*, 2014, **4**, 6239-6245.

73. H. Liu and C. Wong, *Bioorg. Med. Chem.*, 2006, **14**, 7187-7195.

74. M. Imoto, H. Yoshimura, T. Shimamoto, N. Sakaguchi, S. Kusumoto and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 2205-2214.

75. M. Izumi, K. Fukase and S. Kusumoto, *Biosci. Biotechnol. Biochem.*, 2002, **66**, 211-214.

76. F. Javier Munoz, S. Andre, H.-J. Gabius, J. V. Sinisterra, M. J. Hernaiz and R. J. Linhardt, *Green Chem.*, 2009, **11**, 373-379.

77. *US Pat.*, US2010052385, 2011.

78. M. A. Azagarsamy, V. Yesilyurt and S. Thayumanavan, *J. Am. Chem. Soc.*, 2010, **132**, 4550-4551.

79. M. Trester-Zedlitz, K. Kamada, S. K. Burley, D. Fenyö, B. T. Chait and T. W. Muir, *J. Am. Chem. Soc.*, 2003, **125**, 2416-2425.

80. S. Dhar, Z. Liu, J. Thomale, H. Dai and S. J. Lippard, *J. Am. Chem. Soc.*, 2008, **130**, 11467-11476.

81. M. Kita, Y. Hirayama, K. Yoneda, K. Yamagishi, T. Chinen, T. Usui, E. Sumiya, M. Uesugi and H. Kigoshi, *J. Am. Chem. Soc.*, 2013, **135**, 18089-18095.

82. T. Wang, D. Y. W. Ng, Y. Wu, J. Thomas, T. TamTran and T. Weil, *Chem. Commun.*, 2014, **50**, 1116-1118.