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Synthesis and Biological Evaluation of Glycoporphyrins for use as Photodynamic Therapy Agents

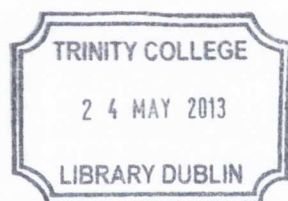


A thesis submitted to the University of Dublin for the degree of
Doctor of Philosophy

By
Robin Daly

Under the supervision of
Dr. Eoin Scanlan

Trinity College Dublin
2013



Thesis 9913

Declaration

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Abstract

Photodynamic therapy (PDT) is a light activated anti-cancer treatment. Reagents for PDT have advanced dramatically since the earliest porphyrin based oligomers. Ultimately the focus is on improving the efficacy of these agents, and the quality of life for the patients. With the current 3rd generation of PDT agents, emphasis is now focused on the improved targeting of these drugs to the required site. The ability of carbohydrates to act both as targeting and solubilising agents, has led to the synthesis and biological testing of a library of glycoporphyrin PDT agents.

Chapter 1 provides an introduction to PDT, and describes how the properties of carbohydrates can be used to improve on current PDT therapeutics. A review of the methods previously employed to prepare carbohydrate functionalised porphyrins, as well as recommendations on future functionalisation strategies is presented.

Chapter 2 describes the development and optimisation of glycoporphyrin synthesis using microwave mediated 'click' chemistry. A library of synthetic glycoporphyrins displaying a diverse set of carbohydrates is presented. Water soluble examples and an analogue displaying a protected Lewis X blood group were prepared. The method of covalent attachment displayed a wide scope, allowing successful introduction of a blood group hexasaccharide, in high yields.

Chapter 3 describes the development of a novel silyl based fucosyl donor. The donor provides α selectivity, but with a protecting group strategy enabling deprotection in the presence of unsaturated bonds. The synthetic scope of the donor was investigated for the synthesis of propargyl substituted α -Fuc-1-6- β -GlcNAc, a bacterial disaccharide with seven fold stronger binding to a fucose binding *R. Solanacearum* lectin, than fucose itself. A water soluble fucose terminating glycoporphyrin was prepared. Application of the new donor was investigated to the synthesis of a fully deprotected propargyl linked Lewis X trisaccharide.

Chapter 4 reports on an improved route to α -fucosylation through the use of a partially protected silyl based fucosyl donor. The reduced steric hindrance associated with this donor allowed the efficient glycosylation of hindered secondary alcohols. Synthesis of a propargyl substituted Lewis X trisaccharide, in yields comparable to that for a per-

benzylated fucosyl donor is reported. Application of this partially protected donor however, provides the advantage of facile deprotection in the presence of an alkyne.

In addition, an investigation was performed on an unusual Fuc-Fuc disaccharide product observed under particular activation conditions with the partially protected donor. The scope and proposed mechanism of a highly novel disaccharide forming reaction is presented.

In chapter 5 a study on the application of porphyrin disaggregation as a method to measure glycoporphyrin-lectin interactions, is reported. The results are analysed in an attempt to understand the targeting ability of glycoporphyrins *in vivo*. Cluster effects for mono-, di- and tri-mannose substituted glycoporphyrins are investigated using the lectin *Con A*. A complimentary set of experiments employing a fucose recognising lectin *Ulex I*, show how interactions between a glycoporphyrin and a lectin can lead to secondary quenching effects by virtue of the orientation of the porphyrin ring.

Chapter 6 details the experimental data required to produce this thesis.

Abbreviations

Å	Angstrom
aq	aqueous
BabA	blood group antigen binding adhesin
br	broad
calcd.	calculated
CHCl ₃	chloroform
CDCl ₃	deuterated chloroform
CRD	carbohydrate recognising domain
Con A	<i>Concanavalin A</i>
COSY	correlation spectroscopy
CuAAC	copper(I)-catalyzed azide-alkyne cycloaddition
d	doublet
DCE	dichloroethane
dd	doublet of doublets
DMAE	2-Dimethyl-amino-ethanol
DCM	dichloromethane
DEPT	distortionless enhancement by polarisation transfer
DMDS	dimethyl disulfide
DMF	<i>N, N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
DPA	diisopropylamino
DPBF	diphenylisobenzylfuran
DTMP	2,6-di- <i>tert</i> -butyl-4-methylpyridine
<i>d</i> ₅ -Pyr	deuterated pyridine
eq	equivalent
EI	electron ionisation
ESI	electrospray ionisation
GP	glycoporphyrin
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
HMBC	heteronuclear multibond correlation
HP	hematoporphyrin
HPMA	hydroxypropyl methacrylamide
HRMS	high resolution mass spectroscopy
HSA	human serum albumin
HSQC	heteronuclear single quantum coherence
Hz	hertz
IG	inverse glycosylation
IR	infra red
<i>J</i>	coupling constant
°C	degrees Celsius

m	multiplet
<i>m</i>	meta
M	mass ion
MALDI	matrix assisted laser desorption ionisation
MeOD	deuterated methanol
MeOH	methanol
MHz	megahertz
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium-bromide
<i>m/z</i>	mass per unit charge
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
<i>o</i>	ortho
O ¹	singlet oxygen
O ³	triplet oxygen
<i>p</i>	para
Pd/C	palladium on activated charcoal
PDT	photodynamic therapy agent
PEGMA	polyethyleneglycolmethacrylate
ppm	parts per million
q	quartet
R _f	retention factor
ROESY	rotating frame Overhauser effect spectroscopy
rt	room temperature
s	singlet
SAR	structure activity relationships
sat.	saturated
S _N Ar	nucleophilic aromatic substitution
STD	saturation transfer difference
t	triplet
TBDMS	<i>tert</i> -butyldimethylsilyl
TBHQ	<i>tert</i> -butyl hydroquinone
TCA	trichloroacetonitrile
TCP	tetrachlorophthalomide protecting group
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THPTA	tris-(hydroxypropyltriazolylmethyl)-amine
TLC	thin layer chromatography
TMU	tetramethylurea
TOF	time of flight analysis
TPPH ₂	tetraphenyl porphyrin
TPPZn	tetraphenyl porphyrinatozinc (II)
μW	microwave

Chapter One

Introduction

Chapter 1 – Introduction

1.1.1 Photodynamic therapy - Towards glycoporphyrin based PDT agents

1.1.2 Background to Photodynamic Therapy

Photodynamic therapy¹ is a treatment where therapeutic agents are used to induce light activated toxicity through the production of singlet oxygen. The requirement of two components; the photosensitizer, and light itself for the therapy to be successful, has allowed chemists and medical practitioners to design and develop a vast range of ideas, resulting in the many clinically approved PDT agents in use today.

Two of the earliest contrasting yet interesting benefits of light based therapies, are Finsen's light, which was used to heal *lupus vulgaris*, and sunlight, for the treatment for neonatal jaundice. Niels Ryberg Finsen won the Nobel Prize in Physiology in 1903 for his discovery that *lupus vulgaris* nodules can be destroyed through a treatment of light. An exact mechanism of action for this treatment was not elucidated until Møller *et al.* discovered the bacteria which caused lupus vulgaris contained coproporphyrin (III) (**1**, Figure 1.1). It was subsequently proposed that light induced singlet oxygen production, results in the death of cells within the lesions.²

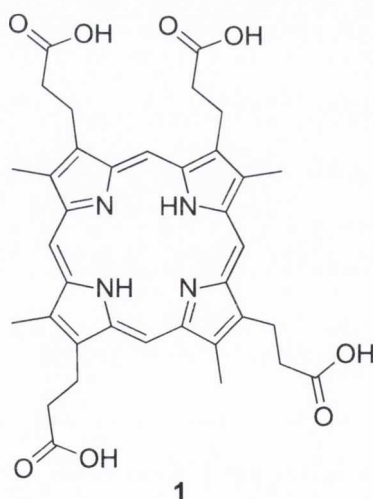
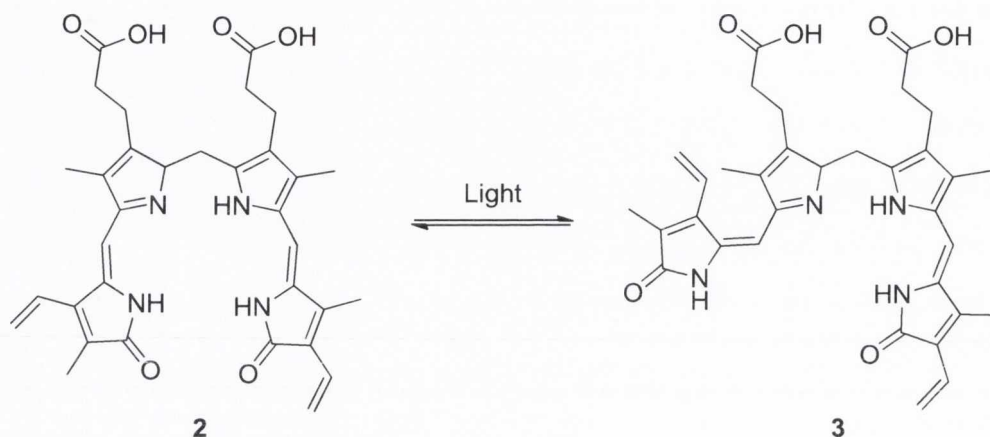


Figure 1.1 - Coproporphyrin (III)

The treatment of neonatal jaundice with light was first detailed by Cremer *et al.* in 1958.³ It was shown that yellowing of the skin disappeared, and the health of jaundiced children improved if they were exposed to sunlight. It has since been confirmed that neonatal jaundice results from higher than optimal levels of cis, cis-bilirubin (**2**, Scheme 1.1) in

newborns. Bilirubin isomer **2**, is insoluble in water due to an internal hydrogen bond network, however, it is normally broken down by gut flora in healthy children. Light promotes the isomerisation of one or both double bonds in **2**, disrupting the hydrogen bonding network and resulting in a water soluble cis, trans-bilirubin (**3**, Scheme 1.1), which can then be excreted.⁴



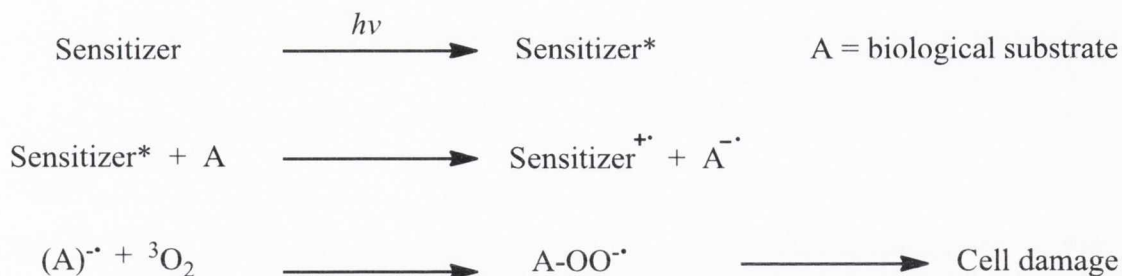
Scheme 1.1 - Bilirubin isomerisation

These examples show the importance of understanding modes of activity in light treatments in order to use this knowledge to create better remedies. The second example above is not strictly PDT but is another example of light inducing a beneficial medical outcome. The common principle operating in these early examples however, is that of light acting on a porphyrin, or porphyrin derived molecule, which is still the main basis for PDT today.

1.1.3 Mechanisms of PDT Activity

Photosensitizer excitation can result in cellular damage through two pathways (Figure 1.2). Type I with the production of superoxy anion radicals, and Type II with the production of singlet oxygen, as described by Crutchley *et al.*⁵

Type I



Type II

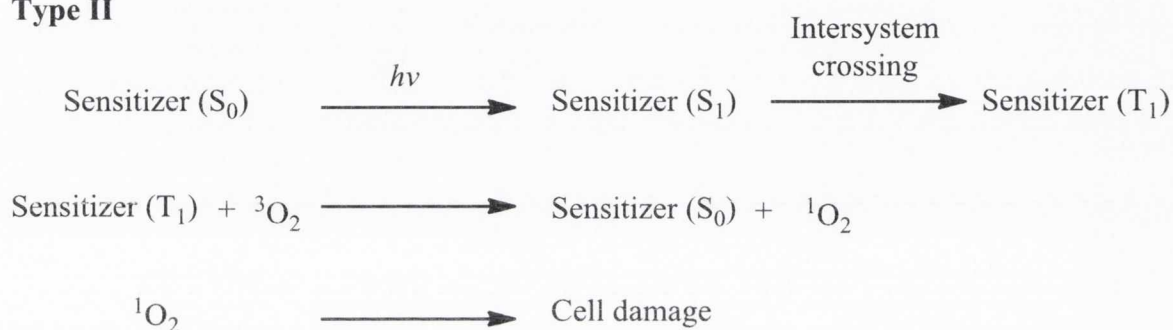


Figure 1.2 - Type I and Type II photosensitization pathways

Evidence for both routes has been presented,⁶ however, more recently the Melnikova⁷ and Hirohara⁸ groups have described experiments which show that type II is the more likely pathway for porphyrin based PDT agents. They demonstrated that porphyrin phototoxicity was prevented by sodium azide which is a known quencher of singlet oxygen, but that mannitol a hydroxyl radical trap had little or no effect. A schematic representation of the Type 2 photochemical reaction pathway is described in a modified version of a Jablonski⁹ diagram (Figure 1.3). Upon excitation by light, the PDT agent can relax either non-radiatively with vibration and heat generation or radiatively via fluorescence. Alternatively, following intersystem conversion to its triplet state, the sensitizer can excite triplet oxygen ${}^3\text{O}_2$ to its singlet state ${}^1\text{O}_2$. Singlet oxygen causes cell damage within 0.02 μm of production, thereby damage is limited to a short radius around the localisation of the photosensitizer.¹⁰ The damage tends to occur through oxidations of unsaturated bonds in various biomolecules, *eg.* lipid membranes, nucleotides, protein damage where amino acids contain unsaturated bonds like tryptophan or histidine. If the damage is suitably catastrophic this will lead to apoptotic or necrotic death giving the compound its therapeutic activity.

Type II

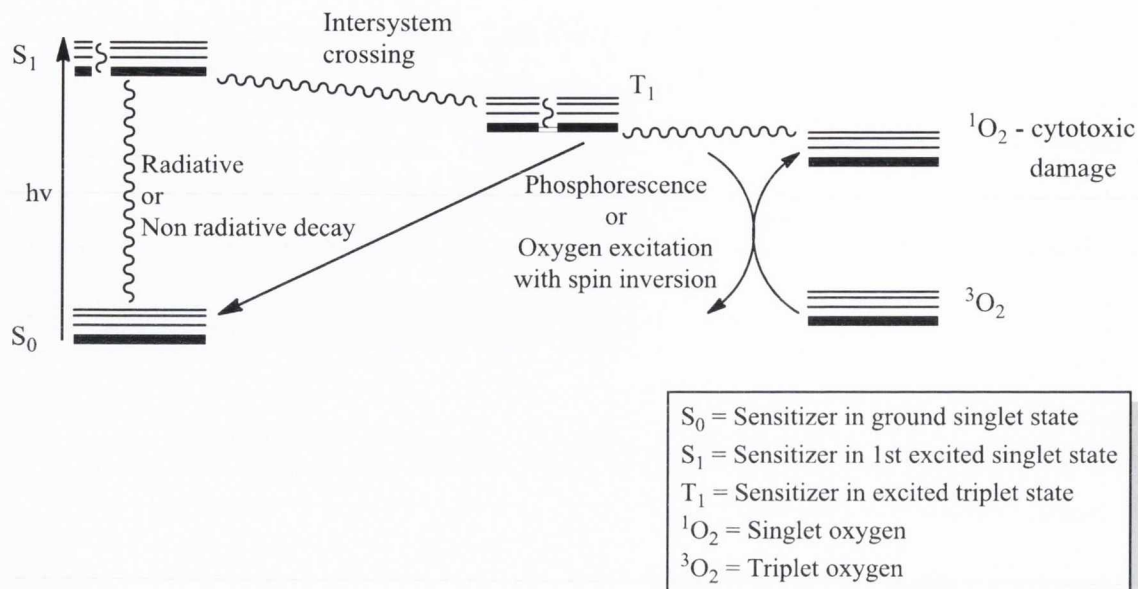


Figure 1.3 - Jablonski diagram for Type II pathway

1.1.4 Photofrin - 1st Generation PDT agent

The properties of an effective PDT agent include, a light absorption profile shifted well into the red region of the spectrum, allowing good light penetration through the skin, high singlet oxygen production, and high target efficacy with little or no off site localisation. In addition, good water solubility for formulation, and fast clearance times for low post treatment photosensitization are required. Finally the compound should exhibit zero dark toxicity. Although a molecule with all of these attributes has not yet been developed, over the past 60 years or so, significant advances towards the goals outlined above have been made.

The first generation clinically approved PDT agent was Photofrin (**4**, Figure 1.4). Photofrin is a polymeric, heterogeneous mixture of porphyrins derived from the treatment of hematoporphyrin with acid, followed by a crude purification.¹¹ It was approved for use on oesophageal cancer¹² by the FDA but from a chemical point of view has a number of disadvantages resulting from its heterogeneity. Also being a porphyrin based drug, it only absorbs light up to about 635 nm, and leads to long post-treatment photosensitization times.

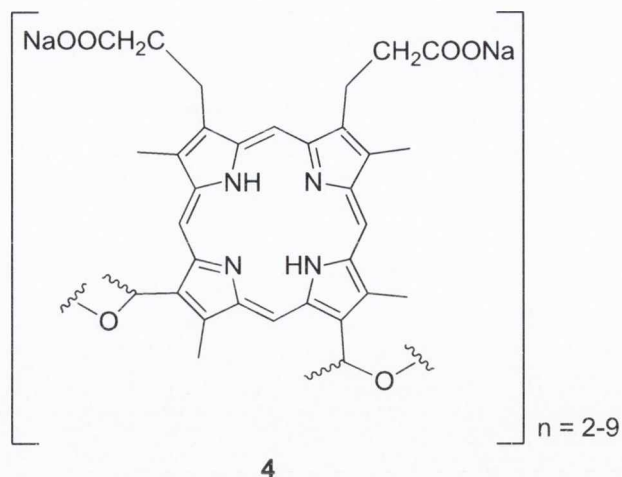


Figure 1.4 - Photofrin (1st generation PDT agent)

1.1.5 2nd Generation PDT treatments and properties

The second generation clinically approved PDT agents (Figure 1.5), sought to improve on absorption wavelengths, water solubility and cancer specificity. The classic example is Foscan, (5, Figure 1.5); approved for use on squamous cell head cancers and neck neoplasms¹³ in the EU. Foscan is a much simpler molecule than Photofrin. It is a synthetic chlorin with a greater absorption co-efficient at longer wavelengths, *circa.* 650 nm, allowing lower doses of the drug and requiring less light for activity. Unfortunately, although selective retention of Foscan by cancerous cells led to some specificity, the low doses of light required for phototoxicity, led to significant post treatment photosensitization, in some cases for up to 3 months.¹⁴

A substantial body of research on 2nd generation PDT agents has focused on modifying the porphyrin core to modulate the optical properties and photosensitizing ability of the PDT agent. These two desirable attributes are linked, as the ability to produce singlet oxygen is related to the triplet lifetime of the PDT agent, which is in turn related to stability of the molecule in the excited state; increased by conjugation. Most of these advances are covered in reviews by Dougherty *et al.*,¹ O'Shea *et al.*,¹⁵ Boyle *et al.*¹⁶ and Senge *et al.*¹⁷ The trend in Q band absorption for 2nd generation PDT agents is shown in Table 1.1.

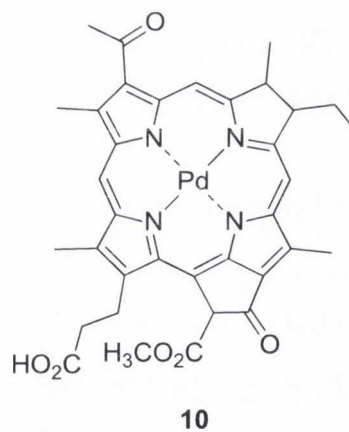
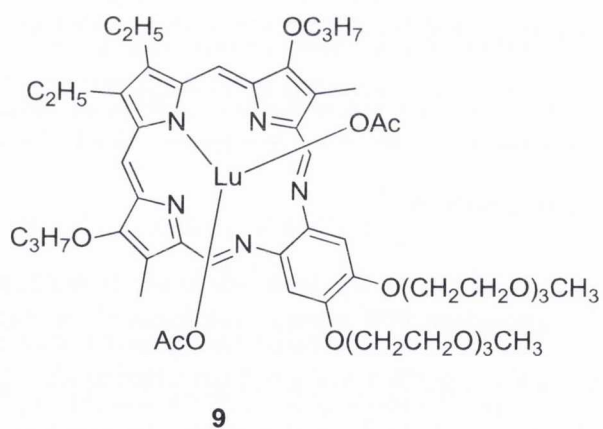
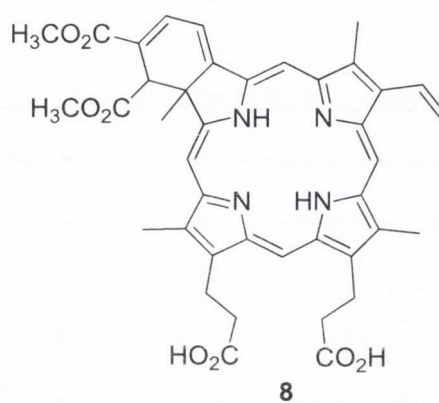
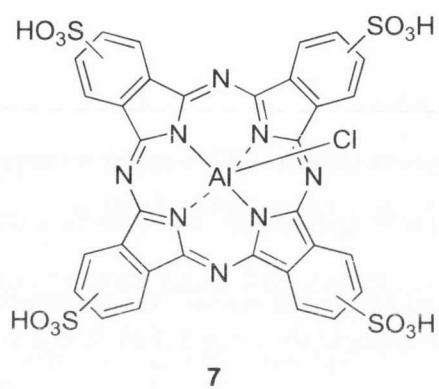
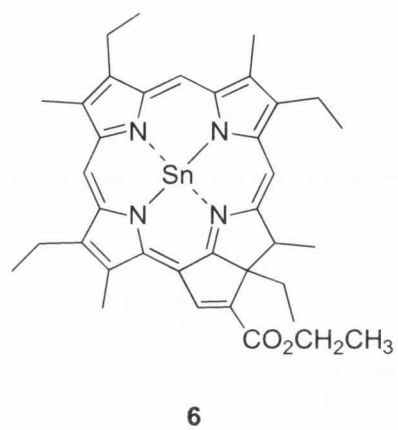
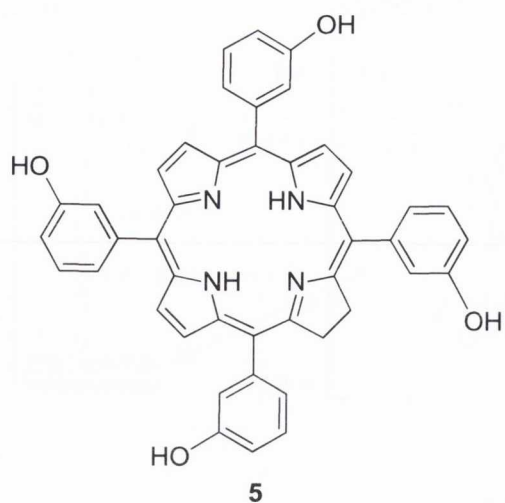


Figure 1.5 - 2nd generation PDT agents

Table 1.1 - Clinical absorption wavelengths for 2nd generation PDT agents

PDT agent (trade name)	Absorption wavelength
(Photofrin) [®] 4	630 nm ¹⁸
Temoporfin (Foscan [®]) 5	650 nm ¹⁷
Tin ethyl etiopurpurin (Pryltin [®]) 6	666 nm ¹⁹
Chloro-alluminium phthalocyanine (ClAlPc) 7	680 nm ²⁰
Verteporfin (Visudyne [®]) 8	689 nm ²¹
Lutetium Texaphyrin (Lutex [®]) 9	732 nm ²²
Pd-bacteriopheophorbide (TOOKAD [®]) 10	764 nm ²³

It can be seen that great advances have been made in red shifting the wavelengths of Q band absorption, considering one of the most simple porphyrins tetraphenylporphyrin has a UV/Vis absorption at 550 nm.

The key requirement of bathochromic shifts in the absorption bands have been achieved through the use of partially reduced porphyrins called chlorins - for example (**5**, Figure 1.5), as well bacteriochlorins, and iso-bacteriochlorins. Other methods to red shift the absorption profile include increased conjugation on the ring, as evident in compounds (**6**, **8**, **10**, Figure 1.5) as well as through increased nitrogen substitution with porphyrazine,²⁴ naphthalocyanines, and the phthalocyanine (**7**, Figure 1.5)²⁵ and finally expanded ring porphyrins - Texaphyrin (**9**, Figure 1.5) and porphycenes.²⁶

Water solubility has been increased through the introduction of functionalities such as carboxylic acids, sulfonic acids, hydroxyl groups and PEG chains to the periphery of the PDT agent.

At Trinity College Dublin, Prof. Senge *et al.* are actively engaged in the production of novel porphyrin featuring fused porphyrin scaffolds and extended π system conjugations.²⁷ These types of compounds are under investigation for their interesting PDT and optical properties with absorption wavelengths of greater than 720 nm an example being (**11**, Figure 1.6).

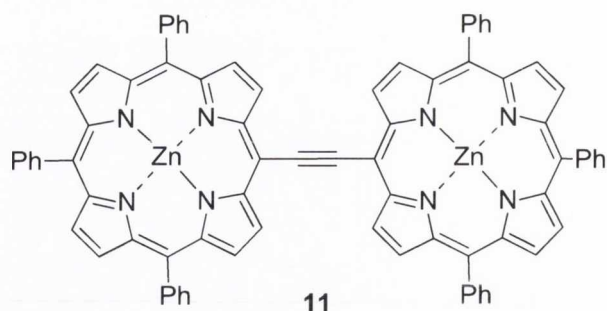


Figure 1.6 - Fused porphyrin dimer

The requirements of red light absorption, high singlet oxygen quantum yields, as well as solubility issues for formulation have all led to significant improvements from 1st and early 2nd generation examples. In the absence of a specific targeting agent however, intracellular localisation depends largely on the polarity, charge or aggregation state of the molecule (Table 1.2).^{17, 28-33}

Table 1.2 - Intracellular location of 1st and 2nd generation PDT agents

PDT Agent	Sub cellular location
Photofrin, 4	Mitochondrial membranes and cytoplasm ²⁸
Foscan, 5	Mitochondria and cytoplasm but not nucleus ⁷
Tin ethyl etiopurpurin, 6	Lysosomes ²⁸
Chloro-aluminium phthalocyanine, 7	Golgi, mitochondria and lysosomal membrane ¹⁷
Verteporfin, 8	Mitochondria ³²
Lutetium Texaphyrin, 9	Lysosomes ²⁸
Pd-bacteriopheophorbide, 10	Cytoplasm, Golgi and diffuse in mitochondria ³³

Localisation sites can depend strongly on the cell type also. For this reason *in vivo* studies are important in identifying the clinically relevant sites for localisation. The troublesome issues of 2nd generation cell specificity and post treatment photosensitization has paved the way for a new generation of targeted PDT agents.

In summary, with the 1st and 2nd generation PDT agents, a general class of drug has been developed, where a patient is administered intravenously, a set time is allowed for cell accumulation, following which light of a wavelength specific to the PDT agent is used to provide a therapeutic response.

1.1.6 3rd Generation PDT treatments and properties

By chemically attaching a targeting agent to a second generation PDT agent the 3rd generation PDT agents were conceived. Reviews on this new class of molecule have been performed by Wohrle *et al.*²⁸, Moser *et al.*³⁴, Boyle *et al.*³⁵, Koonan *et al.*³⁶ and Senge *et al.*¹⁷ These authors are agreed that specific cell targeting with PDT agents, is the next key requirement to improve current treatments. Multiple targeting agents have been employed including; amino acids, folic acid, steroids, LDL and liposomal deliveries, monoclonal antibodies, polymer and nanoparticles based transporters, and finally carbohydrates, in an effort to target photosensitizers to different cell types. Each of these classes of biomolecules has advantages and disadvantages, centred on cost of production and ability to effectively target the final product, as well as effective characterization of the active compound.

1.1.6.1 3rd Generation PDT - Amino acids

Simple amino acid conjugation has been shown to target drugs to particular cells, where they are normally not uptaken.³⁷ Unfortunately, application of this idea to PDT by Krausz *et al.*³⁸, resulted in reduced PDT activity relative to Photofrin, and also a measureable degree of dark toxicity. Short peptide attachments and anti-angiogenic targeting agents for PDT have been reviewed by Olivo *et al.*³⁹ Biological results have been mixed, but given the ease of synthesis of amino acid bearing PDT agents and the plentiful nature of the starting materials, amino acid conjugated PDT agents remain actively studied.

1.1.6.2 3rd Generation PDT - Folic acid

Folic acid as a targeting agent for PDT was investigated by Schneider *et al.*⁴⁰ in a study where a KKB cell line that over expresses folic acid receptors, was treated with a TPPH₂-Folic acid conjugate. After 24 hours, a 7 fold increase in uptake over TPPH₂ was observed, with increased cell death upon irradiation. This result demonstrates that cell targeting through specific biological interactions with over expressed receptors, is a valid drug delivery mechanism for photosensitizers. Knowledge of cancer cell profiles is important in choosing the correct targeting agent.

1.1.6.3 3rd Generation PDT - Steroids

The selective uptake of estradiol conjugated porphyrins over unconjugated porphyrins was reported by Ray *et al.*⁴¹, during investigation of PDT activity in MCF-7 cells, a breast cancer cell type over expressing the estrogen receptor. The response was dose dependant and led to a 2 fold increase in photosensitizer uptake of the modified porphyrin over the unconjugated control. Uptake of both photosensitizers was comparable in the presence of excess estradiol.

1.1.6.4 3rd Generation PDT - Lipids

Many cancer cells over express LDL receptors because of their increased need for cell membrane production. PDT conjugation to LDL, and also formulation of existing agents into liposomes, takes advantage of the increased cell membrane requirement, for rapidly growing cells. Again in the review of delivery for photosensitizers by Konan *et al.*³⁶, a series of different PDT agents demonstrated up to 4 times higher concentration inside cancer cells than normal cells. Foscan itself has been subject to this form of modification as both Fospeg and Foslip. Conflicting reports of the benefits of liposomal formulation seem to depend on the cell type investigated *in vitro*.^{17, 42} Senge *et al.* have reviewed how hydrophobicity⁴³ affects the properties of a PDT agent and the advantages of liposome binding and uptake. This has led to new interpretation of log P requirements for uptake, where higher log P can be tolerated through masking the agent inside a liposome, or by incorporating greater amphiphilicity to the molecule. Further research into this field is required, but ongoing. The advantage of this type of system is that other targeting agents can be co-formulated inside a liposome for increased targeting possibilities, and the non covalent nature of the arrangement allows easier production.

1.1.6.5 3rd generation PDT - Monoclonal antibodies

A very exciting targeting method involving monoclonal antibodies (mAb) is also undergoing an intense investigation. The trend in large pharma R & D towards low dose, high potency drugs has led to the development of a series of blockbuster biologics which have been used in the treatment of heart disease, cancer, inflammation and organ rejection amongst others.⁴⁴ Dissociation constants for these types of molecules can be in the 10⁻¹² M region. The combination of mAb and PDT agents therefore represents a powerful route to specificity. Antibodies against proteins and receptors relevant to cancer

treatment, like epidermal growth factor (EGF and HER2),^{36, 45-47} have been used effectively in 3rd generation PDT agents to give 6 and 7 times greater affinity of the conjugated agent versus the free PDT agent. Longer selective retention times in the tumour cells have also been reported. There are a number of major drawbacks with this type of modification however. The site of attachment on the antibody may affect its activity *in vivo*. Also close proximity of the PDT agent to the protein may hinder its activity through intramolecular quenching processes, although chemical spacer groups are being applied by Phillips *et al.* to circumvent this problem.⁴⁵ Finally, the cost of producing this type of compound is prohibitive for a lot of research groups. Preliminary results would need to show drastic benefits before large scale synthesis is justified, even though mAb targeting abilities may allow much lower concentrations of the drug to be administered. Nevertheless, the ability to raise antibodies against numerous different types of targets allows multiple applications.

1.1.6.6 3rd Generation PDT - Nanoparticles

The attachment of porphyrins to nanoparticles and polymers has also been applied for 3rd generation PDT. Some advantages of this method of delivery are a relatively high drug loading per particle, and that the properties of the nanoparticle can dictate localization, with the PDT agent simply being co-transported. Biodegradable nanoparticles offer the possibility of controlled release.⁴⁸ The Scanlan group through collaboration with Boyle *et al.* have successfully used polyacrylamide nanoparticles as supports for PDT agents, with dual targeting through multifunctionalisation of the polymer.⁴⁹ To date multiple scaffolds have been successfully employed as PDT supports; Poly-vinyl-alcohol, poly-lysine, HPMA and gold to name but a few.³⁶ Nano silica⁵⁰ and PEGMA-co-DPA⁵¹ have been used as a pH dependent delivery method which take advantage of the lower pH often observed in cancer cells to release the agent. The toxicity issue associated generally with nanoparticles is of concern, however, with the increased understanding and development of the science, the use of particle size to determine distribution *in vivo*, shows promise.

Carbohydrate modified 3rd generation PDT agents, are the final class of photosensitizer to be discussed. These PDT agents will be examined in more detail following an introduction to the possible medicinal applications of carbohydrates.

1.2 Carbohydrates as therapeutics

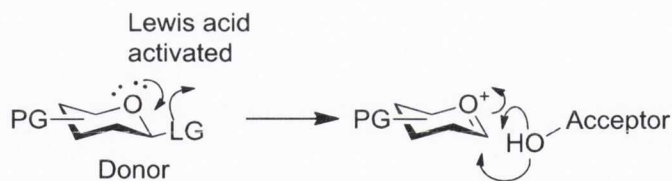
1.2.1 Carbohydrates in biology

Carbohydrates found throughout nature are known to be involved in a wide range of biological processes, from basic food and energy requirements to the complex interactions between a spermatozoa and ovum after fertilisation.⁵² Indeed reviews by Davis *et al.*⁵³ and Dwek *et al.*⁵⁴ have shown carbohydrates to be implicitly involved in neuronal development through sialic acid production on cell surfaces, hormone activities through altered protein folding, immune surveillance/avoidance by viruses and bacteria,⁵⁵ inflammatory responses in blood transfusions, and pathogen to host recognition, for the example binding of influenza and HIV viruses to cell surfaces. As more biologically significant carbohydrate interactions are discovered, the scientific community continues to re-examine the importance of oligosaccharides in nature, with a view towards a greater understanding of these events. Whilst DNA is used to carry our basic genetic information, real time information about the inner workings of a cell can be found by analyzing its oligosaccharide components. Information is generated through the post translational modification of proteins with various carbohydrates. The modification of proteins through glycosylation, can alter their folding patterns, tag proteins for degradation, lead to activation of dormant proteins/enzymes and can tag proteins for transportation.⁵⁶⁻⁵⁷

The chemistry of sugars is very complex due to regio and stereoselectivity requirements. In Nature, carbohydrate synthesis is not templated so a mixture of similar compounds, commonly referred to as glycoforms can be expressed on the surface of cells.

1.2.2 Chemistry of carbohydrates

Both Nature and the carbohydrate chemist encounter the same problem in selectively synthesizing a single analogue from the multitudes of carbohydrate combinations available. The fact that multiple reactive hydroxyl sites exist within each monosaccharide, inevitably leads to difficulty in targeting the required oligosaccharide in a synthetic pathway. For this reason the chemistry of carbohydrates has been historically dominated by protecting group manipulations. Carbohydrate extensions tend to proceed through activation of the anomeric position on a donor to produce an oxocarbenium intermediate, which is glycosylated by an acceptor (Scheme 1.2).



Scheme 1.2 - General glycosylation mechanism

These glycosylations can involve a wide variety of leaving groups, activators, and solvent conditions, all of which can lead to different products. Stereoselective glycosylation to form α or β products can be achieved through neighbouring group participation. For example an equatorial acetyl protecting group in the C-2 position of per-acetylated glucose directs acceptor attack from the β face. Reactivity of carbohydrates in glycosylation can be achieved through variation of the pattern and type of protecting groups used. The effects protecting groups have on glycosylations is known as the armed/disarmed⁵⁸⁻⁵⁹ and even super-armed effect.⁶⁰⁻⁶¹ A comprehensive comparison of these effects on thioglycoside donors was completed by Wong *et al.*⁶², where relative values were assigned to different compounds according to their reactivity, allowing sequential one pot glycosylations. The ability of the protecting group to modulate reactivity is both a combination of the electron withdrawing/donating⁵⁸ tendencies of the protecting group, hyperconjugation into the oxocarbenium ion,⁶³ steric bulk altering intermediate conformations⁶¹ and/or blocking attack from a particular side. In truth all the mechanisms for selectivity are not comprehensively understood.

The choice of leaving group in the anomeric position also plays a key role in the activity of a donor, with oxygen, halide, sulfur, acetimidate and selenium based leaving groups, all providing their own particular benefits and drawbacks.

Finally the choice of Lewis acid and solvent can have a significant effect on glycosylation reactions. Protecting group compatibility with acceptors, donors and activation conditions remains a challenge for carbohydrate chemists. One exciting advance in this field has been chemo-enzymatic synthesis. This is where both chemistry and enzymes are used in the synthesis of target molecules. The interest in using enzymes to aid synthesis is becoming ever more apparent as evident by the very impressive results of the Boons⁶⁴, Wong⁶⁵, Flitsch⁶⁶ and Davis⁶⁷ groups.

1.2.3 Carbohydrate based drugs

The use of carbohydrates directly as drugs, is limited by their pharmacokinetic profiles; Low permeability through the intestinal wall due to high polarity, and fast renal excretion from the blood. However, a wide variety of either carbohydrate containing or derived molecules are used in the treatment of diabetes, influenza, epilepsy and osteoarthritis and others can act as anticoagulants (Heparin), as described in a comprehensive Nature Drug Discovery Review.⁶⁸ A common mechanism of action for some of these drugs is the selective inhibition of enzymes, for example glycosidases in diabetic therapies. The influenza drugs Zanamivir, **13**, (Relenza)⁶⁹ and Oseltamivir phosphate **14**, (Tamiflu; Roche)⁷⁰ were designed from the need to inhibit a neuraminidase enzyme. The enzyme mediates intercellular *influenza* virus spreading, through the hydrolysis of sialic acid residues (**12**, Figure 1.7) on the surface of animal cells. These are examples of carbohydrate mimic drugs. Both companies started with sialic acid, and by removing the non essential functional groups for binding, as well as altering the moieties of the sugar which were easily metabolised, a more metabolically stable drug was prepared (Figure 1.7).

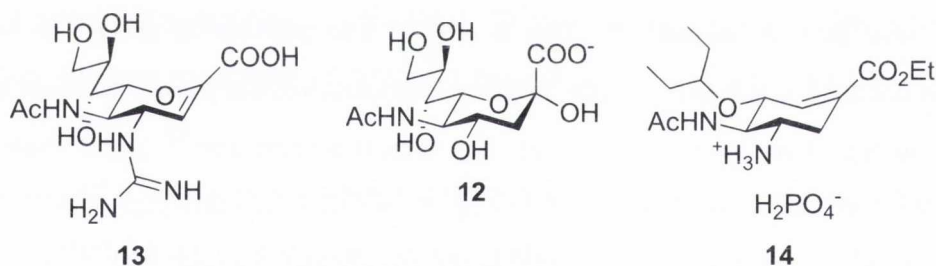


Figure 1.7 - Sialic acid homology to neuraminidase inhibitors

Miglustat (**15**, Figure 1.8), a synthetic alkylated azo sugar, is used as a treatment for Gaucher's disease. Symptoms of this disease result from the build up of glucosylceramide in cells.⁷¹ Abnormal levels of glucosylceramide are implicated in cardiovascular diseases, diabetes and cancer.⁷² This drug inhibits the enzyme glucosylceramide synthase hence alleviating the symptoms caused by excess of the compound.

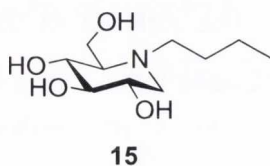


Figure 1.8 - Miglustat

Oligosaccharides do not always require modification to become active therapeutics. Heparin, a carbohydrate polymer with a basic disaccharide repeating unit of sulphated iduronic acid and sulphated glucosamine, and a MW of over 12 kDa, is used as an anti-coagulant. Smaller oligosaccharides, containing the core units of heparin are also clinically approved agents.

Simple sugars too have been used as treatments for certain afflictions. In congenital disorders of glycosylation as described by Jaeken,⁷³ children have defects in their ability to glycosylate certain proteins. These are genetic based diseases, and as *circa*. 1 % of our entire genome is dedicated to glycosylation hardware, there are no doubt many more diseases of this type yet to be identified. The problems originate from faulty cellular machinery in the Golgi apparatus and endoplasmic reticulum; key centres of carbohydrate modifications. Mannose has been successfully used in the treatment of a disease characterized by a deficiency in phosphomannose isomerase.⁷⁴ Mannose (**16**, Figure 1.9) too has been used in the treatment of urinary tract infections through the competitive inhibition of *Escherichia coli* binding to the endothelial wall.⁷⁵

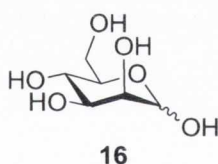


Figure 1.9 - D-Mannose

The use of carbohydrates as potential vaccines has also been the subject of a large body of research. Bacteria, cancer cells, and viruses display distinct carbohydrates on their surfaces. For example the lipopolysaccharide on the surface of *Streptococcus pneumonia* is a key factor in toxicity. Modern vaccinations against pneumonia consist of polysaccharides from the surface of the bacteria conjugated to a carrier protein. Immunity raised to the polysaccharide protects against future infection. However, the bacterium is constantly evolving different serotypes against which the vaccine is not effective. The Oscarson group at UCD, are actively engaged amongst others in the production of a synthetically derived *Streptococcus Pneumonia* vaccine.⁷⁶

So far carbohydrates as drugs have been shown to act through enzyme inhibition, immune response modulation, and through protein binding. There is another class of carbohydrate recognising protein which deserves attention, namely lectins.

1.3 Lectins

Lectins are non-enzymatic proteins containing carbohydrate recognising domains. These domains can be carbohydrate specific but tend to have weak binding affinities; K_d values in the order of 0.1-1 mmol.⁷⁷ Binding affinities can be enhanced through multiple binding of sugars in close proximity to the same lectin, referred to as the multivalent or cluster effect.⁷⁸⁻⁷⁹ The cluster effect results most likely from contributions of the two concepts in Figure 1.10. In model (A, Figure 1.10), when two or more carbohydrates co-operatively bind to two or more binding sites on the protein, the resulting dissociation constant is lowered. Indeed an allosteric effect resulting in protein folding changes upon the binding of one unit, may enhance subsequent binding. Cross linking of lectins may result in lattice formation also.⁸⁰ In model (B, Figure 1.10), if carbohydrates are presented in close proximity, there is an increase in their local concentration at the binding site. With one sugar sitting in the binding site, upon dissociation, there is a greater chance of association from any of the three non bound sugars, as compared to 3 free sugar molecules. The many diverse functions of lectins are still under investigation, and to date, known roles include; cell recognition, host-cell infection, glycoprotein chaperone roles⁸¹ and multiple defence roles in plant and animals.⁸²

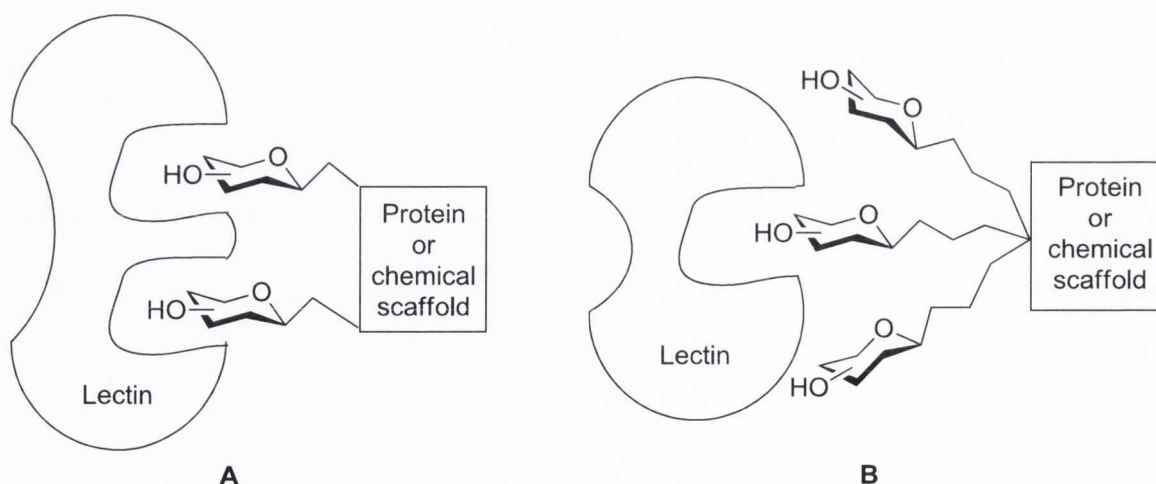


Figure 1.10 - Cluster effect models

1.3.1 Plant lectins

Carbohydrate mediated lectin interactions are involved in many recognition events in plant biology. These proteins can exist as monomeric or heteromeric units, and often require Mg^{2+} or Ca^{2+} ions as co-factors for binding. Plant lectins were first discovered over 100 years ago for their ability to cause hemagglutination⁸³⁻⁸⁴, however, their physiological role in plants is not fully understood. Some common carbohydrate/lectin⁸² recognition pairs are listed in Table 1.3. Many plant derived lectins have been immobilised and can be used for purification of carbohydrates and glycoproteins. Due to their recognition of sugars, plant lectins have proved an invaluable chemical tool. Current methods exploiting sugar-lectin interactions include isothermal calorimetry titration, UV/fluorescent spectrometry using label conjugated lectins, surface plasmon resonance imaging with immobilised lectins, quartz crystal microbalancing on immobilized lectins, hemagglutination assays and co-crystallisations to name but a few.

Table 1.3 - Common plant lectin and carbohydrate pairs

Lectin source	Specificity of sugar
<i>Concanavalin A</i> (Jack Bean)	α -Man, α -Glc
<i>Ulex europaeus</i> (I) (Gorse)	α -Fuc
<i>Ricinus communis</i> (Castor Bean)	β -Gal
<i>Triticum vulgaris</i> (Wheatgerm)	β -GlcNAc
<i>Arachis hypogaea</i> (Peanut)	β -Gal-(1-3)-GalNAc

Nature rarely wastes resources and whereas chemists and biologists have found uses for plant derived lectins, research into their roles in plants is ongoing. As plant defence mechanisms, lectins can produce strong antigenic responses in animals. For example the chemical agent ricin, a lectin derived from the castor bean has been used in biological warfare. Agglutination can also cause animal toxicity so one view could be that plant lectins may have evolved to protect the plant from predators.

Another role of lectins, has been identified through the study of genetically modified plants, and their symbiotic relationships with N_2 fixing bacteria. Bacteria normally reside in nodules on the roots. The interaction of lectins with the rhizobian bacterial surface glycosides have been implicated in host guest recognition which encourages nodule

growth. Once lectin coding genes are altered, a change in the nodulation pattern on plants is observed.⁸⁵

1.3.2 Bacterial and viral lectin interactions

Bacterial and viral infections have processes dictated by lectin carbohydrate interactions. Key examples of these, highlight the possibility of using carbohydrates as antibacterial therapeutics, taking advantage of differential lectin expression.

In Section 1.2.3 describing carbohydrates as therapeutics, the use of mannose as a treatment for urinary tract infection was discussed. The basis for this activity is the expression of a mannose recognising lectin - FimH, on the surface of *E coli* pili. Mannose clusters have been developed as an improved method to saturate FimH. One of the more promising reports is that of the Roy group⁸⁶ who showed a k_d of 0.45 nm for the FimH of *E. Coli*, in a compound containing 4 mannose residues on a tetraphenyl scaffold (**17**, Figure 1.11). This binding represented a 5000 fold increase in specificity over the control mannose, **16**.

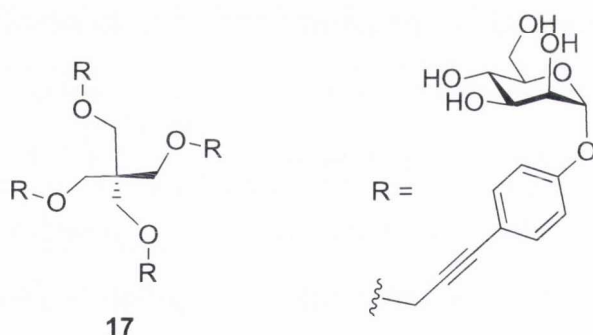


Figure 1.11 - Sub nanomolar FimH affinity

Helicobacter pylori, a bacterium which is the causative agent for stomach and duodenal ulcers, expresses a surface protein called the BabA.⁸⁷ This lectin has a very high affinity for Lewis B (**18**, Figure 1.12), in the order of $1 \times 10^{10} \text{ M}^{-1}$, making it extremely important in bacterial adhesion to the gut wall, ultimately protecting the bacterium from the acidic stomach environment.

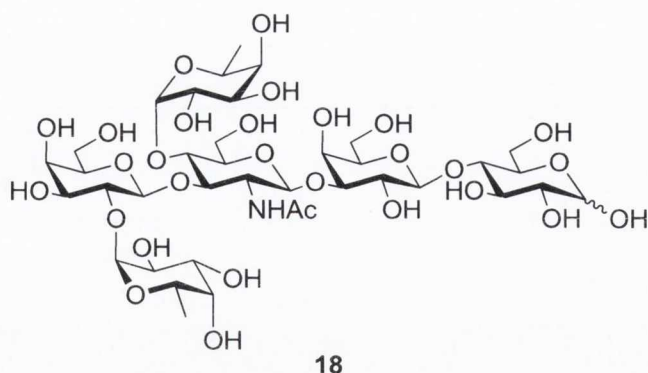


Figure 1.12 - Lewis B hexasaccharide

The importance of carbohydrates in Influenza budding and infection was discussed as a treatment target in Section 1.2.3. However, the initial interactions of influenza with host cells are through the actions of a lectin on its surface, hemagglutinin (HA).⁸⁸ This protein binds to terminal sialic acid residues, leading to endocytosis of the virus by the host cell. Allosteric disruption of sialic acid, binding, has been shown by Russell *et al.* using TBHQ (**19**, Figure 1.13).⁸⁹ Targeting HA's specificity through carbohydrate mimics⁹⁰ could also represent a viable method of influenza treatment. Clustered mimics on chemical scaffolds could encourage virus particles to aggregate, for easier processing by the host's immune defence systems.

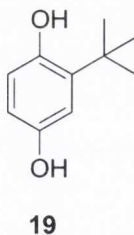


Figure 1.13 - TBHQ - inhibitor of hemagglutinin

1.3.3 Animal lectins

Lectins are not specific to the plant and bacterial worlds however. Animal lectins were first described in extracts from the venom of snakes in 1888 which cause hemagglutination.⁹¹ They tend to have CRD's which are highly conserved⁹² among different animal species, and contain structural homologies with plant lectins, showing important evolutionary conservation requirements. Investigation into the properties of animal lectins has allowed their sub division according to; calcium dependency (C-type

lectins), terminal galactose specific lectins (Galectins), mannose-6-phosphate specific lectins (P-type lectins), and immunoglobulin lectins (I-type lectins). A comprehensive breakdown for the many other subgroups in animal lectin families has been detailed by Esko *et al.*⁹³ Intracellular processing of carbohydrates, and glycoprotein trimming or elongation is performed in the ER and Golgi apparatus. Shuttles between these two structures are mediated by lectins.⁸¹

One example of a key human lectin-carbohydrate interaction, occurs early in the HIV infection cycle. The HIV virus is coated in a glycocalyx which it uses to direct its transport to T cells, its point of viral reproduction. A particular glycoprotein GP120 on the surface of the virus contains a terminal oligomannose region of 9 units, and is recognised by DC-SIGN a lectin on human dendritic cells. The virus is internalised and transported to a T cell for processing only upon the interaction of GP120 with another glycoprotein CD4 on the T cell, a conformational shift in GP120 allows HIV to insert a fusion peptide and infect the host.⁹⁴ DC-SIGN also recognises Lewis X (20, Figure 1.14)⁹⁵ a blood group trisaccharide so Lewis X presenting scaffolds could conceivably compete with the HIV virus to lower the infection rate.

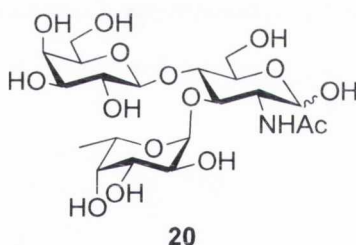


Figure 1.14 - Lewis X trisaccharide

1.3.4 Lectin over expression in tumour cells

1.3.4.1 Galectin-1

The over expression of Galectin-1 is an interesting method for carbohydrate targeting of PDT agents. A number of cancer cell models present this type of overexpression. For example, Griffioen *et al.* demonstrated that Galectin-1 is over expressed during angiogenesis, in cancer cell lines of the human colon, breast, and in a rare bone cancer; Ewing's sarcoma.⁹⁶ All these measurements were taken using normal colon tissue as a control. Gal-1 normally binds terminal β -1-4-Gal and in keeping with previously observed cluster effects, Mayo *et al.*⁹⁷ have shown that this binding is enhanced by large multimeric presentations of β -1-4-Gal. Studies on interrupting Gal-1 binding have led to the

development of Anginex®, a 33 amino acid peptide currently undergoing anti cancer clinical trials.⁹⁸

Furthermore Kiss *et al.*⁹⁹ in a review on the roles of Gal-1 *in vivo*, demonstrated that its over expression on the blood vessel cell walls of lymphomas, plays a role in cancer metastasis, whereas Dian *et al.* identified the over expression of Gal-1 in a laryngeal cell line model.¹⁰⁰

1.3.4.2 Galectin-3

Another lectin in the Galectin family, Gal-3 has been implicated as a reporter of cancerous tissue. In cell studies on thyroid cancer, Bartolazzi *et al.*¹⁰¹ showed that over expression of Gal-3 was a marker for metastasis and it was not expressed in benign thyroid cells. This identifies Gal-3 as an adhesion promoter. Gal-3 has been shown to be over expressed in colon neoplastic lesions by Malorni *et al.*¹⁰² Kuroda *et al.* confirmed Gal-3 over expression in chronic myelogenous leukaemia (CML) cell lines and found during their studies that Gal-3 appeared to promote cell proliferation, led to multidrug resistance and was involved in the cell migration in CML cells.¹⁰³ Antagonists of Gal-3 could therefore be a valid target for CML treatments.

1.3.4.3 E-Selectin

The Lewis X binding lectin E-selectin has been reported to be over expressed on LoVo colon cancer cells and also appears to display a complex relationship with Gal-9.¹⁰⁴ Gal-9 over expression was also seen in this cell line, but only in cases with metastatic regrowth. In this case Gal-9 and E-selectin expression could be used to target LoVo cell lines.

1.3.4.4 Lectin like receptors in retinoblastoma cells

Selective adhesion of neoglycoconjugates to retinoblastoma cells was shown by Gabius *et al.*¹⁰⁵ In their study it was reported that retinoblastoma cells selectively recognised α -Man and β -Gal. Presumably the Gal recognition is through the over expression of a member of the Galectin family. This allowed the targeting of drugs bearing sugars to cancerous cells in this rare form of eye tumour. Further efforts to take advantage of this not fully elaborated lectin based selectivity allowed Maillard *et al.* and Blais *et al.* to selectively target retinoblastoma cells using scaffolds bearing mannose and galactose.¹⁰⁶⁻

1.3.4.5 Sialic acid receptors

Finally sialyl Lewis X has been shown to be a site of adhesion for multiple cancer cell lines.¹⁰⁸ The likelihood that this relationship is lectin based again provides us with more carbohydrate based targeting agents against cancer cells going forward.

1.3.5 Lectins as a target for drugs

In summarising the previous Sections on differential lectin expression, one can see possible applications in many areas from viral and bacterial infections to metastatic tumour treatments. A key factor with the majority of these lectin targets with respect to PDT, is their location *in vivo*. Tumours deep inside tissue and organs will always be difficult targets for PDT agents, however, lectin targets can be reached with modern day key hole surgery utilising fibre optic probes. Examples of accessible regions are the endothelial surfaces on the urethra for *E. coli*, or the stomach wall for *H. Pylori*, blood cells for *H. Influenza*, mucosal barriers for HIV, the trachea, colon, oesophagus, and internal surfaces of blood vessels for cancerous cells.

Having seen the importance of carbohydrates in biology, the utilisation of oligosaccharides to prepare 3rd generation PDT agents is promising. Glycoporphyrins can be defined as porphyrin based molecules which have been modified to contain carbohydrate groups. The vast number of carbohydrates that can possibly be accessed synthetically, allow conceptually the design of one type of drug, with a multitude of different targeting agents. The possibility of a modular approach involving a convergent synthesis of porphyrins and carbohydrates is an exciting prospect. Medicinal chemists could conceivably take a PDT scaffold, functionalise it with a particular carbohydrate, to conveniently direct the drug to the required site. Exploiting the variable lectin recognition patterns and biological distribution could enable the targeting of "Sugar bearing" molecules to animal cells, bacteria and viruses allowing advanced delivery of therapeutics.

1.4 Glycoporphyrins: A review

The potential benefits of carbohydrates as targeting agents have been discussed as part of Section 1.2, however, they are important for two other functions once applied to PDT. Porphyrins, and related aromatic macrocycles, are notoriously insoluble in water. 2nd generation PDT agents required some separate form of polar modification distinct from the goal of improving light absorption at longer wavelengths. As described in Figure 1.5,

extra hydroxyl, carboxylic acids, sulfonic acids or PEG functionalities have been incorporated into the structures, all to improve their solubility. Carbohydrates would have the advantage of providing both a targeting motif as well as an improvement in possible formulation of the final product. The requirement for amphiphilic PDT agents has been described as very beneficial in increasing the possibility of transport through cell membranes.¹⁰⁹ Indeed the drawbacks seen in 1st, 2nd and some 3rd generation PDT agents of up to 90 days post treatment photosensitization, may be alleviated by mimicking some of the body's own metabolism pathways. A common phase II metabolic transformation is glucuronidation, employed to increase polarity for faster clearance times. For this reason glycoporphyrins as PDT agents should demonstrate lower half lives, since they are already glycosylated. Two main considerations for glycoporphyrin synthesis are; which carbohydrates to use, and the functionalisation strategy.

1.4.1 Natural glycoporphyrins as PDT agents

Only limited examples of glycoporphyrins have been isolated from biological systems. These include, a tetrapyrrolic chlorophyll metabolite (**21**, Figure 1.15) discovered during the ripening process of bananas by Krautler *et al.*¹¹⁰, and the Tolyporphins, (**22**, **23**, Figure 1.15), a group of anticancer agents isolated from extracts of an algae *Tolypothrix Nodosa*.^{111,112} The latter displays an unusual 3,6-di-deoxy-galactose moiety with partial acetylation of the C-2 position. Research into the exact biological role of tolyporphins is ongoing, and to date it has been suggested they could function as part of the light harvesting machinery in algae.

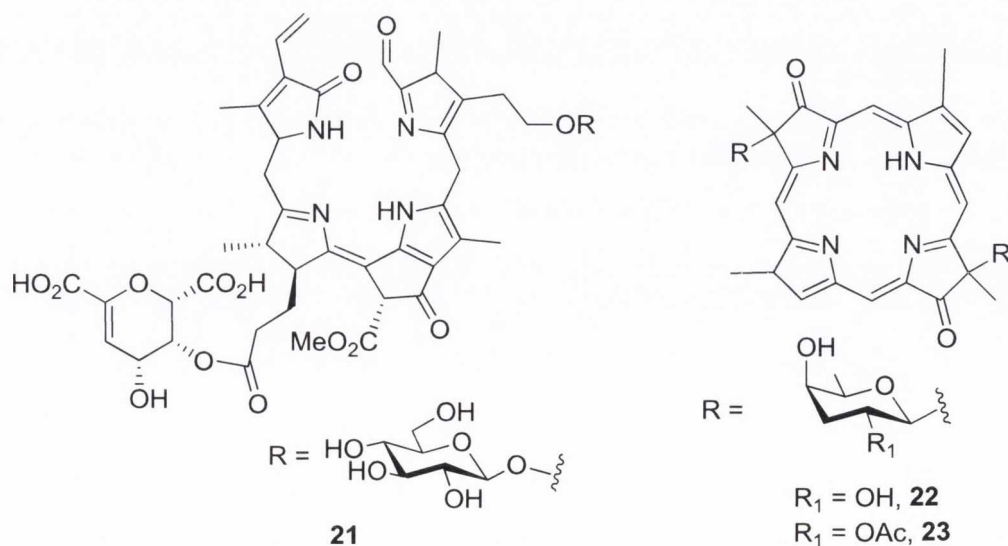


Figure 1.15 - Natural glycosylated porphyrins/derivatives

1.4.2 Semi synthetic and synthetic glycoporphyrins

Synthetic glycoporphyrins as PDT agents have been explored for the last number of decades. The advantages that these compounds have over existing PDT agents are provided by the properties of the carbohydrate used. A multitude of attachment methodologies have been employed to introduce carbohydrates, and these have been detailed in four separate glycoporphyrin reviews, written in 2002, 2007, 2008 and 2009 and a perspective in 2012.¹¹³⁻¹¹⁷ Presented is a summary of the synthetic glycoporphyrins and their conjugation methods, as well as highlights from the glycoporphyrin literature since 2009.

1.4.2.1 Protoporphyrin IX derivatives

As the 1st generation PDT agents were hematoporphyrin derived it was no surprise that the first synthetic glycoporphyrins were glycosylated derivatives thereof. In 1989, Franck *et al.* synthesised di-substituted glycoporphyrins¹¹⁸ containing β linked glucose or galactose, by directly glycosylating primary hydroxyls off the porphyrin (Figure 1.16). In the same paper, the authors showed that the singlet oxygen generation of these compounds was comparable to that of the unglycosylated derivative.

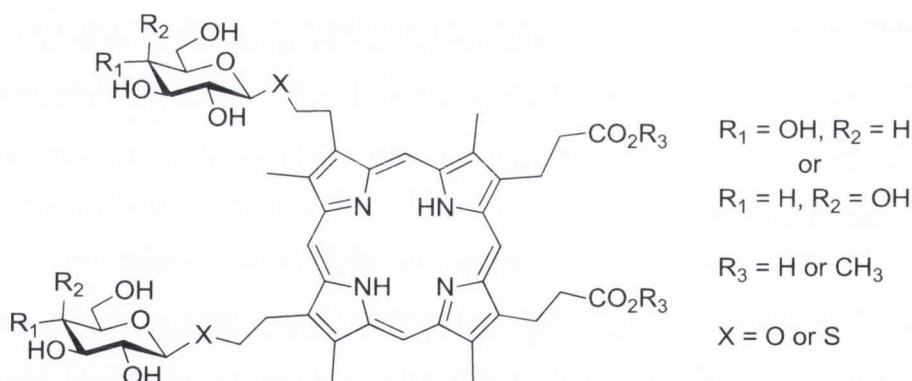


Figure 1.16 - First semi-synthetic glycoporphyrin

Thio linked analogues of Figure 1.16, were prepared in 2001 by Krausz *et al.*¹¹⁹ These compounds were prepared by displacing a bromide on the alkyl chain of the porphyrin with a thio-hemiacetal nucleophile of Glc or Gal. A mixture of α and β anomers were isolated, due to anomerisation of the free thiol starting material. There are biological benefits to non oxygen linked glycosides by virtue of the enhanced enzymatic stability of the glycoporphyrins produced. If PDT targeting is mediated through the sugar it makes

chemical sense to incorporate a carbohydrate moiety which is difficult to cleave enzymatically. The use of halide, thioglycoside and trichloroacetimidate donors to glycosylate free hydroxyls on porphyrins is also a viable route.^{116, 120-121} Here, stereoselectivity must be directed by the protecting group patterns.

Ester linked¹²² cellulose protoporphyrins (**24**, **25**, Figure 1.17), and C-2 amide linked analogues¹²³ of 2-deoxy sugars (**26**, Figure 1.17) have also been prepared. Glycoporphyrin **26**, demonstrated unusual micelle and fibre formation, but despite an interesting and PDT relevant structure, it was not investigated for use as a photosensitizer.

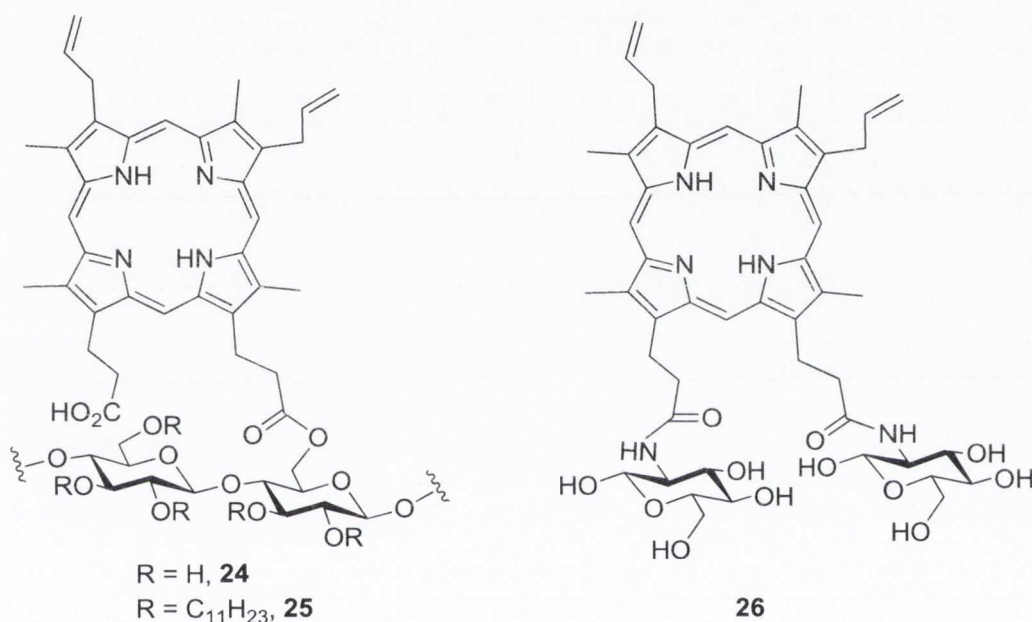


Figure 1.17 - Ester and amide linked protoporphyrin derivatives

1.4.2.2 Synthetic glycoporphyrin - condensation

The early work on carbohydrate functionalised protoporphyrins confirmed singlet oxygen production was not adversely affected by carbohydrate introduction. As the second generation PDT agents became available with their improved red light absorption, chemists turned their attention to alternative methods of attaching sugars to porphyrin scaffolds. Condensation of a pyrrole with a carbohydrate functionalised aldehyde using acid or Lewis acid catalysis followed by oxidation, has been used to make glycosylated porphyrins in one step, adopting the Lindsey,¹²⁴ Rothemund,¹²⁵ or Alder¹²⁶ techniques.

Mixing aldehydes of different proportions allows the synthesis on mono-, di- or trisubstituted glycoporphyrins (Figure 1.18). The carbohydrates are usually protected during condensation and deprotected following the synthesis. Again for the benefit of enzymatic stability, Drain *et al.* prepared a series of C and S linked glycosides attached to

aldehydes for condensation.¹²⁷ Yields in this paper were reported as greater than 50 %, but mean yields from condensation reactions can be of the order of 10 % or so. For this reason only cheap commercially available carbohydrates have been used to synthesise glycoporphyrins through condensation. In general the yields are not high enough to justify the use of complex synthetic oligosaccharides in condensations. The conditions of strong acid or Lewis acid catalysis together with long reaction times are also a drawback, especially if weak glycosidic linkages are present. For complex carbohydrates, alternative means must be used. Impressive attempts have been made for glycoporphyrins synthesised via condensation however, the strapped trehalose glycoporphyrin (**29**, Scheme 1.3)¹²⁸ from di-pyrrole **27**, and a di-aromatic aldehyde spaced using a trehalose, **28**, is one example which would be quite difficult to synthesise by other means due to the labile nature of the glycosidic linkage in trehalose.

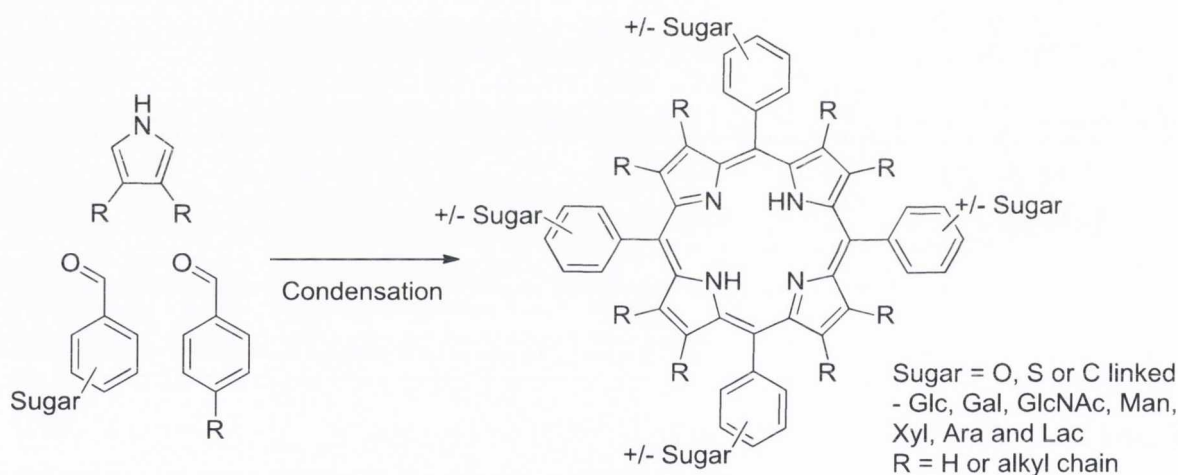
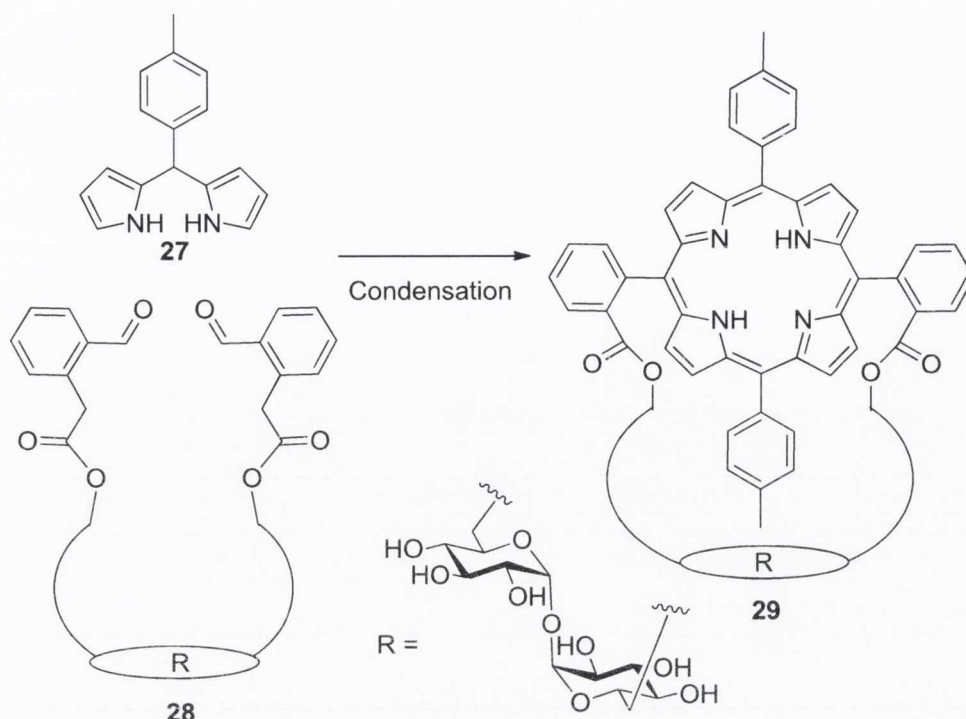


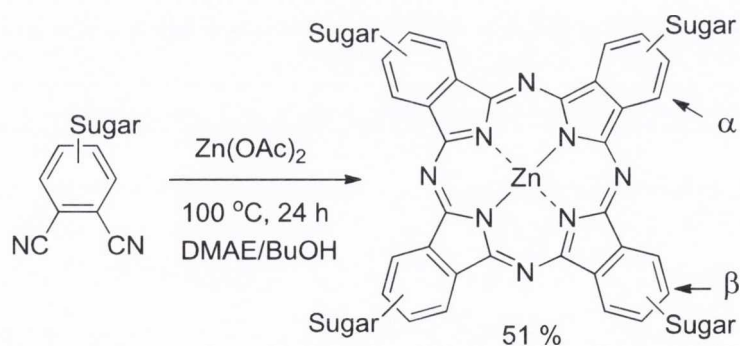
Figure 1.18 - Mixed condensation route to glycoporphyrins



Scheme 1.3 - Strapped glucose trehalose glycoporphyrin synthesis

1.4.2.3 Synthetic glycopthalocyanine - condensation

The use of condensation reactions is more acceptable in the synthesis of glycopthalocyanines as yields are in the order of 50 % or more. Carbohydrate pre-functionalisation of the desired di-cyano benzene is required to prepare general structures such as (Scheme 1.4).¹²⁹⁻¹³⁰



Scheme 1.4 - Regioisomeric glycopthalocyanines preparation

Spacer groups, such as alkyl chains or PEG linkers can be employed between the sugar and the phenyl ring to allow proteins greater accessibility to the sugar. The main drawback with glycopthalocyanine preparation through condensation, is the regioisomeric distribution of the products. This problem is removed if 4,5 or 3,4,5,6

substitution on the benzene core is performed. One such example is the water soluble product (**30**, Figure 1.19) prepared as a mixture of 6-O linked galactose hemiacetals, by Ribeiro *et al.*¹³¹

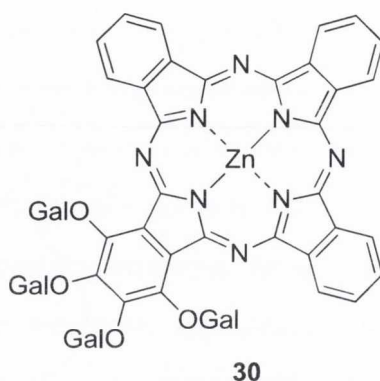


Figure 1.19 - Glycophthalocyanine

1.4.2.4 S_N2 displacement to synthesise O/S linked glycoporphyrins

One issue with the condensation route to carbohydrate substituted porphyrins and phthalocyanines, is the lack of stereoselectivity and formation of regioisomeric products. This is either due to a mixed isomer of the glycoside being used in the synthesis, or through the *in situ* anomerisation when using Lewis acids during condensation in anhydrous conditions. An alternative route widely published in the literature is the nucleophilic displacement of a halide on a suitably functionalised carbohydrate. The advantage from this convergent synthesis is that single isomers can be prepared, one only has to react expensive starting materials near the end of the route, and the yields should be higher. A generic electrophile for this route which is easily prepared in short number of steps is shown in Figure 1.20.

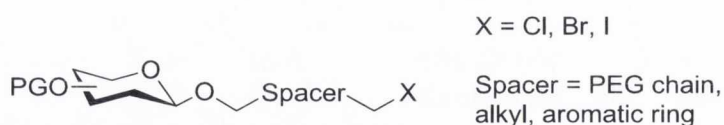


Figure 1.20 - Nucleophilic displacement linkers

Spacers used for nucleophilic displacement glycoporphyrin formation have included O and S linked polyethylene glycols, like the mannose substituted example prepared by Blais *et al.* (**31**, Figure 1.21)^{38, 107}, alkyl chains¹²³ and the cationic example (**32**, Figure 1.21) prepared following the reaction of a pyridine displaying porphyrin with ClAc pre-

functionalised cellulose.¹³² Compound **32** is under investigation for use as an antibacterial PDT thin film. Presumably with its positive charge and interchelation ability, this compound may interact also with DNA although such tests have not yet been carried out. Nucleophilic S_N2 displacement allows the number of carbohydrate substitutions on the porphyrin to be controlled through stoichiometry in the reaction. The length of the chains can easily be tailored to investigate binding affinities, and the method is amenable to the use of complex carbohydrates suitably functionalised in the anomeric position.

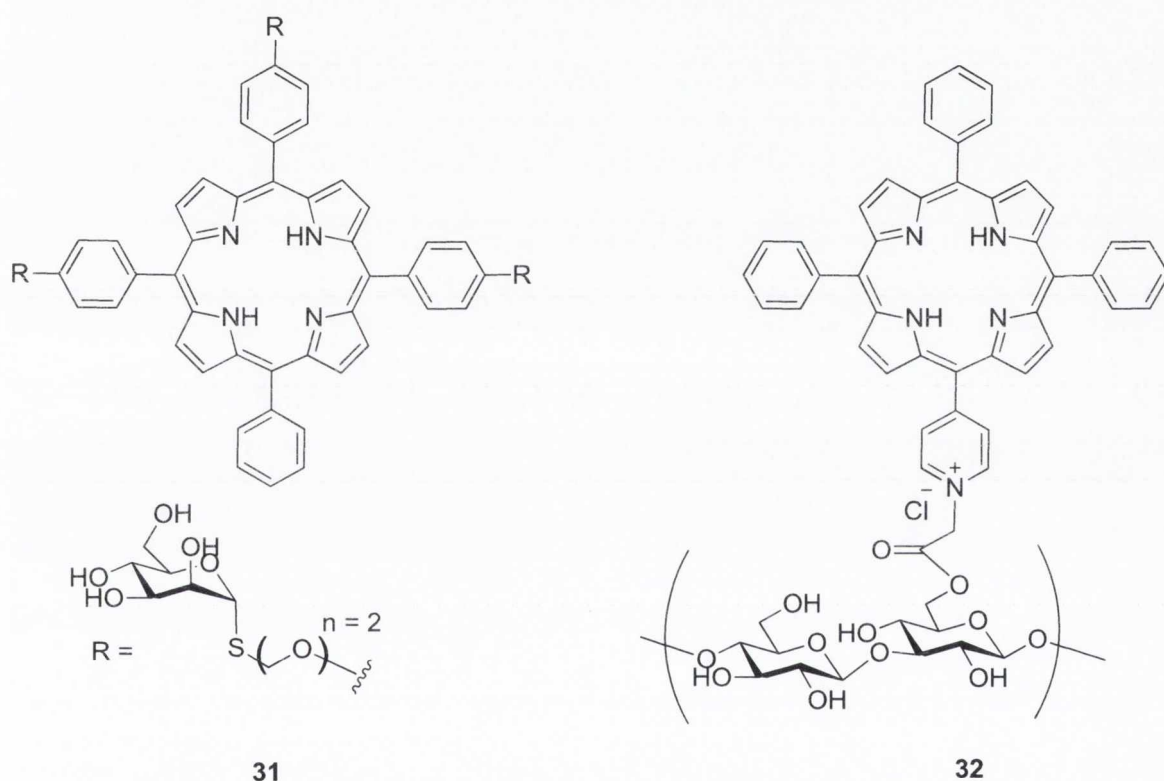


Figure 1.21 - Glycoporphyrins prepared through nucleophilic displacement

1.4.2.5 S_NAr in glycoporphyrin synthesis

The ability to perform nucleophilic aromatic substitution of highly fluoro-substituted aromatic rings has been used to synthesize multifunctionalised glycoporphyrins and glycophtalocyanines. Boyle *et al.* developed a method whereby a nucleophilic thiol displaces the para fluoro group on a pentafluorophenyl substituted porphyrin, providing the cationic, β product (**33**, Figure 1.22).¹³³

Later Cavaleiro *et al.* used S_NAr on a per-fluorinated tetraphenyl porphyrin with the alkoxide of 1,2-3,4 di-isopropylidene protected glucose to ultimately prepare a 6-O linked glycoporphyrin (**34**, Figure 1.22).¹²⁰ Mixed di-, tri-, and tetra substituted examples if this could be prepared by the same route.

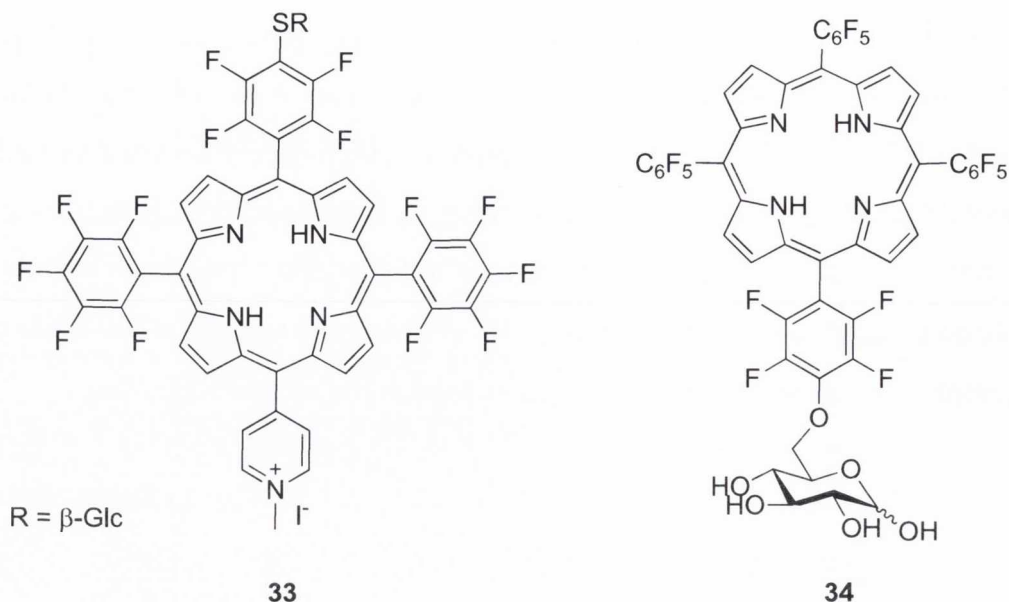


Figure 1.22 - S_NAr on the penta fluorophenyl group

Building on the ideas behind the synthesis of **34**, Hirohara *et al.* altered equivalents of free thioglycoside in the reaction to successfully make the tetra substituted glycoporphyrin (**35**, Figure 1.23).¹³⁴ Less substituted isomers were also prepared, and structure activity relationships of these were investigated for both phototoxicity and uptake.

Recently Drain *et al.* has applied the S_NAr route to the synthesis of an octo-substituted glycoporphyrin (**36**, Figure 1.23), recording an impressive yield of 72 % for the nucleophilic displacement reaction.¹³⁵ Regioisomers are avoided with this route, however, due to significant broadening of the NMR spectra from aggregation, anomeric configurations could not be confirmed.

In conclusion there is little doubt that nucleophilic aromatic substitution of fluorinated porphyrins, provides a viable synthetic route to glycosylated porphyrinoids. The reactions tend to run at ambient temperature and are complete in 1 to 4 days. A base is often required to drive the reaction to completion, depending on the nucleophile. There are limitations to the porphyrin scaffold that can be used however, and the results from ongoing biological studies will provide any cost/benefit analysis in carrying the extra fluorine atoms through to the final product.

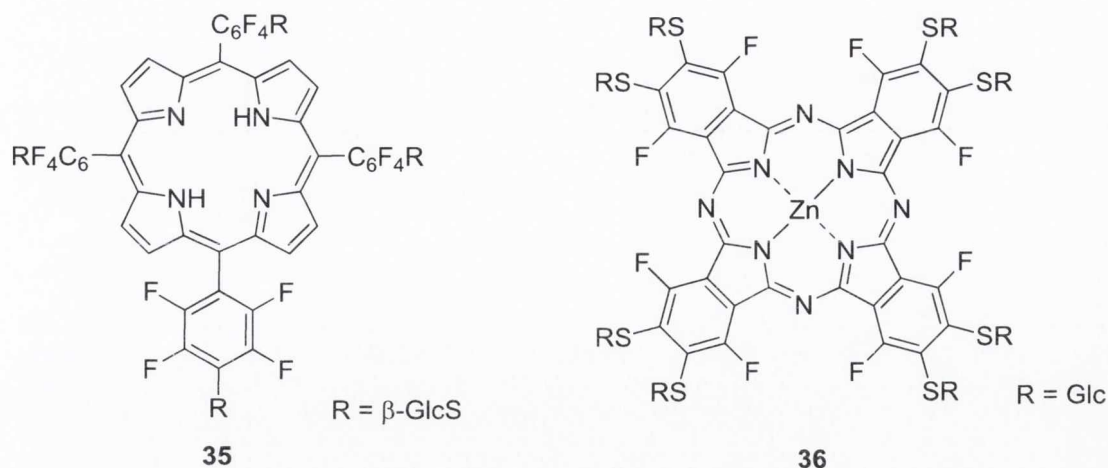


Figure 1.23 - Highly glycosylated S_NAr PDT products

1.4.2.6 Coupling reactions and the synthesis of glycoporphyrinoids

In keeping with the requirement for high yielding conjugation and convergent synthetic routes to glycoporphyrins, it is surprising the lack of coupling reactions employed in their synthesis. It would seem comparatively easy to introduce a substrate for most cross coupling reactions at the reducing end of a complex carbohydrate, and use this to ensure high conjugation yields. Indeed lectin binding studies of monosaccharides vs the same sugar containing an aromatic group in or near the anomeric position have shown a hydrophobic binding site is accessible to increase binding.¹³⁶ This could be an important result to bear in mind. To date only limited examples are available in the literature of glycoporphyrins produced via coupling reactions. Grubbs cross metathesis was employed, to synthesise a glycopurpurinimide¹³⁷ (**37**, Figure 1.24), which could be further functionalised through Diels Alder chemistry. Pandey *et al.* in two more papers¹³⁸⁻¹³⁹ used the same porphyrin derivative to great effect with Pd cross coupling of propargyl Lac, yielding the disaccharide containing PDT agent (**38**, Figure 1.24). Suzuki couplings of a ribose substituted bromo quinolone and boronic ester functionalised TPPZn were successfully employed by Cavaleiro *et al.*, to yield (**39**, Figure 1.24) as an antibiotic with PDT activity.¹⁴⁰

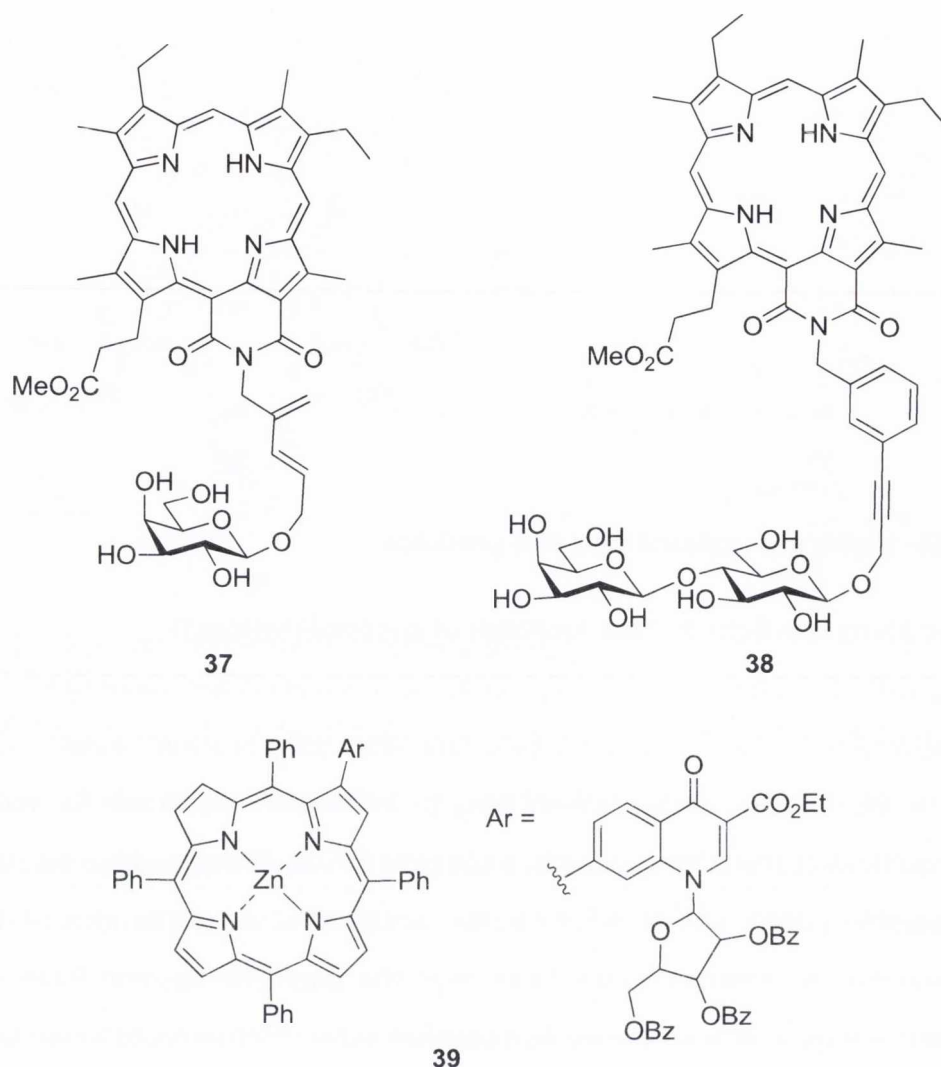


Figure 1.24 - Cross metathesis and Pd cross coupling - glycoporphyrinoid products

1,3 dipolar cycloadditions have been applied to the field of glycoporphyrin synthesis, using suitably functionalised carbohydrate starting materials (Figure 1.25). Galactose nitrones were employed to prepare isoxazolidine fused chlorins, by Dondoni *et al.*¹⁴¹ (**40**, Figure 1.26). This shifted away from the commonly employed meso substitution. The coupling yields reported for compound **40**, were between 18 - 37 %, but the product had favourable optical properties with a red shifted UV/Vis absorption spectrum towards 646 nm. Cavaleiro *et al.* using elegant chemistry, prepared a series of other cycloaddition glycopheophorbide products, employing azomethine ylides¹⁴² (**41**, Figure 1.26), and diazoacetates¹⁴³ (**42-45**, Figure 1.26), containing both O and C linked glycosides.

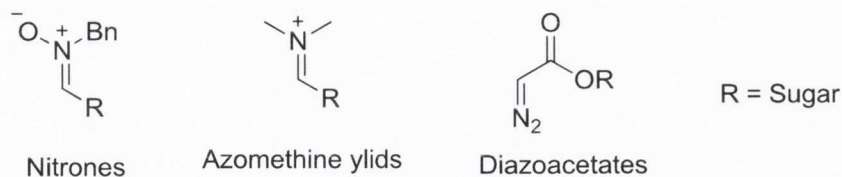


Figure 1.25 - Carbohydrate cycloaddition substrates

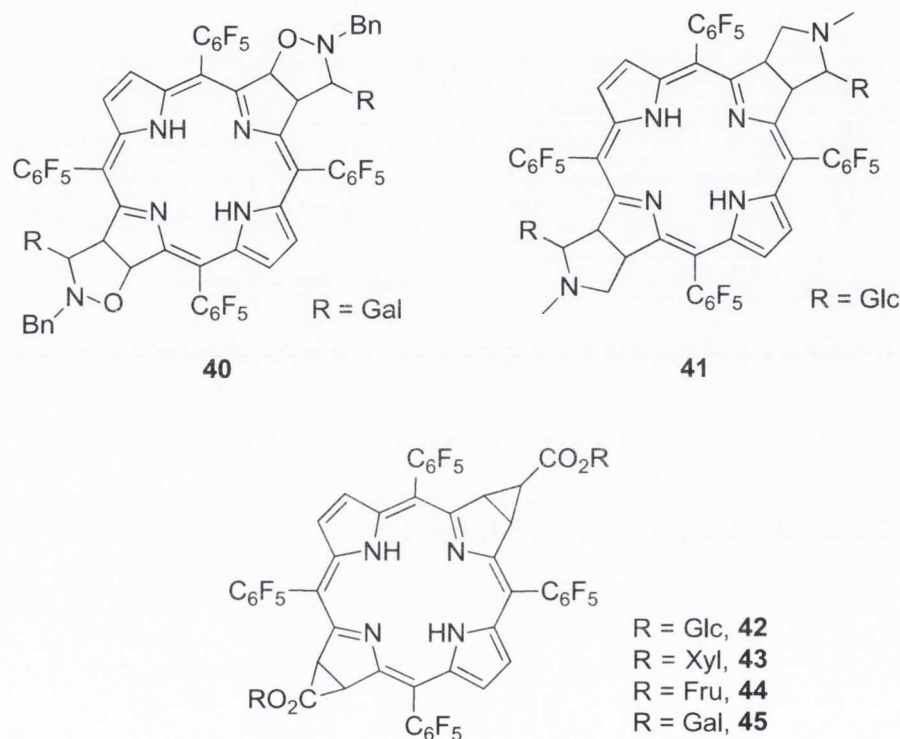


Figure 1.26 - Cycloaddition glycoporphyrinoid products

1.4.2.7 CuAAC “Click” couplings and glycoporphyrin synthesis

The copper (I) catalysed alkyne azide cycloaddition has seen an explosion in citations since its discovery both independently by Sharpless *et al.*¹⁴⁴ and Meldal *et al.*¹⁴⁵ Some of the advantages as applied to the chemistry of porphyrins are discussed by Ahsen *et al.*¹⁴⁶ The benefit of such a clean and high yielding strategy, has led to numerous applications in the field of glycoporphyrin production too, with triazole linked examples at the C-6 and C-1 positions.

The first example of CuAAC in glycoporphyrin synthesis was reported by Grin *et al.* in 2008.¹⁴⁷ Lactose was thermally ligated to a chlorin (**46**, Figure 1.27), in order to take advantage of galectin over expression in cancer cells. Initially Cu (II) insertion was an issue. However, the use of Zn (II) as a protecting group for the porphyrin avoids production of the Cu (II) side product. Zn (II) removal is possible through much milder

acidic conditions than Cu (II) removal. The group later expanded their methodology to include lactose coupled chlorophyll A and bacteriochlorophyll A products.¹⁴⁸⁻¹⁴⁹

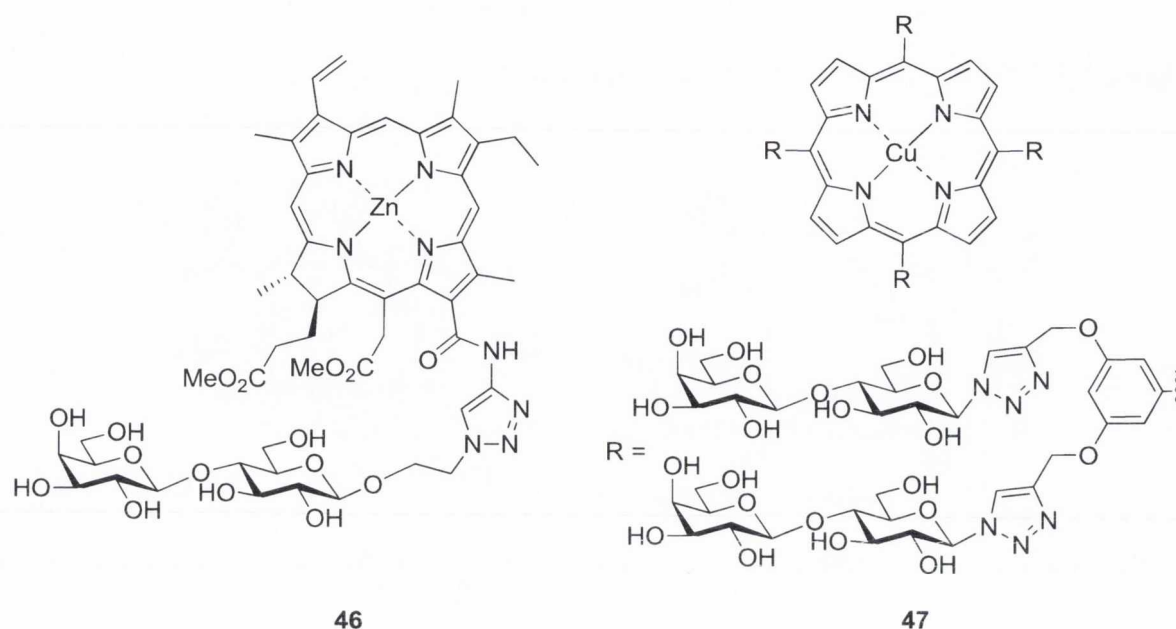
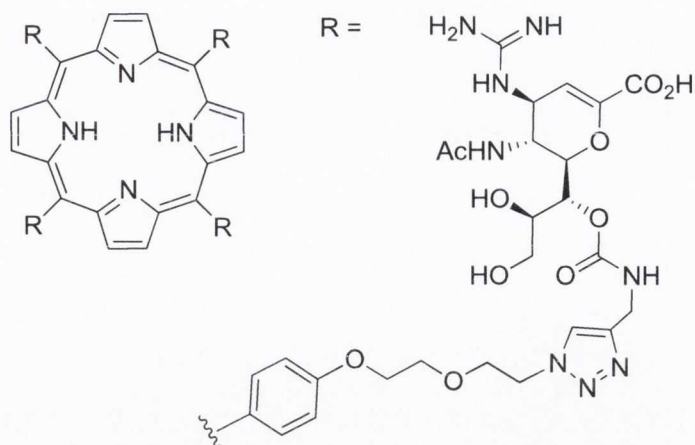


Figure 1.27 - Thermal CuAAC glycoporphyrinoids products

A water soluble octa lactose substituted glycoporphyrin (**47**, Figure 1.27), with Cu (II) in the porphyrin core, was presented by Hasegawa albeit in low yields of 18 %.¹⁵⁰ The group reported difficulty in analysing the compound by NMR in D₂O. Presumably the reasons for this were two fold. Aggregation being one, but also the copper (II) is paramagnetic and its presence would interfere heavily with the NMR analysis of **47**. Given the cluster arrangement of terminal galactose, it would be interesting to investigate how lectins interact with this multi epitope presentation.

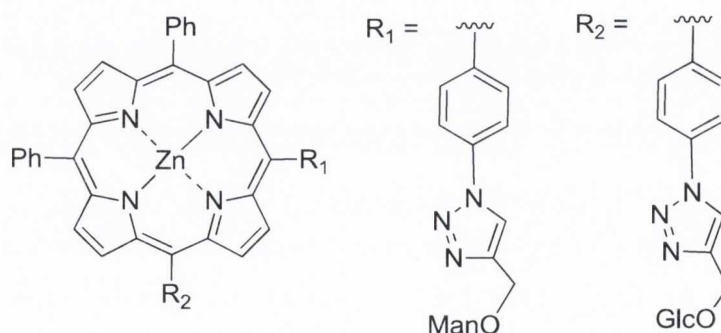
Wong *et al.* were interested in using the proven targeting ability of an existing drug to direct glycoporphyrin structures to their required sites. Using click chemistry they ligated Zanamivir **13**, in 78 % yield¹⁵¹ to prepare the dual agent (**48**, Figure 1.28).



48

Figure 1.28 - Tetra Zanamivir substituted porphyrin

The Scanlan group recently reported the first examples of microwave mediated CuAAC glycoporphyrin synthesis with high yielding reactions (> 90 %) and reaction times of 20 min.¹⁵² A series of acetyl protected mono-, di- and tri- substituted Man, Glc, and GlcNAc examples were prepared and deacetylated using Zemplén conditions. Hetero substitution patterns have been achieved through a two step, sequential click process (49, Figure 1.29). This represents another step towards the goal of high specificity targeting with glycoporphyrins, as different oligosaccharides can now be presented on the same scaffold. To date these are the highest yielding glycoporphyrin procedures, and have since led to an adoption of μ W CuAAC glycoporphyrin synthesis by a number of other groups.



49

Figure 1.29 - Hetero substituted μ W mediated CuAAC glycoporphyrin

Triazole linked glycoporphthalocyanines have also been prepared. Lafont *et al.*¹⁵³ reported a series of water soluble PEG linked sugars, displaying lactose as the most complex carbohydrate (50, Figure 1.30). Schotten *et al.*¹⁵⁴ detailed the octa substituted

phthalocyanines (**51**, **52**, Figure 1.30) also applying CuAAC chemistry. These compounds were prepared for the investigation of both self assembly, and PDT properties. **52**, would make a very interesting galectin probe/binder. Phthalocyanines **50-52** all used the presence of Zn (II), to avoid Cu (II) insertion during the CuAAC reaction.

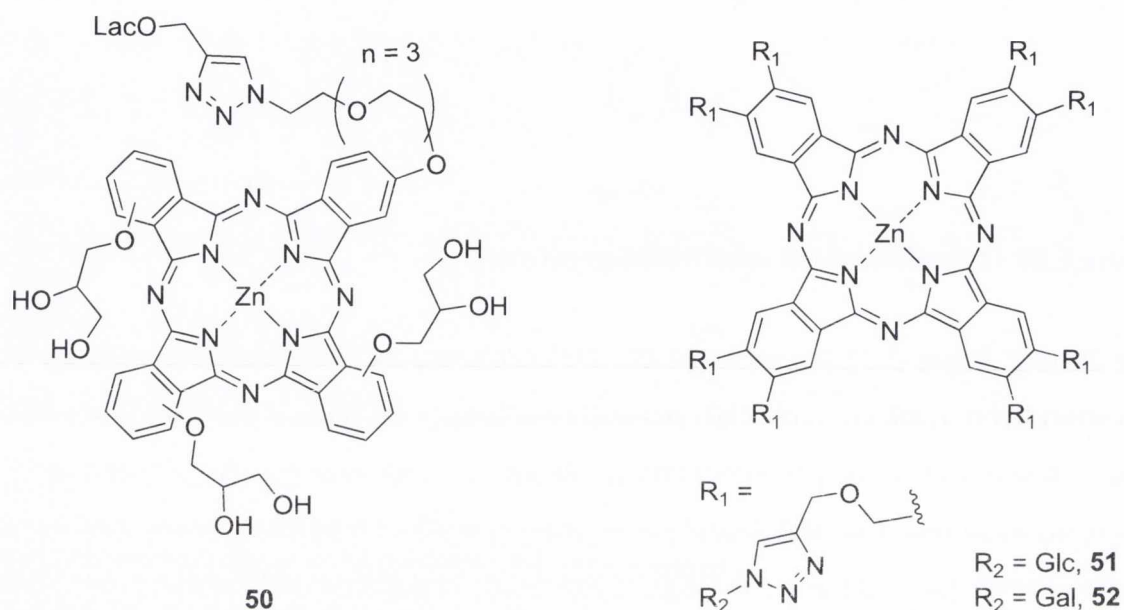


Figure 1.30 - Triazole ligated phthalocyanines

Two of the most recent reports into glycoporphyrin synthesis both employ microwave CuAAC reaction conditions. Maillard *et al.* successfully prepared the 5,10,15 trisubstituted glycoporphyrins (**53**, **54**, Figure 1.31), using the μW CuAAC reaction, and reporting yields of 40 to 80 %.¹⁵⁵ Vidal *et al.* were interested particularly in lectin interactions, and examination of cluster effects, through optimisation of linker length and the choice of linker.¹⁵⁶ The lactose substituted glycoporphyrin (**55**, Figure 1.31) is an example of their more promising porphyrin based lectin binders. Using the CuAAC reaction, both groups have reported marked increases in affinity over the monomeric carbohydrates for their respective lectin targets, and results from glycoporphyrins **53-55** have suggested cluster effects aid in these interactions.

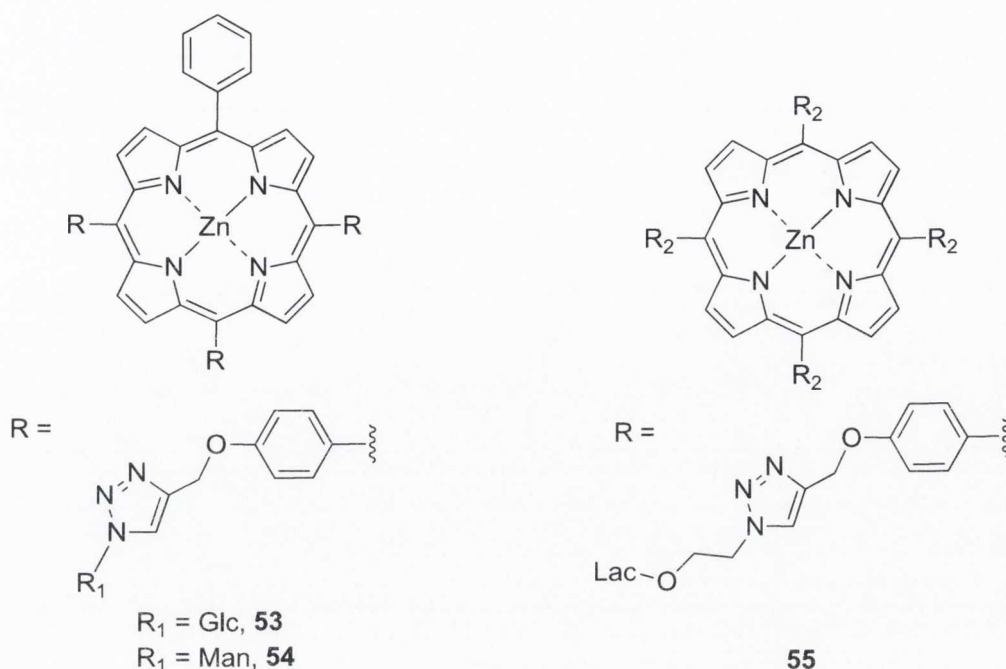


Figure 1.31 - μ Wave CuAAC glycoporphyrin products

In conclusion, CuAAC methods have allowed for high yielding functionalisation of porphyrins with carbohydrates. Advantages of this method are the short reaction times, and relatively clean product, in high yields. To date, the majority of procedures employ protected carbohydrates before covalent attachment to the PDT agent. High yielding glycoporphyrin production from deprotected sugars is a key requirement for the synthesis of examples utilizing more complex carbohydrates.

1.4.2.8 Selected dendrimeric glycoporphyrinoids

Having discussed a series of possible methods of attaching carbohydrates to porphyrin scaffolds and bearing in mind the advantages of cluster effects for increasing binding affinity rates, combinations of both these ideas have also been investigated. Some selected highlights from this style of compound are presented below. Stoddard *et al.* as part of investigations into the redox responses and aggregation effects of glycosylated porphyrins, prepared a dodeca substituted glycoporphyrin (**56**, Figure 1.32).¹⁵⁷ An interesting point they noted during photophysical measurements of **56**, was an inter-molecular interaction in aqueous media which leads to different two lifetimes of excited states upon irradiation. Aggregated glycoporphyrins were shown to present shorter lived excited states following irradiation. As a PDT agent though, **56** does not meet general requirement of amphiphilicity discussed briefly in Section 1.1.6.4.

A highly unusual study conducted by Hayashi *et al.* detailed the incorporation of a galactohemin into myoglobin.¹⁵⁸ A di-galactose substituted amido spacer was prepared which was ligated via an amide coupling to yield a tetra glycosylated porphyrin (**57**, Figure 1.32). Interestingly, **57** could be reintroduced to heme free myoglobin, which incorporated the porphyrin into its own structure. This result is an important point to remember, when considering possible bioavailability issues of glycoporphyrins *in vivo*.

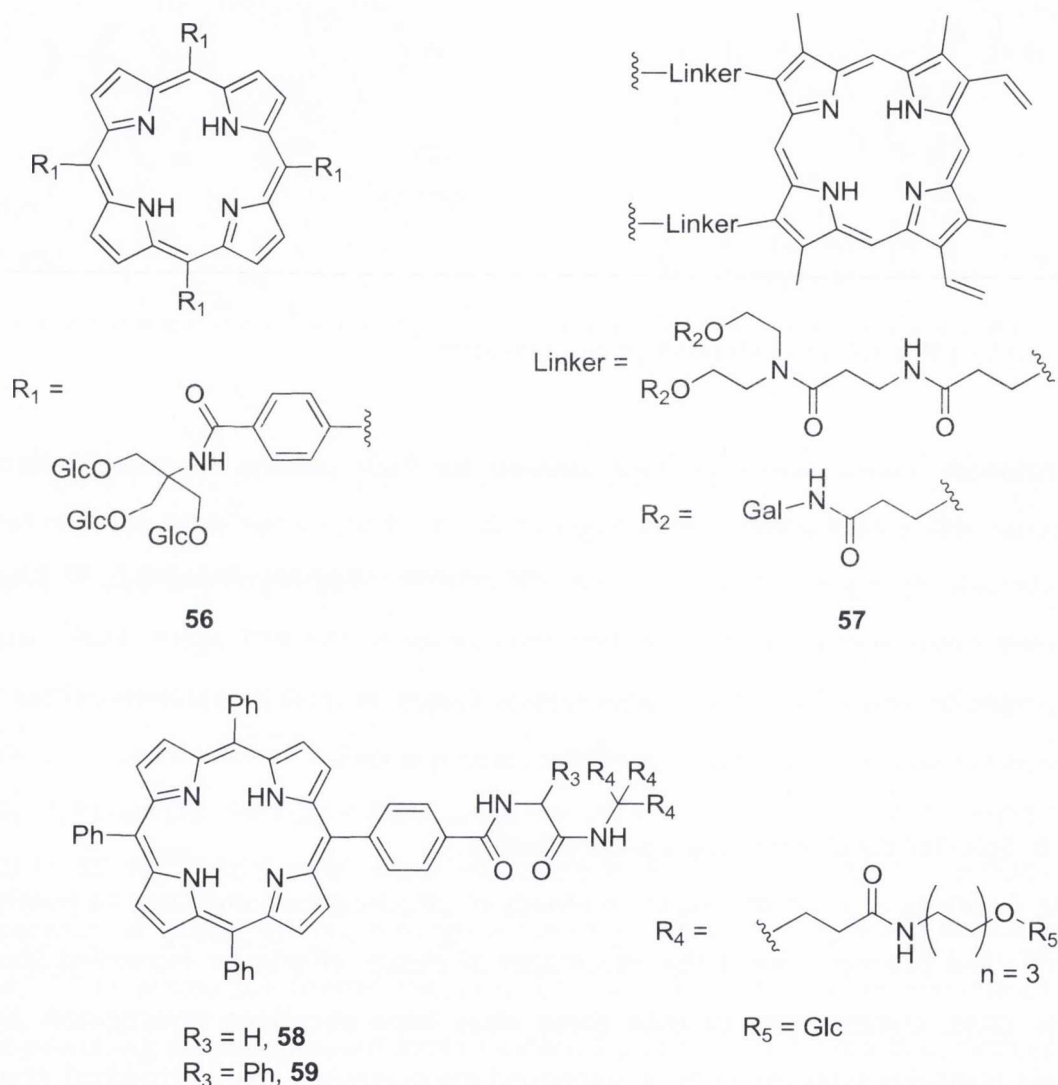


Figure 1.32 - Amide linked porphyrin glycoclusters

Maillard *et al.*¹⁵⁹ in a more recent study, investigated tuning logP of a photosensitizer, by ligating a glycocluster to a porphyrin core through a glycine or phenylalanine moiety (**58**, **59**, Figure 1.32). Amide coupling yields ranged from 32 to 90% in attaching the β -Glc cluster. Analogues of **58** and **59** were also prepared displaying β -Gal and α -Man.

study is required into the choice of linker most suitable to either remain inert, or enhance PDT activity. From a medicinal chemistry point of view it is not unreasonable to think that the spacer groups such as those described in Figure 1.33, could contribute just as much to uptake and localisation as the porphyrin or carbohydrate themselves. A balance needs to be maintained between the complexity of the linker and the advantage provided by its incorporation.

1.4.3 Selected investigations and biological studies of glycoporphyrinoids

The requirements of 3rd generation PDT agents are an improvement in solubility, localisation, and selectivity. A summary of the available biological results for the previously discussed glycoporphyrinoids, is presented below.

Tolyporphyrins (**22** and **23**, Figure 1.15) were tested for PDT activity in a mouse mammary tumour cell line, EMT-6. *In vitro* and *in vivo* imaging confirmed localisation of the PDT agent in the ER and Golgi apparatus.¹⁶² A 5000 fold increase in cell death was reported as compared to Photofrin (**4**, Figure 1.4). The cytotoxicity was attributed to ER damage. ER and Golgi localisation almost certainly results from the cell processing the glycoporphyrin's carbohydrate functionalities. The *in vivo* study reported 10 times less liver localisation as compared to a pheophorbide PDT agent.

The oxygen linked, early generation semi-synthetic GP analogues in Figure 1.16, produced similar $^1\text{O}_2$ profiles to rose Bengal, upon irradiation.¹¹⁸ However, no subsequent PDT studies have yet been published on this series of compounds. The thio analogues (Figure 1.16), were evaluated and were shown to be unaffected by a glycosidase. *In vitro* studies were performed on the chronic leukaemia cell line K562. Interestingly 24 hours after irradiation, the methyl ester analogues were found to be as effective as photofrin, however, following ester hydrolysis, the carboxylic acid analogues were inactive. *In vitro* localisation tests were not performed so it can only be speculated on whether a difference in activity was due to lack of uptake, or localisation.

Thin films of (**25**, Figure 1.17) were evaluated in Petri dishes as bactericidal surfaces. The presence of light inhibited the growth of both *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram-negative).¹²²

The series of tetra substituted C and S linked glycoporphyrins (Figure 1.18), were evaluated for photodynamic activity through an unusual DNA cleavage assay.¹²⁷ Although DNA is not the site of PDT toxicity, $^1\text{O}_2$ induced cleavage could be used to model future

PDT activity. Significant DNA cleavage was not observed, so these compounds were not investigated further. The authors dismiss the $^1\text{O}_2$ production of glycoporphyrins following the poor DNA cleavage. However, there could be another explanation for the disappointing results. DNA interchelation with a porphyrin before $^1\text{O}_2$ production is required for DNA cleavage to be observed. A tetra functionalised porphyrin may not interchelate efficiently due to the steric clashing provided by the carbohydrate groups.

Whilst it is likely the compounds described by (Figure 1.18), do not have the required amphiphilicity to be taken up *in vitro*, the lack of DNA cleavage could be improved through the removal of carbohydrate groups on at least one side of the molecule.

The trans bridged trehalose glycoporphyrin (**29**, Scheme 1.3), was reported to show similar $^1\text{O}_2$ generation to HP, but showed 50 % less toxicity during an *in vitro* study on the K562 cell line.¹²⁸ The ability of the porphyrin to approach the cell membrane before endocytosis could play a role in the lower toxicity, but uptake studies have not yet been reported to confirm this.

Ng *et al.*¹³⁰ investigated the $^1\text{O}_2$ production of mono α , mono β , tetra α and tetra β analogues (Scheme 1.4). Using DPBF as a $^1\text{O}_2$ scavenger, the tetra substituted analogues, having α substitution patterns were reported to provide the highest singlet oxygen generation. Presumably, α substitution disrupted self aggregation. In contrast, the mono substituted compounds performed the best during *in vitro* studies with human colon adenocarcinoma (HT29) and human hepatocarcinoma (HepG2) cells. Further investigation of this through confocal microscopy confirmed that the tetraglycosylated examples were not taken up due to amphiphilic deficiencies.

Glycophthalocyanine (**30**, Figure 1.19), was prepared as a supramolecular tool compound, and despite interesting characteristics, PDT biological evaluation was not performed.

The PEG linked glycoporphyrin (**31**, Figure 1.21), as well as the Man and Gal, O and S linked analogues, illustrate the ability of carbohydrates for cell targeting.^{38, 107} All examples were shown to have similar $^1\text{O}_2$ generation. However, the uptake of a Man substituted example **31**, was five times higher in a human retinoblastoma cells (Y79) as compared to a non mannosylated PEG linker, and two times higher than the Gal analogue. Interestingly the thio linked derivatives showed about 3 times less uptake *in vitro* for the Man example **31**, over the oxygen linked analogue, but similar uptake for both the Gal examples. Co-localisation studies were not performed, but the compounds appeared from confocal microscopy to localise in the perinuclear region and cytoplasm,

and not the nucleus. An active uptake process was reported to be involved, both Gal and Man examples showing 50 % less localisation, if glucose was removed from the cell culture media.

The cationic porphyrin (**32**, Figure 1.21), was prepared as a thin film and evaluated for anti bacterial activity. The authors reported anti-*Staphylococcus aureus* (Gram positive) and anti-*Escherichia coli* (Gram-negative) activity.¹³²

The cationic glycoporphyrin (**33**, Figure 1.22) was reported by Boyle *et al.*¹³³ to demonstrate enhanced mitochondrial localisation properties, and lowering of dark toxicity as compared to a non glycosylated example, in the HT-29 cell line.

Cavaleiro *et al.* compared the antiviral effects of (**34**, Figure 1.22) by evaluating *Herpes simplex 1* and *Herpes simplex 2* infections of Vero cells. Favourable results were found as compared to acyclovir and foscarnet using deprotected sugars in the presence of low levels of white light¹²⁰, and the control cells, prior to *Herpes simplex* infection, showed no phototoxicity. This general effect however, was not specific for either *Herpes simplex 1* or 2, so the compounds are under investigation as antiviral blood purifying agents.

Hirohara *et al.* employing mono to tetra S linked per fluoro glycoporphyrin analogues of (**35**, Figure 1.23) found an unusual result. The 5,15 di-substituted compound showed the best PDT activity with, 3 times the uptake of the 5,10 example in HeLa cells.¹³⁴ This compound is less amphiphilic and would be expected to display lower cellular uptake. Type I versus Type II PDT activity was investigated, with reports of significant Type I contributions, almost equal to the Type II contributions observed for the 5,15 example.

Drain *et al.*¹³⁵ attempted to take advantage of the over expression of glucose transporters by attaching 8 Glc residues to a perfluorophthalocyanine core (**36**, Figure 1.23). $^1\text{O}_2$ production was reported to be comparable at 700 nm to the non-glycosylated analogue. In aqueous media however, severe aggregation occurred, with little or no intracellular fluorescence observed in a breast cancer cell line (MDA-MB-231). However, 4 days after fixing, fluorescence had markedly increased, with the authors citing phthalocyanine disaggregation during storage of the dead cells. The likelihood of Glc being recognised so close to the phthalocyanine core was not discussed, however, further studies incorporating short spacers between the scaffold and the sugar would probably be an advantage.

Pandey *et al.* rationally designed the conjugated glycopurpurinimide (**37**, Figure 1.24), and later showed the enhanced efficacy of Lac substitution over Gal substitution.¹³⁷ Molecular

modelling was employed to confirm the enhanced fit of a purpurinimide displaying Lac over Gal into a binding cleft. Introducing a conjugated diene restricts rotation for decreased entropic penalties upon binding. RIF mouse tumours were successfully treated with both models.

Glyco-purpurinimide (**38**, Figure 1.24), with increased rigidity, showed less PDT activity than **37**, and an uptake study performed on a series of analogues prepared by altering the attachment position of the Gal/Lac around the scaffold, led to an interesting conclusion. It was found that the uptake order of Glc, Gal and Lac substituted examples *in vitro* did not match the *in vivo* efficacy.¹³⁸

Caveleiro *et al.* investigated the antibacterial properties of the Suzuki coupling product, (**39**, Figure 1.24). Having confirmed the $^1\text{O}_2$ production was superior than that of TPPH₂, incubation of these hydroquinoline based glycoporphyrins with *Leishmania braziliensis*, a protozoa responsible for Leishmaniasis led to 30 % inhibition with 80 seconds of irradiation.¹⁴⁰

To date, biological studies on glyco-bacteriochlorins (**40** and **41**, Figure 1.26) have not been completed.¹⁴¹⁻¹⁴²

The glyco-bacteriochlorins (**42-45**, Figure 1.26), showed good $^1\text{O}_2$ generation and during *in vitro* evaluations, appeared to show localisation in both HeLa and (non cancerous) HaCaT cell lines, with no dark toxicity.¹⁴³ Interestingly, the Gal substituted compounds show *ca.* 45 % more toxicity in the HeLa cell line after 15 minutes irradiation at 10^{-6} M.

$^1\text{O}_2$ production of the first 1,2,3-triazole containing glycochlorin (**46**, Figure 1.27), was investigated by Grin *et al.*, who examined the effect of the triazole and Lac substitution. No negative effect on $^1\text{O}_2$ production from either moiety was described, but no *in vitro* studies were subsequently carried out on this compound.¹⁴⁷ Following expansion of this series to bacteriochlorins, the same group reported that Lac substituted scaffolds over other monosaccharides showed the highest PDT activity during *in vitro* screens of a Hep2 cell line.¹⁴⁹

Hasegawa *et al.* reported, that an octo-Lac substituted porphyrin (**47**, Figure 1.27) displays specificity for a *Recinuscomunus agglutinin*, a Lac recognising lectin, but only showed non-specific binding for Con A.¹⁵⁰ It was argued that excessive substitution around the porphyrin is important for efficient lectin binding *in vivo*, in order to disrupt non specific porphyrin binding, but the negative effects on the cellular uptake of such a highly polar molecule were not discussed.

A combination of the anti-influenza and carbohydrate derived drug Zanamivir **13**, and PDT was performed by Wong *et al.*¹⁵¹ The ability of compound (**48**, Figure 1.28), to inhibit neuraminidase, was lowered between 10 and 20 fold, presumably as the structure of **13** has been well optimised to inhibit the enzyme. However, the toxicity in light was over 100 fold better than **13** alone. It was argued that tight binding of the Zanamivir moiety to neuraminidase on the surface of the virus particle, increased the local concentration of the PDT agent, resulting in virus destruction upon irradiation.

Lafont *et al.* synthesised a glyco-phthalocyanine with increased water solubility¹⁵³ through the use of a 1,2 diol moiety, in conjunction with a PEG linker and lactose (**50**, Figure 1.30) to investigate cell selectivity. (**51** and **52**, Figure 1.30) represent a highly functionalised phthalocyanine with a direct sugar to triazole linker, prepared by Schotten *et al.* to investigate lectin and PDT activity,¹⁵⁴ however, to date, biological studies on **50-52** have not been published.

Maillard *et al.* reported the $^1\text{O}_2$ production of (**53** and **54**, Figure 1.31) to be higher than TPPH₂.¹⁵⁵ Cellular studies presented some interesting results. The α -Man analogue **54** was reported to display 5 times more phototoxicity than Glc analogue **53**, in the cell line Y79. This result was positive as the Y79 cell line expresses a Man recognising lectin. In the HT29 cell line, the PDT activity was lower than Y79 for both **53** and **54**. The glycoporphyrin **54**, was surprisingly less active in HT29 cell line than a non triazole containing analogue (**31**, Figure 1.21), previously investigated,¹⁰⁷ however, both **54** and **31** had similar activity in the Y79 cell line. This is the first direct SAR comparison of triazole functionality as compared to a PEG linker. The reason for the lower activity in one cell line is not clear, as both compounds were reported to have similar singlet oxygen production, greater than that of TPPH₂. Obviously the linker plays a role in cell selectivity.

Vidal *et al.* reported elegant lectin binding and hemagglutinin aggregation work on the triazole linked glycoporphyrin (**55**, Figure 1.31), demonstrating the best application yet of glycoporphyrins as lectin binders. Lac displaying, **55**, bound Galectin-1 with a potency 250 times¹⁵⁶ that of Lac alone. However, **55**, demonstrated only 4 times the affinity over Lac for an ECA lectin, (another Lac specific lectin). This data suggests that even amongst lectins which are specific for a particular sugar, additional specificity can be gained. The extrapolation of this finding to the differential lectin expression on cancer, virus or bacterial cells is extremely important. Unfortunately no PDT biological studies have been

carried out with **55** and analogues thereof, as it would be advantageous to analyse results from a large lectin study in tandem with an *in vivo/vitro* examination.

Within the dendrimeric series discussed, Stoddart *et al.* did not carry out biological studies on the glycoporphyrin (**56**, Figure 1.32).

Hayashi *et al.* have not reported any PDT studies on (**57**, Figure 1.32), but their study of the incorporation of galactohemin into myoglobin (Section 1.4.2.8),¹⁵⁸ could have far reaching applications in the study of porphyrin based PDT agents *in vivo*. There are currently no other published studies evaluating the incorporation of glycoporphyrins into myo- or haemoglobins, but the result should be noted when analysing tissue localisation in animal models. If substantial quantities of the PDT agent were encased in large proteins, as described by Hayashi, PDT activity could be rendered obsolete.

(**58**, **59** Figure 1.32) are interesting glycodendrimers, in that they display only 3 carbohydrate residues. Maillard *et al.* reported a stronger affinity for Con A with **58** and **59**, over the 5,10,15 trisubstituted triazole linked α -Man analogue (**54**, Figure 1.31). However, this increased binding did not translate into improved toxicity in the Y79 cell line.¹⁵⁹ In fact, **58** was over 10 times less phototoxic, and **59** was almost 20 times less phototoxic than the 5,10,15 substituted glycoporphyrin (**54**, Figure 1.31), under the same irradiation conditions in the Y79 cell line.^{155, 159} The result implies that Con A is not an adequate model of the α -Man recognising lectin on the surface of Y79 cells, and perhaps some homology models between the two lectins could allow for the better choice of glycoporphyrin spacer required to bridge this gap in knowledge.

Pandey *et al.* presented the elaborate structure (**60**, Figure 1.33), at an international conference on porphyrins and phthalocyanines.¹⁶⁰ It was disclosed that these compounds showed poor cellular uptake. Perhaps the number of redundant atoms in the spacer, and/or the amino thiazolines functionalities are affecting the uptake and localisation. No detailed biological study has been published to date on the results presented at the conference.

The final glycodendrimer to be discussed are the triazine spaced octa β -Gal displaying phthalocyanines (**61**, Figure 1.33). $^1\text{O}_2$ production has been successfully reported and the non specific interaction of **61** with Human serum albumin (HSA) was investigated through tryptophan fluorescence quenching. Using fluorescent titration measurements the authors confirm that **61** does indeed bind to HSA.¹⁶¹ Porphyrin mediated tryptophan quenching, suggested the dendrimers interact near to tryptophan sites on HSA.

Table 1.4 - Summary of PDT localization studies for glycoporphyrins and derivatives

PDT agent	Intracellular localisation	In vivo/in vitro/lectin specificity
(22, Figure 1.15)	Perinuclear region and ER	Lower concentration in liver
(Figure 1.16)	N/A	Slower cell death in K562 than HP
(25, Figure 1.17)	N/A	Anti bacterial Gram +/- activity
(29, Scheme 1.3)	N/A	50 % lower toxicity than HP in K562 cell line
(Scheme 1.4)	Perinuclear organelles, cytoplasm	N/A
(31, Figure 1.21)	Perinuclear region and cytoplasm.	Uptaken by Y79 and HT29
(32, Figure 1.21)	N/A	Anti bacterial Gram +/- activity
(33, Figure 1.22)	Mitochondria	Lower dark toxicity with glycosylation in HT-29 cells
(34, Figure 1.22)	N/A	Antiviral activity in <i>Herpes simplex I & II</i>
(35, Figure 1.23)	N/A	Uptaken by HeLa cells
(36, Figure 1.23)	N/A	Uptaken as nanoaggregates MB-231 cells
(37, Figure 1.24)	Lysosomes and Golgi	Gal 1/3 selectivity, RIF tumour toxicity
(38, Figure 1.24)	Lysosomes and Golgi	Gal 1/3 selectivity, RIF tumour toxicity
(39, Figure 1.24)	N/A	<i>L. braziliensis</i> anti bacterial activity
(42-45, Figure 1.26)	Lysosomes and cell membranes	Toxicity - HeLa > HaCaT
(46, Figure 1.27)	N/A	Hep2 toxicity
(47, Figure 1.27)	N/A	Selective binding of <i>R. agglutinin</i> > Con A

(48, Figure 1.28)	N/A	Influenza toxicity 1000X greater than 13
(54, Figure 1.31)	N/A	Toxicity (25X) Y79 > H29
(55, Figure 1.31)	N/A	Selectivity (250X) greater for galectin-1 than galactose
(57, Figure 1.32)	N/A	Incorporated into myoglobin
(58, Figure 1.32)	N/A	No selectivity of Y79 over H29
(60, Figure 1.33)	N/A	Poor PDT agents, low uptake
(61, Figure 1.33)	N/A	Binds HSA

1.4.4 Glycoporphyrins and phthalocyanines; a summary

A large number of groups have been investigating various methods of linking carbohydrates to a porphyrin scaffold, creating a small library of molecules some of which have been biologically investigated.

The correlated anticancer PDT results are presented in Table 1.4. It is clear that positively charged glycoporphyrins are localised in the mitochondria and that neutral glycoporphyrinoids tend to localise in the Golgi and ER. Cellular uptake of these compounds is only possible if at least one face of the porphyrin is free. A degree of amphiphilicity certainly seems to be required, and this is documented by multiple literature sources. Intracellular selectivity has been demonstrated, but results are currently limited by the availability of model systems, with HeLa, HT-29 and Y79 cell lines predominantly under investigation for *in vitro* studies. The requirement for further *in vitro* and *in vivo* studies is paramount.

To date, methods for the functionalisation of porphyrinoids with carbohydrate moieties have included, esters, amides, glycosylations, nucleophilic substitution, conjugated linkers, cross couplings, and cycloadditions. These linkers have been attached at the β or *meso* positions as well as displaying ortho, meta and para-phenyl positions on porphyrins. α and β substitution patterns on phthalocyanines have also been prepared. The ability to prepare complex glycoporphyrinoids is not in question.

A key issue with the majority of the methods listed above is the poor yield at the point of carbohydrate introduction. For example, condensations can regularly provide optimized yields of less than 10 %, and other methods although higher yielding, involve the use of strong Lewis acids, bases or coupling reagents which often require the carbohydrate to be suitably protected. This is an acceptable synthetic pathway if the carbohydrate used is not difficult to synthesize or is easily accessible. However, as more complex and synthetically expensive carbohydrates are prepared as targeting agents, methods wherein the carbohydrate is reliably introduced late in the synthetic route would be preferable.

Variation in the nature and complexity of the carbohydrate groups is of interest. To date only Glc, Gal, GlcNAc, Man, Xyl, Lyx, Ara, Rib, Maltose, Fructose, cellobiose and Lac, LacNAc, cyclodextrin and cellulose substituted porphyrins have been prepared. This is a reasonably long list, however, all of these can be isolated biologically sources and are relatively cheap mono and disaccharides. Too much selectivity is being expected from such simple carbohydrates. Chemists have yet to apply the above coupling methods to larger more complex carbohydrates which would have a much better chance of targeting, through improved recognition by specific lectins. It is with these requirements in mind that this research has been directed.

1.5 Future explorations of glycoporphyrins

The literature is providing ever more impressive porphyrin scaffolds on which to base PDT agents. A large number of groups are reporting porphyrins and related compounds with improved spectroscopic properties, such as; long wave absorption, and efficient $^1\text{O}_2$ production. There is still scope however, to contribute to the targeting aspect of 3rd generation PDT agents, in particular through the preparation and introduction of more complex carbohydrates.

Cluster effects have been shown to increase carbohydrate-lectin binding, and so variation of the presentation of carbohydrates to investigate regioisomeric effects is important.

Finally, the preferred method of carbohydrate attachment should be mild, high yielding and preferably at the final step, minimising functional group manipulations on a complex oligosaccharide linked glycoporphyrin. The CuAAC reaction satisfies these requirements and will be investigated for the preparation of highly complex glycoporphyrin systems, for anti-cancer and anti-bacterial PDT applications.

Presented is my work together with the help of some gifted collaborators, towards the aforementioned goals.

Chapter 2 details the synthesis of a glycoporphyrin library with new optimised reaction conditions for deprotected sugars, and applications towards the synthesis of highly complex glycoporphyrins.

Chapter 3 details synthetic workarounds for α fucosyl glycosylation in the presence of an alkyne, through the use of a novel silylated fucose donor.

Chapter 4 expands on the silylated fucose donor series, with applications of a partially protected fucose donor, to report a high yielding route to propargyl Lewis X. Other unusual disaccharide producing side reactions of this donor are investigated.

Chapter 5 correlates spectral investigations of glycoporphyrins in aqueous media. Lectin binding fluorescence disaggregation studies are carried out, and results of a biological screen through the help of collaborators in TCD and the IMM.

Chapter 6 contains the experimental procedures

Chapter Two

**A microwave mediated synthesis of a fully
deprotected glycoporphyrin library**

Chapter 2 - A microwave mediated synthesis of a fully deprotected glycoporphyrin library

2.1 Introduction

Both symmetrical and mixed system monosaccharide glycoporphyrins have previously been prepared by the Scanlan group, through the microwave mediated CuAAC reaction.¹⁵² The O-propargyl spacer, which ultimately becomes a triazole ring, orientates the carbohydrate away from the porphyrin, which allows for lectin recognition, but without a large degree of freedom which could lower the strength of binding. The triazole ring may also lead to improved binding; Reymond *et al.* reported a finding that aromatic groups positioned near the anomeric centre, led to increased carbohydrate recognition by lectins, over simple alkyl chain linked examples.¹³⁶ The microwave mediated CuAAC reactions reported, offer moderate to high yields when covalently linking protected sugars to porphyrin cores. However, high yielding applications of CuAAC to glycoporphyrin synthesis with deprotected carbohydrates, have not yet been reported. The ability to employ deprotected oligosaccharides allows carbohydrate functionalisation of porphyrinoids to be performed at the final step, which would avoid protecting group manipulations on the synthetically expensive glycoporphyrin product. The synthesis of O-propargyl linked di- and trisaccharides, could lead to possible chemo-enzymatic glycoporphyrin extension. For example, LacNAc could be enzymatically modified to blood groups H type II, Lewis X and Sialyl Lewis X (Figure 2.1). Examples of the latter have previously been described by Davis *et al.*¹⁶³

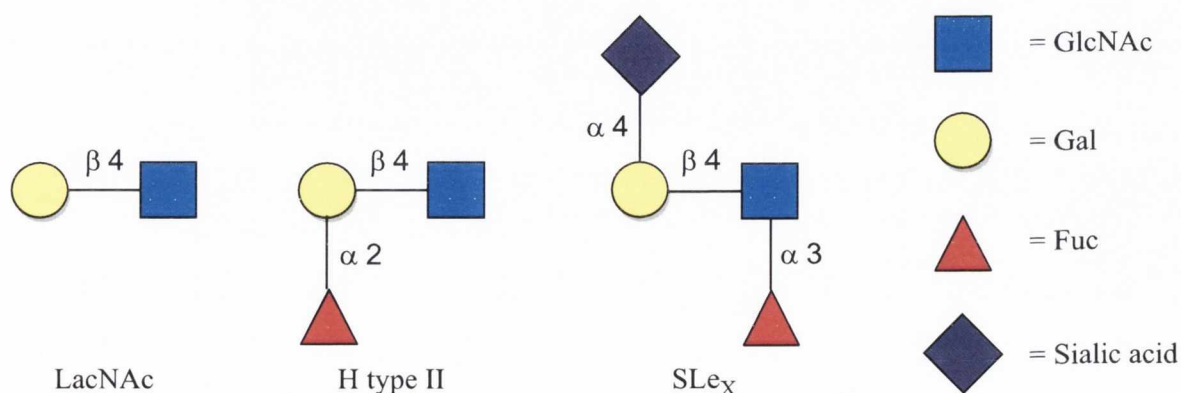
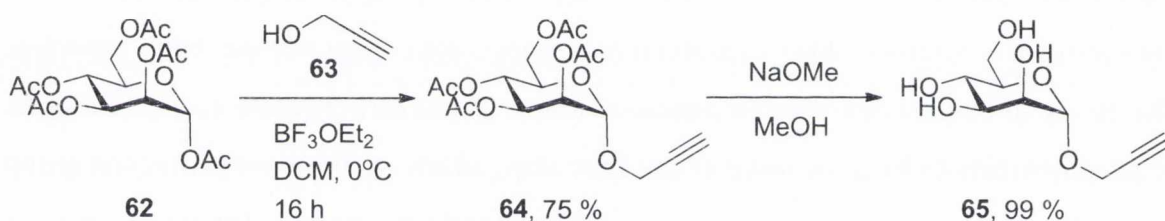


Figure 2.1 - Extended structures related to LacNAc

2.2 O-Propargyl linked carbohydrate synthesis

2.2.1 O-Propargyl monosaccharides synthesis

The O-propargyl group can be introduced at the anomeric position of carbohydrates through glycosylation of propargyl alcohol. Glycosylations directly from a per-acetylated starting donor are the simplest of such preparations (Scheme 2.1). Per-acetylated mannose **62**, was used to glycosylate propargyl alcohol **63**, in the presence of BF_3OEt_2 , providing **64**, in 75 % yield.⁸⁶ Quantitative deacetylation under Zemplén conditions¹⁶⁴ provided the target propargyl mannoside, **65**. The coupling constant of the anomeric proton in **64** at 1.7 Hz (400 MHz, CDCl_3), was inconclusive for a $\text{trans}_{1,2}$ relationship. The dihedral angle between H-1 and H-2 of α or β substituted mannosides, are quite similar, but an X-ray structure (Figure 2.2 - crystal mounted and analysed by Dr. Tom McCabe) as viewed down the C-2 C-1 axis confirmed the α stereochemistry.



Scheme 2.1 - O-Propargyl mannoside synthesis

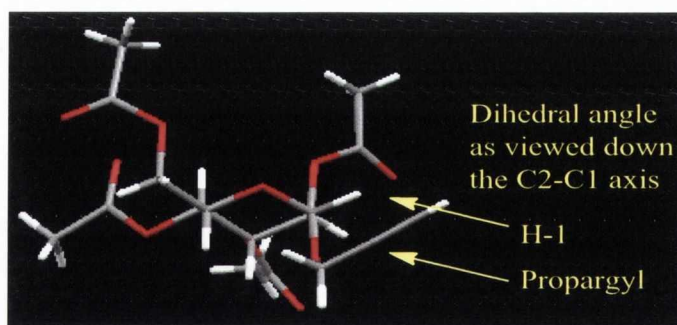
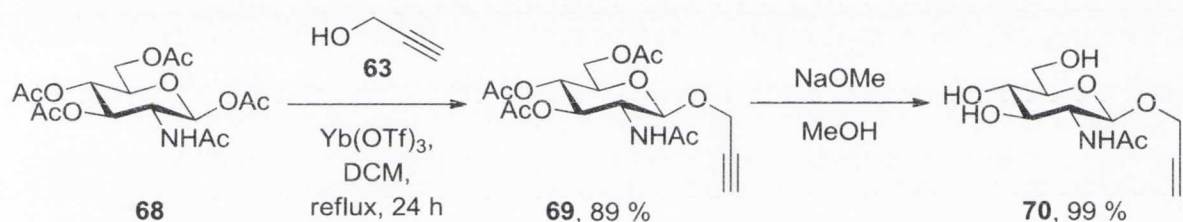


Figure 2.2 - X-ray structure of acetyl protected O-propargyl α -mannose, **64**

An analogous procedure to Scheme 2.1, was carried out using commercially available per-acetylated β -Glc, to yield the acetylated O-propargyl linked β -glucoside intermediate **66**, in 64 %, which was deacetylated to give O-propargyl Glc **67**, in 99 % yield.

The synthesis of O-propargyl substituted GlcNAc **70**, is not as straightforward as the previous two examples. Acetyl directed neighbouring group participation leads to α and β

selectivity in Man and Glc respectively. These reactions proceed via an oxocarbenium ion and subsequent blocking of one face through acetoxonium ion formation. The analogous oxazolinium ion formed when per acetylated GlcNAc **68** is used as a donor, can react further to the oxazoline through the loss of an amido proton. These oxazoline side products lower the reaction yields dramatically, and are normally avoided by protecting the 2-NH₂ as a phthalimide. Yb(OTf)₃ has been used to react these locked oxazolines in the presence of an acceptor.¹⁶⁵ Ultimately Yb(OTf)₃ has been used to form the oxazoline directly from per-acetylated GlcNAc **68**, and then to activate it allowing glycosylation in one pot.¹⁶³ Application of this literature procedure, followed by deacetylation led to the O-propargyl GlcNAc product **70**, (Scheme 2.2) and the completion of the synthesis for the first set of monosaccharide targets.



Scheme 2.2 - O-Propargyl GlcNAc

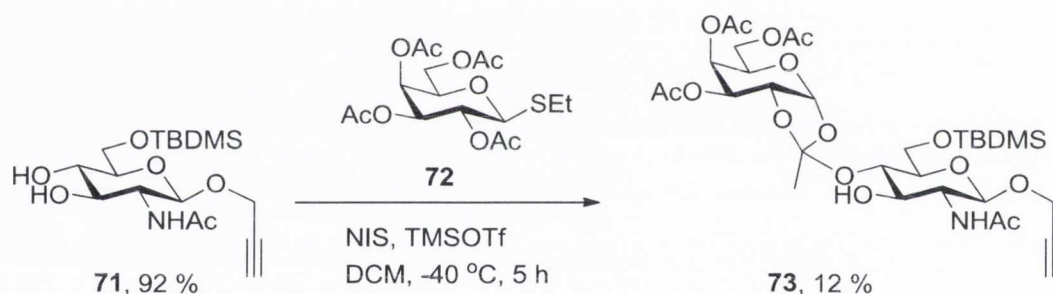
2.2.2 O-Propargyl LacNAc synthesis

As described in chapter 1, to date only cheap, commercially available mono-, disaccharides, and polymers, have been ligated to porphyrin based PDT agents. O-Propargyl substituted LacNAc, as a disaccharide starting material amenable to further transformations into more complex carbohydrates (Figure 2.1), was selected as a valuable target.

2.2.2.1 Attempted route via a galactose thioglycoside donor

Hydrogen bonding between the amide in C-2 and the 3-OH of GlcNAc lowers the ability of the 3-OH to participate in glycosylations. This finding has been previously used to selectively glycosylate the 4-OH position on GlcNAc acceptors, over the 3-OH.¹⁶⁶ The other requirement is a suitable PG at the 6-OH position. The bulky TBDMSCl was used to selectively protect the primary alcohol 6-OH of **70**, to yield acceptor **71**, in a 92 % yield. Imidazole was used as a base but could co-elute during chromatography if care was not taken to ensure its complete removal through aqueous washes during workup.

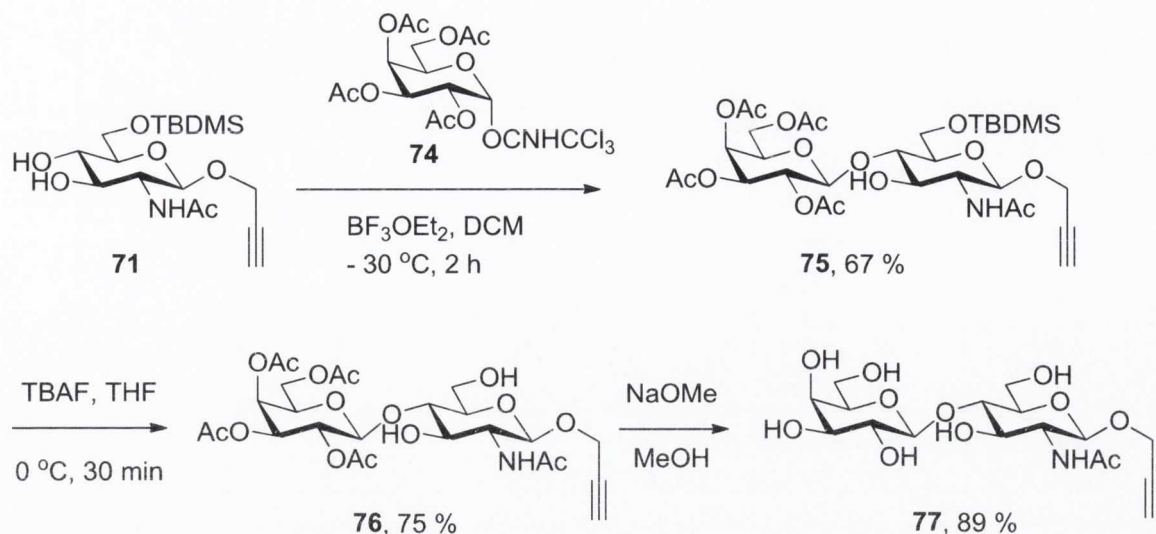
The first donor utilised in O-propargyl substituted LacNAc synthesis, was per-acetylated galactose thioglycoside **72**, which was synthesised according to literature procedure.¹⁶⁷ Glycosylation using the acceptor/donor pair, activating with NIS and TMSOTf, yielded a complex set of products by TLC (Scheme 2.3). Crude mass spectrometry confirmed the required disaccharide mass ion, but upon isolation and characterisation of the main product, the orthoester **73**, was observed in a 12 % yield. Attempts to minimise orthoester formation¹⁶⁸ through longer reaction times of 5 to 16 h, and increased temperature from -40 to -20 and 0 °C resulted in an ever increasing complexity of the side products observed by TLC. Proton NMR analysis (400 MHz, CDCl₃) of the crude material, appeared to show a loss of the alkyne CH as the temperature of the glycosylation was increased, perhaps pointing to an addition of I₂ across the triple bond. However, no such iodinated compounds were isolated. Altering the NIS activation conditions, for dimethyl disulfide - triflic anhydride activation,¹⁶⁹ in order to avoid I₂ issues, did not improve the yield and ultimately this reaction was abandoned.



Scheme 2.3 - LacNAc glycosylation side product - ortho ester formation

2.2.2.2 Alternative route to O-propargyl LacNAc with galactose trichloroacetimidate

An alternative donor galactose trichloroacetimidate **74**, was successfully employed in the synthesis of LacNAc by Davis *et al.*¹⁶³ A slightly modified version of this procedure, (Scheme 2.4), with increased temperature, reduced reaction time, and portionwise addition of donor **72**, improved the glycosylation yield of the required disaccharide **75** from 55 % to 67 %. The β glycosidic linkage in **75** was confirmed with NMR studies (600 MHz, CDCl₃) by a coupling constant of 8.0 Hz, between H-1 (Gal) and H-2 (Gal). The 1-4 linkage was confirmed by HMBC from C-1 (Gal), δ_C 101.3 ppm to H-4 (GlcNAc), δ_H 3.63 ppm, and from H-1 (Gal), δ_H 4.65 ppm to C-4 (GlcNAc) δ_C 80.6 ppm. Subsequent deprotection of the disaccharide **75**, over two steps led to intermediate **76**, and finally **77**, following deacetylation.



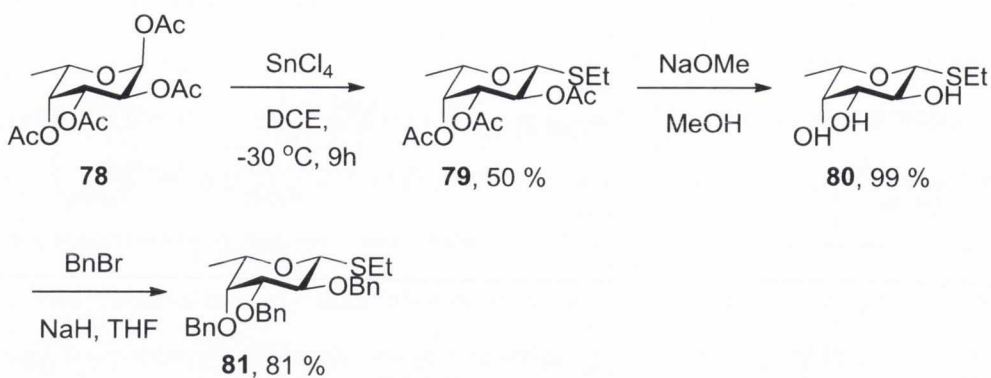
Scheme 2.4 - O-Propargyl LacNAc synthesis

2.2.3 O-Propargyl Lewis X synthesis (Protected)

Interaction of the human lectin DC-Sign with the histo blood group antigen, Lewis X **20**, was described in Section 1.3.3. Incorporation of this oligosaccharide into a PDT agent may contribute to favourable targeting abilities, *in vivo*. Indeed, the terminal Gal moiety on both **77**, and **20**, should lead to Galectin⁹⁹ recognition during biological testing. Therefore the synthesis of O-propargyl Lewis X was attempted, which would yield the most complex carbohydrate targeting agent reported to date on a porphyrin.

2.2.3.1 Fucosylation with a per-benzylated fucose thioglycoside donor

O-Propargyl Lewis X or α -Fuc-(1-3)-(β -Gal-(1-4))- β -GlcNAc-O-propargyl could be prepared from intermediate **75**. The important synthetic requirement of α selectivity for fucose glycosylation meant that a non neighbouring group participating protecting group had to be employed at Fuc O-2. Our first successful route utilised a fucose thioglycoside donor **81** with OBn protecting groups. Donor **81**, had previously been shown in the literature to direct α fucosylation with high stereoselectivity.¹⁶⁶ The synthesis of the fucose donor **81**, is described in Scheme 2.5. One point of interest here was the increase in yield of **81** from 52 % to 82 % after changing the solvent from DMF to THF.¹⁷⁰

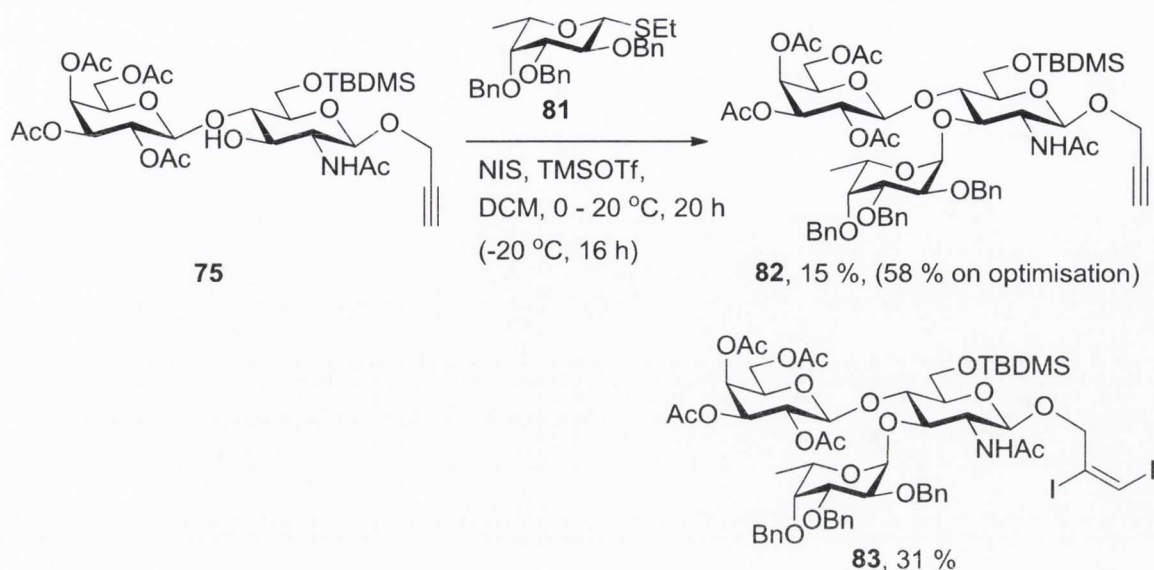


Scheme 2.5 - Fucose donor synthesis

2.2.3.2 Glycosylation with thioglycoside donor **81**

Disaccharide **75**, was glycosylated with benzyl protected fucosyl thioglycoside donor **81**, using NIS and TMSOTf activation (Scheme 2.6). Although problems had occurred previously regarding the stability of alkyne functionalities in the presence of I_2 (Scheme 2.3), it was hoped, that since donor **81**, was armed, that glycosylation would proceed faster than alkyne degradation, minimising side product production. TLC monitoring showed the presence of remaining acceptor **75**, after 6 h with 2 new spots, in the expected region for the product **82**. On quenching of the reaction after 20 h, the two possible product spots had increased in concentration, with the less polar compound in excess. The more polar spot was isolated and confirmed to be protected O-propargyl Lewis X **82**, albeit in a low yield of 15 %. The 1-3 linkage from Fuc to GlcNAc was confirmed by HMBC (600 MHz, CDCl_3) from C-1 (Fuc) δ_{C} 97.4 ppm to H-3 (GlcNAc) at δ_{H} 4.27 ppm. The α linkage between Fuc and GlcNAc was confirmed by a coupling constant of 2.5 Hz between H-1 (Fuc) and H-2 (Fuc).

The product with the slightly less polar R_{f} was purified on silica by column chromatography and analysed by NMR. A large degree of similarity to protected Lewis X **82**, was observed, with one major difference: the alkyne CH δ_{H} 2.40 ppm was absent and a new singlet appeared at δ_{H} 7.12 ppm (Figure 2.3). HRMS confirmed the presence of a diiodinated Lewis X structure **83**, which confirmed earlier suspicions that NIS derived I_2 was reacting with the O-propargyl alkyne. It is likely that these same issues led to the LacNAc synthesis with Gal thioglycoside (Scheme 2.3) being unsuccessful. Previous literature examples exist for direct iodine addition across a triple bond,¹⁷¹ with further evidence for promotion of this reaction through the use of Lewis acid catalysis.¹⁷²



Scheme 2.6 - Lewis X synthesis with NIS activation route A

Having seen that the required product Lewis X glycoside **82**, forms first during this reaction, and as the acceptor **81**, was consumed, more of the iodinated product **83**, was formed, it became clear that the glycosylation was a faster reaction than the iodination. However, as the reaction in Scheme 2.6 proceeds, the I_2 concentration in solution increases with concomitant consumption of donor **81**. Therefore iodination is favoured with longer reaction times. In chapters 3 and 4 of this thesis, work on the stability of the O-propargyl group to I_2 at low temperatures is discussed. It was postulated that lower temperatures with increased equivalents of donor would increase the yields of the desired trisaccharide **82**, in this case also. Indeed the yield of protected Lewis X trisaccharide **82**, was increased to 58 % in 16 h, provided the reaction was run at -20 °C, with 2 equivalents of donor **81**. No appreciable amount of the iodinated compound **83**, was observed by TLC at this temperature.

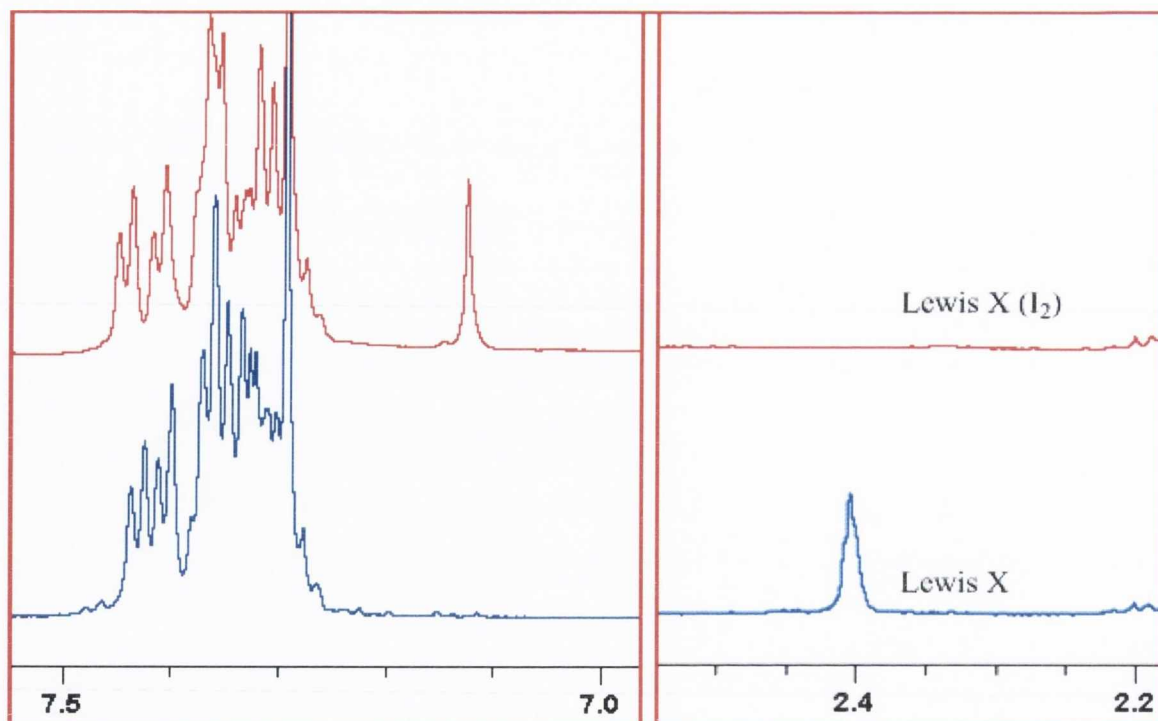
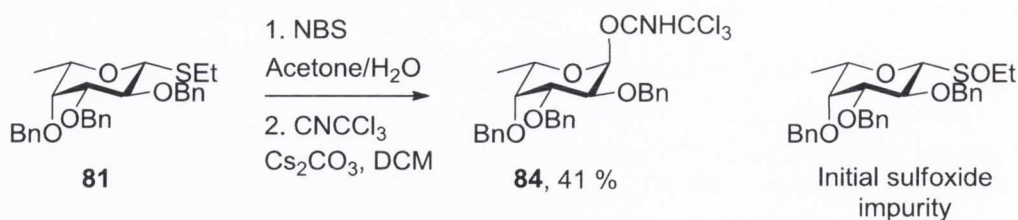


Figure 2.3 - ^1H NMR (600 MHz, CDCl_3) of alkyne (CH), **82**, (red) and di-iodo alkene (CH) **83**, (blue)

2.2.3.3 Fucose trichloroacetimidate preparation

In an attempt to lower the required amount of donor **81**, to reduce the reaction time of Scheme 2.6, and to avoid the iodination issue altogether, the per-benzylated trichloroacetimidate donor **84**, appeared to be a good candidate. Activation of **84**, would not lead to I_2 production, and trichloroacetimidate donors tend to be more reactive than thioglycoside equivalents. The synthesis is described in Scheme 2.7.

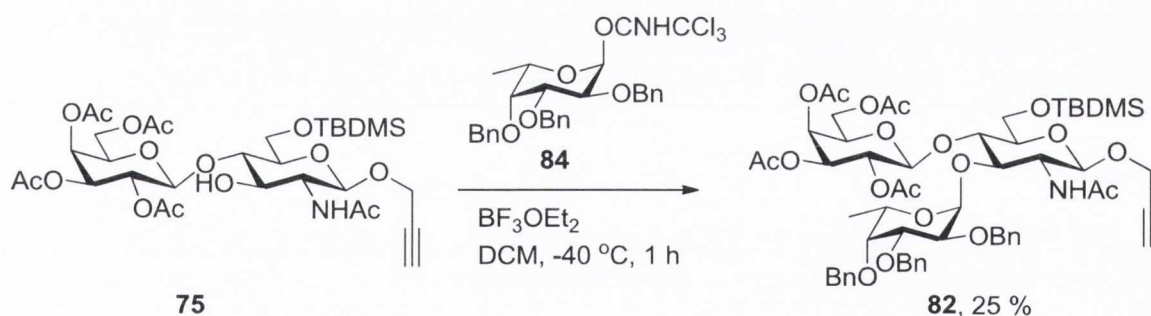


Scheme 2.7 - Synthesis of per-benzylated fucose trichloroacetimidate

Selective hydrolysis of the thioglycoside **81**, using N-bromosuccinimide in acetone/water, provided the crude hemiacetal anomeric mixture, which was used without further purification. Interestingly if the H_2O concentration was too high, evidence for the sulfoxide product was isolated as shown by a characteristic shift of the thioether carbon NMR (100 MHz, CDCl_3) peak in **81** at δ_c 23 ppm to a sulfoxide peak at δ_c 40 ppm in the

crude isolates. In our hands stoichiometric amounts of water gave the best conversion. Initially the trichloroacetimidate was synthesised using the standard procedure¹⁶³ with DBU as a base, yielding no product. Only the hemiacetal starting material was observed by TLC. Given the armed nature of benzylated fucose, it was suspected the donor was forming in solution, but decomposing during work up. For this reason Cs_2CO_3 was used as a base, as it could be removed by filtration, and the excess trichloroacetonitrile could be removed by distillation, followed by rapid filtering through a plug of silica. Donor **84**, was synthesised in 41 % yield, with the remaining intermediate hemiacetal recovered. The donor **84**, proved to be extremely unstable, and needed to be used in glycosylations immediately after production. Decomposition of **84** was observed even if stored dry at $-20\text{ }^\circ\text{C}$.

Unfortunately, glycosylation yields of **82** were only 25 % when 1.5 eq. of donor **84** was used (Scheme 2.8). Taking into account the recovered acceptor starting material **75**, a glycosylation conversion of 91 % was observed, however, the goal of lowering the fucose donor loading was not achieved, and for the best yields of Lewis X trisaccharide **82**, thioglycoside donor **81** should be employed.



Scheme 2.8 - O-Propargyl Lewis X synthesis with a fucose trichloroacetimidate donor

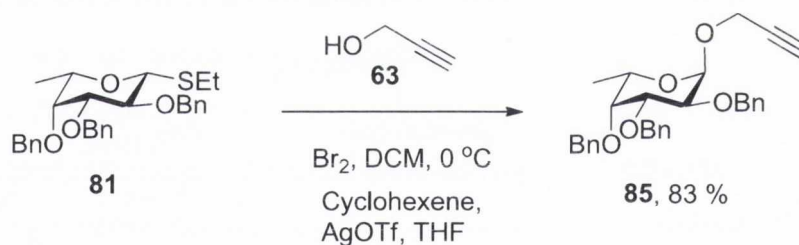
2.2.4 Attempted deprotection of O-Propargyl Lewis X

To enable lectin recognition of Lewis X presented on a porphyrin scaffold, universal deprotection must be performed. Removal of the TBDMS and OAc groups is a trivial process and described during the synthesis of disaccharide (**77**, Scheme 2.4). Removal of the benzyl groups in the presence of an alkyne however, is a difficult step. Due to the presence of the unsaturated propargyl group, standard Pd/C hydrogenation cannot be performed. The use of milder hydrogenation conditions, poisoned catalysts such as

Lindlar's, or Pd/C with BaSO₄ in OBn deprotection is also a common method for the partial reduction of alkynes to alkenes, so is not of practical use. Benzyl deprotection through Pd/C hydrogenation of the protected Lewis X after covalent attachment to a porphyrin, would result in partial reduction of the porphyrin to a chlorin or bacteriochlorin (See later nitro reduction routes in Section 2.3.2), and again this route is not suitable.

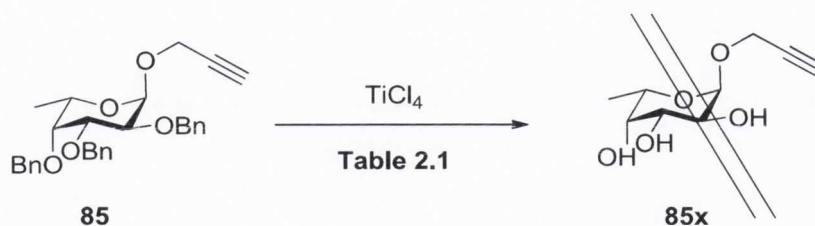
2.2.4.1 Alternative routes to benzyl deprotection

Oxidative or Lewis acids based methods have been previously described for the deprotection of OBn groups, and an investigation of these methods was performed. Test reactions on protected propargyl Lewis X **82**, were not performed due to the limited quantity of this material available. Preliminary tests were performed with fucose thioglycoside **81**, however, concomitant activation of the thioglycoside made results more difficult to interpret. A model system **85**, was prepared (Scheme 2.9), using *in situ* bromination of **81**, followed by a silver (I) salt activation in the presence of acceptor **63**, as optimized by Oscarson *et al.*¹⁷³ Cyclohexene quenches any excess Br₂ so none of the halogen comes into contact with the alkyne.



Scheme 2.9 - Model OBn system for deprotection studies

The attempted debenzoylation of **85** using TiCl₄ (Scheme 2.10), as described by Murali¹⁷⁴ and Kosma¹⁷⁵ failed to provide the desired deprotected product **85x**, under a series of conditions. CHCl₃ as a solvent, led to hydrolysis even at lower temperatures (Table 2.1). TiCl₄ in dioxane appeared a milder method, presumably as a result of Ti(IV) dioxane coordination and no reaction was observed at lower temperatures. In the cases (entry 2-6, Table 2.1), partial hydrolysis at the anomeric centre was observed. Given the polarity of the crude products by TLC, the benzyl protecting groups were also suspected to have been removed, however, this method appears too harsh for the O-propargyl glycoside **85**, and by extension **82**.



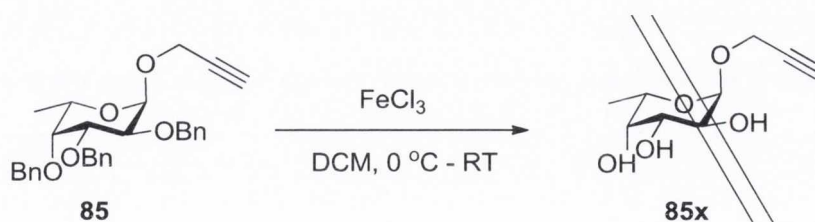
Scheme 2.10 - Attempted TiCl_4 debenzylation

Table 2.1 - Screening of Scheme 2.10 debenzylation conditions

Entry ^a	Solvent	Temperature	Time	Product
1	CHCl_3	0 °C	10 min	No reaction
2	CHCl_3	0 °C	6 h	Hydrolysis
3	Dioxane	20 °C	6 h	No reaction
4	Dioxane	50 °C	6 h	Partial reduction
5	Dioxane	50 °C	16 h	Debenzylation and hydrolysis
6	Dioxane	105 °C	4 h	Debenzylation and hydrolysis

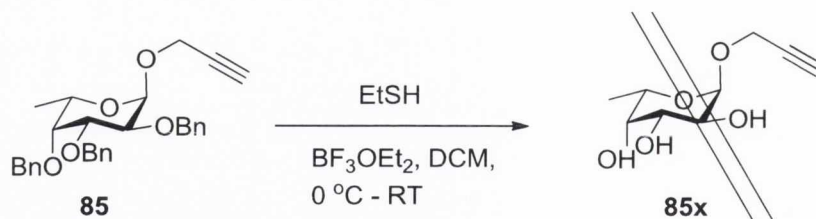
a : TiCl_4 used as a Lewis acid in each case.

Rodebaugh *et al.* successfully employed FeCl_3 as a Lewis acid catalysed route to the deprotection of unhindered benzyl groups.¹⁷⁶ Selective deprotection of OBn protecting groups in the presence of benzoates, esters, amides and alkenes at 0 °C, was reported. Attempts using this method on model compound **85**, provided no deprotected product (Scheme 2.11).



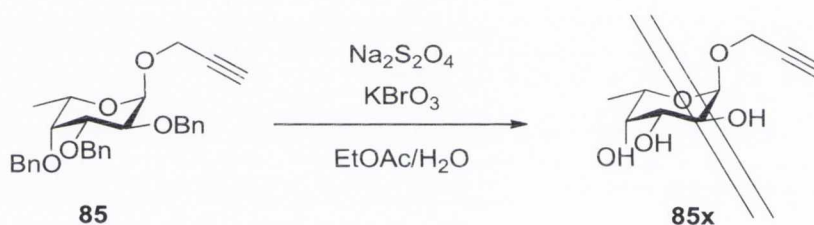
Scheme 2.11 - Attempted FeCl_3 debenzylation

Davis *et al.* reported debenzylated of a C linked glycoside in the presence of BF_3OEt_2 , with EtSH as a solvent.¹⁶³ Efforts at repeating this reaction (Scheme 2.12) led to the curious result of thioglycoside formation. TLC analysis suggested the presence of low quantities of thioglycoside **81**, later confirmed by crude NMR (400 MHz, CDCl_3) investigation. BF_3OEt_2 encourages O-propargyl to act as a leaving group in excess EtSH. Presumably this method is more suitable for C glycosides which are less susceptible to hydrolysis.



Scheme 2.12 - Attempted $\text{BF}_3\text{OEt}_2/\text{EtSH}$ debenzylation

The final attempted debenzylation method, was an oxidative procedure under biphasic conditions, with a slight modification of the reported procedure using $\text{NaBrO}_3/\text{Na}_2\text{SO}_3$.¹⁷⁷ The potassium bromate salt was used instead of the sodium salt, (Scheme 2.13). The reaction unfortunately turned red indicating the production of bromine. Compound **85** was in fact partially debenzylated, but no alkyne peak was visible in the crude NMR (400 MHz, CDCl_3) due to suspected bromination. There is literature precedence in the use of this procedure as a mild brominating agent.¹⁷⁸



Scheme 2.13 - Attempted oxidative debenzylation

Given the unsuccessful attempts at clean deprotection of the OBn groups on the model system **85**, it was proposed that the initial glycoporphyrin formation would display Lewis X with the protecting groups present. This would still represent the most complex glycoporphyrin synthesised from a carbohydrate chemistry point of view, and alternative routes to Lewis X synthesis will be described later in this thesis.

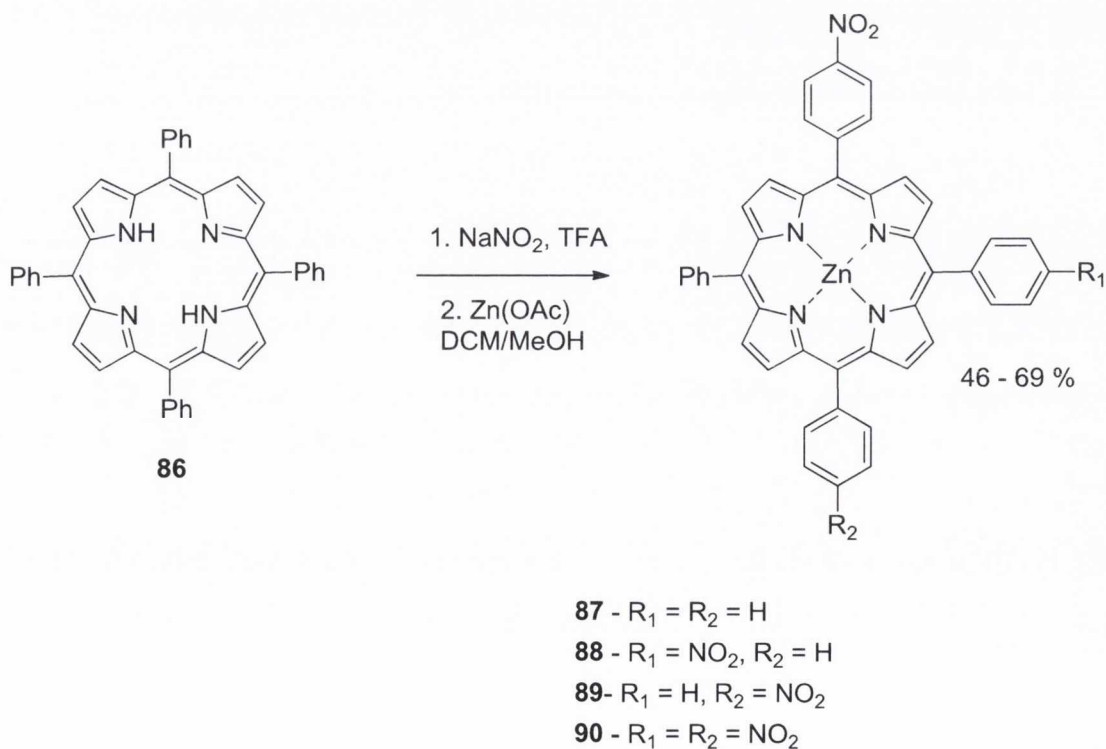
2.3 Azido porphyrin preparation

In an effort to probe the SAR of both amphiphilicity and carbohydrate presentation TPPH₂ **86**, was chosen as a porphyrin scaffold. From review of the previous literature the amphiphilicity provided by at least one unfunctionalised face or corner on the porphyrin would aid cellular uptake.¹⁰⁹ This would leave three other sites where azido functionality could be introduced. As outlined during the introduction, the condensation of suitably functionalised benzaldehydes with pyrrole was one possible route to glycoporphyrins,

however, the use of a common starting material TPPH₂, would allow access to mono- di- and tri- substitution patterns. Synthesis of para substituted 5-mono-, 5,10-di-, 5,15-di- and 5,10,15-tri- azido analogues of tetraphenylporphinatozinc(II) was performed by slightly modifying a procedure reported by Odobel *et al.*,¹⁷⁹ through nitration, reduction to an amine, diazonium intermediate formation and conversion to the azido product.

2.3.1 Selective nitration of TPPH₂

Selective para nitration of TPPH₂ was performed using a literature method.¹⁸⁰ TPPH₂ **86**, was donated by Prof. Senge, TCD. Mono- **87**, di- (2:1 trans **88** : cis **89** as a mixture) and tri-nitro **90** analogues were isolated in 46 %, 69 % (combined) and 50 % yields respectively, with Zinc (II) introduced quantitatively to all isomers in order to prevent Pd insertion during reduction of the nitro group (Scheme 2.14). Equivalents of NaNO₂, and reaction times were altered to give the different products in statistical yields. This method was efficient in that the side products could be recycled, tetraphenylporphyrinatozinc(II) **91**, 42 %, was recovered during synthesis of mono-nitrated porphyrin **87**, and **87** was recovered from di-nitro formation **88** and **89**, etc. The 5,10 and 5,15 di-substituted mixtures **88** and **89**, could not be separated at this stage.



Scheme 2.14 - TPPH₂ nitration

2.3.2 Reduction of nitro porphyrins to amino porphyrins

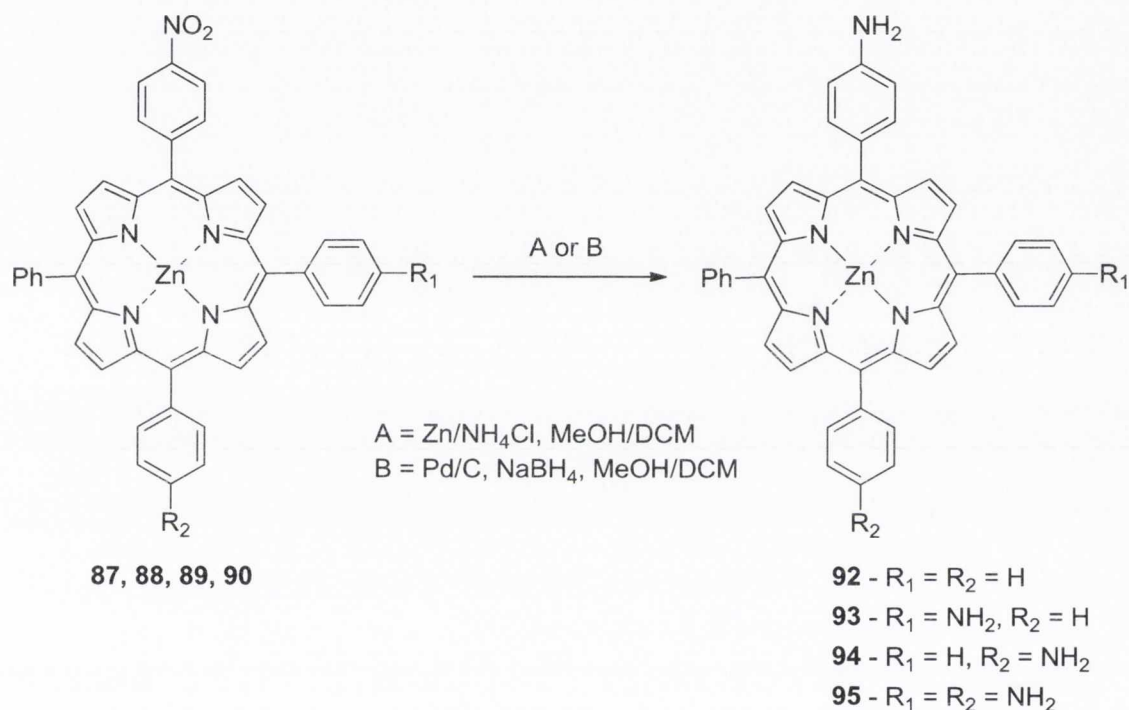
An investigation of three possible nitro reducing methods was performed. Using the mono nitro analogue **87**, reduction was attempted first using H₂ and Pd/C. Mass spectrometry confirmed the presence of the required compound, however, the main product was a bright green compound, assumed to be the chlorin side product. The observed required mass ion for the chlorin during crude HRMS mass spectrometry data supported this assumption. This result confirms the incompatibility of benzyl group deprotection and porphyrins to hydrogenation with Pd/C, as described in Section 2.2.4.

Nitro reduction of **87**, using Zn/NH₄Cl in MeOH/DCM furnished the required compound **92** in 73 % yield. The yield was improved to 83 %, however, using NaBH₄ and Pd/C,¹⁸¹ and this method was subsequently adopted to reduce all nitro analogues as required (Scheme 2.15). The resulting amino compounds had very poor solubility in chlorinated solvents, getting progressively less soluble with increasing amino content, **93**, **94**, and **95**. The presence of co-ordinated zinc metal increases solubility for NMR spectroscopy if deuterated solvents, MeOD or pyridine are added to CDCl₃. The addition of a polar solvent leads to co-ordination of the zinc, increasing the porphyrin's solubility in the mixed solvent system and facilitating easier NMR analysis.

2.3.2.1 Mini study on di-nitro porphyrin cis/trans separation

Fortunately the 5,10 and 5,15 di-amino analogues **93** and **94**, could be separated using column chromatography. A series of mobile phase systems were screened by TLC to maximise separation of the two isomers, whilst minimizing the streaking which occurred on the plate due to the free amino groups. EtOAc:Hexane (1:1) allowed separation of the isomers on low dilutions. However, transferring the method to column chromatography showed significant overlap with an unidentified streaking baseline impurity. Precipitation of products on the column due to poor solubility was also a significant issue. It was noted that THF dissolved higher concentrations of the amino analogues. With this in mind a TLC system of 1:1 THF:Hexane was investigated. Higher loadings were now possible using a THF based system, but the R_f values of the 5,10 and 5,15 di-amino analogues **93** and **94**, were now too similar, resulting in a more difficult separation on silica. Given the apparent improvement in separation using EtOAc, and improved solubility using THF, a combination of both was expected to be optimal. Column chromatography

(THF:EtOAc:Hex, 4:1:5 (v/v/v)) proved successful, allowing the isolation of most of the 5,15 di-amino analogue **94**, at 19 %, and nearly all the 5,10 di-amino **93**, at 61 % yield. The 5,15 di-amino analogue had a lower than optimal isolation (max = 33 %) due to its co-elution in early fractions with an unidentified impurity. As previously described in work by Odobel *et al.*, and confirmed by our experiences, the amino porphyrins are unstable and should be stored in a dark freezer if not in use.¹⁷⁹ This is particularly vital for the 5,15-di-amino example **94**, which appears to break down at a faster rate.

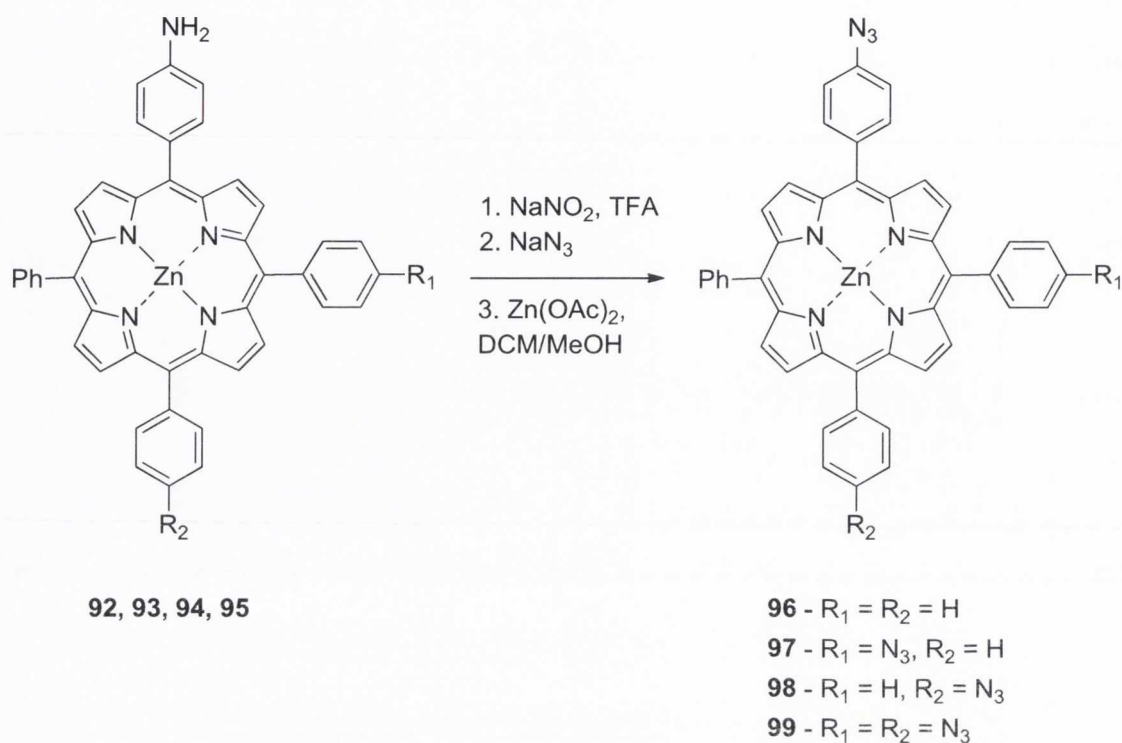


Scheme 2.15 - Reduction of nitro-porphyrins

2.3.3 Azido introduction through diazonium intermediate

Conversion of the amino functionalised zinc porphyrins to the mono- **96**, di- **97** and **98**, and tri- **99** azido zinc analogues was achieved by forming a diazonium ion *in situ* and displacing this with sodium azide as a nucleophile. Trifluoroacetic acid was used as a solvent for this reaction thus the porphyrin was de-metallated as a result. For this reason the final step in the reaction after a mini workup was the reintroduction of zinc (Scheme 2.16). The zinc addition is required in order to avoid the commonly encountered issue of Cu (II) insertion into the porphyrin during later CuAAC reactions, (Section 1.4.2.7). Amino to azido functional group interconversion, proceeded with yields of more than 90 %. The reaction must be performed at 0 °C in order to keep the diazonium intermediate stable, otherwise this is a very robust reaction and is performed in an open air flask. The

solubility of the mono- **96**, di- **97**, **98** and tri- **99** azido porphyrins in CDCl_3 was greatly improved vs the amino analogues, and consequently NMR analysis was simpler.



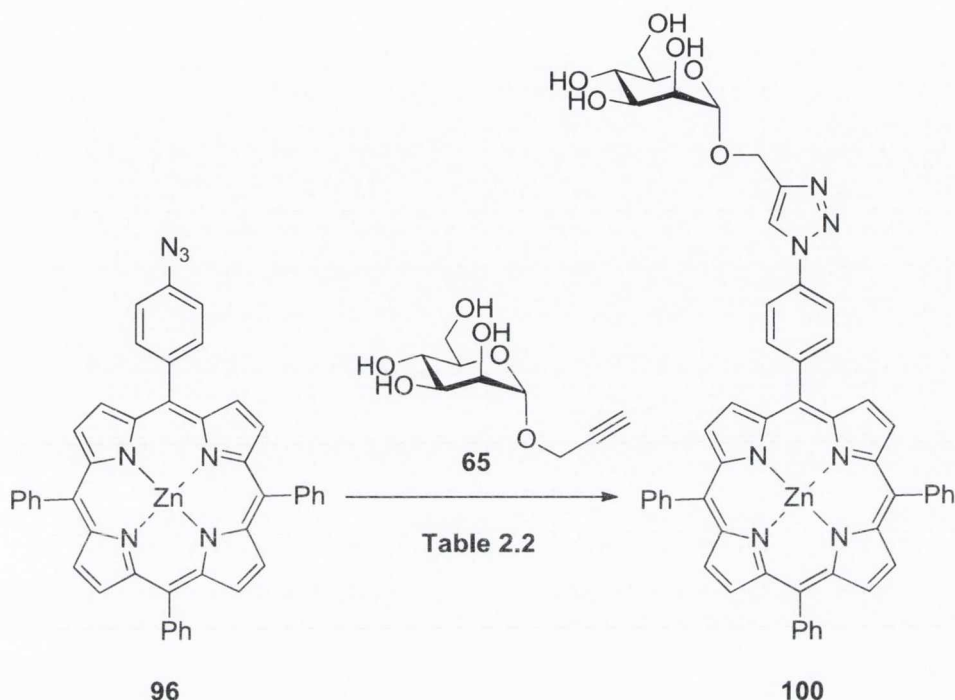
Scheme 2.16 - Azido functionalisation of porphyrins

2.4 Deprotected glycoporphyrin optimisation

The prepared azido porphyrins represent a short synthetic path to a substituted PDT scaffold, which would serve as a model system for carbohydrate based targeting once reacted with suitable alkynes.

Attempts at the synthesis of a deprotected mannosyl glycoporphyrin (Scheme 2.17), using the Scanlan *et al.*¹⁵² procedure developed for acetyl protected monosaccharides were at first unsuccessful (entry 1, Table 2.2). Both porphyrin and carbohydrate starting materials remained unreacted in the microwave vial. An explanation for this result was postulated. It is fair to assume that in a two solvent system comprising of toluene:water (4:1 (v/v)) as used in the original method by Scanlan *et al.*, both the porphyrin and the per-acetylated sugars are dissolved in the organic phase, and water was added to promote toluene heating in the microwave. When using analogous conditions for deprotected sugars, with the carbohydrate dissolving in water, it is likely that both reagents only come into contact at the solvent interface and product formation is minimized. Following from this hypothesis, solvent modification to ensure the reactants occupy the same phase should

improve the yield. Investigation of the various reaction parameters was performed and results of the subsequent optimisation with O-propargyl mannose **65**, and mono-azido porphyrin **96**, are presented in Table 2.2.



Scheme 2.17 - Optimisation of CuAAC reaction with deprotected sugars

The first and most important change to the microwave reaction conditions was the substitution of H₂O with MeOH. A single phase solution would allow the carbohydrate and porphyrin to interact. Following microwave heating, product formation was noted (entry 2, Table 2.2), but the major components were unreacted starting materials. At this point the Cu (I) catalyst was altered. Different Cu (I) sources have been used for the CuAAC reaction, including *in situ* formation through reduction of Cu (II).¹⁸² Cu(MeCN)₄PF₆ is a source Cu (I) soluble in polar organic solvents¹⁸³ and its introduction led to the complete consumption of starting materials. Two spots of product were visible, however, and both appeared to have R_f values in the expected regions for the required product.

Table 2.2 - Optimization table for deprotected click reactions

Entry	Sugar eq.	Catalyst	Ligand	Temp ^a	Solvent	Zn product ^b	Cu product ^b
1	1.30	A (0.3 eq.)	-	140 °C	Toluene:Water (4:1) (1.25 mL)	0 %	0 %
2	1.30	A (0.3 eq.)	-	140 °C	Toluene:MeOH (3:1) (1.33 mL)	5 %	0 %
3	1.30	B (0.5 eq.)	-	140 °C	Toluene:MeOH (3:1) (1.33 mL)	60 %	30 %
4	1.30	B (0.1 eq.)	-	100 °C	Toluene:MeOH (3:1) (0.8 mL)	61 %	0 %
5	1.15	B (0.1 eq.)	THPTA (0.2 eq.)	120 °C	Toluene:MeOH (3:1) (0.8 mL)	87 %	5 %
6	1.10	B (0.15 eq.)	THPTA (0.3 eq.)	115 °C	Toluene:MeOH (3:1) (0.8 mL)	93 %	0 %
7	1.05	B (0.15 eq.)	THPTA (0.3 eq.)	110 °C	Toluene:MeOH (3:1) (0.8 mL)	93 %	0 %
8	1.10*	B (0.2 eq.)	THPTA (0.4 eq.)	110 °C	Toluene:MeOH (3:1) (0.8 mL)	83 %	0 %
9	1.05	B (0.10 eq.)	-	110 °C	Toluene:MeOH (3:1) (0.8 mL)	91 %	0 %
10	1.05	B (0.10 eq.)	-	110 °C	Toluene:MeOH (3:5) (1.6 mL)	89 %	0 %
11	1.05	B (0.10 eq.)	-	110 °C	Toluene:MeOH (1:5) (3 mL)	91 %	0 %

*Per acetylated glucose, Catalyst A = CuCl, Catalyst B = Cu(MeCN)₄PF₆, ^a Time = 20 min,

^b Isolated yield

During TLC staining of the crude products from (Entry 3, Table 2.2), one of the spots turned from red to green whereas the other remained red. This gave a key piece of information as to the possible identity of the second product spot. The molybdenum stain used is made up with dilute sulfuric acid and is highly acidic (pH < 1). At this pH the porphyrin is protonated and the Zn (II) displaced, therefore accounting for the observed colour change from red to green. In this acidic media if a porphyrin does not change colour it is likely that the metal in the centre of the ring is too tightly bound to be removed, this is true with Pd (II) and Cu (II) after coordination to TPPH₂ **86**, for example. Isolation of both products and analysis by HRMS confirmed the Cu (II) insertion was the contaminant (30 %). The main product observed was the Zn (II) containing required glycoporphyrin. NMR analysis is not possible on Cu (II) containing compound as this metal

is paramagnetic. The source of Cu (II) was, of course, the degraded Cu (I) catalyst, and the high temperature of reaction was thought to have encouraged zinc displacement.

(Entries 4-9, Table 2.2) showed that minimization of the Cu (II) substituted product was achieved once reaction temperatures were maintained below 120 °C. The use of a Cu (I) stabilizing ligand THPTA (Figure 2.4)^{184,185} was investigated in (entries 5-8, Table 2.2) but from our results, the addition of this ligand, only increases yield in the range of 2-3 %. Temperature is a more important factor in product formation and the Cu(I) stabilising ligand was not included in later reactions.

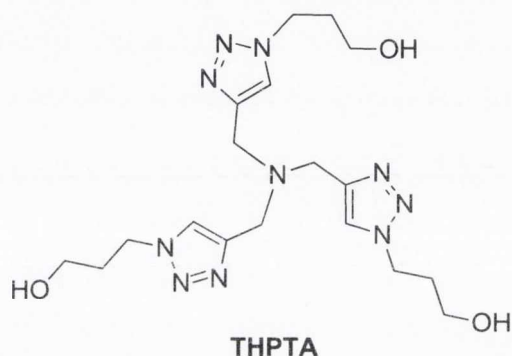


Figure 2.4 - Cu (I) stabilising ligand

(Entries 1-9, Table 2.2) show the efficiency of the reaction as catalyst loadings can be reduced to at least 10 %, and the alkyne can be reduced to 1.05 eq. without affecting the yield. Finally (entries 9-11, Table 2.2) confirm that altering the polarity of the reaction solvent mixtures and dilution does little in the way of affecting the yield. Solvent polarity would be important for multi-functionalised glycoporphyrins, where reaction intermediates could become too polar and precipitate from solution with the higher toluene content.

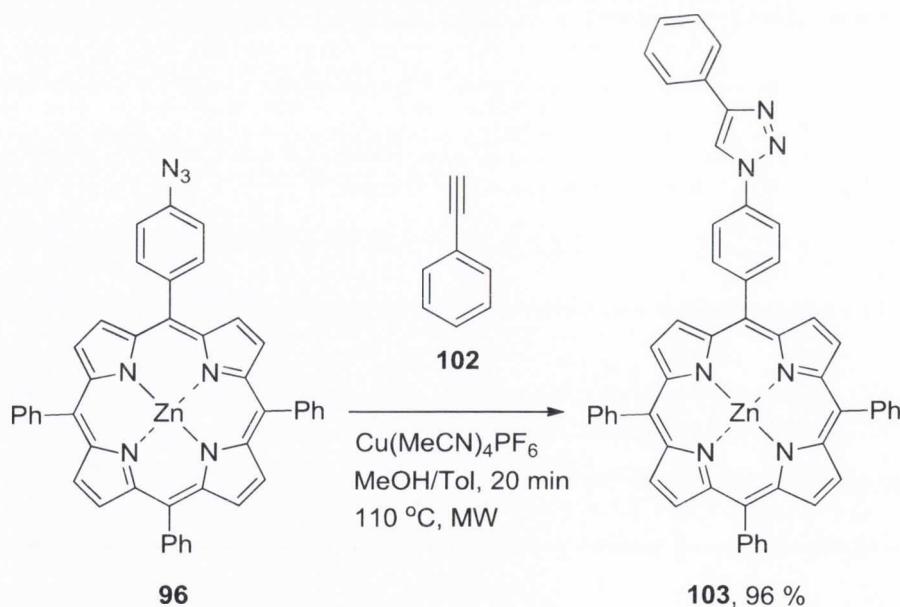
In entry 8, Table 2.2, an acetylated O-propargyl glucose **66** was employed, demonstrating that the new methodology can be applied to both protected and deprotected carbohydrates. This is another advantage over methods previously described in the literature^{147, 150, 152, 155}. The observed yield for the protected glycoporphyrin product **101**, is lower than that previously reported by *circa* 10 %.

In summary, a new method for the production of deprotected monosaccharide glycoporphyrins has been discovered. The yield for this reaction has been optimised from effectively 0 % to in excess of 90 %. The method shows high levels of solvent tolerance

with, 66 % less catalyst and 30 °C lower reaction temperatures than the previous best literature method.¹⁵² Cu (I) stabilising ligands such as THPTA, whilst valuable in other systems, introduce an unnecessary impurity to the reaction and present no tangible advantage in this case.

2.4.1 NMR investigation of triazole regioisomer

One structural aspect of the alkyne/azide cycloaddition reaction required exploration. CuAAC provides the 1,4 regioisomeric product. The cycloaddition reaction heated in the absence of a Cu (I) catalyst provides mixtures of the 1,4 and 1,5 isomers as originally shown by Huisgen.¹⁸⁶ Sharpless *et al.* showed how Cu (I) allowed excellent 1,4 product regioselectivity¹⁴⁴ but this was performed with a catalytic amount of base, albeit at lower temperatures. Since a base was not required in the optimised microwave click reaction (Table 2.2), confirmation of the regioselectivity for the product was required, in order to confirm the need for base can be circumvented though the use of higher temperatures. A model system **103**, with a simple NMR spectrum was synthesised in 96 % yield from mono-azido porphyrin **96**, with phenylacetylene **102**, (Scheme 2.18). NMR analysis (600 MHz, CDCl₃) was then performed to correctly assign the regioisomer.



Scheme 2.18 - Phenyl-triazole-porphyrin synthesis

The two possible regioisomers **103** and **103x**, resulting triazole formation are presented in Figure 2.5. HH COSY analysis allowed the identification of the ortho, meta and para aromatic CH signals - o^I , m^I and o^{II} , m^{II} and p^{II} . 1D NOE experiments monitoring the through space interactions, after irradiation of the 5-triazole CH, would show o^{II} and m^I recognition if **103** was the correct isomer. Isomer **103x**, would only show a positive

interaction with o'' , since, irrespective of how the triazole rotates, the 4-triazole CH is always orientated away from the bridging phenyl, (Figure 2.5). The ROESY spectrum in Figure 2.6, confirmed the expected 1,4 triazole isomer **103**. Irradiation of the 5-triazole-CH at 8.50 ppm, results in two through space resonance peaks corresponding to o'' and m' .

Even if the reaction was not Cu (I) catalysed, **103x** formation would have to proceed through a larger energy barrier resulting from the steric clashing of the phenyl next to the porphyrin ring, and so regioisomer **103**, would still be less favoured.

The 1,4 triazole regioisomer can also be confirmed for glycoporphyrins using the same ROESY based method. In this case positive interactions are seen between the triazole CH to m' and the bridging CH_2 spacer from propargyl group on the carbohydrate.

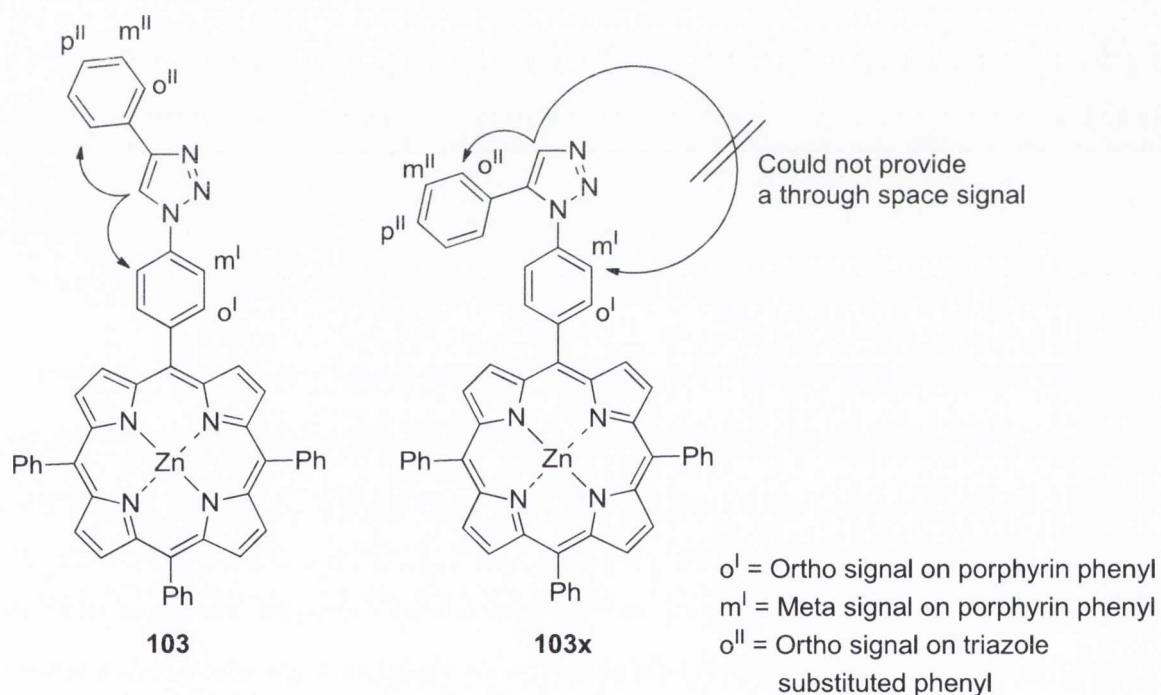


Figure 2.5 - Prediction of NOE through space interactions

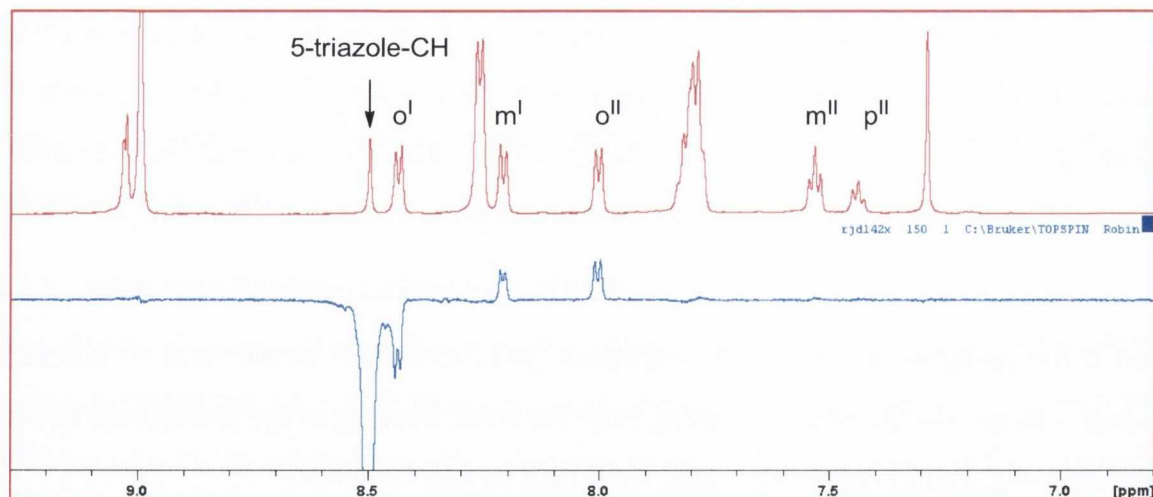


Figure 2.6 - ^1H spectrum + ROESY spectrum overlaid of **103 in CDCl_3 - Irradiation at 8.50 ppm, (600 MHz, CDCl_3)**

2.5 Glycoporphyrin library synthesis

Following optimization of the CuAAC reaction (Table 2.1), confirmation of the regioselectivity (Figure 2.6) and preparation of a series of O-propargyl linked carbohydrates, a small library of glycoporphyrins containing less than 30 examples was prepared using the azidoporphyrins (Scheme 2.16). Included in this series were some protected and mixed system examples, and two non-carbohydrate controls.

2.5.1 Monosubstituted library

The prepared monosubstituted glycoporphyrins is shown in Figure 2.7. 5-Mono-azidoporphyrin **96**, was reacted with a series of previously prepared O-propargyl carbohydrates. The Man substituted product **100**, as already discussed during the optimisation, and analogous examples containing the sugars Glc **104** and per-acetylated Glc **101**, GlcNAc **105**, LacNAc **106** and protected Lewis X trisaccharide **107** were prepared. The propargyl alcohol analogue **108**, was also synthesised originally as a biological control, however, it could not be tested due to inferior solubility. All reactions proceeded in yields greater than 90 %, apart from the acetylated example **101**, and the protected Lewis X example **107**. Some transesterification is likely to reduce the yield in the former with the latter compound showing an impurity, preliminary identified as the protected Lewis X glycoporphyrin with the loss of a TBDMS group, accounting for the lower yield. Interestingly, **106** required the more polar solvent systems (Entry 10 or 11, Table 2.2), as preliminary attempts using the solvent system (Entry 9, Table 2.2), resulted in no reaction

and the recovery of starting materials. At increased levels of toluene O-propargyl LacNAc precipitated from solution. At the time of synthesis, Lewis X was the most complex synthetic carbohydrate attached to a porphyrin reported in the literature. It would have been preferential if the compound could be deprotected, however, efforts to this effect are discussed in later chapters.

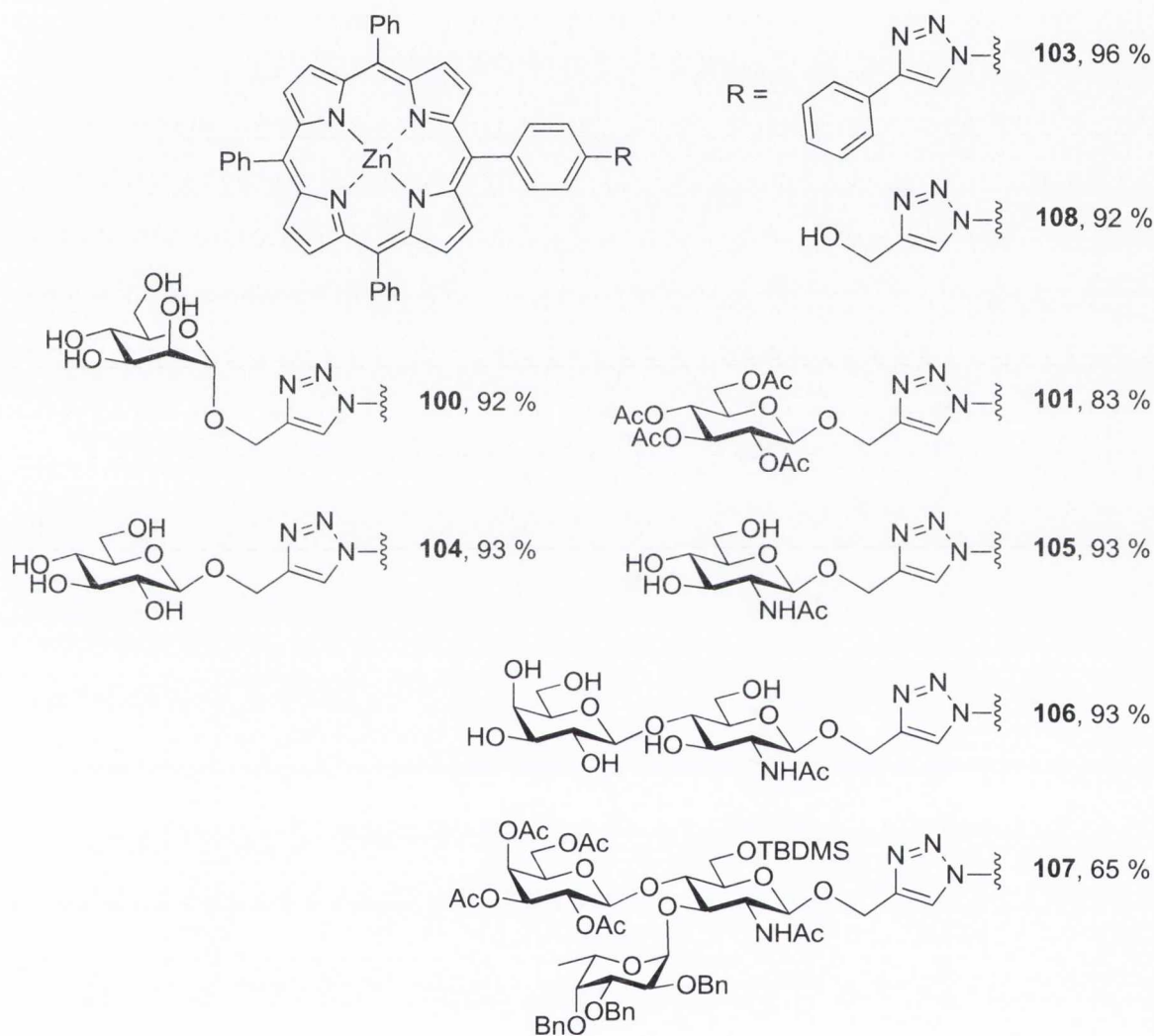


Figure 2.7 - Monosubstituted glycoporphyrins and controls

2.5.1.1 Purification of the mono-substituted glycoporphyrins

The main side products of the CuAAC glycoporphyrin reactions are the spent catalyst ((Cu (II), Cu (I)), 0.05 equivalents of the excess O-propargyl sugar and some amino porphyrin through breakdown of the azido group. Fortunately the large polarity differences and insoluble nature of some of these allows facile normal phase column chromatography, with no other work up apart from solvent removal *in vacuo*. In MeOH:DCM (9:1 (v/v)) or

EtOAc:EtOH:H₂O (7:2:1 (v/v/v)) the monosubstituted glycoporphyrins move at a higher R_f than their respective starting material sugars. To lower the R_f and improve separations EtOH can be replaced with isopropanol. Although not always required, the different phases and solubilities of impurities were used to aid purification in later examples. Prior to characterisation or biological testing, the glycoporphyrins could be freeze dried from H₂O following the addition of minimal MeOH to ensure dissolution.

2.5.1.2 Confirmation of the triazole linkage to carbohydrate with 106

The 1,4 regioisomer of triazole formation was previously confirmed using the phenyl analogue **103**, as a model system. Due to significant overlapping of proton signals in many cases, 2D NMR, especially a high resolution HSQC is vital to the characterisation of glycoporphyrins (Figure 2.7). Using 2D NMR one can also confirm that the carbohydrate is attached to the triazole. The expanded HMBC spectrum of mono-substituted LacNAc glycoporphyrin **106**, is presented in Figure 2.8. The pair of characteristic doublets for the OCH₂ linker, both show long range coupling into three signals: C-1a of LacNAc, 5-triazole-CH and the 4-triazole-C. The use of CDCl₃/MeOD mixture prevents aggregation of the molecule and allows identification of key carbohydrate structural data. MeOD presumably facilitates this through co-ordination of zinc in the porphyrin centre. Lack of aggregation allows us to view other long range through bond couplings: the resonance between the C-4a carbon at δ_C 79.5 ppm and the H-1b signal at δ_H 4.42 ppm.

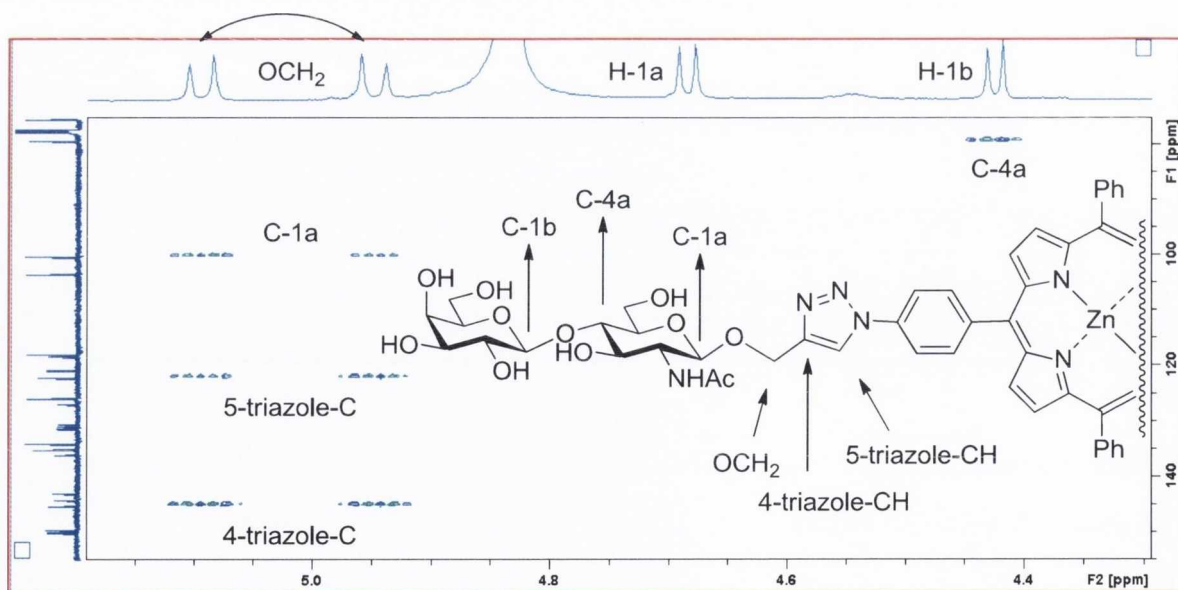


Figure 2.8 - Expanded HMBC spectrum (600 MHz, CDCl₃/MeOD) of **106**

2.5.2 Disubstituted glycoporphyrin library

2.5.2.1 5,10-Disubstituted glycoporphyrins

The prepared *cis* disubstituted glycoporphyrins are shown in Figure 2.9. 5,10-di-azidoporphyrin **97**, was reacted with a series of previously prepared O-propargyl carbohydrates, and the isolated yields of these reactions are shown below. Man **109**, Glc **110**, GlcNAc **111** and LacNAc **112**, analogues were all prepared. The catalyst loading was increased from 0.10 eq. to 0.15 eq., which means these reactions were 25 % more efficient than the monosaccharide synthesis (Figure 2.7), with moderate to high yields in all cases. The carbohydrates were presented symmetrically on the porphyrin, as in the ^1H NMR, an increase in integration was noted but no extra peaks were observed. All di-examples required the more polar solvent system (Entry 10 or 11, Table 2.2), to avoid halting the reaction at partially glycosylated intermediate side products.

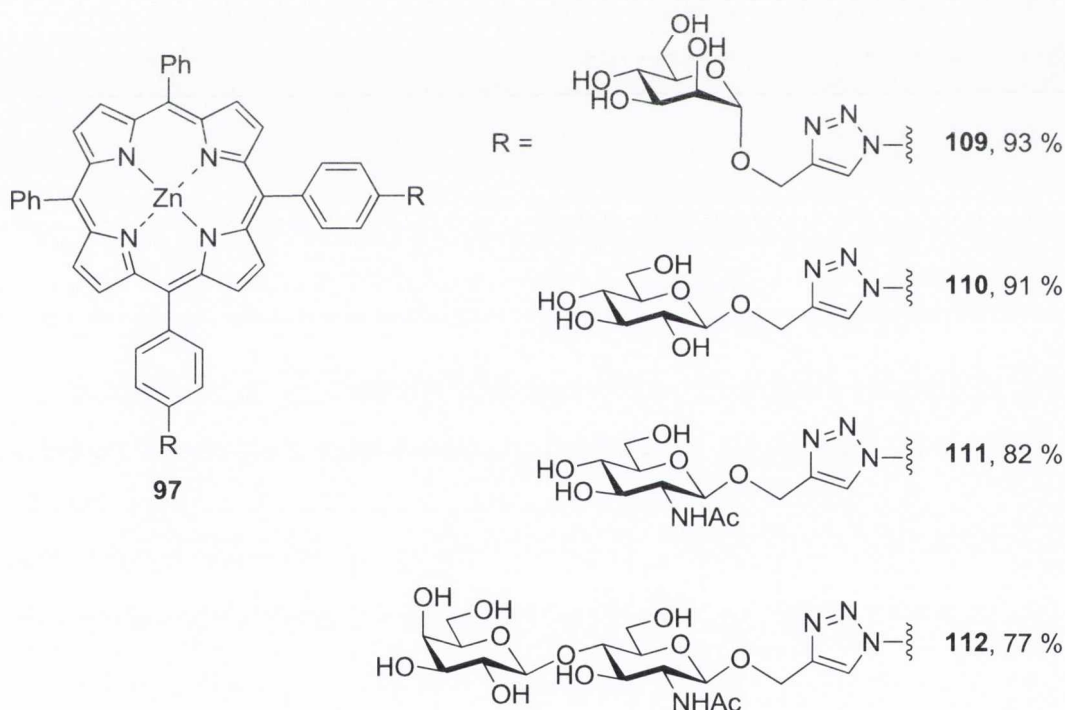


Figure 2.9 - 5,10-Disubstituted glycoporphyrins

The disubstituted glycoporphyrin **112**, was water soluble which fulfils one of the goals of glycoporphyrin PDT synthesis: improved aqueous solubility. The lower isolated yield of **112**, is most likely due to a loss of product adhering to silica during purification. The crude mixture appeared to have the same low level distribution of impurities as the other 5,10

glycoporphyrins (Figure 2.9), indicating no new side product production led to the reduced yields.

2.5.2.2 5,15-Disubstituted glycoporphyrins

The prepared *trans* disubstituted glycoporphyrins are shown in Figure 2.10. 5,15-diazidoporphyrin **98**, was reacted with two previously prepared O-propargyl carbohydrates to prepare Man substituted **113**, and LacNAc substituted **114**. The instability of the 5,15 azido starting material resulted in the lower yields. Additional polar products were seen by TLC if the reaction vessel was placed in light whilst in a concentrated solution for any length of time. Nonetheless the reaction was sufficiently quick and robust and the resulting 5,15-triazole products were stable.

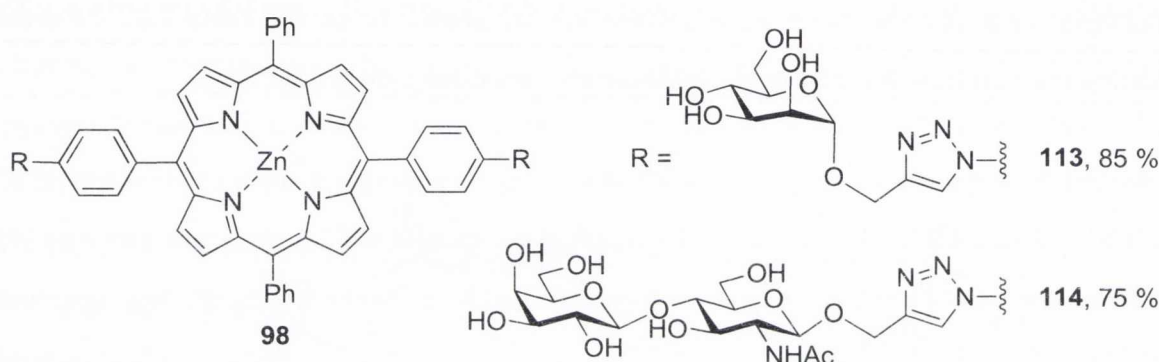


Figure 2.10 - 5,15 - Disubstituted glycoporphyrins

2.5.2.3 Purification of the di-substituted glycoporphyrins

The crude mixtures following the synthesis of di-substituted glycoporphyrins revealed a new impurity profile, albeit in low quantities. Azide breakdown can now lead to two products depending on whether one triazole ring has already been formed. Again no workup other than evaporation and purification with normal phase chromatography was required. The polarity of the mobile phase must be increased from that used for the mono- substituted glycoporphyrin products. In addition, the di-substituted glycoporphyrins are now more polar than their deprotected carbohydrate starting materials. Solvent systems from (EtOAc:EtOH:H₂O, 7:2:1 (v/v/v)) for the monosaccharides towards (EtOAc:EtOH:H₂O, 65:20:15 (v/v/v)) for the LacNAc substituted analogues, allowed effective elution without dissolving the stationary phase. The use of THPTA, although not required, is also highly discouraged at this stage due to the fact that the ligand co-elutes with the 5,10-di-LacNAc glycoporphyrin product **112**. Prior to

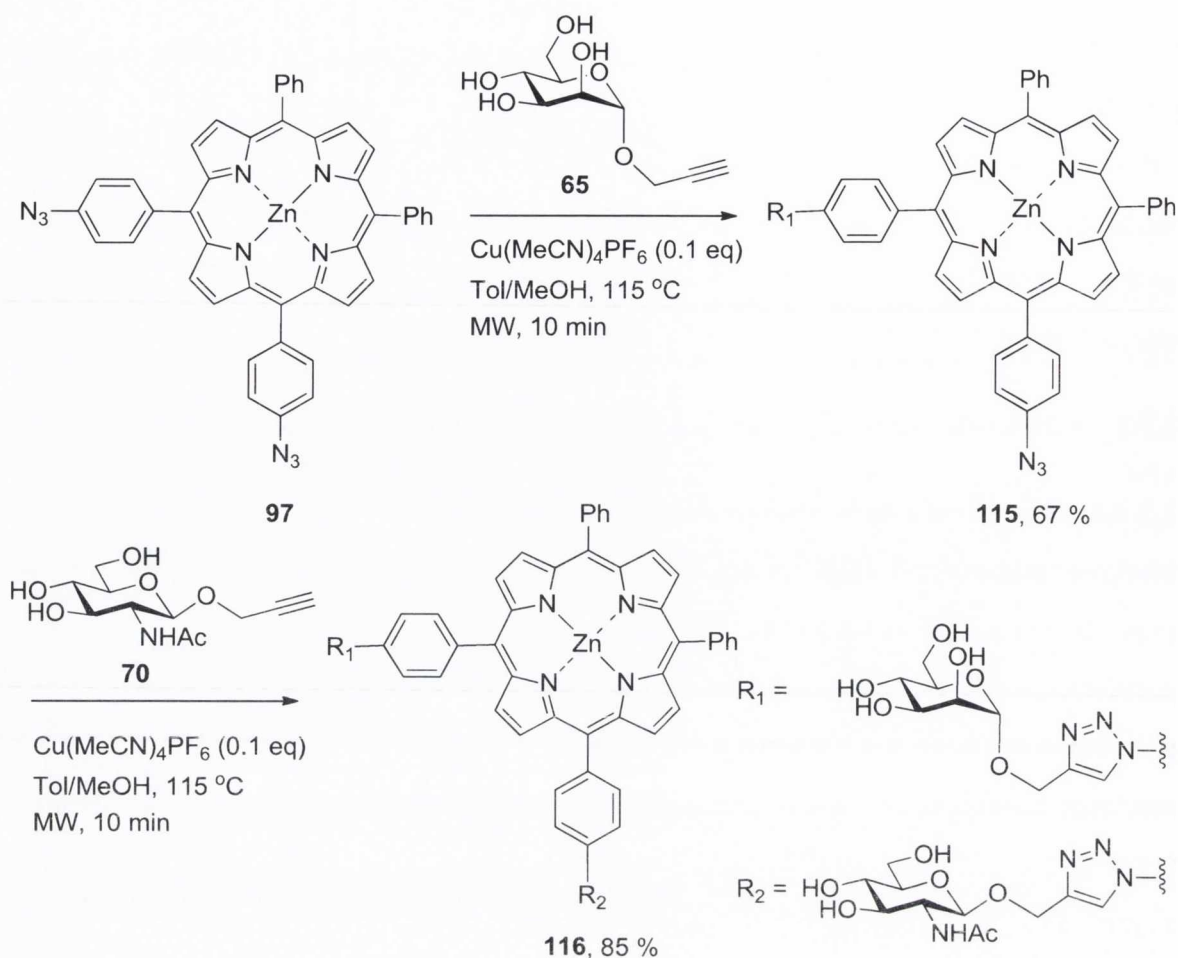
characterisation or biological testing the glycoporphyrins were freeze dried from H₂O directly or after the dropwise addition of MeOH to aid dissolution. 5,15 Di-LacNAc substituted glycoporphyrin **114**, co-eluted with the excess O-propargyl disaccharide **77**, starting material. Fortunately, although the 5,10 analogue **112**, is water soluble, the 5,15 analogue **114**, was not and by simply suspending the crude mixture of **114** in water, and filtering, the carbohydrate impurity was removed in the filtrate.

2.5.3 5,10-Disubstituted mixed system glycoporphyrins

2.5.3.1 Linear route to 5,10-disubstituted mixed systems

Multiple carbohydrate epitopes are displayed in close proximity on the cell surface, and in order to mimic this arrangement the ability to display different oligosaccharides on a porphyrin, would be useful. Scanlan *et al.*¹⁵² prepared the only example of mixed system glycoporphyrins in the literature to date. These were prepared using protected monosaccharides which were subsequently deprotected after conjugation. Presented in Scheme 2.19, is a sequential double click route to mixed systems, using deprotected sugars. Di-azidoporphyrin **97** (3 eq.), were used to provide a statistical mixture of recovered starting material azide **97**, required mono substituted glycoporphyrin **115**, (67 %) and disubstituted **111** (25 %). All three could easily be separated using column chromatography before characterisation. To increase the yield of the intermediate **115**, increased equivalents of the azido porphyrin **97** can be used. Scanlan *et al.* previously reported 4 equivalents of the di-azidoporphyrin **111**, provided a mono azido, mono carbohydrate substituted product in 87 % yields, with acetylated sugars.¹⁵²

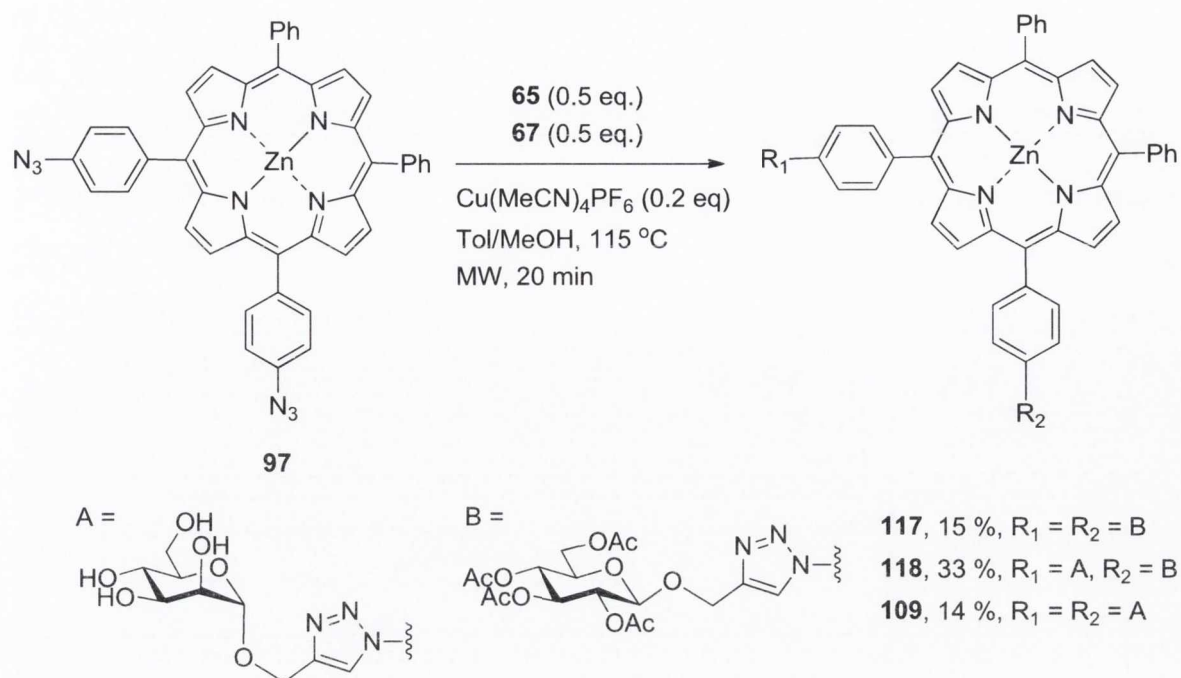
Performing the CuAAC reaction on the intermediate mono-azido **115**, in the presence of another O-propargyl carbohydrate **70**, yielded the mixed system glycoporphyrin product **116** in 85 %. This second stage of the reaction is an improvement on the 53 % literature yield.¹⁵² The order of carbohydrate introduction can be important. For example the difference in ¹H NMR of the intermediate mono glycoporphyrin and the final product di-substituted glycoporphyrin is difficult to observe if GlcNAc was introduced first. However, by introducing the GlcNAc second, as in Scheme 2.19, the appearance of an amide CH₃ in the ¹H NMR is a useful marker that the correct product has been purified before committing resources to a more intensive NMR study.



Scheme 2.19 - 5,10-Disubstituted mixed system - linear route

2.5.3.2 Combinatorial route to 5,10-disubstituted mixed systems

Following on from the ease of purification with normal phase chromatography of the mono-azido intermediate **115**, the option of one pot mixed systems was investigated. Indeed, mixed systems can be synthesised easily, it is the separation which proves problematic. If adequate differences in the polarities of the sugar either exists or was introduced, then a combinatorial method would quickly provide 3 glycoporphyrins per reaction, very useful in the preparation of a larger library. Presented in Scheme 2.20, is a one pot combinatorial double click route to a mixed system. Reacting two carbohydrates **65** and **66** in a 1:1 ratio will theoretically result in three compounds the di-Glc(OAc) glycoporphyrin **117** (previously prepared in the work by Scanlan *et al.*¹⁵²), a mixed system Glc(OAc)/Man glycoporphyrin **118**, and the di-Man glycoporphyrin **109**, in a ratio of 1:2:1. The actual isolated yields of this reaction were lower at 15 %, 33 % and 14 % respectively, however, the result confirmed the combinatorial approach can be employed for larger screens where diverse systems are required.



Scheme 2.20 - 5,10-Disubstituted mixed system - combinatorial route

2.5.4 Trisubstituted glycoporphyrin library

Figure 2.11 shows the prepared trisubstituted glycoporphyrins. 5,10,15-tri-azidoporphyrin **99**, was reacted with a series of previously prepared O-propargyl carbohydrates. Preparation of the Man **119**, Glc **120**, and GlcNAc **121** analogues was straightforward, provided the most polar reaction medium (Entry 11, Table 2.2) was employed. For tri-substitution, the catalyst loading was increased to 20 %, and yields of monosaccharide products were in excess of 70 %. This is comparable to the mono- substituted examples (Figure 2.7), at approximately 90 % conversion per azide functionality. Symmetry was observed in the ^1H NMR spectra with the 5,10,15 presentation of the 3 carbohydrate units, in the α mannose example. A slight complication was observed in the chemical shift for the anomeric proton on the reducing end. Two overlapping signals now appeared for the doublet, in the ratio of 2:1. The 5,15 carbohydrates were presented in the same chemical environment, however, sugar substituted at the 10 position experiences a slight offset. The effect results from a slight difference in electron density on the 10-phenyl ring vs the 5- and 15- phenyl rings, perhaps through offset stacking arrangements in solution, as well as symmetry implications from rotation of the triazole ring on the 10-phenyl ring position.

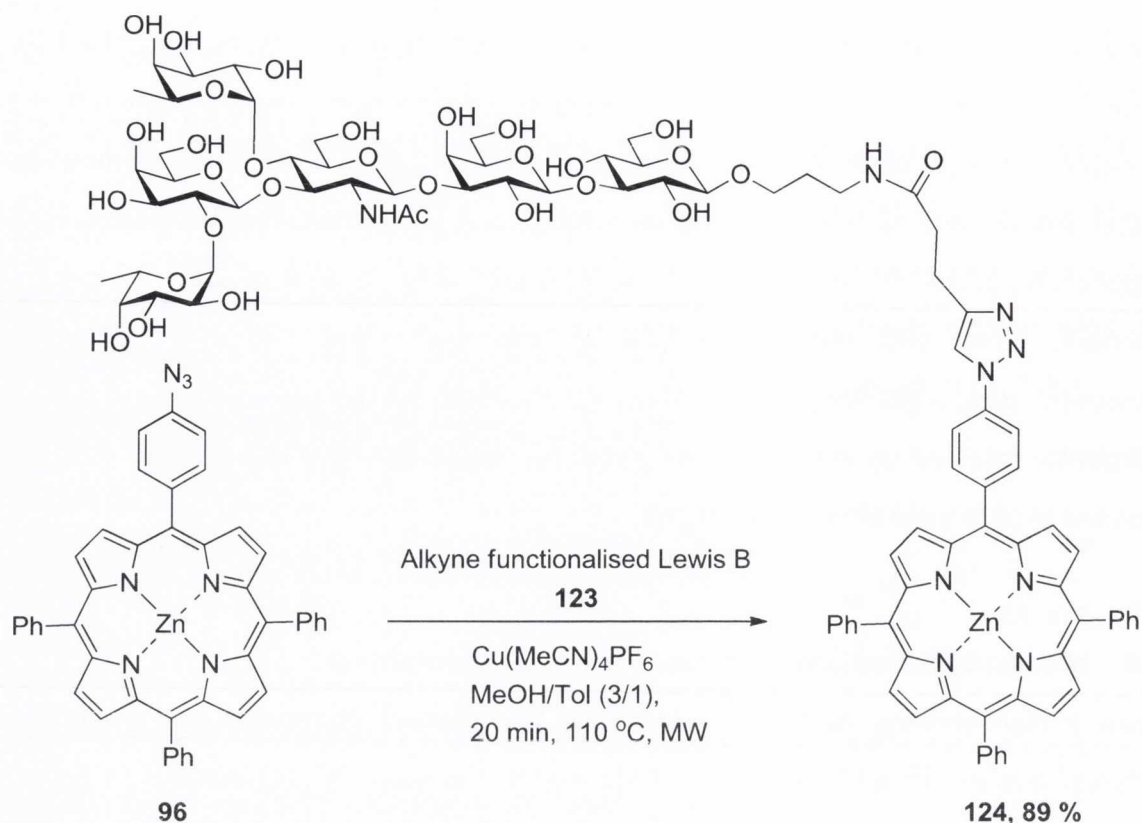
The yield of the tri-LacNAc **122**, derivative was not as high due to a number of factors. It was anticipated that **122**, would be highly water soluble, given the fact that the 5,10

propargyl LacNAc was observed in the aqueous layer, however, by reverse phase TLC analysis, the presence of more than one glycoporphyrin material was observed. It is possible that some of the 4 di-LacNAc mono azido/amino glycoporphyrin impurities were water soluble, and so reverse phase prep MPLC was performed. The 3D structure and aggregation properties of glycoporphyrin **122**, was very unusual in that the required product, moved with the solvent front in both neat water, and neat acetonitrile. However, using a gradient method (MeCN:H₂O, 20-35 %) the required compound was efficiently retained by the stationary phase for separation and the product **122**, was isolated in 56 % yield after freeze drying.

2.6 Monosubstituted Lewis B hexasaccharide glycoporphyrin

With a high yielding route to highly polar deprotected glycoporphyrins developed, interest was expressed by a number of groups in the scope of this method to prepare highly complex glycoporphyrins. Following a collaboration with the Oscarson group, UCD, an alkyne functionalised Lewis B structure **123**, was prepared by Dr. M. Hollinger, UCD.¹⁸⁷ The Lewis B hexasaccharide¹⁸⁷ substituted glycoporphyrin **124**, was successfully prepared in 89 % yield, (Scheme 2.21). The complexity of this deprotected glycoporphyrin brings new possibilities in carbohydrate targeting.

Following demonstration of the versatility of the MW CuAAC procedure, further expansion of the Lewis B series to include 5,10, 5,15 and 5,10,15 trisubstituted Lewis B glycoporphyrins will be completed in due course. Investigations of their application for antibacterial PDT based stomach ulcer treatment will be performed, considering the previously reported K_b in the order of 10^{10} M^{-1} for Lewis B and the *H. pylori* adhesin, (Section 1.3.2).⁸⁷



Scheme 2.21 - Lewis B substituted glycoporphyrin, 124

2.7 Conclusion

An efficient method for the synthesis of O-propargyl carbohydrates has been described. Mono- and di-saccharide examples have been prepared in high yields. The synthesis of O-propargyl Lewis X terminal trisaccharide has been performed. Unfortunately the alkyne/benzyl protecting group removal compatibility issues, led to the incorporation of this compound as a protected sugar.

Improvements to the synthesis of azido functionalised porphyrins have been made. Moreover, relatively simple chromatography conditions have been developed which take advantage of separation and solubility differences of the cis and trans amino porphyrins for easier product separation.

Optimization of a microwave mediated CuAAC reaction has been detailed. Yields of up to 96 % have been recorded using this new method, which leads to products that can be easily purified due to the low level of catalysts used.

Using the prepared carbohydrates and porphyrins and the optimized procedure, a set of glycoporphyrin based PDT agents bearing deprotected sugars was prepared. Of the triazole linked glycoporphyrins detailed in this work, 18 are novel medicinal agents.

The monosaccharides from this work have been reported in a nanoparticle bifunctionalisation paper⁴⁹ through collaboration with Dr. Ross Boyle, from the university of Hull. The disaccharides and trisaccharides from later chapters are part of a paper by the same group, which is currently in preparation.

The optimisation of deprotected glycoporphyrin click chemistry and subsequent glycoporphyrin library synthesis along with biological results following collaboration work described in later chapters, is reported in Chemistry: A European Journal.¹⁸⁸

Application of the optimised CuAAC reaction to the synthesis of a hexasaccharide substituted glycoporphyrin, has confirmed the scope of this route to tolerate highly biologically active carbohydrates, for the synthesis of highly complex glycoporphyrins.

2.8 Future work

The main obstacle encountered in this work was the inability to synthesise deprotected O-propargyl Lewis X. This led to the attachment of Lewis X to a porphyrin as a protected structure. Future studies are required to avoid this synthetic issue and prepare a deprotected Lewis X glycoporphyrin structure. The prepared glycoporphyrins will also be biologically investigated in collaboration with Prof. M. O. Senge, TCD, to understand any SAR from the available library. Insight into the ability of the different carbohydrates to affect uptake, distribution, and toxicity would be valuable information to the field.

Through collaboration with the Oscarson group, the microwave click methodology has been shown to extend to the introduction of hexasaccharide, (Lewis B). Ongoing work on the expansion of this series continues, limited by the ability to prepare Lewis B in higher quantities.

Chapter Three

**Development of a trisilyl fucose donor for the
synthesis of α -fucose oligosaccharides**

Chapter 3 - Development of a trisilyl fucose donor for the synthesis of α -fucose oligosaccharides

3.1 Introduction

3.1.1 Lectin binding and terminal α -fucose containing target molecules

To prepare more efficient PDT therapeutics containing carbohydrates as targeting agents, informative measurements of glycoporphyrin-lectin interactions are required in order to optimise the choice of sugars for attachment. X-ray co-crystallizations are useful tools when available, however, crystallizations give a solid phase snap shot in time, and cannot accurately represent the dynamic processes of carbohydrate-lectin interactions in solution. Efforts at computationally modelling carbohydrate-lectin interactions are becoming more advanced, but remain limited in scope.¹⁸⁹ Researchers rely on a number of other solution and lectin immobilized methods such as isothermal scanning calorimetry, fluorescence emission and subsequent quenching measurements, and surface plasmon resonance techniques. These methods have their advantages and disadvantages. For example the random point of immobilization of a lectin onto a surface may either directly or allosterically affect the binding of a ligand. From some of these methods we may be able to learn about the thermodynamic factors influencing binding and calculate carbohydrate-lectin binding constants, but it will be difficult to identify the important regions of binding on the lectin or the sugar. This information is necessary to design better ligands with more specificity for lectins or cells.

Recent NMR based methods, not only provide carbohydrate-lectin binding constants, but also provide physical information about the binding between a carbohydrate and a lectin. STD NMR can tell us the most important sites for binding on the ligand, and ^1H - ^{15}N HSQC NMR spectroscopy on ^{15}N enriched proteins, in the presence and absence of a ligand, can be used to track the amino acids involved in a binding interaction. Differences observed in ^{15}N chemical shifts, correspond to the amino acids involved in the ligand binding, providing valuable 3D information. Coupled with crystallographic data of the same protein, the actual amino acids involved in interactions can be uncovered. An understanding of the important amino acids involved in lectin binding of carbohydrates, as well as their position on the lectin, would make it possible to design tighter binding ligands. Crowley *et al.* has recently published data on ^1H - ^{15}N HSQC NMR studies:

chemical shift perturbation.¹⁹⁰⁻¹⁹¹ The group has investigated the amino acid structure of a fucose binding lectin *Ralstonia solanacearum*.¹⁹² In a collaboration between both the Scanlan and Crowley groups, a target structure for lectin binding (**125**, Figure 3.1) was designed. This structure is based on a fucose containing disaccharide α -Fuc-(1-6)- β -GlcNAc-O-Me (**126**, Figure 3.1) which has a 6.8 times stronger relative binding to *R. solanacearum* than fucose. If a water soluble glycoporphyrin could be prepared incorporating this molecule into its structure, then NMR experiments could be performed to investigate the different binding sites accessed by such a glycoporphyrin, and how they differ from the free carbohydrate.

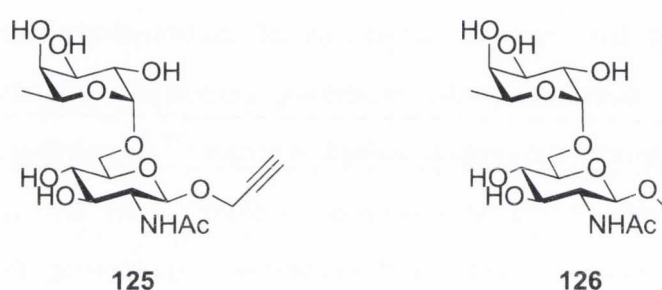


Figure 3.1 - Proposed and known ligands for *R. solanacearum* lectin binding

The synthetic issues with preparation of a **125**, are similar to that noted in Section 2.2.3, mainly the ability to prepare an α linked fucose in the presence of an alkyne. A new method to prepare an α -fucosyl bond which could be deprotected, in the presence of an alkyne was investigated. It was anticipated that this method would have applications in the synthesis of multiple α -fucose targets containing unsaturated bonds. Current synthesis is limited by the ability to remove benzyl protecting groups.

3.1.2 Alternative to OBn protecting groups

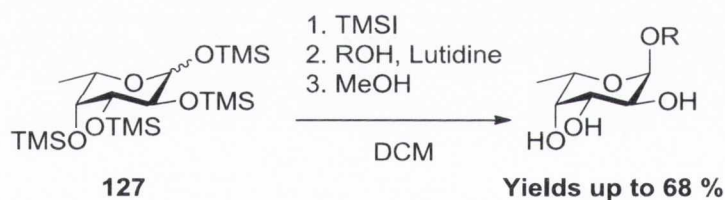
The requirement for α -fucosylation has historically been achieved through the use of non participating protecting groups (Scheme 2.6), and the most frequently used protecting group is the O-benzyl ether.^{166, 193-194} Other variants of the OBn group are known to enable more facile deprotection: the meta-NO₂-benzyl protecting group (Figure 3.2), which can be removed by UV light, and the para-OMe-benzyl protecting group ((Figure 3.2), which can be removed by milder hydrogenation conditions or oxidation.¹⁹⁵



Figure 3.2 - Alternative O-benzyl based protecting groups

Whilst these could be viable options in the synthesis of (**125**, Figure 3.1), only limited investigations on the use of silyl based protecting groups for α -fucosylation, have been reported. Silyl groups can be introduced in high yields (**71**, Scheme 2.3). They can be removed in the presence of unsaturated bonds (Scheme 2.4), and remain orthogonal to other commonly employed protecting groups in carbohydrate chemistry like amides, esters, benzyl ethers, and acetonides, should there be a requirement for further glycosylation of the product glycoside.

The use of a per TMS protected fucose donor has been reported by Hindsgaul *et al.*, (Scheme 3.1).¹⁹⁶ Activation of donor **127**, with TMSI led to α selective fucosylation. The relatively unstable nature of the TMS protecting group, means that further manipulation of the glycosylated product was restricted without further protection of the product.



Scheme 3.1 - Per TMS fucosyl donor activation

A per-TBDMS galactofuranose donor (**128**, Figure 3.3), was investigated by Marino *et al.*¹⁹⁷⁻¹⁹⁸ Donor **128**, was also activated by TMSI, however, the anomeric selectivity of the donor was not exclusive unlike (**127**, Scheme 3.1).

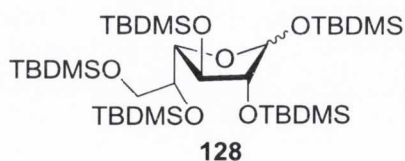


Figure 3.3 - Per TBDMS galactofuranose donor

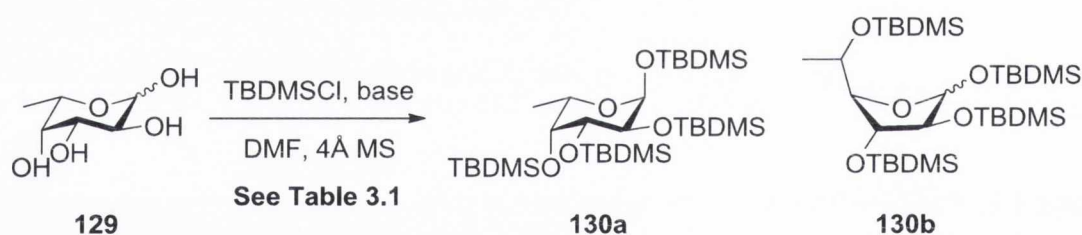
Application of a combination of the strategies used in **127** and **128**, to prepare a per-TBDMS fucose donor, would avoid a hydrogenation reaction in the synthesis of target

disaccharide (**125**, Figure 3.1), and if successful could be extended to O-propargyl Lewis X synthesis. A per-TBDMS fucopyranosyl donor had not been reported to date in the literature.

3.2 Synthesis of per silyl protected fucose donor

3.2.1 Per TBDMS protected fucose donor synthesis

The ability to synthesise in one pot, an alkyne tolerating α specific fucose donor, would provide a much more efficient synthetic route than the current best in class α -fucose donor, per-benzylated thioglycoside (**81**, Scheme 2.5). Attempted synthesis of the donor **130** from the commercially available L-fucose **129**, is shown in Scheme 3.2. The required tetra silyl protected donor **130a**, was not isolated, but rather the furanose isomer **130b**, was observed in up to 85 % yields. The furanose product was assumed to be the most thermodynamically stable, with 3 TBDMS protecting groups on the ring, as opposed to the 4 which would be forced into close proximity in the pyranose isomer **130a**. The structure was confirmed by HMBC NMR studies (Figure 3.4), where a correlation between H-1 to C-3 and C-4, was observed.



Scheme 3.2 - Per TBDMS fucosyl donor synthesis

Table 3.1 - Reaction conditions for attempted per-silylation of fucose

Entry	TBDMSCl	Base	Temperature °C	Time	Yield 130a	Yield 130b
1	5 eq.	Imidazole	40	16 h	Inseparable mixtures of partially protected products	
2	8 eq.	Imidazole	40	48 h	-	70 %
3	8 eq.	2,6 Lutidine, DMAP (cat.)	100	24 h	-	85 %

Investigations on fucofuranoside **130b** or possible applications as a fucosyl furanose donor, were not pursued any further by our group. Later, Ferrières *et al.* independently reported the structure **130b** in 2012, using TBDMSCl to globally protect fucose, with reported yields of 60 %. The application of **130b**, in the synthesis of fucofuranosides under TMSI activating conditions, was also demonstrated.¹⁹⁹

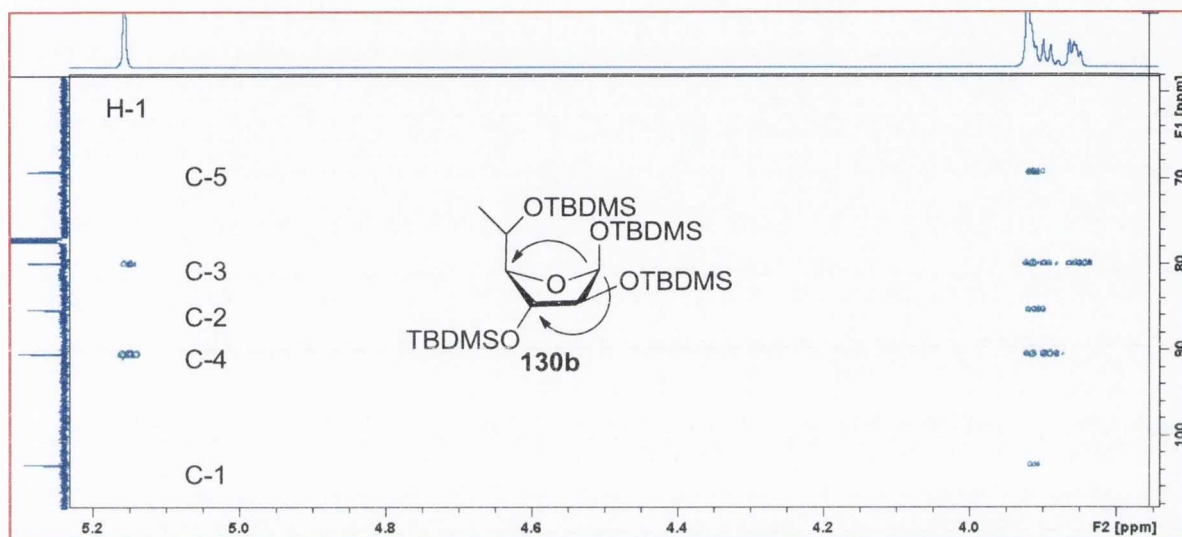


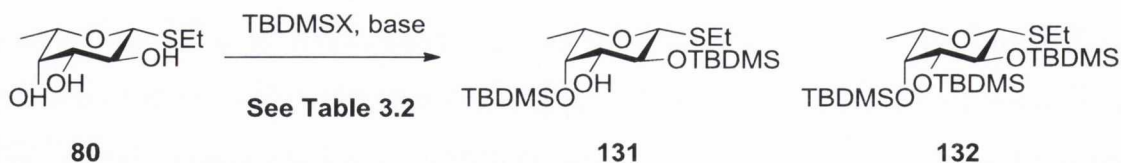
Figure 3.4 - Expanded HMBC (600 MHz) of **130b** showing furanose structure in CDCl_3

3.2.2 Synthesis of a conformationally locked silylated fucopyranoside donor

3.2.2.1 Preliminary issues with silylation leading to a super armed donor

The reason for the successful preparation of TMS protected fucose donor **127**, where **130a** failed, results from the contribution of steric bulk associated with TBDMS protection, which under basic conditions equilibrates to a furanose in order to relieve strain. If fucose was first conformationally locked as a pyranose thioglycoside, and then treated with TBDMSCl, the resulting per-silylated donor would retain the required alkyne orthogonality as outlined in the introduction, (Section 3).

Bols *et al.* have published a series of papers on per-TBDMS pyranoside thioglycoside donors, subsequently coining the term “Super armed,” by virtue of the observed increased reactivity of per-TBDMS donors over other armed donors.^{60-61, 200} Interestingly, amongst all the super armed donors prepared, the fucose example **132**, had not been reported in the literature to date. The previously prepared thioglycoside (**80**, Scheme 2.5), was subjected to a number of silylation conditions, (Scheme 3.3).



Scheme 3.3 - Per TBDMS fucosyl thioglycoside donor synthesis

Table 3.2 - Optimisation of Ethyl-2,3,4-tri-TBDMS-Fuc- β -thioglycoside synthesis

Entry	Reagent	Solvent/Base	Temperature °C	Time	% Yield ^c 131	% Yield ^c 132
1	TBDMSCl ^a	DMF, Imidazole	40	16 h	26	Trace
2	TBDMSCl ^a	DMF, Imidazole	50	48 h	86	5
3	TBDMSCl ^a	DMF, Lutidine, DMAP (cat.)	100	96 h	22	46
4	TBDMSCl ^a	Pyridine, DMAP (cat.)	0-60	24 h	-	83
5	TBDMSOTf ^b	Pyridine, DMAP (cat.)	0-60	36 h	-	97

^a = 5 eq, ^b = 4.5 eq, ^c = isolated yields

The results of a per silylation study on **80**, are presented *vide supra*. (Entries 1-2, Table 3.2), yielded a partially protected thioglycoside **131**, in reasonable yields. This compound will be discussed in chapter 4 due to observed unusual reactivity. (Entries 3-5, Table 3.2) show through variation in temperature, the yield of desired donor **132**, could be increased. A jump in yield was observed when pyridine was substituted as both base and solvent. Finally, following discussions with Dr. Henrik Jensen at the 16th European carbohydrate symposium, Sorrento, TBDMSOTf was employed as the TBDMS source, providing almost quantitative yields of **132**, in multi-gram scale. The use of TBDMSOTf as a more electrophilic source of TBDMS has been well documented for the protection of hindered hydroxyl groups in the literature.⁶⁰

3.2.2.2 Characterisation of novel the fucose donor **132**

NMR spectroscopic analysis of **132** was problematic due to restricted rotation of the bulky TBDMS groups. This steric issue is quite obvious once a molecular model of the donor was prepared. Previous synthesis of "Super-armed" donors required the use of variable temperature NMR in order to speed up the rotation timelines in solution, and resolve any

broadened proton signals.⁶⁰ NMR spectroscopy performed at increased temperature has the effect of averaging out different conformations in solution. The results of variable temperature NMR experiments on **132**, can be seen in Figure 3.5. Finer detail in the proton shifts are observed at higher temperatures.

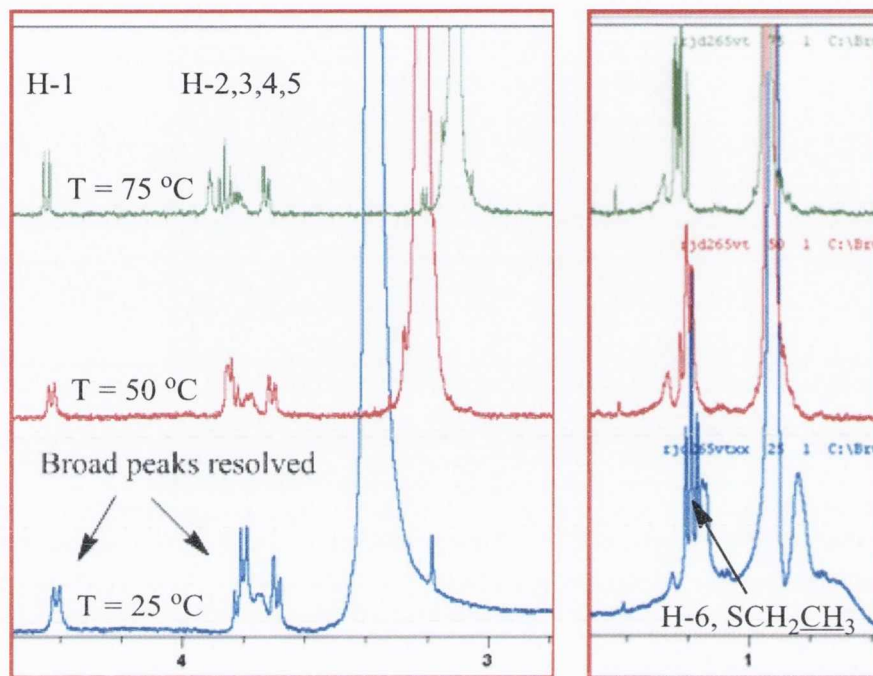


Figure 3.5 - Temperature dependant ^1H NMR (400 MHz) of Tri-TBDMS fucose thioglycoside in d_6 -DMSO

3.2.2.3 ^{13}C NMR problems with donor **132**

Attempted analysis of **132**, by ^{13}C NMR spectroscopy at room temperature, was complicated by significant signal broadening and in some cases the absence of signals altogether, due to restricted rotation issues from the large TBDMS groups. Variable temperature NMR spectroscopy could not be performed in CDCl_3 , due to a limited boiling point, so samples were dissolved in d_6 -DMSO. Even at the elevated temperatures, the solubility of **132**, in d_6 -DMSO was low, and the spectrums recorded were not useful from a signal to noise standpoint. Increasing the amount of material resulted in crystal formation in the NMR tube. Crystalline solids lowered the quality of the spectrum further, as they interfered with the uniformity of the magnetic field. A suitable ^{13}C NMR spectrum could not be attained for **132**, even at raised temperatures.

3.2.3 X-ray structure of fucosyl donor **132**

In the absence of ^{13}C data, another method of characterisation was performed. Large cuboidal crystals of **132**, were grown from 2% Et_2O :Hexane and the resulting X-ray structure (crystal mounted and analysed by Dr. Tom McCabe) is presented in Figure 3.6.

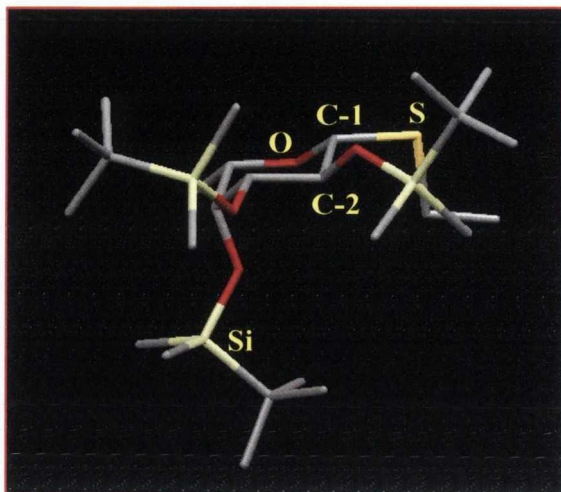


Figure 3.6 - Crystal structure of tri-(*O*-*tert*-butyldimethylsilyl)- β -fucose-thioglycoside, **132**

It was interesting to note that the steric hindrance of the bulky TBDMS protecting groups did not appear to induce ring flipping. The isolated crystals of **132**, are clearly in the $^1\text{C}_4$ conformation. Obviously in solution this may not be the case, but given (Figure 3.6), was the experimentally derived structure, **132**, shall be drawn in the $^1\text{C}_4$ conformation for the duration of this thesis. With a high yielding synthetic route and crystal structure data identifying the compound, the synthetic applications of donor **132** were investigated.

3.3 Selective glycosylation of *O*-propargyl GlcNAc acceptor **70**

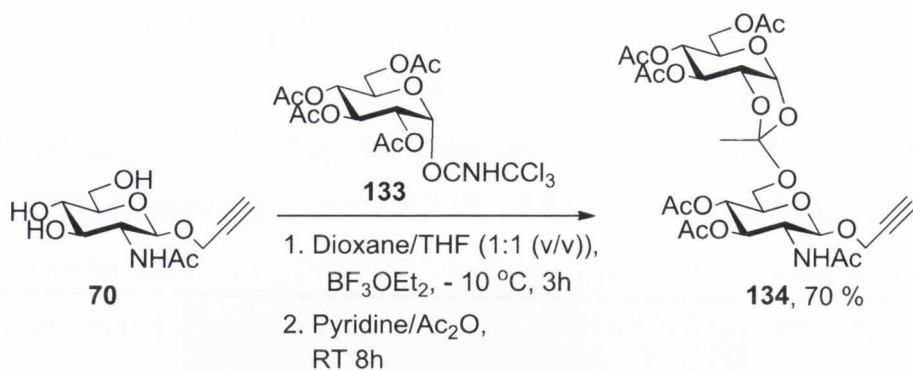
Whilst optimisation of the tri silyl donor **132** was in progress, a separate study on the acceptor (**70**, Scheme 2.2), required for the synthesis of the target disaccharide (**125**, Figure 3.1), was performed. During synthesis of *O*-propargyl-LacNAc (Scheme 2.4), selective glycosylation of the 4-OH over the 3-OH was shown to be efficient. Reactivity differences between primary and secondary hydroxyls allow selective protection of carbohydrates. The exhaustive requirement for protecting group manipulations in carbohydrate chemistry leads to longer synthetic routes. Investigation into selective 6-OH glycosylation of *O*-propargyl GlcNAc acceptor **70**, in the absence of any protecting groups was investigated.

Glucose trichloroacetimidate (**133**, Scheme 3.4), was prepared according to literature procedure.²⁰¹ Cold reaction temperatures are required for selective glycosylations, in order to maximise any differences in reactivity, however, the acceptor **70**, containing three free hydroxyl groups demonstrated low solubility in the chlorinated and ethereal solvents traditionally used for glycosylations. Solubility tests with different solvents showed acceptor **70**, dissolved in dioxane, however, as dioxane freezes at 11 °C, the ability to cool this solvent is limited and a mixed system would be required. A summary of the mixed system solubility study is presented in Table 3.3. If the concentration of THF was increased above 50 %, the acceptor **70**, precipitated from solution below -15 °C.

Table 3.3 - Solubility of acceptor 70 (0.35M) in Dioxane:THF

Entry	Solvent system (v/v)	Solubility of 70 , at 5 °C above solvent mixture melting point	Melting point °C
1	Dioxane : THF (100:0)	Y	11
2	Dioxane : THF (75:25)	Y	1
3	Dioxane : THF (50:50)	Y	-15
4	Dioxane : THF (0:100)	Precipitation	-108

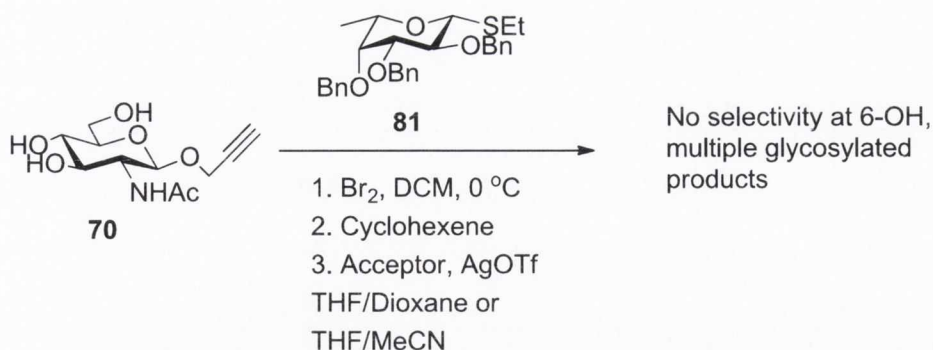
From (Table 3.3) it could be seen that the acceptor **70**, was soluble at a temperature of -10 °C in Dioxane:THF (1:1 (v/v)). Glycosylation of acceptor **70**, with donor **133** activated using BF₃OEt₂, was carried out according to Scheme 3.4. For ease of purification the crude mixture was per-acetylated before the main product, orthoester **134**, was isolated in 70 % yield. This was a positive result insofar as the selectivity for the primary alcohol in **70**, was demonstrated. Given the fact that the per-silylated fucose donor **132**, had no acetyl groups, orthoester formation could not occur. There was one unusual aspect to the characterisation of **134**, that of a long range J_{4,2} coupling of 0.8 Hz on glucose. Obviously the bicyclic ring set up due to the formation of the orthoacetate product **134**, aligns the protons in a manner to allow a long range coupling. This coupling is of a similar order to that reported for a 1,6 anhydrosugar.²⁰²



Scheme 3.4 - Attempted selective glycosylation test on acceptor 70 - (Disarmed)

3.4 Selective glycosylation of O-propargyl GlcNAc acceptor 70 with 81, an armed donor

The test system in Scheme 3.4 may have been a slightly biased reaction, as the donor **133**, was disarmed. This would favour glycosylation of the most reactive hydroxyl. An armed donor would have increased reactivity and may overcome the difference in activation energies for glycosylating a primary hydroxyl over a secondary hydroxyl. In order to test this hypothesis the analogous reaction was performed with donor **81**, (Scheme 3.5). Unfortunately, this reaction resulted in a complex mixture, and it was clear that selectivity for the primary alcohol was lost. This was confirmed when crude HSQC spectra after acetylation, showed glycosylated products having downfield chemical shifts signalling acetylation had occurred on the 6-OH position, as well as acetylated 3-OH and 4-OH positions of the carbohydrate at the reducing end. Acceptor **70**, was found to dissolve in a MeCN:THF (1:1 (v/v)), which could be cooled to -45 °C. Although the colder reaction temperature was expected to increase primary hydroxyl selectivity, no great improvement by TLC was observed. The lack of 6-OH/4-OH selectivity was used by Weimar *et al.*²⁰³ where a 3,4,6-OH deprotected GlcNAc thioglycoside acceptor was used in a convergent synthesis of an OBn-Fuc (1-4 and 1-6) GlcNAc disaccharide, later separated by column chromatography.



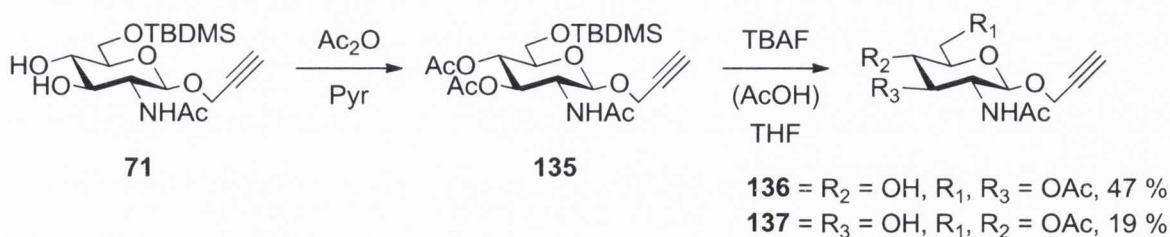
Scheme 3.5 - Attempted selective glycosylation test on 70 - (Armed donor)

Given the lack of selectivity for the primary OH with the armed donor (Scheme 3.5), most closely related to the silyl donor **132**, it was accepted that selective protection of the acceptor **70**, should be carried out.

3.5 Preparation of a 3,4 protected O-propargyl GlcNAc acceptor

3.5.1 Base labile GlcNAc 6-OH protecting group strategy - TBDMS protection

Preparation of a 3,4 selectively protected 6-OH O-propargyl GlcNAc **139**, was attempted from acceptor **71**, according to Scheme 3.6. The route required acetylation and selective TBDMS deprotection. Unfortunately, the basic conditions involved in TBDMS removal using TBAF in THF, resulted in significant acetyl migration and a mixture of two deacetylated regioisomers, **136** and **137** was observed. The issue of OAc migration results from the oxyanion product following TBDMS removal. Literature reports for avoiding this acetyl migration through acetic acid buffering²⁰⁴ lowered the incidence of migration products but as migration still occurred this was not an efficient route.

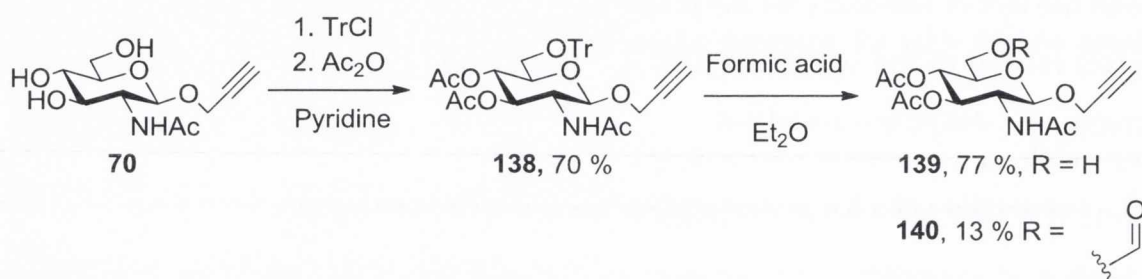


Scheme 3.6 - Base labile GlcNAc 6-OH protection

3.5.2 Acid labile GlcNAc 6-OH protecting group strategy - Trityl protection

Base mediated 6-OTBDMS deprotection was abandoned for an acid labile route which should avoid acetyl migration. Trityl chloride was used in a two step, one pot synthesis of

138 in 70 % yield from **70**, as shown in Scheme 3.7. The one step trityl/acetylation reaction represents a slight improvement on the method published by Szarek *et al.*, which involved the isolation and purification of each intermediate.²⁰⁵ **138**, was selectively deprotected with formic acid¹⁹⁵ to yield acceptor **139**, in 54 % yield over the 3 steps. Isolation of a formate protected side product **140**, was observed, but was easily separated by column chromatography. Attempts to convert the formate side product **140**, into **139**, by refluxing in MeOH led to acetyl migration, and so **140**, production is simply minimized through careful monitoring of the reaction by TLC.

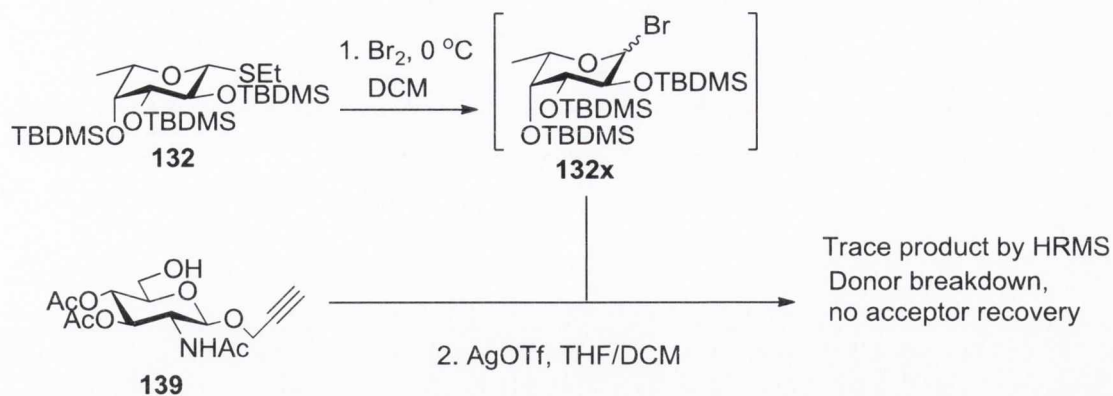


Scheme 3.7 - Acid labile GlcNAc 6-OH protection

3.6 Optimisation of α -Fuc-(1-6)-O-propargyl-GlcNAc activation conditions

3.6.1 Initial attempts at Br₂/AgOTf activated synthesis of target disaccharide **125**

With both the acceptor **139** and donor **132** prepared, their glycosylation was investigated. The presence of two additional acetyl groups allowed the acceptor **139**, to dissolve in DCM, so the need for mixed solvent systems was avoided. Unfortunately, early attempts at the glycosylation were unsuccessful, (Scheme 3.8). Activation of donor **132**, with Br₂ was performed according to literature procedure¹⁷³ and the excess bromine was either quenched with cyclohexene or removed through evaporation. The bromide **132x** was not isolated, but reacted directly with acceptor **139** at -40 °C, followed by silver triflate activation. Complete consumption of the donor **132** had occurred, but the required disaccharide product was only visible in trace amounts by HRMS analysis of the crude reaction mixture.



Scheme 3.8 - Attempted Br_2/AgOTf mediated glycosylation

3.6.2 NMR analysis of donor **132** activation with Br_2

Whilst attempting to improve yields for the glycosylation presented in Scheme 3.8, conveniently, Demchenko *et al.* published a paper using NMR spectroscopy to monitor the α/β interconversion of armed and disarmed thioglycoside derived, glycosyl bromides.²⁰⁶ Concerns following the failure of the reaction in Scheme 3.8 were that over the course of the 90 minute activation period of **132** with Br_2 ,¹⁷³ the TBDMS groups were being removed. This would result in the reaction failing due to donor **132** breakdown, leading to polymerisations in the reaction vessel. Isolation of the **132x**, was not possible due to its instability, however, a similar NMR study to that conducted by Demchenko *et al.*²⁰⁶ monitoring the treatment of **132** with Br_2 , was investigated. Bromine addition to **132**, in distilled CDCl_3 , at -196°C under strictly anhydrous conditions against a positive pressure of nitrogen was performed. A proton spectrum was recorded less than 5 min after Br_2 addition. Overlaid SM, 5 minutes, and 90 minutes post Br_2 addition, NMR spectra are shown in Figure 3.7 and Figure 3.8. The first spectrum shows clean conversion of **132**, to the bromide, with no apparent breakdown after 90 mins at 0°C . In a rescaled section (Figure 3.8), the presence and stability of the TBDMS groups for at least 90 min was confirmed. It was concluded that the formation of the bromide intermediate **132x** was almost instantaneous, and the 90 minutes activation period reported for other donors not required in this case.¹⁷³

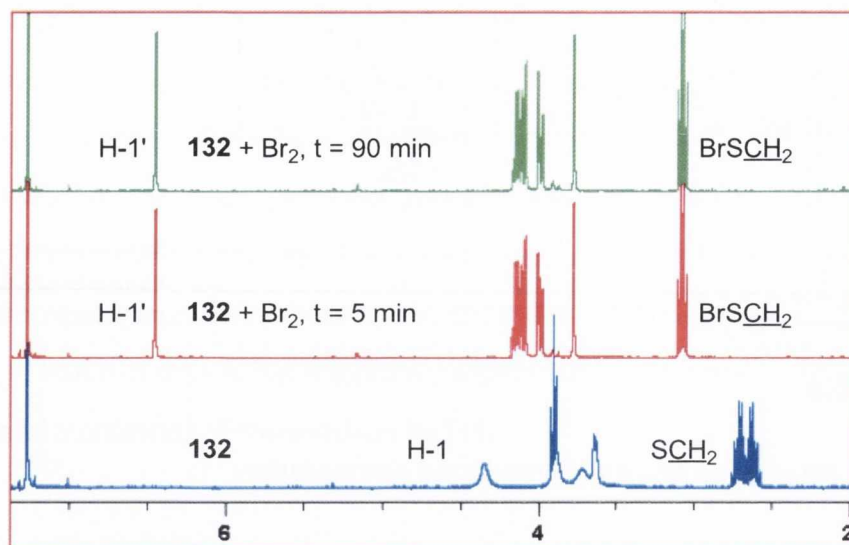


Figure 3.7 - Br₂ addition to **132** in CDCl₃, δ_{H} 6.8 - 2 ppm, (400 MHz)

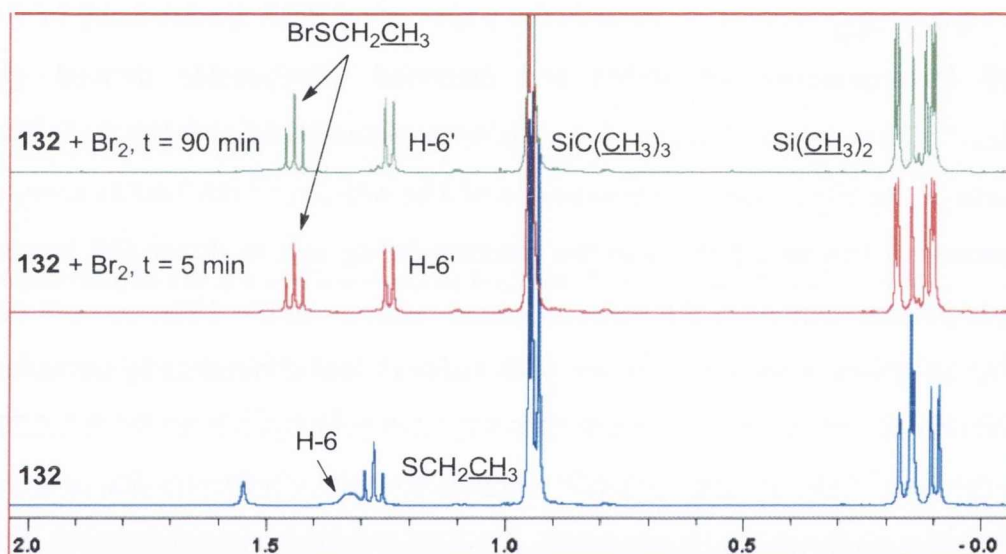


Figure 3.8 - Br₂ addition to **132** in CDCl₃, δ_{H} 2 - 0 ppm, (400 MHz)

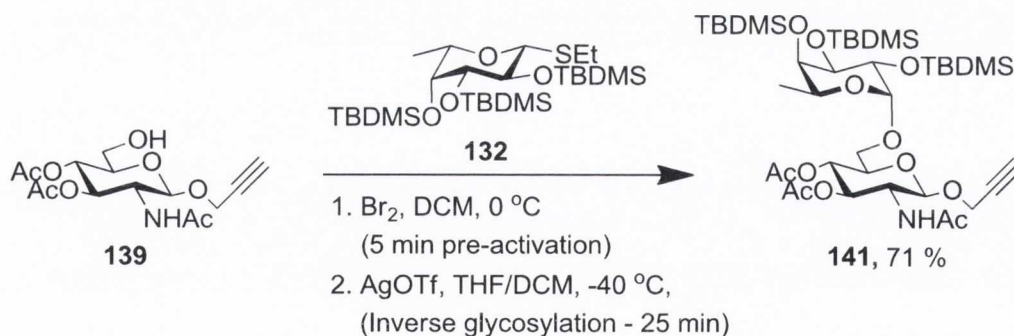
3.6.3 Revisiting the Br₂/AgOTf activation

The NMR studies on Br₂ activation of donor **132**, confirmed the stability of the TBDMS groups during activation, and the intermediate fucosyl bromide **132x**, was present. It was concluded that the problems observed with the glycosylation in Scheme 3.8 must occur, after this step. Reviewing the literature for examples of α -Fuc-(1-6)-GlcNAc synthesis, it became apparent that the α -1-6 linkage is a notoriously weak glycosidic bond.²⁰⁷⁻²⁰⁸ Kunz *et al.* showed the presence of arming groups such as benzyl protection on the fucose, increased the instability of the α -1-6 glycosidic bond.²⁰⁹ Both this group and Weimar *et*

*al.*²⁰³ employed *in situ* anomerisation of fucosyl bromide donors with tert-butylammonium bromide, a mild activating method, taking up to 5 days for donor consumption. The highly reactive nature of the donor **132**, coupled with the weak glycosidic bond in the product, appears to be the main reason for the failure of (Scheme 3.8). Schmidt *et al.* pioneered the inverse glycosylation method, which provided increased glycosylation yields when using highly reactive trichloroacetimidate donors.²¹⁰ Inverse glycosylation therefore seemed appropriate in efforts to prepare α -Fuc-(1-6)-O-propargyl- β -GlcNAc, (**125**, Figure 3.1).

3.6.4 Inverse glycosylation with Br₂/AgOTf activated synthesis of target **125**

In preparation for the inverse glycosylation reaction, addition of AgOTf to the acceptor **139** was performed, to ensure stability of the acceptor. No reaction was observed by TLC and subsequently the glycosylation (Scheme 3.9), was attempted. Inverse glycosylation keeps the acceptor and activator concentrations higher than the donor, encouraging glycosylation of the acceptor before side product formation, or hydrolysis. Fortunately the reaction proceeded very cleanly with the isolation of **141**, in 71 % yield, along with high α selectivity (10.2:1, α : β) and short reaction times (30 min).



Scheme 3.9 - Inverse glycosylation route to **141**

The successful synthesis of **141**, demonstrated the synthetic application of the tri-silyl donor **132**. This donor is important for the introduction of α -fucose in the presence of unsaturated bonds. The use of Br₂ is not ideal however, due to the gradual decrease in pH, of Br₂ over time on storage. Addition of wet bromine leads to partial TBDMS deprotection and lower yields due to contamination from HBr. Investigations into alternative activation methods for donor **132** were performed.

3.6.5 Alternative activations condition and summary table

Table 3.4 presents a screen of alternative activation techniques as applied to the synthesis of disaccharide **141**. A recently discovered thioglycoside activation technique described by Fugedi *et al.*, and gaining acceptance in the literature; 16 synthetic citations since 2010, is the DMDS and Tf₂O method.¹⁶⁹ Attempts using this activator in the absence of a hindered basic buffer, led to degradation and loss of TBDMS groups. Both DTMP and TMU²¹¹ have been used as buffers in order to minimise the loss of acid labile protecting groups. The use of these buffers lead to the required product **141**, in 13 % and 21 % yields respectively, with reasonable anomeric selectivity by NMR.

Activation of **132**, with NIS, once maintained at, or below -20 °C did not lead to iodine addition across the alkyne in **139**. The acceptor **139**, was consumed within 80 minutes, and the product **141**, was isolated in an improved yield of 86 %, with good α anomeric selectivity (8.1:1 α:β). Anomeric mixtures could not be separated with TBDMS protecting groups present, and ratios were determined by NMR. The anomeric mixtures could be separated after a TBDMS to acetyl protecting group interconversion was carried out.

Table 3.4 - Effect of activation conditions on the synthesis of 141

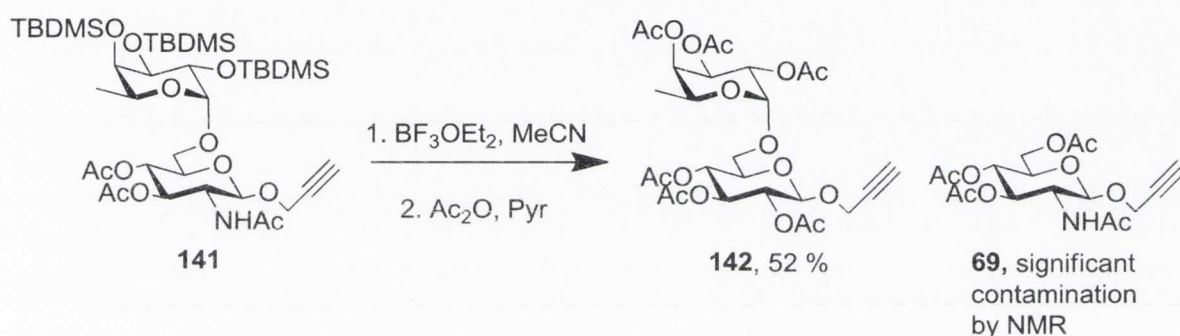
Entry	Activation condition	Temperature °C	Time (Min)	Yield (α:β)
1	DMDS, Tf ₂ O	-40	30	Not isolated
2	DMDS, Tf ₂ O, DTMP	-40	30	13 %, (5.1:1, α:β)
3	DMDS, Tf ₂ O, TMU	-40	30	21 %, (5.4:1, α:β)
4	Br ₂ , AgOTf, cyclohexene	-40	30	Trace
5	Br ₂ , AgOTf ^a	-40	25	71 %, (10.2:1, α:β)
6	NIS, TMSOTf	-20	80	86 %, (8.1:1 α:β)

^a = inverse glycosylation

3.7 Deprotection of protected α-Fuc-(1-6)-O-propargyl-GlcNAc, **141** - BF₃OEt₂

Davis *et al.* published a one pot de-silylation and deacetylation using BF₃OEt₂ in MeCN as a fluoride source, followed by the addition of Na₂CO₃ and MeOH to deacetylate. Application of this procedure on **141**, led to the complete hydrolysis of the α-1-6 glycosidic bond and yielded no deprotected disaccharide.

As a milder alternative, the route was modified to a protecting group interconversion. Following BF_3OEt_2 mediated removal of the TBDMS groups at room temperature, the Lewis acid was quenched with an excess of pyridine and the mixture acetylated overnight to yield **142**, in 52 % (Scheme 3.10). When triethylamine was used to quench the reaction, a lower yield of the acetylated disaccharide **142**, resulted. As could be expected, the Lewis acid also caused partial cleavage of the 1-6 α -glycosidic linkage, resulting in the contaminant **69**. Strictly anhydrous conditions can minimise the observed glycosidic cleavage, however, per-acetylated O-propargyl GlcNAc **69** co-elutes with the required disaccharide **142**, during chromatography and so its production needed to be eliminated.



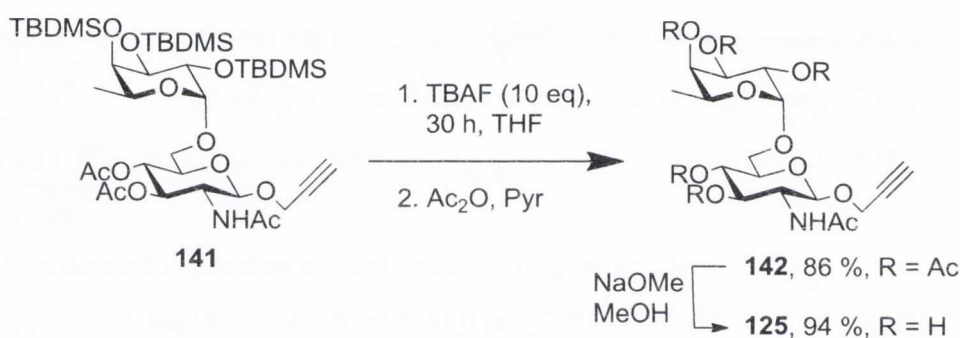
Scheme 3.10 - BF_3OEt_2 mediated TBDMS deprotection of disaccharide **141**

3.8 Deprotection of α -Fuc-(1-6)-O-propargyl GlcNAc, **141** - TBAF

TBAF deprotection is commonly used for the removal of primary hydroxyl TBDMS groups. Attempted deprotection of **141**, with 1.1 eq. of TBAF per TBDMS group at RT did not proceed. Bols *et al.* noted the difficulty in the deprotection of TBDMS groups on super armed donors,⁶⁰ recommending the use of up to 3 and more equivalents of TBAF per TBDMS group. No specified time for the reaction was detailed indicating long reaction times may be required. 3.3 equivalents of TBAF per TBDMS on **141**, were stirred for 16 h. The naked F^- ion in THF is a very strong base and promotes acetyl migration as described in Scheme 3.6. For this reason, the reaction was quenched with the addition of pyridine and acetic anhydride to ensure per-acetylation (Scheme 3.11). Two products were isolated following this reaction, the starting material **141** in 71 % yield, and the required per-acetylated disaccharide **142** in 28 % yield. The $\text{S}_{\text{N}}2$ elimination of a glycoside from a silyl protecting group, proceeds via a proposed penta co-ordinate Si atom. The clashing of multiple TBDMS groups should cause a comparatively high energy barrier for the first

deprotection. Once an initial TBDMS group is removed, the steric bulk should be lowered and due to the large excess of TBAF, subsequent deprotection should occur more readily. Applying this hypothesis, TBAF deprotection was repeated for 30 hours, and following per-acetylation successfully yielded the disaccharide product **142**, in 87 % yield. Deacetylation with NaOMe/MeOH yielded the target structure **125**, in 94 % after freeze drying, (Scheme 3.11).

The successful preparation of **125**, confirms the importance of donor **132**. The terminal fucose disaccharide **125**, could not have been prepared using the traditional fucose donor (**81**, Scheme 2.5), in the presence of an alkyne. In addition, the glycosylation yield of 86 % (Table 3.4), using NIS mediated activation of **132**, is an increase on the reported yields found when employing the per-TMS protected fucosyl donor (**127**, Scheme 3.1).



Scheme 3.11 - TBAF mediated TBDMS deprotection of disaccharide 141

3.9 α -Fuc-(1-6)-O-propargyl-GlcNAc glycoporphyrin synthesis

3.9.1 Monosubstituted α -Fuc-(1-6)-O-propargyl-GlcNAc glycoporphyrin

Given the difficulty in the synthesis of the target disaccharide **125**, a monosubstituted glycoporphyrin was first prepared to ensure the weak α -1-6 glycosidic linkage could tolerate the previously optimized microwave CuAAC conditions. Using the median polarity conditions described by (Entry 10, Table 2.2), the reaction proceeded with a yield of 95 % to give **143**, as a purple solid (Figure 3.9), further demonstrating the mild nature and scope of the optimised CuAAC reaction (Table 2.2). Glycoporphyrin **143**, could be conveniently purified with normal phase chromatography EtOAc:EtOH:H₂O (7:2:1 (v/v/v)). The α -1-6 glycosidic linkage remained stable through preparation and purification.

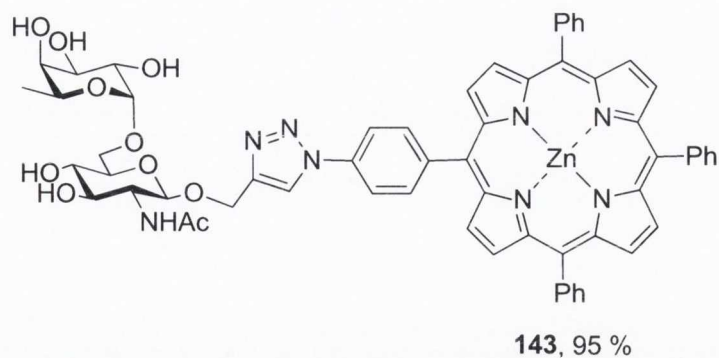


Figure 3.9 - 5-Mono- α -Fuc-(1-6)-O-propargyl-GlcNAc glycoporphyrin, 143

3.9.2 Trisubstituted α -Fuc-(1-6)-O-propargyl-GlcNAc glycoporphyrin synthesis

The successful covalent attachment of disaccharide **125** onto a porphyrin scaffold, (Figure 3.9) confirmed the microwave mediated CuAAC conditions (Table 2.2), did not cleave the weak α -1-6 linkage. Attention now turned to the synthesis of the target tri-substituted glycoporphyrin analogue, for lectin investigations at NUIG. Using the solvent condition (Entry 10, Table 2.2), and a slight excess of the disaccharide **125**, the glycoporphyrin product **144**, was prepared in 79 % yield, (Figure 3.10). Interestingly, unlike the tri-LacNAc substituted glycoporphyrin (**122**, Figure 2.11), which was too polar for normal phase chromatography, the result of ligating the fucose containing disaccharide **125**, is a glycoporphyrin product **144**, containing three less hydroxyl groups. The difference in polarity was sufficient to allow normal phase column chromatography (EtOAc:EtOH:H₂O, 60:30:15 (v/v/v)). Upon removal of all the solvent, gratifyingly, glycoporphyrin **144** was found to be water soluble.

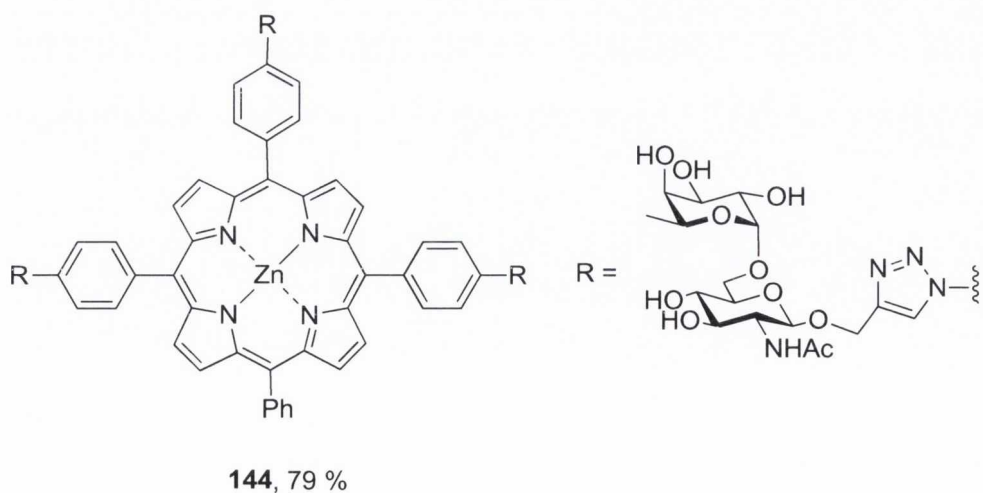


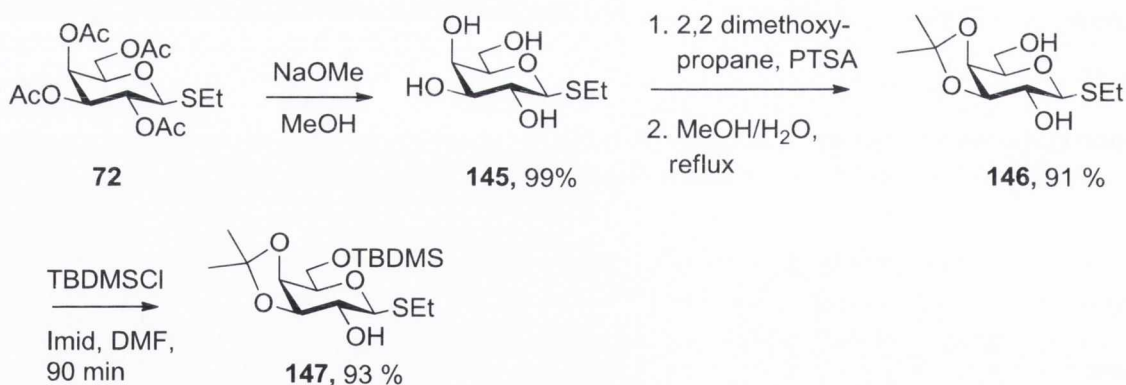
Figure 3.10 - 5,10,15-Tri- α -Fuc-(1-6)-O-propargyl-GlcNAc glycoporphyrin, 144

3.10 Investigations into acceptor scope of the tri-TBDMS fucosyl donor 132

The tri-TBDMS donor **132**, is both super armed by virtue of the TBDMS protection, but also highly hindered as evident from the subsequent difficulty in TBDMS removal. A series of alcohols and carbohydrates were prepared in order to explore the synthetic scope of this new donor.

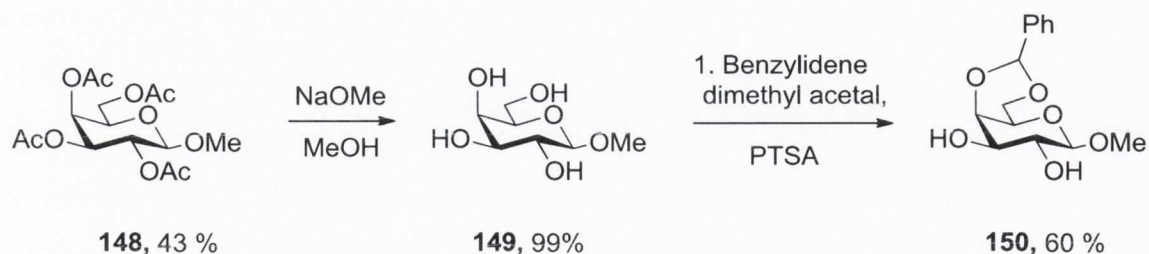
Glycosylation of an unhindered primary alcohol had already be successfully attempted (Table 3.4). Acceptors bearing hindered and unhindered secondary hydroxyls were therefore prepared for investigation.

The thioglycoside acceptor **147**, was prepared (Scheme 3.12), both as an unhindered secondary alcohol acceptor, but also to examine the ability of donor **132** to be selectively activated. Previously prepared galactose thioglycoside **72**, was deacetylated quantitatively to provide **145**, and a selective 3,4 acetonide protection was performed according to the 2 step, one pot procedure reported by Catelani *et al.*, to prepare thioglycoside **146**.²¹² A selective primary TBDMS protection step allowed the isolation of acceptor **147** in 84 % over 4 steps. Competitive activation of donor **132** in the presence of the thioglycoside acceptor **147**, would provide insight into the armed nature of the silylated fucose donor **132**.



Scheme 3.12 - Preparation of thioglycoside acceptor 147

Another galactose based acceptor **150**, was prepared, (Scheme 3.13), to investigate the steric implications of donor **132**. Di-glycosylating **150**, on the two hydroxyls in close proximity with the bulky donor **132**, should prove difficult due to the steric hindrance associated with introduction of the second donor **132**. Any difficulty in performing the second glycosylation may exaggerate selective glycosylation of a given hydroxyl position.



Scheme 3.13 - Preparation of a diol acceptor

Collated results of various glycosylations employing donor **132**, and the highest yielding activation method previously reported for this system (Entry 6, Table 3.4), are shown in Table 3.5. Reactions proceeded in high yields for the less hindered acceptors, with moderate to exclusive α selectivity. As test reactions (entry 1-2, Table 3.5) show, the unhindered primary alcohols, propargyl alcohol **63**, benzyl alcohol **152**, were glycosylated in high yields. There was however, a curious difference in the anomeric selectivity of these two acceptors with the sterically smaller donor providing **151**, as an exclusively α product.

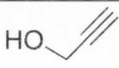
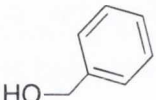
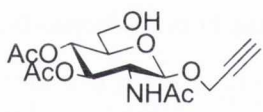
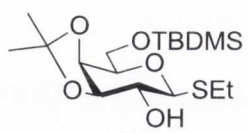
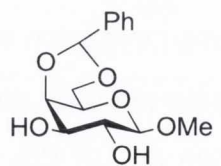
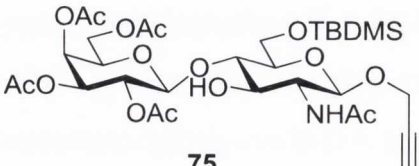
Gratifyingly donor **132**, could be selectively activated in the presence of another thioglycoside, **147**, (entry 4, Table 3.5) and provided a protected terminal disaccharide thioglycoside of the blood group H, in 66 % yield. Thioglycoside acceptor **147**, did not appear in the relative reactivity table reported by Wong *et al.*⁶², however, from review of the protecting groups required to enable a donor to behave as either armed or disarmed, it can be stated that **147**, would show neither qualities, and so could be referred to as non-armed.

Whilst the selective glycosylation of acceptor **150**, to prepare disaccharide **155** (entry 5, Table 3.5), did not provide a high yield, selectivity for the 3-OH, α to 4-O axial group was observed. Hydroxyl groups α to axial protecting groups have been shown to possess increased nucleophilicity over hydroxyls α to equatorial protecting groups, in previous work reported by Lindhurst *et al.*²¹³ The low glycosylation yield of **155**, resulted from the partial loss of the benzyldiene protecting group, perhaps through intermolecular hydrolysis in the presence of a Lewis acid during the glycosylation. Even in strictly anhydrous conditions, each acceptor contains an extra hydroxyl group which could lead to benzyldiene removal. Final confirmation of the 3-O glycosylation regioselectivity, was confirmed when acetylation of **155** gave **155a**, quantitatively.

Accessibility issues for an acceptor hydroxyl, coupled with the large steric bulk of donor **132**, were particularly exaggerated during the attempted re-synthesis of protected O-propargyl Lewis X **156**, (entry 6, Table 3.5). Longer reaction times were required in order to increase the glycosylation yield above 20 %, and observed yields of **156**, were not increased even if reaction continued for up to 16 h. Significant quantities of the unreacted acceptor **75**, could be recovered from the crude reaction mixture, pointing to a lack of accessibility the 3-OH on the acceptor lowering yields, as opposed to side product formation.

An obvious trend is observed throughout series (Table 3.5), where primary hydroxyl acceptors are more easily glycosylated than secondary groups, and the more hindered the alcohol the lower the yield of glycosylated product.

Table 3.5 - Scope of glycosylations with trisilyl donor 132

Entry	Acceptor	Activation Conditions	Yield ($\alpha:\beta$)
1	 63	NIS, TMSOTf, 40 min, -20 °C	151, 86 %, (α)
2	 152	NIS, TMSOTf, 40 min, -20 °C	153, 83 % (3.2:1, $\alpha:\beta$) ^a
3	 139	NIS, TMSOTf, 80 min, -20 °C	141, 86 %, (8.1:1 $\alpha:\beta$) ^a
4	 147	NIS, TMSOTf, 60 min, -30 °C	154, 66%, (α)
5	 150	NIS, TMSOTf, 40 min, -20 °C	155, 31 %, (α) - Glycosylated at 3-OH
6	 75	NIS, TMSOTf, 240 min, -20 °C	156, 24 %, (α), (71% recovered acceptor)

^a = anomeric ratio determined by ¹H NMR

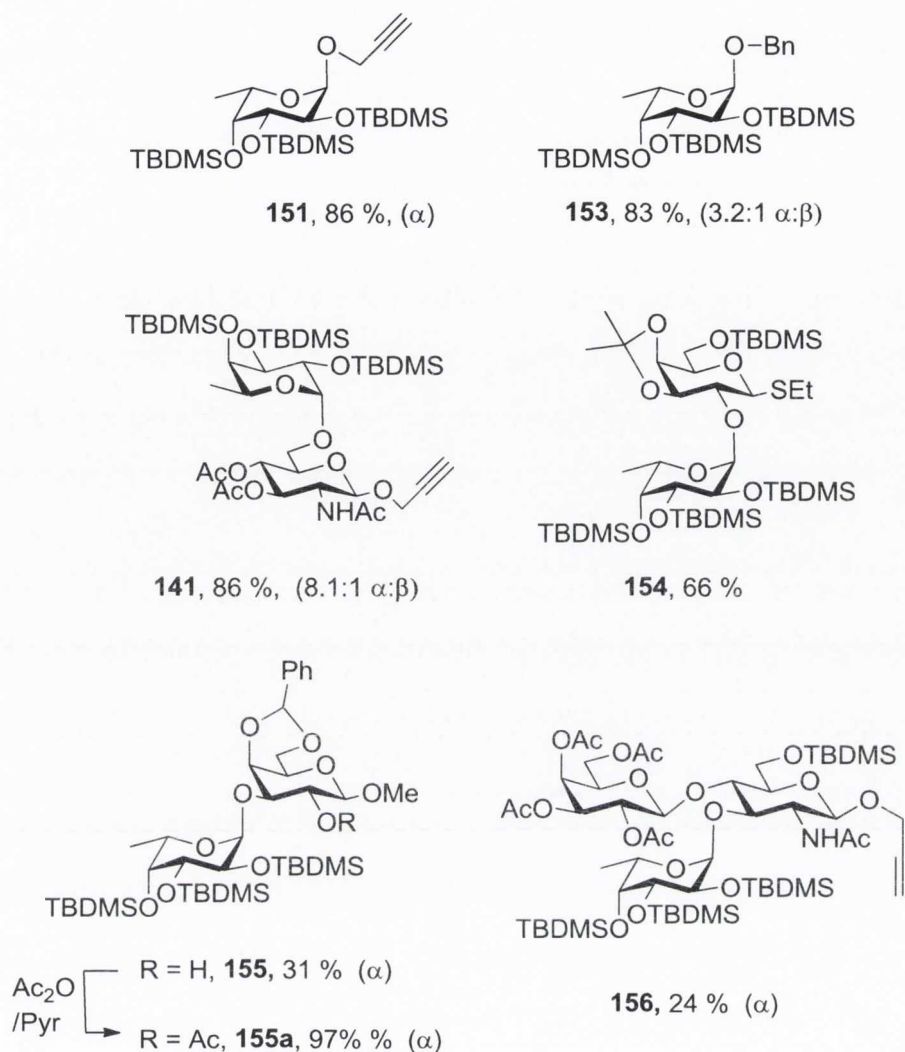


Figure 3.11 - Glycosylated products from Table 3.5

3.11 Suggestions for the low observed yield of protected O-propargyl Lewis X, **156**

In entry 6, Table 3.5, the yield of **156**, was particularly low. Increasing the reaction time, and donor **132**, ratio to 3 eq., did not lead to any measurable increase in yield, although the donor was being consumed. The ultimate fate of the consumed donor **132**, was expected to involve succinimide glycosylation. Evidence for this NIS derived glycosylated succinimide product has been discussed by Bols *et al.*⁶⁰, Davis *et al.*²¹⁴ and Wong *et al.*⁶² All groups have described this reaction to be significant during glycosylations of unreactive acceptors. The patterns displayed in these papers point to the fact that the tri-TBDMS donor **132** is very active, but the 3-OH position in protected LacNAc **75**, is a particularly weak or hindered nucleophile. Crude HRMS confirmed the presence of significant quantities of the proposed succinimide product, which was not isolated.

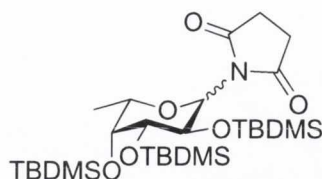
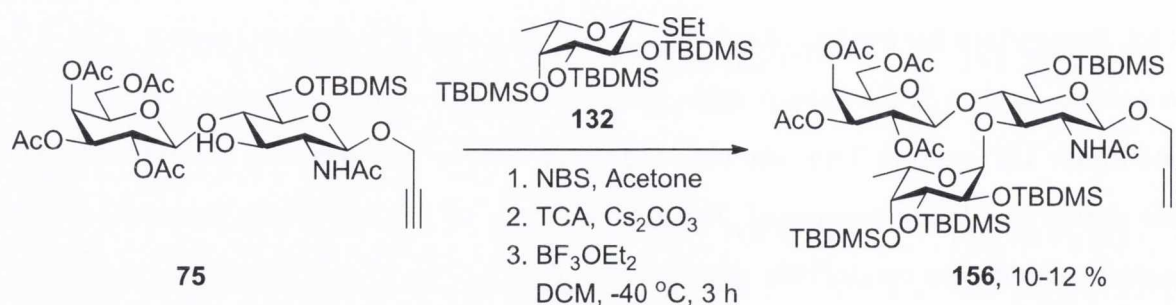


Figure 3.12 - Proposed succinimide side product

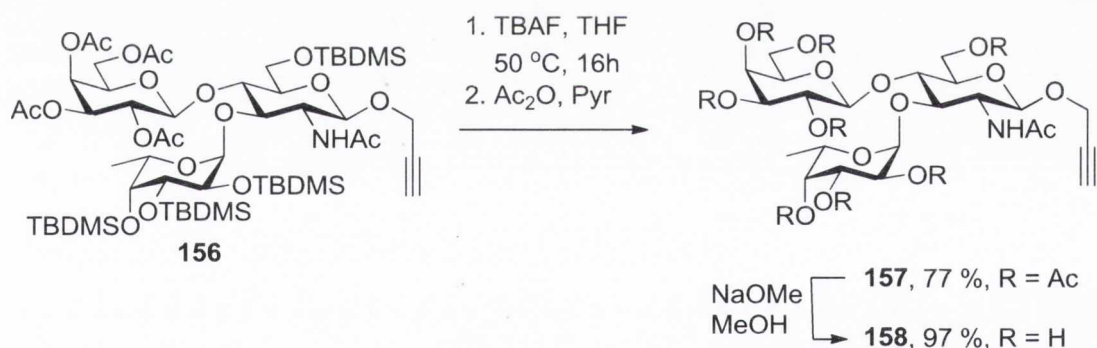
The steric bulk of fucose donor **132**, may also cause difficulty in approaching the free hydroxyl on acceptor **75**, given the close proximity of the Gal moiety in the 4 position. Dwek *et al.*²¹⁵ showed Lewis X (**20**, Figure 1.4), is a very rigid molecule once deprotected. The extra constraints imparted by so many TBDMS groups in close proximity should increase the energy barriers for this glycosylation. Kondo *et al.* showed that the steric bulk around the 3-OH of GlcNAc in Lewis X synthesis, dramatically affects glycosylation yields at that hydroxyl.¹⁹³ The use of alternative activation conditions namely DMS-Tf₂O, and the highly reactive Br₂/AgOTf inverse glycosylation did not improve the yield of **156**, beyond the 24 % found for NIS/TMSOTf. Conversion of donor **132**, to the highly reactive trichloroacetimidate was performed *in situ*, after hydrolysis of **132**, followed by reaction with trichloroacetonitrile and Cs₂CO₃. The trichloroacetimidate was extremely reactive, so the crude material was not purified, but rather used in the glycosylation immediately after filtering of Cs₂CO₃ and distillation of the excess trichloroacetonitrile. The required trisaccharide **156**, was not isolated in higher yields than 10-12 %.



Scheme 3.14 - *In situ* generation of trichloroacetamide towards protected Lewis X synthesis

3.12 Deprotection of protected Lewis X structure 156

The lower yield of TBDMS protected Lewis X **156**, was not ideal, however, if deprotection could be completed, then this would provide an alternative strategy for the synthesis of deprotected propargyl Lewis X, rather than debenzylating as attempted in Section 2.2.4.1. The difficulty in removal of the TBDMS protecting groups from disaccharide (**142**, Scheme 3.11), suggested the greater steric bulk on **156**, would make removal of the TBDMS even more difficult. Initial efforts at an attempted TBDMS to acetyl group interconversion with TBAF treatment for 20 h at room temperature, suggested through crude HRMS that only a single TBDMS group had been removed. Successful deprotection of all TBDMS groups is reported in Scheme 3.15. By increasing the time of reaction to 64 h from 30 h, **157**, could be isolated in 74 % yield. Reaction times could be reduced to 16 h, if the temperature for the treatment with TBAF was increased to 50 °C from RT, with a similar yield of 77 % yield. Quantitative deacetylation, followed by freeze drying allowed the first successful synthesis of O-propargyl Lewis X, **158**, in 11 steps from the respective per-acetylated monosaccharides.



Scheme 3.15 - O-Propargyl Lewis X deprotection

3.13 Conclusion

A novel fucose donor **132**, was been prepared and investigated as a practical intermediate for glycosylations where α -fucose glycosidic linkages are required, whilst tolerating the presence of unsaturated bonds. Donor **132**, was successfully used in the synthesis of a target lectin binding disaccharide, α -Fuc-(1-6)-O-propargyl-GlcNAc, **125**. Applying previously observed substitution patterns on glycoporphyrins to provide for water solubility, a tri-substituted glycoporphyrin **144**, was prepared in high yields for

future NMR investigations of binding studies with a fucose binding lectin from *R. solanacearum*, through collaboration with Dr. Peter Crowley, at NUIG.

Donor **132** was shown to have general applicability, but with lower yields on more hindered alcohols. Its application to O-propargyl Lewis X synthesis (**158**, Scheme 3.15), allowed a circumvention of the debenzylation issues previously observed as a key stumbling block in the conclusions of chapter 2. Unfortunately, the steric bulk of protecting groups on donor **132**, limits the yields reported for the Lewis X trisaccharide **156**. Further improvements are still needed, to allow efficient synthesis of O-propargyl Lewis X **158**, synthesis, before the deprotected trisaccharide can be produced in a suitable scale to incorporate into future glycoporphyrin studies.

Selections of this chapter have been included in a full paper on novel silyl based α selective fucose donors.²¹⁶

3.14 Future work

Lectin binding fluorescence and NMR spectroscopy studies are to be performed on glycoporphyrin **144**. A comparison of these methods will provide insight into whether a complimentary understanding of lectin-carbohydrate binding can be achieved. Investigations into the possible lowering of steric hindrance for similar donors to **132**, in order to increase the yield of secondary alcohol α -fucosylation products, mainly tuned towards an improved synthesis of a deprotected Lewis X would be desirable.

Chapter Four

**Glycosylations using a partially protected
fucosyl donor**

Chapter 4 – Glycosylations using a partially protected fucosyl donor

4.1 Introduction

Protecting carbohydrates during glycosylation reactions is generally essential in order to have any control over product regioselectivity. The introduction of protecting groups, although often unavoidable, is ultimately undesirable, as these groups are generally not part of the final product, and contribute to the lower the atom efficiency of glycosylation processes. Protecting groups can be avoided in cases where high yielding regioselective glycosylations can be applied. For example, the preferential reactivity of primary hydroxyl groups as acceptors, over secondary hydroxyl groups during glycosylation, can be routinely achieved. Previously presented in Scheme 2.4, the selective glycosylation on one of two secondary hydroxyl groups in an acceptor, has been achieved under certain conditions. The next step in complexity would be the use of a partially protected donor. On first viewing this would not seem a likely possibility. The chance of polymerization is greater as donors are often used in excess.

Impressive examples of partially protected donors are presented in Figure 4.1. In 2003 Linhardt *et al.*²¹⁷ struggled with the deprotection of a 2-acetyl group having glycosylated a saponin, but by contrast, found that a protocol involving deacetylation prior to glycosylation and employing a partially protected donor **159**, the reaction proceeded in 45 %. Strategies using partially protected donors, although not commonly employed, have been successfully investigated by a number of other groups. Selected examples include the mannose orthoester donors, **160**, Fraser-Reid *et al.*,²¹⁸ Mycaroside donors, **161-163**, Du *et al.*,²¹⁹⁻²²⁰ and the glucosamine donor, **164**, Walker *et al.*²²¹

In order to minimize the donor polymerization when employing partially protected donors, certain strategies can be used, for example;

- Excess of acceptor
- Glycosylation of a particularly active acceptor (primary hydroxyl)
- Inverse addition glycosylation strategy keeping the acceptor concentration high
- Relying on the steric bulk of the protecting groups on the donor in close proximity to the free hydroxyl, to minimize self polymerisation.

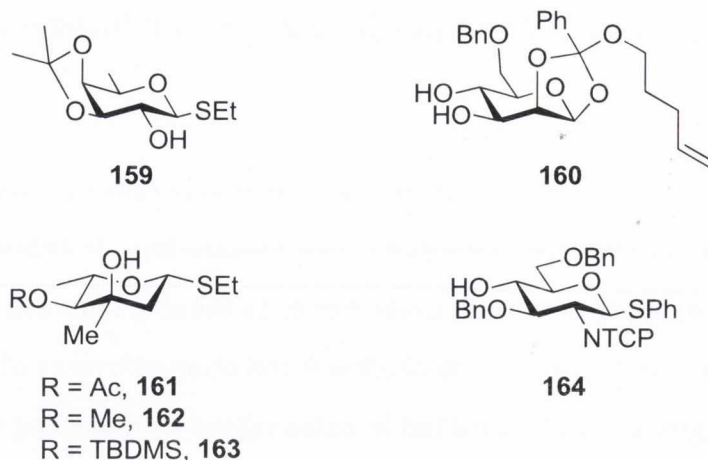


Figure 4.1 - Previously reported partially protected donors

It is with this last strategy in mind that the synthesis of Lewis X was revisited, using the unexpected 2,4 di-TBDMS fucose thioglycoside side product **131**, isolated during optimisation of the tri-silylated fucose donor **132** preparation (Scheme 3.3).

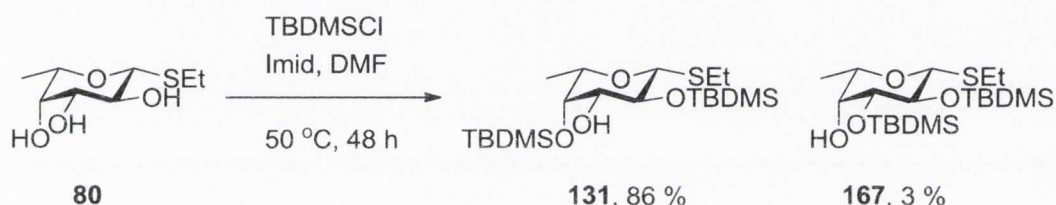
4.2 Partially protected donor ethyl-2,4-di-TBDMS-fucose thioglycoside **131**

4.2.1 Preparation of partially protected fucose donor

During the synthesis of the per TBDMS protected donor (**132**, Scheme 3.3), a 2,4-di-TBDMS side product **131**, was isolated as a clear oil in yields of up to 86 %. The regioisomer displaying 2,3-di-TBDMS substitution **167** was also isolated in a 3 % yield (Scheme 4.1). Whilst **131**, was not the desired compound at the time, it did meet a lot of the requirements for a partially protected donor, and so warranted further investigation. The fact that the 2,4 substitution pattern of **131**, was the main isolated product is interesting in itself. Given the long reaction time required for clean product distribution by TLC, it is likely that this reaction is under thermodynamic control. Kinetically one would expect the axial hydroxyl in the 4 position to be the least reactive, and so either the 2 or 3 OH should be protected first. Literature precedent for imidazole and/or alternative base mediated intramolecular transfer of silyl protecting groups, to neighbouring hydroxyls has been shown by Santos *et al.*²²² and Tadano *et al.*²²³ A similar process is probably responsible for the observed partially protected product from (Scheme 3.3).

One literature precedent for the preparation of a di-TBDMS fucose donor exists as reported by the Du group.²¹⁹ Their protocol reported the use of TBDMSCl and imidazole in

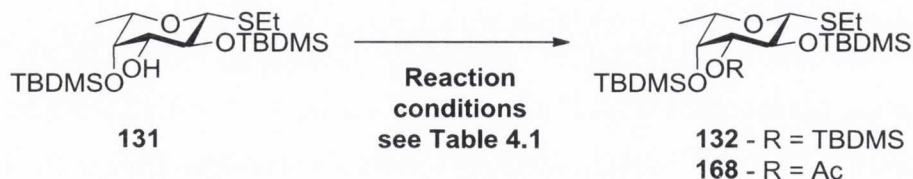
DMF at 40 °C for 12 h, and describes the isolation of a 3,4 di-TBDMS regioisomeric product. The reported characterisation of that product in the literature, actually matches our observed characterisation for **131**. Through high resolution 2D NMR, (HSQC, and selective TOCSY experiments), the structure **131** was unambiguously defined. The hydroxyl was found to be located in the 3-C position and not the 2-C. Although trace amounts of the 2,3 di-TBDMS isomer **167**, were isolated no 3,4 di-TBDMS substituted fucose thioglycoside was observed. It is expected that the reported data for the 3,4 di-TBDMS fucose thioglycoside, described by Du *et al.*²¹⁹, is actually the regioisomer **131**.



Scheme 4.1 - Partially protected Di-TBDMS fucose thioglycoside synthesis

4.2.2 Accessibility of the 3-OH position to protection in partially protected donor **131**

An investigation on the reactivity of the 3-OH in donor **131**, was performed (Scheme 4.2). One of the most simple but useful pieces of data, is the significant sharpening of the 3-OH signal by IR spectroscopy, with an observed IR peak near 3600 cm^{-1} . This finding is consistent with a lack of proton exchange, and lowered H-bonding. A summary of further chemical manipulations on the 3-OH of **131**, are described in Table 4.1.



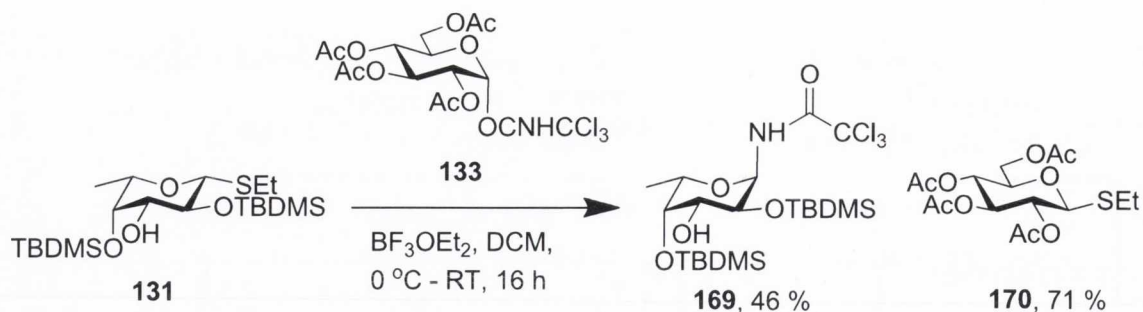
Scheme 4.2 - Accessibility of the 3-OH position on **131**

Table 4.1 - Reactivity table for Scheme 4.2

Entry	Protecting group	Conditions	Yield
1	TBDMSCl	2,6 Lutidine, DMAP, DMF; 24 h, 100 °C	132 - 3 %, 131 - >90 % recovered
2	TBDMSOTf	Pyr, 60 °C, DMAP, 16 h	132 - 8 %, 131 >85 % recovered
3	TBDMSOTf	Pyr, 60 °C, DMAP, 216 h	132 - 5 %, 131 - breakdown
4	Ac ₂ O	Pyridine, DMAP, RT, 18 days	168 , 80 %
5	Ac ₂ O	NaOAc, 90 °C	131 , 99% recovered
6	AcCl	DTMP	131 - Loss of TBDMS groups
7	BnBr	NaH, THF	Silyl migration, 131 - breakdown

As evident from (entries 1-3, Table 4.1), the presence of bulky protecting groups at positions 2 and 4, led to little or no reaction at the 3-OH position in **131**. The acetyl group being substantially less bulky, was expected to have more of an opportunity to react at the 3-OH of **131**. Reported in entry 4, Table 4.1, the acetylated product **168**, can be prepared although it is an incredibly slow reaction. Selected NMR spectra on route to **168**, are shown in Figure 4.2.

The proton NMR spectrum of **131** was completely resolved, which was in contrast to the tri-silyl compound **132**, (Figure 3.7 - 3.8), which showed extensive signal broadening due to restricted rotation of the TBDMS groups. The clearly resolved OH peak near δ_H 2 ppm, is evidence of slow proton exchange, complementing the IR observations, and resulting from the buttressing of neighbouring TBDMS groups. Increasing the temperature to drive acetylation was found only to reduce the yield through thioglycoside breakdown with time. Finally both AcCl and BnBr led to the breakdown and migration of TBDMS protecting groups on the donor. In summary, due to the lack of accessibility to the 3-OH position in **131**, it was concluded that investigation of **131**, as a partially protected donor, was warranted.



Scheme 4.3 - Mixed glycosylation products with 131 as an acceptor

None of the expected disaccharide product was observed or isolated. However, Scheme 4.3 revealed two rearrangement products from this reaction. The observed anomeric group translocation, serves to highlight the lack of reactivity of the 3-OH in **131**. N linked α -trichloroacetamide rearrangements, like product (**169**, Scheme 4.3), have been reported previously.²²⁴⁻²²⁵ Examples have been shown to occur in cases where the acceptor is a poor nucleophile. Similarly, Boons *et al.* previously described the transfer of an anomeric thioether group from acceptor to donor, with reverse transfer and rearrangement of trichloroacetimidate.²²⁶

Analysing the products from (Scheme 4.3), it can be assumed that upon formation of the oxocarbenium resulting from trichloroacetimidate **133**, activation, the lone pair on the sulfur is more accessible than the lone pair on the free hydroxyl. Attack of the glucose oxocarbenium ion by the sulfur atom effectively activates the fucose donor **131**. With elimination of the thioglycoside **170**, trichloroacetamide formed as a result of the trichloroacetimidate activation, then attacks the fucosyl oxocarbenium ion leading to the generation of **169**. Although the reported reaction (Scheme 4.3), led to production of unusual side products, in conclusion the lack of reactivity at the 3-OH position of donor **131** had been confirmed.

4.3 Glycosylation with partially protected 131 - Monomeric products

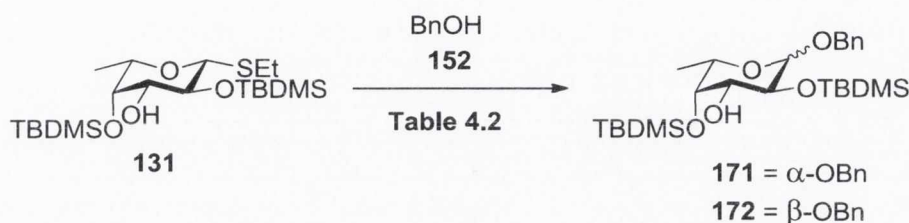
4.3.1 Optimisation of glycosylation on benzyl alcohol with 131

Having previously shown in multiple examples, the lack of accessibility to the 3-OH on **131**, investigation into the use of **131** as donor was performed.

A series of activation conditions were applied with benzyl alcohol as the acceptor (Scheme 4.4), and the results are summarised in Table 4.2. As previously observed with

the tri-TBDMS protected fucose donor (**132**, Table 3.4), NIS/TMSOTf activation gave the highest yields, with similar α/β stereoselectivity.

An unexpected example of fucose dimer formation was noted when Br_2/AgOTf was used to activate donor **131** (entry 1, Table 4.2). Investigations of this reaction are discussed later in Section 4.6.1.



Scheme 4.4 - Test glycosylation with partially protected donor 131

Table 4.2 - Investigation of donor 131 activating conditions

Entry	Donor (eq.)	Activation conditions	Time	Temp	% Yield ($\alpha:\beta$)
1	1	Br_2/AgOTf	25 min	-40 °C	< 10 ^a
2	9	Br_2/AgOTf	25 min	-40 °C	39 (1:2)
3	1	DMDS/ Tf_2O	10 min	-20 °C	42 (6:1)
4	3	DMDS/ Tf_2O	30 min	-40 °C	67 (2.5:1)
5	1	NIS/TMSOTf	150 min	-20 °C	64 (2.7:1)
6	3	NIS/TMSOTf	150 min	-30 °C	88 (2.6:1)

^a = α -Fuc-1-3- α -FucOBn dimers observed

4.3.2 Coupling constants of α/β OBn monosaccharide – Conformation review

An unusually large difference in NMR spectrum between both anomers of the monosaccharide structures **171** and **172**, was noted. A closer investigation of the NMR data was required in order to ascertain if a ring flipping event had occurred, between the two diastereoisomers. Ring flipping or conformational changes in mannose for example, tend to manifest themselves by differing coupling constants between protons of $J_{2,3}$ as axial-equatorial relations become equatorial-axial.²²⁷ An equivalent expected change in fucose would then involve $J_{4,3}$. The relevant NMR data (400 MHz, CDCl_3) for both anomers is presented in Table 4.3.

Table 4.3 - Collated NMR data (400 MHz, CDCl₃) for anomers **171 and **172****

Anomer	(H-1, $J_{1,2}$)	(H-2, $J_{2,3}$)	(H-3, $J_{3,4}$)	(H-4, $J_{4,5}$)	(H-5, $J_{5,6}$)
α	4.83, 3.2 Hz	3.98, 9.7 Hz	3.92, 2.6 Hz	3.88, 0.8 Hz	3.97, 6.5 Hz
β	4.25, 7.5 Hz	3.68, 9.4 Hz	3.46, 2.7 Hz	3.79, br s	3.56, 6.4 Hz

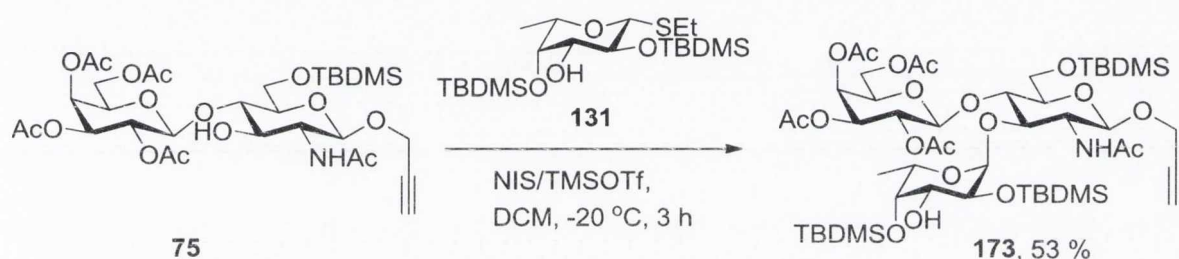
Analysing the NMR shifts from the α anomer **171**, to the β anomer **172**, confirms an offset of between 0.13 and 0.50 ppm, however based on the NMR coupling constant data, it can be seen that only the $J_{1,2}$ constants differ by any major degree, and ring flip is unlikely. A conformational shift may be ignored and both products shall be drawn as 1C_4 . One possible explanation for the downfield shift of the α isomer protons, could be by an intermolecular association in solution which takes place in the β system, but is however, disrupted in the α anomer due to the axial OBn group. A $\beta\beta$ stacking arrangement in solution could alter the experienced magnetic field, pushing signals upfield. As the effect might be blocked due to the α OBn substituent in the $\alpha\alpha$ isomer, then this would shift signals downfield. The crystal structure of tri TBDMS protected fucose thioglycoside **132**, does show a H-1 to H-6 close contact point, which would place a β -OBn substituent over the ring of the neighbouring sugar. Whether these interactions take place or not in solution are more difficult to calculate.

4.4 Application of partially protected donor **131** to the synthesis of Lewis X

4.4.1 Glycosylation of protected LacNAc **75**, with partially protected donor **131**

The hypothesis presented for the lower yield of protected O-propargyl Lewis X (Table 3.5, entry 6) and subsequent difficulty in TBDMS deprotection is discussed in chapter 3, (Figure 3.15), both stemmed from the steric bulk of the tri silyl fucosyl donor **132**. TBDMS groups had the effect of hindering the approach of donor **132**, to the acceptor **75**, during the glycosylation reaction, and also hindering F⁻ approach during the deprotection. Although the di-TBDMS donor **131**, has been shown to display significant steric bulk with regard to access of the 3-OH position, the steric constraints are relative, since the compound physically cannot be as hindered as the tri-TBDMS donor **132**. Application of the partially protected di-silyl donor **131**, to the synthesis of a partially protected O-propargyl Lewis X, **173**, was investigated.

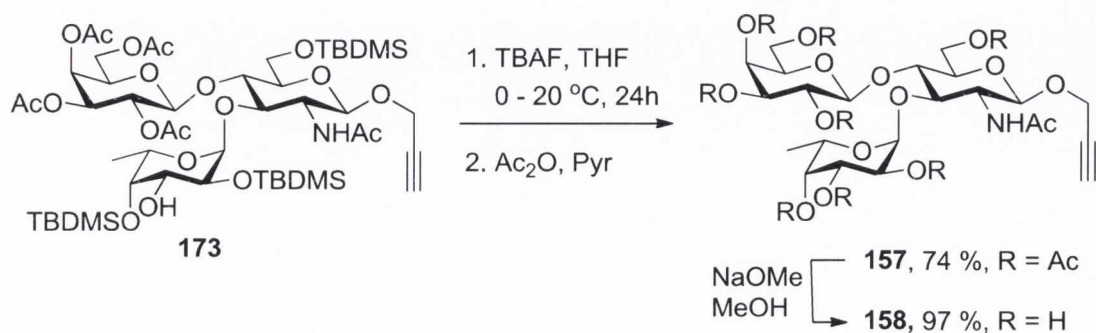
Successful glycosylation of acceptor **75**, with donor **131**, was performed with 1.5 eq. of donor for 40 minutes at -20 °C, leading to the required product **173**, in 42 % yield. Increasing the donor ratio, to 1.8 eq. and reaction time to 3 hours led to the production of **173**, in 53 % yield with complete α selectivity, (Scheme 4.5). This result is comparable to the glycosylation yields observed using the per-benzylated fucose thioglycoside donor **81**, (58 %, Scheme 2.6), but more importantly represents a large increase in yield observed over the equivalent glycosylation employing the tri TBDMS donor **132**, (24 %, Figures 3.11). In conclusion, the reduced steric bulk of donor **131**, does play an important factor in improving Lewis X glycosylation yields.



Scheme 4.5 - Improved route to protected O-propargyl Lewis X, **173**

4.4.2 Deprotection of partially protected O-propargyl Lewis X, **173**

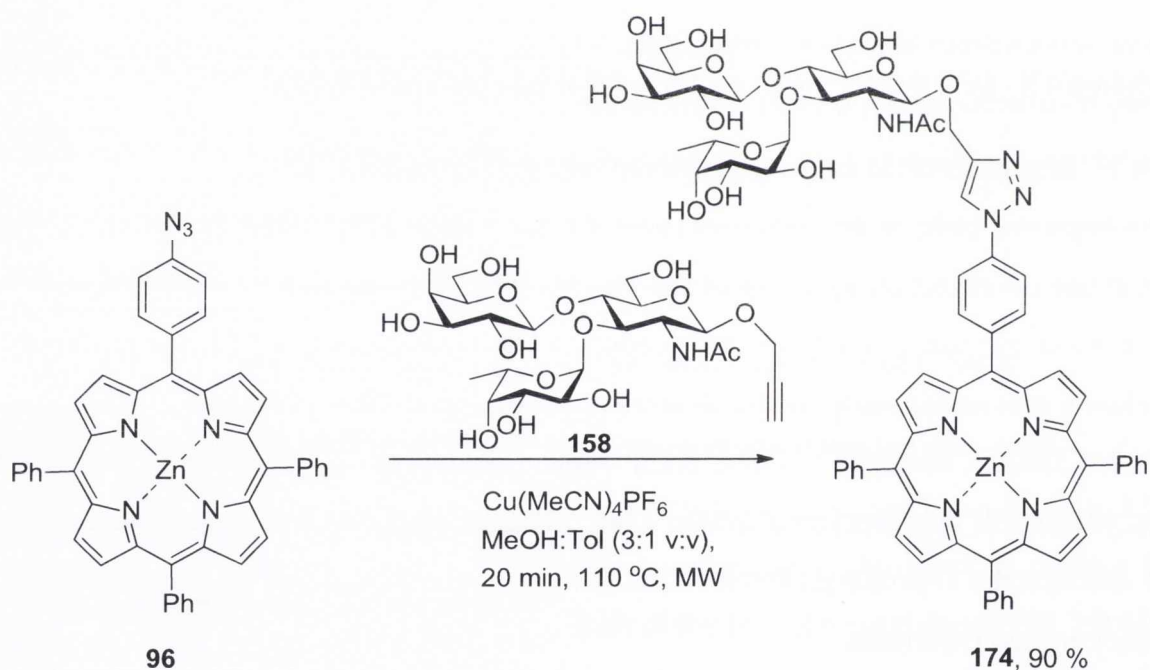
The improved yield of the protected Lewis X trisaccharide **173**, should also translate into an easier deprotection by virtue of the less hindered arrangement of TBDMS groups in **173**. A one pot conversion to the per acetylated O-propargyl Lewis X, **157**, was performed (Scheme 4.6) under much milder deprotection conditions than that required for the tri TBDMS Lewis X analogue (Scheme 3.15). Similar yields were observed and quantitative NaOMe/MeOH deprotection provided **158**.²¹⁶ The successful synthesis and higher yields of deprotected O-propargyl Lewis X **158**, allowed enough material to be prepared for glycoporphyrin synthesis.



Scheme 4.6 - Milder deprotection of partially protected O-propargyl Lewis X, **173**

4.5 First reported deprotected trisaccharide containing glycoporphyrin

Gratifyingly, the CuAAC reaction of mono-azido porphyrin **96**, with deprotected O-propargyl Lewis X **158**, yielded for the first time a deprotected Lewis X containing glycoporphyrin **174**, (Scheme 4.7). The product was isolated in a yield of 90 % following purification by column chromatography (EtOAc:EtOH:H₂O, 7:2:1 (v/v/v)). Glycoporphyrin **174**, was not water soluble, but was sparingly soluble in MeOD allowing NMR characterisation (800 MHz). The possible PDT applications of this compound given its biological importance in recognition events⁹⁵ is exciting. The carbohydrate functionality on this glycoporphyrin is again approaching the level of complexity expected to be required for highly specific cell and lectin binding. It is hoped that future biological investigation of this glycoporphyrin will confirm that there is a clinical advantage in conjugating more biologically active carbohydrates to PDT agents.



Scheme 4.7 - Lewis X substituted glycoporphyrin **174**

4.6 α -Fuc-1-3-Fuc dimer formation

4.6.1 Special case of dimer formation with Br_2/AgOTf activated glycosylation of **131**

As alluded to in entry 1, Table 4.2, the activation of **131**, with Br_2/AgOTf to form the O-benzyl glycosides **171** and **172**, resulted in the unusual dimeric α -Fuc-1-3- α -Fuc products **175** and **176** (Scheme 4.8). Although trace monosaccharide products were observed, the disaccharide products were isolated in suitably high yields to warrant further investigation. The products represent a two step synthesis to Fuc- α -1-3-Fuc from fucose thioglycoside (**80**, Scheme 4.1). The reaction might not have been so unusual were it not for the demonstrated lack of reactivity of the 3-OH as described in Section 4.2.2 and Section 4.2.3. The structure could be confirmed by NMR studies, and following functional group interconversion of TBDMS to OAc, disaccharide **177** was prepared also confirming the α -1-3 linkage, (Scheme 4.8).

The α -Fuc-1-3-Fuc disaccharide is of interest because of its structural homology to the fucose based polymer fucoidan, (Figure 4.3). This polymer has been shown to possess anti-sperm adhesion properties²²⁸ for use as contraceptives, anti-HIV activity²²⁹ *in vitro*, anti-tumourocidal²³⁰ properties, and applications as lower molecular weight heparin mimics.²³¹ Tedious protecting group manipulations are normally required for the synthesis of the α -1-3 fucose linkage. The ability to prepare the disaccharide in such a short route is synthetically useful.

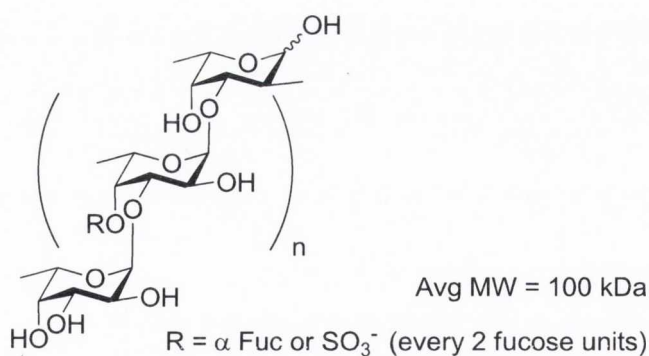
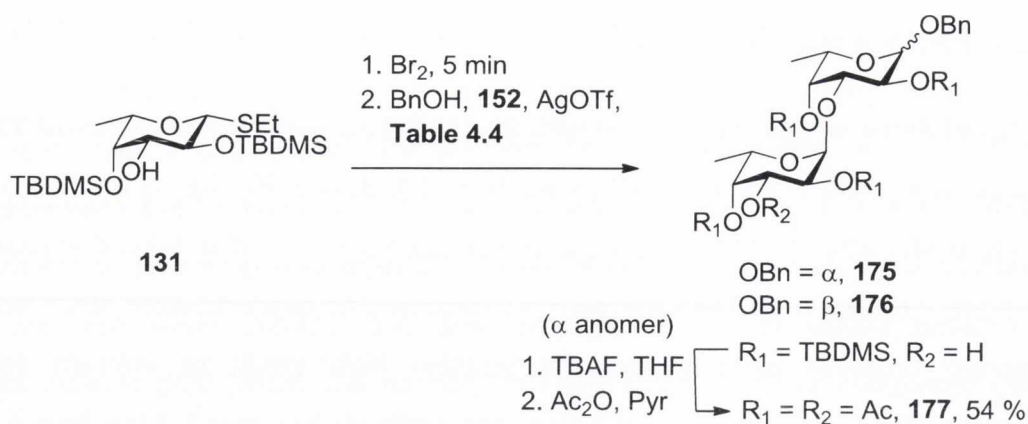


Figure 4.3 - Generic structure of fucoidan



Scheme 4.8 - α-1-3-Fucose disaccharide formation with Br₂/AgOTf activation

Table 4.4 – Affect of activation conditions on fucose disaccharide formation

Entry	Activation conditions	°C ^d	Time (Min)	Fuc-α-1-3-Fuc % yield	Anomeric ratio αα:αβ
1	AgOTf ^a , DCM/THF	0	25	25	2 : 1
2	AgOTf ^a , DCM/THF	-20	25	44	1.2 : 1
3	AgOTf ^a , DCM/THF	-40	25	38	1.4 : 1
4	AgOTf ^a , DCM/THF	-60	25	22	3.9 : 1
5	AgOTf ^a , DCM	-20	25	0 by TLC	N/A
6	AgOTf ^a , THF	-20	25	29	1 : 1.4
7	AgOTf ^a , Et ₂ O	-20	25	14	3.8 : 1
8	AgOTf ^a , DCM/THF	-60	120	20	2.5 : 1
9	AgOTf ^a , DCM/Et ₂ O	-60	120	26	9.3 : 1
10	Ag ₂ CO ₃ ^a , DCM/THF	-20	25	0 by TLC	N/A
11	AgOTf ^b , DCM/THF	-20	25	0 by TLC	N/A
12	AgOTf ^c , DCM/THF	-20	25	26	1.1:1

^a = 2 equivalents, ^b = 0.5 equivalent, ^c = inverse glycosylation,

^d = refers to activation temperature of intermediate, after bromide formation

The reaction conditions for fucose dimer production (Scheme 4.8), were investigated (Table 4.4) to try and gain some more understanding of what was occurring in solution. For mixed solvent systems, the ratio was always DCM:solvent (2:1 (v/v)). Colder reaction temperatures and Et₂O appear to increase the αα stereoselectivity, however, all conditions gave α selectivity at the non reducing end. The use of an alternative silver (I)

source stopped the reaction. Although overall the yields were still quite low, the best reaction conditions (entry 2, Table 4.4) represented an average of 66 % for each glycosylation event, and as some unusual chemistry was occurring, further investigation of the mechanism of reaction was performed.

4.6.2 Order of reaction for α -Fuc 1-3 Fuc - Dimer bromide formation

Since Br_2/AgOTf activation was different from all other activation conditions screened in Table 4.2, in that it proceeded via a two step activation, it was not yet apparent at what stage the Fuc-Fuc glycosylation event was taking place. Again the NMR tracking experiments as detailed by Demchenko *et al.*²⁰⁶ would quickly determine if the monomer fucosyl bromide, or a dimer fucosyl bromide was being carried through to the AgOTf activation step. Given the α selectivity observed, and the possibility of $\text{S}_{\text{N}}2$ on fucose β bromides²³² leading exclusively to α selectivity, it was expected that an $\text{S}_{\text{N}}2$ route via a dimer fucosyl bromide, was the source of the disaccharide products observed in Scheme 4.8. However, upon completing the NMR experiments it can clearly be seen that only the monomeric bromide exists (Figure 4.4).

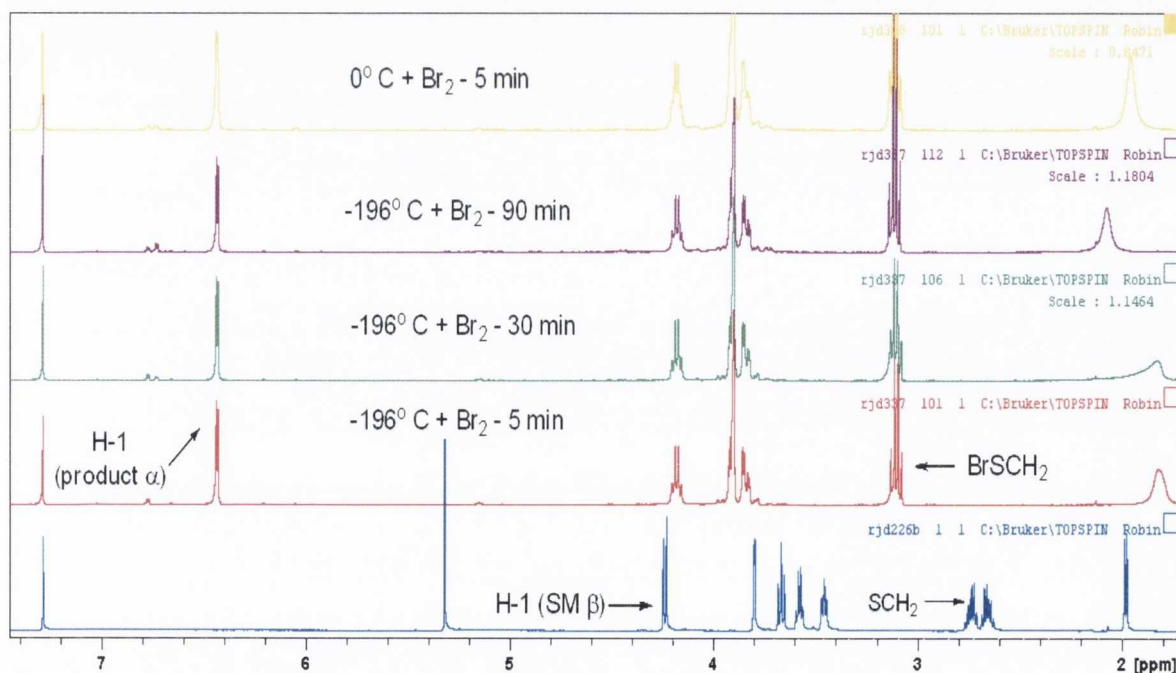
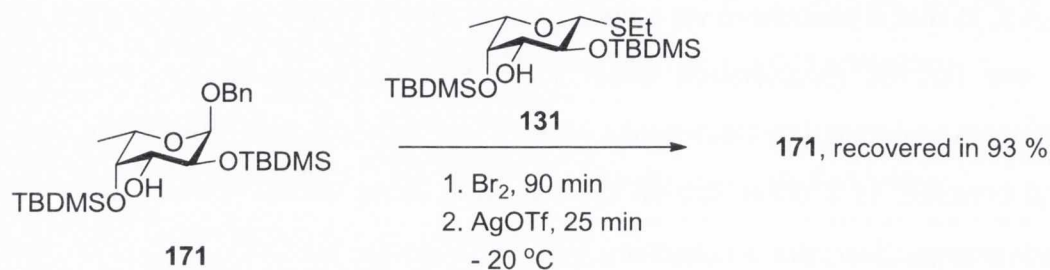


Figure 4.4 - ^1H NMR (400 MHz, CDCl_3) analysis of Br_2 addition to **131**

4.6.3 Order of reaction for α -Fuc 1-3 Fuc - Sequential glycosylation

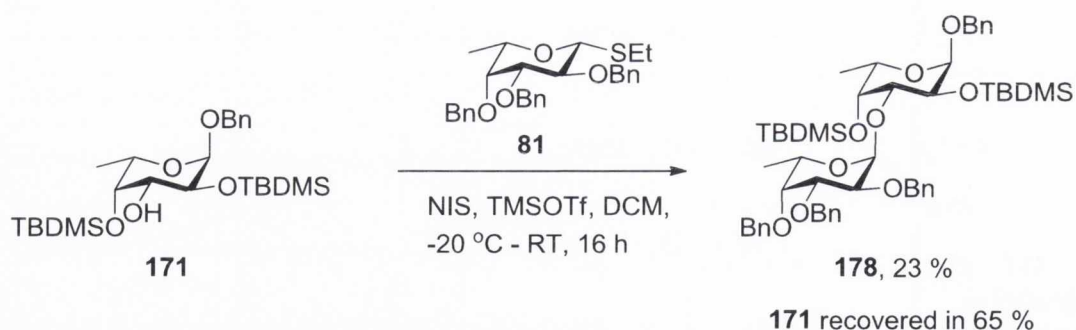
An alternative route proposed leading to the fucose disaccharide, was the formation of the OBn monosaccharide **171** and **172** *in situ*, followed by glycosylation of the 3-OH position to form the disaccharide **175** and **176** respectively. Investigation of this order of

glycosylation reaction was carried out by employing the monosaccharide (**171**, Scheme 4.4) as an acceptor in the conditions which previously formed the α -Fuc-1-3-Fuc disaccharide products (**175** and **176** Scheme 4.8). The results of this investigation are presented in Scheme 4.9. Surprisingly, the majority of the acceptor **171**, was recovered, with complete consumption of the donor **131**. HRMS confirmed the presence of a mass ion corresponding to the monomer, and dimer Fuc hemiacetal.



Scheme 4.9 - Intermediate OBn monosaccharide glycosylation with 131

An investigation employing more forcing glycosylating conditions with an armed per-benzylated donor **81**, is outlined in Scheme 4.10. Under these conditions, the 3-OH of **171**, was accessible a 23 % yield of disaccharide **178**, isolated. However, the majority of the acceptor **171**, was recovered despite the reaction running for 16 h, and warming to RT.



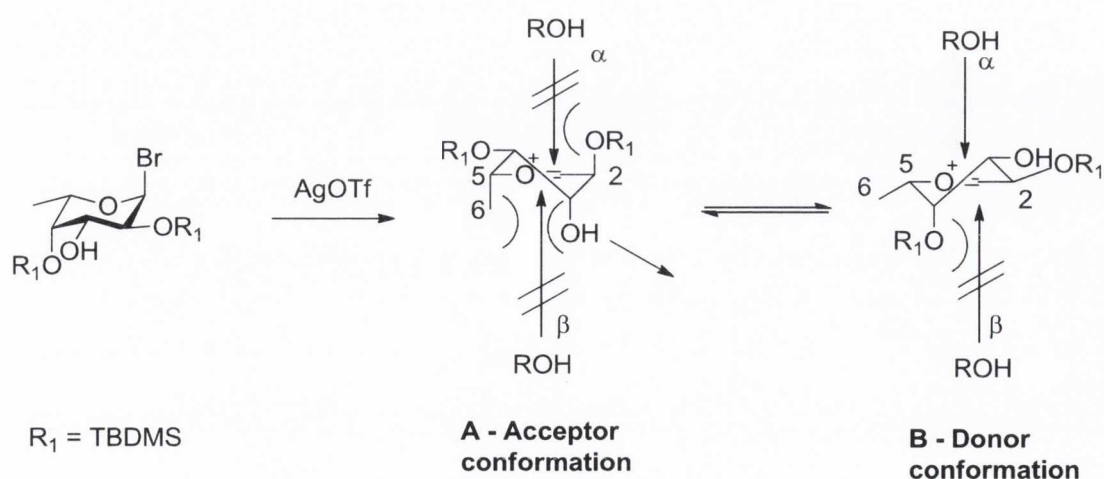
Scheme 4.10 - Intermediate OBn monosaccharide glycosylation with 81

From the observed results, *vide supra*, it appears that the disaccharide products in Scheme 4.8, do not result via an intermediate bromide, nor does it appear to be able to proceed via an intermediate OBn monosaccharide, (Scheme 4.9). It is true that following much longer reaction conditions at much higher temperatures, some 3-OH glycosylated products were observed in Scheme 4.10, but not enough to justify the results in Table 4.2.

4.6.4 Intermediate steric relief - conformational arming

The other possible route to explain the higher than expected yield of the disaccharide products **175** and **176**, would need to take into account the change in conformation of the oxocarbenium ion intermediate, following Ag (I) activation of the intermediate bromide in Scheme 4.8. This could be used to explain an increase in accessibility of the 3-OH.

The conformation of an oxocarbenium ion as proposed by Woods *et al.*²³³ and investigated by Woerpel *et al.*⁶³ is described by a linear arrangement of C-5 - O-5 - C-1 - C-2. As applied to the disaccharide system, (Scheme 4.8), two possible conformations of oxocarbenium ion **A** (⁴H₃) and **B** (³H₄), are shown in Scheme 4.11. Alabugin has described the importance of hyper conjugation in stabilising the oxocarbenium ion.²³⁴ The findings from this paper have been applied by Woerpel *et al.*^{63, 235} to justify the unusual reactivity of 6-deoxy sugars as well as the contribution of protecting groups to both arming and disarming effects. 6-deoxy sugars have no electronegative oxygen atom attached to C-6, and so the protons on C-6 can participate in hyperconjugation, provided these can adopt an axial position, to align the C-6 - H σ orbital, with the C-5 - O⁺ σ^* orbital. Armed donors, containing electron donating protecting groups have more electron density in the C-O bond to contribute via σ bonds to the oxocarbenium ion, than the equivalent C-O bond with an electron withdrawing protecting group.



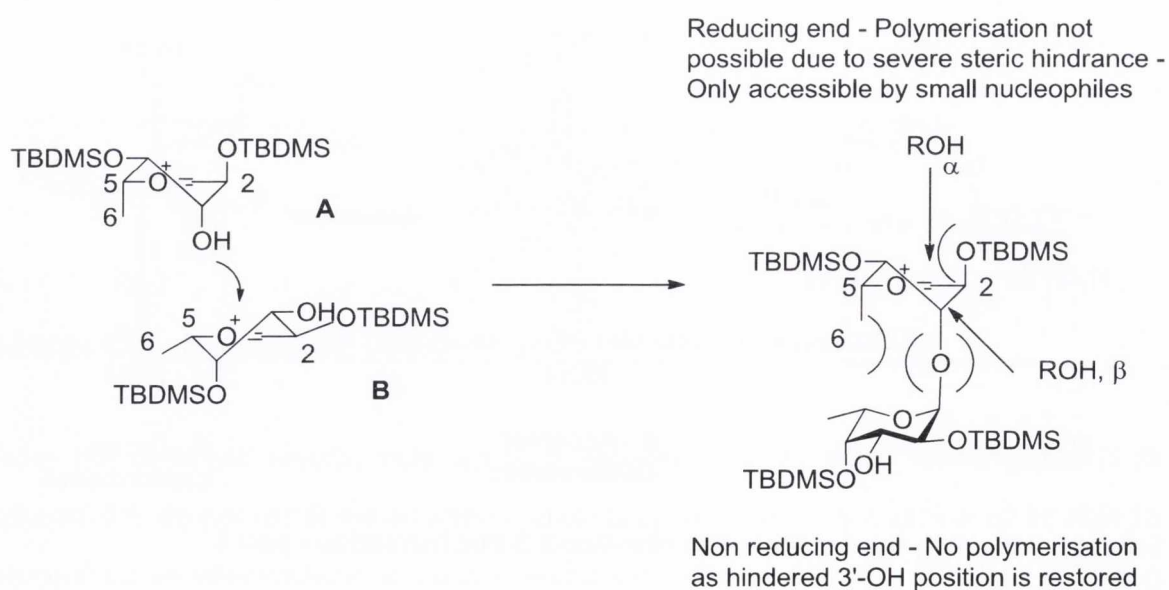
Scheme 4.11 - Proposed mechanism of α -Fuc-1-3-Fuc formation - part 1

The implications of conformational changes on the 3-OH are presented in Scheme 4.12, It can be observed that in **A**, the 3-OH has dropped out of the plane, and is much less hindered. At the same time, the steric interactions of axial groups in the 2, 3, and 6

positions make nucleophilic attack at the oxocarbenium more difficult from both faces. Meanwhile the oxocarbenium ion **B**, is much more accessible from the α face, but the 3-OH is more hindered than in **A**.

Simple molecular dynamic and energy minimisations studies (MM2), produced **A** as the more stable intermediate, most likely due to the hyperconjugation of C-6 - H σ orbital to C-5 - O⁺ σ^* orbital mentioned previously, but more detailed and reliable modelling studies are to be performed in the future.

In order to explain the unusual dimer result, but absence of polymerisations, it must be remembered that the energy barrier between the interconversion of **A** and **B** is likely to be low enough to allow both to exist in solution.^{63, 236} If the accessible oxocarbenium in **B** were to be attacked from its least hindered face, by the less hindered 3-OH of **A**, then the intermediate result would be that shown in Scheme 4.12. It can be seen that in the product, the 3'-OH has now reformed the inaccessible conformation ¹C₄, thereby preventing further reaction at this position (See Scheme 4.2, 4.3, 4.4, 4.10). Meanwhile at the reducing end, the axial 2-OTBDMS group together with the axial 3-O-glycoside and axial 6-CH₃ severely restrict the accessibility of the remaining oxocarbenium, which could now be attacked at the reducing end, by a non-sterically hindered alcohol, over another oxocarbenium ion **A**. Such an attack would provide the experimentally observed α/β mixtures (Table 4.4).



Scheme 4.12 - Proposed mechanism of α -Fuc-1-3- α -Fuc formation - part 2

4.6.5 Critical evaluation of the proposed dimer formation route

The proposed model in Scheme 4.11 and Scheme 4.12, is most likely an over simplification of what is occurring in solution, however, it can explain some of the unusual requirements for the isolated dimer products **175** and **176**. The approach of two oxocarbenium ions should be highly energetically disfavoured, however, counter ions play a large and not entirely understood role in glycosylations,²³⁷ possibly explaining why the reaction failed using a different source of Ag(I) (entry 10, Table 4.4).

The proposed route (Scheme 4.11), would require a large concentration of oxocarbenium ions to be present in solution. Two equivalents of AgOTf were used to activate the fucosyl halide in accordance with literature procedure.¹⁷³ The excess Ag(I) would have the effect of largely increasing the rate of oxocarbenium formation versus a catalytic process. (Entry 11, Table 4.4) investigated the effect of lowering the AgOTf concentration to 0.5 equivalents. The reaction mixture showed a large amount of unidentified products by TLC, and no OBn fucosyl dimer products were observed.

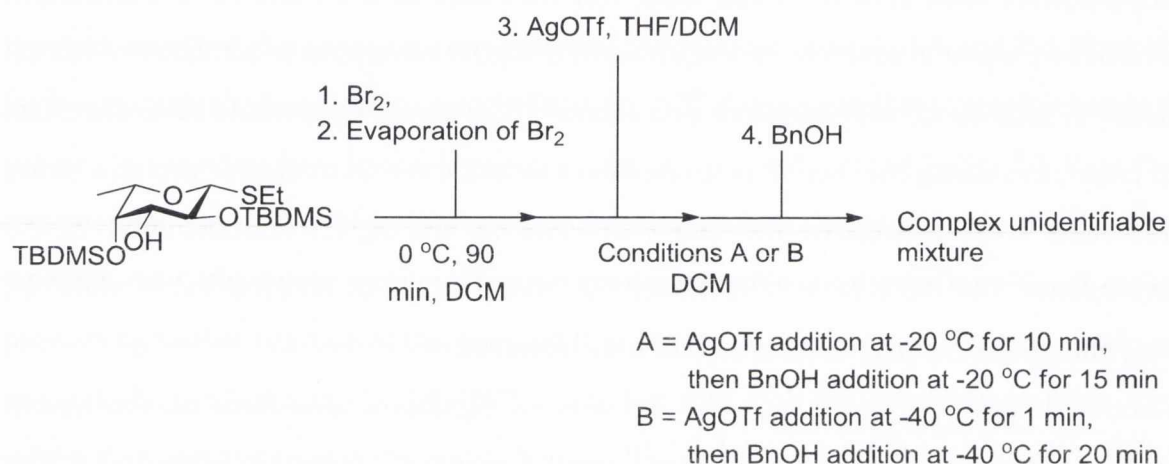
Another unexpected result was the isolation of significant quantities of the fucosyl dimers, after adopting the inverse glycosylation conditions, (Entry 12, Table 4.4). In this case BnOH **152** should remain in excess vs the fucosyl bromide acceptor. It should be expected that this method would prevent any disaccharide product formation, but from the observed results, the rate of dimer formation appears sufficiently high to yield the fucosyl dimer products **175** and **176** in a combined 26 %. The result here may point towards some enhanced nucleophilicity of the 3-OH in conformation (**A**, Scheme 4.11) although justification for this result is difficult to achieve.

4.6.6 Investigation of fucosyl dimer formation through increasing oxocarbenium ion interactions

4.6.6.1 AgOTf with delayed BnOH addition

If the key step requires an oxocarbenium ion like species approaching another oxocarbenium ion, (Scheme 4.12), then a valid attempt to increase the α -Fuc-1-3- α -Fuc disaccharide yield, would be to remove the competing acceptor during initial activation. A key factor would be whether the intermediate oxocarbenium ion fucose dimer would tolerate these conditions.

Delayed addition of benzyl alcohol acceptor **152**, with Br₂/AgOTf activation was attempted (Scheme 4.13). The ability to control the oxocarbenium formation, was not possible either at -20 °C for 10 mins, or -40 °C for 1 minute, before BnOH **152**, addition. A complex mixture of products emerged. Presumably the oxocarbenium intermediate is too reactive with silver activation, in the absence of added acceptors. Perhaps a glycosyl triflate is formed and no glycosylation occurs on the addition of **152**, before the mixture breaks down upon workup.

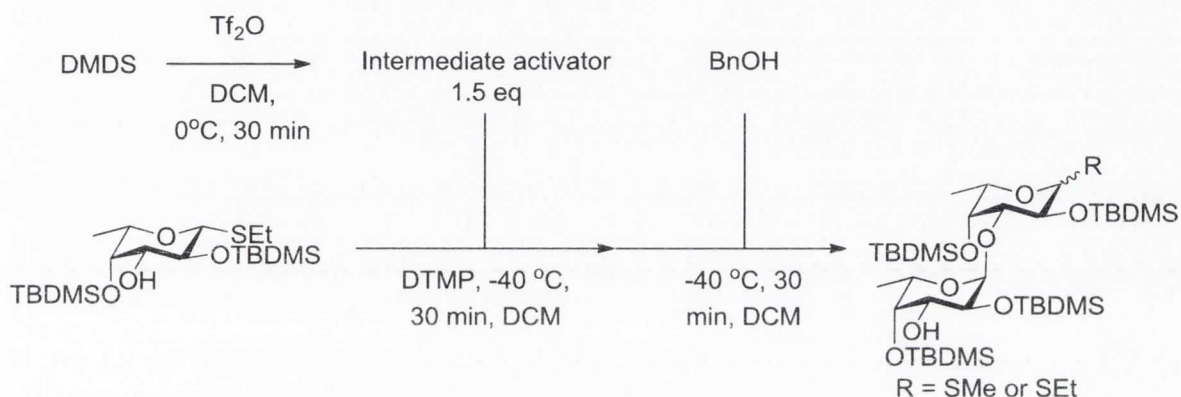


Scheme 4.13 - Delayed acceptor addition AgOTf

4.6.6.2 DMDS-Tf₂O with delayed BnOH addition

Given the high reactivity of the AgOTf based activation, slower and less reactive activation conditions were expected to be beneficial in the delayed acceptor addition experiments. NIS activation prior to BnOH **152**, addition was not attempted as the succinimide formed during the activation process, would be glycosylated by the fucosyl oxocarbenium ion, as previously reported in the literature.^{62, 214} DMDS-Tf₂O activation with DTMP buffering however, might be useful as the side products from activation should not be reactive. Another delayed addition experiment was set up in Scheme 4.14. Initial results appeared promising by TLC analysis of the crude mixture. However, upon isolation of what appeared to be a single spot by TLC, a complex mixture containing SMe and SEt Fuc-Fuc disaccharides was observed. These products could not be separated, and due to overlapping complexity of NMR, were only observed during HRMS analysis. The α and β thioglycosides were isolated as inseparable mixtures in yields approaching 50 % yield. The fact that evidence for the SMe Fuc-Fuc disaccharide product was observed, meant either the dimethyl disulfide, or the mixed ethyl methyl thioether, is acting as a nucleophile and

regenerating a thioglycoside before the BnOH **152**, can be added. It could be argued that because Fuc-Fuc formation is observed, it may be proceeding through an oxocarbenium oxocarbenium intermediate as alluded to in Br₂/AgOTf case (Section 4.6.4), but the excess activator is used up during the regeneration of the thioglycoside halting the glycosylation, prior to BnOH **152** addition. Repeating the reaction in Scheme 4.14, using an extra equivalent of DMDS-Tf₂O activator, after BnOH **152**, addition, led to the observation of significant baseline impurities and reaction failure by TLC.



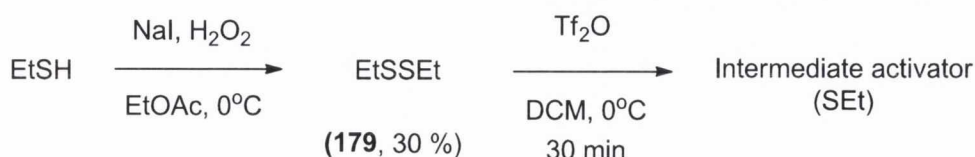
Scheme 4.14 - Delayed acceptor addition DMDS-TfO₂

4.6.7 Disaccharide thioglycoside reformation

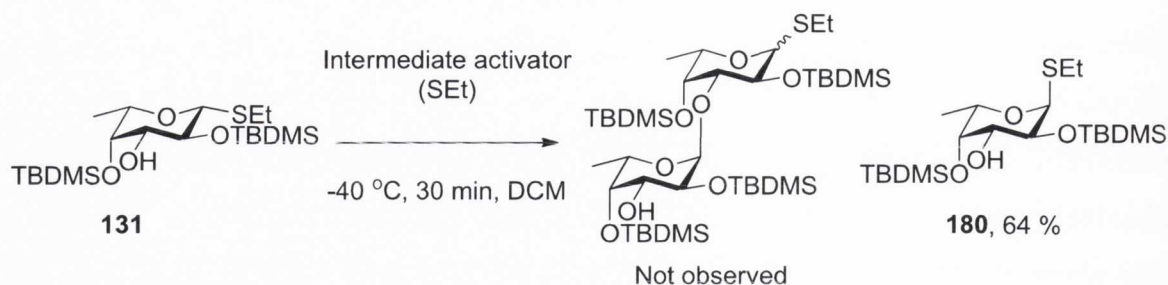
In an effort to gain some useful synthetic methodology out of the unusual but crude mixed thioglycoside formation (Scheme 4.14), the activation conditions were reviewed. If diethyl disulfide (**179**, Scheme 4.15), instead of dimethyl disulfide was reacted with Tf₂O, and this intermediate used to activate the di-TBDMS donor **131**, then the crude reaction mixture should only contain α and β ethyl thioglycosides, which at least, may be characterised as a mixture.

4.6.8 Synthesis of diethyl disulfide and use in thioglycoside **131**, activation

Diethyl disulfide was prepared through the oxidation of ethane thiol by H₂O₂ in the presence of NaI, as described by Kiriara *et al.*, to yield the required disulfide **179**.²³⁸



Scheme 4.15 - Diethyl disulfide synthesis and activator formation



Scheme 4.16 – Attempted disaccharide donor synthesis

The effect on substituting dimethyl disulfide with diethyl disulfide, (Scheme 4.16), whilst expected to be negligible, was in fact quite important. The main product isolated was the Fuc- α -SEt monomer, **180**, in 65 % yield, and the recovered donor **131**, in 14 % yield. The anomeric coupling constant (^1H NMR, 400 MHz, CDCl_3) was larger than expected at $J_{1,2} = 5.0$ Hz, however, the rest of the coupling constants matched that of the similar α anomer, (Table 4.3). It is possible that hyperconjugation could stabilise the intermediate activator (disulfide-triflate)²³⁹ in the ethyl case but less so in the methyl case. Another factor would be the increased nucleophilicity of ethyl disulfide over methyl disulfide, once an oxocarbenium ion of the donor had formed. This epimerisation reaction, whilst being mechanistically and synthetically significant in its own right, was not useful towards disaccharide formation. In conclusion it appears that efficient Fuc-Fuc disaccharide formation can only be achieved through the use of Br_2 and AgOTf activation, as this is the only system where a competing nucleophile is not produced during the activation process.

Our proposed mechanism (Scheme 4.11) and (Scheme 4.12) would be encouraged to occur with halophilic silver activation, as no competing nucleophiles are created; NIS activation creates succinimide, DMDS clearly creates disulfides and thioethers. In addition, Ag(I) activations are extremely fast, requiring much shorter reaction times than other activation routes (Table 4.2). Finally, NIS activation of thioglycoside uses a catalytic amount of Lewis acid, so the oxocarbenium ion concentrations in solution would be lower accounting for the observed high yielding monosaccharide products in entries 5-6, Table 4.2.

4.7 Applications of di-fucose methodology

The particularly tight window of conditions for which the dimer reaction (Scheme 4.8) appears to proceed is a drawback of the methodology, but given the quite unusual lack of glycosylation beyond disaccharide formation, some strict conformational requirements must be adhered to. (Table 4.5) shows a summary of the best yielding conditions when altering the acceptors used in the α -Fuc-1-3-Fuc disaccharide production, together with a sharp drop off in activity, as the acceptor becomes more sterically hindered.

4.7.1 Expansion of α -Fuc-1-3-Fuc beyond aglycans - Further steric constraints

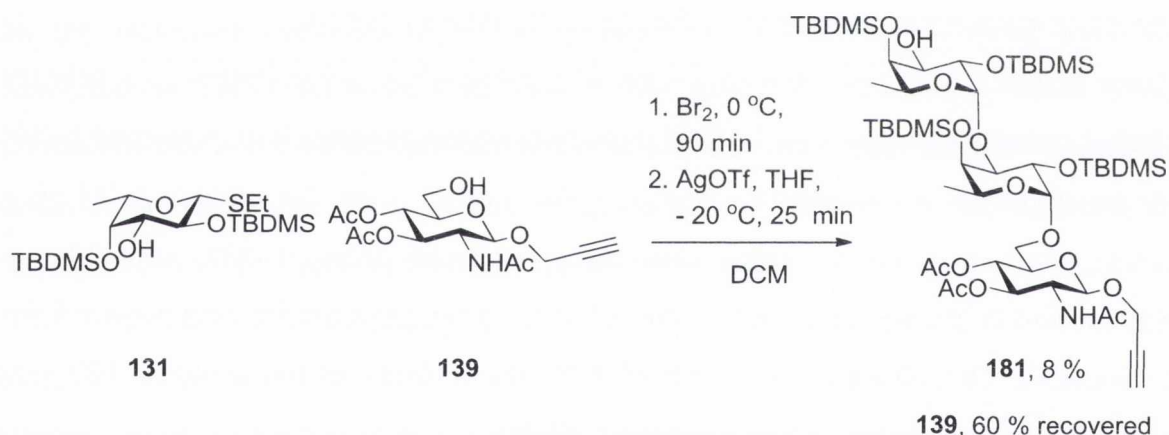
Propargyl alcohol **63**, could be successfully di-fucosylated using the Br₂/AgOTf activation conditions and partially protected donor **131**. (Entry 1, Table 4.5). The anomeric ratios at the reducing end were different to that observed in Scheme 4.8, and if the proposed mechanistic model (Scheme 4.12) was consulted, attack of **63** from the α anomeric face should be less hindered, considering the glycoside in the 3 position.

(Entry 3, Table 4.5) shows the expansion of Fuc-Fuc dimer glycosylation to a primary alcohol carbohydrate (Scheme 4.17). If steric bulk on the acceptor is an important factor for glycosylation at the reducing end of α -Fuc-1-3-Fuc, then the primary 6-OH on a carbohydrate may be accessible. Previously prepared acceptor **139**, containing a deprotected 6-OH was subjected to the α -Fuc-(1-3)-Fuc glycosylation and provided the trisaccharide **181**, albeit in low yields of 8 %. The majority of the acceptor **139**, was recovered pointing to the lack of steric compatibility.

(Entry 4, Table 4.5) shows the complete halting of reducing end glycosylation when a secondary alcohol is glycosylated. A glycosylation using the partially protected LacNAc acceptor **75** was attempted, but no isolated tetrasaccharide was observed. Although not conclusive proof of the unusual proposed route (Scheme 4.12), the lower yields observed in Table 4.5, with increasing steric bulk of the acceptor, would be predicted by this mechanism.

Table 4.5 - Modified acceptor glycosylations with 131

Entry	°C (B)	Time Min	Activation conditions, Acceptor	α -Fuc(1-3)Fuc-acceptor yield	Anomeric ratio $\alpha\alpha:\alpha\beta$
1	0, -20	90, 25	AgOTf, DCM/THF (2:1) Propargyl alcohol 63 , (1 eq.)	53 %	5.6 : 1
2	0, -20	90, 25	AgOTf, DCM/THF (2:1) Benzyl alcohol 152 , (1 eq.)	44 %	1.2 : 1
3	0, -20	90, 25	AgOTf, DCM/THF (2:1) Primary OH sugar - 139 , (1 eq.)	8 %, (60 % acceptor recovered)	α
4	0, -20	90, 25	AgOTf, DCM/THF (2:1) Secondary OH sugar - 75 (1 eq.)	0 %, (93 % acceptor recovered)	N/A



Scheme 4.17 - One pot trisaccharide using 2,4 di TBDMS fucosyl donor

4.8 Conclusion

The steric hindrance imparted by the 2,4-di-TBDMS protecting groups on donor **131**, allow controllable monosaccharide glycosylations to occur, when a catalytic amount of activator is employed. Although **131** is a sterically large donor, it is physically smaller than the tri-TBDMS donor **132**, previously employed to prepare TBDMS protected O-propargyl Lewis X **156**, (Table 3.5). This difference in steric bulk between both donors, has allowed the increase in yield for the partially protected O-propargyl Lewis X, **173**, to 58 %, and consequently, much milder deprotection. This route through partial protection, increases the glycosylation yield, and with only 2 TBDMS groups present, requires less reagents for

deprotection, representing a much more efficient route to O-propargyl Lewis X, **158**. Upon deprotection of O-propargyl Lewis X, the synthesis another complex glycoporphyrin, displaying a biologically active blood group sugar **174**, was reported.

An unusual side reaction was investigated, which may have uncovered a novel route to disaccharide formation. It is proposed that an oxocarbenium induced conformational shift opens a partially protected hydroxyl to glycosylation. The reaction has a low scope currently, but as understanding of the mechanism proceeds, and with the development of different protecting group strategies, an improvement in yields is hoped.

This chapter shows the double edged sword of serendipity. The partially protected donor **131**, was not a targeted compound, but ended up having applications which allowed the efficient synthesis of the Lewis X blood group, through a lowering of steric bulk vital for the successful glycosylation of LacNAc acceptor, **75**. However, donor **131**, also revealed an unusual set of side reactions, which under particular activation conditions lead to disaccharide formation.

Selections of this chapter have been included in a full paper on novel silyl based α selective fucose donors.²¹⁶

4.9 Future work

The O-propargyl Lewis X synthesis has been improved, and a reliable moderate to high yielding route to the O-propargyl substituted blood group sugar has been developed. Biological evaluation of the Lewis X substituted glycoporphyrin will now be of high interest, as well as the effects Lewis X has on intracellular and *in vivo* localisation. Enzymatic sialylation of the Lewis X glycoporphyrin would add another degree of complexity to the glycoporphyrin targeting ability, and warrants investigation.

As regards the α -Fuc-1-3-Fuc disaccharide formation, preliminary investigations have revealed the novel synthetic applications of a partially protected fucosyl donor. In light of the results presented in Chapter 4, further investigations involving carbohydrate derivatives with similar 2,3,4-equatorial-equatorial-axial hydroxyls such as Gal, are warranted. Similarly, derivatives containing axial-equatorial-equatorial hydroxyl groups such as Rha and Man should also provide additional interesting data.

A computational investigation of the proposed mechanism for the formation of the α -Fuc-1-3-Fuc disaccharide moiety may clarify current understanding of the model.

Chapter Five

Glycoporphyrin-lectin interaction and biological studies

Chapter 5 – Glycoporphyrin-lectin interaction and biological studies

5.1 Introduction

5.1.1 Measuring carbohydrate lectin interactions

The importance of lectins in plants and animals, and their ability to recognise specific carbohydrates was detailed in Section 1.3. Methods such as surface plasmon resonance,⁸⁶ atomic force microscopy,²⁴⁰ and quartz crystal microbalancing,¹⁰⁶ have all been successfully used to probe lectin, carbohydrate, and glycoporphyrin interactions. These methods involve the adsorption or chemical bonding of either the protein or the ligand on to a solid phase. A drawback of these surface based techniques was described in Section 3.1, where the random nature of solid surface binding, could result in the partial blocking or allosteric interference of a carbohydrate binding site. Such anomalies can lead to inaccurate binding results and false negatives. Solution phase measurements are more likely to mimic biological binding events, and investigations on ligand-lectin binding have been performed using isothermal titration calorimetry,²⁴¹ NMR,¹⁹⁰ and fluorescent emission²⁴² based techniques. Examples of solution based lectin carbohydrate binding measurements are also described in the introduction chapter, (Section 1.3).

5.2 Hydrophobic interactions of glycoporphyrins in aqueous media

The intrinsic amphiphilic properties of glycoporphyrins, can make them difficult to handle, but this property can also be used to a chemist's advantage when studying lectin interactions. The aggregation of porphyrins in organic solvents upon the addition of water is a well known phenomenon,²⁴³ and results from porphyrins π stacking in order to minimise hydrophobic repulsion in aqueous solution. With suitable functionalisation, porphyrins can become water soluble and so subsequent behaviour in aqueous media is of interest. Previously prepared water soluble glycoporphyrins, have already shown unusual amphiphilic properties during purification; for example, tri-LacNAc glycoporphyrin **122**, (Section 2.5.4.1).

5.2.1 NMR analysis of water soluble di- and tri-LacNAc glycoporphyrins in D₂O

One of the initial goals in preparing glycoporphyrins was to improve water solubility, for improved formulation. The water soluble examples, di- and trisubstituted LacNAc, **112**

and **122**, were of particular interest for this reason. Understanding the orientation or physical presentation of the carbohydrate moiety on the LacNAc glycoporphyrins in aqueous solution was important, in order to investigate their ability to interact with a lectin *in vitro/vivo*. Any significant changes in observed chemical shifts during NMR spectroscopy, may point towards the modes of aggregation, and could provide information on the accessibility of the sugar region in aqueous media. ^1H NMR spectra (600 MHz) of **112** and **122**, in D_2O , overlaid against the same sample in $\text{CDCl}_3/\text{MeOD}$ or $\text{CDCl}_3/d_5\text{-Pyr}$ are presented in Figure 5.1 and Figure 5.2. It can be seen, that aggregation of both glycoporphyrins in D_2O , has made the NMR spectrum uncharacterisable. A series of 10 fold dilutions in order to minimise aggregation, as well as the addition of MeOD up to a concentration of 10 %, did not resolve the aqueous spectra.

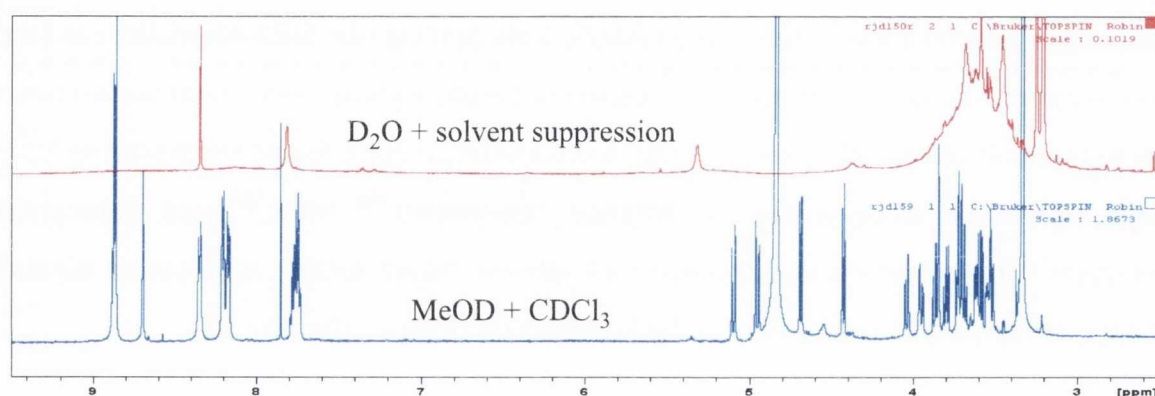


Figure 5.1 - Overlaid ^1H NMR (600 MHz) spectra of **112**, in $\text{D}_2\text{O}/\text{MeOD}$ and $\text{MeOD}/\text{CDCl}_3$

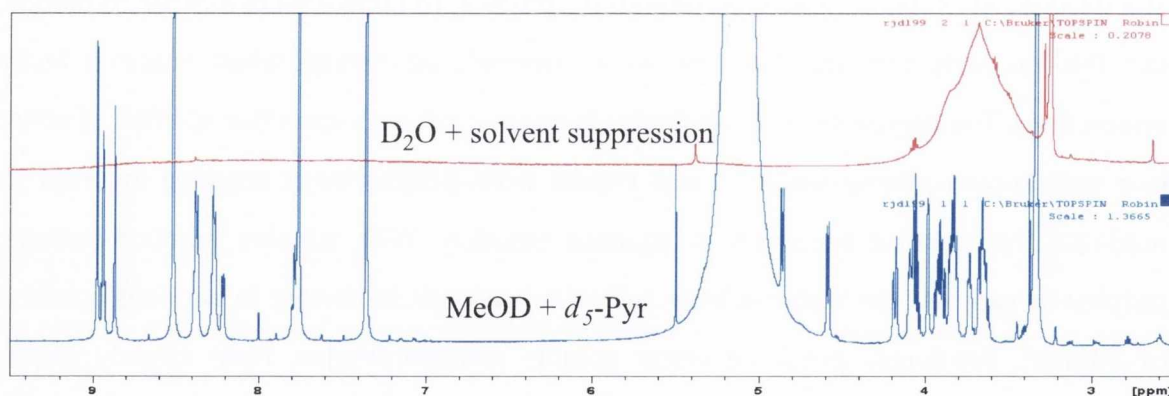


Figure 5.2 - Overlaid ^1H NMR (600 MHz) spectra of **122**, in $\text{D}_2\text{O}/\text{MeOD}$ and $\text{MeOD}/d_5\text{-Pyr}$

5.2.2 UV/Vis spectroscopy of glycoporphyrins in aqueous media

UV/Vis analysis was performed on the aqueous solutions of the glycoporphyrins to ensure they were not finely dispersed aggregated suspensions. Overlaid UV/Vis spectra of **122** (1×10^{-5} M) in $\text{MeOH}/\text{CHCl}_3$ (blue) and water (Red), are presented in Figure 5.3. The large

broadening of the Soret band at 423 nm, is a characteristic reporter signal for porphyrin aggregation.²⁴³ Liposomal formulation of PDT agents has been shown to increase cancer cell targeting (Section 1.1.5.4). If glycoporphyrin aggregation led to micelle formation, these compounds may demonstrate unusual uptake profiles through a combination of micelle and carbohydrate targeting properties. Through collaboration with Dr. Carsten Ehrhardt, TCD, an investigation of micelle formation using dynamic light scattering experiments, and surface tension measurements, was not successful in providing a critical micelle concentration. It appears that the glycoporphyrins displaying this range of amphiphilicity, exist as non-micellular aggregates in solution. Ongoing research is underway in the Scanlan group, to create more amphiphilic glycoporphyrins which may encourage organisation into micelles in aqueous solution.

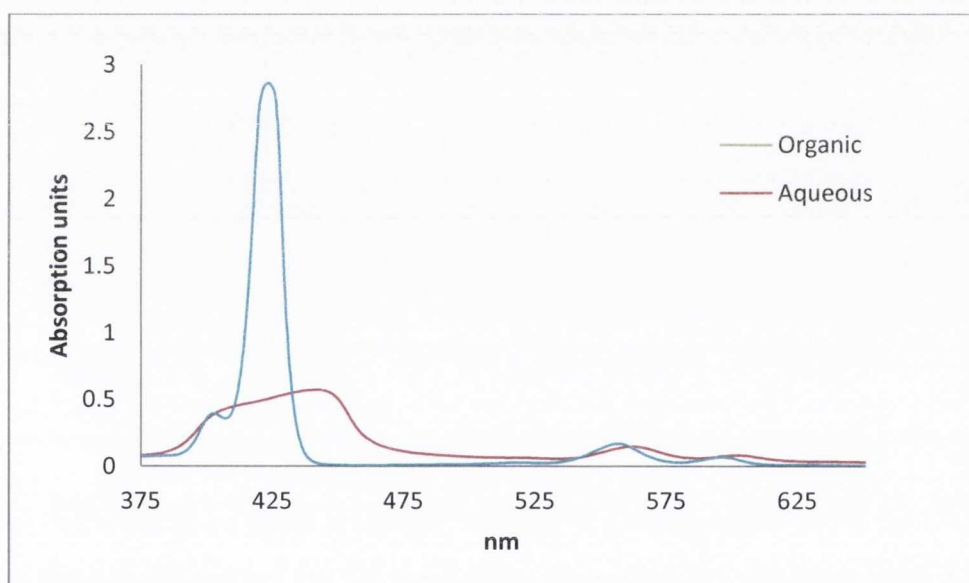


Figure 5.3 - UV/Vis absorption of 122 at (1×10^{-5} M) in $\text{CDCl}_3/\text{MeOH}$ (Blue) and H_2O (Red)

5.3 Investigations of aqueous aggregation and fluorescence quenching with Con A

Quenching of fluorescent emission can occur if the fluorescent molecule comes into close contact with another functionality that can absorb energy.²⁴⁴⁻²⁴⁶ Fluorescent quenching has previously been used to probe ligand macromolecule binding, where a decrease in fluorescence emission from a chromophore, occurs following a binding event with the macromolecule.²⁴⁶⁻²⁴⁷

Aggregation of the glycoporphyrins **112** and **122**, has been confirmed in aqueous media, (Figure 5.1-3). Further confirmation of aggregation can be shown if Beer Lamberts law does not hold for the aqueous systems, and will be investigated in future studies.

Quenching requires the close proximity of two molecules, so self quenching of glycoporphyrin fluorescence would already be occurring in aqueous media. Maillard *et al.* investigated the increase in fluorescence emission from a self quenched glycoporphyrin (**58**, Figure 1.32), in aqueous media, upon the addition of a lectin.²⁴² Interaction of a glycoporphyrin with a protein results in molecular recognition, disaggregation of the porphyrin, and consequently an increase in the observed fluorescence emission (Figure 5.4). Disaggregation depends on the ability of a lectin to recognise the carbohydrate displayed on the glycoporphyrin. As a general rule, disaggregation can then be measured by the change in the fluorescent emission observed, with respect to the concentration of lectin added. Maillard *et al.* had prepared the mannosylated glycoporphyrin dendrimer **58**, to target a retinoblastoma cell line,¹⁰⁵ and so Con A, an α -mannose specific lectin, was chosen as a model for cellular targeting.

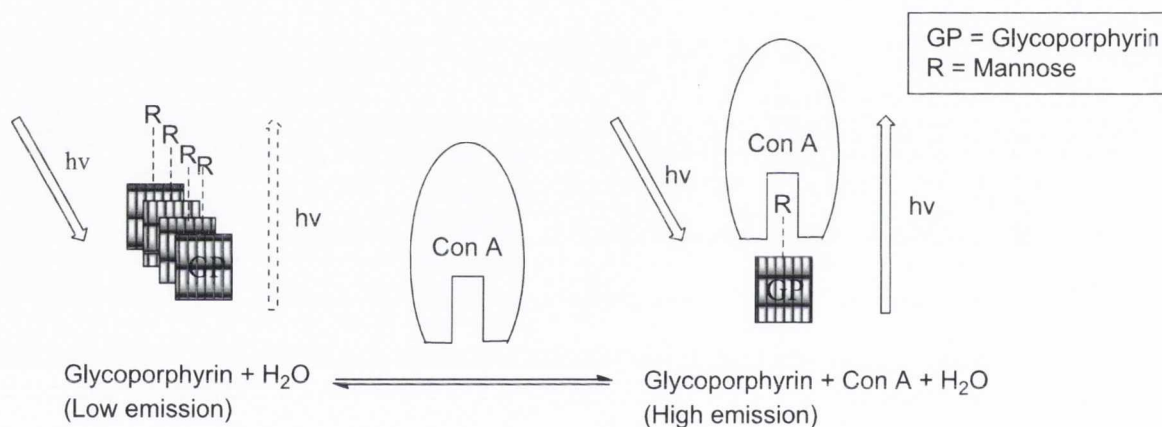


Figure 5.4 - Disaggregation leading to fluorescence

In a direct comparison to the experiment presented in Figure 5.4, the mono-, di-, and tri-mannosylated glycoporphyrins (**100**, **109**, **113**, **119**, Section 2.5), were selected to investigate Con A binding. The regioisomeric presentation of α -mannose units, could allow probing of any cluster effects specific to the triazole substituted glycoporphyrins. Con A exists as a tetramer at pH = 6.5, with each monomer containing a binding site for one α mannose unit.²⁴⁸ The distances between the binding sites of the Con A lectin, are estimated to be approximately 60 Å.²⁴⁹ Molecular modelling of 5,10 di-mannose substituted glycoporphyrin **109**, showed a distance of approximately 17 Å between the two carbohydrate regions. With this calculated distance, the carbohydrate units should

not be able to span two binding sites on the one tetramer. However, local concentration effects or lectin-lectin bridging may occur.

5.3.1 Confirmation of aggregation for the mannose substituted glycoporphyrins

In order to confirm aggregation of glycoporphyrins **100**, **109**, **113** and **119** in water, stock solutions (2×10^{-4} M) in MeOH:Pyridine (49:1 (v/v)), were diluted to (1×10^{-5} M) with DCM or H₂O respectively, (Figure 5.5). The organic solutions showed very minor differences in absorption irrespective of the mannose substitution pattern. There was a red shift of about 3-4 nm for the Soret band in organic solution, as compared to Figure 5.3, readily explained through combinations of the presence of pyridine interacting with the zinc centre, and the changes in pH.²⁵⁰⁻²⁵¹ In aqueous media, aggregation was clearly observed for all four glycoporphyrins, in particular the mono substituted glycoporphyrin **100**, which displayed a much lower absorption from 390nm to 430nm. It appears that two or more carbohydrate moieties alter the aggregation profile in aqueous media enough to result in an increase in absorption of the Soret band.

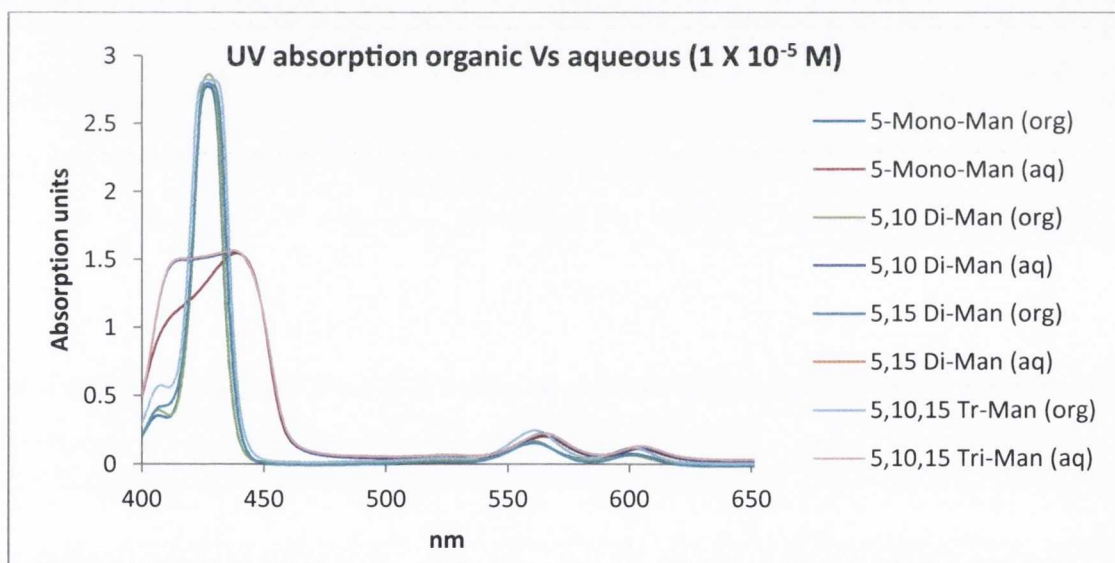


Figure 5.5 - Overlaid UV/Vis spectra in organic and 95% aqueous media

5.3.2 Concanavalin A lectin mediated disaggregation study

Having confirmed the aggregation of the mannose substituted glycoporphyrins in aqueous media, binding experiments were set up according to the Maillard literature procedure.²⁴² Stock solutions of each glycoporphyrin (2.0×10^{-4} M) were prepared in MeOH:Pyridine (49:1 (v/v)). All stock solutions were diluted to (2.0×10^{-7} M) with an aqueous buffer (pH = 6.5), containing HEPES (1.0×10^{-2} M), NaCl (1.5×10^{-1} M), CaCl₂ (1.0×10^{-3} M), and NiCl₂ (1.0×10^{-3} M), prior to measurements. Metal ions are required to

occupy centres near the carbohydrate binding site for optimal lectin carbohydrate interactions.²⁴¹ Diluted glycoporphyrin solutions (1 mL) were added to variable concentrations of Con A solutions, in aqueous buffer (1 mL), providing a final glycoporphyrin concentration of (1.0×10^{-7} M), with Con A ratios varied from 0 - 1000 equivalents. The mixtures were equilibrated for 2 h at 37 °C with gentle agitation, before fluorescent spectra were recorded.

5.3.2.1 Mono-mannose substituted glycoporphyrin, 100

The normalised fluorescent emission spectra for glycoporphyrin **100**, following incubation with increasing concentrations of Con A, and exciting at 423 nm, are shown in Figure 5.7. Disaggregation occurred in the presence of the lectin, as reported by Maillard²⁴² and a subsequent binding curve could be plotted from the change in fluorescence at 650 nm, (Figure 5.8). The unusual behaviour of spectra maxima divergence between 609 nm and 659 nm was not immediately clear. A binding curve (dotted line) was fitted to the data for 659 nm, as per the equation (Figure 5.6.)²⁴², where F_0 is the fluorescent emission in absence of the lectin, and $F_{\max}$ is the maximum observed fluorescent emission observed after Con A saturation.²⁴²

$$F = F_0 + \frac{(F_{\max} - F_0)K_b[\text{Con A}]}{1 + K_b[\text{Con A}]}$$

Figure 5.6 - Equation for plotting best fit of lectin binding

For the mono-substituted glycoporphyrin **100**, a $K_b = 3.7 \times 10^5 \text{ M}^{-1}$ was observed. This value is comparable to that of other mannose substituted glycoporphyrins,²⁴² reported in the order of 10^5 M^{-1} . It is also 10 fold higher than the reported Con A binding of α -OMe-Man.²⁴⁸ One unusual aspect to the fluorescent data recorded, was the lowering of observed fluorescence emission of **100** at the upper end of concentrations of Con A added. A suggested rationale for this observation was the ability for the bound glycoporphyrin to become quenched by the excess lectin present. If disaggregation occurs as a result of the carbohydrate being bound, removing the porphyrin from an aggregate, then fluorescence increases. However, if the concentration of added lectin reaches a saturation point, the extra protein may start to form non-specific interactions with the porphyrin, and fluorescent resonance energy transfer could lead quenching. The lectin concentration that $F_{\max}$ is observed at, is proportional to the binding constant. The actual

change in fluorescence from F_0 to $F_{\max}$, is a combination of disaggregation effects coupled with any quenching processes induced by virtue of binding the lectin.

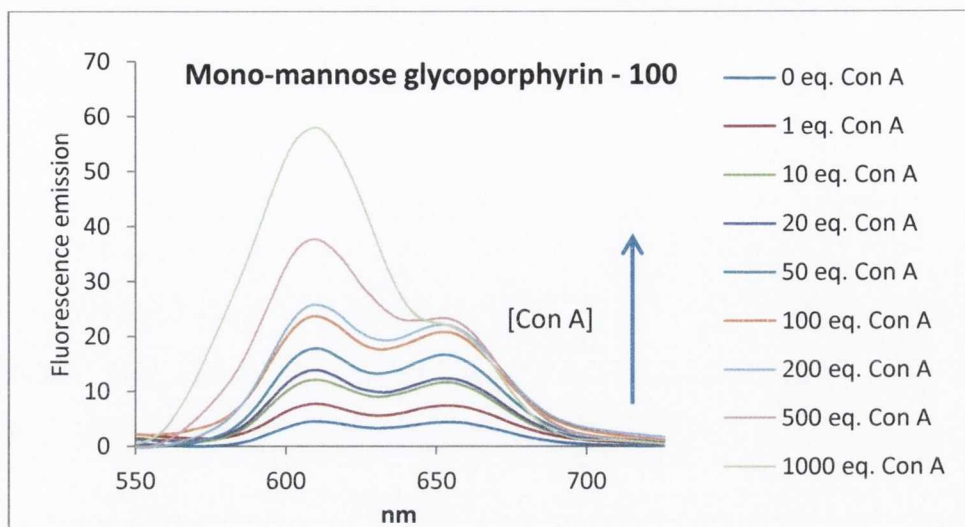


Figure 5.7 - Δ Fluorescent emission of 100 with increasing [Con A]

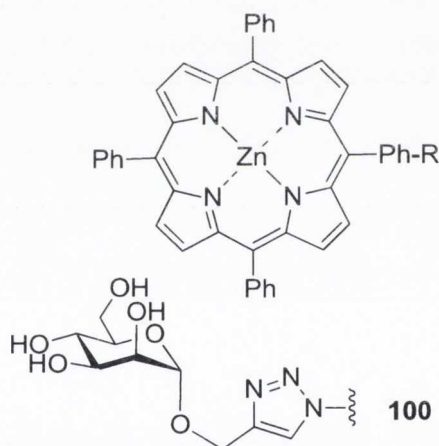
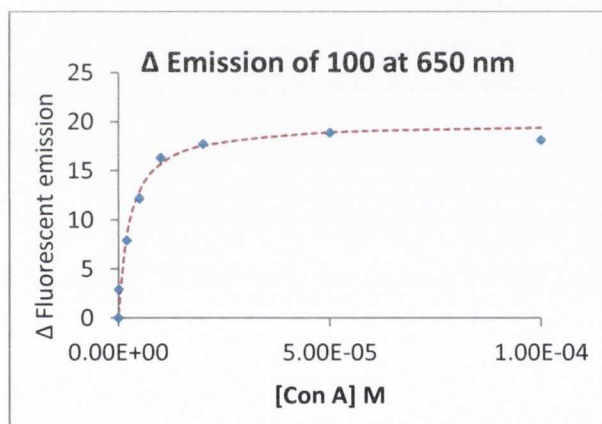


Figure 5.8 - Δ Fluorescent emission of 100 at 650 nm vs [Con A] M

5.3.3 Concanavalin A study on 5,10 and 5,15 di-mannose substituted glycoporphyrins

5.3.3.1 Concanavalin A study on 5,10 di-mannose substituted glycoporphyrin

The equivalent study for 5,10 di-substituted glycoporphyrin **109**, is presented in Figure 5.9. A marked difference in the fluorescent emission was observed, compared to the mono-substituted example (Figure 5.7). Fluorescent emission for **109**, was higher in the absence of any lectin, due to altered aggregation, as supported by the UV/Vis spectra (Figure 5.5). There was also a 9 nm red shift in the maximum emission. A biphasic binding process was observed, as fluorescence increased sharply up to about 20 eq. of Con A,

where the graph plateaus, before an increase in fluorescence is observed again, at a notably different rate, (Figure 5.10). This two part curve appeared consistently when emission studies were repeated, and appears unique to the 5,10 presentation of α -mannose. It is currently hypothesised that at lower concentrations of Con A, a single tetramer of the lectin is bound per glycoporphyrin and both carbohydrates are involved in co-operative binding, cluster effects. However, as the higher equivalents of Con A are introduced, the glycoporphyrins may start bridging pairs of Con A tetramers, which would lead to different effects on the porphyrin fluorescence. A single approximate binding curve, with a $K_b = 3.3 \times 10^5 \text{ M}^{-1}$ (dotted line) was fitted for reference only. Binding at low concentrations of Con A, is certainly tighter than this reference K_b as evident by the sharp increase in fluorescence, but not as tight at the higher concentration of Con A.

Literature precedent of biphasic lectin binding curves, suggest two or more binding events to be occurring.²⁵²⁻²⁵³ With this idea in mind, it is also possible that the 5,10 di-substitution pattern disrupts the Con A tetramer quaternary structure at low concentrations of the lectin. In this case Con A could exist as a mixture of monomer or dimers bridged by the glycoporphyrin. As the concentration of Con A was increased, then tetramers would be reformed. Maillard *et al.*²⁴² and Rusin *et al.*²⁵⁴ in their respective Con A binding studies, make the assumption that at higher concentrations of Con A, only one porphyrin binds a Con A molecule, and suggest the unlikelihood of multiple porphyrins binding Con A. However, neither take into account the corollary is also possible. At low concentration of protein, multiple Con A monomers could interact with a single porphyrin through bridging, and at higher concentrations of protein non-specific interactions with the porphyrin ring could occur. Non specific porphyrin/protein binding constants have previously been described in the literature. For example, Crowley *et al.* reported a water soluble sulphated porphyrin interacting with cytochrome c, providing a K_b in the order of 10^4 M^{-1} .^{191, 255-256}

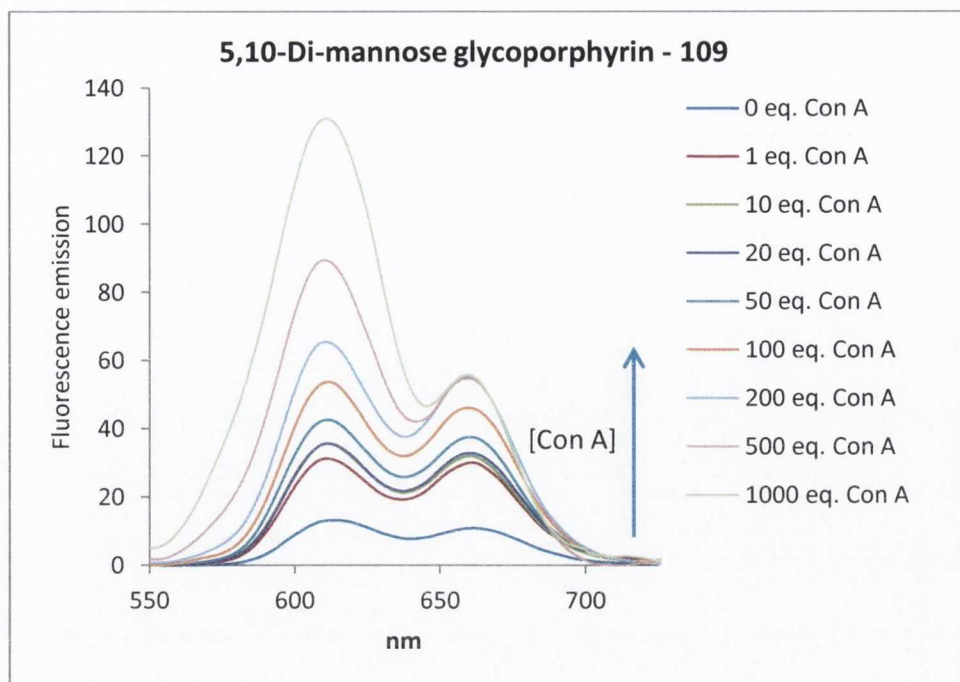


Figure 5.9 - Δ Fluorescent emission of **109** with increasing [Con A]

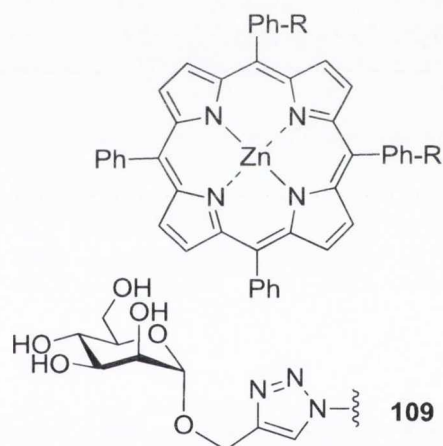
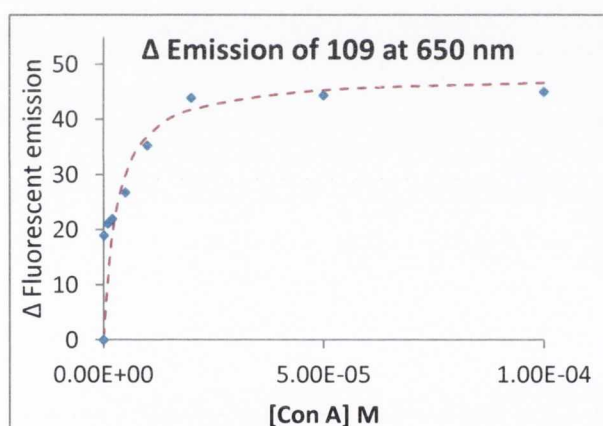


Figure 5.10 - Δ Fluorescent emission of **109** at 659 nm vs [Con A] M

5.3.3.2 Concanavalin A study on 5,15 di-mannose substituted glycoporphyrin

The equivalent study for 5,15 substituted glycoporphyrin **113**, is presented in Figure 5.11. The overall change in fluorescence from 0 equivalents of Con A to 1000 equivalents of Con A is almost identical to (**109**, Figure 5.9). The rate of change with respect to Con A concentration differs from significantly from **109**, and a best fit of $K_b = 6.0 \times 10^5 \text{ M}^{-1}$ (dotted line), for the observed change in emission with respect to Con A concentration, was fitted for **113**, in Figure 5.12. A biphasic curve was not observed for **113**, and the increase in fluorescence noted following the addition of just one equivalent of Con A was

markedly lower than presented in Figure 5.9. This divergence again points to the interesting macroscopic binding effects specific to the 5,10 substitution pattern at lower concentrations of Con A. Above 100 equivalents of Con A, non-specific binding or bridging does appear to be occurring for **113**, with a slight decrease in fluorescence, similar to that in Figure 5.8.

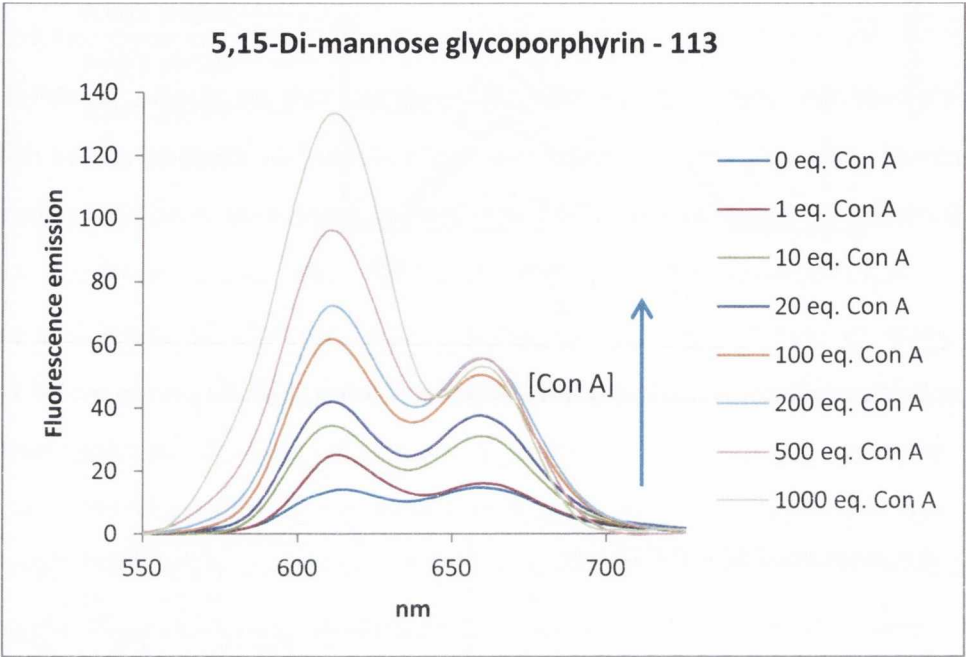


Figure 5.11 - Δ Fluorescent emission of 113 with increasing [Con A]

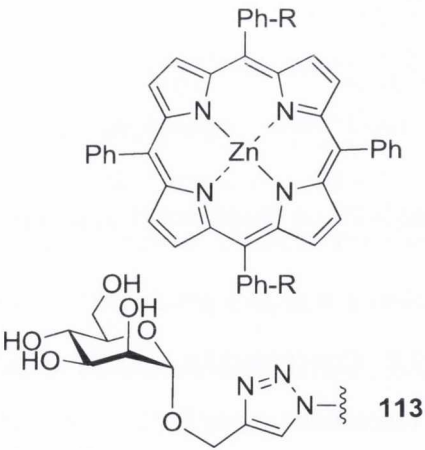
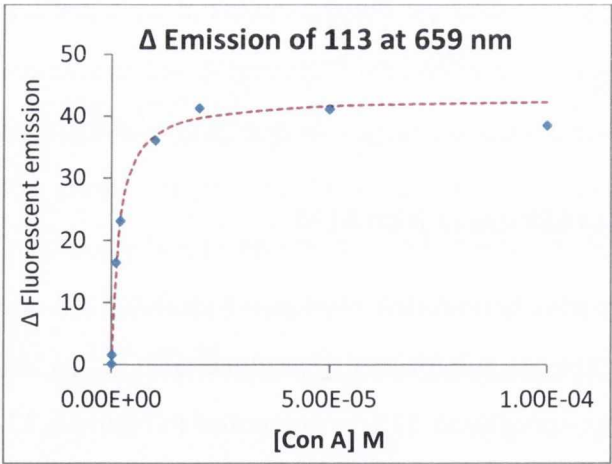


Figure 5.12 - Δ Fluorescent emission of 113 at 659 nm vs [Con A] M

5.3.3.3 Concanavalin A study on 5,10,15 tri-mannose substituted glycoporphyrin

The last example in the series of mannosylated glycoporphyrin Con A binding studies, is reported in Figure 5.13. Once again the observed fluorescence in the absence of Con A is

higher than the di- and mono- substituted analogues. This observation can be justified by the lower self quenching of **119**, through disrupted aggregation from the increased carbohydrate content. Interestingly, although the increase in fluorescence by emission units is obviously larger, a plot of the change in emission at 660 nm, versus Con A concentration (Figure 5.14), shows a best fit of $K_b = 1.45 \times 10^5 \text{ M}^{-1}$ (dotted line), which is actually lower than the previous three examples. Although an unusual observation, there are two important conclusions to take from it. Firstly, the porphyrin core itself must play some useful interaction along with carbohydrate binding for the mono- and di-substituted examples. Literature examples exist, where lectins have been shown to display an increased affinity for carbohydrates with hydrophobic functionalities near the anomeric centre, over carbohydrates not functionalised in this manner. For example, Reymond *et al.* reported up to a 160 fold increase in affinity of *Pseudomonas aeruginosa* for galactose dendrimers containing an aromatic ring, over the non-aromatic containing equivalent.¹³⁶ Assuming a co-operative hydrophobic interaction was involved in glycoporphyrin binding, it could be disrupted through the presence of additional polar moieties not involved in binding. The lower binding affinity provides evidence that simply increasing the carbohydrate capacity on a scaffold does not automatically impart additional recognition.

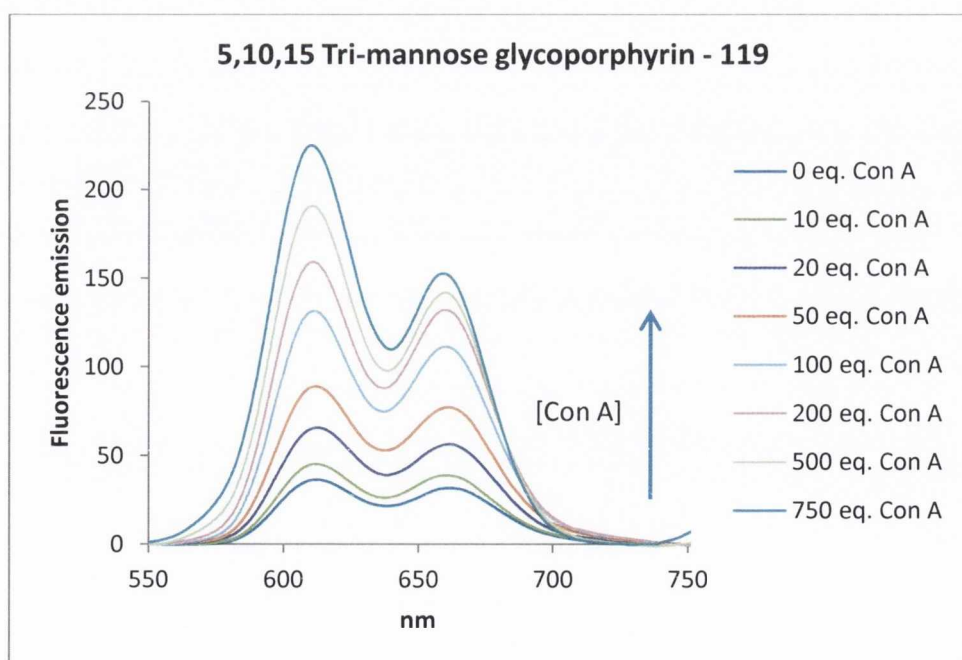


Figure 5.13 - Δ Fluorescent emission of 119 with increasing [Con A]

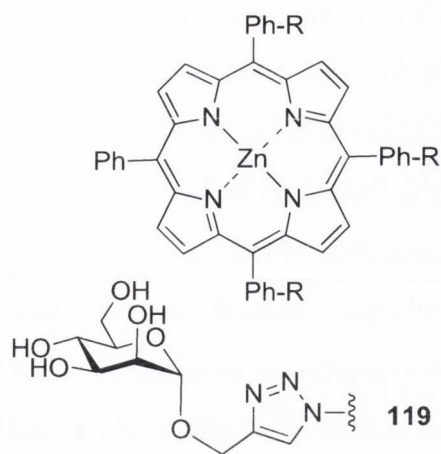
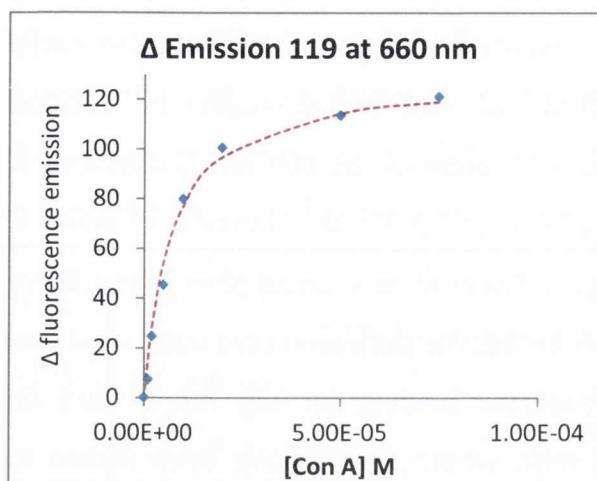


Figure 5.14 - Δ Fluorescent emission of 119 at 660 nm vs [Con A] M

5.3.4 Concanavalin A control study on tetraphenylporphyrin

In order to compare and contrast non-carbohydrate dependant disaggregation, a Con A binding control experiment was performed using TPPZn **91**, (Figure 5.15). A best fit binding (dotted line) of emission at 605 nm gave a $K_b = 1.25 \times 10^4 \text{ M}^{-1}$, over 10 fold lower than the lowest observed glycoporphyrin binding interactions. The result reveals a measure of the non-specific interactions with Con A during disaggregation of TPPZn **91**, but also confirms the selectivity for Con A provided by mannose substitution. The observed non-specific K_b in Figure 5.15, is in a similar range to that reported by Maillard *et al.*²⁴², for their study on Con A binding of a PEG linked tetraphenylporphyrin, $K_b = 1.8 \times 10^4 \text{ M}^{-1}$. Crowley *et al.* showed cytochrome to porphyrin non-specific interactions to be in the order of 10^4 M^{-1} .¹⁹¹ The increase in TPPZn fluorescence had not reached F_{max} after incubation with 1000 equivalents of Con A confirming an obvious difference in binding of TPPZn versus the glycoporphyrins previously described.

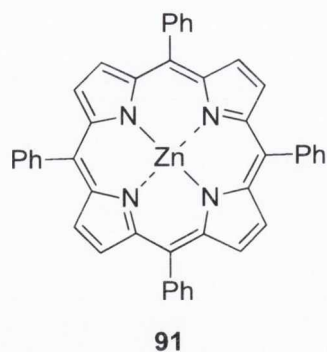
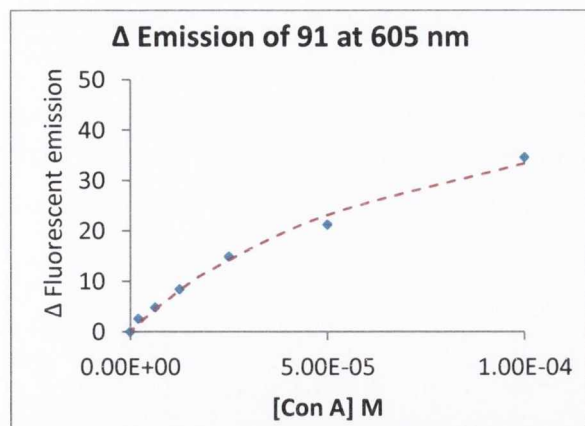


Figure 5.15 - Δ Fluorescent emission of 91 at 605 nm vs [Con A] M

5.3.5 Comparison of binding curves for control (TPPZn), mono-, di-, and tri-mannose substituted glycoporphyrins

A very interesting outcome is noted when the fluorescent emission of the tetraphenyl porphyrin control and all mannose substituted glycoporphyrins versus Con A concentration are overlaid, (Figure 5.16). There are stark differences in disaggregation, through increases in fluorescence, but the finding that total F_0 to F_{max} is not necessarily proportional to the K_b of the glycoporphyrin/lectin interaction, is an interesting divergence. The derived k_b values for all 5 examples tested are displayed in Table 5.1. Interaction of Con A specifically through mannose can be confirmed for the tested glycoporphyrins, but at low and high lectin concentration ratios, non trivial binding processes contribute measurably to the observed fluorescence. The non-specific quenching and biphasic curves observed, must relate to changes in the Con A quaternary structure. The contribution of non-specific porphyrin interactions at higher concentrations of Con A for the tri-substituted example **119**, is particularly clear given the similarities above 100 equivalents of Con A to the equivalent TPPZn binding curve (Figure 5.16).

Cluster effects were unlikely to be observed in this study as the distance between Con A binding sites was too large to be chelated by the 5,10 disubstituted glycoporphyrins **109**. Gratifyingly, the increase in binding for the 5,15 disubstituted analogue is over 300 times that of mannose alone, demonstrating the possible advantages of carbohydrates as targeting agents for porphyrins. Future applications of the mannose substituted glycoporphyrin series to *E. Coli* specific binding, would be interesting. Anti bacterial PDT

could be very selective in this case, given the distance between carbohydrate binding domains on the Fimb H is only 10 Å,⁸⁶ a distance easily spanned by both **109** and **119**

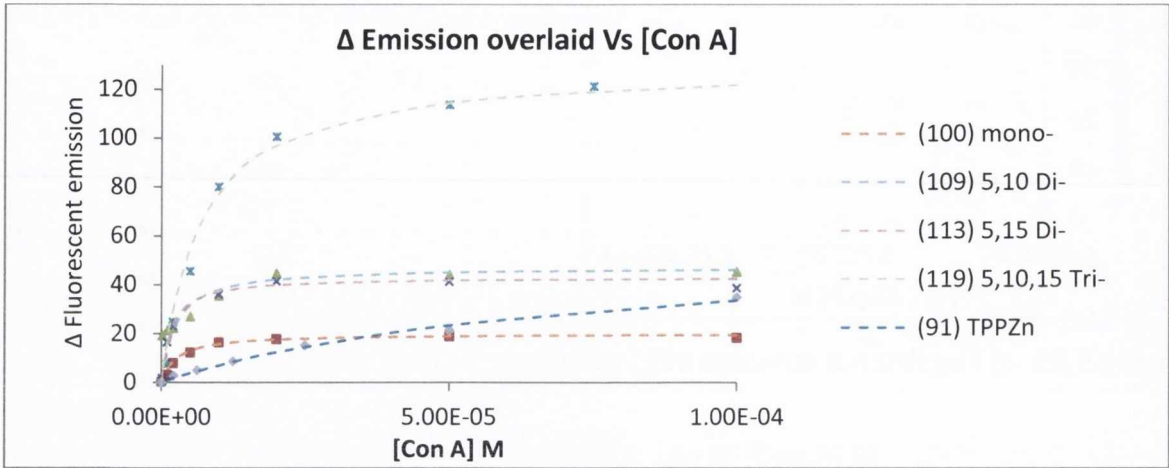


Figure 5.16 - Comparison of emission vs [Con A] overlaid

Table 5.1 - Reported and observed Con A binding constants

Ligand	Observed K_b for Con A ^a
α -OMe-Mannose ²⁵⁷	$0.8 (\pm 0.02) \times 10^4 \text{ M}^{-1}$
TPPZn, 91	$1.3 (\pm 0.04) \times 10^4 \text{ M}^{-1}$
5-Mono-mannose glycoporphyrin, 100	$3.7 (\pm 0.19) \times 10^5 \text{ M}^{-1}$
5,10-Di-mannose glycoporphyrin, 109	$3.3 (\pm 0.27) \times 10^5 \text{ M}^{-1}$
5,15-Di-mannose glycoporphyrin, 113	$6.0 (\pm 0.11) \times 10^5 \text{ M}^{-1}$
5,10,15-Tri-mannose glycoporphyrin, 119	$1.5 (\pm 0.08) \times 10^5 \text{ M}^{-1}$

^a Figures in parenthesis represent the standard deviation of the observed K_b from replicated experiments

5.4 Applications of aggregation in fluorescence quenching with Ulex I

The Con A binding studies with mannose substituted glycoporphyrins, demonstrated that the fluorescent based analysis of glycoporphyrin Con A binding, is transferable across a series of glycoporphyrin models. To date Con A has been the only lectin reported for glycoporphyrin disaggregation studies. We were interested in applying the fluorescent disaggregation methodology, to other lectin/carbohydrate pairs. In chapter 3, a terminal α -fucose disaccharide (**125**, Scheme 3.11) was prepared for NMR based titration studies on *R. Solanacearum*. Analysis of lectin mediated glycoporphyrin disaggregation with a fucose recognising lectin, would provide complimentary data for the proposed NMR study. A direct comparison using the fucose binding lectin of interest from *R.*

Solanacearum was not possible, due to difficulties in accessing the lectin, however, *Ulex europaeus* agglutinin I, an alternative fucose binding lectin was employed.

Ulex I has a molecular weight of 63,000 and is made up of two similar sized subunits.²⁵⁸ Due to its specificity for α -fucose, the lectin is used as the standard method for identification of the Bombay type blood group, particular to a human phenotype not expressing fucose on ABO blood groups.²⁵⁹

5.4.1 *Ulex* I and tri- α -Fuc-(1-6)-GlcNAc - glycoporphyrin, **144**

The results for a fluorescent disaggregation binding study on glycoporphyrin **144**, displaying the α -Fuc-1-6- β -GlcNAc residue, with *Ulex* I are shown in Figure 5.17. A plot of the change in emission at 659 nm against *Ulex* I concentration is provided in Figure 5.18. The first interesting point to note was the concentration of *Ulex* I required to reach $F_{\max}$, approximately 20 equivalents. On closer examination of the curve it demonstrates much more complicated kinetics, with at least 2 different processes taking place. Similar to the abnormalities observed in Figure 5.10, at low concentrations of *Ulex* I, a markedly different binding curve is observed, with fluorescent emission actually decreasing upon the addition of 1 equivalent of *Ulex* I. Dimers of *Ulex* I are thought to be the active quaternary structure,²⁵⁸ so perhaps **144**, is interacting with a monomer structure or bridging multiple structures resulting in its own quenching through energy transfer. From 1 to 20 equivalents of the lectin, a sharp increase in the slope is observed, followed by a tailing off of fluorescence, as concentrations of *Ulex* I approach 100 equivalents. A representative best fit binding curve was plotted (dotted line) with a $K_b = 2.1 \times 10^6 \text{ M}^{-1}$ (Figure 5.18). This binding is in the order of 10 times stronger than the binding of trimannose glycoporphyrin **119**, for Con A. Irrespective of the different lectins investigated, native ligands for wild type lectins are likely to be oligosaccharide chains, from glycoproteins, so it is not surprising that a disaccharide is bound more efficiently than a monosaccharide, as observed in Figure 5.18. For example *R. Solanacearum* binds α -Fuc-(1-6)-GlcNAc-OMe, with a K_b of 6.8 times higher than α -fucose-OMe alone. Unfortunately no literature reference for the equivalent relative affinities of *Ulex* I was found.

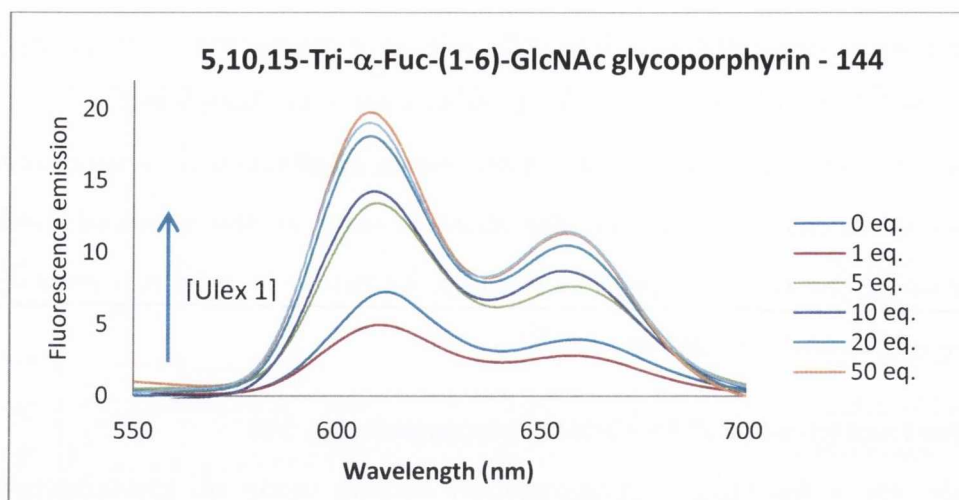


Figure 5.17 - Δ Fluorescent emission of 144 with increasing [Ulex I]

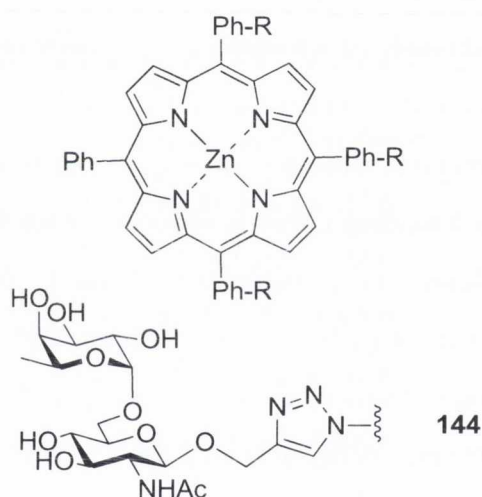
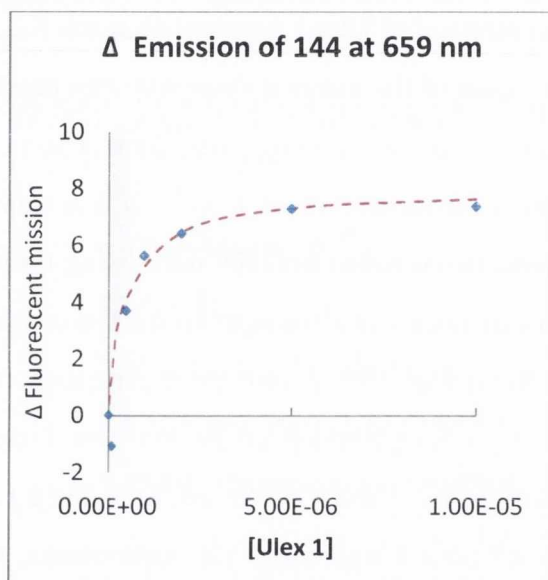


Figure 5.18 - Δ Fluorescent emission of 144 at 659 nm vs [Ulex I] M

5.4.2 Disaggregation leading to alternative fluorescent quenching

In order to justify the comparatively low overall change in emission ($F_{\max} - F_0$) of **144**, following disaggregation, a preliminary rationale is presented. Delbaere *et al.* and Imberty *et al.* have shown the 3-OH and 4-OH positions of fucose to be vital in Ulex I binding, and removal or protecting of either of these sites, results in no interaction with Ulex I.²⁶⁰⁻²⁶¹ One important aspect not addressed in the glycoporphyrin based literature, is the orientation of the porphyrin during the act of carbohydrate-lectin interaction. Important effects on the presentation and orientation of the porphyrin can be observed through the $F_{\max} - F_0$ value, which may have consequences for activity in PDT. The chemical environment experienced by the porphyrin section of a glycoporphyrin following lectin

interactions, can depend on how the carbohydrate approaches the binding domain of the lectin. If carbohydrate binding, forces the porphyrin ring into close proximity with the surface of the lectin, then although porphyrin disaggregation would result, competing quenching processes could occur to the surface of the lectin Figure 5.19. The observed increase in fluorescence emission upon disaggregation could be dampened as observed in Figure 5.17. Comparing this system to the large increase in fluorescence observed in Figure 5.13, for α -mannose substituted glycoporphyrins, and the same simple model could be applied (Figure 5.20). α vs β linkages to the porphyrin may play a role in encouraging quenching interactions.

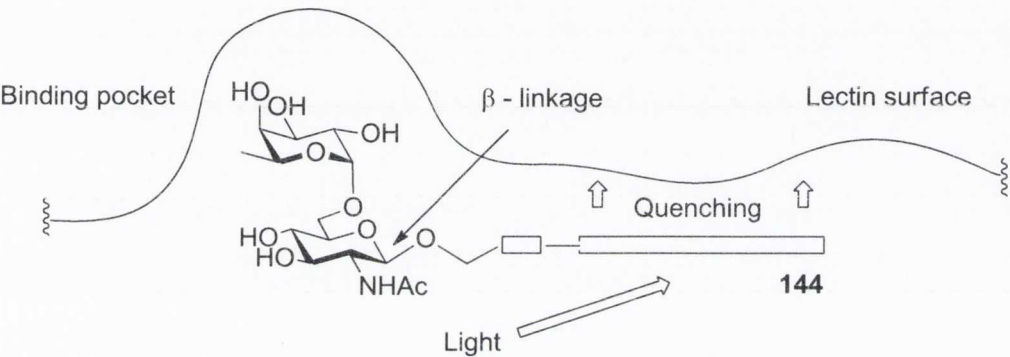


Figure 5.19 - Model binding conditions resulting in quenching of fluorescence

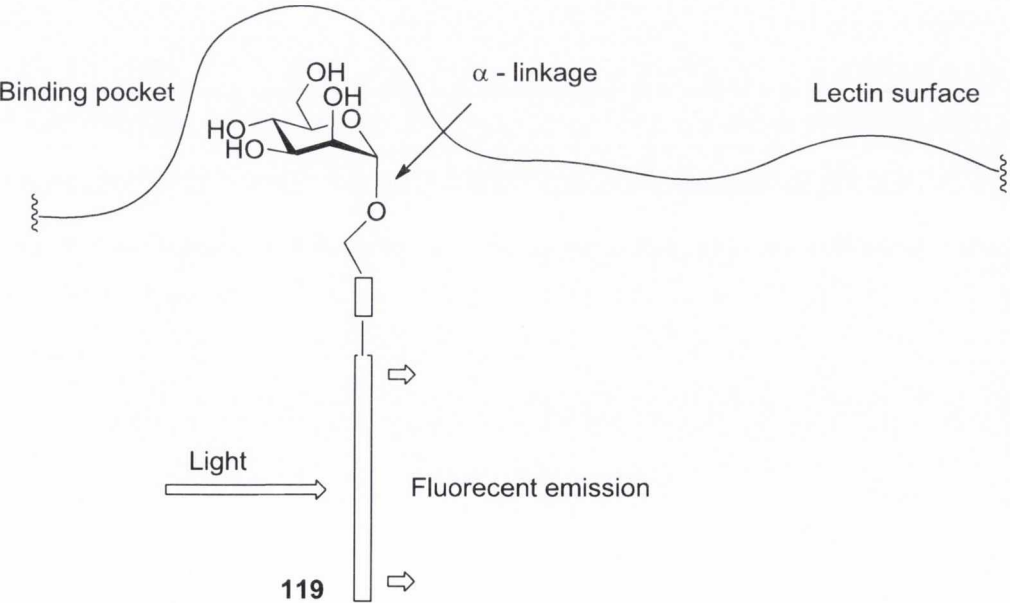


Figure 5.20 - Model binding conditions resulting in increase of fluorescence

Direct evidence for the kind of interactions in the above figures, is difficult without crystal structures showing close atom-atom contact points between both the carbohydrate and the lectin, and between the porphyrin scaffold and neighbouring sites on the lectin. Insight could also be gained through the NMR based techniques, if lectin to porphyrin through space interactions were observed.

5.4.3 Con A with tri- α -Fuc-(1-6)-O-propargyl-GlcNAc-glycoporphyrin

Having shown the molecular recognition of two different lectins for glycoporphyrins, a direct comparison of the Ulex I and Con A binding, using the fluorescent disaggregation model would prove the principle that carbohydrates could direct glycoporphyrins, to a protein target.

The interaction of **144**, with Con A was investigated (Figure 5.21). A plot of the change in emission at 659 nm against the protein concentration is shown in Figure 5.22. From the increase in fluorescence emission, as compared to the Ulex I equivalent (Figure 5.17), it can be observed that the orientation of the carbohydrate upon binding, places the porphyrin in a different chemical environment, and there is less of an interaction between the protein surface and the porphyrin. However, as the β glycosidic linkage remains, the increased fluorescence observed in Figure 5.21, is vastly lower than the increase observed for the Con A interaction with a tri-Man substituted glycoporphyrin (**119**, Figure 5.13). At lower concentrations of Con A, a decrease in fluorescence is not reported, and even at 1000 equivalents of Con A, $F_{\max}$ was not yet observed. From the change in fluorescent emission observed, (Figure 5.22), a case of two overlaid binding curves, as opposed to biphasic binding appears to be reported. Up to around 200 equivalents of Con A, a proposed carbohydrate recognition event is being observed, however, the latter part of the curve is more akin to a non-specific interaction, similar to that reported with TPPZn (**91**, Figure 5.15). The fitted line for the former was plotted at an estimated $K_b = 4.5 \times 10^5 \text{ M}^{-1}$.

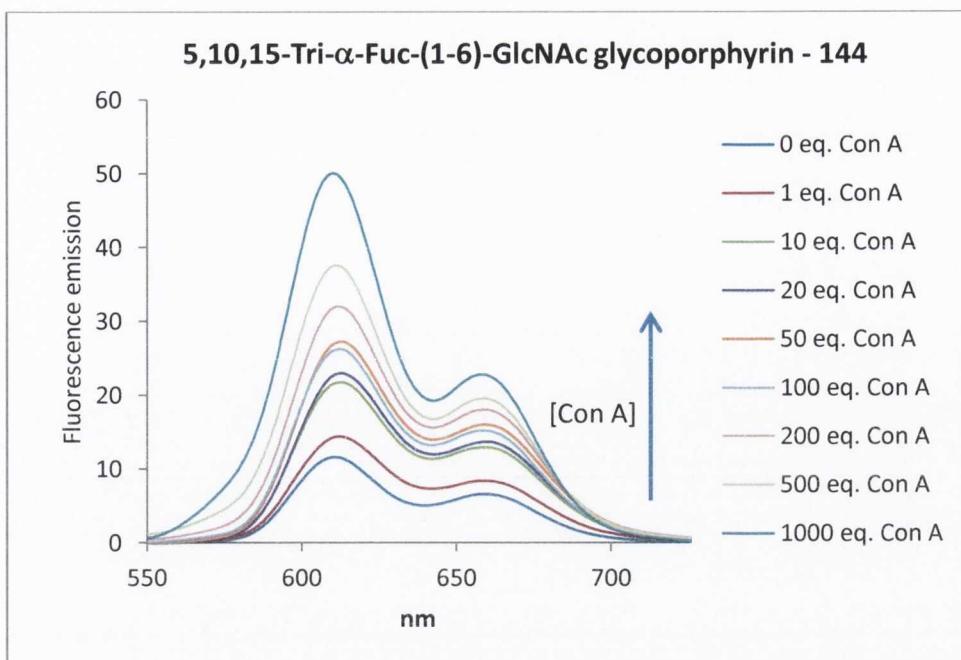


Figure 5.21 - Δ Fluorescent emission of 144 with increasing [Con A]

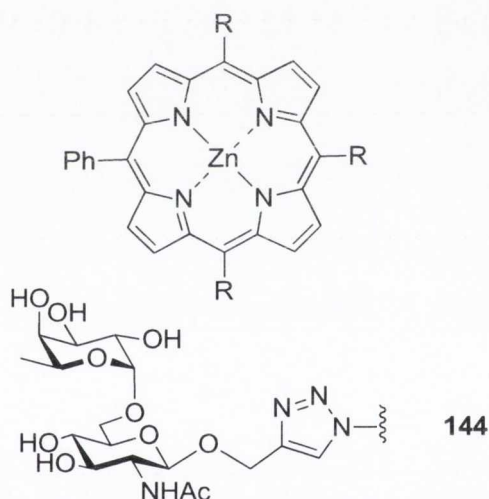
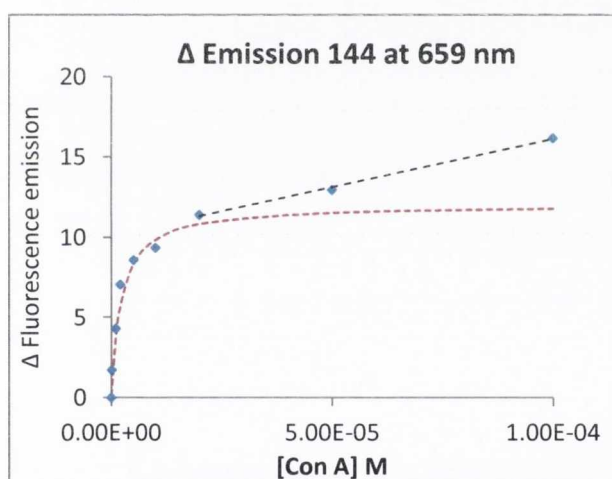


Figure 5.22 - Δ Fluorescent emission of 144 at 659 nm vs [Con A] M

5.4.4 Overlay of binding curves for binding curves for Ulex I vs Con A

A comparison of the change in fluorescent emission upon disaggregation, for the interaction of **144** with both Con A and Ulex I, is presented in Figure 5.23. It was disappointing that the difference in binding was not more obvious, however, from our studies on various glycoporphyrin lectin interactions it can be seen that the promiscuous nature of the porphyrin-protein, non-specific binding, appears to provide a baseline recognition event in many cases. Nonetheless, (Table 5.2) demonstrates the higher selectivity of Ulex I over Con A, for the fucose presenting glycoporphyrin **144**. Over the series of disaggregation experiments, enhanced affinity over the respective

carbohydrates has been demonstrated by the glycoporphyrins. Extrapolation of this idea to cellular binding confirms the ability of one glycoporphyrin to distinguish between two different proteins, and is a positive result for the future selective targeting of PDT agents *in vivo* with carbohydrates.

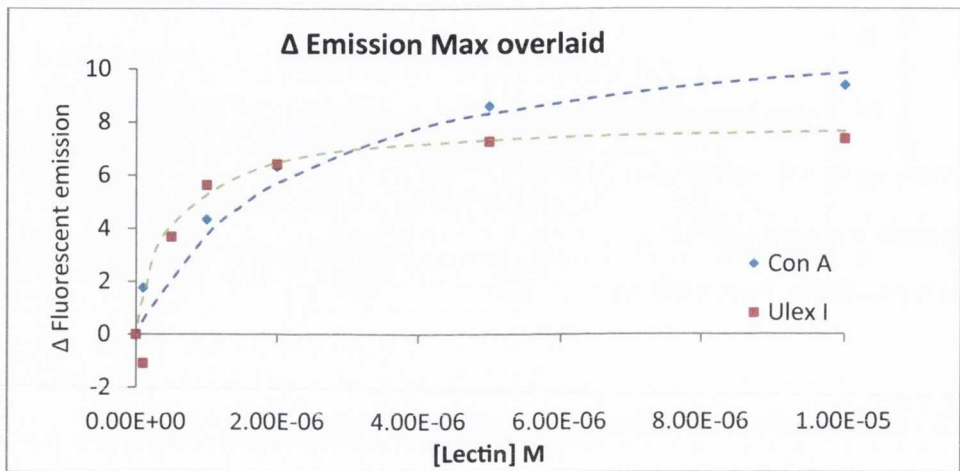


Figure 5.23 - Comparison of emission for 144 vs [Con A] and [Ulex I]

Table 5.2 - Observed lectin binding constants for glycoporphyrin - 144

Ligand	Observed K_b for Con A	Observed K_b for Ulex I
5,10,15-Tri- α -Fuc-(1-6)-GlcNAc glycoporphyrin - 144	$4.5 (\pm 0.50) \times 10^5 \text{ M}^{-1}$	$2.1 (\pm 0.08) \times 10^6 \text{ M}^{-1}$

^a Figures in parenthesis represent the standard deviation of the observed K_b from replicated experiments

5.5 ¹⁵N enriched HSQC with *R. Solanacearum* fucose binding lectin

To date only preliminary studies on the interaction of the fucose binding lectin from *R. Solanacearum* can be reported. A control experiment was performed by Mr. Pawel Antonik, of the Crowley group, NUIG, investigating the change in chemical shifts observed upon addition of α -Fuc-(1-6)-O-propargyl-GlcNAc (**125**, Figure 3.1), as compared to O-propargyl LacNAc (**77**, Scheme 2.4). The conditions were analogous experiment to that previously reported by the Crowley group.¹⁹¹ No interaction was observed for the Gal terminating example (**77**), but a significant altering of the ¹H-¹⁵N shifts was observed at amino acid sites presumed to represent key interactions in the carbohydrate recognising domain. The analogous glycoporphyrin interactions will be investigated in due course with tri-terminal Fuc displaying (**144**, Figure 3.10) in order to quantify the effect of presenting this disaccharide on a scaffold. It is hoped this experiment will provide insight

into the hypothesis described in Figure 5.19, where porphyrin quenching effects may be observed through allosteric shifting of signals in the HSQC spectrum.

5.6 Biological evaluation of early glycoporphyrin library

In order to understand the *in vitro* uptake and localisation properties of the triazole linked glycoporphyrins, a preliminary biological study was performed on mono- (**100**, **104**, **105**, **106**, Figure 2.7)), di- (**109**, **110**, **111**, **112**, **113**, **114**, **116**, Figure 2.9, Figure 2.10, Scheme 2.19)), and tri- (**119**, **120**, **121**, **122**, Figure 2.11), substituted glycoporphyrins. My own contribution to the biological studies was under the guidance Gisela Vaz, who completed the majority of the study as part of her thesis for the degree, doctor of philosophy in biochemistry, at the Institute of Molecular Medicine (IMM), St. James Hospital, Dublin. The work was the result of a collaboration between Prof. M. Senge (TCD), and Dr. T. Davies (IMM), and Dr. E. M. Scanlan. As the majority of the physical testing was performed by Mrs. Vaz, only a summary of the results attained are presented. The complete combination of the biological testing has been published alongside the glycoporphyrin synthesis.¹⁸⁸

As PDT treatment is currently used for oesophageal cancer the human oesophageal squamous cell carcinoma cell line OE21, was chosen for *in vitro* studies.²⁶² As a healthy cell reference, the Het 1a cell line was also investigated for three 5,10-disubstituted glycoporphyrins (**112**, **110** and **116**). To the best of our knowledge, neither of these cell lines demonstrate lectins specific for the recognition of carbohydrates, but nonetheless, the OE21 cell line remains an interesting clinically relevant biological model for uptake studies. The second generation photosensitizer, Foscan (**5**, Figure 1.5) was supplied by Prof. Mathias Senge, and was used as a positive control in all experiments.

5.6.1 Glycoporphyrin formulation

During the formulation process for *in vitro* testing of medicinal compounds, sterility must be ensured. Bacterial and virus particle filters can be employed but there is anecdotal evidence that aggregated material can often clog these filters. Stock solutions containing an ethanol concentration of over 50 % are considered sterile. For this reason, the clinically approved method of administering Foscan from a stock solution of 1 mM made up in, (ethanol:propylene glycol, 4:6 (v/v)) was altered to a formulation of (ethanol:propylene

glycol, 6:4 (v/v)), before samples were diluted using a sterile phosphate buffer to 6 μ M, 4 μ M, 2 μ M, and 1 μ M.

Following 24 h storage of the diluted glycoporphyrin solutions at 37 °C, the mono-substituted examples contained traces of particulate matter. Although this could be re-dispersed upon sonication, the danger of aggregate precipitation falsely affecting the uptake and localisation studies meant that only the di- and tri- substituted glycoporphyrins were examined *in vitro*.

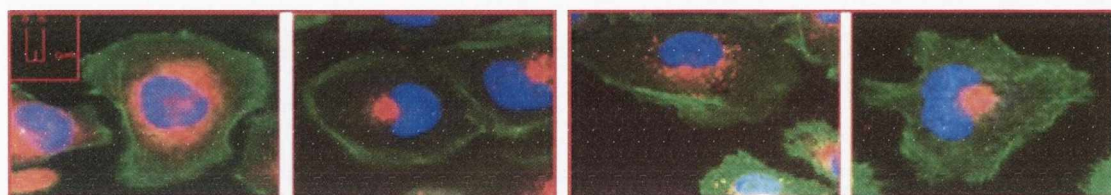
5.7 Cellular uptake and localisation

5.7.1 Investigation of Foscan and a glycoporphyrin sample set - OE21

A test set of three 5,10-disubstituted glycoporphyrins ((**112**, **110** and **116**)) and the control Foscan (**5**) were investigated at 3 μ M, in the OE21 and Het 1a cell lines. Uptake was seen in all four compounds using a GE In Cell fluorescent analyser. Fluorescent imaging results of the OE21 series following cell fixation, are displayed in Figure 5.24.

Differences in localisation can be observed across the series shown (**A - D**, Figure 5.24). Foscan, appears to localise generally in the cytoplasm. Both **112** (**B**, Figure 5.24) and **116** (**D**, Figure 5.24), appear to localise in the Golgi apparatus and/or the Endoplasmic reticulum. In contrast, **110** (**C**, Figure 5.24) appears to have been packaged into discrete organelles, either endosomes or lysosomes. Co-localisation studies with organelle tracker would be required to confirm these localisation sites, however, from the images currently available, the morphology of distribution is consistent with those sites previously described for glycoporphyrins in Chapter 1 (Section 1.3). Variation of intracellular localisation is of particular interest, as it is clearly resulting from differential carbohydrate recognition by the cell. The common monosaccharide to **112** and **116**, is GlcNAc, which is sulfonated by GlcNAc-6-sulfotransferase in the Golgi apparatus,²⁶³ however, GlcNAc presentation in LacNAc is quite different to free GlcNAc, and sulfation tends to occur at the non reducing end of oligosaccharides. The observed cell morphology (**D**, Figure 5.24), is particularly consistent with transport to the nuclear region of the cell. The blebbing shape, opposite the organelle containing the glycoporphyrin, along with the fact that the nucleus has been pushed into a bean shape, suggest cytoskeleton changes. It is possible that all 3 glycoporphyrins in Figure 5.24, are following the same metabolism path, transport to the Golgi, processing and distribution out of the cell in endosomes. If this is so, then in the case of **110** (**C**, Figure 5.24) faster processing due to a terminal Glc could

be under observation. Unfortunately with just single time point experiments, it cannot be conclusively confirmed how respective localisation came to pass. Multiple time point experiments, or even real time imaging of cellular uptake would be greatly informative in tracking the nuances of differential carbohydrate presentation.



A - Foscan

B - 112

C - 110

D - 116

5,10-Di LacNAc

5,10-Di Glc

5-GlcNAc-10-Man

Red = Porphyrin, Green = FITC-Phalloidin (Actin stain), Blue = Hoechst (Nucleus stain)

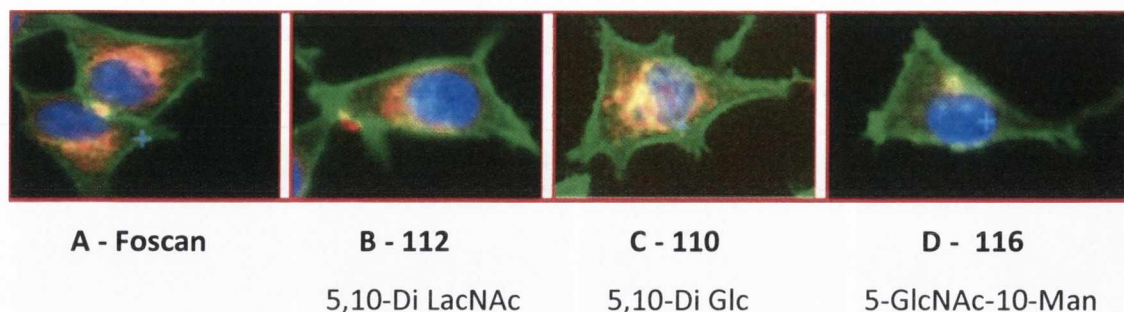
Figure 5.24 - Overlaid fluorescence emission from OE21 cells at 3 μ M (Dark)

A common assay used to quantify cell death is the MTT assay. This assay quantifies mitochondrial activity through the conversion of a tetrazole based compound into a purple formazan crystal, which can be analysed using UV plate readers. It is important that PDT agents do not have any underlying toxicity. Gratifyingly, no dark toxicity was observed in the glycoporphyrin series by MTT assay. The morphology of the cells appeared normal and stable after incubation with the glycoporphyrins, and the cells did not appear to show any signs of distress. This finding is of significant importance in PDT, as agents cannot be cytotoxic in the absence of light.

5.7.2 Investigation of Foscan and a glycoporphyrin sample set - Het 1a

Uptake and localisation images from the non-cancerous Het 1a cell model, following cell fixation, are displayed in Figure 5.25. Although, Foscan **5**, appears to be generally localised inside the cell, compounds **112** (**B**, Figure 5.25) and **110** (**D**, Figure 5.25) appear to have a much more diffuse presence, certainly as compared to **116** (**C**, Figure 5.25). Glucose as a targeting agent plays a more important role in the Het 1a cell line, and certainly seems to have resulted in a much higher uptake. The fact some that the triazole glycoporphyrins have shown intracellular selectivity, and increased uptake in a cancerous cell model over a non cancerous cell model, was a key early finding in the study. Enhancement of this observed selectivity, through incubation of glycoporphyrins with

specific lectin over expressing cell lines, will be investigated in due course. The differences in cellular and intracellular localisation provided justification for *in vivo* studies.



Red = Porphyrin, Green = FITC-Phalloidin (Actin stain), Blue = Hoechst (Nucleus stain)

Figure 5.25 - Overlaid fluorescence emission from Het 1 A cells at 3 μ M (Dark)

5.7.3 SAR trends in glycoporphyrin uptake.

In order to quickly screen the libraries of PDT agents for effectiveness, an automated study was performed on the entire library to search for any SAR between the site and type of carbohydrate displayed and cellular uptake.

As the mono-substituted glycoporphyrins were not fully soluble during formulation, they were not included in this SAR study. The compiled cellular uptake data, as measured by the intensity of the glycoporphyrin photosensitizer emission from within the OE21 cell is shown in Figure 5.26.¹⁸⁸ 5,10 disubstituted glycoporphyrins **109**, **110**, **111**, **112**, and **116**, showed the greatest uptake. Interestingly, the 5,15 di-substituted glycoporphyrins **113** and **114** showed little or no uptake, along with all of the tri-substituted glycoporphyrins. Differences in amphiphilicity are suggested as the reasoning for this unusual finding. The 5,10 substitution pattern makes two hydrophobic edges of the porphyrin available to the cell membrane, to aid insertion into the cell membrane during endocytosis. The requirement to balance hydrophobicity in order to achieve uptake is a standard theme in medicinal chemistry, and has been discussed as applied to porphyrins by Senge *et al.*²⁷ and Roeder *et al.*¹⁰⁹ Blais *et al.* have demonstrated the uptake of tri-substituted glycoporphyrins as being superior to mono- and di-substituted analogues.¹⁰⁵ However, these carbohydrates were spaced further from the porphyrin core on PEG linkers, which have a degree of flexibility, placing the sugar unit away from the cell membrane as the porphyrin approaches, allowing uptake. More rigid glycoporphyrin linkers like the triazole spaced glycoporphyrins detailed in this thesis, are more restricted in their movements,

and so the arrangement of the carbohydrates around the porphyrin core, is more important.

Another consideration is the difference in fluorescent emission depending on local glycoporphyrin interactions, as described in the Ulex I vs Con A studies, (Section 5.3). Using fluorescence to measure uptake needs to be interpreted with particular care, since cases where low uptake, as defined by fluorescent emission, could actually describe very high uptake conditions, with an equally high competing protein to porphyrin quenching process. For this reason complimentary tests such as light mediated cell toxicity were performed, which could also provide information about whether the porphyrins were uptaken.

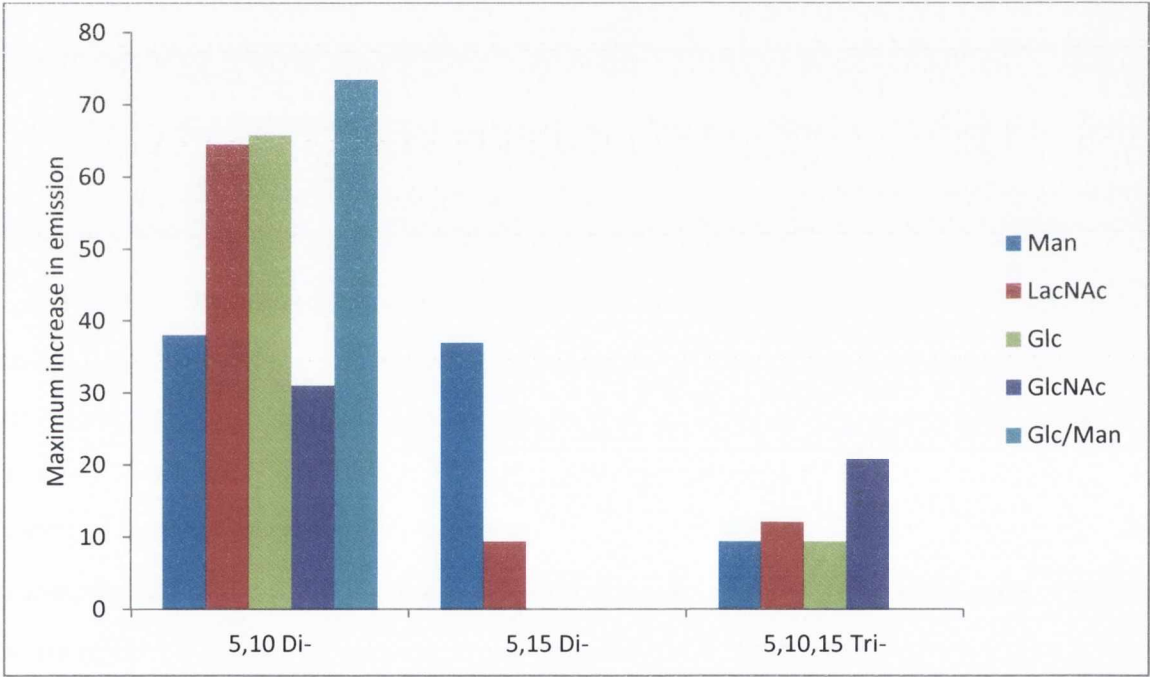


Figure 5.26 - Δ fluorescence emission at 620 nm from control in OE21 cell model

5.7.4 Light toxicity - General MTT assay results

Light toxicity of the biologically tested glycoporphyrins was evaluated through an MTT assay. This mitochondrial viability assay, was performed 4 hours following irradiation of the OE21 cells. Foscan (5), showed a dose dependant response, with complete cellular destruction at 3 μ M, after only 2 minutes irradiation.

Surprisingly, no significant cell death as measured by mitochondrial viability was observed at this time point, in any of the glycoporphyrins.¹⁸⁸ The lack of toxicity was of particular

interest, considering (Figure 5.26), clearly shows the uptake of at least the 5,10 di-substituted glycoporphyrins.

There are a number of possible reasons for this low phototoxicity. Firstly, the triazole linked glycoporphyrins may be poor producers of singlet oxygen, so although uptaken and localisation was observed, the compounds remained inert in the cell. Another possibility was that the compounds did produce singlet oxygen, but that the rate of production was not high enough to induce cell death. This possibility is likely, since porphyrins have been shown to be less efficient singlet oxygen producers than chlorins and bacteriochlorins.^{5,}

²⁶⁴⁻²⁶⁵ The original goal of this research project was to use porphyrins as scaffolds with which to quantify intracellular localisation effects of carbohydrates. An easy workaround to increase PDT activity *in vitro* would be to select glycoporphyrins known to be uptaken, and prepare the chlorin analogues.

The last possibility was that the site of localisation leads to cell death through a different route which would be missed by an MTT assay conducted immediately after irradiation. Longer lag times before conducting the MTT assay would confirm this hypothesis. Due to Foscan localisation in the mitochondria, it is not surprising that a cell viability test, which measures mitochondrial activity, would show a very fast response. However, if intracellular damage of a PDT agent was centred at the Golgi/ER, perhaps the result could be cytotoxicity, but over a longer time period. Recently, Wlodkowica *et al.* discussed the possibility of inducing cell death through alternative organelle targeting *in vitro*.²⁶⁶ One of the key targets discussed in their paper was that of toxicity through Golgi/ER destruction. Caspases present in the Golgi apparatus can lead to apoptotic cell death, if the integrity of the Golgi apparatus is disrupted. Interestingly, below a certain threshold of Golgi stress, pro-survival mechanisms can also be triggered.

5.7.5 Light toxicity morphology changes

Having quantified the low cell death from the glycoporphyrin series through an MTT assay, whilst also collecting data confirming glycoporphyrin uptake was observed in the OE21 cell line, (Figure 5.24), a repeat microscopic analysis was performed following irradiation. The results of this investigation are shown in Figure 5.27. In example **A**, where the cell was irradiated in the absence of a photosensitizer as a control, the normal, healthy cell morphology was defined. Clearly, in the case of Foscan (**5**), these cells have been completely destroyed. The cytoplasm volume has reduced considerably, and the

nucleus has shrunk. The observations in **B**, Figure 5.27, are complemented by the lack of cell viability from the MTT assay results.

(**C**, **D**, **E**, Figure 5.27), can be directly compared to (**B**, **C**, **D**, Figure 5.24), where it is evident from all three that morphological changes have occurred following irradiation. The cytoplasm volumes have decreased, however, not to the extent of Foscan (**5**, **B**, Figure 5.27). The image of **112** (**C**, Figure 5.27) now shows some diffuse localisation in the cytoplasm, not present before irradiation. The neat compartmentalisation of **110**, before irradiation, now appears to be broken up somewhat (**D**, Figure 5.27). A diffuse localisation in the cytoplasm is observed in **116** (**E**, Figure 5.27), unlike the tight Golgi localisation observed prior to irradiation. PDT responses are observed in all GP cases, however, whether the response is pro-survival or apoptotic so far, remains unclear.

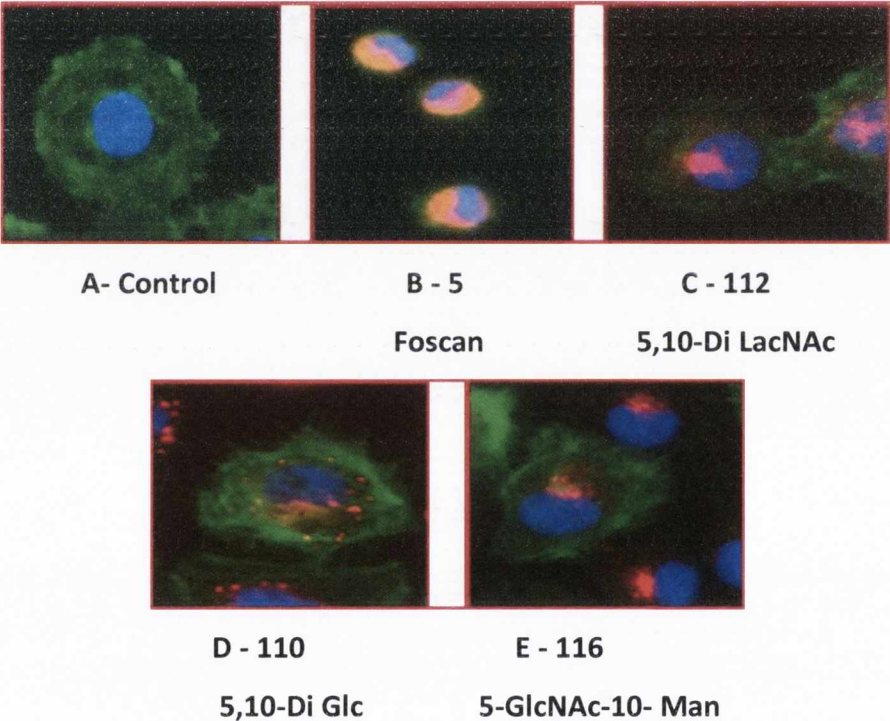


Figure 5.27 - Overlaid fluorescence emission from OE21 cells at 3 μ M after irradiation

5.7.6 Summary of preliminary biological evaluation

From the biological results presented above, a SAR pattern has emerged showing 5,10 di-substitution, is clearly the best regioisomer for cellular uptake on this system. Intracellular localisation has been observed to be directed by the carbohydrate, although the exact mechanism of this has yet to be elucidated. Provisionally, cell toxicity of glycoporphyrins is very low when applying clinically relevant Foscan irradiation timelines, although cell

viability 24 hours post irradiation would be interesting, it has not yet been performed. Although cell toxicity was not achieved in this study, cell morphology changes offer promise for the future biological evaluation, where under more forcing irradiation conditions, with increased light doses being applied to the cell, a successful result is expected.

5.8 Relative singlet oxygen production: A study on triazole containing glycoporphyrins

The lack of PDT induced toxicity from the glycoporphyrin study was surprising given the confirmed cellular uptake of certain examples. Investigating the relative singlet oxygen production of a triazole linked glycoporphyrin as compared to our positive control, Foscan is a valuable experiment in justifying the sub optimal PDT activity. Noted cell morphology changes following irradiation of the glycoporphyrin analogues, led us to believe singlet oxygen was being produced, but perhaps production was too low to result in cell death. Maillard *et al.* has previously described the lower phototoxicity of triazole linked glycoporphyrins and performed relative $^1\text{O}_2$ production measurements to investigate a possible cause.¹⁵⁵

5.8.1 DPBF degradation in organic solvent

A relative $^1\text{O}_2$ production test was performed, using the photo-degradation of DPBF,¹⁵ in the presence of a photosensitizer, and a homemade white light source with an output of 415 W/m^2 . A UV/Vis spectrum of DPBF at $5 \times 10^{-5} \text{ M}$, in DMF is shown in Figure 5.28. The 5,10-di-lactosamine substituted glycoporphyrin **112**, which showed high uptake in the cell content assay, but which also has the highest carbohydrate content, was taken as reference for the triazole containing glycoporphyrins. Relative $^1\text{O}_2$ production versus Foscan in DMF was recorded by monitoring the change in UV/Vis absorption of DPBF, at 411 nm, and the results are presented in Figure 5.29. Singlet oxygen was produced by Foscan at approximately 1.8 times a greater rate than glycoporphyrin **112**. This is a very useful result, as the lack in cell toxicity (Section 5.7.4), is more likely a product of low $^1\text{O}_2$ production, as opposed to the absence of $^1\text{O}_2$ production. The low level of $^1\text{O}_2$ production could be theoretically increased through longer irradiation timelines. If this were the case, then the glycoporphyrins tested in Figure 5.27 could still be clinically useful for PDT applications. From the results of Figure 5.29, an irradiation time of at least four minutes would be recommended during repeated biological studies with the uptaken compounds, to produce a similar quantity of $^1\text{O}_2$.

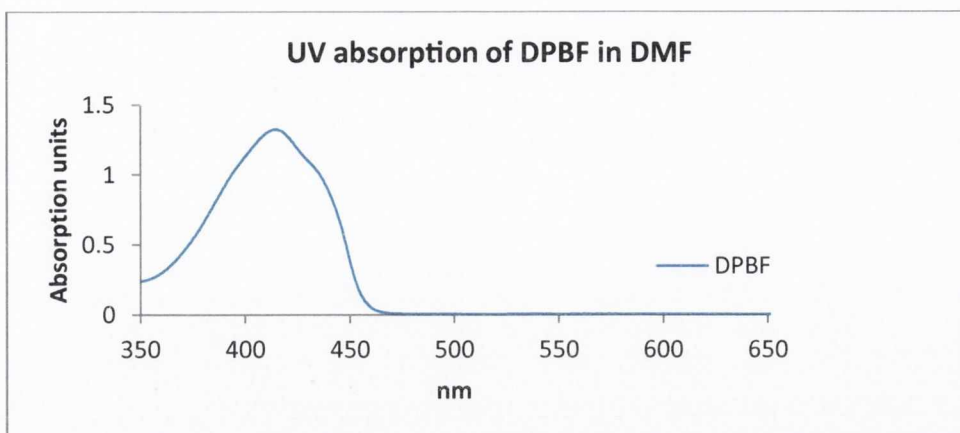


Figure 5.28 - UV/Vis absorption of DPBF in DMF

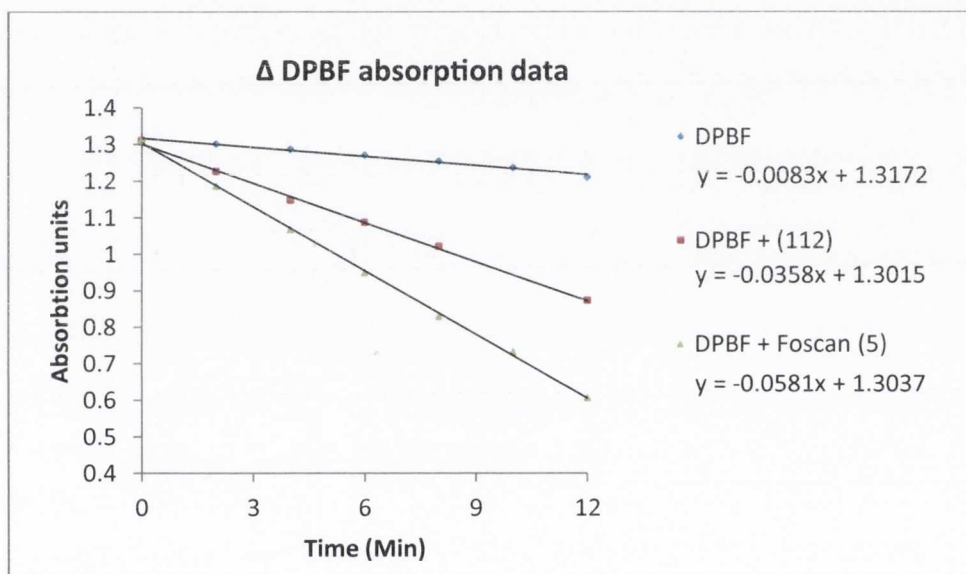


Figure 5.29 - $^1\text{O}_2$ production in DMF as a function of DPBF absorbance

5.8.2 DPBF degradation in aqueous media

Triazole linked glycoporphyrins produce $^1\text{O}_2$ at about half the rate of Foscan. Reductions in $^1\text{O}_2$ production of 50 % by photosensitizers in aqueous media has been previously described in the literature.⁵ Performing $^1\text{O}_2$ studies in aqueous media is a more valid test to monitor *in vitro* $^1\text{O}_2$ production, especially if aggregation of the triazole linked glycoporphyrins leads to no $^1\text{O}_2$. The UV/Vis absorption spectrum of DPBF in DMF:H₂O (1:1 (v/v)) is shown in Figure 5.30.

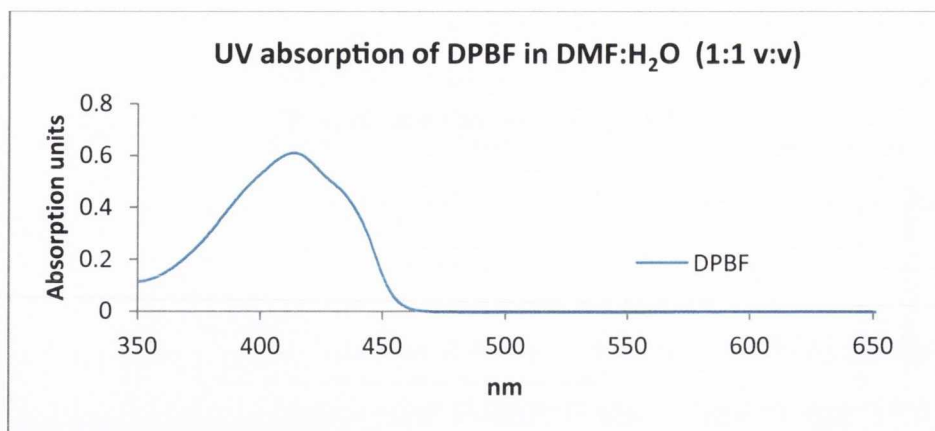


Figure 5.30 - UV/Vis absorption of DPBF in DMF:H₂O (1:1 (v/v))

A lower maximum absorption of DPBF resulting from aggregation is observed, with about half the absorption as compared to Figure 5.28. In an effort to understand the singlet oxygen production ability of aggregated glycoporphyrins, a photodegradation experiment of DPBF in DMF:H₂O (1:1 (v/v)) was performed (Figure 5.31).

Foscan **5**, remained a more efficient producer of ¹O₂. Taking the control slopes into account, **112**, produces ¹O₂ at a rate of 1.9 times less than Foscan, a value largely comparable to the equivalent test in DMF, (Figure 5.29). The advantage of having this information is that both in organic and aqueous solutions, the relative ability of triazole linked glycoporphyrins and Foscan to produce ¹O₂ is comparable. The rate of ¹O₂ production for both the reference glycoporphyrin and Foscan is lower in aqueous media, but since the relative rate of ¹O₂ production is equivalent, the presence of a triazole ring is not any more detrimental in aqueous media than in organic media.

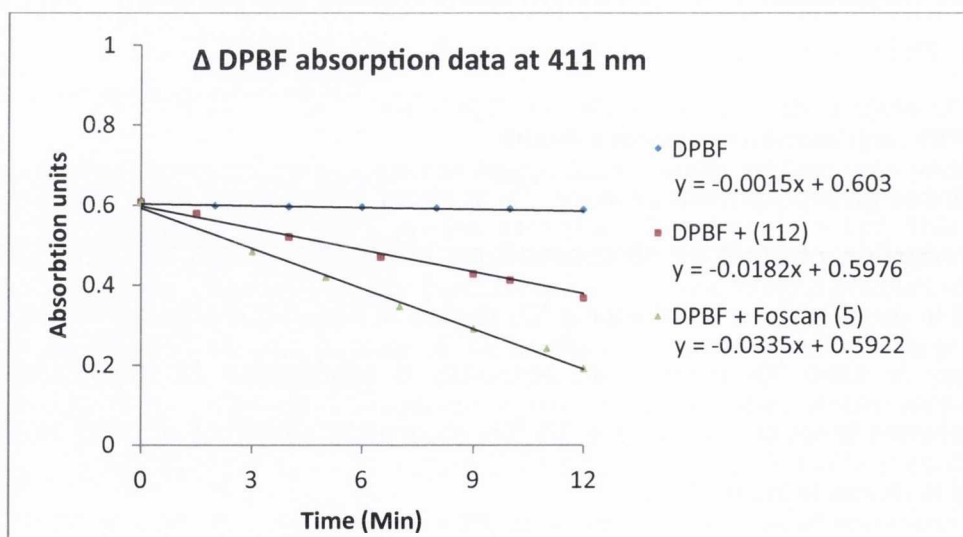


Figure 5.31 - ¹O₂ production in DMF:H₂O (1:1 (v/v))

The ability to conduct this test exclusively in water is limited by the insolubility of DPBF and Foscan in H₂O. However, it is clear from (Figure 5.31), that increased aggregation (which was also shown in Figure 5.5), in aqueous media leads to a decrease in ¹O₂ production.

It has also been shown that significant disaggregation is observed upon the binding of a mannose and fucose substituted glycoporphyrins with Con A, (Figure 5.13), and Ulex I (Figure 5.17). Wang *et al.* has reported similar disaggregation of porphyrins, with fetal bovine serum.²⁶⁷ It may be possible that protein mediated disaggregation could selectively activate glycoporphyrin agents *in vitro*, where binding of a particular lectin would increase the ¹O₂ production. With the same logic, PDT agents would remain aggregated, and thereby would have an inherent lowered ability to produce ¹O₂ when an agent is not bound by a carbohydrate recognising lectin. It is therefore possible to take advantage of glycoporphyrin disaggregation, to aid cellular targeting, and lower off site post treatment photosensitization. The disaggregation principle, has been successfully used to switch on and off fluorescence of nanoparticles, inside and outside cells by O'Shea *et al.*²⁶⁸

5.9 Conclusion

In conclusion, the effectiveness of glycoporphyrin aggregation and subsequent lectin mediated disaggregation was successfully used in identifying porphyrin structures with the optimum carbohydrate distribution for cellular uptake. For Con A, the 5,10-disubstituted Mannose analogue gave the best observed interactions, especially at lower concentrations of the lectin. Non-trivial binding kinetics are observed in some cases, so macroscopic binding constants should be fitted for general comparisons. The increased ability for more complex carbohydrates, mimicking the natural ligand for a lectin, was demonstrated by the Ulex I study. Interestingly, an unusual difference in the overall maximum fluorescence increase for different glycoporphyrins, has led to the proposal that conformational factors, regarding how a porphyrin presents a sugar to a binding pocket, can lead to lectin porphyrin quenching. These studies will hopefully be complemented by future NMR ¹⁵N enriched HSQC studies, which could identify porphyrin interactions.

Biological studies were successful in providing SAR information on cell uptake, with the recommendation that 5,10 di-substituted examples should be employed and synthesised

for future investigations. It appears this substitution pattern provides a “sweet spot” in amphiphilicity for the OE21 and Het 1a, cell lines investigated so far.

From our singlet oxygen production studies, it can be concluded that the irradiation timelines for PDT activity (Figure 5.27), were too low. Indeed an increase in irradiation time could be a useful quality. Foscan, whilst a clinically approved PDT agent, could almost be described as being too active. Two minute irradiation timelines in the clinic lead to short operations, but the offsite localisation means that post treatment photosensitisation is a real issue (see 90 days, Section 1.4). By using a less efficient PDT agent, and requiring 10 minutes of irradiation to induce cell death, then post treatment photosensitisation from off target localisation would be reduced in a relative manner. The strict requirements of covering of skin *etc.* may not be as penal.

Separately the lack of mitochondrial localisation observed for the uptaken glycoporphyrins, will almost certainly lead to alternative pathways of cell death, once activated. These compounds will certainly be investigated for future *in vitro* studies. The possibility of lectin mediated On/Off switching of PDT activity is another advantage. Offsite localisation may not be as detrimental if the PDT agent has a substantially lower PDT effect as an aggregate.

5.10 Future work

Further biological testing should be performed with both longer irradiation times, and increased recovery time, before MTT assay is performed. This will allow the cytotoxic potential of the prepared glycoporphyrins to be more effectively observed, and may even lead to an observation of localisation specific cell death pathways.

Cross referencing of lectin over expression in available cell lines, against the carbohydrates attached to our glycoporphyrin library, should achieve more specific cell targeting.

Having shown the intracellular uptake of the 5,10 series, it would be interesting to prepare the chlorin analogues, for direct PDT comparisons. In order to increase the level of complexity even further, perhaps the enzymatic extension carbohydrate chains on glycoporphyrins could be performed.

Should these glycoporphyrins remain non phototoxic and inert, even at longer irradiation timelines, then their use as intracellular probes, given the fluorescent properties and the observed intracellular localisation of the glycoporphyrins, is another viable application.

Chapter Six

Experimental data

Chapter 6 – Experimental data

6.1.1 Experimental data - General Information

Unless otherwise stated; specific rotation was recorded in a Rudolph research autopol IV polarimeter, infrared spectra (IR) were recorded on a Perkin Elmer spectrometer. UV/Vis spectra were recorded with a Perkin Elmer Lambda 35UV/Vis spectrometer. Fluorescence spectroscopy was recorded with a Perkin Elmer LS55 Fluorescence Spectrometer. Microwave reactions were carried out in a Biotage Initiator microwave reactor. A Brüker Avance 400, and an Agilent 400 spectrometer were employed for ^1H (400.13 MHz) and ^{13}C (100.61 MHz) NMR spectra, a Brüker Ultrashield 600 spectrometer was employed for ^1H (600.13 MHz) and ^{13}C (150.90 MHz) NMR spectra, and an Agilent 800 spectrometer was employed for ^1H (799.73 MHz) NMR spectra. Resonances δ , are in ppm units downfield from an internal reference in CDCl_3 ($\delta_{\text{H}} = 7.26$ ppm, $\delta_{\text{C}} = 77.0$ ppm), MeOH ($\delta_{\text{H}} = 3.31$ ppm, $\delta_{\text{C}} = 49.0$ ppm), D_2O ($\delta_{\text{H}} = 4.79$ ppm). For oligosaccharides the notation a, b, c.... refers to the monosaccharide from the reducing end. Mass spectrometry analysis was performed with a Q-ToF Premier Waters Maldi-quadrupole time-of-flight (Q-ToF) mass spectrometer equipped with Z-spray electrospray ionization (ESI) and matrix assisted laser desorption ionisation (MALDI) sources. X-ray crystallography was performed with a Brüker SMART APEX diffractometer. Silica gel Florisil (200 mesh; Aldrich) was used for column chromatography. Mobile phases are described as (v/v) for both isocratic and gradient systems. Pre-packed Biotage C_{18} cartridges were used for reverse phase chromatography. Analytical thin-layer chromatography was performed using Merck 60 F_{254} silica gel (pre-coated sheets, 0.2 mm thick, 20 cm x 20 cm) and visualised by UV irradiation or molybdenum staining. DCM, MeOH, THF and toluene were dried over flame dried 3 Å or 4 Å sieves. Dimethylformamide (DMF), triethylamine (Et_3N) and trifluoroacetic acid (TFA) were used dry from sure/seal bottles. Other reagents were purchased from an industrial supplier. Lectins were sourced as lyophilised powders from Sigma Aldrich or Megazyme Ltd.

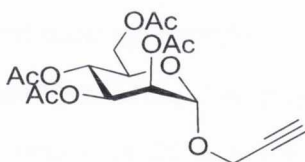
6.2 Experimental data for Chapter 2

6.2.1 General procedure for glycosylation of propargyl alcohol with per acetylated carbohydrate

To the per-acetylated carbohydrate and propargyl alcohol **63**, (4 eq.), in a flame dried 100 mL flask under N₂, was added anhydrous DCM (9 mL/g). The mixture was cooled to 0 °C, and BF₃OEt₂ (5 eq.) was added. The mixture was stirred for 16 h. The reaction was quenched with sat. aqueous NaHCO₃ solution (20 mL) and diluted with DCM, and the organic layer separated. The aqueous layer was washed with DCM (20 mL) and the combined organic layers washed with H₂O (10 mL), dried over MgSO₄ and filtered. The solvent was removed and the oily residue purified by column chromatography (EtOAc:Hexane, 1:1 (v/v)) to yield the product.

6.2.2 General procedure for carbohydrate deacetylation

To a stirred solution of the acetylated carbohydrate in MeOH (20 mL/g) was added sodium methoxide (0.1 eq). The mixture was stirred at 20 °C until full conversion was determined by TLC (MeOH:EtOAc, 1:9 (v/v)). The mixture was neutralized using Dowex resin and filtered through a plug of celite. The solvent was removed *in vacuo* to yield the product.



6.2.3 Propargyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside, **64**

The reaction of propargyl alcohol **63** (0.3 mL, 5.17 mmol), per-acetylated mannose **62** (500 mg, 1.29 mmol) and BF₃OEt₂ (0.8 mL, 6.47 mmol) in DCM (10 mL) was carried out as described in the general glycosylation procedure (Section 6.2.1), to yield the product **64**, as a white solid, (373 mg, 75 %);

Analytical data was in good agreement with literature example.⁸⁶

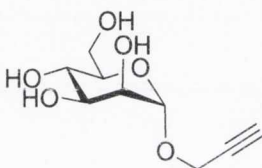
$[\alpha]_D^{22} = 71^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 g cm⁻¹ in CHCl₃);

$\nu_{\max}$ (thin film) 3300 cm⁻¹ (C≡CH), 2990 cm⁻¹ (CH), 1742 cm⁻¹ (C=O);

¹H NMR (400 MHz, CDCl₃) δ 5.37 (1H, dd, $J_{3,4} = 9.6$ Hz, $J_{3,2} = 3.0$ Hz, H-3), 5.33 (1H, m, H-4), 5.31 (1H, dd, $J_{2,3} = 3.0$ Hz, $J_{2,1} = 1.7$ Hz, H-2), 5.06 (1H, d, $J_{1,2} = 1.7$ Hz, H-1), 4.30 (1H, dd, $J_{6,6'} = 12.2$ Hz, $J_{6,5} = 5.1$ Hz, H-6), 4.29 (2H, d, $J = 2.4$ Hz, $\underline{\text{CH}_2\text{C}\equiv\text{CH}}$), 4.13 (1H, dd, $J_{6',6} = 12.2$ Hz, $J_{6',5} = 2.1$ Hz, H-6'), 4.05 (1H, m, H-5), 2.49 (1H, t, $J = 2.5$ Hz, $-\text{C}\equiv\text{CH}$), 2.17, 2.11, 2.05, 2.00 (3H, s, CH₃);

¹³C NMR (100 MHz, CDCl₃) δ 172.2, 171.5, 171.4, 171.3 (C=O), 97.7 (C-1), 79.4 ($\text{CH}_2\text{C}\equiv\text{CH}$), 77.2 ($\text{C}\equiv\text{CH}$), 70.8 (C-2), 70.5 (C-5), 70.4 (C-3), 67.5 (C-4), 63.8 (C-6), 56.5 ($\underline{\text{CH}_2\text{C}\equiv\text{C}}$), 22.4, 22.3, 22.2, 22.2 (CH₃);

M/z HRMS (ESI-TOF) calcd. for C₁₇H₂₂O₁₀Na = 409.1111 (M+Na)⁺. Found = 409.1111.



6.2.4 Propargyl- α -D-mannopyranoside, **65**

Per acetylated glycoside **64** (500 mg, 1.29 mmol), and NaOMe (7 mg, 0.13 mmol) in MeOH (10 mL) were reacted according to the general deacetylation procedure (Section 6.2.2), to yield the product **65** as a white solid, (279 mg, 99 %);

Analytical data was in good agreement with literature example.²⁶⁹

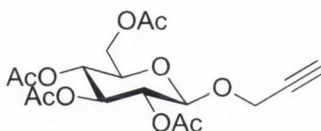
$[\alpha]_{\text{D}}^{22} = 137^\circ$ (deg cm³ g⁻¹ dm⁻¹) ($c = 0.1$ g cm⁻¹ in MeOH);

ν_{max} (thin film) 3282 cm⁻¹ (OH), 2927 cm⁻¹ (CH), 2120 cm⁻¹ ($\text{C}\equiv\text{CH}$);

¹H NMR (400 MHz, MeOD) δ 4.97 (1H, d, $J_{1,2} = 1.4$ Hz, H-1), 4.28 (2H, d, $J = 2.5$ Hz, $\underline{\text{CH}_2\text{C}\equiv\text{CH}}$), 3.84 (1H, dd, $J_{6',6} = 11.8$ Hz, $J_{6',5} = 1.9$ Hz, H-6'), 3.79 (1 H, dd, $J_{2,3} = 3.1$ Hz, $J_{2,1} = 1.5$ Hz, H-2), 3.70 (1H, dd, $J_{6,6'} = 11.8$ Hz, $J_{6,5} = 5.7$ Hz, H-6), 3.67 (1H, dd, $J_{3,4} = 9.3$ Hz, $J_{3,2} = 3.1$ Hz, H-3), 3.62 (1H, dd, $J_{4,3} = J_{4,5} = 9.4$ Hz, H-4), 3.51 (1H, m, H-5), , 2.87 (1H, t, $J = 2.5$ Hz, $-\text{C}\equiv\text{CH}$);

¹³C NMR (100 MHz, MeOD) δ 96.4 (C-1), 76.6 ($-\text{C}\equiv\text{CH}$), 72.5 ($-\text{C}\equiv\text{CH}$), 71.7 (C-5), 69.0 (C-3), 68.6 (C-2), 65.0 (C-4), 59.4 (C-6), 51.3 ($\underline{\text{CH}_2\text{C}\equiv\text{C}}$);

M/z HRMS (ESI-TOF) calcd. for C₉H₁₄O₆Na = 241.0688 (M+Na)⁺. Found = 241.0689.



6.2.5 Propargyl-2,3,4,6-tetra-O-acetyl- β -D-glucopyranoside, **66**

Propargyl alcohol **63** (1.37 mL, 22.56 mmol), per-acetylated glucose, (2.2 g, 5.64 mmol) and BF_3OEt_2 (3.54 mL, 28.19 mmol) were reacted according to the general glycosylation procedure (Section 6.2.1), to yield the product **66**, as a white solid, (1.40 g, 64 %);

Analytical data was in good agreement with literature example.²⁷⁰

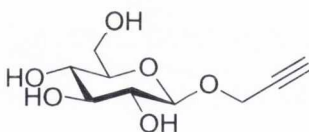
$[\alpha]_D^{22} = -49^\circ$ (deg $\text{cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.1 \text{ g cm}^{-1}$ in CHCl_3);

ν_{max} (thin film) 3300 cm^{-1} ($\text{C}\equiv\text{CH}$), 2958 cm^{-1} (CH), 1734 cm^{-1} (C=O);

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.27 (1H, d, $J = 9.0 \text{ Hz}$, H-3), 5.13 (1H, dd, $J_{4,3} = J_{4,5} = 9.3 \text{ Hz}$, H-4), 5.04 (1H, app t, $J_{2,3} = J_{2,1} = 9.0 \text{ Hz}$, H-2), 4.80 (1H, d, $J_{1,2} = 8.9 \text{ Hz}$, H-1), 4.39 (2H, d, $J = 1.7 \text{ Hz}$, $\text{CH}_2\text{C}\equiv\text{C}$), 4.30 (1H, dd, $J_{6,5} = 4.6 \text{ Hz}$, $J_{6,6'} = 12.3 \text{ Hz}$, H-6), 4.18 (1H, dd, $J_{6',6} = 11.9 \text{ Hz}$, $J_{6',5} = 1.9 \text{ Hz}$, H-6'), 3.75 (1H, m, H-5), 2.49 (1H, t, $J = 1.7 \text{ Hz}$, t, $-\text{C}\equiv\text{CH}$), 2.12, 2.09, 2.05, 2.03 (3H, s, CH_3);

$^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 170.3, 169.8, 169.0, 168.9 (C=O), 97.6 (C-1), 77.6 ($-\text{C}\equiv\text{CH}$), 75.0 ($-\text{C}\equiv\text{CH}$), 72.3 (C-3), 71.4 (C-5), 70.5 (C-2), 67.8 (C-4), 61.3 (C-6), 55.5 ($\text{CH}_2\text{C}\equiv\text{CH}$), 20.3, 20.3, 20.2, 20.2 (CH_3);

M/z HRMS (ESI-TOF) calcd. for $\text{C}_{17}\text{H}_{22}\text{O}_{10}\text{Na} = 409.1111$ ($M+\text{Na}$)⁺. Found = 409.1119.



6.2.6 Propargyl- α -D-glucopyranoside, **67**

Per acetylated glycoside **66** (200 mg, 0.52 mmol) and NaOMe (3 mg, 0.05 mmol) in MeOH (5 mL) were reacted according to the general deacetylation procedure (Section 6.2.2), to yield the product **65** as a white solid, (111 mg, 98 %);

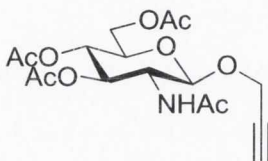
Analytical data was in good agreement with literature example.²⁷¹

$[\alpha]_D^{22} = -59^\circ$ (deg $\text{cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.1 \text{ g cm}^{-1}$ in MeOH);

ν_{max} (thin film) cm^{-1} 3305 cm^{-1} (OH), 2962 cm^{-1} (CH), 2120 cm^{-1} ($\text{C}\equiv\text{CH}$);

$^1\text{H NMR}$ (400 MHz, MeOD) δ 4.50 (1H, d, $J_{1,2} = 7.9 \text{ Hz}$, H-1), 4.44 (2H, d, $J = 2.2 \text{ Hz}$, $\text{CH}_2\text{C}\equiv\text{C}$), 3.88 (1H, dd, $J_{6',6} = 12.1 \text{ Hz}$, $J_{6',5} = 1.9 \text{ Hz}$, H-6'), 3.71 (1H, dd, $J_{6,6'} = 12.0 \text{ Hz}$, $J_{6,5} =$

5.0 Hz, H-6), 3.41 (1H, t, $J_{3,4} = J_{3,2} = 8.5$ Hz, H-3), 3.35 (1H, t, $J_{4,3} = J_{4,5} = 8.5$ Hz, H-4), 3.33 (1H, m, H-5), 3.26 (1H, dd, $J_{2,3} = 8.5$ Hz, $J_{2,1} = 7.9$ Hz, H-2), 2.57 (1H, t, $J = 2.2$ Hz, $\text{C}\equiv\text{CH}$);
 ^{13}C NMR (100 MHz, MeOD) δ 101.6 (C-1), 79.5 ($\text{C}\equiv\text{CH}$), 77.4 (C-3), 77.3 (C-5), 75.8 ($\text{C}\equiv\text{CH}$), 74.2 (C-2), 71.0 (C-4), 62.3 (C-6), 56.4 ($\text{CH}_2\text{C}\equiv\text{CH}$);
 M/z HRMS (ESI-TOF) calcd. for $\text{C}_9\text{H}_{14}\text{O}_6\text{Na} = 241.0688$ (M+Na) $^+$. Found = 241.0685.



6.2.7 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-O-propargyl- β -D-glucopyranose, 69

To *N*-acetylated glucosamine **68** (905 mg, 2.32 mmol) and propargyl alcohol **63** (261 mg, 4.64 mmol) under N_2 was added anhydrous DCM (20 mL). $\text{Yb}(\text{OTf})_3$ (610 mg, 0.98 mmol) was added to the stirred solution and the mixture heated to a reflux for 24 h. The mixture was filtered through a plug of celite rinsing with DCM (2 x 10 mL). The organic layer was washed with deionised H_2O (20 mL), dried over MgSO_4 , filtered and the solvent removed *in vacuo*. The mixture was purified by column chromatography (EtOAc) to yield the product **69** as a white foam (815 mg, 89 % yield);

Analytical data was in good agreement with literature example.¹⁶³

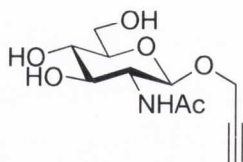
$[\alpha]_{\text{D}}^{20} = -39.0^\circ$ (deg $\text{cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.1 \text{ g cm}^{-1}$ in CHCl_3);

ν_{max} (thin film) 3279 cm^{-1} ($\text{C}\equiv\text{CH}$), 3101 cm^{-1} (NH), 2940 cm^{-1} (CH), 1736 cm^{-1} (C=O), 1647 cm^{-1} ($\text{NHC}=\text{O}$);

^1H NMR (400 MHz, CDCl_3) δ 5.85 (1H, d, $J = 8.9$ Hz, NH), 5.29 (1H, dd, $J_{3,2} = 10.3$ Hz, $J_{3,4} = 9.5$ Hz, H-3), 5.09 (1H, app t, $J_{4,3} = J_{4,5} = 9.5$ Hz, H-4), 4.87 (1H, d, $J_{1,2} = 8.3$ Hz, H-1), 4.38 (2H, d, $J = 2.3$ Hz, $\text{CH}_2\text{C}\equiv\text{CH}$), 4.28 (1H, dd, $J_{6,6'} = 12.3$ Hz, $J_{6,5} = 4.6$ Hz, H-6), 4.15 (1H, dd, $J_{6',6} = 12.3$ Hz, $J_{6',5} = 2.3$ Hz, H-6'), 3.96 (1H, m, H-2), 3.75 (1H, m, H-5), 2.50 (1H, t, $J = 2.3$ Hz, $\text{CH}_2\text{C}\equiv\text{CH}$), 2.09, 2.05, 2.04, 1.97 (3H, s, CH_3);

^{13}C NMR (100 MHz, CDCl_3) δ 170.5, 170.3, 170.1, 168.9 (C=O), 97.9 (C-1), 78.0 ($\text{CH}_2\text{C}\equiv\text{CH}$), 74.9 ($\text{CH}_2\text{C}\equiv\text{CH}$), 71.9 (C-3), 71.4 (C-5), 68.0 (C-4), 61.5 (C-6), 55.5 ($\text{CH}_2\text{C}\equiv\text{CH}$), 53.7 (C-2), 22.9, 20.3, 20.2, 20.1 (CH_3);

M/z HRMS (ESI-TOF) calcd. for $\text{C}_{17}\text{H}_{23}\text{NO}_9\text{Na} = 408.1271$ (M+Na) $^+$. Found 408.1264.



6.2.8 2-Acetamido-2-deoxy-1-O-propargyl-β-D-glucopyranose, **70**

Per acetylated glycoside **69** (1.00 g, 2.58 mmol), and NaOMe (15 mg, 0.25 mmol) in MeOH (20 mL) were reacted according to the general deacetylation procedure (Section 6.2.2), to yield the product **70** as a white solid (660 mg, 99 %);

Analytical data was in good agreement with literature example.¹⁶³

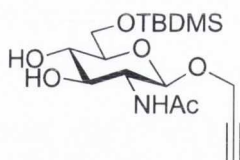
$[\alpha]_D^{20} = -54^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 g cm⁻¹ in MeOH);

$\nu_{\max}$ (thin film) 3285 cm⁻¹ (OH), 2927 cm⁻¹ (CH), 1644 cm⁻¹ (NHC=O);

¹H NMR (400 MHz, MeOD) δ 4.62 (1H, d, $J_{1,2} = 8.4$ Hz, H-1), 4.39 (2H, m, CH₂C≡CH), 3.91 (1H, dd, $J_{6,6'} = 12.1$ Hz, $J_{6',5} = 2.1$ Hz, H-6'), 3.69 (2H, m, H-2, H-6), 3.51 (1H, dd, $J_{3,4} = 10.2$ Hz, $J_{3,2} = 8.2$ Hz, H-3), 3.31 (2H, m, H-4, H-5), 2.88 (1H, t, $J = 2.4$ Hz, CH₂C≡CH), 2.01 (3H, s, CH₃);

¹³C NMR (100 MHz, MeOD) δ 172.5 (C=O), 99.1 (C-1), 78.6 (CH₂C≡CH), 76.7 (C-5), 74.8 (CH₂C≡CH), 74.6 (C-3), 70.7 (C-4), 61.3 (C-6), 55.7 (C-2), 55.1 (CH₂C≡CH), 21.6 (CH₃);

M/z HRMS (ESI-TOF) calcd. for C₁₇H₂₃NO₉Na = 282.0954 (M+Na)⁺. Found 282.0956.



6.2.9 2-Acetamido-2-deoxy-6-O-*tert*-butyldimethylsilyl-1-O-propargyl-β-D-glucopyranose, **71**

Glycoside **70** (0.54 g, 2.08 mmol) and imidazole (0.35 g, 5.21 mmol) were dissolved in anhydrous DMF (20 mL) at 30 °C containing pre-activated 4 Å molecular sieves. TBDMSCI (0.34 g, 2.29 mmol) was added portionwise over 1h. The mixture was stirred for 1h. The reaction was quenched with MeOH (10 mL) and the solvent removed *in vacuo*. The mixture was dissolved in EtOAc (30 mL) and washed with deionised H₂O (8 x 5 mL), dried over MgSO₄ and filtered. The solvent was removed *in vacuo* and the pale yellow oil was purified by column (MeOH:EtOAc, 0:19 to 1:19 (v/v)) to yield the product **71** as an off white solid, (713 mg, 92 %);

Analytical data was in good agreement with literature example.¹⁶³

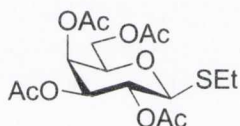
$[\alpha]_D^{20} = -63^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 g cm⁻¹ in CHCl₃);

$\nu_{\max}$ (thin film) 3356 cm⁻¹ (OH, NH), 2935 cm⁻¹ (CH), 1655 cm⁻¹ (NHC=O);

¹H NMR (400 MHz, MeOD) δ 4.62 (1H, d, $J_{1,2} = 8.3$ Hz, H-1), 4.36 (2H, d, $J = 2.3$ Hz, CH₂C $\equiv$ CH), 4.01 (1H, dd, $J_{6',6} = 11.3$ Hz, $J_{6',5} = 1.8$ Hz, H-6'), 3.85 (1H, dd, $J_{6,6'} = 11.3$ Hz, $J_{6,5} = 5.3$ Hz, H-6), 3.66 (1H, dd, $J_{2,3} = 10.1$ Hz, $J_{2,1} = 8.3$ Hz, H-2), 3.49 (1H, dd, $J_{3,2} = 10.1$ Hz, $J_{3,4} = 8.7$ Hz, H-3), 3.38 (1H, dd, $J_{4,5} = 9.2$ Hz, $J_{4,3} = 8.7$ Hz, H-4), 3.29 (1H, ddd, $J_{5,4} = 9.3$ Hz, $J_{5,6} = 5.3$ Hz, $J_{5,6'} = 1.9$ Hz, H-5), 2.88 (1H, t, $J = 2.4$ Hz, CH₂C $\equiv$ CH), 2.01 (3H, s, CH₃), 0.95 (9H, s, SiC(CH₃)₃), 0.13, 0.12 (3H, s, Si(CH₃)₂);

¹³C NMR (100 MHz, MeOD) δ 172.4 (C=O), 98.8 (C-1), 78.4 (CH₂C $\equiv$ CH), 76.8 (C-5), 74.9 (CH₂C $\equiv$ CH), 74.7 (C-3), 70.4 (C-4), 62.6 (C-6), 55.7 (C-2), 54.8 (CH₂C $\equiv$ CH), 25.0 (SiC(CH₃)₃), 21.6 (CH₃), 17.9 (SiC(CH₃)₃), -6.3, -6.5 (Si(CH₃)₂);

M/z HRMS (ES⁺) calcd. for C₁₇H₃₁NO₆SiNa = 396.1818 (M+Na)⁺. Found 396.1824.



6.2.10 Ethyl-2,3,4,6-tetra-O-acetyl-1-thio- β -D-galactopyranoside, **72**

To a stirred solution of 1,2,3,4,6-penta-O-acetyl- β -D galactopyranoside (6.16 g, 15.78 mmol) in anhydrous DCE (50 mL) containing pre-activated 4 Å molecular sieves, at -30 °C was added ethanethiol (2.31 mL, 31.25 mmol). The mixture was stirred under N₂ for 10 min. Tin tetrachloride (1.83 mL, 16.61 mmol) was added dropwise and the solution stirred at -30 °C for 4 h. The crude reaction mixture was added dropwise to sat. aqueous NaHCO₃ solution (40 mL) and the mixture stirred for 16 h. The mixture was filtered through a plug of celite. The organic layer was washed with deionised H₂O, dried over MgSO₄ and filtered. The solvent was removed *in vacuo*, and the product precipitated from diethyl ether/hexane to yield **72**, as a white solid, (4.83 g, 78 %);

Analytical data was in good agreement with literature example.¹⁶⁷

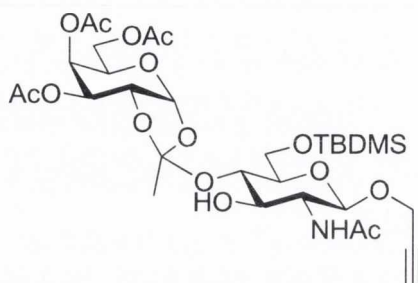
$[\alpha]_D^{20} = -8^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 g cm⁻¹ in CHCl₃);

$\nu_{\max}$ (thin film) 2939 cm⁻¹ (CH), 1738 cm⁻¹ (C=O);

¹H NMR (400 MHz CDCl₃) δ 5.44 (1H dd, $J_{4,3} = 3.4$ Hz, $J_{4,5} = 0.6$ Hz, H-4), 5.25 (1H, app t, $J_{2,1} = J_{2,3} = 10.0$ Hz, H-2), 5.06 (1H, dd, $J_{3,2} = 10.0$ Hz, $J_{3,4} = 3.4$ Hz, H-3), 4.51 (1H, d, $J_{1,2} = 10.0$ Hz, H-1), 4.18 (2H, m, H-6, H-6'), 3.95 (1H, m, H-5), 2.74 (2H, m, SCH₂CH₃), 2.17, 2.09, 2.07, 2.01 (CH₃), 1.25 (3H, t, $J = 7.4$ Hz, SCH₂CH₃);

^{13}C NMR (100 MHz, CDCl_3) δ 169.9, 169.8, 169.7, 169.2 (C=O), 83.6 (C-1), 73.9 (C-5), 71.5 (C-3), 66.8 (C-4), 66.7 (C-2), 61.0 (C-6), 23.9 (SCH_2CH_3), 20.4, 20.3, 20.3, 20.2 (CH_3), 14.4, SCH_2CH_3);

M/z HRMS (ESI-TOF) calcd. for $\text{C}_{16}\text{H}_{24}\text{O}_9\text{SNa} = 415.1039$ ($M+\text{Na}$) $^+$. Found 415.1039.



6.2.11 2-Acetamido-2-deoxy-1-O-propargyl-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranose)-1/2-4)- β -D-glucopyranose orthoester, **73**

Acceptor **71** (100 mg, 0.27 mmol) and donor **72** (105 mg, 0.27 mmol) were dissolved in dry DCM (3 mL). The solvent was removed *in vacuo*, co-evaporating with toluene. The mixture of sugars was dried under vacuum for 3 h, before addition of anhydrous DCM (5 mL) containing pre-activated 3 Å molecular sieves, under N_2 . The mixture was cooled to -40°C . NIS (84 mg, 0.37 mmol) was added followed by TMS.OTF (cat.) and the mixture was stirred for 5 h at -40°C . The mixture was quenched through the addition of Et_3N (1 mL). The solvent was removed *in vacuo*, and mixture purified by column chromatography ($\text{EtOAc}:\text{Hex}$, 2:3 to 19:1 (v/v)) to yield the product **73** as a colourless oil (23 mg, 12 % yield);

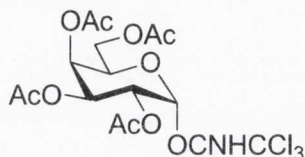
$[\alpha]_D^{20} = -47^\circ$ ($\text{deg cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.1 \text{ g cm}^{-1}$ in CHCl_3);

ν_{max} (thin film) 3450 (OH), 2941 cm^{-1} (CH), 1747 cm^{-1} (C=O), 1654 cm^{-1} (NHC=O);

^1H NMR (600 MHz, CDCl_3) δ 5.95 (1H, d, $J_{1,2} = 4.7 \text{ Hz}$, H-1b), 5.65 (1H, d, $J = 7.7 \text{ Hz}$, NH), 5.48 (1H, dd, $J_{4,3} = 2.7 \text{ Hz}$, $J_{4,5} = 2.5 \text{ Hz}$, H-4b), 5.03 (1H, dd, $J_{3,2} = 6.7 \text{ Hz}$, $J_{3,4} = 2.7 \text{ Hz}$, H-3b), 4.78 (1H, d, $J_{1,2} = 8.2 \text{ Hz}$, H-1a), 4.49 (1H, dd, $J_{2,3} = 6.7 \text{ Hz}$, $J_{2,1} = 4.8 \text{ Hz}$, H-2b), 4.38 (2H, dd, $J_{H,H'} = 5.6 \text{ Hz}$, $J = 2 \text{ Hz}$, $\text{CH}_2\text{C}\equiv\text{C}$), 4.33 (1H, ddd, $J_{5,6} = 8.9 \text{ Hz}$, $J_{5,6'} = 6.5 \text{ Hz}$, $J_{5,4} = 2.4 \text{ Hz}$, H-5b), 4.14 (2H, m, H-6b), 4.07 (1H, s, OH), 3.90 (1H, dd, $J_{6',6} = 11.0 \text{ Hz}$, $J_{6',5} = 1.3 \text{ Hz}$, H-6'a), 3.82 (2H, m, H-6a, H-3a), 3.63 (2H, m, H-4a, H-2a), 3.35 (1H, m, H-5a), 2.46 (1H, t, $J = 2 \text{ Hz}$, $-\text{C}\equiv\text{CH}$), 2.15, 2.07, 2.07 (3H, s, CH_3), 2.06 (3H, s, CH_3 (NHAc)), 1.76 (3H, s, $\text{C(O)}_3(\text{CH}_3)$), 0.92 (9H, s, $\text{Si}(\text{CH}_3)_3$), 0.10, 0.09 (3H, s, $\text{Si}(\text{CH}_3)_2$);

^{13}C NMR (150 MHz, CDCl_3) δ 171.0 (NHC=O), 170.4, 170.1, 169.7 (C=O), 122.2 ($\text{C(O)}_3\text{CH}_3$), 97.6 (C-1a), 97.6 (C-1b), 78.8 ($\text{C}\equiv\text{CH}$), 75.4 (C-5a), 75.1 (C-2b), 75.0 ($\text{C}\equiv\text{C-H}$), 73.3 (C-3a), 72.5 (C-4a), 71.5 (C-3b), 69.6 (C-5b), 66.1 (C-4b), 61.8 (C-6a), 61.0 (C-6b), 56.4 (C-2a), 55.3 ($\text{CH}_2\text{C}\equiv\text{C}$), 26.1 ($\text{C(O)}_3\text{CH}_3$), 25.9 ($\text{SiC(CH}_3)_3$), 23.6 (NHCOCH_3), 20.6, 20.6, 20.5 (CH_3), 18.4 ($\text{SiC(CH}_3)_3$), -5.1, -5.4 ($\text{Si(CH}_3)_2$);

M/z HRMS (ESI-TOF) calcd. for ($\text{C}_{31}\text{H}_{49}\text{NO}_{15}\text{SiNa}$) = 726.2769 ($\text{M}+\text{Na}$)⁺. Found 726.2789.



6.2.12 2,3,4,6-Tetra-O-acetyl- β -D-galactopyranoside-trichloroacetimidate, **74**

To a stirred solution of 1,2,3,4,6-penta-O-acetyl- β -D-galactopyranoside (3.00 g, 7.69 mmol) in THF (20 mL) was added benzylamine (1.69 mL, 15.38 mmol). The mixture was stirred at 20 °C for 16 h. The solvent was removed *in vacuo* and the mixture was diluted with DCM (30 mL). The organic layer was washed with HCl (1M, 10 mL), then sat. aqueous NaHCO_3 solution (30 mL), and deionised H_2O (20 mL). The organic layer was dried over MgSO_4 , filtered and the solvent removed *in vacuo*. The crude, dried anomeric mixture was dissolved in anhydrous DCM (20 mL) under N_2 . Trichloroacetonitrile (7.95 mL, 91.6 mmol) was added and the mixture cooled to 0 °C. A catalytic amount of DBU (0.1 mL) was added and the mixture stirred for 7 h whilst warming to 20 °C. The mixture was filtered through a plug of silica and the solvent removed *in vacuo*. The product was purified by column chromatography (EtOAc:Hexane, 2:8 to 3:7 (v/v) to yield the product **74**, as a white foam (3.00 g, 77 % yield);

Analytical data was in good agreement with literature example.¹⁶³

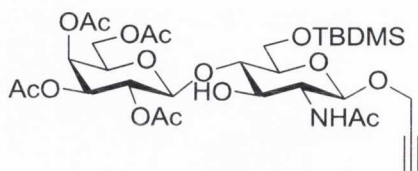
$[\alpha]_{\text{D}}^{20} = 164^\circ$ ($\text{deg cm}^3 \text{ g}^{-1} \text{ dm}^{-1}$) ($c = 0.1 \text{ g cm}^{-1}$ in CHCl_3);

ν_{max} (thin film) 3286 cm^{-1} (NH), 2927 cm^{-1} (CH), 1742 cm^{-1} (C=O), 1678 cm^{-1} (C=N);

^1H NMR (400 MHz, CDCl_3) δ 8.69 (1H, s, NH), 6.62 (1H, d, $J_{1,2} = 3.5 \text{ Hz}$, H-1), 5.59 (1H, dd, $J_{4,3} = 2.9 \text{ Hz}$, $J_{4,5} = 1.1 \text{ Hz}$, H-4), 5.45 (1H, dd, $J_{3,2} = 10.8 \text{ Hz}$, $J_{3,4} = 3.0 \text{ Hz}$, H-3), 5.39 (1H, dd, $J_{2,3} = 10.8 \text{ Hz}$, $J_{2,1} = 3.5 \text{ Hz}$, H-2), 4.46 (1H, dt, $J_{5,6} = 6.5 \text{ Hz}$, $J_{5,4} = 1.1 \text{ Hz}$, H-5), 4.19 (1H, dd, $J_{6,6'} = 11.4 \text{ Hz}$, $J_{6,5} = 6.5 \text{ Hz}$, H-6), 4.10 (1H, dd, $J_{6',6} = 11.4 \text{ Hz}$, $J_{6',5} = 6.5 \text{ Hz}$, H-6'), 2.19, 2.06, 2.05, 2.04 (3H, s, CH_3);

^{13}C NMR (100 MHz, CDCl_3) δ 169.9, 169.7, 169.6, 169.5, (C=O), 160.5 (C=NH), 93.1 (C-1), 90.3 (CCl_3), 68.5 (C-5), 67.1 (C-3), 66.9 (C-4), 66.4 (C-2), 60.8 (C-6), 20.2, 20.2, 20.2, 20.1 (CH_3);

M/z HRMS (ESI-TOF) calcd. for $(\text{C}_{16}\text{H}_{20}\text{NO}_{10}\text{NaCl}_3) = 514.0050 (\text{M}+\text{Na})^+$. Found 514.0048.



6.2.13 O-(2,3,4,6-Tetra-O-acetyl- β -D-galactopyranosyl)-(1-4)-2-acetamido-2-deoxy-6-O-*tert*-butyldimethylsilyl-1-O-propargyl- β -D-glucopyranose, **75**

Acceptor **71** (600 mg, 1.61 mmol) and donor **74** (950 mg, 1.93 mmol) were dissolved in anhydrous DCM (3 mL). The solvent was removed *in vacuo*, co-evaporating with toluene (3 x 10 mL). The mixture of sugars was dried under vacuum for 1 h. DCM (5 mL) containing pre-activated 3 Å molecular sieves was added to the mixture of sugars, under N_2 . The mixture was cooled to $-30\text{ }^\circ\text{C}$. BF_3OEt_2 (313 μL , 1.93 mmol) was added and the mixture stirred for 1 h. Donor **74** (95 mg, 0.19 mmol) was added and the mixture stirred for a further 1 h. The reaction was diluted with DCM (10 mL) and quenched through the addition of sat. aqueous NaHCO_3 solution (4 mL). The mixture was filtered through a plug of celite and the organic layer extracted. The organic layer was dried over MgSO_4 , filtered and the solvent was removed *in vacuo*. The product was purified by column chromatography ($\text{EtOAc}:\text{Hex}$, 9:1 to 9:0 (v/v)) to yield **75**, as an off white solid, (755 mg, 67 % yield);

Analytical data was in good agreement with literature example.¹⁶³

$[\alpha]_{\text{D}}^{20} = -26\text{ }^\circ (\text{deg cm}^3 \text{ g}^{-1} \text{ dm}^{-1})$ ($c = 0.1 \text{ g cm}^{-1}$ in CHCl_3);

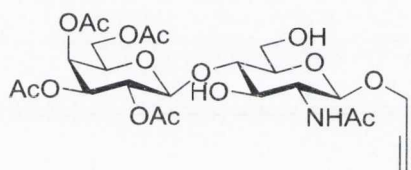
ν_{max} (thin film) 3480 cm^{-1} (OH), 3279 cm^{-1} ($\text{C}\equiv\text{CH}$), 2941 cm^{-1} (CH), 1748 cm^{-1} (C=O), 1657 cm^{-1} ($\text{NHC}=\text{O}$);

^1H NMR (600 MHz, CDCl_3) δ 5.58 (1H, d, $J = 7.6 \text{ Hz}$, NH), 5.41 (1H, d, $J_{4,3} = 2.9 \text{ Hz}$, H-4b), 5.24 (1H, dd, $J_{2,3} = 10.3 \text{ Hz}$, $J_{2,1} = 8.2 \text{ Hz}$, H-2b), 5.01 (1H, dd, $J_{3,2} = 10.4 \text{ Hz}$, $J_{3,4} = 3.0 \text{ Hz}$, H-3b), 4.87 (1H, d, $J_{1,2} = 8.0 \text{ Hz}$, H-1a), 4.65 (1H, d, $J_{1,2} = 8.0 \text{ Hz}$, H-1b), 4.37 (2H, m, $\text{CH}_2\text{C}\equiv\text{CH}$), 4.17 (2H, m, H-6b), 4.00 (1H, m, H-5b), 3.98 (1H, m, H-3a), 3.87 (1H, d, $J_{6',6} = 11.5 \text{ Hz}$, H-6'a), 3.76 (1H, dd, $J_{6,6'} = 11.4$, $J_{6,5} = 3.2 \text{ Hz}$, H-6a), 3.63 (1H, app t, $J_{4,3} = J_{4,5} = 8.9$

H, H-4a), 3.53 (1H, m, H-2a), 3.41 (1H, d, $J_{5,4} = 8.9$ Hz, H-5a), 2.46 (1H, s, $\text{CH}_2\text{C}\equiv\text{CH}$), 2.18, 2.09, 2.08, 2.04, 2.00 (3H, s, CH_3), 0.93 (9H, s, $(\text{SiC}(\text{CH}_3)_3)$), 0.11, 0.10 (3H, s, $\text{Si}(\text{CH}_3)_2$);

^{13}C NMR (150 MHz, CDCl_3) δ 170.4 (CONH), 170.3, 169.9, 169.8, 169.1 (C=O), 101.3 (C-1b), 97.5 (C-1a), 80.6 (C-4a), 78.61 ($\text{CH}_2\text{C}\equiv\text{CH}$), 74.8 ($\text{CH}_2\text{C}\equiv\text{CH}$), 74.6 (C-5a), 71.7 (C-3a), 71.09 (C-5b), 70.7 (C-3b), 68.7 (C-2b), 66.7 (C-4b), 61.3 (C-6b), 61.2 (C-6a), 56.1 (C-2a), 55.2 ($\text{CH}_2\text{C}\equiv\text{CH}$), 25.7 ($\text{SiC}(\text{CH}_3)_3$), 23.5 (NHCOCH_3), 20.5, 20.4, 20.3, 20.3 (CH_3), 18.1 ($\text{SiC}(\text{CH}_3)_3$), -5.1, -5.4 ($\text{Si}(\text{CH}_3)_2$), (a = GlcNAc, b = Gal), Long range coupling between H1-gal/C4-GlcNAc in the HMBC spectrum confirms the glycosidic linkage to be 1-4;

M/z HRMS (ESI-TOF) calcd. for $(\text{C}_{31}\text{H}_{49}\text{NO}_{15}\text{SiNa}) = 726.2769$ (M+Na) $^+$. Found 726.2775.



6.2.14 2-Acetamido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)-1-O-propargyl- β -D-glucopyranose, **76**

To a solution of glycoside **75** (200 mg, 0.28 mmol) in THF (3 mL) was added TBAF 1M in THF (0.71 mL, 0.71 mmol) at 0 °C. The mixture was stirred for 30 min whilst warming to 20 °C. Deionised H_2O (10 mL) was added and the product extracted with EtOAc (3 x 25 mL). The organic layer was dried over MgSO_4 , filtered and the solvent evaporated. The product was purified by column chromatography (MeOH:EtOAc, 1:20 (v/v)) to yield the product **76** as a clear oil (125 mg, 75 %);

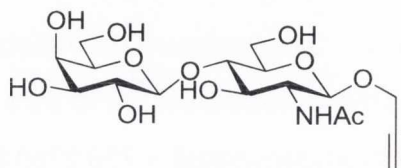
$[\alpha]_D^{20} = -19^\circ$ (deg $\text{cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.1 \text{ g cm}^{-1}$ in CHCl_3);

ν_{max} (thin film) 3450 cm^{-1} (OH), 3281 cm^{-1} ($\text{C}\equiv\text{CH}$), 2943 cm^{-1} (CH), 1741 cm^{-1} (C=O), 1659 cm^{-1} (NHC=O);

^1H NMR (600 MHz, CDCl_3) δ 5.84 (1H, d, $J = 6.2$ Hz, NH), 5.39 (1H, d, $J_{4,3} = 3.1$ Hz, H-4b), 4.94 (1H, dd, $J_{3,2} = 9.8$ Hz, $J_{3,4} = 3.1$ Hz, H-3b), 4.89 (1H, d, $J_{1,2} = 8.4$ Hz, H-1a), 4.56 (1H, d, $J_{6',6} = 11.9$ Hz, H-6'a), 4.53 (1H, d, $J_{1,2} = 7.5$ Hz, H-1b), 4.41 (2H, m, $\text{CH}_2\text{C}\equiv\text{CH}$), 4.29 (1H, dd, $J_{6',6} = 12.0$ Hz, $J_{6,5} = 4.9$ Hz, H-6a), 4.14 (2H, m, H-6b), 3.98 (1H, m, H-3a), 3.98 (1H, m, H-5b), 3.90 (1H, app t, $J_{2,3} = J_{2,1} = 8.8$ Hz, H-2b), 3.62 (1H, m, H-5a), 3.60 (1H, m, H-4a), 3.51 (1H, m, H-2a), 2.53 (1H, s, $\text{CH}_2\text{C}\equiv\text{CH}$), 2.17, 2.12, 2.07, 2.06, 2.06 (3H, s, CH_3);

¹³C NMR (150 MHz, CDCl₃) δ 171.6, 170.8, 170.1, 169.9, 169.6 (C=O), 102.9 (C-1b), 97.2 (C-1a), 78.6 (C-4a), 78.1 (CH₂C≡CH), 75.0 (CH₂C≡CH), 72.8 (C-5a), 72.6 (C-3a), 71.9 (C-5b), 70.9 (C-3b), 67.4 (C-2b), 66.6 (C-4b), 62.8 (C-6a), 61.2 (C-6b), 56.6 (C-2a), 55.5 (CH₂C≡CH), 23.0 (CH₃CONH), 20.5, 20.3, 20.2, 20.1 (CH₃);

M/z HRMS (ESI-TOF) calcd. for (C₂₅H₃₅NO₁₅Na) = 612.1904 (M+Na)⁺. Found 612.1915.



6.2.15 2-Acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-1-O-propargyl-β-D-glucopyranose, (also O-propargyl LacNAc), **77**

Per acetylated glycoside **76** (80 mg, 0.14 mmol), and NaOMe (0.7 mg, 0.01 mmol) in MeOH (3 mL) were reacted according to the general deacetylation procedure (Section 6.2.2), followed by freeze drying to yield the product **77**, as a white solid, (51 mg, 89 %);

Analytical data was in good agreement with literature example.¹⁶³

[α]_D²⁰ = -60 ° (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 g cm⁻¹ in MeOH);

ν_{max} (thin film) 3330 cm⁻¹ (OH), 3261 (C≡CH), 2929 cm⁻¹ (CH), 1646 (NHC=O);

¹H NMR (600 MHz, MeOD) δ 4.65 (1H, d, *J*_{1,2} = 8.3 Hz, H-1a), 4.40 (1H, d, *J*_{1,2} = 7.8 Hz, H-1b), 4.38 (2H, t, *J* = 2.5 Hz, CH₂C≡CH), 3.95 (1H, dd, *J*_{6',6} = 12.0 Hz, *J*_{6',5} = 2.3 Hz, H-6a'), 3.87 (1H, dd, *J*_{6,6'} = 12.0 Hz, *J*_{6,5} = 4.2 Hz, H-6a), 3.83 (1H, d, *J*_{4,3} = 3.1 Hz, H-4b), 3.78 (1H, dd, *J*_{6,6'} = 11.3 Hz, *J*_{6,5} = 7.7 Hz, H-6b), 3.75 (1H, dd, *J*_{2,3} = 10.1 Hz, *J*_{2,1} = 8.4 Hz, H-2a), 3.71 (1H, dd, *J*_{6',6} = 11.4 Hz, *J*_{6',5} = 4.6 Hz, H-6b'), 3.69 (1H, dd, *J*_{3,2} = 10.2 Hz, *J*_{3,4} = 8.7 Hz, H-3a), 3.63 (1H, app t, *J*_{4,3} = *J*_{4,5} = 8.7 Hz, H-4a), 3.61 (1H, m, H-5b), 3.56 (1H, dd, *J*_{2,3} = 9.6 Hz, *J*_{2,1} = 8.2 Hz, H-2b), 3.50 (1H, dd, *J*_{3,2} = 9.7 Hz, *J*_{3,4} = 3.1 Hz, H-3b), 3.44 (1H, m, H-5a), 2.87 (1H, t, *J* = 2.5 Hz, CH₂C≡CH), 2.00 (3H, s, CH₃);

¹³C NMR (150 MHz, MeOD) δ 172.1 (C=O), 103.5 (C-1b), 98.9 (C-1a), 79.3 (C-4a), 78.4 (CH₂C≡CH), 75.6 (C-5b), 75.1 (C-5a), 74.6 (CH₂C≡CH), 73.3 (C-3b), 72.6 (C-3a), 71.0 (C-2b), 68.8 (C-4b), 60.9 (C-6b), 60.3 (C-6a), 54.9 (CH₂C≡CH), 54.9 (C-2a), 21.4 (CH₃);

M/z HRMS (ESI-TOF) calcd. for C₁₇H₂₇NO₁₁Na = 444.1482 (M+Na)⁺. Found = 444.1476.



6.2.16 Ethyl-2,3,4-tri-O-acetyl-1-thio- β -L-fucopyranoside, **79**

To a stirred solution of 1,2,3,4-tetra-O-acetyl- α -L-fucopyranoside, **78** (5.00 g, 15.05 mmol) in anhydrous DCE (50 mL) containing pre-activated 4 Å molecular sieves, was added ethane thiol (2.17 mL, 30.10 mmol). The mixture was stirred under N_2 for 30 min. The solution was cooled to $-30\text{ }^\circ\text{C}$ and $SnCl_4$ (1.93 mL, 15.2 mmol) was added over 20 min. The mixture was stirred at $-30\text{ }^\circ\text{C}$ for 9 h. The crude reaction mixture was added dropwise to sat. aqueous $NaHCO_3$ solution (40 mL) and the mixture stirred for 48 h. The mixture was filtered through a plug of celite and the organic layer was separated. The organic layer was washed with deionised H_2O (20 mL) before drying over $MgSO_4$. The organic layer was filtered and the solvent removed *in vacuo*. The mixture was loaded in dry silica and purified by column chromatography (EtOAc:Hexane, 1:4 (v/v)) to give a mixture of recovered starting material (706 mg, 14 %) and the required product **79**, as a white solid, (2.60 g, 52 %);

Analytical data was in good agreement with literature example.²⁷²

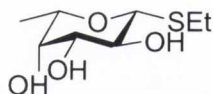
$[\alpha]_D^{20} = 7^\circ$ (deg $cm^3\text{ g}^{-1}\text{ dm}^{-1}$) ($c = 0.1\text{ g cm}^{-1}$ in $CHCl_3$);

$\nu_{\max}$ (thin film) 2985 cm^{-1} (CH), 1741 cm^{-1} (C=O);

1H NMR (400 MHz, $CDCl_3$) δ 5.28 (1H, dd, $J_{4,3} = 3.4\text{ Hz}$, $J_{4,5} = 1.0\text{ Hz}$, H-4), 5.25 (1H, app t, $J_{2,1} = J_{2,3} = 9.9\text{ Hz}$, H-2), 5.05 (1H, dd, $J_{3,2} = 10\text{ Hz}$, $J_{3,4} = 3.4\text{ Hz}$, H-3), 4.46 (1H, d, $J_{1,2} = 9.9\text{ Hz}$, H-1), 3.83 (1H, dq, $J_{5,6} = 6.3\text{ Hz}$, $J_{5,4} = 1.0\text{ Hz}$, H-5), 2.74 (2H, m, SCH_2CH_3), 2.18, 2.07, 1.99 (3H, s, CH_3), 1.28 (3H, t, $J = 7.5\text{ Hz}$, SCH_2CH_3), 1.22 (3H, d, $J_{6,5} = 6.3\text{ Hz}$, H-6);

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 169.7, 169.3 ($\underline{C=O}$), 83.1 (C-1), 72.7 (C-5), 71.9 (C-3), 69.9 (C-4), 66.8 (C-2), 23.7 (SCH_2CH_3), 20.4, 20.3, 20.2 (CH_3), 15.9 (C-6) 14.3 (SCH_2CH_3);

M/z HRMS (ESI-TOF) calcd. for $(C_{14}H_{22}O_7SNa)^+ = 357.0984$ ($M+Na$) $^+$. Found 357.0988.



6.2.17 Ethyl-1-thio- β -L-fucopyranoside, **80**

Per acetylated glycoside **79** (230 mg, 0.69 mmol), and NaOMe (3.2 mg, 0.07 mmol) in MeOH (10 mL) were reacted according to the general deacetylation procedure (Section 6.2.2), to yield the product **80**, as a white solid, (1.37 g, 99 %);

Analytical data was in good agreement with literature example.²⁷²

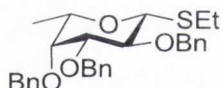
$[\alpha]_D^{20} = 43^\circ$ (deg cm³ g⁻¹ dm⁻¹) ($c = 0.1$ g cm⁻¹ in MeOH);

$\nu_{\max}$ (thin film) 3360 cm⁻¹ (OH), 2933 (CH);

¹H NMR (400 MHz, MeOD) δ 4.32 (1H, d, $J_{1,2} = 9.2$ Hz, H-1), 3.67 (2H, m, H-4, H-5), 3.53 (1H, dd, $J_{2,3} = 9.6$ Hz, $J_{2,1} = 9.2$ Hz, H-2), 3.48 (1H, dd, $J_{3,2} = 9.6$ Hz, $J_{3,4} = 3.2$ Hz, H-3), 2.73 (2H, m, S-CH₂CH₃), 1.30 (3H, t, $J = 7.6$ Hz, S-CH₂CH₃), 1.27 (3H, d, $J_{6,5} = 6.5$ Hz, H-6);

¹³C NMR (100 MHz, MeOD) δ 85.8 (C-1), 75.0 (C-3), 74.6 (C-5), 71.8 (C-4), 69.8 (C-2), 23.6 (S-CH₂CH₃), 15.6 (C-6), 14.1 (S-CH₂CH₃).

M/z HRMS (ESI-TOF) calcd. for (C₈H₁₆O₄SN_a) = 231.0667 (M+Na)⁺. Found 231.0656.



6.2.18 Ethyl-2,3,4-tri-O-benzyl-1-thio- β -L-fucopyranoside, **81**

To a stirred solution of **80** (1.30 g, 6.24 mmol) in dry THF (75 mL) was added sodium hydride (0.75 g, 31.21 mmol). The solution was stirred for 10 min. BnBr (3.71 mL, 31.21 mmol) and the mixture stirred at 35 °C for 20 h. The reaction was quenched with the addition of MeOH (20 mL) over ice and the solvent removed *in vacuo*. The residue was purified by column chromatography (EtOAc:Hexane, 9:1 (v/v)) to yield the required product **81** as a white solid, (2.40 g, 81 %);

Analytical data was in good agreement with literature example.²⁷³

$[\alpha]_D^{20} = 11^\circ$ (deg cm³ g⁻¹ dm⁻¹) ($c = 0.1$ g cm⁻¹ in CHCl₃);

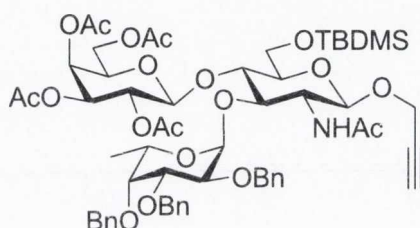
$\nu_{\max}$ (thin film) 3031 cm⁻¹ (CH);

¹H NMR (400 MHz, CDCl₃) δ 7.40 (15H, m, Ar CH), 5.02 (1H, d, $J = 11.8$ Hz, Ph-CH(H)), 4.92 (1H, d, $J = 10.3$ Hz, Ph-CH(H)), 4.83 (1H, d, $J = 10.3$ Hz, Ph-CH(H)), 4.81 (1H, d, $J = 11.8$ Hz, Ph-CH(H)), 4.76 (1H, d, $J = 11.8$ Hz, Ph-CH(H)), 4.73 (1H, d, $J = 11.8$ Hz, Ph-CH(H)), 4.42 (1H, d, $J_{1,2} = 9.3$ Hz, H-1), 3.85 (1H, app t, $J_{2,1} = J_{2,3} = 9.3$ Hz, H-2), 3.64 (1H, dd, $J_{4,3} = 2.6$ Hz, $J_{4,5}$

= 0.4 Hz, H-4), 3.59 (1H, dd, $J_{3,2} = 9.3$ Hz, $J_{3,4} = 2.8$ Hz, H-3), 3.51 (1H, dq, $J_{5,6} = 6.5$ Hz, $J_{5,4} = 0.4$ Hz, H-5), 2.77 (2H, m, SCH_2CH_3), 1.33 (3H, t, $J = 7.5$ Hz, SCH_2CH_3), 1.23 (3H, d, $J_{6,5} = 6.5$ Hz, H-6);

^{13}C NMR (100 MHz, CDCl_3) δ 138.2, 138.0, 137.9 (Ar $\underline{\text{C}}$), 128.0, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.3, 127.2, 127.1, 127.1, 127.0, (Ar $\underline{\text{CH}}$), 84.5 (C-1), 84.0 (C-3), 77.9 (C-2), 75.9 (C-4), 75.3 (CH_2), 74.1 (C-5), 74.0 (CH_2), 72.4 (CH_2), 24.2 (SCH_2CH_3), 16.8 (C-6), 14.6 (SCH_2CH_3);

M/z HRMS (ESI-TOF) calcd. for $(\text{C}_{29}\text{H}_{34}\text{O}_4\text{SNa}) = 501.2076$ ($M+\text{Na}$) $^+$. Found 501.2067.



6.2.19 Propargy-((2,3,4,6-Tetra-O-acetyl- β -D-galactopyranosyl)-(1-4))-((2,3,4-Tri-O-benzyl- α -L-fucopyranosyl)-(1-3))-2-acetamido-2-deoxy-6-O-tert-

butyldimethylsilyl- β -D-glucopyranoside, (also protected O-propargyl Lewis X), **82**

Acceptor **76** (50 mg, 0.071 mmol) and donor **81** (71 mg, 0.142 mmol) were dissolved in DCM (3 mL). The solvent was removed *in vacuo*, co-evaporating with toluene. The mixture of sugars was dried under vacuum for 3 h. The mixture was dissolved in DCM (3 mL) containing pre-activated 3 Å molecular sieves under N_2 . The mixture was cooled to -20 °C. NIS (32 mg, 0.142 mmol) and TMSOTf (cat.) were added and the mixture stirred for 16 h at -20 °C. The mixture was quenched through the addition of Et_3N (0.5 mL) and diluted with DCM (10 mL). The mixture was washed with sat. aqueous $\text{Na}_2\text{S}_2\text{O}_3$ solution (5 mL) and deionised H_2O . The organic layer was dried over MgSO_4 , filtered and the solvent was removed *in vacuo*. Purification by column chromatography ($\text{EtOAc}:\text{Hex}$, 1:1 (v/v)) yielded the product **82** as a white solid, (46 mg, 58 %);

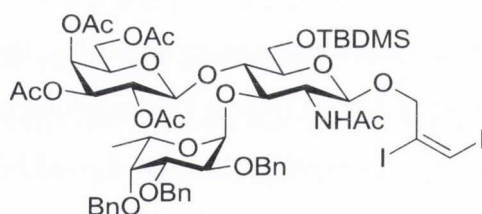
$[\alpha]_{\text{D}}^{20} = -32^\circ$ ($\text{deg cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.2 \text{ g cm}^{-1}$ in CHCl_3);

ν_{max} (thin film) 3272 cm^{-1} ($\text{C}\equiv\text{CH}$), 3030 cm^{-1} (CH), 1753 cm^{-1} (C=O), 1661 cm^{-1} (NHC=O);

^1H NMR (600 MHz, CDCl_3) δ 7.37 (15H, m, Ar CH), 5.85 (1H, d, $J = 7.6$ Hz, NH), 5.40 (1H, d, $J_{4,3} = 2.9$ Hz, H-4b), 5.15 (1H, d, $J_{1,2} = 2.5$ Hz, H-1c), 5.14 (1H, dd, $J_{2,3} = 10.2$ Hz, $J_{2,1} = 8.9$ Hz, H-2b), 5.04 (1H, d, $J_{1,2} = 6.5$ Hz, H-1a), 5.00 (1H, d, $J = 11.8$ Hz, $\text{PhCH}(\text{H})$), 4.99 (1H, dd, $J_{3,2} = 10.2$ Hz, $J_{3,4} = 3.6$ Hz, H-3b), 4.88 (1H, d, $J = 11.7$ Hz, $\text{PhCH}(\text{H})$), 4.87 (1H, d, $J = 11.5$ Hz,

PhCH(H)), 4.82 (1H, d, $J = 11.5$ Hz, PhCH(H)), 4.80 (1H, d, $J_{1,2} = 8.8$ Hz, 1-b), 4.78 (1H, d, $J = 11.7$ Hz, PhCH(H)), 4.72 (1H, d, $J = 11.8$ Hz, PhCH(H)), 4.48 (1H, q, $J = 6.5$ Hz, H-5c), 4.27 (1H, m, H-3a), 4.26 (2H, s, CH₂C≡CH), 4.20 (1H, dd, $J_{6,6'} = 10.8$ Hz, $J_{6,5} = 8.1$ Hz, H-6b), 4.18 (1H, dd, $J_{3,2} = 10.2$ Hz, $J_{3,4} = 3.7$ Hz, H-3c), 4.07 (1H, dd, $J_{6',6} = 10.8$ Hz, $J_{6',5} = 6.0$ Hz, H-6'b), 3.98 (1H, dd, $J_{2,3} = 10.2$ Hz, $J_{2,1} = 2.3$ Hz, H-2c), 3.96 (2H, m, H-4a, H-6a), 3.89 (1H, dd, $J_{6',6} = 11.1$ Hz, $J_{6',5} = 2.4$ Hz, H-6'a), 3.85 (1H, m, H-5b), 3.74 (1H, br s, H-4c), 3.53 (1H, m, H-2a), 3.40 (1H, m, H-5a), 2.40 (1H, s, CH₂C≡CH), 2.08, 2.05, 2.00, 1.98 (3H, s, CH₃), 1.82 (3H, s, NHAc), 1.24 (3H, d, $J_{6,5} = 6.4$ Hz, H-6c), 0.94 (9H, s, SiC(CH₃)₃), 0.11, 0.10 (3H, s, Si(CH₃)₂); ¹³C NMR (150 MHz, CDCl₃) δ 170.2 (NHC=O), 170.1, 170.0, 169.9, 169.3 (C=O), 138.9, 138.8, 138.7 (Ar C), 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 127.9, 127.9, 127.7, 127.5, 127.3, 127.1, 127.1 (Ar CH), 99.5 (C-1b), 97.4 (C-1c), 97.3 (C-1a), 80.0 (C-2c), 79.0 (CH₂C≡CH), 77.2 (C-4c), 76.4 (C-3c), 75.5 (C-5a), 74.6 (CH₂C≡CH), 74.4, 73.7 (Ph-CH₂), 73.5 (C-4a), 73.3 (C-3a), 72.6 (Ph-CH₂), 70.8 (C-3b), 70.7 (C-5b), 68.9 (C-2b), 66.9 (C-4b), 66.5 (C-5c), 61.4 (C-6a), 60.6 (C-6b), 55.7 (C-2a), 55.3 (CH₂C≡CH), 25.9, 25.9, 25.9 (SiC(CH₃)₃), 23.3 (NHCOCH₃), 20.8, 20.6, 20.6, 20.5 (COCH₃), 18.2 (SiC(CH₃)₃), 16.8 (C-6c), -4.9, -5.3 (Si(CH₃)₂), (a = GlcNAc, b = Gal, c = Fuc);

M/z HRMS (ESI-TOF) calcd. for (C₅₈H₇₇NO₁₉SiNa) = 1142.4757 (M+Na)⁺. Found 1142.4736.



6.2.20 ((2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1-4))-((2,3,4-O-benzyl-α-L-fucopyranoside)-(1-3))-2-acetamido-2-deoxy-6-O-*tert*-butyldimethylsilyl-1-O-(2,3-diiodo-allyl)-β-D-glucopyranose, 83

Acceptor **76** (40 mg, 0.057 mmol) and donor **81** (51 mg, 0.107 mmol) were dissolved in DCM (3 mL). The solvent was removed *in vacuo*, co-evaporating with toluene. The mixture of sugars was dried under vacuum for 3 h. The mixture was dissolved in DCM (3 mL) containing activated 3 Å molecular sieves under N₂. The mixture was cooled to 0 °C. NIS (24 mg, 0.107 mmol) and TMSOTf (cat.) were added and the mixture stirred whilst

warming to RT over 16 h. The mixture was quenched through the addition of Et₃N (0.5 mL) and diluted with DCM (10 mL). The mixture was washed with sat. aqueous Na₂S₂O₃ solution (5 mL) and deionised H₂O. The organic layer was dried over MgSO₄, filtered and the solvent was removed *in vacuo*. Purification by column chromatography (EtOAc:Hex, 4:6 (v/v)) yielded the product **83** as a white solid, (27 mg, 35 %)

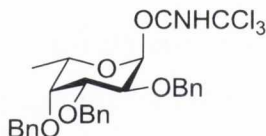
$[\alpha]_D^{20} = -61^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 g cm⁻¹ in CHCl₃);

$\nu_{\max}$ (thin film) 3250 cm⁻¹ (NH), 2927 cm⁻¹ (CH), 1751 (C=O), 1674 cm⁻¹ (NHC=O);

¹H NMR (600 MHz, CDCl₃) δ 7.37 (15H, m, Ar CH), 7.13 (1H, s, -C=CH), 5.75 (1H, d, *J* = 7.9 Hz, NH), 5.40 (1H, d, *J*_{4,3} = 2.6 Hz, H-4b), 5.14 (2H, m, H-1c, H-2B), 5.00 (1H, d, *J*_{4,3} = 11.7 Hz, PhCH(H)), 4.97 (1H, dd, *J*_{3,2} = 10.6 Hz, *J*_{3,4} = 3.8 Hz, H-3b), 4.91 (1H, d, *J* = 12.0 Hz, PhCH(H)), 4.88 (1H, *J*_{1,2} = 8.0 Hz, H-1b), 4.88 (1H, d, *J*_{1,2} = 7.1 Hz, H-1a), 4.86 (2H, d, *J* = 7.2 Hz, PhCH₂), 4.77 (1H, d, *J* = 12.1 Hz, PhCH(H)), 4.73 (1H, d, *J* = 11.7 Hz, PhCH(H)), 4.58 (1H, q, *J* = 6.5 Hz, H-5c), 4.42 (2H, s, OCH₂Cl=CH), 4.32 (1H, app t, *J*_{3,2} = *J*_{3,4} = 8.0 Hz, H-3a), 4.21 (1H, dd, *J*_{6,6'} = 10.8 Hz, *J*_{6,5} = 8.0 Hz, H-6b), 4.18 (1H, dd, *J*_{3,2} = 9.9 Hz, *J*_{3,4} = 3.3 Hz, H-3c), 4.07 (1H, dd, *J*_{6',6} = 11.0 Hz, *J*_{6',5} = 5.9 Hz, H-6'b), 4.01 (1H, dd, *J*_{2,3} = 10.2 Hz, *J*_{2,1} = 2.3 Hz, H-2c), 3.99 (1H, m, H-6a), 3.96 (1H, app t, *J*_{4,3} = *J*_{4,5} = 8.2, H-4a), 3.93 (1H, m, H-6'a), 3.85 (1H, m, H-5b), 3.76 (1H, br s, H-4c), 3.44 (1H, m, H-2a), 3.35 (1H, m, H-5a), 2.08, 2.05, 1.99, 1.94 (3H, s, OAc), 1.78 (3H, s, NHAc), 1.26 (3H, d, *J*_{6,5} = 6.4 Hz, H-6c), 0.96 (9H, s, SiC(CH₃)₃), 0.15, 0.15 (3H, s, Si(CH₃)₂);

¹³C NMR (150 MHz, CDCl₃) δ 170.2 (NHC=O), 170.1, 170.1, 169.9, 169.2 (C=O), 138.9, 138.7, 138.6 (Ar C), 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 127.6, 127.6, 127.6, 127.5, 127.3, 127.1, 127.1 (Ar CH), 99.7 (C-1b), 97.7 (C-1c), 97.3 (C-1a), 88.0 (-Cl=CH), 82.7 (-Cl=CH), 80.3 (C-2c), 77.2 (C-4c), 76.6 (C-3c), 75.5 (C-5a), 74.8 (CH₂Cl=CH), 74.4, 74.0 (PhCH₂), 73.8 (C-4a), 73.5 (C-3a), 72.6 (PhCH₂), 71.0 (C-3b), 70.8 (C-5b), 69.0 (C-2b), 67.0 (C-4b), 66.6 (C-5c), 61.2 (C-6a), 60.6 (C-6b), 57.2 (C-2a), 26.1, (SiC(CH₃)₃), 23.8 (NHCOCH₃), 20.8, 20.7, 20.7, 20.7 (CH₃), 18.2 (SiC(CH₃)), 16.9 (C-6c), -4.9, -5.1 (Si(CH₃)₂, (a = GlcNAc, b = Gal, c = Fuc);

***M/z* HRMS (ESI-TOF)** calcd. for (C₅₈H₇₇I₂NO₁₉SiNa) = 1396.2846 (M+Na)⁺. Found = 1396.2231



6.2.21 2,3,4-Tri-O-benzyl- α -L-fucopyranoside-trichloroacetimidate, **84**

To thioglycoside **81** (75 mg, 0.16 mmol), in anhydrous acetone (2 mL), was added deionised H₂O (4 μ L) and NBS (232 mg, 1.30 mmol). The solution was stirred for 5 min before it was quenched with sat. aqueous NaHCO₃ solution (5 mL). The mixture was diluted with DCM (10 mL) and the organic layer collected. The organic layer was washed sequentially with sat. aqueous Na₂S₂O₃ solution (5 mL) and deionised H₂O (5 mL). The organic layer was dried over MgSO₄, filtered and the solvent removed *in vacuo*. The crude anomeric mixture was dissolved in DCM (2 mL). Trichloroacetonitrile (162 μ L, 1.60 mmol) and Cs₂CO₃ (7 mg, 0.03 mmol) were added and the mixture stirred at 20 °C under N₂ for 6 h. The mixture was filtered through a plug of celite and the solvent removed *in vacuo*. The residue was purified by column chromatography (EtOAc:Hex, 1:4 (v/v)), to yield the product **84** as an off white solid, (37 mg, 41 %);

Analytical data was in good agreement with literature example.²⁷⁴

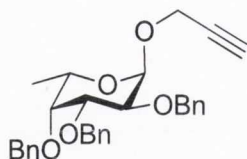
$[\alpha]_D^{20} = -74^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 g cm⁻¹ in CHCl₃);

$\nu_{\max}$ (thin film) 3335 cm⁻¹ (NH), 2931 cm⁻¹, 1728 (C=O), 1667 (C=N);

¹H NMR (600 MHz, CDCl₃) δ 8.54 (1H, s, NH), 7.35 (15H, m, Ar CH) 6.55 (1H, d, $J_{1,2} = 3.5$ Hz, H-1), 5.05 (1H, d, $J = 11.7$ Hz, Ph-CH(H)), 4.90 (1H, d, $J = 12.0$ Hz, Ph-CH(H)), 4.79 (1H, d, $J = 11.7$ Hz, Ph-CH(H)), 4.79 (1H, d, $J = 11.1$ Hz, Ph-CH(H)), 4.78 (1H, d, $J = 11.2$ Hz, Ph-CH(H)), 4.71 (1H, d, $J = 11.5$ Hz, Ph-CH(H)), 4.28 (1H, dd, $J_{2,3} = 10.1$ Hz, $J_{2,1} = 3.5$ Hz, H-2), 4.12 (1H, dq, $J_{5,6} = 6.3$ Hz, $J_{5,4} = 1$ Hz, H-5), 4.06 (1H, dd, $J_{3,2} = 10.1$ Hz, $J_{3,4} = 2.8$ Hz, H-3), 3.75 (1H, dd, $J_{4,3} = 2.7$ Hz, $J_{4,5} = 1$ Hz, H-4), 1.19 (3H, d, $J_{6,5} = 6.5$ Hz, H-6);

¹³C NMR (150 MHz, CDCl₃) δ 161.3 (C=NH), 138.6, 138.5, 138.4 (Ar C), 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.3, 127.7, 127.7, 127.7, 127.6, 127.5, 127.5, 127.5 (Ar CH), 95.3 (C-1), 91.6 (CCl₃), 78.3 (C-3), 77.3 (C-4), 75.8 (C-2), 74.9, 73.2, 72.8 (CH₂), 69.6 (C-5), 16.7 (C-6);

M/z HRMS (ESI-TOF) calcd. for (C₂₉H₃₀NO₅Cl₃Na) = 600.1087 (M+Na)⁺. Found 600.1119.



6.2.22 2,3,4-Tri-O-benzyl-1-O-propargyl- α -L-fucopyranose, **85**

To a stirred solution of donor **81** (100 mg, 0.21 mmol) in DCM at 0 °C under N₂ was added Br₂ (108 μ L, 2.10 mmol). The mixture was stirred at 0 °C for 90 min. Cyclohexene was added dropwise until the red colour disappeared and the mixture was cooled to -20 °C. Propargyl alcohol **63** (24 μ L, 0.42 mmol), and AgOTf (110 mg, 0.42 mmol) was added and the mixture stirred for 40 min. The reaction was quenched with Et₃N (1 mL), and the mixture filtered through a plug of celite. The solvent was removed and the mixture purified by column chromatography (EtOAc:Hex, 9:1 (v/v)) to yield the product **85**, as a clear oil (82 mg, 83 %);

$[\alpha]_D^{20} = 12^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 g cm⁻¹ in CHCl₃);

$\nu_{\max}$ (thin film) 3301 cm⁻¹ (C $\equiv$ CH), 3031 cm⁻¹, 2959 cm⁻¹ (CH);

¹H NMR (600 MHz, MeOD) δ 7.45 - 7.25 (15H, m, Ar CH), 5.09 (1H, d, $J_{1,2} = 3.5$ Hz, H-1), 4.92 (1H, d, $J = 11.3$ Hz, PhCH(H)), 4.87 (1H, br s, PhCH(H)), 4.79 (1H, d, $J = 4.0$ Hz, PhCH(H)), 4.79 (1H, d, $J = 11.1$ Hz, PhCH(H)), 4.74 (2H, br s, PhCH₂), 4.63 (1H, d, $J = 11.5$ Hz, PhCH(H)), 4.26 (2H, s, OCH₂), 4.01 (1H, dd, $J_{2,3} = 10.4$ Hz, $J_{2,1} = 3.4$ Hz, H-2), 3.95 (2H, m, H-5, H-3), 3.83 (1H, d, $J_{4,3} = 2.4$ Hz, H-4), 2.92 (1H, br s, C $\equiv$ CH), 1.17 (3H, d, $J_{6,5} = 6.7$ Hz, H-6);

¹³C NMR (150 MHz, MeOD) δ 139.2, 139.0, 138.7 (Ar C), 128.4, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 127.8, 127.8, 127.8, 127.7, 127.6 (Ar CH), 96.2 (C-1), 79.0 (C-3), 78.4 (C-4), 76.1 (C-2), 75.3, 72.9, 72.9 (CH₂), 67.3 (C-5), 54.3 (OCH₂), 15.8 (C-6);

M/z HRMS (ESI-TOF) calcd. for (C₃₀H₃₂O₅Na) = 495.2147 (M+Na)⁺. Found 495.2140.

6.2.23 General procedures for the preparation of azido substituted porphyrins

All azidophenylporphyrins (mono, 5,10-bis, 5,15-bis, and 5,10,15 tris-) were synthesised according to the general methods described below. The reference for this material can be found - E. M. Scanlan, M. O. Senge, A. Corral, C. C. Heindl and O. B. Locos, *European Journal of Organic Chemistry*, **2010**, 1026-1028 – Supplementary information.

6.2.23.1 General mono-, di-, tri- nitration method

To a stirred solution of 5,10,15,20 tetraphenyl porphyrin (1 eq.) in TFA (16mg/mL), was added NaNO_2 (Mono - 1.8 eq., Di - 8 eq., Tri - 36 eq.). The mixture was stirred for (Mono - 4.5 min, Di - 2 min, Tri - 60 min) before being quenched with deionised water (2 volumes). DCM was added and the organic layer separated. The aqueous layer was back extracted with DCM. The combined green organic extracts were washed with sat. NaHCO_3 solution so that a red colour was observed. The organic layer was collected and the solvent removed in vacuo. The crude nitro porphyrin was purified by column chromatography (DCM:Hexane, 1:1 (v/v) to 1:0 (v/v)) to yield the product as a purple solid which was re-dissolved in DCM (10mg/mL). A solution of Zn(OAc)_2 (4 eq.) in MeOH (70 mg/mL) was added and the solution was stirred for 1 h. The solvent was removed in vacuo. The residue was re-dissolved in DCM (10 mg/mL) and the mixture filtered through a small plug of silica. The solvent was removed in vacuo to yield a deep purple solid.

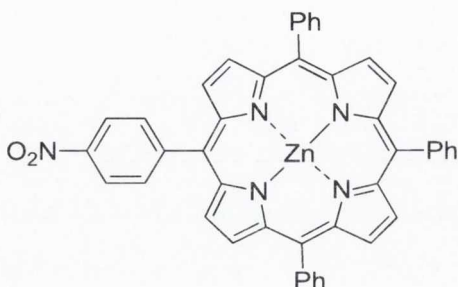
6.2.23.2 General nitroporphyrin reduction method

The previously prepared nitroporphyrin (1 eq.) was dissolved in DCM:MeOH (1:1 (v/v)) (10 mg/mL) and 10% Pd/C (40% (w/w)) was added. The suspension was deoxygenated with an N_2 bubbler for 10 min. NaBH_4 (10 eq. per NO_2 group) was added portionwise over 30 min, and the mixture was stirred under N_2 for 90 min. The mixture was filtered through a plug of celite and the solvent removed. The crude material was dissolved in DCM and filtered through a plug of silica. The solvent was removed in vacuo to yield a crude purple solid, which may be purified by column chromatography.

6.2.23.3 General diazotization route to azide substituted porphyrin

To a stirred solution of the previously prepared amino-porphyrin (1 eq.) in TFA (50 mg/mL) at 0°C was added a solution of NaNO_2 (3 eq. per amino group) in deionized water (30 mg/mL). The mixture was stirred for 10 min at 0°C . A solution of NaN_3 (5 eq. per amino group) in deionized water (50 mg/mL) was added dropwise and the reaction stirred for 1h. The reaction was diluted with deionised water (2 volumes) and the product was extracted with DCM. The organic layers were combined and washed with sat. NaHCO_3 and deionised H_2O . The organic layer was dried over Na_2SO_4 , filtered and the solvent removed in vacuo to yield a crude solid. The crude product was filtered through a plug of silica in DCM. The solution was concentrated in vacuo to yield a purple solid which was re-dissolved in DCM (10mg/mL). A solution of Zn(OAc)_2 (4 eq.) in MeOH (70 mg/mL) was

added and the solution was stirred for 1 h. The solvent was removed in vacuo. The residue was re-dissolved in DCM (10 mg/mL) and the mixture filtered through a small plug of silica. The solvent was removed in vacuo to yield a deep purple solid. Further purification through column chromatography if required.



6.2.24 5-(4-Nitrophenyl)-10,15,20-trisphenyl-porphyrinatozinc(II), **87**

Tetraphenylporphyrin **86** (200 mg, 0.33 mmol) was reacted according to the general nitration method (Section 6.2.23.1), to yield the product **87** as a purple solid, (118 mg, 46 %);

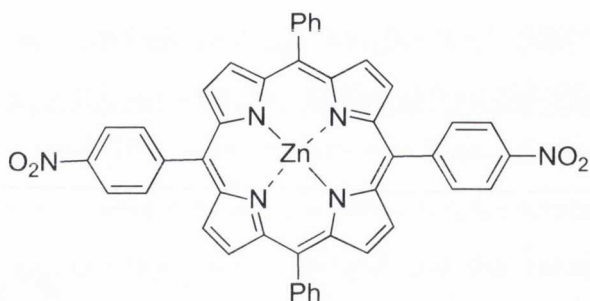
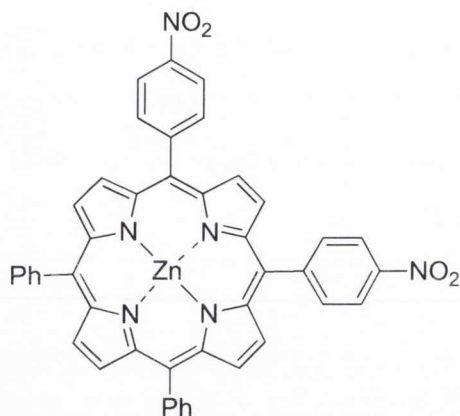
Analytical data was in good agreement with literature example.¹⁵²

ν_{max} (thin film) 3030 cm^{-1} (CH), 1520 cm^{-1} , 1350 cm^{-1} (N=O),

^1H NMR (400 MHz, CDCl_3) δ 9.03 (2H, d, J = 4.6 Hz, β -H), 9.00 (4H, s, β -H), 8.87 (2H, d, J = 4.6 Hz, β -H), 8.66 (2H, d, J = 8.4 Hz, m -Ph), 8.43 (2H, d, J = 8.4 Hz, o -Ph), 8.24 (6H, d, J = 7.2 Hz, o -Ph), 7.80 (9H, m, m , p -Ph);

^{13}C NMR (100 MHz, CDCl_3) 150.1, 150.1, 150.0, 150.0, 149.8, 149.8 (Ar α -C), 149.5 (Ar C- NO_2), 148.7, 148.7 (Ar α -C) 147.1, 142.1, 142.0, 142.0 (Ar C), 134.6, 134.6, 134.0, 134.0, 134.0, 134.0, 134.0, 134.0, (Ar CH), 132.2, 132.2, 131.9, 131.9, 131.8, 131.8, 130.6, 130.6 (Ar β -CH) 127.2, 127.2, 126.2, 126.2, 126.2, 126.2, 126.2, 126.2, 126.2 (Ar CH), 121.5 (Ar meso C), 121.3, 121.3 (Ar CH), 121.2, 121.2, 117.3 (Ar meso C);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{44}\text{H}_{27}\text{N}_5\text{O}_2\text{Zn}$ = 721.1456, (M)⁺. Found = 721.1487.



6.2.25 5,10-(4-Dinitrophenyl)-15,20-diphenyl-porphyrinatozinc(II), 88

and 5,15-(4-Dinitrophenyl)-10,20-diphenyl-porphyrinatozinc(II), 89

Tetraphenylporphyrin **86** (200 mg, 0.33 mmol) was reacted according to the general nitration method (Section 6.2.23.1), to yield the products **88** and **89** (2:1 mixture) as purple solids, (162 mg, 69 % yield);

Analytical data was in good agreement with literature example.¹⁵²

6.2.26 5,10-(4-Dinitrophenyl)-15,20-diphenyl-porphyrinatozinc(II), 88

$\nu_{\max}$ (thin film) 3051 cm^{-1} (CH), 1591 cm^{-1} , 1511 cm^{-1} , 1335 cm^{-1} (N=O);

^1H NMR (400 MHz, CDCl_3) δ 9.04 (2H, d, $J = 4.6$ Hz, β -H), 9.01 (2H, s, β -H), 8.91 (2H, s, β -H), 8.88 (2H, d, $J = 4.6$ Hz, β -H), 8.66 (4H, m, m -Ph), 8.43 (4H, d, $J = 8.4$ Hz, o -Ph), 8.25 (4H, m, o -Ph), 7.80 (6H, m, m , p -Ph);

^{13}C NMR (100 MHz, CDCl_3) 150.2, 150.2, 149.9, 149.9, 149.6, 149.6, 148.9, 148.9 (Ar α -C), 148.6, 148.6 (Ar C- NO_2), 147.1, 147.1, 142.1, 142.1 (Ar C), 134.6, 134.6, 134.6, 134.6, 134.0, 134.0, 134.0, 134.0 (Ar CH), 132.4, 132.4, 132.0, 132.0, 131.1, 131.1, 130.7, 130.7 (Ar β -CH), 127.2, 127.2, 126.2, 126.2, 126.2, 126.2 (Ar CH) 121.7, 121.7 (Ar meso C), 121.2, 121.2, 121.2, 121.2 (Ar CH), 117.4, 117.4 (Ar meso C);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{44}\text{H}_{26}\text{N}_6\text{O}_4\text{Zn} = 766.1367$, (M)⁺. Found = 766.1332.

6.2.27 5,15-(4-Dinitrophenyl)-10,20-diphenyl-porphyrinatozinc(II), 89

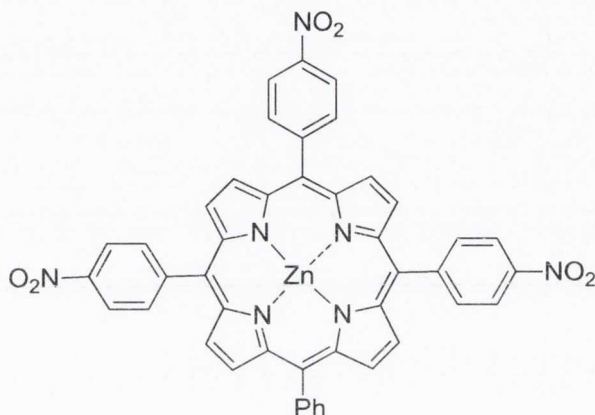
Analytical data was in good agreement with literature example.¹⁵²

$\nu_{\max}$ (thin film) 3051 cm^{-1} (CH), 1591 cm^{-1} , 1511 cm^{-1} , 1335 cm^{-1} ;

^1H NMR (400 MHz, CDCl_3) δ 9.04 (4H, d, $J = 4.6$ Hz, β -H), 8.89 (4H, d, $J = 4.6$ Hz, β -H), 8.66 (4H, m, m -Ph), 8.43 (4H, d, $J = 8.4$ Hz, o -Ph), 8.25 (4H, m, o -Ph), 7.80 (6H, m, m , p -Ph);

^{13}C NMR (100 MHz, CDCl_3) 150.0, 150.0, 150.0, 150.0, 148.8, 148.8, 148.8, 148.8 (Ar α -C), 148.6, 148.6 (Ar C- NO_2), 147.1, 147.1, 142.1, 142.1 (Ar C), 134.6, 134.6, 134.6, 134.6, 134.0, 134.0, 134.0, 134.0 (Ar CH), 132.4, 132.4, 132.4, 132.4, 130.8, 130.8, 130.8, 130.8 (Ar β -CH), 127.2, 127.2, 126.2, 126.2, 126.2, 126.2 (Ar CH) 121.7, 121.7 (Ar meso C), 121.2, 121.2, 121.2, 121.2 (Ar CH), 117.4, 117.4 (Ar meso C);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{44}\text{H}_{26}\text{N}_6\text{O}_4\text{Zn}$ = 766.1367, (M) $^+$. Found = 766.1332.



6.2.28 5,10,15-(4-Tri-nitrophenyl)-20-phenyl-porphyrinatozinc(II), 90

Tetraphenylporphyrin **86** (200 mg, 0.33 mmol) was reacted according to the general nitration method (Section 6.2.23.1), to yield the product **90**, as a purple solid, (123 mg, 50 % yield);

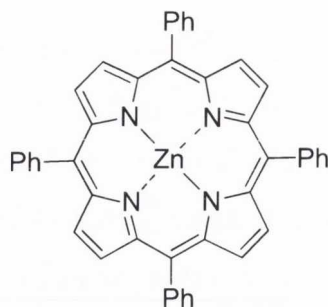
Analytical data was in good agreement with literature example.¹⁵²

ν_{max} (thin film) 3033 cm^{-1} (CH), 1594 cm^{-1} , 1517 cm^{-1} , 1344 cm^{-1} (NO);

^1H NMR (600 MHz, CDCl_3 + MeOD) δ 8.92 (2H, d, J = 4.5 Hz, β -H), 8.81 (4H, s, β -H), 8.77 (2H, d, J = 4.5 Hz, β -H), 8.61 (6H, m, m -Ph), 8.38 (6H, m, o -Ph), 8.18 (2H, m, o -Ph), 7.76 (3H, m, m , p -Ph);

^{13}C NMR (125 MHz, CDCl_3 + MeOD) 149.6, 149.6, 149.2, 149.2, 148.4, 148.4, 148.3, 148.3 (Ar α -C), 148.1, 148.1, 148.1 (Ar C- NO_2), 146.5, 146.5, 146.5, 141.7 (Ar C), 134.0, 134.0, 134.0, 134.0, 134.0, 134.0, 133.4, 133.4 (Ar CH), 131.8, 131.8, 130.6, 130.6, 130.5, 130.5, 130.2, 130.2 (Ar β -CH), 126.6, 125.5, 125.5 (Ar CH), 121.3 (Ar meso C), 122.5, 122.5, 122.5, 122.5 (Ar CH), 117.4, 117.4, 117.0 (Ar meso-C);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{44}\text{H}_{25}\text{N}_7\text{O}_6\text{Zn}$ = 811.1158, (M) $^+$. Found = 811.1168.



6.2.29 5,10,15,20-Tetraphenylporphyrinatozinc(II), 91

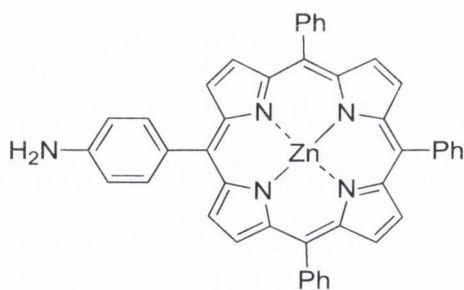
To a solution of tetraphenylporphyrin **86** (50 mg, 0.08 mmol) in DCM (10 mL) was added a solution of $\text{Zn}(\text{OAc})_2$ (60 mg, 0.33 mmol) in MeOH (2 mL). The mixture was stirred for 4 h. The solvent was removed *in vacuo* and the residue dissolved in DCM (5 mL). The suspension was filtered through a plug of silica washing with DCM (50 mL) and the solvent was removed to yield the product **91**, as a purple solid (54 mg, 99 %);

ν_{max} (thin film) 3053 cm^{-1} (CH);

^1H NMR (400 MHz, CDCl_3) δ 9.00 (8H, s, β -H), 8.28 (8H, d, $J = 7.5\text{ Hz}$, *o*-Ph), 7.80 (12H, m, *m*, *p*-Ph);

^{13}C NMR (100 MHz, CDCl_3) 150.3 (Ar α -C), 142.8 (Ar C), 134.5 (Ar CH), 132.0 (Ar β -C), 127.5 (Ar CH) 121.2 (Ar meso C), 121.3, 121.3 (Ar CH) 121.2 (Ar meso C);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{44}\text{H}_{28}\text{N}_4\text{Zn} = 676.1605$, (M) $^+$. Found = 676.1602.



6.2.30 5-(4-Aminophenyl)-10,15,20-trisphenyl-porphyrinatozinc(II), 92

Mono nitro substituted tetraphenyl porphyrin **87** (200 mg, 0.27 mmol) was reacted according to general nitro reduction method (Section 6.2.23.2), to yield the product **92** as a purple solid, (160 mg, 83 %);

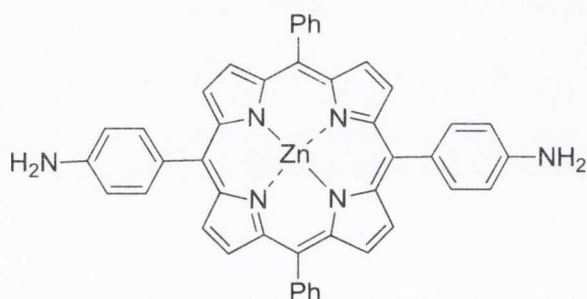
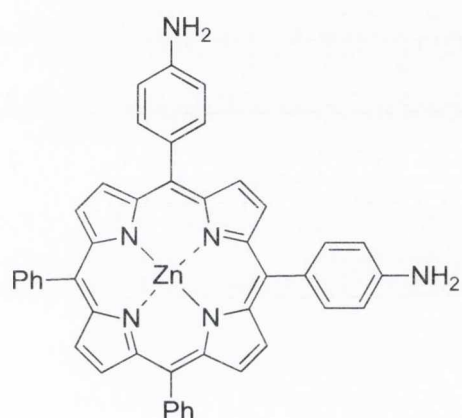
Analytical data was in good agreement with literature example.¹⁵²

ν_{max} (thin film) 3350 cm^{-1} (NH_2), 3043 cm^{-1} (CH);

¹H NMR (400 MHz, CDCl₃ + MeOD) δ 8.93 (2H, d, *J* = 4.6 Hz, β-H), 8.86 (2H, m, β-H), 8.85 (4H, s, β-H), 8.19 (6H, d, *J* = 8.3 Hz, *o*-Ph), 7.94 (2H, d, *J* = 8.0 Hz, *o*-Ph), 7.72 (9H, m, *m*, *p*-Ph), 6.95 (2H, d, *J* = 8.0 Hz, *m*-Ph);

¹³C NMR (100 MHz, CDCl₃ + MeOD) 150.0, 150.0, 150.0, 150.0, 150.0, 150.0 (Ar α-C), 149.9 (Ar C-NH₂), 149.8, 149.8 (Ar α-C), 136.7, 136.7, 136.7 (Ar C), 135.5, 135.5, 134.4, 134.4, 134.4, 134.4, 134.4 (Ar CH), 131.7, 131.7, 131.5, 131.5, 131.4, 131.4, 131.3, 131.3 (Ar β-CH) 127.1 (Ar C), 127.0, 127.0, 127.0, 126.2, 126.2, 126.2, 126.2, 126.2, 126.2, (Ar CH) 121.4, 121.4, 121.4, 118.5 (Ar meso C), 113.5, 113.5 (Ar CH);

***M/z* HRMS (ESI-TOF)** calcd. for C₄₄H₃₀N₅Zn = 692.1793, (M+H)⁺. Found = 692.1777.



6.2.31 5,10-(4-Diaminophenyl)-15,20-diphenyl-porphyrinatozinc(II), 93

and 5,15-(4-Diaminophenyl)-10,20-diphenyl-porphyrinatozinc(II), 94

The mixture of di-nitro substituted tetraphenyl porphyrins **88** and **89** (2:1 mixture, 520 mg, 0.68 mmol) were reacted according to general nitro reduction method (Section 6.2.23.2), then purified by column chromatography (THF:EtOAc:Hex, 4:1:5 (v/v/v)) to yield product **93** as a purple solid, (294 mg, 61 % yield) and product **94** as a purple solid, (89 mg, 19 % yield) respectively.

Analytical data was in good agreement with literature example.¹⁵²

5,10-(4-Diaminophenyl)-15,20-diphenyl-porphyrinatozinc(II), 93

***v*_{max} (thin film)** 3335 cm⁻¹ (NH₂), 3032 cm⁻¹ (CH);

¹H NMR (400 MHz, CDCl₃ + MeOD) δ 8.96 (2H, d, *J* = 4.6 Hz, β-H), 8.95 (2H, s, β-H), 8.90 (2H, s, β-H), 8.88 (2H, d, *J* = 4.6 Hz, β-H), 8.23 (4H, d, *J* = 7.2 Hz, *o*-Ph), 7.93 (4H, m, *o*-Ph), 7.73 (6H, m, *m*, *p*-Ph), 6.80 (4H, m, *m*-Ph);

¹³C NMR (100 MHz, CDCl₃ + MeOD) δ 150.5, 150.5, 150.5, 150.5, 149.9, 149.9, 149.9, 149.9 (Ar α-C), 143.5, 143.5 (Ar C-NH₂), 138.2, 138.2 (Ar C), 135.4, 135.4, 135.4, 135.4, 134.5, 134.5, 134.5, 134.5 (Ar CH), 131.4, 131.4, 131.3, 131.3, 131.2, 131.2, 131.1, 131.1 (Ar β-CH), 127.0, 127.0, 126.2, 126.2, 126.2, 126.2 (Ar CH), 125.6, 125.6 (Ar C), 123.4, 123.4, 120.2, 120.2 (Ar meso C), 113.6, 113.6, 113.6, 113.6 (Ar CH);

M/z HRMS (ESI-TOF) calcd. for C₄₄H₂₆N₆O₄Zn = 707.1902, (M+H)⁺. Found = 707.1921.

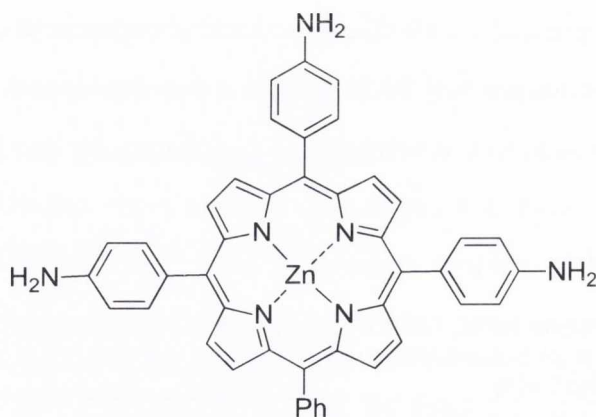
5,15-(4-Diaminophenyl)-10,20-diphenyl-porphyrinatozinc(II), **94**

ν_{max} (thin film) 3335 cm⁻¹ (NH₂), 3033 cm⁻¹ (CH);

¹H NMR (400 MHz, d₅-Pyr + CDCl₃) δ 8.89 (4H, d, *J* = 4.6 Hz, β-H), 8.66 (4H, d, *J* = 4.6 Hz, β-H), 7.90 (4H, m, *o*-Ph), 7.75 (4H, d, *J* = 8.2 Hz, *o*-Ph), 7.75 (6H, m, *m*, *p*-Ph), 6.87 (4H, m, *m*-Ph);

¹³C NMR (100 MHz, d₅-Pyr + CDCl₃) δ 152.1, 152.1, 152.1, 152.1, 150.8, 150.8, 150.8, 150.8 (Ar α-C), 149.7, 149.7 (Ar C-NH₂), 138.9, 138.9 (Ar C), 135.7, 135.7, 135.7, 135.7, 134.4, 134.4, 134.4, 134.4 (Ar CH), 131.9, 131.9, 131.9, 131.9, 131.2, 131.2, 131.2, 131.2 (Ar β-CH), 128.3, 128.3, 128.3, 128.3, 126.9, 126.9, (Ar CH), 126.1, 126.1 (Ar C), 121.7, 121.7, 120.2, 120.2 (Ar meso C), 112.6, 112.6, 112.6, 112.6 (Ar CH);

M/z HRMS (ESI-TOF) calcd. for C₄₄H₃₁N₆Zn = 707.1902, (M+H)⁺. Found = 707.1901.



6.2.32 5,10,15-(4-Triaminophenyl)-20-diphenyl-porphyrinatozinc(II), **95**

Tri nitro substituted tetraphenyl porphyrin **89** (360 mg, 0.44 mmol) was reacted according to general nitro reduction method (Section 6.2.23.2) and purified by column chromatography (THF:EtOAc:Hex, 4:1:2 (v/v/v)) to yield the product **95**, as a green/purple solid, (278 mg, 87 %);

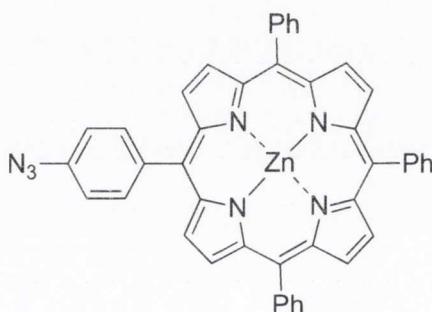
Analytical data was in good agreement with literature example.¹⁵²

$\nu_{\max}$ (thin film) 3357 cm^{-1} (NH_2);

^1H NMR (400 MHz, CDCl_3 + MeOD) δ 8.89 (4H, s, β -H), 8.88 (2H, d, J = 4.6 Hz, β -H), 8.78 (2H, d, J = 4.6 Hz, β -H), 8.16 (2H, m, *o*-Ph), 7.94 (6H, m, *o*-Ph), 7.69 (6H, m, *m*, *p*-Ph), 7.06 (6H, m, *m*-Ph);

^{13}C NMR (100 MHz, CDCl_3 + MeOD) δ 150.7, 150.7, 149.8, 149.8, 149.7, 149.7, 149.2, 149.2 (Ar α -C) 144.9, 144.9, 144.9 (Ar C- NH_2), 141.1 (Ar C), 135.7, 135.7, 134.8, 134.8, 134.8, 134.8, 134.8, 134.8 (Ar CH), 130.8, 130.8, 130.7, 130.7, 130.6, 130.6, 130.5, 130.5 (Ar β -CH), 127.6, 127.6, 126.4 (Ar CH), 125.5, 125.5, 125.5 (Ar C), 120.2, 119.9, 119.9, 119.2 (Ar meso C), 112.9, 112.9, 112.9, 112.9, 112.9, 112.9 (Ar CH);

M/z HRMS (MALDI-TOF+) calcd. for $\text{C}_{44}\text{H}_{31}\text{N}_7\text{Zn}$ = 721.1932 (M)⁺. Found 721.1922



6.2.33 5-(4-Azidophenyl)-10,15,20-trisphenylporphyrinatozinc(II), **96**

Mono amino substituted tetraphenyl porphyrin **92** (374 mg, 0.54 mmol), was reacted according to general diazotization to azide method (Section 6.2.23.3) and purified by column chromatography (THF:Hexane, 1:5 (v/v)) to yield the product **96**, as a purple solid, (288 mg, 74 %);

Analytical data was in good agreement with literature example.¹⁵²

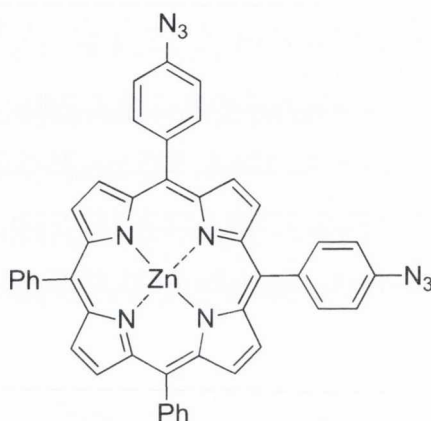
$\nu_{\max}$ (thin film) 3035 cm^{-1} (CH), 2121 cm^{-1} , 2082 cm^{-1} (N_3);

^1H NMR (400 MHz, CDCl_3) δ 8.99 (2H, d, J = 4.6 Hz, β -H), 8.97 (4H, s, β -H), 8.96 (2H, d, J = 4.6 Hz, β -H), 8.24 (6H, m, *o*-Ph), 8.23 (2H, m, *o*-Ph) 7.78 (9H, m, *m*, *p*-Ph), 7.44 (2H, d, J = 8.1 Hz, *m*-Ph);

^{13}C NMR (100 MHz, CDCl_3) 150.3, 150.3, 150.3, 150.3, 150.2, 150.2, 150.1, 150.1 (Ar α -C), 142.7, 142.7, 142.7 (Ar C), 139.6 (Ar C- N_3), 139.6 (Ar C), 135.6, 135.6, 134.4, 134.4, 134.4, 134.4, 134.4, 134.4 (Ar CH), 132.2, 132.2, 131.1, 131.1, 131.1, 132.0, 131.7, 131.7 (Ar β -

CH), 127.6, 127.6, 127.6 (Ar, CH), 126.6, 126.6, 126.6, 126.6, 126.6, 126.6 (Ar CH), 121.3, 121.3, 121.3, 119.9 (Ar meso C), 117.3, 117.3 (Ar CH);

M/z HRMS (MALDI-TOF) calcd. for $C_{44}H_{27}N_7Zn = 717.1619 (M)^+$. Found = 717.1633



6.2.34 5,10-(4-Diazophenyl)-15,20-diphenyl-porphyrinatozinc(II), **97**

Di amino substituted tetraphenyl porphyrin **93** (294 mg, 0.61 mmol), was reacted according to general diazotization to azide method (Section 6.2.23.3) and purified by column chromatography (THF:Hexane, 1:5 (v/v)) to yield the product **97**, as a purple solid, (230 mg, 73 %);

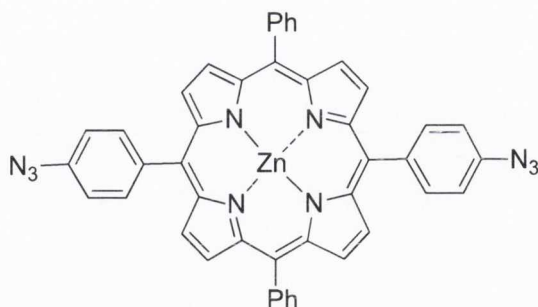
Analytical data was in good agreement with literature example.¹⁵²

$\nu_{\max}$ (thin film) 3030 cm^{-1} (CH), 2118 cm^{-1} , 2082 cm^{-1} (N_3);

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 8.98 (2H, d, $J = 4.6$ Hz, $\beta\text{-H}$), 8.97 (2H, s, $\beta\text{-H}$), 8.96 (2H, s, $\beta\text{-H}$), 8.94 (2H, d, $J = 4.6$ Hz, $\beta\text{-H}$), 8.24 (4H, m, $o\text{-Ph}$), 8.22 (4H, m, $o\text{-Ph}$), 7.78 (6H, m, $m, p\text{-Ph}$), 7.42 (4H, d, $J = 8.2$ Hz, $m\text{-Ph}$);

$^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 150.2, 150.2, 150.1, 150.1, 150.0, 150.0, 149.9, 149.9 (Ar $\alpha\text{-C}$), 143.4, 143.4 (Ar C), 140.3, 140.3 (Ar C- N_3), 139.3, 139.3 (Ar C), 135.7, 135.7, 135.7, 135.7, 134.6, 134.6, 134.6, 134.6 (Ar CH), 131.9, 131.9, 131.8, 131.8, 131.5, 131.5, 131.4, 131.4 (Ar $\beta\text{-CH}$), 127.3, 127.3, 126.4, 126.4, 126.4, 126.4 (Ar CH), 121.0, 121.0, 119.4, 119.4 (Ar meso C), 117.1, 117.1, 117.1, 117.1 (Ar CH);

M/z HRMS (MALDI-TOF) calcd. for $C_{44}H_{26}N_{10}Zn = 758.1633 (M)^+$. Found = 758.1654.



6.2.35 5,15-(4-Diazenidophenyl)-10,20-diphenylporphyrinatozinc(II), **98**

Di amino substituted tetraphenyl porphyrin **94** (89 mg, 0.13 mmol), was reacted according to general diazotization to azide method (Section 6.2.23.3) and purified by column chromatography (THF:Hexane, 1:4 (v/v)) to yield the product **98**, as a purple solid, (48 mg, 50 %);

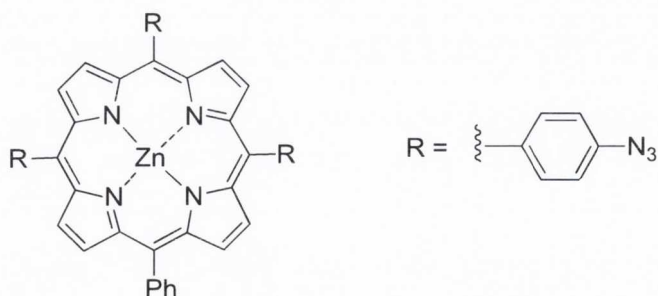
Analytical data was in good agreement with literature example.¹⁵²

$\nu_{\max}$ (thin film) 3034 cm^{-1} (CH), 2119, 2083 cm^{-1} ;

^1H NMR (400 MHz, CDCl_3) δ 8.98 (4H, d, $J = 4.6$ Hz, β -H), 8.95 (4H, d, $J = 4.6$ Hz, β -H), 8.24 (4H, m, *o*-Ph), 8.22 (4H, m, *o*-Ph), 7.79 (6H, m, *m*, *p*-Ph), 7.44 (4H, d, $J = 8.0$ Hz, *m*-Ph);

^{13}C NMR (100 MHz, CDCl_3) δ 150.3, 150.3, 150.3, 150.3, 150.2, 150.2, 150.2, 150.2 (Ar α -C), 142.7, 142.7 (Ar C), 139.6, 139.6 (Ar C- N_3), 137.2, 137.2 (Ar C), 135.6, 135.6, 135.6, 135.6, 134.4, 134.4, 134.4, 134.4 (Ar CH), 1312.2, 132.2, 132.2, 132.2, 131.7, 131.7, 131.7, 131.7 (Ar β -CH), 127.6, 127.6, 126.6, 126.6, 126.6, 126.6 (Ar CH), 124.2, 121.3, 121.3, 119.9 (Ar meso C), 117.3, 117.3, 117.3, 117.3 (Ar CH);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{44}\text{H}_{26}\text{N}_{10}\text{Zn} = 758.1633$ (M)⁺. Found = 758.1636



6.2.36 5,10,15-(4-Triazidophenyl)-20-phenylporphyrinatozinc(II), **99**

Tri amino substituted tetraphenyl porphyrin **95** (43 mg, 0.06 mmol) was reacted according to general diazotization to azide method (Section 6.2.23.3) and purified by column chromatography (DCM:Hexane, 1:1 (v/v)) to yield the product **99**, as a purple solid, (41 mg, 87 %);

Analytical data was in good agreement with literature example.¹⁵²

(ν_{max} (thin film) 3029 cm^{-1} (CH), 2123 cm^{-1} , 2086 cm^{-1} (N_3);

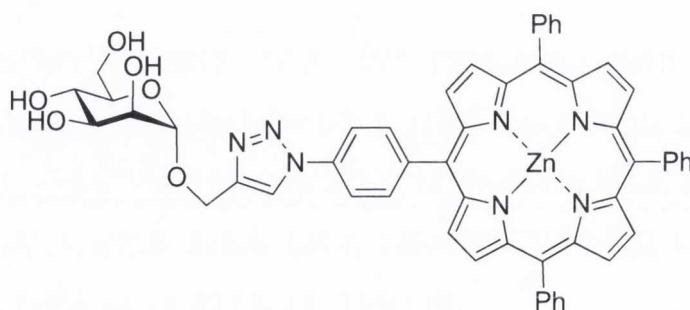
^1H NMR (400 MHz, CDCl_3 + MeOD) δ 8.82 (2H, d, J = 4.6 Hz, β -H), 8.80 (4H, s, β -H), 8.78 (2H, d, J = 4.6 Hz, β -H), 8.12 (2H, m, o -Ph), 8.12 (6H, d, J = 8.2 Hz, o -Ph), 7.67 (3H, m, m , p -Ph), 7.34 (6H, d, J = 8.2 Hz, m -Ph);

^{13}C NMR (100 MHz, CDCl_3) δ 150.1, 150.1, 150.0, 150.0, 149.9, 149.9, 149.9 (Ar α -C), 143.1 (Ar C), 140.1, 140.1, 140.1 (Ar C- N_3), 139.3, 139.3, 139.3 (Ar C), 135.6, 135.6, 135.6, 135.6, 135.6, 135.6, 134.4, 134.4 (Ar CH), 131.8, 131.8, 131.4, 131.4, 131.4, 131.4, 131.3, 131.3 (Ar β -CH), 127.2, 126.3, 126.3 (Ar CH), 120.9, 119.5, 119.5, 119.4 (Ar meso C), 117.0, 117.0, 117.0, 117.0, 117.0, 117.0;

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{44}\text{H}_{25}\text{N}_{13}\text{Zn}$ = 799.1647 (M)⁺. Found = 799.1683.

6.2.37 General glycoporphyrin procedure for mono-, di- and trisubstituted triazole linked glycoporphyrins:

Mono- **96**, di- **97**, **98**, or tri- **99**, azido porphyrin (0.02 mmol), O-propargyl linked carbohydrate (0.021 mmol per azide) and $\text{Cu}(\text{MeCN})_4\text{PF}_6$ (10 % - mono, 15 % - di, 20 % - tri) were dissolved in solvent system A (Toluene (0.6 mL) and MeOH (0.2 mL), solvent system B (Toluene (0.6 mL) and MeOH (1.0 mL) or solvent system C (Toluene (0.5 mL) and MeOH (2.5 mL). The mixture was heated to 110 °C for 20 min in a microwave reactor. The solvent was removed *in vacuo*, and the product purified by column chromatography to yield a bright purple solid.



6.2.38 5-(4-(4'-[(α -D-Mannopyranosyl)oxymethyl]-1- N -1,2,3-triazol-1'-yl)phenyl)-10,15,20-trisphenyl-porphyrinatozinc(II), **100**

Mono azido substituted porphyrin **96** (15 mg, 0.02 mmol) and propargyl functionalised mannose **65** (5 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system A, and the crude material purified by column

chromatography (EtOAc:EtOH:H₂O, 7:2:1 (v/v/v)) to yield the product **100**, as a purple solid, (18 mg, 92 %);

R_f = 0.36 (DCM:MeOH, 9:1 (v/v));

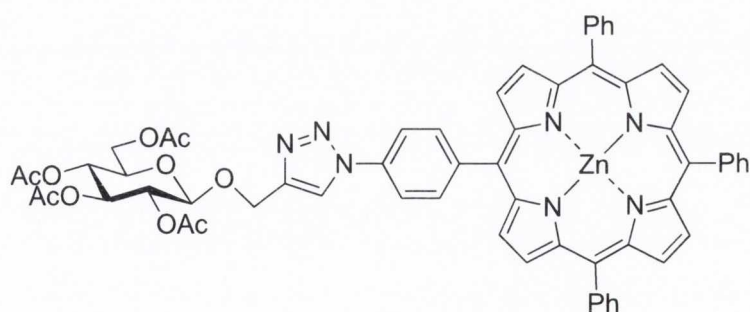
$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at c = 0.01 in CH₂Cl₂/MeOH 1:1);

$\nu_{\max}$ (thin film) 3362 cm⁻¹ (OH), 2922 cm⁻¹ (CH);

¹H NMR (400 MHz, CDCl₃ + 5 % MeOD) δ 8.82 (2H, d, J = 4.5 Hz, β -H), 8.78 (4H, s, β -H), 8.76 (2H, d, J = 4.5 Hz, β -H), 8.36 (1H, s, 5-triazole-H), 8.29 (2H, d, J = 7.8 Hz, o -Ph), 8.11 (6H, d, J = 6.7 Hz, o -Ph), 8.02 (2H, d, J = 7.8 Hz, o -Ph), 7.64 (9H, m, m , p -Ph), 4.93 (1H, br s, H-1), 4.87 (1H, d, J = 12.3 Hz, OCH(H)), 4.72 (1H, d, J = 12.3 Hz, OCH(H)), 3.85 (1H, dd, $J_{2,3}$ = 3.1 Hz, $J_{2,1}$ = 1.4 Hz, H-2), 3.80 (1H, dd, $J_{6',6}$ = 12.1 Hz, $J_{6',5}$ = 2.8 Hz, H-6'), 3.76 (1H, dd, $J_{6,6'}$ = 12.1 Hz, $J_{6,5}$ = 4.6 Hz, H-6), 3.72 (1H, dd, $J_{3,4}$ = 9.3 Hz, $J_{3,2}$ = 3.2 Hz, 3.66 (1H, m, H-4), 3.59 (1H, m, H-5);

¹³C NMR (150 MHz, CDCl₃ + 5 % MeOD) δ 149.3, 149.3, 149.2, 149.2, 149.1, 149.1, 148.6, 148.6 (Ar α -C), 143.9, 143.7, 142.3, 142.3, 142.3, 135.1 (Ar C), 134.6, 134.6, 133.5, 133.5, 133.5, 133.5, 133.5, 133.5 (Ar CH), 131.0, 131.0, 130.8, 130.8, 130.7, 130.7, 130.0, 130.0 (Ar β -CH), 126.3, 126.3, 126.3, 125.4, 125.4, 125.4, 125.4, 125.4, 125.4 (Ar CH), 121.0 (5-triazole-C), 120.1, 119.9, 119.9 (Ar C), 117.6, 117.6 (Ar CH), 117.2 (Ar C), 98.4 (C-1), 71.9 (C-5), 70.4 (C-3), 69.6 (C-2), 66.3 (C-4), 60.7 (C-6), 58.9 (CH₂);

M/z HRMS (MALDI-TOF) calcd. for C₅₃H₄₁N₇O₆Zn = 935.2410 (M)⁺. Found 935.2366.



6.2.39 5-(4-(4'-[(2,3,4,6-Tetra-O-acetyl- β -D-glucopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-10,15,20-trisphenyl-porphyrinatozinc(II), **101**

Mono azido substituted porphyrin **96** (15 mg, 0.02 mmol) and propargyl functionalised per OAc glucose **66** (8.1 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system A, and the crude material purified by column chromatography (MeOH:DCM, 1:49 (v/v)) to yield the product **101** as a purple solid, (19 mg, 83 %);

$R_f = 0.67$ (DCM/MeOH 49:1 (v/v));

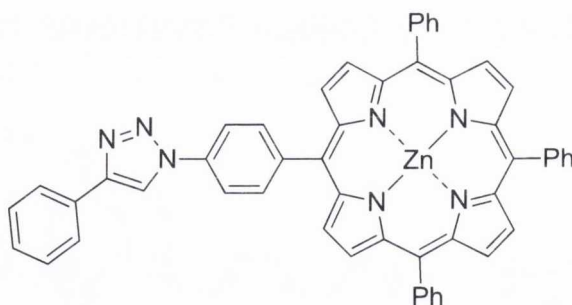
$[\alpha]_D^{20} = \text{N/A}$ (Sample too dark for analysis at $c = 0.01$ in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1);

ν_{max} (thin film) 3053 cm^{-1} , 2932 cm^{-1} (CH), 1737 cm^{-1} (C=O);

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 9.00 (2H, d, $J = 4.1$ Hz, $\beta\text{-H}$), 8.99 (4H, s, $\beta\text{-H}$), 8.94 (2H, d, $J = 4.1$ Hz, $\beta\text{-H}$), 8.39 (2H, d, $J = 8.1$ Hz, $o\text{-Ph}$), 8.25 (6H, d, $J = 6.3$ Hz, $o\text{-Ph}$), 8.14 (1H, s, 5-triazole-C), 8.02 (2H, d, $J = 8.1$ Hz, $m\text{-Ph}$), 7.79 (9H, m, m , $p\text{-Ph}$), 5.26 (1H, dd, $J_{3,4} = J_{3,2} = 9.5$ Hz, H-3), 5.14 (1H, dd, $J_{4,3} = J_{4,5} = 9.5$ Hz, H-4), 5.07 (1H, dd, $J_{2,3} = 9.5$, $J_{2,1} = 8.0$ Hz, H-2), 4.78 (1H, d, $J = 12.4$ Hz, $\text{OCH}(\text{H})$), 4.69 (1H, d, $J_{1,2} = 7.9$ Hz, H-1), 4.61 (2H, d, $J = 12.4$, $\text{OCH}(\text{H})$), 4.31 (1H, dd, $J_{6,6'} = 12.4$ Hz, $J_{6,5} = 4.5$ Hz, H-6), 4.19 (1H, dd, $J_{6',6} = 12.4$ Hz, $J_{6',5} = 1.9$ Hz, H-6'), 3.78 (1H, m, H-5), 2.13, 2.07, 2.06, 2.04 (3H, s, CH_3);

$^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 170.2, 169.8, 169.0, 168.9 (C=O), 149.9, 149.9, 149.9, 149.8, 149.8, 149.2, 149.2 (Ar $\alpha\text{-C}$), 143.8, 143.6, 142.4, 142.4, 142.4, 135.4 (Ar C), 134.9, 134.9, 134.0, 134.0, 134.0, 134.0, 133.0, 133.0 (Ar CH), 131.9, 131.9, 131.7, 131.7, 131.6, 131.6, 130.8, 130.8 (Ar $\beta\text{-CH}$), 127.1, 127.1, 127.1, 126.1, 126.1, 126.1, 126.1, 126.1, 126.1 (Ar CH), 121.0, 120.9, 120.9 (Ar C), 120.0 (5-triazole-C), 118.2 (Ar C), 117.8, 117.8 (Ar CH), 99.7 (C-1), 72.6 (C-3), 71.4 (C-2), 70.7 (C-5), 67.7 (C-4), 61.9 (OCH_2), 61.3 (C-6);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{61}\text{H}_{49}\text{N}_7\text{O}_{10}\text{Zn} = 1103.2832$ (M) $^+$. Found 1103.2832.



6.2.40 5-(4-(4'-[Phenyl]-1-N-1,2,3-triazol-1'-yl)-phenyl)-10,15,20-trisphenylporphyrinato-zinc(II), **103**

Mono azido substituted porphyrin **96** (15 mg, 0.02 mmol) and phenylacetylene **102** (2.1 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system A, and the crude material purified by column chromatography (DCM) to yield the product **103**, as a purple solid, (17 mg, 96 %);

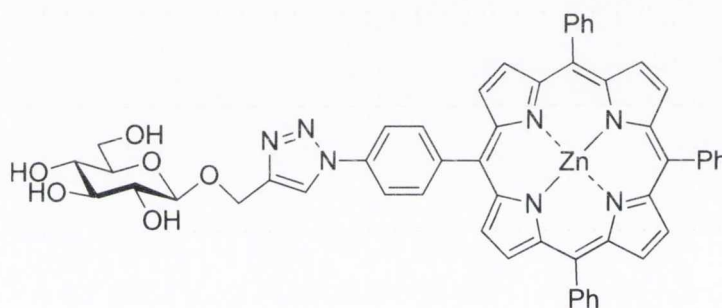
$R_f = 0.24$ (DCM);

ν_{max} (thin film) 2921 cm^{-1} (CH);

¹H NMR (400 MHz, CDCl₃) δ 9.03 (2H, d, *J* = 4.3 Hz, β-H), 8.99 (6H, br s, β-H), 8.48 (1H, s, 5-triazole-H), 8.43 (2H, d, *J* = 7.8 Hz, *o*-Ph), 8.26 (6H, d, *J* = 6.4 Hz, *o*-Ph), 8.20 (2H, d, *J* = 7.8 Hz, *m*-Ph), 7.97 (2H, d, *J* = 7.5 Hz, *o*-Ph'), 7.79 (9H, m, *m*, *p*-Ph), 7.52 (2H, t, *J* = 7.5 Hz, *m*-Ph'), 7.43 (1H, t, *J* = 7.5 Hz, *p*-Ph');

¹³C NMR (100 MHz, CDCl₃) δ 150.0, 150.0, 149.9, 149.9, 148.8, 148.8, 149.3, 149.3 (Ar α-C), 148.2 (4-triazole-C), 143.2, 142.2, 142.2, 142.2, 136.0 (Ar C), 135.0, 135.0, 134.0, 134.0, 134.0, 134.0, 134.0 (Ar CH), 131.9, 131.9, 131.8, 131.8, 131.7, 131.7, 131.0, 131.0 (Ar β-CH), 129.7 (Ar C), 128.5, 128.5, 128.1, 127.1, 127.1, 127.1, 126.2, 126.2, 126.2, 126.2, 126.2, 125.4, 125.4 (Ar CH), 121.1, 120.9, 120.9, 118.3 (Ar C), 118.2, 118.2 (Ar CH), 117.4 (5-triazole-C);

***M/z* HRMS (MALDI-TOF)** calcd. for C₅₂H₂₃N₇Zn = 819.2089 (*M*)⁺. Found 819.2079.



6.2.41 5-(4-(4'-[(β-D-glucopyranosyl)oxymethyl]-1-N-1,2,3-triazol-1'-yl)phenyl)-10,15,20-trisphenylporphyrinatozinc(II), **104**

Mono azido substituted porphyrin **96** (15 mg, 0.02 mmol) and propargyl functionalised glucose **67** (5 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system A, and the crude material purified by column chromatography (EtOAc:EtOH:H₂O, 7:2:1 (v/v/v)) to yield the product **104**, as a purple solid, (18 mg, 93 %)

R_f = 0.29 (DCM:MeOH, 9:1 (v/v));

[α]_D²⁰ = N/A (Sample too dark for analysis at *c* = 0.01 in CH₂Cl₂/MeOH 1:1);

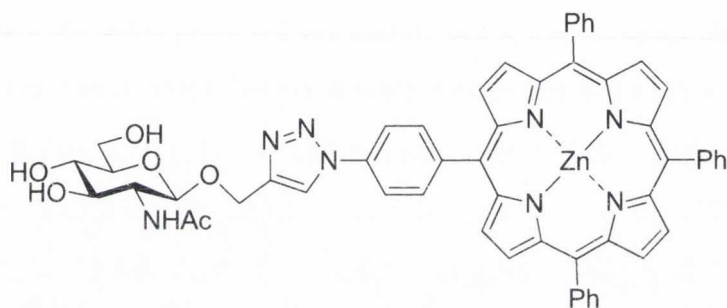
*ν*_{max} (thin film) 3346 cm⁻¹ (OH), 2922 cm⁻¹ (CH);

¹H NMR (400 MHz, CDCl₃ + 10 % MeOD) δ 8.87 (2H, d, *J* = 4.5 Hz, β-H), 8.84 (4H, s, β-H), 8.82 (2H, d, *J* = 4.5 Hz, β-H), 8.51 (1H, s, 5-triazole-H), 8.36 (2H, d, *J* = 8.0 Hz, *o*-Ph), 8.17 (6H, d, *J* = 6.8 Hz, *o*-Ph), 8.07 (2H, d, *J* = 8.0 Hz, *m*-Ph), 7.69 (9H, m, *m*, *p*-Ph), 5.09 (1H, d, *J*

= 12.3 Hz, OCH(H)), 4.91 (1H, d, $J = 12.3$ Hz, OCH(H)), 4.47 (1H, d, $J_{1,2} = 7.6$ Hz, H-1), 3.90 (1H, dd, $J_{6',6} = 11.4$ Hz, H-6'), 3.76 (1H, dd, $J_{6,6'} = 11.8$ Hz, $J_{6,5} = 4.9$ Hz, H-6), 3.42 (2H, m, H-3, H-4), 3.36 (1H, m, H-2), 3.30 (1H, m, H-5);

^{13}C NMR (100 MHz, $\text{CDCl}_3 + 10\% \text{ MeOD}$) δ 150.2, 150.2, 150.1, 150.1, 150.0, 150.0, 149.5, 149.5 (Ar α -C), 145.0, 144.6, 143.2, 143.2, 143.2, 136.0 (Ar C), 135.5, 135.5, 134.5, 134.5, 134.5, 134.5, 134.5 (Ar CH), 131.9, 131.9, 131.8, 131.8, 131.7, 131.7, 130.9, 130.9 (Ar β -CH), 127.3, 127.3, 127.3, 126.3, 126.3, 126.3, 126.3, 126.3, 126.3 (Ar CH), 121.8 (5-triazole-C), 121.1, 120.8, 120.8 (Ar C), 118.5, 118.5 (Ar CH), 118.2 (Ar C), 102.2 (C-1), 76.3 (C-3), 76.1 (C-5), 73.5 (C-2), 70.0 (C-4), 62.1 (OCH_2), 61.7 (C-6);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{53}\text{H}_{41}\text{N}_7\text{O}_6\text{Zn} = 935.2410$ (M)⁺. Found 935.2405.



6.2.42 5-(4-(4'-[(2-Acetamido-2-deoxy- β -D-glucopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-10,15,20-trisphenyl-porphyrinatozinc(II), **105**

Mono azido substituted porphyrin **96** (15 mg, 0.02 mmol) and propargyl functionalised GlcNAc **70** (6 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system A and the crude material purified by column chromatography ($\text{MeOH}:\text{DCM}$, 1:9 (v/v)) to yield the product **105**, as a purple solid, (19 mg, 93 %);

$R_f = 0.15$ ($\text{DCM}:\text{MeOH}$, 9:1 (v/v));

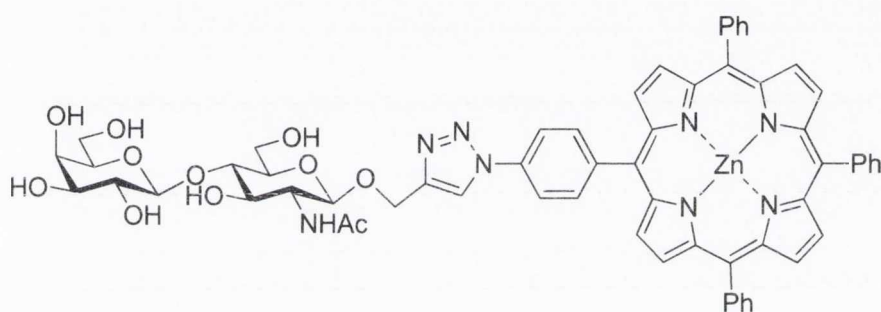
$[\alpha]_D^{20} = \text{N/A}$ (Sample too dark for analysis at $c = 0.01$ in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1);

ν_{max} (thin film) 3321 cm^{-1} (OH), 1636 cm^{-1} (NHC=O);

^1H NMR (600 MHz, $\text{CDCl}_3 + 5\% \text{ MeOD}$) δ 8.87 (2H, d, $J = 4.6$ Hz, β -H), 8.86 (4H, s, β -H), 8.83 (2H, d, $J = 4.6$ Hz, β -H), 8.35 (1H, s, 5-triazole-H), 8.33 (2H, d, $J = 7.7$ Hz, *o*-Ph), 8.17 (6H, d, $J = 6.6$ Hz, *o*-Ph), 8.03 (2H, d, $J = 7.7$ Hz, *m*-Ph), 7.68 (9H, m, *m*, *p*-Ph), 4.98 (1H, d, $J = 12.6$ Hz, OCH(H)), 4.78 (1H, d, $J = 12.6$ Hz, OCH(H)), 4.65 (1H, d, $J_{1,2} = 7.9$ Hz, H-1), 3.89 (1H, br d, $J_{6',6} = 11.9$ Hz, H-6'), 3.79 (1H, dd, $J_{6,6'} = 12.0$ Hz, $J_{6,5} = 4.1$ Hz, H-6), 3.69 (1H, dd, $J_{2,3} = J_{2,1} = 8.1$ Hz, H-2), 3.50 (2H, m, H-3, H-4), 3.35 (1H, m, H-5), 1.99 (3H, s, CH_3);

^{13}C NMR (150 MHz, CDCl_3 + 5 % MeOD) δ 173.0 (C=O), 150.0, 150.0, 149.9, 149.9, 148.8, 148.8, 149.3, 149.3 (Ar α -C), 144.9, 144.4, 143.0, 143.0, 143.0, 135.8 (Ar C), 135.5, 135.5, 134.3, 134.3, 134.3, 134.3, 134.3, 134.3 (Ar CH), 131.8, 131.8, 131.6, 131.6, 131.5, 131.5, 130.8, 130.8 (Ar β -CH), 127.1, 127.1, 127.1, 126.1, 126.1, 126.1, 126.1, 126.1, 126.1 (Ar CH), 121.8 (Ar C), 120.9 (5-triazole-C), 120.6, 120.6 (Ar C), 118.3, 118.3 (Ar CH), 118.0 (Ar C), 100.3 (C-1), 75.9 (C-5), 75.2 (C-3), 70.8 (C-4), 61.8 (OCH_2), 61.6 (C-6), 56.3 (C-6), 22.5 (CH_3);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{55}\text{H}_{44}\text{N}_8\text{O}_6\text{Zn}$ = 976.2675 (M)⁺. Found 976.2656.



6.2.43 5-(4'-[(2-Acetamido-2-deoxy-4-O-(β -D-galactopyranosyl)- β -D-glucopyranoside)-oxymethyl]-1-N-1,2,3-triazol-1'-yl)phenyl)-10,15,20-trisphenylporphyrinatozinc(II), **106**

Mono azido substituted porphyrin **96** (15 mg, 0.02 mmol) and propargyl functionalised LacNAc **77** (9 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography (EtOAc:isop:H₂O, 7:2:0.8 (v/v/v)) to yield the product **106**, as a purple solid, (22 mg, 93 %);

R_f = 0.34 (EtOAc:EtOH:H₂O, 7:2:1 (v/v/v));

$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at c = 0.01 in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1);

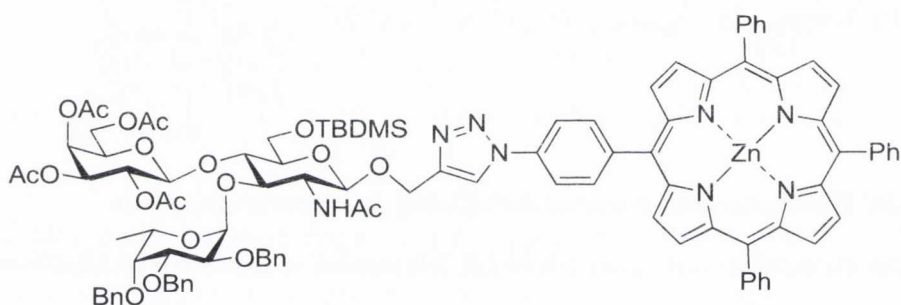
ν_{max} (thin film) 3374 cm^{-1} (OH), 2950 cm^{-1} (CH), 1643 cm^{-1} (NHC=O),

^1H NMR (600 MHz, MeOD_3 + 10 % CDCl_3) δ 8.88 (2H, d, J = 4.7 Hz, β -H), 8.87 (2H, d, J = 4.9 Hz, β -H), 8.85 (4H, s, β -H) 8.74 (1H, s, 5-triazole-H), 8.43 (2H, d, J = 8.1 Hz, o -Ph), 8.27 (2H, d, J = 8.1 Hz, m -Ph), 8.21 (2H, d, J = 7.2 Hz, o -Ph), 7.77 (9H, m, m , p -Ph), 5.11 (1H, d, J = 12.5 Hz, $\text{OCH}(\text{H})$), 4.95 (1H, d, J = 12.5 Hz, $\text{OCH}(\text{H})$), 4.69 (1H, d, J = 8.1 Hz, H-1a), 4.42 (1H, d, $J_{1,2}$ = 7.7 Hz, H-1b), 4.04 (1H, dd, $J_{6',6}$ = 12.2 Hz, $J_{6',5}$ = 2.3 Hz, H-6a'), 3.95 (1H, dd, $J_{6,6'}$ = 12.2 Hz, $J_{6,5}$ = 4.1 Hz, H-6a), 3.86 (1H, dd, $J_{2,1}$ = $J_{2,3}$ = 9.0 Hz, H-2a), 3.85 (1H, d, $J_{4,3}$ =

3.5 Hz, H-4b), 3.80 (1H, dd, $J_{6,6'} = 11.5$ Hz, $J_{6,5} = 7.3$ Hz, H-6b), 3.73 (2H, m, H-6b', H-3a), 3.69 (1H, dd, $J_{4,3} = J_{4,5} = 8.7$ Hz, H-4a), 3.61 (1H, m, H-5b), 3.59 (1H, dd, $J_{2,3} = 9.0$ Hz, $J_{2,1} = 7.7$ Hz, H-2b), 3.54 (1H, m, H-5a) 3.51 (1H, dd, $J_{3,2} = 9.3$ Hz, $J_{3,4} = 3.3$ Hz, H-3b), 2.02 (3H, s, CH₃);

¹³C NMR (150 MHz, MeOD + 10 % CDCl₃) δ 172.2, (C=O), 150.0, 150.0, 150.0, 150.0, 149.9, 149.9, 149.5, 149.5 (Ar α-C), 145.3, 144.4, 143.3, 143.3, 143.3, 136.1 (Ar C), 135.2, 135.2, 134.1, 134.1, 134.1, 134.1, 134.1, 134.1 (Ar CH), 131.3, 131.3, 131.1, 131.1, 131.0, 131.0, 130.5, 130.5 (Ar β-CH), 126.9, 126.9, 126.9, 125.9, 125.9, 125.9, 125.9, 125.9, 125.9 (Ar CH), 122.2 (5-triazole-CH), 120.7, 120.5, 120.5, 118.1 (Ar C), 117.9, 117.9, (Ar CH), 103.6 (C-1b), 100.4 (C-1a), 79.4 (C-4a), 75.6 (C-5b), 75.2 (C-5a), 73.3 (C-3b), 72.6 (C-3a), 71.1 (C-2b), 68.8 (C-4b), 61.4 (OCH₂), 61.0 (C-6b), 60.5 (C-6a), 55.2 (C-2a), 21.6 (CH₃);

M/z HRMS (MALDI-TOF) calcd. for C₆₁H₅₄N₈O₁₁Zn = 1138.3204 (M)⁺. Found = 1138.3208.



6.2.44 5-(4'-[[(O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1-4))-((2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3))-2-acetamido-2-deoxy-6-O-tert-butyl-dimethylsilyl-β-D-glucopyranoside)-1-oxymethyl]-1-N-1,2,3-triazol-1'-yl)phenyl]-10,15,20-triphenyl-porphyrinatozinc(II), 107

Mono azido substituted porphyrin **96** (15 mg, 0.02 mmol) and propargyl functionalised protected Lewis X trisaccharide **82** (20 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system A, and the crude material purified by column chromatography (EtOAc:Pet Ether, 15:85 (v/v)) to yield the product **107**, as a purple solid, (25 mg, 65 %);

$R_f = 0.69$ (Pet Ether:EtOAc, 3:1 (v/v));

$[\alpha]_D^{20} = \text{N/A}$ (Sample too dark for analysis at $c = 0.01$ in CH₂Cl₂/MeOH 1:1);

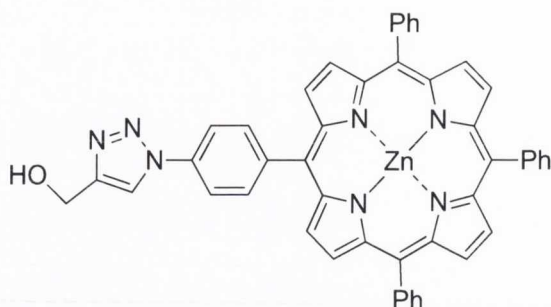
ν_{max} (thin film) 3300 cm⁻¹ (NH), 2927 cm⁻¹ (CH), 1751 cm⁻¹ (C=O), 1648 cm⁻¹ (NHC=O);

¹H NMR (600 MHz, MeOD) δ 8.85 (6H, m, β-H), 8.80 (2H, d, $J = 4.5$ Hz, β-H), 8.55 (1H, s, 5-triazole-H), 8.25 (2H, d, $J = 7.7$ Hz, *o*-Ph), 8.21 (6H, d, $J = 7.7$ Hz, *o*-Ph), 8.07 (2H, d, $J = 7.7$

Hz, *m*-Ph), 7.76 (9H, m, *m*, *p*-Ph), 7.41 (2H, d, *J* = 7.1 Hz, *o*-Ph (Fuc)), 7.36 (2H, d, *J* = 7.1 Hz, *o*-Ph (Fuc)), 7.26 (10H, m, *o*, *m*, *p*-Ph (Fuc)), 7.14 (1H, t, *J* = 7.1 Hz, *p*-Ph (Fuc)), 5.40 (1H, d, *J*_{4,3} = 3.4 Hz, H-4 (Gal)), 5.37 (1H, d, *J*_{1,2} = 2.9 Hz, H-1 (Fuc)), 5.15 (1H, dd, *J*_{2,3} = 10.3 Hz, *J*_{2,1} = 8.3 Hz, H-2 (Gal)), 5.05 (1H, d, *J*_{1,2} = 8.3 Hz, H-1 (Gal)), 5.04 (1H, d, *J* = 12.4 Hz, OCH(H) (Linker)), 5.02 (1H, dd, *J*_{3,2} = 10.4 Hz, *J*_{3,4} = 3.5 Hz, H-3b), 4.90 (1H, d, *J* = 11.3 Hz, PhCH(H) (Fuc)), 4.89 (1H, d, *J* = 12.4 Hz, OCH(H) (Linker)), 4.83 (4H, m, 3 x PhCH(H), H-5 (Fuc)), 4.70 (2H, m, H-1 (GlcNAc), PhCH(H) (Fuc)), 4.60 (1H, d, *J* = 11.7 Hz, PhCH(H) (Fuc)), 4.32 (1H, dd, *J*_{6,6'} = 10.8 Hz, *J*_{6,5} = 7.4 Hz, H-6 (Gal)), 4.07 (7H, m, H-2, H-4, H-6, H-6', (GlcNAc), H-6' (Gal), H-2, H-3 (Fuc)), 4.00 (1H, m, H-5 (Gal)), 3.83 (2H, m, H-4 (Fuc), H-3 (GlcNAc)), 3.47 (1H, m, H-5a), 2.09, 1.99, 1.99 (3H, s, CH₃), 1.98 (3H, s, NHAc (CH₃)), 1.97 (3H, s, CH₃), 1.28 (3H, d, *J*_{6,5} = 6.6 Hz, H-6 (Fuc)), 1.00 (9H, s, SiC(CH₃)₃), 0.20, 0.10 (3H, s, Si(CH₃)₂);

¹³C NMR (150 MHz, MeOD) δ 172.4 (NHCO), 170.9, 170.5, 170.4, 169.9 (C=O), 150.6, 150.6, 150.5, 150.5, 150.5, 150.5, 150.0, 150.0 (Ar α-C), 146.1 (4-triazole-C), 144.8, 143.8, 143.8, 143.8 (Ar C), 139.3, 139.0, 138.6 (Ar C (Fuc)), 136.5 (Ar C), 135.6, 135.6, 135.6, 134.6, 134.6, 134.6, 134.6, 134.6, 134.6 (Ar CH), 131.8, 131.8, 131.6, 131.6, 131.6, 131.6, 131.0, 131.0 (Ar β-CH), 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.3, 128.2, 128.2, 127.7, 127.6 (Ar CH (Fuc)), 127.4, 127.4, 127.4 (Ar CH), 127.2, 127.2, 127.2 (Ar CH (Fuc)), 126.5, 126.5, 126.5, 126.5, 126.5, 126.5 (Ar CH), 122.3 (5-triazole-CH), 121.2, 121.1, 121.1, 118.7 (Ar C), 118.4, 118.4 (Ar CH), 100.9 (C-1 (GlcNAc)), 100.2 (C-1 (Gal)), 96.7 (C-1 (Fuc)), 79.3 (C-3(Fuc)), 78.2 (C-3 (GlcNAc)), 76.1 (C-2 (Fuc)), 76.0 (C-5 (GlcNAc)), 74.8 (Ph-CH₂ (Fuc)), 74.4 (C-4 (GlcNAc)), 72.8, 72.5 (Ph-CH₂ (Fuc)), 71.4 (C-3 (Gal)), 71.1 (C-5 (Gal)), 70.2 (C-4 (Fuc)), 69.5 (C-2 (Gal)), 67.5 (C-4 (Gal)), 66.6 (C-5 (Fuc)), 62.1 (OCHH (Linker)), 61.5 (C-6 (GlcNAc)), 61.0 (C-6 (Gal)), 56.4 (C-2 (GlcNAc)), 25.5, 25.5, 25.5 (SiC(CH₃)₃), 22.4 (NHOCCH₃), 19.9, 19.7, 19.6, 19.4 (CH₃), 18.3 (SiC(CH₃)₃), 13.4 (C-6 (Fuc)), -5.6, -6.1 (Si(CH₃)₂);

***M/z* HRMS (MALDI-TOF)** calcd. for C₁₀₂H₁₀₄N₈O₁₉SiZn = 1836.6478 (M)⁺. Found = 1836.6567.



6.2.45 5-(4-(4'-[Hydroxymethyl]-1-*N*,1,2,3-triazol-1'-yl)-phenyl)-10,15,20-trisphenyl-porphyrinato-zinc(II), **108**

Mono azido substituted porphyrin **96** (15 mg, 0.02 mmol) and propargyl alcohol **63** (1.16 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system A, and the crude material purified by column chromatography (MeOH:DCM, 1:19 (v/v)) to yield the product **108**, as a purple solid, (15 mg, 92 %);

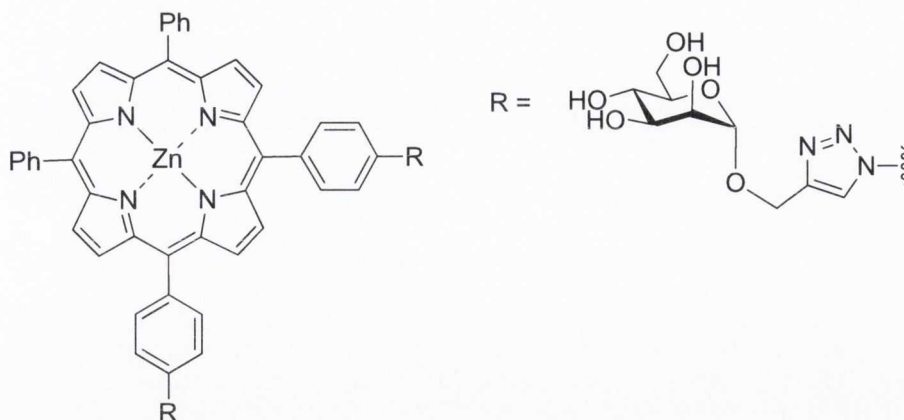
$R_f = 0.39$ (DCM/MeOH 19:1 (v/v));

$\nu_{\max}$ (thin film) 3350 cm^{-1} (OH), 2922 cm^{-1} (CH);

^1H NMR (400 MHz, $\text{CDCl}_3 + 10\%$ MeOD) δ 8.87 (2H, d, $J = 4.6$ Hz, β -H), 8.85 (4H, s, β -H), 8.83 (2H, d, $J = 4.6$ Hz, β -H), 8.38 (1H, s, 5-triazole-H), 8.36 (2H, d, $J = 8.4$ Hz, *o*-Ph), 8.18 (6H, d, $J = 7.4$ Hz, *o*-Ph), 8.09 (2H, d, $J = 8.5$ Hz, *m*-Ph), 7.69 (9H, m, *m*, *p*-Ph), 4.87 (2H s, CH_2);

^{13}C NMR (100 MHz, $\text{CDCl}_3 + 10\%$ MeOD) δ 154.1, 154.1, 154.1, 154.1, 153.9, 153.9, 153.5, 153.5 (Ar α -C), 152.9 (4-triazole-C), 148.5, 147.2, 147.2, 147.2, 140.0 (Ar C), 139.5, 139.5 (Ar CH), 138.4, 138.4, 138.4, 138.4, 138.4, 138.4 (Ar CH), 135.8, 135.8, 135.6, 135.6, 135.6, 135.6, 135.0, 135.0 (β -CH), 131.2, 131.2, 131.2, 130.2, 130.2, 130.2, 130.2, 130.2, 130.2 (Ar CH), 124.8, 124.8, 124.8, 124.8 (Ar C), 122.4, 122.4 (Ar CH), 117.9 (5-triazole-C), 56.0 (CH_2);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{47}\text{H}_{31}\text{N}_7\text{ONa} = 773.1882$ (M) $^+$. Found 773.1863.



6.2.46 5,10-Bis-(4-(4'-[(α -D-mannopyranosyl)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-15,20-diphenylporphyrinatozinc(II), **109**

Di azido substituted porphyrin **97** (16 mg, 0.02 mmol) and propargyl functionalised mannose **65** (10 mg, 0.04 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography (EtOAc:isopropanol:H₂O, 7:2:0.8 (v/v/v)) to yield the product **109**, as a purple solid, (23 mg, 93 %);

R_f = 0.23 (EtOAc:isopropanol:H₂O, 7:2:0.8 (v/v/v));

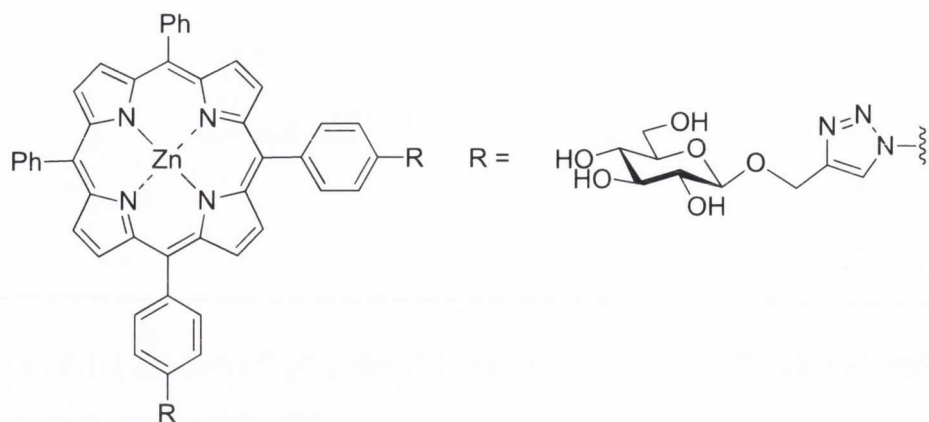
$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at c = 0.01 in CH₂Cl₂/MeOH 1:1);

$\nu_{\max}$ (thin film) 3282 cm⁻¹ (OH), 2923 cm⁻¹ (CH);

¹H NMR (600 MHz, MeOD + 10 % CDCl₃) δ 8.85 (2H, s, β -H), 8.84 (2H, d, J = 4.6 Hz, β -H), 8.75 (4H, br s, β -H), 8.38 (2H, s, 5-triazole-H), 8.15 (4H, d, J = 7.0 Hz, *o*-Ph), 8.10 (4H, br s, *o*-Ph), 7.87 (4H, br s, *m*-Ph), 7.72 (2H, m, *p*-Ph), 7.67 (4H, m, *m*-Ph), 4.99 (2H, br s, H-1), 4.91 (2H, d, J = 12.3 Hz, OCH(H)), 4.75 (2H, d (H₂O overlap), J = 12.1 Hz, OCH(H)), 3.94 (2H, m, $J_{6',5}$ = 1.8 Hz, H-6'), 3.93 (2H, m, H-2), 3.74 (2H, dd, $J_{6,6'}$ = 11.8 Hz, $J_{6,5}$ = 5.0 Hz, H-6), 3.82 (2H, m, H-3), 3.72 (4H, m, H-4, H-5);

¹³C NMR (150 MHz, MeOD + 10 % CDCl₃) δ 150.1, 150.1, 150.0, 150.0, 149.6, 149.6, 149.4, 149.4 (Ar α -C), 144.7, 144.7, 144.1, 144.1, 143.0, 143.0, 135.8, 135.8 (Ar C), 135.0, 135.0, 135.0, 134.1, 134.1, 134.1, 134.1 (Ar CH), 131.7, 131.7, 131.5, 131.5, 131.0, 131.0, 130.8, 130.8 (Ar β -CH), 127.0, 127.0, 126.0, 126.0, 126.0, 126.0 (Ar CH), 122.1, 122.1 (5-triazole-C), 121.0, 121.0, 118.3, 118.3 (Ar C), 117.9, 117.9, 117.9, 117.9 (Ar CH), 99.3, 99.3 (C-1), 73.3, 73.3 (C-5), 71.1, 71.1 (C-3), 70.5, 70.5 (C-2), 67.1, 67.1 (C-4), 61.5, 61.5 (C-6), 59.3, 59.3 (OCH₂);

***M/z* HRMS (MALDI-TOF)** calcd. for C₆₂H₅₄N₁₀O₁₂Zn = 1194.3214 (M)⁺. Found 1194.3215



6.2.47 5,10-Bis-(4-(4'-[(β -D-glucopyranosyl)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-15,20-diphenyl-porphyrinatozinc(II), **110**

Di azido substituted porphyrin **97** (16 mg, 0.02 mmol) propargyl functionalised glucose **67** (10 mg, 0.04 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography (EtOAc:Isopropanol:H₂O, 7:2:1 (v/v/v)) to yield the product **110**, as a purple solid, (23 mg, 91 %);

R_f = 0.25 (EtOAc:Isopropanol:H₂O, 7:2:0.8 (v/v/v));

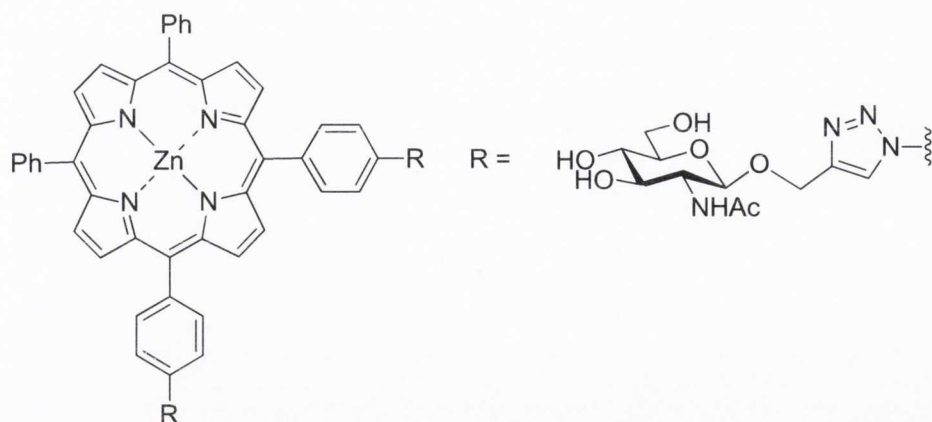
$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at c = 0.01 in CH₂Cl₂/MeOH 1:1);

$\nu_{\max}$ (thin film) 3336 cm⁻¹ (OH), 2990 cm⁻¹ (CH);

¹H NMR (600 MHz, MeOD + 10 % CDCl₃) δ 8.84 (4H, br s, β -H), 8.79 (4H, br s, β -H), 8.53 (2H, s, 5-triazole-H), 8.21 (4H, d, J = 7.6 Hz, *o*-Ph), 8.16 (4H, br s, *o*-Ph), 7.99 (4H, d, J = 7.6 Hz, *m*-Ph), 7.70 (6H, m, *m*, *p*-Ph), 5.02 (2H, d, J = 12.1 Hz, OCH(H)), 4.90 (2H, d, J = 12.2 Hz, OCH(H)), 4.45 (2H, d, $J_{1,2}$ = 7.7 Hz, H-1), 3.91 (2H, dd, $J_{6',6}$ = 12.0 Hz, H-6'), 3.74 (2H, dd, $J_{6,6'}$ = 12.1 Hz, $J_{6,5}$ = 5.7 Hz, H-6), 3.44 (2H, dd, $J_{3,2} = J_{3,4}$ = 9.03, H-3), 3.37 (2H, m, H-4), 3.35 (2H, m, H-2), 3.34 (2H, m, H-5);

¹³C NMR (150 MHz, MeOD + 10 % CDCl₃) δ 150.1, 150.1, 150.0, 150.0, 149.6, 149.6, 149.5, 149.5 (Ar α -C), 145.2, 145.2, 144.1, 144.1, 143.1, 143.1, 135.9, 135.9 (Ar C), 135.1, 135.1, 135.1, 134.1, 134.1, 134.1, 134.1 (Ar CH), 131.7, 131.7, 131.4, 131.4, 130.9, 130.9, 130.7, 130.7 (Ar β -CH) 126.9, 126.9, 125.9, 125.9, 125.9, 125.9 (Ar CH), 122.1, 122.1 (5-triazole-C), 120.9, 120.9, 118.3, 118.3 (Ar C), 117.9, 117.9, 117.9, 117.9 (Ar CH), 102.3, 102.3 (C-1), 76.4, 76.4 (C-5), 76.4, 76.4 (C-3), 73.5, 73.5 (C-2), 70.1, 70.1 (C-4), 61.7, 61.7 (OCH₂), 61.3, 61.3 (C-6);

M/z HRMS (MALDI-TOF) calcd. for C₆₂H₅₄N₁₀O₁₂Zn = 1194.3214 (M)⁺. Found 1194.3231.



6.2.48 5,10-Bis-(4-(4'-[(2-acetamido-2-deoxy- β -D-glucopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-15,20-diphenylporphyrinatozinc(II), **111**

Di azido substituted porphyrin **97** (16 mg, 0.02 mmol) and propargyl functionalised GlcNAc **70** (12 mg, 0.04 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography (EtOAc:EtOH:H₂O, 7:2:1 (v/v/v)) to yield the product **111**, as a purple solid, (22 mg, 82 %);

R_f = 0.3 (EtOAc:EtOH:H₂O, 7:2:1 (v/v/v))

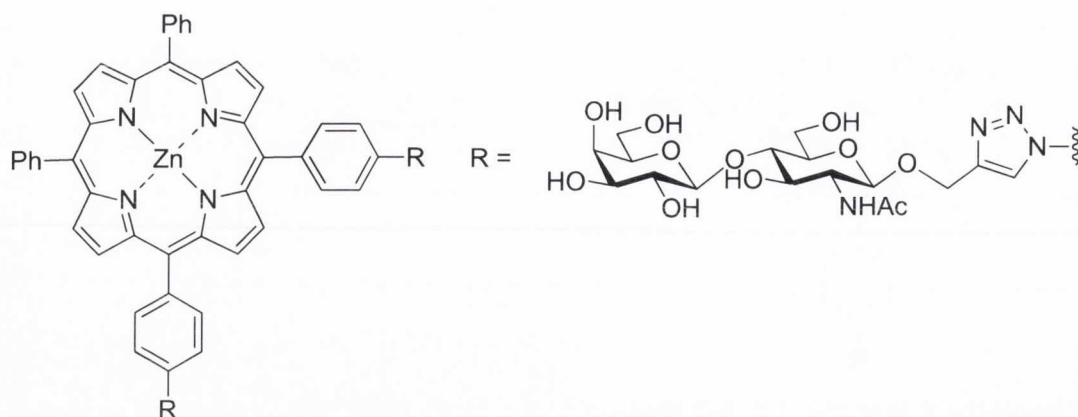
$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at c = 0.01 in CH₂Cl₂/MeOH 1:1);

$\nu_{\max}$ (thin film) 3366 cm⁻¹ (OH), 1647 cm⁻¹ (NHC=O);

¹H NMR (600 MHz, MeOD + 5 % CDCl₃) δ 8.88 (2H, d, J = 4.6 Hz, β -H), 8.87 (2H, s, β -H), 8.86 (2H, s, β -H), 8.85 (2H, d, J = 4.7 Hz, β -H), 8.70 (2H, s, 5-triazole-H), 8.33 (4H, d, J = 8.1 Hz, *o*-Ph), 8.20 (4H, d, J = 7.2 Hz, *o*-Ph), 8.16 (4H, d, J = 8.1 Hz, *m*-Ph), 7.75 (6H, m, *m*, *p*-Ph), 5.11 (2H, d, J = 12.3 Hz, OCH(H)), 4.96 (2H, d, J = 12.4 Hz, OCH(H)), 4.68 (2H, d, $J_{1,2}$ = 8.5 Hz, H-1), 3.99 (1H, dd, $J_{6',6}$ = 12.1 Hz, $J_{6',5}$ = 1.4 Hz, H-6'), 3.80 (2H, m, H-6), 3.78 (2H, dd, $J_{2,3}$ = 10.2, $J_{2,1}$ = 8.4 Hz, H-2), 3.55 (2H, dd, $J_{3,4}$ = 10.5 Hz, $J_{3,2}$ = 8.5 Hz, H-3), 3.42 (4H, m, H-4, H-5), 2.03 (6H, s, CH₃);

¹³C NMR (150 MHz, MeOD + 5 % CDCl₃) δ 172.4, 172.4 (C=O), 150.1, 150.1, 150.0, 150.0, 149.6, 149.6, 149.5, 149.5 (Ar α -C), 145.3, 145.3, 144.2, 144.2, 143.1, 143.1, 136.1, 136.1 (Ar C), 135.2, 135.2, 135.2, 135.2, 134.1, 134.1, 134.1, 134.1 (Ar CH), 131.6, 131.6, 131.3, 131.3, 130.9, 130.9, 130.7, 130.7 (Ar β -CH), 126.9, 126.9, 125.9, 125.9, 125.9, 125.9 (Ar CH), 122.3 (5-triazole-C), 120.9, 120.9, 118.4, 118.4 (Ar C), 117.9, 117.9, 117.9, 117.9 (Ar CH), 100.4, 100.4 (C-1), 76.6, 76.6 (C-5), 74.6, 74.6 (C-3), 70.7, 70.7 (C-4), 61.4, 61.4 (OCH₂), 61.4, 61.4 (C-6), 55.9, 55.9 (C-2), 21.7, 21.7 (CH₃);

M/z HRMS (MALDI-TOF) calcd. for C₆₆H₆₀N₁₂O₁₂Zn = 1276.3745 (M)⁺. Found 1276.3737



6.2.49 5,10-Bis-(4-(4'-[(2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside)oxymethyl]-1-N-1,2,3-triazol-1'-yl)phenyl)-15,20-diphenylporphyrinatozinc(II), **112**

Di azido substituted porphyrin **97** (16 mg, 0.02 mmol) and propargyl functionalised LacNAc **77** (18 mg, 0.04 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography (EtOAc:EtOH:H₂O, 65:20:15 (v/v/v)) to yield the product **112**, as a purple solid, (26 mg, 77 %);

$R_f = 0.15$ (EtOAc:EtOH:H₂O, 65:22:15 (v/v/v));

$[\alpha]_D^{20} = \text{N/A}$ (Sample too dark for analysis at $c = 0.01$ in CH₂Cl₂/MeOH 1:1);

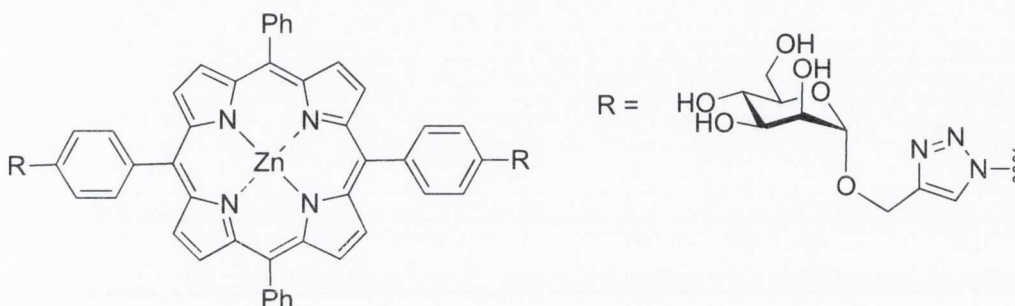
ν_{max} (thin film) 3353 cm⁻¹ (OH, NH), 1640 cm⁻¹ (NHC=O);

¹H NMR (600 MHz, MeOD + 5 % CDCl₃) δ 8.87 (8H, m, β -H), 8.69 (2H, s, 5-triazole-H), 8.34 (4H, d, $J = 7.7$ Hz, *o*-Ph), 8.20 (4H, d, $J = 6.7$ Hz, *o*-Ph), 8.17 (4H, d, $J = 7.8$ Hz, *m*-Ph), 7.76 (6H, m, *m*, *p*-Ph), 5.09 (2H, d, $J = 12.4$ Hz, OCH(H)), 4.95 (2H, d, $J = 12.4$ Hz, OCH(H)), 4.68 (2H, d, $J = 8.3$ Hz, H-1a), 4.42 (1H, d, $J_{1,2} = 7.7$ Hz, H-1b), 4.03 (2H, dd, $J_{6',6} = 12.2$ Hz, $J_{6',5} = 2.4$ Hz, H-6a'), 3.95 (2H, dd, $J_{6,6'} = 12.3$ Hz, $J_{6,5} = 4.5$ Hz, H-6a), 3.86 (2H, dd, $J_{2,1} = J_{2,3} = 9.1$ Hz, H-2a), 3.84 (2H, d, $J_{4,3} = 3.7$ Hz, H-4b), 3.80 (2H, dd, $J_{6,6'} = 11.6$ Hz, $J_{6,5} = 7.9$ Hz, H-6b), 3.73 (2H, m, H-6b'), 3.72 (2H, m, H-3a), 3.68 (2H, m, H-4a), 3.61 (2H, m, H-5b), 3.59 (2H, dd, $J_{2,3} = 9.1$ Hz, $J_{2,1} = 7.5$ Hz, H-2b), 3.55, (2H, m, H-5a) 3.51 (2H, dd, $J_{3,2} = 9.2$ Hz, $J_{3,4} = 3.2$ Hz, H-3b), 2.02 (6H, s, CH₃);

¹³C NMR (150 MHz, MeOD + 5 % CDCl₃) δ 172.2, 172.2 (C=O), 150.1, 150.1, 150.0, 150.0, 149.6, 149.6, 149.5, 149.5 (Ar α -C), 145.3, 145.3 (4-triazole-C), 144.2, 144.2, 143.1, 143.1, 136.1, 136.1 (Ar C), 135.2, 135.2, 135.2, 135.2, 134.1, 134.1, 134.1, 134.1 (Ar CH), 131.5, 131.5, 131.3, 131.3, 130.9, 130.9, 130.7, 130.7 (Ar β -CH), 126.9, 126.9, 125.9, 125.9,

125.9, 125.9 (Ar CH), 122.2, 122.2 (5-triazole-CH), 120.9, 120.9, 118.4, 118.4 (Ar C), 117.9, 117.9, 117.9 (Ar CH), 103.6, 103.6 (C-1b), 100.4, 100.4 (C-1a), 79.5, 79.5 (C-4a), 75.6, 75.6 (C-5b), 75.2, 75.2 (C-5a), 73.3, 73.3 (C-3b), 72.6, 72.6 (C-3a), 71.1, 71.1 (C-2b), 68.8, 68.8 (C-4b), 61.4, 61.4 (OCH₂), 61.0, 61.0 (C-6b), 60.5, 60.5 (C-6a), 55.2, 55.2 (C-2a), 21.6, 21.6 (CH₃);

M/z HRMS (MALDI-TOF) calcd. for C₇₈H₈₀N₁₂O₂₂Zn = 1600.4802 (M)⁺. Found = 1600.4767.



6.2.50 5,15-Bis-(4-(4'-[(α -D-mannopyranosyl)oxymethyl]-1-N-1,2,3-triazol-1'-yl)phenyl)-10,20-diphenylporphyrinatozinc(II), **113**

Di azido substituted porphyrin **98** (16 mg, 0.02 mmol) and propargyl functionalised mannose **65** (10 mg, 0.04 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography (EtOAc:isopropanol:H₂O, 7:2:0.5 (v/v/v)) to yield the product **113**, as a purple solid, (21 mg, 85%);

R_f = 0.15 (EtOAc:isop:H₂O, 7:2:0.5 (v/v/v)),

$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at c = 0.01 in CH₂Cl₂/MeOH 1:1);

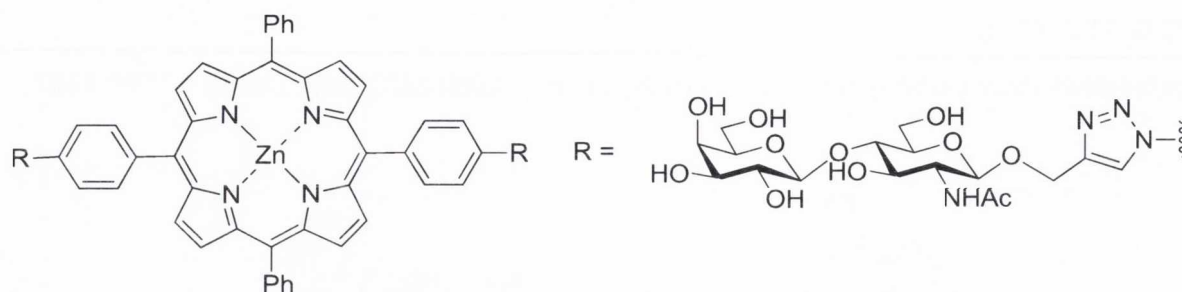
$\nu_{\max}$ (thin film) 3363 cm⁻¹ (OH), 2923 cm⁻¹ (CH);

¹H NMR (600 MHz, MeOD + 10 % CDCl₃) δ 8.87 (4H, d, J = 4.3 Hz, β -H), 8.81 (4H, d, J = 4.2 Hz, β -H), 8.55 (2H, s, 5-triazole-H), 8.26 (4H, d, J = 7.7 Hz, o -Ph), 8.18 (4H, d, J = 7.1 Hz, o -Ph), 8.04 (4H, d, J = 7.7 Hz, m -Ph), 7.74 (6H, m, m , p -Ph), 5.01 (2H, br s, H-1), 4.97 (2H, d, J = 12.3 Hz, OCH(H)), 4.82 (2H, d, J = 12.3 Hz, OCH(H)), 3.94 (4H, m, H-6', H-2), 3.83 (4H, m, H-6, H-3), 3.72 (4H, m, H-4, H-5);

¹³C NMR (150 MHz, MeOD + 10 % CDCl₃) δ 150.1, 150.1, 150.1, 150.1, 149.6, 149.6, 149.6, 149.6 (Ar α -C), 144.8, 144.8, 144.3, 144.3, 143.1, 143.1, 135.9, 135.9 (Ar C), 135.0, 135.0, 135.0, 134.2, 134.2, 134.2, 134.2 (Ar CH), 131.7, 131.7, 131.7, 131.7, 131.9, 131.9, 131.9, 131.9 (Ar β -CH), 127.1, 127.1, 126.1, 126.1, 126.1, 126.1 (Ar CH), 122.1, 122.1 (5-triazole-C), 120.9, 120.9, 118.5, 118.5 (Ar C), 118.1, 118.1, 118.1, 118.1 (Ar CH),

99.4, 99.4 (C-1), 73.3, 73.3 (C-5), 71.1, 71.1 (C-3), 70.5, 70.5 (C-2), 67.2, 67.2 (C-4), 61.6, 61.6 (C-6), 59.5, 59.5 (OCH₂);

M/z HRMS (MALDI-TOF) calcd. for C₆₂H₅₄N₁₀O₁₂Zn = 1194.3214 (M)⁺. Found 1194.3223



6.2.51 5,15-Bis-(4-(4'-[(2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-10,20-diphenylporphyrinatozinc(II), **114**

Di azido substituted porphyrin **98** (16 mg, 0.02 mmol) and propargyl functionalised LacNAc **77** (18 mg, 0.04 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography (EtOAc:EtOH:H₂O, 65:20:15 (v/v/v)) to yield the product **114**, as a purple solid, (25 mg, 75%);

R_f = 0.22 (EtOAc:EtOH:H₂O, 65:20:15 (v/v/v));

$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at $c = 0.01$ in CH₂Cl₂/MeOH 1:1);

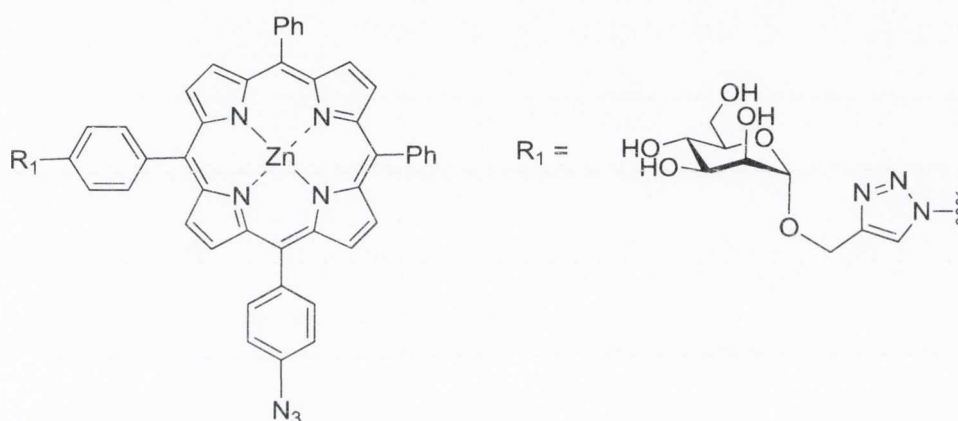
$\nu_{\max}$ (thin film) 3370 cm⁻¹ (OH), 2922 cm⁻¹ (CH), 1638 cm⁻¹ (NHC=O);

¹H NMR (600 MHz, MeOD + 5 % CDCl₃) δ 8.89 (4H, d, $J = 4.4$ Hz, β -H), 8.88 (4H, d, $J = 4.5$ Hz, β -H), 8.73 (2H, s, 5-triazole-H), 8.40 (4H, d, $J = 7.9$ Hz, *o*-Ph), 8.22 (8H, m, *m*, *o*-Ph), 7.76 (6H, m, *m*, *p*-Ph), 5.13 (2H, d, $J = 12.5$ Hz, OCH(H)), 4.97 (2H, d, $J = 12.5$ Hz, OCH(H)), 4.71 (2H, d, $J = 8.4$ Hz, H-1a), 4.42 (2H, d, $J_{1,2} = 7.6$ Hz, H-1b), 4.04 (2H, dd, $J_{6',6} = 12.3$ Hz, $J_{6',5} = 2.2$ Hz, H-6a'), 3.95 (2H, dd, $J_{6,6'} = 12.3$ Hz, $J_{6,5} = 4.6$ Hz, H-6a), 3.87 (2H, dd, $J_{2,1} = J_{2,3} = 9.0$ Hz, H-2a), 3.85 (2H, d, $J_{4,3} = 3.3$ Hz, H-4b), 3.81 (2H, dd, $J_{6,6'} = 11.6$ Hz, $J_{6,5} = 7.2$ Hz, H-6b), 3.73 (4H, m, H-6b', H-3a), 3.69 (2H, app t, $J_{4,3} = J_{4,5} = 8.7$ Hz, H-4a), 3.61 (2H, m, H-5b), 3.59 (2H, dd, $J_{2,3} = 9.5$ Hz, $J_{2,1} = 7.8$ Hz, H-2b), 3.54, (2H, m, H-5a), 3.52 (2H, dd, $J_{3,2} = 9.6$ Hz, $J_{3,4} = 3.2$ Hz, H-3b), 2.03 (6H, s, CH₃);

¹³C NMR (150 MHz, MeOD + 5 % CDCl₃) δ 172.2, (C=O), 150.1, 150.1, 150.1, 150.1, 149.6, 149.6, 149.6 (Ar α -C), 145.4, 145.4, 143.3, 143.3, 143.1, 143.1, 136.1, 136.1 (Ar C),

135.3, 135.3, 135.3, 135.3, 134.2, 134.2, 134.2, 134.2 (Ar CH), 131.6, 131.6, 131.6, 131.6, 130.8, 130.8, 130.8, 130.8 (Ar β -CH), 127.0, 127.0, 125.9, 125.9, 125.9, 125.9 (Ar CH), 122.3 (5-triazole-CH), 120.8, 120.8, 118.5, 118.5 (Ar C), 118.1, 118.1, 118.1, 118.1 (Ar CH), 103.6, 103.6 (C-1b), 100.5, 100.5 (C-1a), 79.6, 79.6 (C-4a), 75.6, 75.6 (C-5b), 75.2, 75.2 (C-5a), 73.3, 73.3 (C-3b), 72.6, 72.6 (C-3a), 71.1, 71.1 (C-2b), 68.8, 68.8 (C-4b), 61.6, 61.6 (OCH₂), 61.1, 61.1 (C-6b), 60.7, 60.7 (C-6a), 55.2, 55.2 (C-2a), 21.8, 21.8 (CH₃);

***M/z* HRMS (MALDI-TOF)** calcd. for C₇₈H₈₀N₁₂O₂₂ZnNa = 1623.4699 (M+Na)⁺. Found = 1623.4661.



6.2.52 5-(4-Azidophenyl)-10-(4-(4'-[(α -D-mannopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-15,20-diphenylporphyrinatozinc(II), **115**

Di azido substituted porphyrin **97** (52 mg, 0.07 mmol) propargyl functionalised mannose **65** (5 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B for 10 minutes, and the crude material purified by column chromatography (EtOAc:isopropanol:H₂O, 7:2:1 (v/v/v)) to yield the product **115**, as a purple solid (15 mg, 67 %); (1.7 eq. of SM recovered, 15% disubstituted product recovered);

R_f = 0.27 (EtOAc:EtOH:H₂O, 7:2:1 (v/v/v));

$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at $c = 0.01$ in CH₂Cl₂/MeOH 1:1);

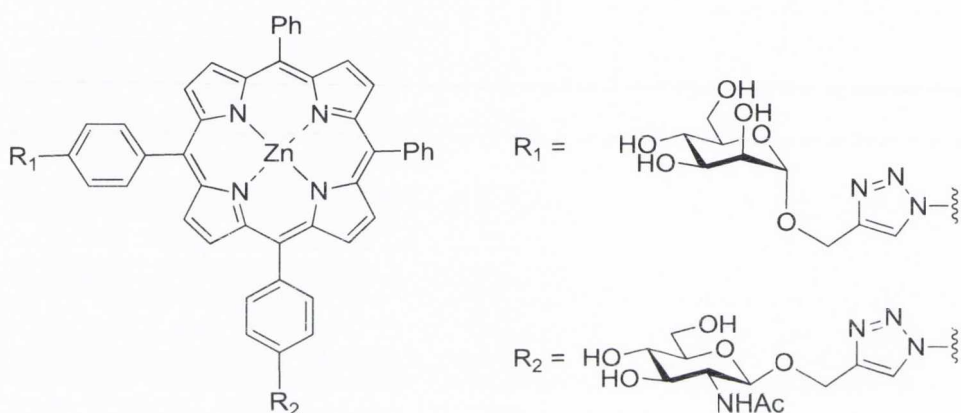
$\nu_{\max}$ (thin film) 3392 cm⁻¹ (OH), 2925 cm⁻¹ (CH), 2124 cm⁻¹ (N₃);

¹H NMR (600 MHz, CDCl₃ + 50 % MeOD) δ 8.77 (8H, m, β -H), 8.42 (1H, s, 5-triazole-H), 8.27 (2H, d, $J = 7.4$ Hz, *o*-Ph (Man)), 8.08 (6H, m, *o*-Ph, *o*-Ph(N₃)), 8.02 (2H, d, $J = 7.4$ Hz, *m*-Ph), 7.61 (6H, m, *m*, *p*-Ph), 7.29 (2H, d, $J = 7.4$ Hz, *m*-Ph(N₃)), 4.90 (1H, br s, H-1), 4.86 (1H, d, $J = 12.5$ Hz, OCH(H)), 4.71 (1H, d, $J = 12.2$ Hz, OCH(H)), 3.83 (1H, br s, H-2), 3.79 (1H, d,

$J_{6',6} = 11.4$ Hz, H-6'), 3.74 (1H, dd, $J_{6,6'} = 11.5$ Hz, $J_{6,5} = 4.7$ Hz, H-6), 3.71 (1H, m, H-3), 3.62 (1H, m, H-4), 3.59 (1H, m, H-5);

^{13}C NMR (150 MHz, CDCl_3 + 50 % MeOD) δ 150.1, 150.1, 150.0, 149.9, 149.9, 149.8, 149.5, 149.4 (Ar α -C), 144.8, 144.5, 143.1, 143.1, 140.0, 139.2, 135.9 (Ar C), 135.4, 135.4, 135.3, 135.3, 134.3, 134.3, 134.3, 134.3 (Ar CH), 131.8, 131.7, 131.6, 131.5, 131.4, 131.1, 130.9, 130.8 (Ar β -CH), 127.1, 127.1, 126.1, 126.1, 126.1, 126.1 (Ar CH), 122.1 (5-triazole-C), 120.9, 120.8, 119.3 (Ar meso C), 118.3, 118.3 (Ar CH), 118.1 (Ar meso C), 116.8, 116.8 (Ar CH), 99.2 (C-1), 72.9 (C-5), 71.1 (C-3), 70.4 (C-2), 67.2 (C-4), 61.5 (C-6), 59.6 (OCH_2);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{53}\text{H}_{40}\text{N}_{10}\text{O}_{10}\text{Zn} = 976.2424$ (M)⁺. Found 976.2383.



6.2.53 5-((4-(4'-[(2-Acetamido-2-deoxy- β -D-glucopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-10-(4-(4'-[(α -D-mannopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-15,20-diphenylporphyrinatozinc(II), **116**

Mono glycosylated glyco-azido porphyrin **115** (18 mg, 0.02 mmol) and propargyl functionalised GlcNAc **70** (6 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography ($\text{EtOAc}:\text{EtOH}:\text{H}_2\text{O}$, 7:2:1 (v/v/v)) to yield the product **116**, as a purple solid (19.5 mg, 85 %);

$R_f = 0.19$ ($\text{EtOAc}:\text{EtOH}:\text{H}_2\text{O}$, 7:2:1 (v/v/v));

$[\alpha]_D^{20} = \text{N/A}$ (Sample too dark for analysis at $c = 0.01$ in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1);

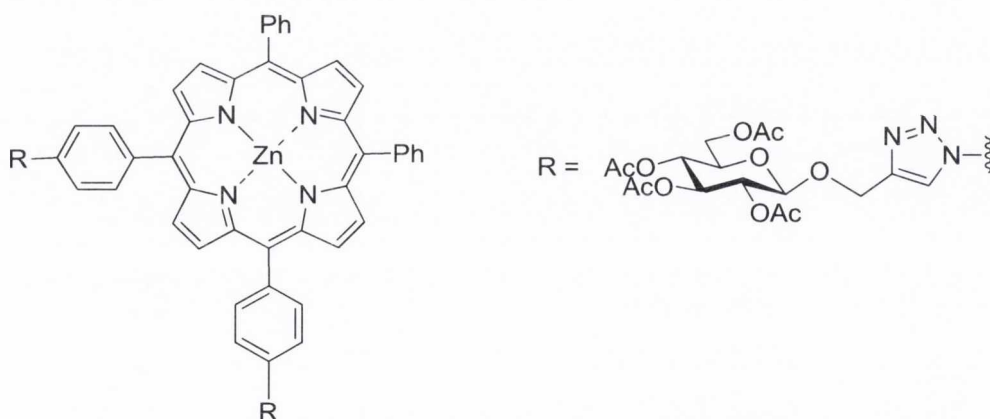
ν_{max} (thin film) 3371 cm^{-1} (OH), 2927 cm^{-1} (CH), 1640 cm^{-1} (C=O);

^1H NMR (600 MHz, MeOD + 10 % CDCl_3) δ 8.85 (4H, m, β -H), 8.80 (2H, m, β -H), 8.76 (2H, m, β -H), 8.59 (1H, s, 5-triazole-H (GlcNAc)), 8.49 (1H, s, 5-triazole-H (Man)), 8.24 (2H, d, $J = 7.3$ Hz, *o*-Ph(GlcNAc)), 8.16 (4H, $J = 6.6$ Hz, *o*-Ph), 8.14 (2H, d, $J = 7.4$ Hz, *o*-Ph (Man)), 8.05

(2H, d, $J = 7.2$ Hz, *m*-Ph (GlcNAc)), 7.92 (2H, d, $J = 7.4$ Hz, *m*-Ph (Man)), 7.71 (6H, m, *m*, *p*-Ph), 5.08 (1H, d, $J = 12.3$ Hz, OCH(H) (GlcNAc)), 5.00 (1H, br s, H-1 (Man)), 4.93 (2H, d, $J = 12.0$ Hz, OCH(H) (GlcNAc), OCH(H) (Man)), 4.77 (1H, d, $J = 12.3$ Hz, OCH(H) (Man)), 4.67 (1H, d, $J_{1,2} = 8.2$ Hz, H-1 (GlcNAc), 4.52 (1H, br s, NH), 3.99 (1H, d, $J_{6',6} = 11.7$ Hz, H-6' (GlcNAc)), 3.94 (1H, d, $J_{6',6} = 12.0$ Hz, H-6' (Man)), 3.92 (1H, m, H-2 (Man)), 3.81 (1H, dd, $J_{6,6'} = 12.0$ Hz, $J_{6,5} = 4.0$ Hz, H-6 (Man)), 3.80 (2H, m, H-6 (GlcNAc), H-3 (Man)), 3.77 (1H, m, H-2 (GlcNAc)), 3.71 (2H, m, H-4 (Man), H-5 (Man)), 3.55 (1H, m, H-3 (GlcNAc)), 3.41 (2H, m, H-4 (GlcNAc), H-5 (GlcNAc)), 2.01 (3H, s, CH₃ (GlcNAc));

¹³C NMR (150 MHz, MeOD + 10 % CDCl₃) δ 172.4 (C=O), 150.1, 150.1, 149.9, 149.9, 149.6, 149.6, 149.5, 149.4 (Ar α -C), 145.3, 144.8 (4-triazole-C), 144.1, 144.1, 143.1, 143.1, 136.0, 135.9 (Ar C), 135.1, 135.1, 135.0, 135.0, 134.1, 134.1, 134.1, 134.1 (Ar CH), 131.6, 131.6, 131.3, 131.3, 130.9, 130.9, 130.7, 130.7 (Ar β -CH), 126.9, 126.9, 125.9, 125.9, 125.9, 125.9 (Ar CH), 122.1 (5-triazole-CH (GlcNAc)), 122.1 (5-triazole-CH (Man)), 120.9, 120.9, 118.3, 118.3 (Ar meso C), 117.9, 117.9, 117.8, 117.8 (Ar CH), 100.4 (C-1 (GlcNAc)), 99.4 (C-1 (Man)), 76.6 (C-5 (GlcNAc)), 74.5 (C-3 (GlcNAc)), 73.4 (C-5 (Man)), 71.0 (C-3 (Man)), 70.7 (C-2 (GlcNAc)), 70.5 (C-4 (GlcNAc)), 67.2 (C-4 (Man)), 61.5 (C-6 (Man)), 61.4 (C-6 (GlcNAc)), 61.4 (OCH₂ (GlcNAc)), 59.3 (OCH₂ (Man)), 55.9 (C-2 (GlcNAc)), 21.7 (CH₃);

***M/z* HRMS (MALDI-TOF)** calcd. for C₆₄H₅₇N₁₁O₁₂Zn = 1235.3480 (M)⁺. Found 1235.3436.



6.2.54 5,10-(Di(4-(4'-[(2,3,4,6-tetra-O-acetyl- β -D-glucopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-15,20-diphenylporphyrinatozinc(II), **117**

Di azido substituted porphyrin **97** (22 mg, 0.03 mmol), propargyl substituted per OAc glucose **66** (13 mg, 0.03 mmol) and propargyl substituted mannose **65** (8 mg, 0.03 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in

solvent system B, and the crude material purified by column chromatography (DCM:MeOH, 12:1 (v/v)) to yield the product **117**, as purple solid (7 mg, 15 %);

$R_f = 0.64$ (DCM:MeOH, 9:1 (v/v));

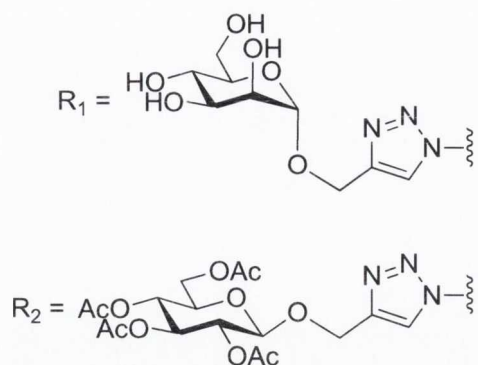
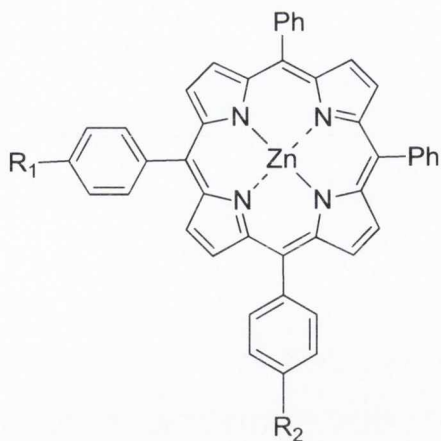
$[\alpha]_D^{20} = \text{N/A}$ (Sample too dark for analysis at $c = 0.01$ in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1);

ν_{max} (thin film) 2964 cm^{-1} (CH), 1744 cm^{-1} (C=O);

$^1\text{H NMR}$ (600 MHz, CDCl_3) δ 8.93 (2H, d, $J = 4.6\text{ Hz}$, $\beta\text{-H}$), 8.92 (2H, s, $\beta\text{-H}$), 8.90 (2H, s, $\beta\text{-H}$), 8.89 (2H, d, $J = 4.6\text{ Hz}$, $\beta\text{-H}$), 8.39 (4H, d, $J = 8.1\text{ Hz}$, $o\text{-Ph}$), 8.31 (2H, s, 5-triazole-H), 8.22 (4H, d, $J = 7.6\text{ Hz}$, $o\text{-Ph-H}$), 8.13 (2H, d, $J = 8.1\text{ Hz}$, $m\text{-Ph}$), 7.77 (6H, m, $m, p\text{-Ph}$), 5.31 (2H, t, $J_{3,2} = J_{3,4} = 9.1\text{ Hz}$, H-3), 5.19 (2H, app t, $J_{4,3} = J_{4,5} = 9.7\text{ Hz}$, H-4), 5.18 (2H, d, $J = 12.6\text{ Hz}$, $\text{OCH}(\text{H})$), 5.13 (2H, dd, $J_{3,2} = 8.9\text{ Hz}$, $J_{2,1} = 7.9\text{ Hz}$, H-2), 4.86 (2H, d, $J = 12.6\text{ Hz}$, $\text{OCH}(\text{H})$), 4.82 (2H, d, $J_{2,1} = 7.9\text{ Hz}$, H-1), 4.35 (2H, dd, $J_{6,6'} = 12.5\text{ Hz}$, $J_{6,5} = 4.8\text{ Hz}$, H-6), 4.22 (2H, dd, $J_{6,6'} = 12.5\text{ Hz}$, $J_{6',5} = 2.3\text{ Hz}$, H-6'), 3.85 (2H, ddd, $J_{5,4} = 9.7\text{ Hz}$, $J_{5,6} = 4.8\text{ Hz}$, $J_{5,6'} = 2.3\text{ Hz}$, H-5), 2.16, 2.11, 2.08, 2.05 (6H, s, CH_3);

$^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 170.2, 169.8, 169.0, 168.9 (C=O), 149.9, 149.9, 149.9, 149.8, 149.8, 149.2, 149.2 (Ar $\alpha\text{-C}$), 143.8, 143.6, 142.4, 142.4, 142.4, 135.4 (Ar C), 134.9, 134.9, 134.0, 134.0, 134.0, 134.0, 134.0, 133.0 (Ar CH), 131.9, 131.9, 131.7, 131.7, 131.6, 131.6, 130.8, 130.8 (Ar $\beta\text{-CH}$), 127.1, 127.1, 127.1, 126.1, 126.1, 126.1, 126.1, 126.1, 126.1 (Ar CH), 121.0, 120.9, 120.9 (Ar C), 120.0 (5-triazole-C), 118.2 (Ar C), 117.8, 117.8 (Ar CH), 99.7 (C-1), 72.6 (C-3), 71.4 (C-2), 70.7 (C-5), 67.7 (C-4), 61.9 (OCH_2), 61.3 (C-6);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{78}\text{H}_{70}\text{N}_{10}\text{O}_{20}\text{Zn} = 1530.4059$ (M) $^+$. Found 1530.4042.



6.2.55 5-((4'-[(2,3,4,6-Tetra-O-acetyl- β -D-glucopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-10-(4'-[(α -D-mannopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-15,20-diphenylporphyrinatozinc(II), **118**

Di azido substituted porphyrin **97** (22 mg, 0.03 mmol), propargyl substituted per OAc glucose **66** (13 mg, 0.03 mmol) and propargyl substituted mannose **65** (8 mg, 0.03 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography (DCM:MeOH, 9:1 (v/v)) to yield the product **118**, as purple solid (13.3 mg, 33 %);

R_f = 0.21 (DCM:MeOH, 9:1 (v/v));

$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at c = 0.01 in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1);

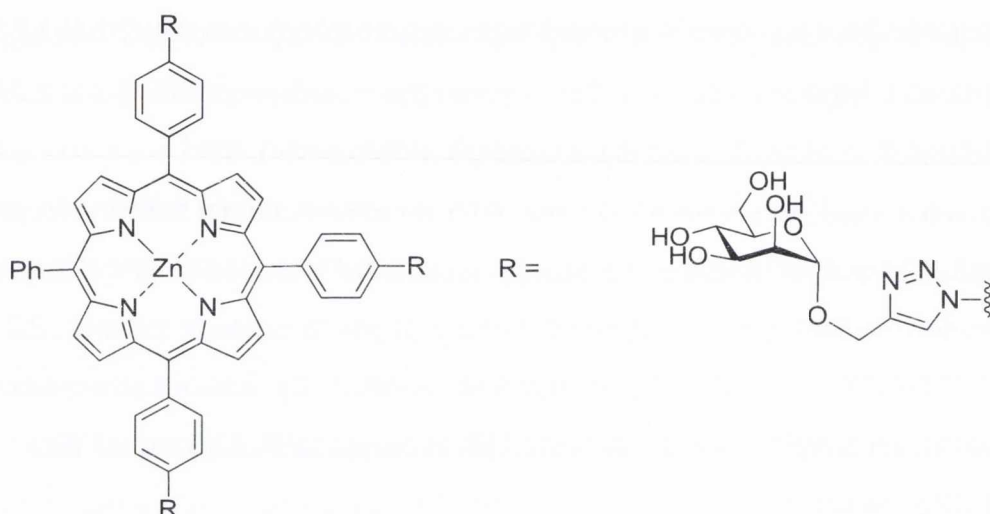
ν_{max} (thin film) 3385 cm^{-1} (OH), 2922 cm^{-1} (CH), 1755 cm^{-1} (C=O);

^1H NMR (600 MHz, MeOD + 30 % CDCl_3) δ 8.88 (8H, m, β -H), 8.82 (1H, s, 5-triazole-H), 8.48 (1H, s, 5-triazole-H), 8.39 (2H, d, J = 7.1 Hz, *o*-Ph), 8.36 (2H, J = 7.8 Hz, *o*-Ph), 8.23 (4H, d, J = 7.6 Hz, *o*-Ph), 8.20 (2H, d, J = 7.1 Hz, *m*-Ph), 8.14 (2H, d, J = 7.7 Hz, *m*-Ph), 7.79 (6H, m, *m*, *p*-Ph), 5.25 (1H, app t, $J_{3,4} = J_{3,2} = 9.6$ Hz, H-3 (Glc)), 5.06 (1H, app t, $J_{4,3} = J_{4,5} = 9.6$ Hz, H-4 (Glc)), 5.03 (1H, s, H-1 (Man)), 5.02 (1H, d, J = 12.0 Hz, OCH(H)), 4.96 (1H, dd, $J_{2,3} = 9.6$ Hz, $J_{2,1} = 8.5$ Hz), 4.90 (2H, under solvent, OCH₂) (Glc)), 4.89 (H, d, J = 12.0 Hz, OCH(H) (Man)), 4.72 (1H, d, $J_{1,2} = 8.2$ Hz, H-1 (Glc)), 4.72 (1H, d, $J_{1,2} = 8.4$ Hz, H-1 (Glu)), 4.22 (1H, dd, $J_{6,6'} = 11.8$ Hz, $J_{6,5} = 3.8$ Hz, H-6 (Glc)), 4.06 (1H, d, $J_{6',6} = 12.0$ Hz, H-6' (Glu)), 3.95 (1H, dd, $J_{6',6} = 12.3$ Hz, $J_{6',5} = 1.9$ Hz, H-6' (Man)), 3.93 (1H, dd, $J_{2,3} = 2.8$ Hz, $J_{2,1} = 1.6$ Hz, H-2 (Man)), 3.80 (2H, m, H-3 (Man)), 3.70 (3H, m, H-5 (Glc), H-5 (Man), H-4 (Man)), 2.09, 2.07, 2.05, 2.00 (3H, s, CH₃ (Glc));

^{13}C NMR (150 MHz, MeOD + 30 % CDCl_3) δ 171.5, 170.8, 170.1, 169.7 (C=O), 150.1, 150.1, 150.0, 150.0, 149.7, 149.7, 149.5, 149.5 (Ar α -C), 144.9, 144.7 (4-triazole-C), 144.3, 144.2,

143.1, 143.1, 136.2, 136.1 (Ar C), 135.2, 135.2, 135.1, 135.1, 134.1, 134.1, 134.1, 134.1 (Ar CH), 131.5, 131.5, 131.3, 131.3, 130.9, 130.9, 130.7, 130.7 (Ar β -CH), 127.0, 127.0, 126.0, 126.0, 126.0 (Ar CH), 122.3 (5-triazole-CH), 121.0 (5-triazole-CH), 120.9, 120.9, 118.3, 118.3 (Ar meso C), 118.1, 118.1, 118.1, 118.1 (Ar CH), 99.6 (C-1 (Glc)), 99.5 (C-1 (Man)), 73.6 (C-5 (Man)), 72.7 (C-3 Glc), 71.3 (C-5 (Glc)), 71.2 (C-2 (Glc)), 71.0 (C-3 (Man)), 70.5 (C-2 (Man)), 68.1 (C-4 (Glc)), 67.3, C-4 (Man)), 61.7 (OCH₂ (Glc)), 61.5 (C-6 (Man)), 61.3 (C-6 (Glc)), 59.4 (OCH₂ (Man)), 19.4, 19.2, 19.1, 19.1 (CH₃);

M/z HRMS (MALDI-TOF) calcd. for C₆₄H₅₇N₁₁O₁₂Zn = 1362.3637 (M)⁺. Found 1362.3596.



6.2.56 5,10,15-Tris-(4-(4'-[(α -D-mannopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-20-diphenylporphyrinatozinc(II), **119**

Tri azido substituted porphyrin **99** (18 mg, 0.02 mmol) and propargyl functionalised mannose **65** (15 mg, 0.07 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography (EtOAc:EtOH:H₂O, 68:20:12 (v/v/v)) to yield the product **119**, as a purple solid (26 mg, 81 %);

R_f = 0.16 (EtOAc:EtOH:H₂O, 65:20:15 (v/v/v));

$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at c = 0.01 in CH₂Cl₂/MeOH 1:1);

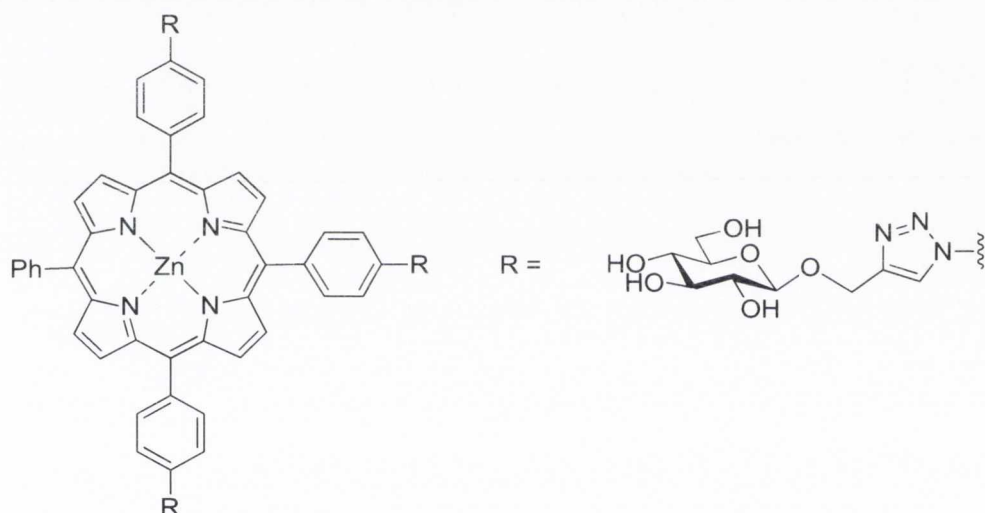
$\nu_{\max}$ (thin film) 3323 cm⁻¹ (OH), 2924 cm⁻¹ (CH);

¹H NMR (600 MHz, MeOD + 20 % CDCl₃) δ 8.89 (8H, m, β -H), 8.78 (3H, s, 5-triazole-H), 8.36 (6H, m, *o*-Ph), 8.22 (2H, d, J = 7.7 Hz, *o*-Ph), 8.21 (6H, m, *m*-Ph), 7.77 (3H, m, *m*, *p*-Ph), 5.03 (3H, br s, H-1), 5.02 (3H, d, J = 12.3 Hz, OCH(H)), 4.87 (3H, d, J = 12.3 Hz,

OCH(H)), 3.95 (3H, m, H-6'), 3.94 (3H, m, H-2), 3.83 (3H, m, H-6), 3.82 (3H, m, H-3), 3.72 (6H, m, H-4, H-5);

^{13}C NMR (150 MHz, MeOD + 20 % CDCl_3) δ 150.2, 150.2, 149.7, 149.7, 149.7, 149.7, 149.6, 149.6 (Ar α -C), 144.9, 144.9, 144.9 (4-triazole-C), 144.1, 144.1, 144.1, 143.0, 136.1, 136.1, 136.1 (Ar C), 135.1, 135.1, 135.1, 135.1, 135.1, 135.1, 134.1, 134.1 (Ar CH), 131.7, 131.7, 131.2, 131.2, 131.1, 131.1, 130.9, 130.9 (Ar β -CH), 127.1, 126.0, 126.0 (Ar CH), 122.3, 122.3, 122.3 (5-triazole-C), 121.3, 118.8, 118.8, 118.6 (Ar C), 118.0, 118.0, 118.0, 118.0, 118.0, 118.0 (Ar CH), 99.4, 99.4, 99.4 (C-1), 73.5, 73.5, 73.5 (C-5), 71.0, 71.0, 71.0 (C-3), 70.5, 70.5, 70.5 (C-2), 67.2, 67.2, 67.2 (C-4), 61.6, 61.6, 61.6 (C-6), 59.4, 59.4, 59.4 (OCH_2);

M/z HRMS (MALDI-TOF) calcd. for $\text{C}_{71}\text{H}_{67}\text{N}_{13}\text{O}_{18}\text{NaZn}$ = 1476.3916 ($M+\text{Na}$) $^+$. Found 1476.3857.



6.2.57 5,10,15-Tris-(4-(4'-[(β -D-glucopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-20-diphenylporphyrinatozinc(II), **120**

Tri azido substituted porphyrin **99** (18 mg, 0.02 mmol) and propargyl functionalised glucose **67** (15 mg, 0.07 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography ($\text{EtOAc}:\text{EtOH}:\text{H}_2\text{O}$, 7:2:1 (v/v/v)) to yield the product **120**, a purple solid (23 mg, 70 %);

R_f = 0.14 ($\text{EtOAc}:\text{EtOH}:\text{H}_2\text{O}$, 7:2:1 (v/v/v));

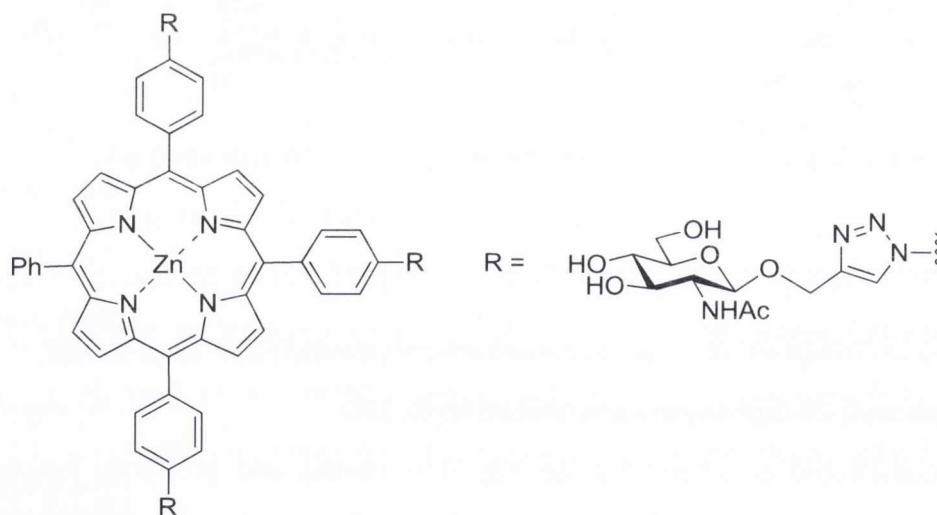
$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at c = 0.01 in $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 1:1);

ν_{max} (thin film) 3305 cm^{-1} (OH), 2921 cm^{-1} (CH);

¹H NMR (600 MHz, MeOD + 15 % CDCl₃) δ 8.89 (8H, m, β-H) , 8.80 (3H, s, 5-triazole-H), 8.36 (6H, d, *J* = 7.7 Hz, *o*-Ph), 8.21 (2H, m, *o*-Ph), 8.20 (6H, d, *J* = 7.8 Hz, *m*-Ph), 7.78 (3H, m, *m*, *p*-Ph), 5.14 (2H, d, *J* = 12.3 Hz, OCH(H)), 5.13 (1H, d, *J* = 12.3 Hz, OCH(H)), 5.01 (2H, d, *J* = 12.4 Hz, OCH(H)), 5.00 (1H, d, *J* = 12.4 Hz, OCH(H)), 4.53 (2H, d, *J*_{1,2} = 7.7 Hz, H-1), 4.52 (1H, d, *J*_{1,2} = 7.7 Hz, H-1'), 3.96 (3H, dd, *J*_{6',6} = 11.9 Hz, *J*_{6',5} = 1.8 Hz, H-6'), 3.75 (3H, m, H-6), 3.43 (3H, app t, *J*_{3,4} = *J*_{3,2} = 8.5 Hz, H-3), 3.37 (6H, m, H-4, H-5), 3.35 (3H, m, H-2);

¹³C NMR (150 MHz, MeOD + 15 % CDCl₃) δ 150.7, 150.7, 150.3, 150.3, 150.2, 150.2, 150.1, 150.1 (Ar α-C), 145.9, 145.9, 145.9 (4-triazole-C), 144.6, 144.6, 144.6, 143.5, 136.7, 136.7, 136.7 (Ar C), 135.6, 135.6, 135.6, 135.6, 135.6, 135.6, 134.6, 134.6 (Ar CH), 132.1, 132.1, 131.6, 131.6, 131.5, 131.5, 131.4, 131.4 (Ar β-CH) 127.6, 126.5, 126.5 (Ar CH), 122.8, 122.8, 122.8 (5-triazole-C), 121.7, 119.3, 119.3, 119.1 (Ar C), 118.5, 118.5, 118.5, 118.5, 118.5, 118.5 (Ar CH) , 102.9, 102.9, 102.9 (C-1), 77.1, 77.1, 77.1 (C-5), 77.0, 77.0, 77.0 (C-3), 74.1, 74.1, 74.1 (C-2) , 70.7, 70.7, 70.7 (C-4) , 62.2, 62.2, 62.2 (OCH₂) , 61.8, 61.8, 61.8 (C-6);

***M/z* HRMS (MALDI-TOF)** calcd. for C₇₁H₆₇N₁₃O₁₈ZnNa = 1476.3916 (*M*+Na)⁺. Found = 1476.3948.



6.2.58 5,10,15-Tris-(4-(4'-[(2-acetamido-2-deoxy-β-D-glucopyranoside)oxymethyl]-1-*N*-1,2,3-triazol-1'-yl)phenyl)-20-diphenylporphyrinatozinc(II), **121**

Tri azido substituted porphyrin **99** (18 mg, 0.02 mmol) and propargyl functionalised GlcNAc **70** (18 mg, 0.07 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography (EtOAc:EtOH:H₂O, 65:20:15 (v/v/v)) to yield the product **121**, as a purple solid (25 mg, 70 %);

$R_f = 0.15$ (EtOAc:EtOH:H₂O, 65:20:15 (v/v/v));

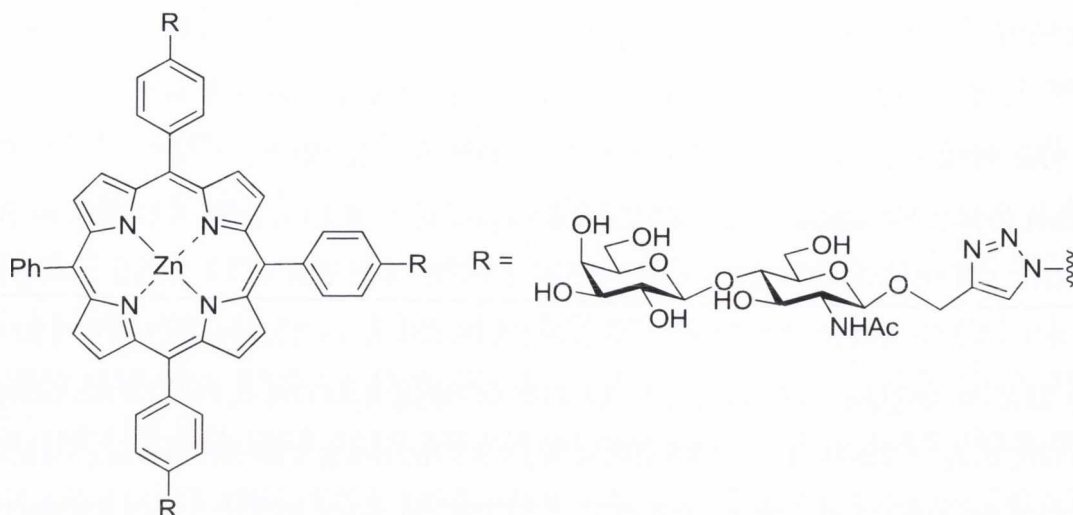
$[\alpha]_D^{20} = \text{N/A}$ (Sample too dark for analysis at $c = 0.01$ in CH₂Cl₂/MeOH 1:1);

ν_{max} (thin film) 3372 cm⁻¹ (OH), 2924 cm⁻¹ (CH), 1646 cm⁻¹ (CONH);

¹H NMR (600 MHz, MeOD + 15 % CDCl₃) δ 8.84 (2H, d, $J = 4.5$ Hz, β -H), 8.81 (6H, m, β -H), 8.66 (3H, s, 5-triazole-H), 8.25 (6H, d, $J = 6.7$ Hz, *o*-Ph), 8.15 (2H, d, $J = 7.2$ Hz, *o*-Ph), 8.09 (6H, d, $J = 6.7$ Hz, *m*-Ph), 7.71 (3H, m, *m*, *p*-Ph), 5.08 (2H, d, $J = 12.2$ Hz, OCH(H)), 5.07 (1H, d, $J = 12.2$ Hz, OCH(H)), 4.94 (2H, d, $J = 12.3$ Hz, OCH(H)), 4.93 (1H, d, $J = 12.3$ Hz, OCH(H)), 4.66 (2H, d, $J_{1,2} = 8.5$ Hz, H-1), 4.65 (1H, d, $J_{1,2} = 8.5$ Hz, H-1'), 3.96 (3H, dd, $J_{6',6} = 11.9$ Hz, $J_{6',5} = 1.8$ Hz, H-6'), 3.76 (6H, m, H-6, H-2), 3.52 (3H, dd, $J_{3,4} = 10.1$ Hz, $J_{3,2} = 8.4$ Hz, H-3), 3.39 (6H, m, H-4, H-5), 2.00 (6H, s, CH₃), 1.99 (3H, s, CH₃);

¹³C NMR (150 MHz, MeOD + 15 % CDCl₃) δ 172.4, 172.4, 172.4 (C=O), 150.2, 150.2, 149.7, 149.7, 149.7, 149.7, 149.6, 149.6 (Ar α -C), 145.3, 145.3, 145.3 (4-triazole-C), 144.0, 144.0, 144.0, 143.0, 136.1, 136.1, 136.1 (Ar C), 135.1, 135.1, 135.1, 135.1, 135.1, 135.1, 134.1, 134.1 (Ar CH), 131.7, 131.7, 131.1, 131.1, 131.0, 131.0, 130.9, 130.9 (Ar β -CH) 127.0, 126.0, 126.0 (Ar CH), 122.3, 122.3, 122.3 (5-triazole-C), 121.4, 118.8, 118.8, 118.6 (Ar C), 117.9, 117.9, 117.9, 117.9, 117.9, 117.9 (Ar CH), 100.4, 100.4, 100.4 (C-1), 76.6, 76.6, 76.6 (C-5), 74.5, 74.5, 74.5 (C-3), 70.7, 70.7, 70.7 (C-4), 61.4, 61.4, 61.4 (C-6), 61.4, 61.4, 61.4 (OCH₂), 55.9, 55.9, 55.9 (C-2), 21.6, 21.6, 21.6 (CH₃);

M/z HRMS (MALDI-TOF) calcd. for C₇₇H₇₆N₁₆O₁₈Zn = 1576.4815, (M+Na)⁺. Found 1576.4819.



6.2.59 5,10,15-Tris-(4-(4'-[(2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside)oxymethyl]-1-N-1,2,3-triazol-1'-yl)phenyl)-20-phenyl-porphyrinatozinc(II), **122**

Tri azido substituted porphyrin **99** (20 mg, 0.03 mmol) and propargyl functionalised LacNAc **77** (28 mg, 0.08 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system C, and the crude material purified by reverse phase column chromatography (MeCN:H₂O, 1:4 to 7:13 (v/v)) to yield a the product **122**, as a purple solid 27 mg, 56 % yield;

$R_f = 0.11$ (MeCN:H₂O, 2:8 v/v, reverse phase C₁₈ silica);

$[\alpha]_D^{20} = \text{N/A}$ (Sample too dark for analysis at $c = 0.01$ in MeOH/H₂O 1:1);

ν_{max} (thin film) 3310 cm⁻¹ (OH), 2905 cm⁻¹ (CH), 1651 cm⁻¹ (NHC=O);

¹H NMR (600 MHz, MeOD + 15 % *d*₅-Pyr) δ 8.97 (4H, br s, β-H), 8.93 (4H, m, β-H), 8.87 (3H, s, 5-triazole-H), 8.37 (6H, d, $J = 7.0$ Hz, *o*-Ph), 8.26 (6H, d, $J = 7.2$ Hz, *m*-Ph), 8.21 (2H, d, $J = 6.5$ Hz, *o*-Ph), 7.80 (1H, m, *p*-Ph), 7.79 (2H, m, *m*-Ph), 5.18 (3H, d, (MeOD overlap), OCH(H)), 5.04 (3H, d, $J = 12.6$ Hz, OCH(H)), 4.86 (2H, d, $J_{1,2} = 8.4$ Hz, H-1a), 4.85 (1H, d, $J_{1,2} = 8.4$ Hz, H-1a), 4.58 (2H, d, $J_{1,2} = 7.8$ Hz, H-1b), 4.58 (1H, d, $J_{1,2} = 7.8$ Hz, H-1b), 4.18 (3H, dd, $J_{6',6} = 12.2$ Hz, $J_{6',5} = 2.1$ Hz, H-6a'), 4.09 (3H, m, H-6a), 3.95 (3H, m, H-2a), 3.99 (3H, d, $J_{4,3} = 2.8$ Hz, H-4b), 3.93 (2H, dd, $J_{6,6'} = 11.3$ Hz, $J_{6,5} = 7.3$ Hz, H-6b), 3.92 (1H, dd, $J_{6,6'} = 11.3$ Hz, $J_{6,5} = 7.3$ Hz, H-6b), 3.89 (3H, m, H-3a), 3.84 (3H, m, H-6b'), 3.82 (6H, m, H-4a, H-2b), 3.73 (3H, m, H-5b), 3.66 (3H, dd, $J_{3,2} = 9.7$ Hz, $J_{3,4} = 3.0$ Hz, H-3b), 3.64 (3H, m, H-5a), 2.03 (6H, s, CH₃), 2.02 (3H, s, CH₃);

¹³C NMR (150 MHz, MeOD + 15 % *d*₅-Pyr) δ 172.0, 172.0, 172.0 (C=O), 150.3, 150.3, 149.8, 149.8, 149.8, 149.8, 149.7, 149.7 (Ar α-C), 145.6, 145.6, 145.6 (4-triazole-C), 143.7,

$R_f = 0.15$ (EtOAc:EtOH:H₂O, 6:3:2 (v/v/v));

$[\alpha]_D^{20} = \text{N/A}$ (Sample too dark for analysis at $c = 0.01$ in CH₂Cl₂/MeOH 1:1);

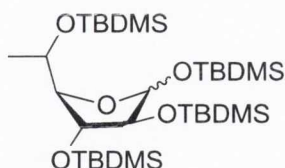
ν_{max} (thin film) 3377 cm⁻¹ (OH), 2927 cm⁻¹ (CH), 1642 cm⁻¹ (C=O);

¹H NMR (800 MHz, MeOD) δ 8.85 (2H, d, $J = 4.6$ Hz, β -H), 8.84 (2H, d, $J = 4.6$ Hz, β -H), 8.82 (4H, s, β -H), 8.49 (1H, s, 5-triazole-H), 8.34 (2H, d, $J = 7.4$ Hz, *o*-Ph), 8.18 (8H, m, *o*, *m*-Ph), 7.75 (9H, m, *m*, *p*-Ph), 5.09 (1H, d, $J_{1,2} = 2.5$ Hz, H-1f), 5.01 (1H, d, $J_{1,2} = 3.7$ Hz, H-1-e), 4.79 (1H, q, $J_{6,5} = 6.5$ Hz, H-5e), 4.56 (1H, br s, NH-linker), 4.54 (1H, d, $J_{1,2} = 7.4$ Hz, H-1d), 4.51 (1H, d, $J_{1,2} = 8.5$ Hz, H-1c), 4.34 (1H, q, $J_{6,5} = 6.5$ Hz, H-5f), 4.28 (1H, d, $J_{1,2} = 7.8$ Hz, H-1a), 4.20 (1H, d, $J_{1,2} = 7.8$ Hz, H-1b), 3.98 (1H, app t, $J_{3,2} = J_{3,4} = 9.8$ Hz, H-3c), 3.89 (3H, m, H-6c, H-6a, OCH(H)CH₂CH₂N), 3.82 (5H, m, H-2c, H-6'c, H-6'a, H-4b, H-3d), 3.76 (1H, dd, $J_{6',6} = 11.6$ Hz, $J_{6',5} = 4.4$ Hz, H-6'd), 3.73 (2H, m, H-2e, H-4d), 3.70 (2H, m, H-4c, H-3e), 3.68 (2H, m, H-4e, H-2f), 3.66 (1H, br s, H-3f), 3.60 (4H, m, H-6d, OCH(H)CH₂CH₂N, H-4f, H-2d), 3.49 (3H, m, H-6'b, H-2b, H-3a), 3.44 (2H, m, H-4a, H-3b), 3.36 (5H, m, OCH₂CH₂CH₂N, H-5a, H-5d, H-5c), 3.29 (1H, m, H-6b), 3.24 (1H, dd, $J_{2,3} = 8.5$ Hz, $J_{2,1} = 7.9$ Hz, H-2a), 3.18 (2H, t, $J = 7.5$ Hz, COCH₂CH₂), 3.17 (1H, m, H-5b), 2.71 (2H, t, $J = 7.6$ Hz, COCH₂CH₂), 1.95 (3H, s, COCH₃), 1.79 (2H, quintet, $J = 6.2$ Hz, OCH₂CH₂CH₂N), 1.24 (3H, d, $J_{6,5} = 6.6$ Hz, H-6e), 1.22 (3H, d, $J_{6,5} = 6.6$ Hz, H-6f);

¹³C NMR (150 MHz, MeOH) δ 173.6 (COCH₂CH₂), 172.9 (COCH₃), 172.2, (C=O), 150.6, 150.6, 150.5, 150.5, 150.5, 150.5, 150.1, 150.1 (Ar α -C), 147.9 (4-triazole-C), 144.8, 143.8, 143.8, 143.8, 136.8 (Ar C), 135.8, 135.8, 134.7, 134.7, 134.6, 134.6, 134.6, 134.6 (Ar CH), 131.9, 131.9, 131.6, 131.6, 131.6, 131.6, 130.1, 130.1 (Ar β -CH), 127.5, 127.5, 127.5, 126.5, 126.5, 126.5, 126.5, 126.5, 126.5, 126.5 (Ar CH), 121.2 (Ar C), 121.1 (5-triazole-CH), 121.0, 121.0 (Ar C), 118.8 (Ar C), 118.5, 118.5 (Ar CH), 103.9 (C-1b), 103.8 (C-1c), 103.2 (C-1a), 101.4 (C-1d), 100.6 (C-1f), 98.6 (C-1e), 82.2 (C-3b), 79.3 (C-4a), 77.4 (C-2d), 76.5 (C-5c), 75.8 (C-5d), 75.6 (C-5a), 75.4 (C-3a, C-5b), 75.3 (C-3c), 74.9 (C-4f), 73.8 (C-2a), 72.9 (C-3f), 72.8 (C-3e), 72.4 (C-4c), 70.7 (C-2b), 70.5 (C-2f), 70.4 (C-3d), 69.5 (C-4e), 69.3 (C-4d), 69.1 (C-2e), 68.9 (C-4b), 67.5 (OCH₂CH₂CH₂N), 66.8 (C-5e), 66.3 (C-5f), 61.9 (C-6d), 61.2 (C-6b), 61.0 (C-6a), 60.6 (C-6c), 56.3 (C-2c), 36.8 (OCH₂CH₂CH₂N), 35.5 (COCH₂CH₂), 29.3 (OCH₂CH₂CH₂N), 22.2 (COCH₃), 21.8 ((COCH₂CH₂), 15.6 (C-6e), 15.5 (C-6f);

M/z HRMS (MALDI-TOF) calcd. for C₉₀H₁₀₃N₉O₃₀Zn = 1853.6102, (M)⁺. Found = 1853.6102.

6.3 Experimental data for Chapter 3



6.3.1 1,2,3,5-Tetra-O-*tert*-butyldimethylsilyl -α-L-fucofuranoside, 130b

To a stirred solution of L-fucopyranoside **129** (0.20 g, 3.84 mmol) in DMF (5 mL) was added 2,6-lutidine (1.20 mL, 12.10 mmol) and DMAP (0.05g). A solution of TBDMSCl (1.47 g, 9.76 mmol) in DCE (5 mL) was added and the mixture stirred at 100 °C with condenser attached for 24 h under N₂. The mixture was quenched with the addition of deionised H₂O (40 mL) over ice, and the product diluted with EtOAc (150 mL). The organic layer was collected and washed with brine, HCL (1M), sat. aqueous NaHCO₃ solution and dried over MgSO₄. The mixture was filtered, and the solvent removed *in vacuo*. The crude material was purified by column chromatography (EtOAc:Hex, 1:99 (v/v)) to yield the product **130b**, as a clear oil (643 mg, 85 %);

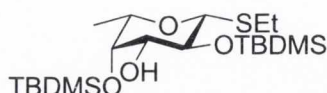
$[\alpha]_D^{22} = 54^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CHCl₃);

$\nu_{\max}$ (thin film) 2929 cm⁻¹ (CH);

¹H NMR (600 MHz, CDCl₃) δ 5.16 (1H, s, H-1), 3.92 (2H, m, H-2, H-3), 3.88 (1H, m, H-5), 3.85 (1H, dd, $J_{4,5} = 5.5$ Hz, $J_{4,3} = 3.5$ Hz, H-4), 1.19 (3H, d, $J_{6,5} = 6$ Hz, H-6), 0.92, 0.91, 0.90, 0.90 (9H, s, SiC(CH₃)₃), 0.12, 0.12, 0.10, 0.10, 0.10, 0.10, 0.09, 0.08 (3H, s, Si(CH₃));

¹³C NMR (150 MHz, CDCl₃) δ 103.3 (C-1) 90.3 (C-4), 85.2 (C-2), 79.8 (C-3), 69.1 (C-5), 26.0, 25.8, 25.7, 25.7 (SiC(CH₃)₃), -4.2, -4.2, -4.4, -4.4, -4.5, -4.6, -4.8, -5.2 (Si(CH₃));

M/z HRMS (ESI-TOF) calcd. for C₃₀H₆₈O₅NaSi₄ = 643.4042 (M+Na)⁺. Found = 643.4022.



6.3.2 Ethyl-2,4-di-O-*tert*-butyldimethylsilyl -1-thio-α-L-fucopyranoside, 131

To a solution of ethyl-1-thio-β-L-fucopyranoside **80** (1.3 g, 1.63 mmol) and imidazole (1.47 g, 21.98 mmol) in DMF (10 mL) was added TBDMSCl (1.47 g, 9.77 mmol). The mixture was heated to 50 °C for 48 h. The reaction was cooled to RT and quenched over ice with deionised H₂O (10 mL). The mixture was diluted with EtOAc (100 mL) and sequentially washed with brine (3 X 100 mL), deionised H₂O (2 X 100 mL) and dried over MgSO₄. The

mixture was filtered and the solvent removed *in vacuo*. The crude material was purified by column chromatography (Et₂O:Hexane, 1:25 (v/v)) to yield the product **131**, as a yellow oil (2.34 g, 86 %);

$[\alpha]_D^{20} = 78^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3602 cm⁻¹ (OH), 2928 cm⁻¹ (CH);

¹H NMR (600 MHz, CDCl₃) δ 4.24 (1H, d, $J_{1,2} = 9.1$ Hz, H-1), 3.80 (1H, d, $J_{4,5} = 2.7$ Hz, H-4), 3.67 (1H, app t, $J_{2,3} = J_{2,1} = 8.9$ Hz, H-2), 3.57 (1H, q, $J_{5,6} = 6.4$ Hz, H-5), 3.46 (1H, m, H-3), 2.74 (1H, m, SCH(H)), 2.65 (1H, m, SCH(H)), 1.98 (1H, d, $J = 6.7$ Hz, OH), 1.30 (3H, t, $J = 7.5$ Hz, SCH₂CH₃), 1.35 (3H, d, $J_{6,5} = 6.3$ Hz, H-6), 0.96 (9H, s, SiC(CH₃)₃), 0.94 (9H, s, SiC(CH₃)₃), 0.20, 0.16, 0.15, 0.11 (3H, s, Si(CH₃)₂);

¹³C NMR (150 MHz, CDCl₃) δ 85.7 (C-1), 76.8 (C-3), 74.9 (C-5), 73.5 (C-4), 71.9 (C-2), 26.1, 26.0 (SiC(CH₃)₃), 23.9 (SCH₂CH₃), 18.4, 18.4 (SiC(CH₃)₃), 17.7 (C-6), 14.9 (SCH₂CH₃), -3.9, -4.0, -4.1, -4.2 (Si(CH₃)₂);

M/z HRMS (ESI-TOF) calcd. for C₂₀H₄₄O₄NaSSi₂ = 459.2397 (M+Na)⁺. Found = 459.2401.



6.3.3 Ethyl-2,3,4-tri-O-tert-butyldimethylsilyl-1-thio- α -L-fucopyranoside, **132**

To a solution of 1-ethylthio- β -L-fucopyranoside **80** (0.87 g, 4.20 mmol) and DMAP (50 mg), in pyridine (10 mL) was added TBDMSOTf (5.00 g, 18.92 mmol), at 0 °C under N₂. The mixture was stirred for 5 min before heating to 60 °C for 36 h. The reaction was cooled over ice and quenched with deionised H₂O (10 mL). The mixture was diluted with EtOAc (50 mL) and washed sequentially with brine (100 mL), 10% CuSO₄ (2 X 50 mL) and deionised H₂O (2 X 50 mL). The organic layer was dried over MgSO₄, filtered and the solvent removed *in vacuo*. The product was purified by column chromatography (EtOAc:Hex, 1:49 (v/v)) and recrystallized from Et₂O:Hex (1:49 (v/v)) to yield the product **132**, as a white crystalline solid (2.2 g, 97 %);

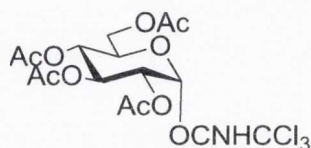
$[\alpha]_D^{20}$ (deg cm³ g⁻¹ dm⁻¹) = 65 ° (c = 0.1 in CH₃Cl);

Mp = 62-63 °C;

$\nu_{\max}$ (thin film) 2929 cm⁻¹ (CH);

¹H NMR (400 MHz, DMSO 75 °C) δ 4.43 (1H, d, $J_{1,2} = 7.2$ Hz, H-1), 3.90 (1H, br s, H-4), 3.85 (1H, app t, $J_{2,3} = J_{2,1} = 7.2$ Hz, H-2), 3.81 (1H, m, H-5), 3.71 (1H, dd, $J_{3,2} = 7.1$ Hz, $J_{3,4} = 1.9$

Hz, H-3), 2.60 (2H, m, SCH_2CH_3), 1.23 (3H, d, $J_{6,5} = 4.4$ Hz, H-6), 1.21 (3H, t, $J = 7.5$ Hz, SCH_2CH_3), 0.95, 0.94, 0.92 (9H, s, $\text{Si}(\text{CH}_3)_3$), 0.15, 0.14, 0.14, 0.14, 0.10, 0.09 ($\text{Si}(\text{CH}_3)_2$); ^{13}C NMR (150 MHz, CDCl_3) δ (Signals too broad for full characterisation); M/z HRMS (ESI-TOF) calcd. for $\text{C}_{26}\text{H}_{58}\text{O}_4\text{NaSSi}_3 = 573.3261$ ($\text{M}+\text{Na}$) $^+$. Found = 573.3257.



6.3.4 2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl-trichloroacetimidate, **133**

To a stirred solution of 1,2,3,4,6-penta-O-acetyl- β -D-glucopyranoside (1.5 g, 3.84 mmol) in THF (10 mL) was added benzylamine (1.05 mL, 7.68 mmol). The mixture was stirred at 20 °C for 16 h. The solvent volume was reduced under vacuum and the mixture was diluted with EtOAc (30 mL). The organic layer was washed sequentially with 1M HCl (3 X 10 mL), sat. aqueous NaHCO_3 solution (10 mL) and deionised H_2O (20 mL). The organic layer was dried over MgSO_4 filtered and the solvent removed *in vacuo*. The crude anomeric mixture was used dissolved in DCM (5 mL) and trichloroacetonitrile (3.7 mL, 37 mmol) was added. The mixture was cooled to 0 °C and DBU (0.1 mL) added. The mixture was stirred over ice for 2 h, and allowed to warm to RT for 4 h under N_2 . The mixture was diluted with DCM (20 mL) and filtered through a plug of silica. The solvent was removed *in vacuo* and the product purified by column chromatography (EtOAc:Hex, 3:7 (v/v)) to yield the product **133**, as a white foam (670 mg, 35 %);

Analytical data was in good agreement with literature example.²⁷⁵

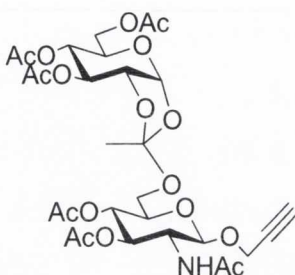
$[\alpha]_{\text{D}}^{20} = 60^\circ$ (deg $\text{cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.1$ in CHCl_3);

ν_{max} (thin film) 2928 cm^{-1} (CH), 1741 cm^{-1} (C=O), 1641 cm^{-1} (C=N);

^1H NMR (400 MHz, CDCl_3) δ 8.71 (1H, s, NH), 6.57 (1H, d, $J_{1,2} = 3.9$ Hz, H-1), 5.57 (1H app t, $J_{3,4} = J_{3,2} = 9.9$ Hz, H-3), 5.19 (1H, app t, $J_{4,3} = J_{4,5} = 9.9$ Hz, H-4), 5.14 (1H, dd, $J_{2,3} = 10.1$ Hz, $J_{2,1} = 3.7$ Hz, H-2), 4.28 (1H, dd, $J_{6,6'} = 12.3$ Hz, $J_{6,5} = 4.0$ Hz, H-6), 4.22 (1H, ddd, $J_{5,4} = 10.1$ Hz, $J_{5,6} = 4.0$ Hz, $J_{5,6'} = 1.9$ Hz, H-5), 4.13 (1H, dd, $J_{6',6} = 12.3$ Hz, $J_{6',5} = 2.0$ Hz, H-6'), 2.09, 2.06, 2.04, 2.03 (3H, s, CH_3);

^{13}C NMR (100 MHz, CDCl_3) δ 170.6, 170.0, 169.9, 169.5 (C=O), 160.8 ($\text{C}=\text{NH}$), 92.9 (C-1), 90.7 ($\text{C}=\text{CH}_2$), 70.0 (C-5), 69.8 (C-3), 69.7 (C-2), 67.7 (C-4), 61.4 (C-6), 20.7, 20.7, 20.6, 20.5 (CH_3);

M/z HRMS (ESI-TOF) calcd. for $\text{C}_{16}\text{H}_{20}\text{NO}_{10}\text{NaCl}_3 = 514.0050$ ($\text{M}+\text{Na}$) $^+$. Found = 514.0052.



6.3.5 (2,3,4,6-Tetra-O-acetyl-glucopyranosyl)-(1/2-6)-2-acetamindo-2-deoxy-3,4-di-O-acetyl-O-propargyl- β -glucopyranose-orthoester, **134**

A mixture of acceptor **70** (100 mg, 0.39 mmol), donor **133** (209 mg, 0.42 mmol), were dissolved in 1,4-dioxane (9 mL), containing pre-activated 3 Å ms under N_2 . Anhydrous THF (6 mL) was added. The suspension was cooled to $-10\text{ }^\circ\text{C}$ and BF_3OEt_2 (57 μL , 0.46 mmol) was added. The mixture was stirred for 90 min before quenching with sat. aqueous NaHCO_3 solution (10 mL). The mixture was filtered through a plug of celite and extracted with EtOAc. The solvent was removed *in vacuo* and the crude material dissolved in pyridine (2 mL) and Ac_2O (2 mL) at $0\text{ }^\circ\text{C}$. The mixture was stirred for 16 h and worked up according to standard acetylation conditions. The crude material was purified by column chromatography (Hex:EtOAc, 8:2 (v/v)) to yield the product **134**, as an amorphous solid (182 mg, 70 % yield);

$[\alpha]_{\text{D}}^{20}$ (deg $\text{cm}^3 \text{g}^{-1} \text{dm}^{-1}$) = -77 ° ($c = 0.1$ in CH_2Cl_2);

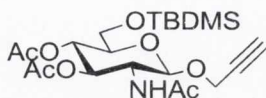
ν_{max} (thin film) 3273 cm^{-1} ($\text{C}\equiv\text{CH}$), 2931 cm^{-1} (CH), 1732 cm^{-1} (C=O);

^1H NMR (400 MHz, CDCl_3) δ 5.74 (1H, d, $J_{1,2} = 5.0\text{ Hz}$, H-1B), 5.53 (1H, d, $J = 8.7\text{ Hz}$, NH), 5.27 (1H, dd, $J_{3,2} = 10.8\text{ Hz}$, $J_{3,4} = 9.6\text{ Hz}$, H-3A), 5.19 (1H, app t $J_{3,4} = J_{3,2} = 2.9\text{ Hz}$, H-3B), 5.04 (1H, app t, $J_{4,3} = J_{4,5} = 9.4\text{ Hz}$, H-4A), 4.92 (1H, ddd, $J_{4,5} = 9.6\text{ Hz}$, $J_{4,3} = 2.8\text{ Hz}$, $J_{4,2} = 0.8\text{ Hz}$, H-4B), 4.83 (1H, d, $J_{1,2} = 8.4\text{ Hz}$, H-1A), 4.38 (2H, m, OCH_2), 4.32 (1H, ddd, $J_{2,1} = 5.2\text{ Hz}$, $J_{2,3} = 2.9\text{ Hz}$, $J_{2,4} = 0.9\text{ Hz}$, H-2B), 4.21 (2H, m, H-6A), 3.93 (2H, m, H-2A, H-5B), 3.65 (1H, m, H-5A), 3.62 (2H, m, H-6B), 2.49 (1H, app t, $J = 2.3\text{ Hz}$, $\text{C}\equiv\text{CH}$), 2.15, 2.12, 2.11, 2.05, 2.05 (CH_3CO), 1.98 (3H, NHCOCH_3), 1.72 ($\text{CH}_3\text{C}(\text{O})_3$);

^{13}C NMR (100 MHz, CDCl_3) δ 170.6, 170.3, 169.8, 169.2, 169.1, 168.8 (C=O), 120.7 ($\text{CH}_3\text{C}(\text{O})_3$), 97.8 (C-1A), 96.6 (C-1B), 78.1 ($\text{C}\equiv\text{CH}$), 74.9 ($\text{C}\equiv\text{CH}$), 72.4 (C-2B), 72.2 (C-5A),

72.0 (C-3A), 69.5 (C-6A), 68.7 (C-4A), 67.7 (C-4B), 66.5 (C-5B), 62.6 (C-6A), 62.2 (C-6B), 55.4 (OCH₂), 53.8 (C-2A), 22.9 (NHCOCH₃), 20.4, 20.4, 20.3, 20.2, 20.2 (COCH₃), 20.0 (CH₃C(O)₃);

M/z HRMS (ESI-TOF) calcd. for C₂₉H₃₉NO₁₇Na = 696.2116 (M+Na)⁺. Found = 696.2134.



6.3.6 Propargyl-2-acetamido-3,4-di-O-acetyl-2-deoxy-6-O-*tert*-butyldimethylsilyl- β -D-glucopyranoside, **135**

To a solution of partially protected glycoside **70** (220 mg, 0.289 mmol) in pyridine (0.5 mL) at 0 °C was added Ac₂O (0.5 mL). The mixture was stirred for 4 h. The reaction was quenched with deionised H₂O (2 mL) and diluted with EtOAc (10 mL). The organic layer was washed sequentially with brine (10 mL), 10 % CuSO₄ solution (2 X 10 mL) and deionised H₂O (10 mL). The organic layer was dried over MgSO₄, filtered and the solvent removed *in vacuo* to yield the product **135**, as a white amorphous solid (267 mg, 99 % yield);

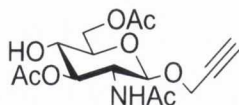
$[\alpha]_D^{20} = -24^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3260 cm⁻¹ (NH, C $\equiv$ CH), 2930 cm⁻¹ (CH), 1746 cm⁻¹ (C=O), 1654 (NHC=O);

¹H NMR (400 MHz, CDCl₃) δ 5.49 (1H, d, J = 8.9 Hz, NH), 5.24 (1H, dd, J_{3,2} = 10.5 Hz, J_{3,4} = 9.2 Hz, H-3), 5.04 (1H, app t, J_{4,3} = J_{4,5} = 9.4 Hz, H-4), 4.79 (1H, d, J_{1,2} = 8.6 Hz, H-1), 4.41 (2H, d, J = 2.4 Hz, OCH₂), 3.98 (1H, d, app t, J_{2,3} = 10.5 Hz, J_{2,NH} = J_{2,1} = 8.8 Hz, H-2), 3.74 (1H, dd, J_{6',6} = 11.6 Hz, J_{6',5} = 2.9 Hz, H-6'), 3.71 (1H, dd, J_{6,6'} = 11.5 Hz, J_{6,5} = 5 Hz, H-6), 3.57 (1H, ddd, J_{5,4} = 9.6 Hz, J_{5,6} = 5.0 Hz, J_{5,6'} = 2.9 Hz, H-5), 2.47 (1H, t, J = 2.5 Hz, C $\equiv$ CH), 2.06, 2.05 (3H, s, CH₃), 1.99 (3H, s, NHCOCH₃), 0.91 (9H, s, SiC(CH₃)₃), 0.08, 0.07 (3H, s, Si(CH₃)₂);

¹³C NMR (100 MHz, CDCl₃) δ 171.2, 170.2, 169.3 (C=O), 98.2 (C-1), 78.6 (C $\equiv$ CH), 75.2 (C $\equiv$ CH), 74.9 (C-5), 72.9 (C-3), 69.0 (C-4), 62.5 (C-6), 55.5 (OCH₂), 54.2 (C-2), 25.8 (SiC(CH₃)₃), 23.4 (CH₃), 20.7, 20.7 (CH₃), 18.3 (SiC(CH₃)₃), -5.4, -5.4 (Si(CH₃)₂);

M/z HRMS (ESI-TOF) calcd. for C₂₁H₃₅NO₈NaSi = 480.2030 (M+Na)⁺. Found = 480.2039.



6.3.7 Propargyl-2-acetamido-3,6-di-O-acetyl-2-deoxy- β -D-glucopyranoside, **136**

To a stirred solution of globally protected glycoside **135** (260 mg, 0.57 mmol) in THF (5 mL) at 0 °C was added acetic acid (32 μ L, 1.14 mmol) and 1 M TBAF (1.13 mL, 1.14 mmol). The mixture was stirred at 0 °C for 2 h. The reaction was diluted with EtOAc (10 mL) and washed with brine (10 mL) and deionised H₂O (10 mL). The organic layer was dried over MgSO₄ filtered and the solvent removed *in vacuo*. The crude material was purified by column chromatography (MeOH:EtOAc, 1:9 (v/v)) to yield the product **136**, as a clear oil (92 mg, 47 %);

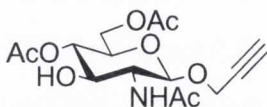
$[\alpha]_D^{20}$ (deg cm³ g⁻¹ dm⁻¹) = -35 ° (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3330 cm⁻¹ (OH), 3274 cm⁻¹ (C $\equiv$ CH), 3100 cm⁻¹ (NH), 2923 cm⁻¹ (CH), 1725 cm⁻¹ (C=O), 1659 cm⁻¹ (NHC=O);

¹H NMR (600 MHz, CDCl₃) δ 5.90 (1H, d, J = 9.2 Hz, NH), 5.14 (1H, app t, J_{3,4} = J_{3,2} = 8.8 Hz, H-3), 4.78 (1H, d, J_{1,2} = 8.4 Hz, H-1), 4.47 (1H, dd, J_{6,6'} = 12.2 Hz, J_{6,5} = 2.5 Hz, H-6), 4.38 (3H, m, OCH₂, H-6'), 3.94 (1H, m, H-2), 3.71 (2H, m, H-4, H-5), 3.44 (1H, br s, OH), 2.48 (1H, br s, C $\equiv$ CH), 2.14, 2.12 (3H, s, CH₃), 1.98 (3H, s, NHCOCH₃);

¹³C NMR (150 MHz, CDCl₃) δ 171.8, 171.6, 170.4 (C=O), 99.4 (C-1), 78.6 (C $\equiv$ CH), 75.0 (C $\equiv$ CH), 74.9 (C-3), 74.0 (C-5), 68.6 (C-4), 62.8 (C-6), 55.6 (OCH₂), 50.8 (C-2), 23.2, 20.8, 20.7 (CH₃);

M/z HRMS (ESI-TOF) calcd. for C₁₅H₂₁NO₈Na = 366.1165 (M+Na)⁺. Found = 366.1183.



6.3.8 Propargyl-2-acetamido-4,6-di-O-acetyl-2-deoxy- β -D-glucopyranoside, **137**

To a stirred solution of globally protected glycoside **135** (260 mg, 0.57 mmol) in THF (5 mL) at 0 °C was added acetic acid (32 μ L, 1.14 mmol) and 1 M TBAF (1.13 mL, 1.14 mmol). The mixture was stirred at 0 °C for 2 h. The reaction was diluted with EtOAc (10 mL) and washed with brine (10 mL) and deionised H₂O (10 mL). The organic layer was dried over MgSO₄ filtered and the solvent removed *in vacuo*. The crude material was purified by column chromatography (MeOH:EtOAc, 1:9 (v/v)) to yield the product **137**, as a clear oil (37 mg, 19 % yield);

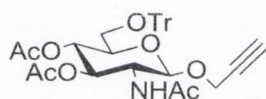
$[\alpha]_D^{20} = -51^\circ$ (deg cm³ g⁻¹ dm⁻¹) = -51° (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3350 cm⁻¹ (OH), 3305 cm⁻¹ (C≡CH), 3100 cm⁻¹ (NH), 2990 cm⁻¹ (CH), 1750 cm⁻¹ (C=O), 1650 (NHC=O);

¹H NMR (600 MHz, CDCl₃) δ 5.88 (1H, d, J = 4.7 Hz, NH), 4.98 (1H, app t, J_{4,3} = J_{4,5} = 9.3 Hz, H-4), 4.81 (1H, d, J_{1,2} = 8.2 Hz, H-1), 4.75 (1H, br s, OH), 4.43 (2H, dd, J = 15.9 Hz, J = 2.1 Hz, OCH₂), 4.30 (1H, dd, J_{6,6'} = 12.5 Hz, J_{6,5} = 5.0 Hz, H-6), 4.16 (1H, dd, J_{6',6} = 12.5 Hz, J_{6',5} = 2.1 Hz, H-6'), 3.98 (1H, app t, J_{3,4} = J_{3,2} = 9.3 Hz, H-3), 3.69 (H, m, H-5), 3.55 (1H, m, C-2), 2.55 (1H, t, J = 2.4 Hz, C≡CH), 2.13, 2.10, 2.08 (3H, s, CH₃);

¹³C NMR (150 MHz, CDCl₃) δ 172.5, 170.8, 170.3 (C=O), 97.7 (C-1), 78.5 (C≡CH), 75.7 (C≡CH), 73.1 (C-3), 72.5 (C-5), 71.1 (C-4), 62.2 (C-6), 58.4 (C=2), 56.0 (OCH₂), 23.5, 20.9, 20.8 (CH₃);

M/z HRMS (ESI-TOF) calcd. for C₁₅H₂₁NO₈Na = 366.1165 (M+Na)⁺. Found = 366.1160.



6.3.9 Propargyl-2-acetamido-3,4-di-O-acetyl-2-deoxy-6-O-triphenylmethyl- β -D-glucopyranoside, **138**

To a mixture of propargyl substituted GlcNAc **70** (300 mg, 1.16 mmol) and trityl chloride (387 mg, 1.39 mmol) was added pyridine (5 mL). The solution was heated to 90 °C for 5 h. The reaction was cooled over ice and Ac₂O (0.43 mL, 4.64 mmol) was added. The mixture was stirred for 2 h. The reaction was quenched with H₂O (10 mL) and diluted with EtOAc (30 mL). The organic layer was washed sequentially with brine (10 mL), 10 % CuSO₄ solution (2 X 20 mL) and H₂O (10 mL). The organic layer was dried over MgSO₄, filtered and the solvent removed *in vacuo*. The crude material was purified using column chromatography (EtOAc:Hex, 8:2 (v/v)) to yield the product **138**, as a white amorphous solid (477 mg, 70 % yield);

$[\alpha]_D^{20}$ (deg cm³ g⁻¹ dm⁻¹) = -28° (c = 0.1 in CH₃Cl);

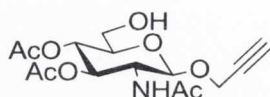
$\nu_{\max}$ (thin film) 3276 cm⁻¹ (C≡CH), 3250 cm⁻¹ (NH), 3091 cm⁻¹ (Ar CH), 2932 cm⁻¹ (CH), 1743 cm⁻¹ (C=O), 1654 cm⁻¹ (NHC=O);

¹H NMR (400 MHz, CDCl₃) δ 7.48 (6H, d, J = 7.8 Hz, o-Ph), 7.32 (6H, t, J = 7.9 Hz, m-Ph), 7.25 (3H, t, J = 7.5 Hz, p-Ph), 5.49 (1H, d, J = 9.1 Hz, NH), 5.20 (2H, m, H-3, H-4), 4.84 (1H, d, J_{1,2} = 8.6 Hz, H-1), 4.50 (2H, d, J = 2.1 Hz, OCH₂), 4.10 (1H, m, H-2), 3.61 (1H, m, H-5),

3.27 (1H, dd, $J_{6',6} = 10.5$ Hz, $J_{6',5} = 1.8$ Hz, H-6'), 3.13 (1H, dd, $J_{6,6'} = 10.4$ Hz, $J_{6,5} = 4.8$ Hz, H-6), 2.51 (1H, t, $J = 2.2$ Hz, C≡CH), 2.05, 2.01 (3H, s, CH₃), 1.76 (NHCOCH₃);

¹³C NMR (100 MHz, CDCl₃) δ 171.3, 170.3, 168.9 (C=O), 143.6, 143.6, 143.6, (Ar C), 128.7, 128.7, 128.7, 128.7, 128.7 (Ar CH), 127.8, 127.8, 127.8, 127.8, 127.8, 127.8 (Ar CH), 127.0, 127.0, 127.0 (Ar CH), 98.3 (C-1), 86.6 (C(Ph)₃), 78.6 (C≡CH), 75.3 (C≡CH), 73.6 (C-5), 72.9 (C-3), 68.7 (C-4), 62.0 (C-6), 55.4 (OCH₂), 54.3 (C-2), 23.4, 20.8, 20.4 (CH₃);

M/z HRMS (ESI-TOF) calcd. for C₃₄H₃₅NO₈Na = 608.2260 (M+Na)⁺. Found = 608.2253.



6.3.10 Propargyl-2-acetamido-3,4-di-O-acetyl-2-deoxy-β-D-glucopyranoside, **139**

A mixture of Et₂O/Formic (1:1 (v/v)) (6 mL) was added to globally protected glycoside **138** (470 mg, 0.8 mmol) and the mixture stirred at RT for 2 h. The solvent was removed *in vacuo* co-evaporating with toluene/MeOH. The crude material was loaded in DCM and purified by column chromatography (EtOAc:Hexane, 4:1 (v/v)) to yield the product **139**, as a white amorphous solid (213 mg, 77 %);

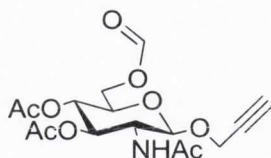
$[\alpha]_D^{20} = -60^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3460 cm⁻¹ (OH), 3268 cm⁻¹ (C≡CH), 3089 cm⁻¹ (NH), 2939 cm⁻¹ (CH), 1745 cm⁻¹ (C=O), 1650 cm⁻¹ (NHC=O);

¹H NMR (400 MHz, CDCl₃) δ 5.59 (1H, d, $J = 9.4$ Hz, NH), 5.34 (1H, app t, $J_{3,4} = J_{3,2} = 9.6$ Hz, H-3), 5.06 (1H, app t, $J_{4,3} = J_{4,5} = 9.6$ Hz, H-4), 4.88 (1H, d, $J_{1,2} = 8.7$ Hz, H-1), 4.42 (2H, br s, OCH₂), 4.10 (1H, app q, $J_{2,1} = J_{2,H} = J_{2,3} = 9.3$ Hz, H-2), , 3.78 (1H, d, $J_{6',6} = 12.2$ Hz, H-6'), 3.63 (1H, dd, $J_{6,6'} = 12.1$ Hz, $J_{6,5} = 3.9$ Hz, H-6), 3.57 (1H, m, H-5), 2.50 (1H, s, C≡CH), 2.09, 2.07 (3H, s, CH₃), 1.99 (NHCOCH₃);

¹³C NMR (100 MHz, CDCl₃) δ 170.6, 169.9, 169.8 (C=O), 98.1 (C-1), 78.2 (C≡CH), 74.9 (C≡CH), 73.8 (C-5), 71.8 (C-3), 68.3 (C-4), 60.8 (C-6), 55.6 (OCH₂), 53.9 (C-2), 22.9, 20.3, 20.3 (CH₃);

M/z HRMS (ESI-TOF) calcd. for C₁₅H₂₁NO₈Na = 366.1165 (M+Na)⁺. Found = 366.1183.



6.3.11 2-Acetamido-3,4-di-O-acetyl-2-deoxy-6-O-formate-1-O-propargyl- β -D-glucopyranose, **140**

Side product isolated during the synthesis of glycoside **139**, and purified by column chromatography (EtOAc:Hexane, 4:1 (v/v)) to yield the side product **140**, as a white amorphous solid (40 mg, 13 %);

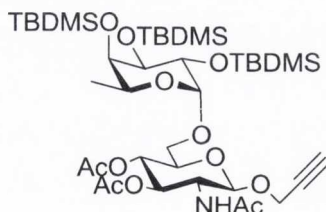
$[\alpha]_D^{22} = -41^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3274 cm⁻¹ (C $\equiv$ CH), 3086 cm⁻¹ (NH), 2950 cm⁻¹ (CH), 1745 cm⁻¹ (C=O), 1722 cm⁻¹ (HC=O), 1655 cm⁻¹ (NHC=O);

¹H NMR (400 MHz) δ 8.06 (1H, s, HC=O), 5.57 (1H, d, J = 8.6 Hz, NH), 5.31 (1H, dd, J_{3,2} = 10.6 Hz, J_{3,4} = 9.6 Hz, H-3), 5.06 (1H, app t, J_{4,3} = J_{4,5} = 9.9 Hz, H-4), 4.88 (1H, d, J_{1,2} = 8.6 Hz, H-1), 4.37 (2H, d, J = 2.1 Hz, OCH₂), 4.29 (2H, d, J_{6,5} = 3.6 Hz, H-6), 3.91 (1H, m, H-2), 3.76 (1H, dt, J_{5,4} = 9.9 Hz, J_{5,6} = 3.6 Hz, H-5), 2.46 (1H, t, J = 2.3 Hz, C $\equiv$ CH), 2.04, 2.03 (3H, s, CH₃), 1.96 (NHCOCH₃);

¹³C NMR (100 MHz, CDCl₃) δ 170.9, 170.3, 169.4 (C=O), 160.4 (HC=O), 98.2 (C-1), 78.4 (C $\equiv$ CH), 75.5 (C $\equiv$ CH), 72.2 (C-3), 71.8 (C-5), 68.6 (C-4), 61.5 (C-6), 55.9 (OCH₂), 54.3 (C-2), 23.4, 20.7, 20.6 (CH₃);

M/z HRMS (ESI-TOF) calcd. for C₁₆H₂₁NO₉Na = 394.1114 (M+Na)⁺. Found = 394.1117



6.3.12 O-(2,3,4-O-Tert-butyldimethylsilyl- α -L-fucopyranosyl)-(1-6)-2-acetamido-3,4-di-O-acetyl-2-deoxy-1-O-propargyl- β -D-glucopyranose, **141**

To a stirred solution of GlcNAc acceptor **139** (100 mg, 0.29 mmol), fucose donor **132** (400 mg, 0.44 mmol) and NIS (98 mg, 0.44 mmol) in DCM (15 mL) with activated 3 Å ms at -20 °C under N₂, was added a catalytic amount of TMSOTf. The solution was stirred at -20 °C for 4 h before quenching with Et₃N (0.5 mL) and diluting with DCM (20 mL). The organic layer was washed with sat. aqueous Na₂S₂O₃ solution (2 X 10 mL), deionised H₂O (10 mL).

The organic layer was dried over MgSO_4 , filtered and the solvent removed *in vacuo*. The crude mixture was purified by column chromatography (EtOAc:Hex, 3:2 (v/v)) to yield the product **141**, as an amorphous white solid (208 mg, 86 %);

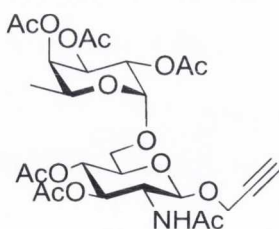
$[\alpha]_D^{22} = -55^\circ \text{ deg cm}^3 \text{ g}^{-1} \text{ dm}^{-1}$ ($c = 0.1$ in CH_3Cl);

ν_{max} (thin film) 3286 cm^{-1} ($\text{C}\equiv\text{CH}$), 3100 cm^{-1} (NH), 2931 cm^{-1} (CH), 1754 cm^{-1} (C=O), 1661 cm^{-1} (NHC=O);

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.48 (1H, d, $J = 8.8 \text{ Hz}$, NH), 5.22 (1H, app t, $J_{3,2} = 10.2 \text{ Hz}$, $J_{3,4} = 9.4 \text{ Hz}$, H-3A), 4.96 (1H, app t, $J_{4,3} = J_{4,5} = 9.6 \text{ Hz}$, H-4A), 4.78 (1H, d, $J_{1,2} = 8.4 \text{ Hz}$, H-1A), 4.7 (1H, d, $J_{1,2} = 2.8 \text{ Hz}$, H-1B), 4.36 (2H, d, $J = 2.2 \text{ Hz}$, OCH_2), 4.01 (1H, br d, $J_{3,2} = 7.4 \text{ Hz}$, H-3B), 3.94 (3H, br m, H-2A, H-2B, H-5B), 3.79 (1H, br s, H-4B), 3.71 (1H, dd, $J_{6',6} = 11.4 \text{ Hz}$, $J_{6',5} = 1.6 \text{ Hz}$, H-6'A), 3.70 (1H, m, 5-A), 3.58 (1H, dd, $J_{6,6'} = 11.6 \text{ Hz}$, $J_{6,5} = 6.4 \text{ Hz}$, H-6A), 2.42 (1H, t, $J = 2.2 \text{ Hz}$, $\text{C}\equiv\text{CH}$), 2.02, 2.00 (3H, s, CH_3), 1.96 (NHCOCH_3), 1.18 (3H, d, $J_{6,5} = 6.5 \text{ Hz}$, H-6B), 0.92, 0.91, 0.89 (9H, s, $\text{SiC}(\text{CH}_3)_3$), 0.13, 0.12, 0.09, 0.06, 0.06, 0.05 ($\text{Si}(\text{CH}_3)_2$);

$^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 171.1, 170.3, 169.2 (C=O), 99.1 (C-1B), 98.0 (C-1A), 78.6 ($\text{C}\equiv\text{CH}$), 75.2 ($\text{C}\equiv\text{CH}$), 73.6 (C-5A), 73.4 (C-4B), 72.8 (C-3A), 72.4 (C-2B), 70.3 (C-3B), 69.2 (C-4A), 68.9 (C-5B), 67.2 (C-6A), 55.4 (OCH_2), 54.2 (C-2A), 26.5, 26.1, 26.1 ($\text{SiC}(\text{CH}_3)_3$), 23.4, (NHCOCH_3), 20.3, 20.3 (CH_3), 18.7, 18.5, 18.3, ($\text{SiC}(\text{CH}_3)_2$), 16.6 (C-6B), -3.6, -4.0, -4.2, -4.3, -4.6 ($\text{Si}(\text{CH}_3)_2$);

M/z HRMS (ESI-TOF) calcd. for $\text{C}_{39}\text{H}_{73}\text{NO}_{12}\text{NaSi}_3 = 854.4338$ ($\text{M}+\text{Na}$) $^+$. Found = 854.4331.



6.3.13 2-Acetamido-3,4-di-O-acetyl-2-deoxy-6-O-(2,3,4-tri-O-acetyl- α -L-fucopyranosyl)-1-O-propargyl- β -D-glucopyranose, **142**

To a solution of disaccharide **141** (450 mg, 0.54 mmol) in THF (10 mL) was added TBAF (1M in THF) (5.4 mL, 5.4 mmol) at 0°C . The mixture was allowed to warm to RT and stirred for 30 h. The solvent volume was reduced *in vacuo* and the residue dissolved in pyridine (3 mL). Ac_2O (1.5 mL) was added at 0°C and the mixture was stirred for 16 h, warming to RT. The reaction was quenched by the addition of MeOH (1 mL) followed by dilution with EtOAc (30 mL). The organic layer was washed with H_2O , 10 % CuSO_4 solution,

H₂O, dried over MgSO₄, filtered and the solvent removed *in vacuo*. The crude material was purified by column chromatography (EtOAc:Hex, 9:1 (v/v)) to yield the product **142**, as a white foam (285 mg, 86 %);

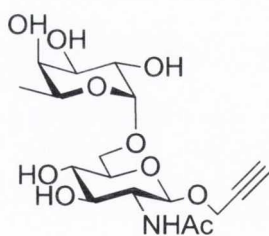
$[\alpha]_D^{20} = -120^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3276 cm⁻¹ (C≡CH), 3083 cm⁻¹, (NH), 2927 cm⁻¹ (CH), 2115 cm⁻¹ (C≡CH), 1741 (C=O), 1655 (C=ONH);

¹H NMR (600 MHz, CDCl₃) δ 5.50 (1H, d, *J* = 8.9 Hz, NH), 5.36 (1H, dd, *J*_{3,2} = 10.6 Hz, *J*_{3,4} = 3.3 Hz, H-3B), 5.32 (1H, d, *J*_{4,3} = 3.4 Hz, H-4B), 5.31 (1H, app t, *J*_{3,2} = *J*_{3,4} = 9.9 Hz, H-3A), 5.13 (1H, dd, *J*_{2,3} = 10.5 Hz, *J*_{2,1} = 3.5 Hz, H-2B), 5.09 (1H, d, *J*_{1,2} = 3.6 Hz, H-1B), 5.07 (1H, app t, *J*_{4,3} = *J*_{4,5} = 9.5 Hz, H-4A), 4.87 (1H, d, *J*_{1,2} = 8.3 Hz, H-1), 4.40 (2H, m, OCH₂), 4.18 (1H, m, H-5B), 3.93 (1H, m, H-2A), 3.76 (1H, *J*_{6',6} = 11.4 Hz, *J*_{6,5} = 2.2 Hz, H-6'A), 3.70 (1H, m, H-5A), 3.59 (1H, dd, *J*_{6,6'} = 11.6 Hz, *J*_{6,5} = 5.1 Hz, H-6A), 2.50 (1H, t, *J* = 1.9 Hz, -C≡CH), 2.18, 2.13, 2.05, 2.05, 2.01, 1.99 (3H, s, CH₃), 1.16 (3H, d, *J*_{6,5} = 6.6 Hz, H-6B);

¹³C NMR (150 MHz, CDCl₃) δ 170.9, 170.6, 170.6, 170.2, 169.9, 169.3 (C=OCH₃), 98.2 (C-1A), 96.7 (C-1B), 78.6 (C≡CH), 75.3 (C≡CH), 73.2 (C-5A), 72.5 (C-3A), 71.0 (C-4B), 69.0 (C-4A), 68.0 (C-2B), 67.9 (C-3B), 66.5 (C-6A), 64.5 (C-5B), 55.7 (CH₂C≡CH), 54.3 (C-2A), 23.3 (NHCOCH₃), 20.8, 20.7, 20.7, 20.6, 20.6 (COCH₃), 15.9 (C-6B);

M/z HRMS (ESI-TOF) calcd. for C₂₇H₃₇NO₁₅Na = 638.2061, (M+Na)⁺. Found = 638.2051.



6.3.14 2-Acetamido-2-deoxy-6-O-(α-L-fucopyranosyl)-1-O-propargyl-β-D-glucopyranose, **125**

Per acetylated disaccharide **142** (108 mg, 0.16 mmol) and NaOMe (1 mg, 0.02 mmol) in MeOH (3 mL) were reacted according to the general deacetylation procedure (Section 6.2.2), followed by freeze drying to yield the product **125** as a white solid (67 mg, 94 %);

$[\alpha]_D^{20} = -83^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in MeOH);

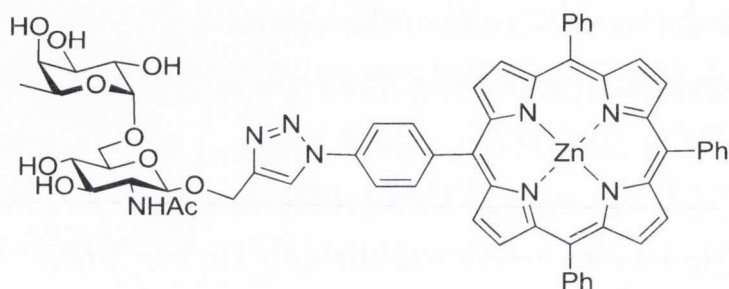
$\nu_{\max}$ (thin film) 3284 cm⁻¹ (OH), 3924 cm⁻¹ (CH), cm⁻¹, 1648 cm⁻¹ (C=ONH);

¹H NMR (600 MHz, MeOD) δ 4.86 (1H, d, *J*_{1,2} = 2.9 Hz, H-1B), 4.62 (1H, d, *J*_{1,2} = 8.5 Hz, H-1A), 4.35 (2H, d, *J* = 2.0 Hz, OCH₂), 4.11 (1H, q, *J*_{5,6} = 6.5 Hz, H-5B), 3.96 (1H, d, *J*_{6',6} = 11.3

Hz, H-6'A), 3.77 (3H, m, H-6A, H-3B, H-2B), 3.69 (1H, br s, H-4B), 3.68 (1H, app t, $J_{2,3} = J_{2,1} = 9.0$ Hz, H-2A), 3.51 (1H, m, H-3A), 3.43 (2H, m, H-4A, H-5A), 2.88 (1H, t, $J = 2.0$ Hz, $\text{-C}\equiv\text{CH}$), 2.00 (3H, s, CH_3), 1.24 (3H, d, $J_{6,5} = 6.6$ Hz, H-6B);

^{13}C NMR (150 MHz, CDCl_3) δ 172.8 (C=O), 99.9 (C-1B), 99.5 (C-1A), 78.99 ($\text{-C}\equiv\text{CH}$), 75.9 (C-5A), 75.2 ($\text{C}\equiv\text{CH}$), 74.6 (C-3A), 72.7 (C-4B), 70.8 (C-4A), 70.7 (C-3B), 69.1 (C-2B), 67.1 (C-6A), 66.6 (C-5B), 56.2 (C-2A), 55.4 (OCH_2), 21.9 (CH_3), 15.7 (C-6B);

M/z HRMS (ESI-TOF) calcd. for $\text{C}_{17}\text{H}_{27}\text{NO}_{10}\text{Na} = 428.1533$, ($\text{M}+\text{Na}$) $^+$. Found = 428.1532.



6.3.15 5-(4-(4'-[(2-Acetamido-2-deoxy-6-O-(α -L-fucopyranosyl)- β -D-glucopyranoside-oxy-methyl)-1-N-1,2,3-triazol-1'-yl]phenyl)-10,15,20-trisphenyl-porphyrinatozinc(II), **143**

Mono azido substituted porphyrin **96** (15 mg, 0.02 mmol) and propargyl functionalised disaccharide **125** (9 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography ($\text{EtOAc}:\text{EtOH}:\text{H}_2\text{O}$, 7:2:1 (v/v/v)) to yield the product **143**, as a purple solid, (23 mg, 95 %);

$R_f = 0.39$ ($\text{EtOAc}:\text{EtOH}:\text{H}_2\text{O}$, 7:2:1 (v/v/v));

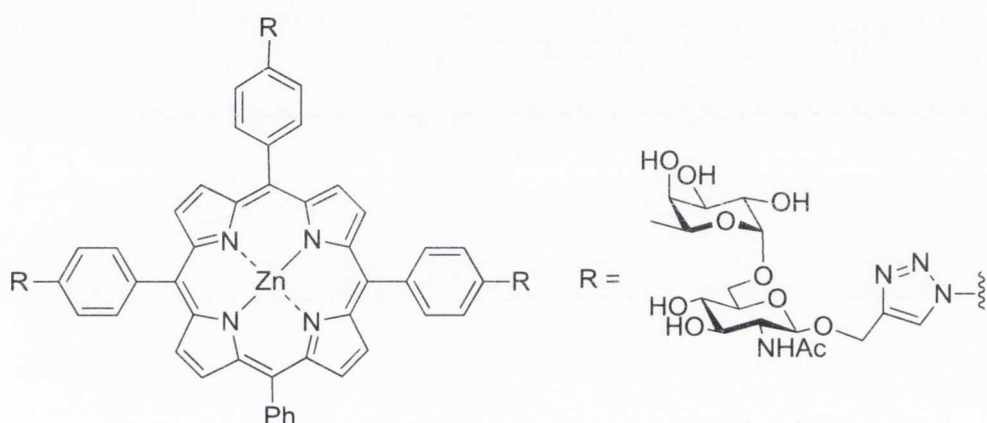
$[\alpha]_D^{20} = \text{N/A}$ (Sample too dark for analysis at $c = 0.01$ in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:1);

ν_{max} (thin film) 3322 cm^{-1} (OH), 2923 cm^{-1} (CH) 1648 cm^{-1} (C=ONH);

^1H NMR (600 MHz, $\text{MeOD} + 25\%$ d_5 -Pyr) δ 8.68 (8H, m, β -H), 8.66 (1H, s, 5-triazole-H), 8.15 (2H, m, o-Ph), 8.05 (2H, m, m-Ph), 7.99 (6H, m, o-Ph), 7.56 (9H, m, m, p-Ph), 4.96 (1H, d, $J = 12.2$ Hz, $\text{OCH}(\text{H})$), 4.87 (2H, m, (under solvent), $\text{OCH}(\text{H})$), H-1B), 4.61 (1H, d, $J_{1,2} = 8.3$ Hz, H-1A), 4.00 (1H, q, $J_{5,6} = 6.5$ Hz, H-5B), 3.97 (1H, d, $J_{6',6} = 11.9$ Hz, H-6'A), 3.79 (3H, br m, H-2A, H-2B, H-3B), 3.69 (1H, dd, $J_{6,6'} = 11.9$ Hz, $J_{6,5} = 5.9$ Hz, H-6A), 3.59 (1H, br s, H-4B), 3.53 (1H, app t, $J_{3,2} = J_{3,4} = 8.2$ Hz, H-3A), 3.47 (1H, m, H-5A), 3.43 (1H, app t, $J_{4,3} = J_{4,5} = 8.7$ Hz, H-4A), 1.83 (3H, s, CH_3), 1.12 (3H, d, $J_{6,5} = 6.5$ Hz, H-6B);

^{13}C NMR (100 MHz, MeOD + 25 % *d5*-Pyr) δ 172.5 (C=O), 150.5, 150.5, 150.4, 150.4, 150.4, 150.4, 150.0, 150.0 (Ar α -C), 145.7, 144.4, 143.5, 143.5, 143.5, 136.3 (Ar C), 135.7, 135.7, 134.6, 134.6, 134.6, 134.6, 134.6, 134.6 (Ar CH), 132.1, 132.1, 131.9, 131.9, 131.8, 131.8, 131.4, 131.4 (Ar β -CH), 127.5, 127.5, 127.5, 126.5, 126.5, 126.5, 126.5, 126.5, 126.5 (Ar CH), 122.8 (5-triazole-C), 121.3 (Ar C), 120.2, 120.2, 118.9 (Ar C), 118.5, 118.5 (Ar CH), 100.7 (C-1B), 100.3 (C-1A), 76.2 (C-5A), 75.0 (C-3A), 72.7 (C-4B), 71.2 (C-4A), 70.8 (C-3B), 69.3 (C-2B), 67.6 (C-6A), 66.7 (C-5B), 61.7 (OCH₂), 56.5 (C-2A), 22.3 (CH₃), 16.0 (C-6B);

M/z HRMS (MALDI-TOF) calcd. for C₆₁H₅₄N₈O₁₀ZnNa = 1122.3254, (M+Na)⁺. Found = 1122.3235.



6.3.16 5,10,15-Tris-(4-(4'-[(2-acetamido-2-deoxy-6-O-(α -L-fucopyranosyl)- β -D-glucopyranoside-oxymethyl]-1-N-1,2,3-triazol-1'-yl)phenyl)-20-phenylporphyrinatozinc(II), **144**

Tri azido substituted porphyrin **99** (18 mg, 0.02 mmol) and propargyl functionalised disaccharide **125** (27 mg, 0.06 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system B, and the crude material purified by column chromatography (EtOAc:EtOH:H₂O, 60:30:15 (v/v/v)) to yield the product **144**, as a purple solid, (54 mg, 79 %);

R_f = 0.27 (EtOAc:EtOH:H₂O, 60:30:15 (v/v/v));

$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at c = 0.01 in CH₂Cl₂/MeOH 1:1);

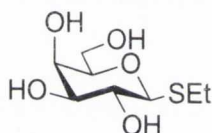
$\nu_{\max}$ (thin film) 3295 cm⁻¹ (OH), 2922 cm⁻¹ (CH) 1647 cm⁻¹ (C=ONH);

^1H NMR (600 MHz, MeOD + 20 % *d5*-Pyr) δ 8.76 (4H, br s, β -H), 8.72 (8H, br s, β -H), 8.67 (3H, s, 5-triazole-H), 8.15 (6H, d, J = 6.7 Hz, o-Ph), 8.05 (6H, m, m-Ph), 8.00 (2H, m, o-Ph), 7.56 (3H, m, m, p-Ph), 4.97 (3H, d, J = 12.1 Hz, OCH(H)), 4.87 (6H, m, (under solvent), OCH(H)), H-1B), 4.61 (3H, d, $J_{1,2}$ = 8.1 Hz, H-1A), 3.97 (6H, m, H-5B, H-6'), 3.79 (9H, br m,

H-2A, H-2B, H-3B), 3.69 (3H, dd, $J_{6,6'} = 11.0$ Hz, $J_{6,5} = 4.9$ Hz, H-6A), 3.57 (3H, br s, H-4B), 3.53 (3H, app t, $J_{3,2} = J_{3,4} = 8.5$ Hz, H-3A), 3.47 (3H, m, H-5A), 3.43 (3H, app t, $J_{4,3} = J_{4,5} = 8.9$ Hz, H-4A), 1.81 (9H, s, CH₃), 1.12 (9H, d, $J_{6,5} = 6.3$ Hz, H-6B);

¹³C NMR (100 MHz, MeOD + 20 % d₅-Pyr) δ 172.5, 172.5, 172.5, (C=O), 150.6, 150.6, 150.4, 150.4, 150.25, 150.25, 150.2, 150.2, (Ar α-C), 145.8, 145.8, 145.8, 144.1, 144.1, 144.1, 143.4, 136.3, 136.3, 136.3 (Ar C), 135.7, 135.7, 135.7, 135.7, 135.7, 135.7, 134.7, 134.7 (Ar CH), 132.3, 132.3, 131.9, 131.9, 131.8, 131.8, 131.6, 131.6 (Ar β-CH), 127.6, 126.6, 126.6 (Ar CH), 122.8, 122.8, 122.8, (5-triazole-C), 121.7, 119.6, 119.6, 119.4, (Ar C), 118.5, 118.5, 118.5, 118.5, 118.5, 118.5, (Ar CH), 100.7, 100.7, 100.7, (C-1B), 100.3, 100.3, 100.3, (C-1A), 76.3, 76.3, 76.3, (C-5A), 75.0, 75.0, 75.0, (C-3A), 72.7, 72.7, 72.7, (C-4B), 71.2, 71.2, 71.2, (C-4A), 70.8, 70.8, 70.8, (C-3B), 69.3, 69.3, 69.3, (C-2B), 67.6, 67.6, 67.6, (C-6A), 66.7, 66.7, 66.7, (C-5B), 61.8, 61.8, 61.8, (OCH₂), 56.5, 56.5, 56.5, (C-2A), 22.3, 22.3, 22.3, (CH₃), 16.0, 16.0, 16.0, (C-6B);

M/z HRMS (ESI-TOF) calcd. for C₉₅H₁₀₆N₁₆O₃₀Zn = 2014.6552, (M)⁺. Found = 2014.6539.



6.3.17 Ethyl-1-thio-β-D-galactopyranoside, **145**

Per acetylated glycoside **72** (3.50 g, 8.92 mmol) and NaOMe (53 mg, 0.89 mmol) in MeOH (40 mL) were reacted according to the general deacetylation procedure (Section 6.2.2), to yield the product **145**, as a white solid (2.00g, 99 %);

Analytical data was in good agreement with literature example.²⁷⁶

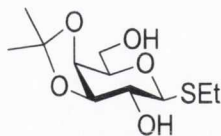
[α]_D²⁰ (deg cm³ g⁻¹ dm⁻¹) = -24 ° (c = 0.1 g cm⁻¹ in MeOH);

ν_{max} (thin film) 3397 cm⁻¹ (OH), 2980 cm⁻¹ (CH);

¹H NMR (400 MHz, MeOD) δ 4.34 (1H, d, $J_{1,2} = 9.5$ Hz, H-1), 3.90 (1H, dd $J_{4,3} = 3.1$ Hz, $J_{4,5} = 0.7$ Hz, H-4), 3.76 (1H, dd, $J_{6,6'} = 11.4$ Hz, $J_{6,5} = 6.8$ Hz, H-6), 3.70 (1H, dd, $J_{6',6} = 11.4$ Hz, $J_{6',5} = 5.3$ Hz, H-6'), 3.57 (1H, t, $J_{2,1} = J_{2,3} = 9.3$ Hz, H-2), 3.55 (1H, m, H-5), 3.48 (1H, dd, $J_{3,2} = 9.3$ Hz, $J_{3,4} = 3.1$ Hz, H-3), 2.77 (2H, m, S-CH₂CH₃), 1.31 (3H, t, $J = 7.5$ Hz, (S-CH₂CH₃));

¹³C NMR (100 MHz, MeOD) δ 86.1 (C-1), 79.2 (C-5), 74.9 (C-3), 70.0 (C-2), 69.1 (C-4), 61.2 (C-6), 23.5 ((S-CH₂CH₃), 14.1 ((S-CH₂CH₃));

M/z HRMS (ESI-TOF) calcd. for C₁₈H₁₆O₅SNa = 247.0616 (M+Na)⁺. Found 247.0610.



6.3.18 Ethyl-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside, **146**

To a stirred solution of thioglycoside **145** (1.00 g, 4.46 mmol) in 2,2-dimethoxymethane (45 mL) under N₂ was added PTSA monohydrate (0.037 g, 0.19 mmol) and the mixture stirred under N₂ for 60 h. Et₃N (0.25 mL, 1.90 mmol) was added to quench the reaction. The solvent was removed *in vacuo*, co-evaporating with toluene (3 X 10 mL). The crude oil was dissolved in MeOH:H₂O (10:1 (v/v)) (77 mL) and the solution refluxed for 4 h. The solvent was removed *in vacuo* and the residue purified by column chromatography (MeOH/EtOAc, 0:1 to 1:24 (v/v)) to yield the product **146**, as a white solid (1.07g, 91 %);

Analytical data was in good agreement with literature example.²⁷⁶

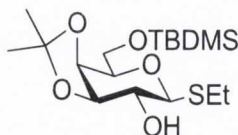
$[\alpha]_D^{20}$ (deg cm³ g⁻¹ dm⁻¹) = 34 ° (c = 0.1 g cm⁻¹ in MeOH);

$\nu_{\max}$ (thin film) 3177 cm⁻¹ (OH); 2940 cm⁻¹ (CH);

¹H NMR (400 MHz, MeOD) δ 4.36 (1H, d, $J_{1,2}$ = 10.1 Hz, H-1), 4.27 (1H, dd $J_{4,3}$ = 5.7 Hz, $J_{4,5}$ = 2.3 Hz, H-4), 4.03 (1H, dd, $J_{3,2}$ = 6.9 Hz, $J_{3,4}$ = 5.7 Hz, H-3), 3.86 (1H, ddd, $J_{5,6}$ = 7.2 Hz, $J_{5,6'}$ = 5.3 Hz, $J_{5,4}$ = 2.2 Hz, H-5), 3.79 (1H, dd, $J_{6,6'}$ = 11.4 Hz, $J_{6,5}$ = 7.3 Hz, H-6), 3.74 (1H, dd, $J_{6',6}$ = 11.4 Hz, $J_{6',5}$ = 5.3 Hz, H-6'), 3.47 (1H, dd, $J_{2,1}$ = 10.1 Hz, $J_{2,3}$ = 6.9 Hz, H-2), 2.74 (2H, m, S-CH₂CH₃), 1.49 (3H, s, CH₃), 1.35 (3H, s, CH₃) 1.31 (3H, t, J = 7.4 Hz, S-CH₂CH₃);

¹³C NMR (100 MHz, MeOD) δ 109.9 ((CH₃)₂C), 85.1 (C-1), 80.2 (C-3), 77.5 (C-5), 74.3 (C-4), 72.4 (C-2), 61.6 (C-6), 27.5 ((CH₃)₂C), 25.6 ((CH₃)₂C), 23.7 (S-CH₂CH₃), 14.4 ((S-CH₂CH₃);

M/z HRMS (ESI-TOF) calcd. for C₁₁H₂₀O₅SNa = 287.0929 (M+Na)⁺. Found 287.0920.



6.3.19 Ethyl-6-tert-butyltrimethylsilyl-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside, **147**

To a solution of partially protected thioglycoside **146** (900 mg, 3.41 mmol) and imidazole (579 mg, 8.51 mmol) in anhydrous DMF (25 mL) at 30 °C containing pre-activated 4 Å molecular sieves (4.00g) was added TBDMSCl (565 mg, 3.75 mmol). The mixture was stirred for 30 min under N₂. Imidazole (347 mg, 5.11 mmol) and TBDMSCl (307 mg, 2.04 mmol) were added portionwise over the next 1 h. The reaction was quenched with MeOH

(20 mL) and the mixture stirred for 20 min. The mixture was filtered and the solvent volume reduced *in vacuo*. The remaining oil was dissolved in EtOAc (30 mL) and washed with brine (5 X 30 mL) to remove DMF. The organic layer was dried over MgSO₄, filtered and the solvent removed *in vacuo*. The product was purified by column chromatography (EtOAc:Hexane, 2:8 to 1:1 (v/v)) to yield the product **147**, as an off white oil (1.2 g, 93%);

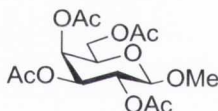
$[\alpha]_D^{20} = -12^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.05 g cm⁻¹ in MeOH);

$\nu_{\max}$ (thin film) 3448 cm⁻¹ (OH); 2955 cm⁻¹ (CH);

¹H NMR (400 MHz, MeOD) δ 4.36 (1H, d, $J_{1,2} = 10.1$ Hz, H-1), 4.27 (1H, dd, $J_{4,3} = 5.6$ Hz, $J_{4,5} = 2.0$ Hz, H-4), 4.01 (1H, dd, $J_{3,2} = 7.0$ Hz, $J_{3,4} = 5.5$ Hz, H-3), 3.90-3.82 (3H, m, H-5, H-6, H-6'), 3.46 (1H, dd, $J_{2,1} = 10.1$ Hz, $J_{2,3} = 7.2$ Hz, H-2), 2.73 (2H, m, SCH₂CH₃), 1.49 (3H, s, CH₃), 1.35 (3H, s, CH₃), 1.30 (3H, t, $J = 7.4$ Hz, SCH₂CH₃), 0.93 (9H, s, SiC(CH₃)₃), 0.12 (6H, s, Si(CH₃)₂);

¹³C NMR (100 MHz, MeOD) δ 108.9 ((CH₃)₂C=), 84.2 (C-1), 79.3 (C-3), 76.5 (C-5), 73.2 (C-4), 71.6 (C-2), 61.8 (C-6), 26.7, 24.8 ((CH₃)₂C=), 24.5 (SiC(CH₃)₃), 22.9 (SCH₂CH₃), 17.3 SiC(CH₃)₃, 13.8 (SCH₂CH₃), -7.0, -7.2 (Si(CH₃)₂);

M/z HRMS (ESI-TOF) calcd. for C₁₇H₃₄O₅NaSSi = 401.1794 (M+Na)⁺. Found 401.1789.



6.3.20 Methyl-2,3,4,6-O-acetyl-β-D-galactopyranoside, **148**

To a stirred solution of 1,2,3,4,6-penta-O-acetyl galactopyranoside (1.00 g, 2.56 mmol) and MeOH (0.41 mL, 10.24 mmol) in DCM (5 mL) was added BF₃OEt₂ (1.58 mL, 6.4 mmol) at -20 °C, under argon. The mixture was stirred for 16 h, warming to 0 °C. The reaction was quenched with the addition of Et₃N (1 mL) and the diluted with DCM (10 mL). The organic layer was washed with NaHCO₃ solution, dried over MgSO₄ filtered and the solvent removed *in vacuo*. The crude material was purified by column chromatography (EtOAc:Hex, 1:1 (v/v)) to yield the required product **148**, as a clear oil (400 mg, 43 %);

Analytical data was in good agreement with literature example.²⁷⁷

$[\alpha]_D^{20} = -18^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₂Cl₃);

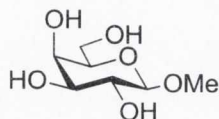
$\nu_{\max}$ (thin film) 2942 cm⁻¹ (CH), 1741 cm⁻¹ (C=O);

¹H NMR (400 MHz, CDCl₃) δ 5.39 (1H, dd, $J_{4,3} = 3.3$ Hz, $J_{4,5} = 0.7$ Hz, H-4), 5.20 (1H, dd, $J_{2,3} = 10.5$ Hz, $J_{2,1} = 8.0$ Hz, H-2), 5.01 (1H, dd, $J_{3,2} = 10.4$ Hz, $J_{3,4} = 3.4$ Hz, H-3), 4.39 (1H, d, $J_{1,2} =$

8.0 Hz, H-1), 4.20 (1H, dd, $J_{6',6} = 11.3$ Hz, $J_{6',5} = 6.5$ Hz, H-6'), 4.13 (1H, dd, $J_{6,6'} = 11.3$ Hz, $J_{6,5} = 6.8$ Hz, H-6), 3.90 (1H, dt, $J_{5,6} = 6.7$ Hz, $J_{5,4} = 0.8$ Hz, H-5), 3.52 (3H, s, OCH₃), 2.15, 2.06, 2.05, 1.98 (CH₃);

¹³C NMR (100 MHz, CDCl₃) δ 170.4, 170.2, 170.1, 169.4 (C=O), 102.1 (C-1), 70.9 (C-3), 70.6 (C-5), 68.8 (C-2), 67.0 (C-4), 61.3 (C-6), 57.0 (OMe), 20.8, 20.6, 20.6, 20.5 (CH₃);

M/z HRMS (ESI-TOF) calcd. for C₁₅H₂₂O₁₀Na = 385.1111, (M+Na)⁺. Found = 385.1125.



6.3.21 Methyl-β-D-galactopyranoside, 149

Per acetylated glycoside **148** (390 mg, 1.08 mmol) and NaOMe (5 mg, 0.1 mmol) in MeOH (10 mL) were reacted according to the general deacetylation procedure (Section 6.2.2), to yield the product **149**, as a white solid (207 mg, 99 %);

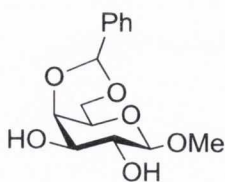
$[\alpha]_D^{20} = -24^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in MeOH);

$\nu_{\max}$ (thin film) 3243 cm⁻¹ (OH), 2935 cm⁻¹ (CH);

¹H NMR (400 MHz, MeOD) δ 4.17 (1H, d, $J_{1,2} = 7.2$ Hz, H-1), 3.87 (1H, dd, $J_{4,3} = 3.0$ Hz, $J_{4,5} = 0.8$ Hz, H-4), 3.79 (2H, m, H-6'), 3.57-3.52, (3H, m, H-5, H-2), 3.50 (1H, dd, $J_{3,2} = 9.3$ Hz, $J_{3,4} = 3.0$ Hz, H-3);

¹³C NMR (100 MHz, MeOD) δ 104.6 (C-1), 75.2 (C-5), 73.6 (C-3), 71.1 (C-2), 68.9 (C-4), 61.1 (C-6), 55.8 (OMe);

M/z HRMS (ESI-TOF) calcd. for C₇H₁₃O₆ = 193.0712, (M)⁻. Found = 193.0709.



6.3.22 Methyl-4,6-O-benzylidene-β-D-galactopyranoside, 150

To a stirred solution of **149** (215 mg, 1.10 mmol) and PTSA (21 mg, 0.11 mmol) in MeCN (3 mL) was added benzylidene dimethoxyacetal (0.19 mL, 1.32 mmol). The mixture was heated to 40 °C for 16 h. The reaction was neutralized with Et₃N and the solvent removed *in vacuo*. The crude mixture was purified using column chromatography (MeOH:EtOAc, 2:23 (v/v)) to yield the product **150**, as a white solid (187 mg, 60 %);

Analytical data was in good agreement with literature example.²⁷⁸

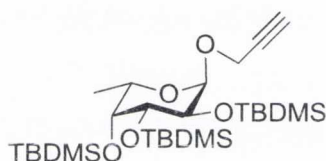
$[\alpha]_D^{20} = -50^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in MeOH);

$\nu_{\max}$ (thin film) 3350 cm⁻¹ (OH), 2858 cm⁻¹ (CH);

¹H NMR (400 MHz, CDCl₃) δ 7.49 (2H, m, Ar CH), 7.35 (3H, m, Ar CH), 5.54 (1H, s, -CHPh), 4.33 (1, dd, $J_{6',6} = 12.5$ Hz, $J_{6',5} = 1.3$ Hz, H-6'), 4.19 (1H, d, $J_{1,2} = 7.6$ Hz, H-1), 4.19 (1H, dd, $J_{4,3} = 3.8$ Hz, $J_{4,5} = 0.5$ Hz, H-4), 4.07 (1H, dd, $J_{6,6'} = 12.5$ Hz, $J_{6,5} = 1.8$ Hz, H-6), 3.74 (1H, dd, $J_{2,3} = 9.6$ Hz, $J_{2,1} = 7.5$ Hz, H-2), 3.67 (1H, dd, $J_{3,2} = 9.6$ Hz, $J_{3,4} = 3.7$ Hz, H-3), 3.57 (3H, s, OMe), 3.45 (1H, br m, H-5), 2.53 (2H, br s, OH);

¹³C NMR (100 MHz, CDCl₃) δ 137.4 (Ar C), 129.2 (Ar CH), 128.2 (Ar CH), 126.4 (Ar CH), 103.8 (C-1), 101.4 (PhCHOO), 75.3 (C-4), 72.7 (C-3), 71.8 (C-2), 69.7 (C-6), 66.7 (C-5), 57.1 (OCH₃);

M/z HRMS (ESI-TOF) calcd. for C₁₄H₁₈O₆Na = 305.1001, (M+Na)⁺. Found = 305.1007.



6.3.23 2,3,4-Tri-O-tert-butyldimethylsilyl-1-O-propargyl- α -L-fucopyranose, **151**

The trisilyl fucose donor **132** (60 mg, 0.11 mmol), acceptor propargyl alcohol **63** (8.16 μ l, 0.14 mmol), NIS (27 mg, 0.12 mmol) and catalytic TMSOTf were reacted according to the procedure for Section 6.3.12, at -20 °C for 40 min. The crude material was purified by column chromatography (EtOAc:Hex, 1:19 (v/v)) to yield the required product **151**, as a clear oil (51 mg, 86 %);

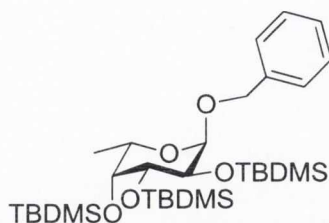
$[\alpha]_D^{20} = -30^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₂Cl₃);

$\nu_{\max}$ (thin film) 3313 cm⁻¹ (C $\equiv$ CH), 2886 cm⁻¹ (CH);

¹H NMR (400 MHz, CDCl₃) δ 4.99 (1H, d $J_{1,2} = 3.2$ Hz, H-1), 4.25 (2H, m, OCH₂), 4.11 (1H, dd, $J_{2,3} = 9.2$ Hz, $J_{2,1} = 3.0$ Hz, H-2), 3.95 (1H, dd, $J_{3,2} = 9.4$ Hz, $J_{3,4} = 2.2$ Hz), 3.89 (1H, br q, $J_{5,6} = 6.6$ Hz, H-5), 3.77 (1H, br s, H-4), 2.37 (1H, t, $J = 2.3$ Hz, C $\equiv$ CH), 1.20 (3H, d, $J_{6,5} = 6.6$ Hz, H-6), 0.94, 0.93, 0.92 (3H, s, SiC(CH₃)₃), 0.16, 0.14, 0.12, 0.10, 0.09, 0.08 (3H, s, Si(CH₃)₂);

¹³C NMR (100 MHz, CDCl₃) δ (C-1-6 not visible, due to broadening) 79.7 (-C $\equiv$ CH), 74.0 (-C $\equiv$ CH), 54.4 (OCH₂), 26.6, 26.2, 26.1 (SiC(CH₃)₃), 18.8, 18.6, 18.3 (SiC(CH₃)₃), -3.4, -3.9, -4.1, -4.2, -4.6, -4.6 (Si(CH₃)₂);

M/z HRMS (ESI-TOF) calcd. for $C_{27}H_{56}O_5NaSi_3$ = 567.3333, $(M+Na)^+$. Found = 567.3351.



6.3.24 Benzyl-2,3,4-Tri-O-*tert*-butyldimethylsilyl- α -L-fucopyranoside, **153**

The trisilyl fucose donor **132** (60 mg, 0.11 mmol), acceptor benzyl alcohol **63** (15 μ l, 0.14 mmol), NIS (27 mg, 0.12 mmol) and catalytic TMSOTf were reacted according to the procedure for Section 6.3.12, at -20°C for 40 min. The crude material was purified by column chromatography (EtOAc:Hex, 1:19 (v/v)) to yield the required product **153**, as a clear oil (54 mg, 83 % (3:2 α : β));

α anomer characterised.

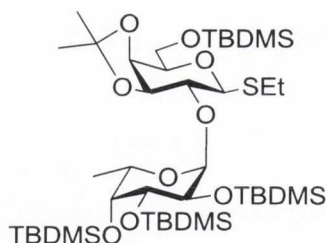
$[\alpha]_D^{20} = -38^\circ$ (deg $\text{cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.1$ in CH_2Cl_3)

ν_{max} (thin film) 2953 cm^{-1} (CH);

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.40 - 7.28 (5H, m, Ar CH), 4.86 (1H, d, $J_{1,2} = 2.9 \text{ Hz}$, H-1), 4.73 (1H, d, $J = 12.4 \text{ Hz}$, OCHH), 4.52 (1H, d, $J = 12.4 \text{ Hz}$, OCHH), 4.12 (1 H, dd, $J_{2,3} = 9.5 \text{ Hz}$, $J_{2,1} = 2.5 \text{ Hz}$, H-2), 4.08 (1H, dd, $J_{3,2} = 9.4 \text{ Hz}$, $J_{3,4} = 1.8 \text{ Hz}$, H-4), 3.90 (1H, q, $J_{5,6} = 6.6 \text{ Hz}$, H-5), 3.77 (1H, br s, H-4), 1.16 (3H, d, $J_{6,5} = 6.5 \text{ Hz}$, H-6), 0.95, 0.93, 0.90 ($\text{SiC}(\text{CH}_3)_3$), 0.16, 0.15, 0.12, 0.08, 0.06, 0.05 ($\text{Si}(\text{CH}_3)_2$);

$^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 138.5 (Ar C), 138.2 127.3 (Ar CH), 99.2 (C-1), 75.8 (C-4), 72.3 (C-3), 70.1 (C-2), 69.3 (OCH₂), 68.4 (C-5), 26.6, 26.2, 26.1 ($\text{SiC}(\text{CH}_3)_3$), 18.8, 18.6, 18.3 ($\text{SiC}(\text{CH}_3)_3$), 16.9 (C-6), -3.5, -3.9, -4.1, -4.3, -4.6, 4.6 ($\text{Si}(\text{CH}_3)_2$);

M/z HRMS (ESI-TOF) calcd. for $C_{31}H_{60}O_5NaSi_3$ = 619.3646, $(M+Na)^+$. Found = 619.3636.



6.3.25 Ethyl-O-(2,3,4-Tri-O-*tert*-butyldimethylsilyl- α -L-fucopyranoside)-(1-2)-6-*tert*-butyldimethylsilyl-3,4-O-isopropylidene-1-thio- β -D-galactopyranoside, **154**

The trisilyl fucose donor **132** (245 mg, 0.45 mmol), acceptor **147** (130 mg, 0.34 mmol), NIS (77 mg, 0.34 mmol) and catalytic TMSOTf in DCM (5 mL) were reacted according to the procedure for Section 6.3.12, at $-30\text{ }^{\circ}\text{C}$ for 60 min. The crude material was purified by column chromatography (EtOAc:Hex, 1:24 (v/v)) to yield the product **154**, as a clear oil (198 mg, 66 %);

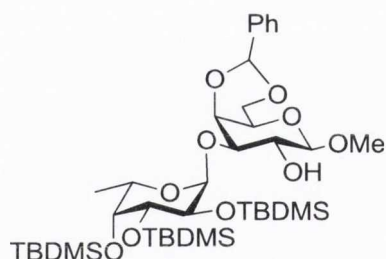
$[\alpha]_{\text{D}}^{20} = -25\text{ }^{\circ}$ (deg $\text{cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.1$ in CH_2Cl_3);

ν_{max} (thin film) 2929 cm^{-1} (CH);

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.27 (1H, d, $J_{1,2} = 1.7\text{ Hz}$, H-1B), 4.39 (1H, d, $J_{1,2} = 9.4\text{ Hz}$, H-1A), 4.19 (3H, m, H-5B, H-4A, H-3A), 4.12 (2H, br s, H-2B, H-3B), 3.89 (1H, dd, $J_{6,6'} = 10.1\text{ Hz}$, $J_{6,5} = 7.1\text{ Hz}$, H-6A), 3.8 (1H, dd, $J_{6',6} = 10.0\text{ Hz}$, $J_{6',5} = 5.7\text{ Hz}$, H-6'A), 3.79 (1H, dd, $J_{5,6} = 10.5\text{ Hz}$, $J_{5,4} = 1.6\text{ Hz}$, H-5A), 3.79 (1H, br s, H-4B), 3.73 (1H, dd, $J_{2,1} = 9.4\text{ Hz}$, $J_{2,3} = 5.9\text{ Hz}$, H-2A), 2.78 (1H, dq, $J_{\text{gem}} = 12.8\text{ Hz}$, $J = 7.9\text{ Hz}$, SCH(H)), 2.68 (1H, dq, $J_{\text{gem}} = 12.8\text{ Hz}$, $J = 7.5\text{ Hz}$, SCH(H)), 1.15, 1.30 (3H, s, $\text{C}(\text{CH}_3)_2$), 1.29 (2H, t, $J = 7.3\text{ Hz}$, SCH_2CH_3), 1.16 (3H, d, $J_{6,5} = 6.3\text{ Hz}$, H-6B), 0.96, 0.95, 0.95, 0.92 ($\text{SiC}(\text{CH}_3)_3$), 0.16, 0.16, 0.13, 0.13, 0.11, 0.10, 0.10, 0.90 ($\text{Si}(\text{CH}_3)_2$);

$^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 109.6 ($\text{C}(\text{CH}_3)_2$), 97.1 (C-1B), 83.2 (C-1A), 79.3 (C-3A), 76.9 (C-5A), 76.8 (C-4B), 74.1 (C-2A), 73.0 (C-4A), 71.6 (C-3B), 69.5 (C-2B), 68.7 (C-5B), 62.1 (C-6A), 27.7 ($\text{CCH}_3(\text{CH}_3)$), 26.5, 26.2, 26.1 ($\text{SiC}(\text{CH}_3)_3$), 25.9 ($\text{CCH}_3(\text{CH}_3)$), 25.7 ($\text{SiC}(\text{CH}_3)_3$), 24.0 (SCH_2CH_3), 18.8, 18.5, 18.2, 18.1 ($\text{SiC}(\text{CH}_3)_3$), 16.8 (C-6B), 14.9 (SCH_2CH_3), -3.7, -4.2, -4.3, -4.4, -4.6, -4.6, -5.5, -5.7 ($\text{Si}(\text{CH}_3)_2$);

M/z HRMS (ESI-TOF) calcd. for $\text{C}_{41}\text{H}_{86}\text{O}_9\text{SSi}_4\text{Na} = 889.4967$, $(\text{M}+\text{Na})^+$. Found = 889.4956.



6.3.26 O-(2,3,4-Tri-O-*tert*-butyldimethylsilyl- α -L-fucopyranosyl)-(1-3)-4,6-O-benzylidene-1-methyl- β -D-galactopyranose, **155**

The trisilyl fucose donor **132** (234 mg, 0.42 mmol), acceptor **150** (120 mg, 0.43 mmol), NIS (98 g, 0.43 mmol) and catalytic TMSOTf were reacted according to the procedure for Section 6.3.12, at -20 °C for 40 min. The crude material was purified by column chromatography (EtOAc:Hex, 2:3 (v/v)) to yield the product **155**, as a clear oil (104 mg, 31 %);

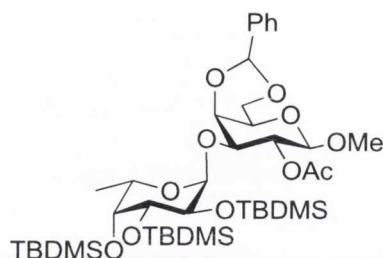
$[\alpha]_D^{20} = -18^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.01 in CH₂Cl₃);

$\nu_{\max}$ (thin film) 3574 cm⁻¹ (OH), 2931 cm⁻¹ (CH);

¹H NMR (100 MHz, CDCl₃) δ 7.50 (2H, m, Ar CH), 7.37 (3H, m, Ar CH), 5.55 (1H, s, PhCHO₂), 4.96 (1H, d, $J_{1,2} = 2.8$ Hz, H-1b), 4.38 (2H, m, H-1a, H-6'a), 4.35 (1H, d, $J_{4,3} = 3.3$ Hz, H-4a), 4.19 (1H, m, H-5b), 4.16 (1H, m, H-2b), 4.13 (1H, dd, $J_{3,2} = 8.1$ Hz, $J_{3,4} = 1.7$ Hz, H-3b), 4.10 (1H, dd, $J_{6,6'} = 10.7$ Hz, $J_{6,5} = 1.7$ Hz, H-6a), 4.00 (1H, dd, $J_{2,3} = 9.5$ Hz, $J_{2,1} = 7.9$ Hz, H-2a), 3.69 (1H, br s, H-4b), 3.61 (3H, s, OCH₃), 3.49 (2H, m, H-5a, H-3a) 1.06 (3H, d, $J_{6,5} = 6.6$ Hz, H-6b), 0.95, 0.94, 0.93 (9H, s, SiC(CH₃)₃), 0.16, 0.14, 0.13, 0.13, 0.12, 0.06 (3H, s, Si(CH₃)₂);

¹³C NMR (100 MHz, CDCl₃) δ 137.8 (Ar C), 128.8, 128.1, 128.1, 125.9, 125.9 (Ar CH), 103.3 (C-1a), 103.1 (C-1b), 100.7 (PhCHO₂), 83.0 (C-3a), 75.9 (C-4b), 75.2 (C-4a), 71.8 (C-3b), 70.2 (C-5b), 69.4 (C-6a), 69.4 (C-2a), 69.0 (C-2b), 64.5 (C-5a), 56.7 (OCH₃), 26.7, 26.4, 26.1 (SiC(CH₃)₃), 19.0, 18.6, 18.5 (SiC(CH₃)₃), 17.2 (C-6), -3.2, -3.9, -3.9, -4.3, -4.4, -4.8 (Si(CH₃)₂);

M/z HRMS (ESI-TOF) Calcd. for C₃₈H₇₀O₁₀Si₃Na = 793.4175, (M+Na)⁺. Found = 793.4193.



6.3.27 O-(2,3,4-Tri-O-*tert*-butyldimethylsilyl- α -L-fucopyranosyl)-(1-3)-2-acetyl-4,6-O-benzylidene-1-methyl- β -D-galactopyranose, **155a**

Disaccharide **155** (25 mg, 0.03 mmol), Ac₂O (excess, 0.8 mL) and pyridine (1 mL) were stirred at RT for 16 h. The mixture was quenched with the addition of deionised water (2 mL). The aqueous layer was extracted with EtOAc (3 X 3 mL), and the organic layer washed with CuSO₄ (10 % solution) until the purple pyridine complex was no longer observed. The organic layer was dried over MgSO₄, filtered and the solvent removed *in vacuo* to yield the product **155a**, as a clear oil (25 mg, 97 %);

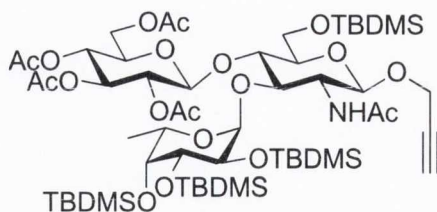
$[\alpha]_D^{20} = -36^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.01 in CH₂Cl₃);

$\nu_{\max}$ (thin film) 2931 cm⁻¹ (CH), 1753 cm⁻¹ (C=O);

¹H NMR (400 MHz, CDCl₃) δ 7.56 (2H, m, Ar CH), 7.40 (3H, m, Ar CH), 5.57 (1H, s, PhCH₂O₂), 5.48 (1H, dd, $J_{2,3} = 9.9$ Hz, $J_{2,1} = 8.1$ Hz, H-2a), 4.89 (1H, d, $J_{1,2} = 2.9$ Hz, H-1b), 4.50 (1H, d, $J_{4,3} = 2.9$ Hz, H-4a), 4.38 (1H, d, $J_{1,2} = 8.0$ Hz, H-1a), 4.34 (1H, dd, $J_{6',6} = 12.1$ Hz, $J_{6',5} = 1.2$ Hz, H-6'a), 4.09 (3H, m, H-5b, H-2b, H-6a), 3.97 (1H, dd, $J_{3,2} = 9.5$ Hz, $J_{3,4} = 1.7$ Hz, H-3b), 3.63 (1H, br s, H-4b), 3.61 (1H, dd, $J_{3,2} = 10.6$ Hz, $J_{3,4} = 3.3$ Hz, H-3a), 3.54 (3H, s, OCH₃), 3.48 (1H, br s, H-5a), 2.12 (3H, s, CH₃ (OAc)), 0.95, 0.92, 0.92 (9H, s, SiC(CH₃)₃), 0.90 (3H, d, $J_{6,5} = 6.8$ Hz, H-6b), 0.13, 0.12, 0.11, 0.11, 0.10, 0.02 (3H, s, Si(CH₃)₂);

¹³C NMR (100 MHz, CDCl₃) δ 169.42 (C=O), 137.7 (Ar C), 129.0, 128.2, 128.2, 126.4, 126.4 (Ar CH), 102.0 (C-1b), 101.7 (C-1a), 101.3 (PhCH₂O₂), 80.6 (C-3a), 76.5 (C-4b), 75.3 (C-4a), 71.6 (C-3b), 70.2 (C-5b), 69.2 (C-6a), 69.1 (C-2b), 68.5 (C-2a), 66.4 (C-5a), 55.7 (OCH₃), 26.6, 26.3, 26.2 (SiC(CH₃)₃), 21.3 (CH₃), 18.8, 18.6, 18.6 (SiC(CH₃)₃), 16.9 (C-6), -3.3, -3.7, -4.2, -4.3, -4.6, -4.7 (Si(CH₃)₂);

M/z HRMS (ESI-TOF) calcd. for C₄₀H₇₂O₁₁NaSi₃ = 835.4280, (M+Na)⁺. Found = 835.4260.



6.3.28 O-(2,3,4,6-Tetra-O-acetyl- β -D-galactopyranosyl)-(1-4)-(2,3,4-tri-O-*tert*-butyldimethylsilyl- α -L-fucopyranosyl)-(1-3)-2-acetamido-2-deoxy-6-O-*tert*-butyldimethylsilyl-1-O-propargyl- β -D-glucopyranose, 156

The trisilyl fucose donor **132** (109 mg, 0.12 mmol), acceptor **76** (90 mg, 0.128 mmol), NIS (43 mg, 0.12 mmol) and catalytic TMSOTf were reacted according to the procedure for Section 6.3.12, at $-20\text{ }^{\circ}\text{C}$ for 4 h. The crude material was purified by column chromatography (EtOAc:Hex, 1:1 (v/v)) to yield the product **156**, as a white solid (41 mg, 24 %);

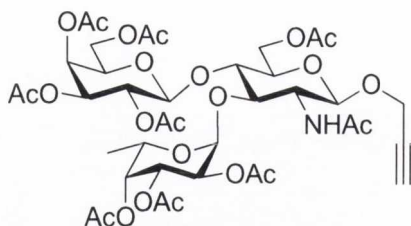
$[\alpha]_{\text{D}}^{20} = -67^{\circ}$ (deg cm³ g⁻¹ dm⁻¹) ($c = 0.01$ in CH₂Cl₃);

ν_{max} (thin film) 3407 cm⁻¹ (NH), 3296 cm⁻¹ (C $\equiv$ CH), 2930 cm⁻¹ (CH), 1751 cm⁻¹ (C=O), 1678 cm⁻¹ (C=ONH);

¹H NMR (400 MHz, CDCl₃) δ 6.36 (1H, d, $J = 9.9$ Hz, NH), 5.41 (1H, d, $J_{4,3} = 3.2$ Hz, H-4b), 5.16 (1H, dd, $J_{2,3} = 10.3$ Hz, $J_{2,1} = 7.7$ Hz, H-2b), 5.03 (1H, dd, $J_{3,2} = 10.6$ Hz, $J_{3,4} = 3.5$ Hz, H-3b), 4.90 (1H, br s, H-1c), 4.67 (1H, d, $J_{1,2} = 2.0$ Hz, H-1a), 4.41 (1H, d, $J_{1,2} = 7.8$ Hz, H-1b), 4.32 (1H, br d, $J = 10$ Hz, H-2a), 4.25 (2H, m, OCH₂), 4.20 (1H, m, H-6b), 4.08 (4H, m, H-6'b, H-6a, H-5c, H-5b), 3.94 (1H, m, H-4c), 3.89 (3H, m, H-2c, H-3c, H-5a), 3.84 (1H, app t, $J_{3,2} = J_{3,4} = 6.1$ Hz, H-3a), 3.76 (1H, dd, $J_{6'6} = 10.1$ Hz, $J_{6'5} = 5.2$ Hz, H-6'a), 3.64 (1H, dd, $J_{4,5} = 9.8$ Hz, $J_{4,3} = 5.8$ Hz, H-4a), 2.36 (1H, t, $J = 2.3$ Hz, C $\equiv$ CH), 2.18, 2.12, 2.07, 2.04, 2.01 (3H, s, CH₃), 1.17 (3H, br s, H-6c), 0.95, 0.93, 0.93, 0.92 (9H, s, SiC(CH₃)₃), 0.14, 0.13, 0.13, 0.12, 0.11, 0.10, 0.08, 0.06 (Si(CH₃)₂);

¹³C NMR (100 MHz, CDCl₃) δ (C-1c-6c not visible, due to broadening), 99.2 (C-1b), 98.0 (C-1a), 79.1 (C $\equiv$ CH), 76.2 (C-4a), 74.9 (C-5a), 74.3 (C $\equiv$ CH), 72.3 (C-4c), 71.0 (C-3a), 70.2 (C-3b), 68.8 (C-2b), 66.7 (C-4b), 62.7 (C-6a), 61.0 (C-6b), 54.8 (OCH₂), 48.3 (C-2a), 26.6, 26.4, 26.1, 25.8 (SiC(CH₃)₃), 22.9 (NHCOCH₃), 21.0, 20.7, 20.6, 20.6 (CH₃), 18.7, 18.6, 18.2, 18.0 (SiC(CH₃)₃), -4.0, -4.0, -4.2, -4.7, -5.0, -5.2, -5.2 (Si(CH₃)₂), (a = GlcNAc, b = Gal, c = Fuc);

M/z HRMS (ESI-TOF) calcd. for C₅₅H₁₀₁NO₁₉NaSi₄ = 1214.5943, (M+Na)⁺. Found = 1214.5894.



6.3.29 O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1-4)-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1-3)-2-acetamido-2-deoxy-6-O-acetyl-1-O-propargyl-β-D-glucopyranose, 157

To a solution of protected trisaccharide **156** (24 mg, 0.02 mmol) in THF (2.5 mL) was added TBAF (1M in THF) (0.3 mL, 0.30 mmol) at 0 °C. The mixture was heated to 50 °C and stirred for 16 h. The solvent volume was reduced *in vacuo* and the residue dissolved in pyridine (1 mL). Ac₂O (1 mL) was added at 0 °C and the mixture was stirred for 16 h, warming to RT. The reaction was quenched by the addition of MeOH (1 mL) followed by dilution with EtOAc (30 mL). The organic layer was washed with H₂O, 10 % CuSO₄ solution, H₂O, dried over MgSO₄, filtered and the solvent removed *in vacuo*. The crude material was purified by column chromatography (EtOAc:Hex, 4:1 (v/v)) to yield the product **157**, as a white solid (14 mg, 77 %);

$[\alpha]_D^{20} = 77^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.01 in CHCl₃);

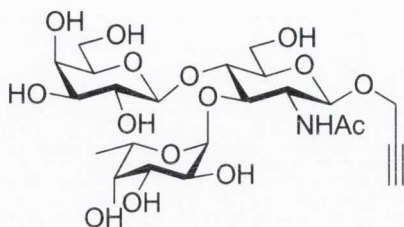
$\nu_{\max}$ (thin film) 3300 cm⁻¹ (NH), 3281 cm⁻¹ (C≡CH), 2927 cm⁻¹ (CH), 1740 cm⁻¹ (C=O), 1672 cm⁻¹ (C=ONH);

¹H NMR (600 MHz, CDCl₃) δ 5.52 (1H, d, *J* = 8.8 Hz, NH), 5.46 (1H, d, *J*_{1,2} = 3.8 Hz, H-1c), 5.44 (1H, d, *J*_{4,3} = 3.3 Hz, H-4b), 5.39 (1H, d, *J*_{4,3} = 3.0 Hz, H-4c), 5.23 (1H, dd, *J*_{3,2} = 10.9 Hz, *J*_{3,4} = 3.1 Hz, H-3c), 5.12 (1H, d, *J*_{2,3} = 10.4 Hz, *J*_{2,1} = 8.2 Hz, H-2b), 5.05 (1H, dd, *J*_{2,3} = 10.8 Hz, *J*_{2,1} = 3.8 Hz, H-2c), 5.01 (1H, dd, *J*_{3,2} = 10.4 Hz, *J*_{3,4} = 3.5 Hz, H-3b), 4.82 (1H, m, H-5c), 4.81 (1H, d, *J*_{1,2} = 7.3 Hz, H-1a), 4.63 (1H, dd, *J*_{6',6} = 12.1 Hz, *J*_{6',5} = 2.8 Hz, H-6'a), 4.49 (1H, dd, *J*_{6',6} = 11.9 Hz, *J*_{6',5} = 5.6 Hz, H-6'b), 4.49 (1H, d, *J*_{1,2} = 8.0 Hz, H-1b), 4.33 (2H, m, OCH₂), 4.30 (1H, *J*_{6,6'} = 7.5 Hz, *J*_{6,5} = 11.8 Hz, H-6b), 4.19 (1H, dd, *J*_{6,6'} = 11.9 Hz, *J*_{6,5} = 4.9 Hz, H-6a), 4.13 (1H, m, H-3a), 3.90 (1H, t, *J*_{5,6} = 6.8 Hz, H-5b), 3.87 (1H, m, H-5a), 3.83 (1H, m, H-2a), 3.62 (1H, m, H-4a), 2.45 (1H, *J* = 2.2 Hz, C≡CH), 2.22, 2.17, 2.16, 2.13, 2.10, 2.19, 2.02, 2.00, 1.99 (3H, s, CH₃), 1.23 (3H, d, *J*_{6,5} = 6.6 Hz, H-6b), (a = GlcNAc, b = Gal, c = Fuc);

¹³C NMR (150 MHz, CDCl₃) δ 171.1, 170.8, 170.7, 170.6, 170.4, 170.3, 170.03, 169.87, 169.3 (C=O), 100.4 (C-1b), 97.8 (C-1a), 95.1 (C-1c), 78.6 (C≡CH), 75.2 (C≡CH), 74.2 (C-5a), 72.9 (C-4a), 72.9 (C-3a), 71.3 (C-4c), 71.0 (C-5b), 70.8 (C-3b), 68.9 (C-2b), 68.1 (C-2c), 68.0

(C-3c), 66.6 (C-4b), 64.2 (C-5c), 61.9 (C-6a), 60.7 (C-6b), 55.8 (C-6b), 55.0 (C-2a), 23.5 (NHCOCH₃), 21.0, 20.9, 20.8, 20.8, 20.7, 20.7, 20.6, 20.6 (CH₃), 15.8 (C-6c);

M/z HRMS (ESI-TOF) calcd. for C₃₉H₅₃NO₂₃Na = 926.2906, (M+Na)⁺. Found = 926.2895.



6.3.30 2-Acetamido-2-deoxy-β-D-galactopyranosyl-(1-4)-(-α-L-fucopyranosyl)-(1-3))-1-O-propargyl-β-D-glucopyranose, **158**

Per acetylated trisaccharide **157** (132 mg, 0.15 mmol) and NaOMe (6.3 mg, 0.02 mmol) in MeOH (10 mL) was reacted according to the general deacetylation procedure (Section 6.2.2), followed by freeze drying to yield the product **158**, as a white solid (72 mg, 87 %);

[α]_D²⁰ = 35 ° (deg cm³ g⁻¹ dm⁻¹) (c = 0.01 in CH₂Cl₂/MeOH 1:1);

ν_{max} (thin film) 3274 cm⁻¹ (OH), 2924 cm⁻¹ (CH), 1646 cm⁻¹ (C=ONH)

¹H NMR (600 MHz, CDCl₃) δ 5.05 (1H, d, *J*_{1,2} = 3.6 Hz, H-1c), 4.86 (1H, m, H-5c), 4.69 (1H, d, *J*_{1,2} = 7.6 Hz, H-1a), 4.46 (1H, d, *J*_{1,2} = 7.6 Hz, H-1b), 4.37 (2H, s, OCH₂), 3.95 (2H, br s, C-6a), 3.92 (3H, m, H-2a, H-5b, H-4a), 3.88 (1H, dd, *J*_{3,2} = 10.5 Hz, *J*_{3,4} = 3.3 Hz, H-3c), 3.82 (1H, d, *J*_{4,3} = 2.5 Hz, H-4b), 3.79 (1H, dd, *J*_{6,6'} = 11.2 Hz, *J*_{6,5} = 6.9 Hz, H-6b), 3.74 (1H, d, *J*_{4,3} = 3.0 Hz, H-4c), 3.69 (1H, dd, *J*_{6',6} = 11.5 Hz, *J*_{6',5} = 5.1 Hz, H-6'b), 3.65 (1H, dd, *J*_{2,3} = 10.2 Hz, *J*_{2,1} = 3.5 Hz, H-2c), 3.53 (1H, *J*_{2,3} = 9.6 Hz, *J*_{2,1} = 7.3 Hz, H-2b), 3.48 (1H, dd, *J*_{3,2} = 9.7 Hz, *J*_{3,4} = 2.8 Hz, H-3b), 3.46 (2H, m, H-5a, H-3a), 2.88 (1H, br s, -C≡CH), 1.99 (3H, s, CH₃), 1.20 (3H, d, *J*_{6,5} = 6.6 Hz, H-6c);

¹³C NMR (150 MHz, CDCl₃) δ 172.9 (C=O), 102.9 (C-1b), 99.3 (C-1c), 99.0 (C-1c), 78.9 (-C≡CH), 76.5 (C-3a), 75.6 (C-5a), 75.5 (-C≡CH), 75.2 (C-4a), 74.2 (C-5b), 73.9 (C-3b), 72.7 (C-4c), 71.7 (C-2b), 70.2 (C-3c), 69.0 (C-2c), 68.9 (C-4b), 66.7 (C-5c), 61.8 (C-6b), 60.3 (C-6a), 56.2 (C-2a), 55.4 (OCH₂), 22.0 (CH₃), 15.6 (C-6c), (a = GlcNAc, b = Gal, c = Fuc);

M/z HRMS (ESI-TOF) calcd. for C₂₃H₃₇NO₁₅Na = 590.2061, (M+Na)⁺. Found = 590.2055.

6.4 Experimental data for chapter 4



6.4.1 Ethyl-2,3-di-O-*tert*-butyldimethylsilyl-1-thio- α -L-fucopyranoside, **167**

Side product observed during the synthesis of di-silyl fucose thioglycoside donor **131** (Section 6.3.2). The crude material was purified by column chromatography (Et₂O:Hexane, 1:23 (v/v)) to yield the product **167**, as a clear oil (80 mg, 3 % yield);

$[\alpha]_D^{20} = 39^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3492 cm⁻¹ (OH), 2929 cm⁻¹ (CH);

¹H NMR (600 MHz, CDCl₃) δ 4.28 (1H, d, $J_{1,2} = 8.8$ Hz, H-1), 3.69 (1H, app t, $J_{2,3} = J_{2,1} = 8.3$ Hz, H-2), 3.67 (1H, m, H-4), 3.65 (1H, dd, $J_{3,2} = 8.0$ Hz, $J_{3,4} = 2.0$ Hz, H-3), 3.60 (1H, q, $J_{5,6} = 6.5$ Hz, H-5), 2.70 (2H, m, SCH₂CH₃), 2.35 (1H, d, $J = 1.5$ Hz, OH), 1.36 (3H, d, $J_{6,5} = 6.5$ Hz, H-6), 1.29 (3H, t, $J = 7.4$ Hz, SCH₂CH₃), 0.97, (9H, s, SiC(CH₃)₃), 0.94 (9H, s, SiC(CH₃)₃), 0.22, 0.18, 0.15, 0.11 (3H, s, Si(CH₃)₂);

¹³NMR (150 MHz, CDCl₃) δ 86.4 (C-1), 77.8 (C-3), 73.9 (C-5), 72.9 (C-4), 71.4 (C-2), 26.4, 26.3 (SiC(CH₃)₃), 24.8 (SCH₂CH₃), 18.3, 18.2 (SiC(CH₃)₃), 16.7 (C-6), 14.8 (SCH₂CH₃), -2.1, -3.4, -3.4, -4.0 (Si(CH₃)₂);

M/z HRMS (ESI-TOF) calcd. for C₂₀H₄₄O₄NaSSi₂ = 459.2397 (M+Na)⁺. Found = 459.2392.



6.4.2 Ethyl-3-O-acetyl-2,4-di-O-*tert*-butyldimethylsilyl-1-thio- α -L-fucopyranoside, **168**

To a stirred solution of thioglycoside **167** (50 mg, 0.11 mmol) in pyridine (2 mL) was added Ac₂O (2 mL) at 0 °C. The mixture was stirred at RT for 18 days. The reaction was quenched with deionised H₂O (1 mL) and diluted with EtOAc (10 mL). The crude mixture was washed sequentially with brine (10 mL), 10 % CuSO₄ solution (2 X 5 mL) and deionised H₂O (10 mL). The organic layer was dried over MgSO₄, filtered and the solvent removed *in vacuo*. The crude material was purified using column chromatography (EtOAc:Hexane, 3:97 (v/v)) to yield the product **168**, as an oily wax (43 mg, 80 %);

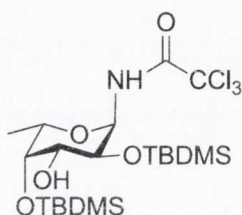
$[\alpha]_D^{20} = 53^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.01 in CH₃Cl);

$\nu_{\max}$ (thin film) 2929 cm⁻¹ (CH), 1746 cm⁻¹ (C=O);

^1H NMR (600 MHz, CDCl_3) δ 4.75 (1H, dd, $J_{3,2} = 9.4$ Hz, $J_{3,4} = 2.2$ Hz, H-3), 4.30 (1H, d, $J_{1,2} = 9.1$ Hz, H-1), 3.98 (1H, app t, $J_{2,1} = J_{2,3} = 9.3$ Hz, H-2), 3.89 (1H, d, $J_{4,3} = 1.9$ Hz, H-4), 3.67 (1H, q, $J_{5,6} = 6.4$ Hz, H-5), 2.75 (1H, m, $\text{SCH}(\text{H})\text{CH}_3$), 2.67 (1H, m, $\text{SCH}(\text{H})\text{CH}_3$), 2.15 (3H, s, CH_3), 1.30 (3H, t, $J = 7.2$ Hz, SCH_2CH_3), 1.23 (3H, d, $J_{6,5} = 6.3$ Hz, H-6), 0.97, 0.90 (9H, s, $\text{Si}(\text{CH}_3)_3$), 0.20, 0.09, 0.07, 0.06 (3H, s, $\text{Si}(\text{CH}_3)_2$);

^{13}C NMR (150 MHz, CDCl_3) δ 170.6 (C=O), 86.2 (C-1), 78.9 (C-3), 74.5 (C-5), 71.8 (C-4), 68.2 (C-2), 25.9, 25.9 ($\text{Si}(\text{CH}_3)_3$), 23.8 (SCH_2CH_3), 21.9 (COCH_3), 18.3, 18.2 ($\text{Si}(\text{CH}_3)_3$), 17.6 (C-6), 14.9 (SCH_2CH_3), -3.9, -4.0, -4.1, -4.5 ($\text{Si}(\text{CH}_3)_2$);

M/z HRMS (ESI-TOF) calcd. for $\text{C}_{22}\text{H}_{46}\text{O}_5\text{NaSSi}_2 = 501.2502$ ($\text{M}+\text{Na}$) $^+$. Found = 501.2512.



6.4.3 1-N-Trichloroacetamido-2,4-di-O-*tert*-butyldimethylsilyl- α -L-fucopyranose, **169**

To a stirred solution of thioglycoside acceptor **133** (100 mg, 0.22 mmol) and trichloroacetimidate donor **131** (135 mg, 0.27 mmol) in DCM (3 mL) with pre-activated 3 Å ms, at 0 °C was added BF_3OEt_2 (37 μL , 0.27 mmol). The reaction was stirred for 16 h warming to RT before quenching with sat. aqueous NaHCO_3 solution (5 mL). The reaction was diluted with DCM (10 mL) and filtered through a plug of celite. The solvent was removed *in vacuo* and purified using column chromatography (EtOAc :Hexane, 1:49 (v/v)) to yield the product **169**, as an off white amorphous solid (57 mg, 46 % yield);

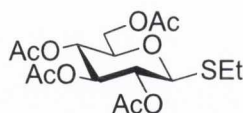
$[\alpha]_{\text{D}}^{20} = 49^\circ$ (deg $\text{cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.1$ in CH_3Cl);

ν_{max} (thin film) 3501 cm^{-1} (OH), 3389 cm^{-1} (NH), 2929 cm^{-1} (CH), 1708 cm^{-1} (C=O);

^1H NMR (400 MHz, CDCl_3) δ 7.27 (1H, d, $J = 6.4$ Hz, NH), 5.54 (1H, d, $J_{1,2} = 4.2$ Hz, H-1), 4.10 (1H, dd, $J_{2,3} = 7.3$ Hz, $J_{2,1} = 4.2$ Hz, H-2), 3.95 (1H, app t, $J_{4,3} = J_{4,5} = 2.9$ Hz, H-5), 3.91 (1H, dq, $J_{5,6} = 6.5$ Hz, $J_{5,4} = 2.8$ Hz, H-5), 3.69 (1H, dd, $J_{3,2} = 7.6$ Hz, $J_{3,4} = 3.0$ Hz, H-3), 1.33 (3H, d, $J_{6,5} = 6.4$ Hz, H-6), 0.96, 0.94 (9H, s, $\text{Si}(\text{CH}_3)_3$), 0.17, 0.15, 0.14, 0.13 (3H, s, $\text{Si}(\text{CH}_3)_2$);

^{13}C NMR (100 MHz, CDCl_3) δ 162.1 (C=O), 98.2 (COCCl_3), 76.6 (C-1), 72.2 (C-3), 70.6 (C-4), 69.8 (C-5), 69.1 (C-2), 25.9, 25.7 ($\text{Si}(\text{CH}_3)_3$), 18.0, 17.7 ($\text{Si}(\text{CH}_3)_3$), 16.0 (C-6), -4.3, -4.6, -4.7, -4.7 ($\text{Si}(\text{CH}_3)_2$);

M/z HRMS (ESI-TOF) calcd. for $\text{C}_{20}\text{H}_{40}\text{NO}_5\text{NaSi}_2\text{Cl}_3 = 558.1408$ ($\text{M}+\text{Na}$) $^+$. Found = 558.1414.



6.4.4 Ethyl-2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside, **170**

Second product isolated during the attempted glycosylation in Section 6.4.3. The crude material was purified using column chromatography (EtOAc:Hexane, 1:49 (v/v)) to yield the product **170**, as an off white amorphous solid (64 mg, 71 % yield);

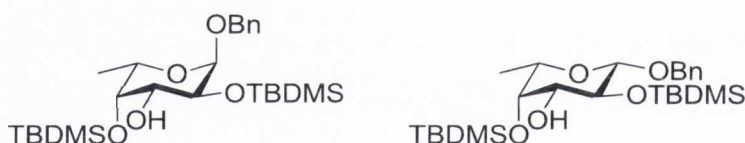
$[\alpha]_D^{20} = -22^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 2964 cm⁻¹ (CH), 1737 cm⁻¹ (C=O);

¹H NMR (400 MHz, CDCl₃) δ 5.24 (1H, app t, $J_{3,2} = J_{3,4} = 9.5$ Hz, H-3), 5.10 (1H app t, $J_{4,3} = J_{4,5} = 9.8$ Hz, H-4), 5.05 (1H, app t, $J_{2,3} = J_{2,1} = 9.5$ Hz, H-2), 4.51 (1H, d, $J_{1,2} = 9.8$ Hz, H-1), 4.26 (1H, dd, $J_{6,6'} = 12.4$ Hz, $J_{6,5} = 4.9$ Hz, H-6), 4.15 (1H, dd, $J_{6',6} = 12.3$ Hz, $J_{6',5} = 2.2$ Hz, H-6'), 3.73 (1H, ddd, $J_{5,4} = 10.0$ Hz, $J_{5,6} = 4.8$ Hz, $J_{5,6'} = 2.3$ Hz, H-5), 2.72 (2H, m, SCH₂CH₃), 2.09, 2.08, 2.04, 2.03 (3H, s, CH₃), 1.29 (3H, t, J = 7.4 Hz, SCH₂CH₃);

¹³C NMR (100 MHz, CDCl₃) δ 170.2, 169.9, 169.0, 169.0 (C=O), 83.1 (C-1), 75.4 (C-5), 73.4 (C-3), 69.3 (C-2), 67.8 (C-4), 61.7 (C-6), 23.7 (SCH₂CH₃), 20.3, 20.3, 20.2, 20.1 (CH₃), 14.4 (SCH₂CH₃);

M/z HRMS (ESI-TOF) calcd. for C₁₆H₂₄O₉SN_a = 415.1039 (M+Na)⁺. Found = 415.1054.



6.4.5 2,4-Di-O-*tert*-butyldimethylsilyl-1-O-benzyl-α-L-fucopyranose, **171**

and 2,4-Di-O-*tert*-butyldimethylsilyl-1-O-benzyl-β-L-fucopyranose, **172**

The di-silyl fucose donor **131** (150 mg, 0.32 mmol), benzyl alcohol **152** (0.1 mL, 0.96 mmol), NIS (81 mg, 0.36 mmol) and catalytic TMSOTf were reacted according to the procedure for Section 6.3.12 at -30 °C for 150 min. The crude material was purified by column chromatography (DCM:Hexane, 3:2 (v/v)), to yield the product **171** and **172**, as clear oils (145 mg, 88 % (2.6:1, α:β);

α anomer characterisation, **171**

$[\alpha]_D^{20} = 37^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3599 cm^{-1} (OH), 2929 cm^{-1} (CH);

^1H NMR (400 MHz, CDCl_3) δ 7.40 (2H, d, $J = 7.8$ Hz, *o*-Ph), 7.35-7.32 (3H, m, *m,p*-Ph), 4.83 (1H, d, $J_{1,2} = 3.2$ Hz, H-1), 4.71 (1H, d, $J = 12.1$ Hz, PhCH₂), 4.57 (1H, d, $J = 12.1$ Hz, PhCH₂), 3.98 (1H, dd, $J_{2,3} = 9.7$ Hz, $J_{2,1} = 3.4$ Hz, H-2), 3.97 (1H, q, $J_{5,6} = 6.5$ Hz, H-5), 3.92 (1H, m, H-3), 3.88 (1H, dd, $J_{4,3} = 2.6$ Hz, $J_{4,5} = 0.8$ Hz, H-4), 1.98 (1H, br s, OH), 1.20 (3H, d, $J_{6,5} = 6.6$ Hz, H-6), 0.96, 0.92 (Si C(CH₃)₃), 0.16, 0.12, 0.08, 0.01 (3H, s, Si(CH₃)₂);

^{13}C NMR (100 MHz, CDCl_3) δ 137.5 (Ar C), 128.4 (Ar CH), 128.3 (Ar CH), 127.7 (Ar CH), 97.9 (C-1), 73.5 (C-4), 70.6 (C-3), 70.7 (C-2), 69.2 (OCH₂Ph), 67.2 (C-5), 26.1, 25.8 (SiC(CH₃)₃), 18.5, 18.2 (SiC(CH₃)), 17.2 (C-6), -4.0, -4.5, -4.5, -4.7 (Si(CH₃));

M/z HRMS (ESI-TOF) calcd. for C₂₅H₄₆O₅NaSi₂ = 505.2782, (M+Na)⁺. Found = 505.2777.

β anomer characterisation, **172**

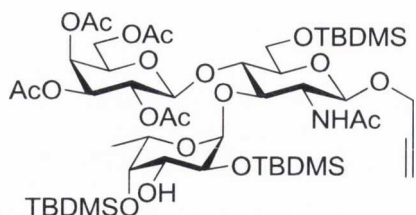
$[\alpha]_{\text{D}}^{20} = 20^\circ$ (deg $\text{cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.1$ in CH₃Cl);

$\nu_{\max}$ (thin film) 3595 cm^{-1} (OH), 2929 cm^{-1} (CH);

^1H NMR (400 MHz, CDCl_3) δ 7.40 (2H, d, $J = 7.3$ Hz, *o*-Ph), 7.35 (2H, t, $J = 7.2$ Hz, *m*-Ph), 7.31 (1H, t, $J = 7.1$ Hz, *p*-Ph), 4.93 d, $J = 11.8$ Hz, OCH(H)Ph), 4.61 (1H, d, $J = 11.8$ Hz, PhCH(H)), 4.25 (1H, $J_{1,2} = 7.5$ Hz, H-1), 3.79 (1H, dd, $J_{4,3} = 2.7$ Hz, H-4), 3.68 (1H, dd, $J_{2,3} = 9.4$ Hz, $J_{2,1} = 7.3$ Hz, H-2), 3.56 (1H, q, $J_{5,6} = 6.5$ Hz, H-5), 3.46 (1H, m, H-3), 2.02 (1H, d, $J = 4.7$ Hz, OH), 1.29 (3H, d, $J_{6,5} = 6.4$ Hz, H-6), 0.98, 0.90 (9H, s, Si C(CH₃)₃), 0.16, 0.13, 0.12, 0.06 (3H, s, Si(CH₃)₂);

^{13}C NMR (100 MHz, CDCl_3) δ 137.5 (Ar C), 128.3 (Ar CH), 128.1 (Ar CH), 127.5 (Ar CH), 102.0 (C-1), 75.5 (C-3), 73.0 (C-4), 72.7 (C-2), 71.2 (C-5), 70.5 (OCH₂Ph), 26.1, 25.9 (SiC(CH₃)₃), 18.6, 18.2 (SiC(CH₃)), 17.3 (C-6), -4.1, -4.1, -4.2, -4.7 (Si(CH₃));

M/z HRMS (ESI-TOF) calcd. for C₂₅H₄₆O₅NaSi₂ = 505.2782, (M+Na)⁺. Found = 505.2786.



6.4.6 O-(2,3,4,6-Tetra-O-acetyl- β -D-galactopyranosyl)-(1-4))-O-(2,4-di-O-*tert*-butyldimethylsilyl- α -L-fucopyranosyl)-(1-3))-2-acetamido-2-deoxy-6-O-*tert*-butyldimethylsilyl-1-O-propargyl- β -D-glucopyranose, **173**

To a stirred solution of disaccharide acceptor **76** (225 mg, 0.32 mmol), thioglycoside donor **131** (251 mg, 0.57 mmol), and NIS (108 mg, 0.57 mmol) in DCM (5 mL) at $-20\text{ }^{\circ}\text{C}$ under argon, was added TMSOTf (cat.). The mixture was stirred at $-20\text{ }^{\circ}\text{C}$ for 3 h. The reaction was quenched with the addition of Et_3N (0.5 mL) and diluted with DCM (10 mL). The organic layer was washed with sat. aqueous $\text{Na}_2\text{S}_2\text{O}_3$ solution (10 mL), deionised H_2O (10 mL), dried over MgSO_4 , filtered and the solvent removed *in vacuo*. The crude material was purified by column chromatography (EtOAc:Hex, 1:3 to 1:1 (v/v)) to yield the product **173**, as a white solid (182 mg, 53 %);

$[\alpha]_{\text{D}}^{20} = -54\text{ }^{\circ}$ (deg $\text{cm}^3\text{ g}^{-1}\text{ dm}^{-1}$) ($c = 0.1$ in CH_3Cl);

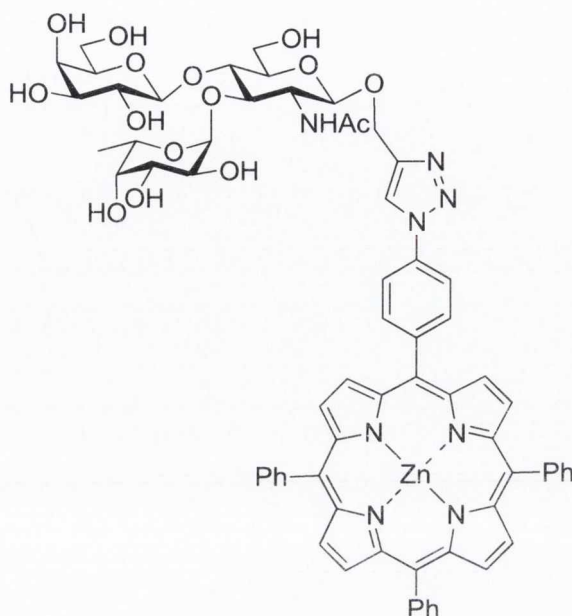
ν_{max} (thin film) 3598 cm^{-1} (OH), 3335 cm^{-1} (NH), 3328 cm^{-1} ($\text{C}\equiv\text{CH}$), 2930 cm^{-1} (CH), 1754 cm^{-1} (C=O), 1663 cm^{-1} ($\text{C}=\text{ONH}$);

$^1\text{H NMR}$ (600 MHz, CDCl_3) δ 6.04 (1H, $J = 8.6\text{ Hz}$, NH), 5.38 (1H, d, $J_{4,3} = 3.0\text{ Hz}$, H-4b), 5.10 (1H, dd, $J_{2,3} = 10.6\text{ Hz}$, $J_{2,1} = 7.7\text{ Hz}$, H-2b), 5.05 (1H, d, $J_{1,2} = 3.4\text{ Hz}$, H-1c), 5.00 (1H, dd, $J_{3,2} = 10.6\text{ Hz}$, $J_{3,4} = 3.3\text{ Hz}$, H-3b), 4.69 (1H, d, $J_{1,2} = 6.0\text{ Hz}$, H-1a), 4.61 (1H, d, $J_{1,2} = 8.0\text{ Hz}$, H-1b), 4.26 (2H, d, $J = 2.0\text{ Hz}$, OCH_2), 4.14 (1H, dd, $J_{6,6'} = 11.4\text{ Hz}$, $J_{6,5} = 7.5\text{ Hz}$, H-6b), 4.13 (1H, m, H-5c), 4.10 (1H, dd, $J_{6',6} = 11.4\text{ Hz}$, $J_{6',5} = 6.4\text{ Hz}$, H-6'b), 4.04 (1H, app t, $J_{3,4} = J_{3,2} = 6.0\text{ Hz}$, H-3a), 3.95 (1H, app t, $J_{4,3} = J_{4,5} = 5.7\text{ Hz}$, H-4a), 3.92 (2H, m, H-6a, H-2c), 3.89 (1H, m, H-2a), 3.82 (2H, m, H-5b, H-4c), 3.78 (2H, m, H-6'a, H-3c), 3.45 (1H, m, H-5a), 2.36 (1H, br s, $-\text{C}\equiv\text{CH}$), 2.17, 2.07, 2.02, 2.01, 1.98 (3H, s, CH_3), 1.91 (1H, d, $J = 4.2\text{ Hz}$, OH), 1.16 (3H, d, $J_{6,5} = 6.2\text{ Hz}$, H-6c), 0.92, 0.92, 0.90 (9H, s, $\text{SiC}(\text{CH}_3)_3$), 0.13, 0.11, 0.11, 0.09, 0.08, 0.07 ($\text{Si}(\text{CH}_3)_2$);

$^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 170.2, 170.0, 169.9, 169.7, 169.7 (C=O), 99.12 (C-1b), 98.0 (C-1a), 97.8 (C-1c), 78.9 ($-\text{C}\equiv\text{CH}$), 75.7 (C-5a), 74.4 ($-\text{C}\equiv\text{CH}$), 73.8 (C-3c), 73.0, 73.0 (C-3a, C-4a), 70.7 (C-4c), 70.4 (C-3b), 70.3 (C-2c), 69.9 (C-5b), 68.9 (C-2b), 67.3 (C-5c), 66.8 (C-4b), 61.6 (C-6a), 60.9 (C-6b), 54.7 (OCH_2), 52.5 (C-2a), 25.9, 25.8, 25.7 ($\text{SiC}(\text{CH}_3)_3$), 23.3

(NHCOCH₃), 20.7, 20.6, 20.5, 20.4 (CH₃), 18.4, 18.0, 18.0 (SiC(CH₃)₃), 17.1 (C-6c), -4.0, -4.4, -4.7, -4.9, -5.3, -5.4 (Si(CH₃)₂), (a = GlcNAc, b = Gal, c = Fuc);

M/z HRMS (ESI-TOF) calcd. for C₄₉H₈₇O₁₉NaSi₃ = 1100.5078 (M+Na)⁺. Found = 1100.5076.



6.4.7 5-(4-(4'-[[(2-Acetamido-2-deoxy-(O-(α -L-fucopyranosyl)-(1-3))-(O-(β -D-galactopyranosyl)-(1-4)) β -D-glucopyranoside)-1-oxymethyl]-1-N-1,2,3-triazol-1'-yl)phenyl)-10,15,20-triphenyl-porphyrinatozinc(II), **174**

Mono azido porphyrin **96** (15 mg, 0.02 mmol) and propargyl functionalized Lewis X **158** (12 mg, 0.02 mmol) were reacted according to the general glycoporphyrin procedure (Section 6.2.37) in solvent system C, and the crude material purified by column chromatography (EtOAc:EtOH:H₂O, 7:2:1 (v/v/v)) to yield the product **174**, as a purple solid, (24 mg, 90 %);

R_f = 0.43 (EtOAc:EtOH:H₂O, 7:2:1 (v/v/v));

$[\alpha]_D^{20}$ = N/A (Sample too dark for analysis at c = 0.01 in CH₂Cl₂/MeOH 1:1);

$\nu_{\max}$ (thin film) 3322 cm⁻¹ (OH), 2924 cm⁻¹ (CH), 1646 cm⁻¹ (C=ONH);

¹H NMR (800 MHz, MeOD) δ 8.86 (2H, d, J = 4.5 Hz, β -H), 8.85 (2H, d, J = 4.4 Hz, β -H), 8.82 (4H, s, β -H), 8.81 (1H, s, 5-triazole-H), 8.40 (2H, d, J = 7.7 Hz, o-Ph), 8.25 (2H, d, J = 7.7 Hz, m-Ph), 8.19 (6H, d, J = 6.6 Hz, o-Ph), 7.76 (9H, m, m, p-Ph), 5.08 (1H, d, J = 12.2 Hz, OCH(H)), 5.05 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1c), 4.95 (1H, d, J = 12.4 Hz, OCH(H)), 4.82 (1H, m, H-5c), 4.73 (1H, d, $J_{1,2}$ = 7.7 Hz, H-1a), 4.46 (1H, d, $J_{1,2}$ = 7.7 Hz, H-1b), 4.03 (1H, $J_{6'6}$ = 12.3 Hz, $J_{6'5}$ = 2.2 Hz, H-6'a), 4.00 (1H, $J_{6,6'}$ = 12.3 Hz, $J_{6,5}$ = 3.9 Hz, H-6'a), 3.99 (1H, m, H-2a), 3.95 (2H, m, H-5b, H-4a), 3.87 (1H, $J_{3,2}$ = 10.2 Hz, $J_{3,4}$ = 2.9 Hz, H-3c), 3.79 (1H, d, $J_{4,3}$ = 2.9 Hz, H-

4b), 3.77 (1H, dd, $J_{6,6'} = 11.6$ Hz, $J_{6,5} = 6.9$ Hz, H-6b), 3.72 (1H, d, $J_{4,3} = 2.5$ Hz, H-4c), 3.67 (1H, dd, $J_{6',6} = 11.7$ Hz, $J_{6',5} = 5.2$ Hz, H-6'b), 3.64 (1H, dd, $J_{2,3} = 10.3$ Hz, $J_{2,1} = 4.0$ Hz, H-2c), 3.55 (1H, m, H-3a), 3.52 (1H, $J_{2,3} = 9.5$ Hz, $J_{2,1} = 7.7$ Hz, H-2b), 3.47 (2H, m, H-3b, H-5a), 1.98 (3H, s, CH₃), 1.18 (3H, d, $J_{6,5} = 6.7$ Hz, H-6c);

¹³C NMR (150 MHz, MeOD) δ 171.4 (C=O), 149.1, 149.1, 140.0, 140.0, 149.0, 149.0, 148.5, 148.5 (Ar α -C), 144.3, 143.4, 142.3, 142.3, 142.3, 135.2 (Ar C), 134.2, 134.2, 133.1, 133.1, 133.1, 133.1, 133.1, 133.1 (Ar CH), 130.3, 130.3, 130.1, 130.1, 130.1, 130.1, 129.5, 129.5 (Ar β -CH), 126.0, 126.0, 126.0, 125.0, 125.0, 125.0, 125.0, 125.0, 125.0 (Ar CH), 121.4 (5-triazole-C), 119.7, 119.5, 119.5, 117.2 (Ar C), 117.1, 117.1 (Ar CH), 101.5 (C-1b), 99.0 (C-1a), 97.9 (C-1c), 75.1 (C-3a), 74.2 (C-5a), 74.1 (C-4a), 72.8 (C-5b), 72.4 (C-3b), 71.2 (C-4c), 70.3 (C-2b), 68.7 (C-3c), 67.6 (C-2c), 67.5 (C-4b), 65.2 (C-5c), 60.3 (C-6b), 60.3 (OCH₂), 59.0 (C-6a), 55.0 (C-2a), 20.6 (CH₃), 14.1 (C-6c), (a = GlcNAc, b = Gal, c = Fuc);

M/z HRMS (MAILDI-TOF) calcd. for C₆₇H₆₄N₈O₁₅Na = 1284.3783, (M)⁺. Found = 1284.3812.



6.4.8 O-(2,4-Di-O-*tert*-butyldimethylsilyl- α -L-fucopyranosyl)-2,4-di-O-*tert*-butyldimethyl-silyl-1-O-benzyl- α -L-fucopyranose, 175
and O-(2,4-di-O-*tert*-butyldimethylsilyl- α -L-fucopyranosyl)-2,4-di-O-*tert*-butyldimethylsilyl-1-O-benzyl- β -L-fucopyranose, 176

Thioglycoside donor **131** (120 mg, 0.28 mmol), was dried under vacuum for 12 h, before dissolving in DCM (2 mL) containing pre activated 3 Å ms under N₂. The mixture was cooled to 0 °C and Br₂ (104 μ L, 2.80 mmol) was added. The mixture was stirred under N₂ for 5 min at 0 °C before the bromine was removed *in vacuo*, co-evaporating with DCM and toluene. The mixture was re-dissolved in anhydrous DCM, and BnOH **152** (29 μ L, 0.28 mmol) was added. The temperature lowered to -20 °C. AgOTf (141 mg, 0.55 mmol) in dry THF (1 mL) was cooled to -20 °C, and added. The mixture was stirred in the dark for 25 min before quenching with Et₃N (0.5 mL), and stirring for 20 min. The mixture was diluted with DCM (10 mL) and filtered through a plug of celite. The organic layer was washed with

sat. aqueous NaHCO₃ solution (5 mL), deionised H₂O (3 mL) then dried over MgSO₄. The mixture was filtered and the solvent removed *in vacuo*. The mixture was purified by column chromatography (Et₂O:Hex, 1:19(v/v)) to yield the mixture of disaccharide products **175** (28 mg, 24 % yield), and **176** (23 mg, 20 % yield) as clear oils;

$\alpha\alpha$ anomer characterised, **175**

$[\alpha]_D^{20} = 64^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3590 cm⁻¹ (OH), 2928 cm⁻¹ (CH);

¹H NMR (600 MHz, CDCl₃) δ 7.38 (2H, d, *J* = 7.2 Hz, o-Ph), 7.33 (2H, t, *J* = 7.2 Hz, m-Ph), 7.28 (1H, t, *J* = 7.2 Hz, p-Ph), 5.16 (1H, d, *J*_{1,2} = 3.0 Hz, H-1B), 4.85 (1H, d, *J*_{1,2} = 3.4 Hz, H-1A), 4.66 (1H, d, *J* = 11.8 Hz, OCHH), 4.51 (1H, d, *J* = 11.8 Hz, OCHH), 4.15 (1H, dd, *J*_{2,3} = 10.0 Hz, *J*_{2,1} = 3.3 Hz, H-2A), 4.10 (1H, q, *J*_{5,6} = 6.5 Hz, H-5B), 4.05 (1H, dd, *J*_{3,2} = 10.0 Hz, *J*_{3,4} = 2.0 Hz, H-3A), 3.99 (1H, br s, H-4A), 3.94 (1H, ddd, *J*_{3,2} = 9.3 Hz, *J*_{3,OH} = 5.2 Hz, *J*_{3,4} = 1.7 Hz, H-3B), 3.88 (2H, m, H-5A, H-2B), 3.81 (1H, d, *J*_{4,3} = 1.6 Hz, H-4B), 1.76 (1H, *J* = 5.2 Hz, 3B-OH), 1.15 (3H, d, *J*_{6,5} = 6.4 Hz, H-6A), 1.14 (3H, d, *J*_{6,5} = 6.4 Hz, H-6B), 0.96 (18H, s, SiC(CH₃)₃), 0.91, 0.86 (9H, SiC(CH₃)₃), 0.16, 0.16, 0.13, 0.11, 0.11, 0.11, 0.04, 0.04 ((3H, s, Si(CH₃)₂);

¹³C NMR (150 MHz, CDCl₃) δ 138.0 (Ar C), 128.2 (Ar CH), 128.1 (Ar CH), 127.1 (Ar CH), 100.4 (C-1B), 99.1 (C-1A), 77.7 (C-3A), 74.3 (C-4A), 74.0 (C-4B), 72.0 (C-2B), 70.5 (C-3 B), 70.4 (C-2A), 69.6 (PhCH₂O), 68.1 (C-5B), 68.0 (C-5A), 26.1, 26.1, 26.0, 25.9 (SiC(CH₃)₃), 18.6, 18.5, 18.1, 18.1 (SiC(CH₃)₃), 17.2 (C-6B), 17.0 (C-6A), -3.8, -3.9, -4.0, -4.1, -4.4, -4.7, -4.7, -4.8, -4.8 Si(CH₃);

M/z HRMS (ESI-TOF) calcd. for C₄₃H₈₄O₉NaSi₄ = 879.5090, (M+Na)⁺. Found = 879.5068.

$\alpha\beta$ anomer characterised, **176**

$[\alpha]_D^{20} = 92^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

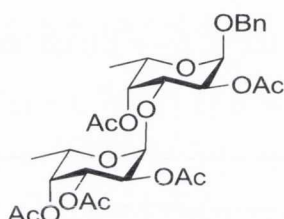
$\nu_{\max}$ (thin film) 3599 cm⁻¹ (OH), 2929 cm⁻¹ (CH);

¹H NMR (600 MHz, CDCl₃) δ 7.39 (2H, d, *J* = 7.2 Hz, o-Ph), 7.34 (2H, t, *J* = 7.2 Hz, m-Ph), 7.30 (1H, t, *J* = 7.2 Hz, p-Ph), 5.07 (1H, d, *J*_{1,2} = 2.0 Hz, H-1B), 4.92 (1H, d, *J* = 11.6 Hz, PhCH(H)), 4.52 (1H, d, *J* = 11.6 Hz, PhCH(H)), 4.29 (1H, br s, H-1A), 4.11 (1H, q, *J*_{5,6} = 6.8 Hz, H-5B), 4.08 (1H, br s, H-4A), 3.92 (3H, m, H-3B, 2B, 5A), 3.76 (1H, br s, H-4B), 3.62 (2H, m, H-2A, 3A), 1.73 (1H, br s, OH), 1.32 (3H, br s, H-6A), 1.15 (3H, d, *J*_{6,5} = 6.7 Hz, H-6B), 0.97,

(18H, s, SiC(CH₃)₃), 0.94, 0.89 (9H, s, Si C(CH₃)₃), 0.15, 0.15, 0.14, 0.13, 0.12, 0.11, 0.11, 0.05 (3H, s, Si(CH₃)₂);

¹³C NMR (150 MHz, CDCl₃) δ 137.9 (Ar C), 128.2 (Ar CH), 128.0 (Ar CH), 127.3 (Ar CH), 102.6 (C-1A), 101.0 (C-1B), 81.5 (C-3A), 74.0-72.5 (br, C-4A), 72.3 (C-2B), 74.0 (C-4B), 72.0 (C-2A), 71.7 (C-5A), 70.4 (C-3B), 70.2 (OCH₂Ph), 68.0 (C-5B), 26.1, 26.1, 26.1, 26.0 (SiC(CH₃)₃), 18.6, 18.5, 18.3, 18.1 (SiC(CH₃)), 17.3 (C-6A), 17.2 (C-6B), -3.6, -3.9, -4.0, -4.0, -4.2, -4.4, -4.4, -4.6 (Si(CH₃));

M/z HRMS (ESI-TOF) calcd. for C₄₃H₈₄O₉NaSi₄ = 879.5090, (M+Na)⁺. Found = 879.5063.



6.4.9 O-(2,3,4-Tri-O-acetyl- α -L-fucopyranosyl)-2,4-di-O-acetyl-1-O-benzyl- α -L-fucopyranose, **177**

To a solution of disaccharide **175** (50 mg, 0.06 mmol) in THF (2 mL) at 0 °C, was added TBAF (0.70 mL, 1M). The mixture was stirred whilst warming to RT for 12 h. The solvent was removed *in vacuo* and the mixture dissolved in pyridine (1 mL). The mixture was cooled to 0 °C and Ac₂O (1 mL) was added. The mixture was stirred warming to RT for 12 h. The reaction was quenched with the addition of MeOH (1 mL), and the mixture diluted with EtOAc (15 mL). The organic layer was washed with HCl (1M, 5 mL), CuSO₄ (10 % aqueous, 2 X 10 mL) and deionised H₂O (10 mL). The organic layer was dried over MgSO₄, filtered and the solvent removed *in vacuo*. The crude residue was purified by column chromatography (EtOAc:Hex, 3:7 (v/v)), to yield the product **177**, as a clear oil, (29 mg, 81 %);

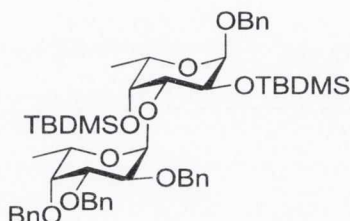
$[\alpha]_D^{20} = -121^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 2926 cm⁻¹ (CH), 1741 cm⁻¹ (C=O);

¹H NMR (600 MHz, CDCl₃) δ 7.37 (2H, m, Ar CH), 7.33 (3H, m, Ar CH), 5.31 (1H, br s, H-4b), 5.26 (2H, m, H-2b, H-3b), 5.25 (1H, d, $J_{4,3} = 2.7$ Hz, H-4a), 5.20 (1H, d, $J_{1,2} = 3.6$ Hz, H-1a), 5.17 (1H, d, $J_{1,2} = 2.0$ Hz, H-1b), 5.09 (1H, dd, $J_{2,3} = 10.7$ Hz, $J_{2,1} = 3.7$ Hz, H-2a), 4.72 (1H, d, $J = 12.0$ Hz, PhCH(H)), 4.56 (1H, d, $J = 12.0$ Hz, PhCH(H)), 4.41 (1H, q, $J_{5,6} = 6.5$ Hz, H-5b), 4.34 (1H, dd, $J_{3,2} = 10.8$ Hz, $J_{3,4} = 3.0$ Hz, H-3a), 4.09 (1H, q, $J_{5,6} = 6.5$ Hz, H-5a), 2.19, 2.18, 2.09, 2.09, 1.98 (CH₃), 1.19 (C-6b), 1.11 (C-6a);

¹³C NMR (150 MHz, CDCl₃) δ 170.7, 170.4, 170.3, 170.1, 170.1 (C=O), 137.4 (Ar C), 128.4, 127.9, 127.6 (Ar CH), 95.5 (C-1a), 93.6 (C-1b), 71.2 (C-4b), 69.9 (PhCH₂), 69.7 (C-3a), 69.8 (C-2a), 69.7 (C-4a), 67.8 (C-3b), 67.1 (C-2b), 65.0 (C-5a), 64.6 (C-5b), 20.8, 20.8, 20.7, 20.6, 20.6 (CH₃), 16.1 (C-6a), 15.8 (C-6b);

M/z HRMS (ESI-TOF) calcd. for C₂₉H₃₈O₁₄Na = 633.2159, (M+Na)⁺. Found = 633.2137.



6.4.10 O-(2,3,4-Tri-O-benzyl-α-L-fucopyranosyl)-2,4-(Di-O-tert-butyldimethylsilyl)-1-O-benzyl-α-L-fucopyranose, **178**

To a solution of acceptor **171** (50 mg, 0.10 mmol), thioglycoside donor **81** (59 mg, 0.12 mmol) and NIS (33mg, 0.14 mmol) in DCM at -20 °C under N₂, was added TMSOTf (cat.). The mixture was stirred for 16 h, warming to RT, before the addition of Et₃N (0.50 mL). The mixture was diluted with DCM (10 mL), and washed with sat. aqueous Na₂S₂O₃ solution (5 mL) and deionised H₂O (5 mL). The organic layer was dried over MgSO₄, filtered and the solvent removed *in vacuo*. The crude mixture was purified by column chromatography (EtOAc:Hexane, 1:11 (v/v)) to yield the product **178**, as a clear oil (21 mg, 23%);

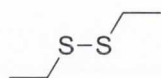
[α]_D²⁰ = 32 ° (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

ν_{max} (thin film) 2529 cm⁻¹ (CH);

¹H NMR (400 MHz, CDCl₃) δ 7.40-7.24 (20H, m, Ar CH), 5.22 (1H, br s, H-1b), 4.99 (1H, d, *J* = 11.8 Hz, (PhCH(H))), 4.87 (1H, d, *J* = 10.8 Hz, (PhCH(H))), 4.82 (1H, d, *J*_{1,2} = 2.9 Hz, H-1a), 4.81 (1H, d, *J* = 12.3 Hz, (PhCH(H))), 4.76 (1H, d, *J* = 12.3 Hz, (PhCH(H))), 4.70 (1H, d, *J* = 12.2 Hz, (PhCH(H))), 4.69 (1H, d, *J* = 11.6 Hz, (PhCH(H))), 4.68 (1H, d, *J* = 11.0 Hz, (PhCH(H))), 4.14 (2H, m, H-5b, H-2a), 4.07 (3H, m, H-2b, H-3a, H-3b), 3.87 (1H, br s, H-4a), 3.69 (1H, br s, H-4b), 3.67 (1H, q, *J*_{5,6} = 6.3 Hz, H-5-a), 1.12 (3H, d, *J*_{6,5} = 6.5 Hz, H-6b), 1.04 (3H, d, *J*_{6,5} = 6.4 Hz, H-6a), 0.89, 0.88 (9H, s, Si(CH₃)₃), 0.08, 0.08, 0.03, 0.01 (Si(CH₃)₂);

¹³C NMR (100 MHz, CDCl₃) δ 138.4, 138.3, 138.1, 137.7 (Ar C), 127.9, 127.8, 127.7, 127.6, 127.6, 127.6, 127.4, 127.2, 127.0, 127.0, 126.9, 126.8, 126.8 (Ar CH), 98.2 (C-1a), 97.0 (C-1b), 79.1 (C-3a), 77.2 (C-4b), 76.8 (C-2b), 75.5 (C-3b), 74.3 (PhCH₂), 73.4 (PhCH₂), 72.1

(PhCH₂), 72.1 (C-4a), 69.0 (C-2a), 68.9 (PhCH₂), 67.7 (C-5a), 66.5 (C-5b), 22.6, 22.5 (SiC(CH₃)₃), 18.0, 17.6 (SiC(CH₃)₃), 16.4 (C-6a), 16.1 (C-6b), -4.4, -4.8, -5.0, -5.4 (Si(CH₃)₂);
M/z HRMS (ESI-TOF) calcd. for C₅₂H₇₄O₉NaSi₂ = 921.4769, (M+Na)⁺. Found = 921.4785.



6.4.11 Di-ethyl disulfide, 179

To a suspension of ethane thiol (1 mL, 13.4 mmol) and NaI (20 mg) in EtOAc (20 mL) at 0 °C, was added H₂O₂ (1.43 mL, 13.5 mmol). The mixture was stirred warming to room temperature for 16 h. The organic layer was washed with H₂O (5 mL), dried over MgSO₄, filtered and the solvent carefully removed under reduced pressure to yield the required product **179**, as a clear oil (0.25 mL, 26 %);

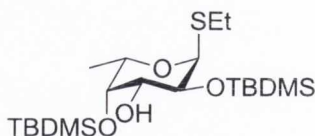
Analytical data was in good agreement with literature example.²⁷⁹

$\nu_{\max}$ (thin film) 2964, 2926 cm⁻¹ (CH);

¹H NMR (400 MHz, CDCl₃) δ 2.73 (4H, q, *J* = 7.4 Hz, SCH₂CH₃), 1.35 (6H, t, *J* = 7.3 Hz, SCH₂CH₃);

¹³C NMR (100 MHz, CDCl₃) δ 32.5 (SCH₂CH₃), 14.0 (SCH₂CH₃);

M/z HRMS (EI-TOF) calcd. for C₄H₁₀S₂ = 122.0224, (M)⁺. Found = 122.0219.



6.4.12 Ethyl-2,4-di-O-tert-butyldimethylsilyl-1-thio-α-L-fucopyranoside, 180

The thioglycoside donor **131** (105 mg, 0.24 mmol) and DTMP (49 mg, 0.24 mmol) were dissolved in DCM at -40 °C, under N₂. Diethyl disulfide **179** (49 mg, 0.40 mmol), and Tf₂O (101 mg, 0.36 mmol) were premixed under N₂ for 30 min at 0 °C and added to the solution in DCM (1 mL). The reaction was stirred for 1 h before quenching with Et₃N (0.3 mL), and the mixture diluted with DCM (10 mL). The organic layer was washed with HCl (1 M, 5 mL), sat. aqueous NaHCO₃ solution (5 mL) and deionised H₂O. The organic layer was dried over MgSO₄, filtered and the solvent removed *in vacuo*. The mixture was purified by column chromatography (DCM:Hex, 1:1 (v/v)), to yield the product **180**, as a clear oil, (65 mg, 64 %);

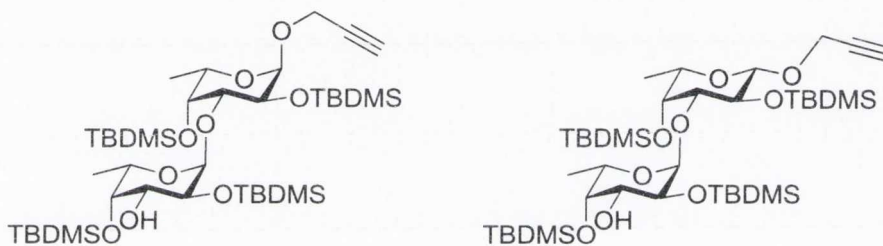
[α]_D²⁰ = -14 ° (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3598 cm^{-1} (OH), 2929 cm^{-1} (CH);

^1H NMR (400 MHz, CDCl_3) δ 5.34 (1H, d, $J_{1,2} = 5.0$ Hz, H-1), 4.26 (1H, q, $J_{5,6} = 6.8$ Hz, H-5), 4.19 (1H, dd, $J_{2,3} = 9.4$ Hz, $J_{2,1} = 5.2$ Hz, H-2), 3.86 (1H, dd, $J_{4,3} = 2.7$ Hz, $J_{4,5} = 0.5$ Hz, H-4), 3.72 (1H, m, H-3), 2.61 (1H, m, $\text{SCH}(\text{H})\text{CH}_3$), 2.50 (1H, m, $\text{SCH}(\text{H})\text{CH}_3$), 2.04 (1H, d, $J = 2.8$ Hz, OH), 1.29 (3H, t, $J = 7.1$ Hz, SCH_2CH_3), 1.23 (3H, d, $J_{6,5} = 6.7$ Hz, H-6), 0.96, 0.95 ($\text{SiC}(\text{CH}_3)_3$), 0.16, 0.14, 0.13, 0.12 ($\text{Si}(\text{CH}_3)_2$);

^{13}C NMR (100 MHz, CDCl_3) δ 85.1 (C-1), 73.0 (C-4), 72.0 (C-3), 69.9 (C-2), 67.3 (C-5), 26.1, 25.8 ($\text{SiC}(\text{CH}_3)_3$), 23.7 (SCH_2CH_3), 18.5, 18.4 ($\text{SiC}(\text{CH}_3)_3$), 17.1 (C-6), 15.0 (SCH_2CH_3), -4.0, -4.5, -4.5, -4.7 ($\text{Si}(\text{CH}_3)_2$);

M/z HRMS (ESI-TOF) calcd. for $\text{C}_{20}\text{H}_{44}\text{O}_4\text{NaSi}_2\text{S} = 459.2397$, $(\text{M}+\text{Na})^+$. Found = 459.2397.



6.4.13 O-(2,4-Di-O-*tert*-butyldimethylsilyl- α -L-fucopyranosyl)-2,4-di-O-*tert*-butyldimethylsilyl-1-O-propargyl- α -L-fucopyranose, **181**

and

O-(2,4-Di-O-*tert*-butyldimethylsilyl- α -L-fucopyranosyl)-2,4-di-O-*tert*-butyldimethylsilyl-1-O-propargyl- β -L-fucopyranose **182**

Thioglycoside donor **131** (120 mg, 0.28 mmol), Br_2 (104 μL , 2.80 mmol) and propargyl alcohol **63** (15 μL , 0.28 mmol) were reacted according to procedure described in Section 6.4.8. The crude material was purified by column chromatography (EtOAc :Hexane, 1:49 (v/v)) to yield product **181**, as a clear oil, (50 mg, 45 %) and the product **182**, as a clear oil (9 mg, 8 %);

$\alpha\alpha$ anomer characterised, **181**

$[\alpha]_{\text{D}}^{20} = -76^\circ$ ($\text{deg cm}^3 \text{g}^{-1} \text{dm}^{-1}$) ($c = 0.1$ in CH_3Cl);

$\nu_{\max}$ (thin film) 3599 cm^{-1} (OH), 2929 cm^{-1} (CH);

^1H NMR (600 MHz, CDCl_3) δ 5.13 (1H, d, $J_{1,2} = 3.1$ Hz, H-1B), 4.97 (1H, d, $J_{1,2} = 3.7$ Hz, H-1A), 4.24 (2H OCH₂), 4.16 (1H, dd, $J_{2,3} = 10.1$ Hz, $J_{2,1} = 3.5$ Hz, H-2A), 4.10 (1H, q, $J_{5,6} = 6.6$ Hz, H-5B), 4.01 (1H, br s, H-4A), 4.00 (1H, dd, $J_{3,2} = 9.9$ Hz, $J_{3,4} = 2.2$ Hz, H-3A), 3.94 (1H, ddd, $J_{3,2} = 9.6$ Hz, $J_{3,\text{OH}} = 6.0$ Hz, $J_{3,4} = 2.9$ Hz, H-3B), 3.90 (1H, dd, $J_{3,2} = 10.0$ Hz, $J_{3,4} = 3.1$ Hz,

H-2B) 3.87 (1H, q, $J_{5,6} = 6.6$ Hz, H-5A), 3.81 (1H, d, $J_{4,3} = 1.9$ Hz, H-4B), 2.36 (1H, t, $J = 2.4$ Hz C $\equiv$ CH), 1.76 (1H, d, $J = 6.0$ Hz, OH), 1.17 (3H, d, $J_{6,5} = 6.8$ Hz, H-6A), 1.14 (3H, d, $J_{6,5} = 6.8$ Hz, H-6B), 0.96, 0.95, 0.94, 0.93 (9H, s, SiC(CH₃)₃), 0.16, 0.15, 0.15, 0.15, 0.15, 0.11, 0.11, 0.11 (3H, s, Si(CH₃)₂);

¹³C NMR (150 MHz, CDCl₃) δ 100.8 (C-1B), 98.0 (C-1A), 79.6 (C $\equiv$ CH), 77.9 (C-3A), 74.3 (C-4A), 74.1 (C $\equiv$ CH), 74.0 (C-4B), 72.1 (C-2B), 70.5 (C-3B), 70.0 (C-2A), 68.3 (C-5A), 68.2 (C-5B), 54.2 (OCH₂), 26.1, 26.1, 26.1, 26.0 (SiC(CH₃)₃), 18.6, 18.6, 18.5, 18.5, 18.3, 18.3, 18.2, 18.2 (SiC(CH₃)₃), 17.3 (C-6B), 16.9 (C-6A), -3.8, -3.9, -3.9, -4.2, -4.4, -4.7, -4.7, -4.9 (Si(CH₃)₂);

M/z HRMS (ESI-TOF) calcd. for C₃₉H₈₀O₉NaSi₄ = 827.4777 (M+Na)⁺. Found = 827.4770.

$\alpha\beta$ anomer characterised, **182**

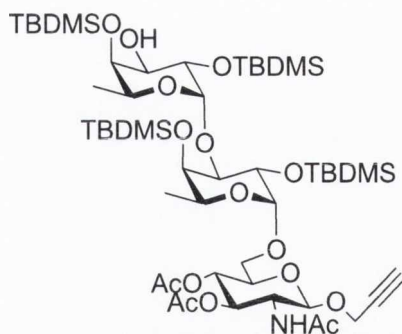
$[\alpha]_D^{20} = -57^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3587 cm⁻¹ (OH), 2931 cm⁻¹ (CH);

¹H NMR (400 MHz, CDCl₃) δ 5.07 (1H, d, $J_{1,2} = 2.8$ Hz, H-1b), 4.37 (1H, dd, $J = 15.5$ Hz, $J = 2.4$ Hz, OCH(H)) 4.34 (1H, d, $J_{1,2} = 6.5$ Hz, H-1a), 4.31 (1H, dd, $J = 15.6$ Hz, $J = 2.2$ Hz, OCH(H)), 4.10 (1H, q, $J_{5,6} = 6.6$ Hz, H-5b), 4.03 (1H, br s, H-4a), 3.97 (1H, ddd, $J_{3,2} = 10.0$ Hz, $J_{3,OH} = 6.5$ Hz, $J_{3,4} = 2.6$ Hz, H-3b), 3.92 (1H, dd, $J_{2,3} = 10.1$ Hz, $J_{2,1} = 3.1$ Hz, H-2b), 3.85 (1H, dd, $J_{2,3} = 8.9$ Hz, $J_{2,1} = 6.4$ Hz, H-2a), 3.81 (1H, br s, H-4b), 3.60 (1H, dd, $J_{3,2} = 8.6$ Hz, $J_{3,4} = 1.2$ Hz, H-3a), 3.57 (1H, m, H-5a), 2.39 (-C $\equiv$ CH), 1.24 (3H, d, $J_{6,5} = 6.8$ Hz, C-6a), 1.15 (3H, d, $J_{6,5} = 6.8$ Hz, C-6b), 1.00, 0.95, 0.95, 0.94 (9H, s, SiC(CH₃)₃), 0.15, 0.15, 0.14, 0.14, 0.14, 0.14, 0.12, 0.12 (3H, s, Si(CH₃)₂);

¹³C NMR (100 MHz, CDCl₃) δ 101.4 (C-1a), 101.0 (C-1b), 82.1 (C-3a), 78.8 (-C $\equiv$ CH), 74.1 (C-4b), 72.5 (C-4a), 72.1 (C-2b), 71.9 (C-2a), 70.1 (C-3b), 68.0 (C-5b), 55.0 (OCH₂), 26.0, 25.9, 25.9, 25.7 (SiC(CH₃)₃), 18.5, 18.4, 18.4, 18.53, 18.1, 18.1, 18.0, 18.0 (SiC(CH₃)₃), 17.8, 17.8 (C-6b, C-6a) -3.7, -3.7, -3.9, -4.1, -4.2, -4.5, -4.6, -4.7 (Si(CH₃)₂);

M/z HRMS (ESI-TOF) calcd. for C₃₉H₈₀O₉NaSi₄ = 827.4763 (M+Na)⁺. Found = 827.4736.



6.4.14 2-Acetamido-2-deoxy-3,4-di-O-acetyl-6-O-(3-O-(2,4-di-O-*tert*-butyldimethylsilyl)- α -L-fucopyranosyl)-2,4-di-O-*tert*-butyldimethylsilyl- α -L-fucopyranosyl)-1-O-propargyl- β -D-glucopyranose, 183

Thioglycoside donor **131** (128 mg, 0.30 mmol), Br₂ (104 μ L, 2.80 mmol) and acceptor **139** (100 mg, 0.30) were reacted according to procedure described in Section 6.4.8. The crude material was purified by column chromatography (EtOAc:Hex, 1:1 (v/v)) to yield the product as a clear oil (13 mg, 8 %);

$[\alpha]_D^{20} = -83^\circ$ (deg cm³ g⁻¹ dm⁻¹) (c = 0.1 in CH₃Cl);

$\nu_{\max}$ (thin film) 3530 cm⁻¹ (OH), 3276 cm⁻¹ (C $\equiv$ CH), 3035 cm⁻¹ (NH), 2929 cm⁻¹ (CH), 1753 cm⁻¹ C=O, 1663 cm⁻¹ (NHC=O);

¹H NMR (600 MHz, CDCl₃) δ 5.39 (1H, d, J = 9.1 Hz, NH), 5.24 (1H, app t, $J_{3,4} = J_{3,2} = 9.8$ Hz, H-3A), 5.10 (1H, d, $J_{1,2} = 2.3$ Hz, H-1C), 5.00 (1H, app t $J_{4,3} = J_{4,5} = 9.6$ Hz, H-4A), 4.76 (1H, d, $J_{1,2} = 8.4$ Hz, H-1A), 4.74 (1H, d, $J_{1,2} = 3.0$ Hz, H-1B), 4.37 (2H, m, CH₂C $\equiv$ CH), 4.08 (1H, dd, $J_{2,3} = 9.6$ Hz, $J_{2,1} = 2.7$ Hz, H-2B), 4.06 (2H, m, H-5C, H-4B), 4.00 (1H, app q, $J_{2,NH} = J_{2,1} = J_{2,3} = 8.9$ Hz, H-2A), 3.93 (4H, m, H-3B, H-3C, H-2C, H-5B), 3.83 (1H, br s, H-4C), 3.75 (1H, br d, $J_{6',6} = 11.6$ Hz, H-6'A), 3.73 (1H, m, H-5A), 3.60 (1H, dd, $J_{6,6'} = 11.5$ Hz, $J_{6,5} = 5.8$ Hz, H-6A), 2.46 (1H, s, C $\equiv$ CH), 2.05, 2.03, 1.98 (3H, s, COCH₃), 1.79 (1H, d, J = 4.5 Hz, 3C-OH), 1.19 (3H, d, $J_{6,5} = 6.4$ Hz, H-6B), 1.15 (3H, d, $J_{6,5} = 6.4$ Hz, H-6C), 0.97, 0.97, 0.95, 0.92 (9H, s, SiC(CH₃)₃), 0.20, 0.17, 0.16, 0.15, 0.13, 0.11, 0.11, 0.10 (3H, s, SiCH₃);

¹³C NMR (150 MHz, CDCl₃) δ 171.0, 169.9, 168.9 (C=O), 100.6 (C-1C), 99.0 (C-1B), 98.2 (C-1A), 78.7 (HC $\equiv$ C), 77.6 (C-3B), 74.9 (C $\equiv$ CH), 73.8 (C-4C), 73.4 (C-5A), 73.3 (C-4B), 72.7 (C-3A), 71.6 (C-3C), 70.3 (C-2C), 70.1 (C-2B), 68.9 (C-4A), 68.5 (C-5B), 68.0 (C-5C), 66.8 (C-6A), 55.4 (CH₂C $\equiv$ CH), 54.0 (C-2A), 25.9, 25.9, 25.9, 25.8 (SiC(CH₃)₃), 23.2 (NHCOCH₃), 20.5, 20.5 (COCH₃), 18.4, 18.3, 18.2, 18.0 (SiC(CH₃)₃), 17.1 (C-6C), 16.6 (C-6B), -3.6, -4.0, -4.0, -4.4, -4.6, -4.9, -4.9, -4.9 (SiCH₃);

M/z HRMS (ESI-TOF) calcd. for C₅₁H₉₆NO₁₆Si₄ = 1090.5806, (M-H)⁻. Found = 1090.5806.

6.5 Experimental data for Chapter 5

6.5.1 General procedure for lectin binding fluorescence studies with glycoporphyrins²⁴²

Stock solutions of the glycoporphyrin (1.0×10^{-4} M) were prepared in MeOH:Pyridine (49:1 (v/v)) and stored in the freezer. All stock solutions were diluted to (2×10^{-7} M) with an aqueous buffer, containing HEPES (1.0×10^{-2} M), NaCl (1.5×10^{-1} M), CaCl_2 (1.0×10^{-3} M), NiCl_2 (1.0×10^{-3} M), prior to use. Diluted glycoporphyrin solutions (1 mL) were added to variable concentrations of lectin solutions, in aqueous buffer (1 mL). Final glycoporphyrin concentrations of (1.0×10^{-7} M) with varied lectin ratios were equilibrated for 2 h at 37 °C under gentle agitation, before fluorescent spectra were recorded.

6.5.2 General procedure for biological evaluation of glycoporphyrin library

Experimental as per literature presentation.¹⁸⁸

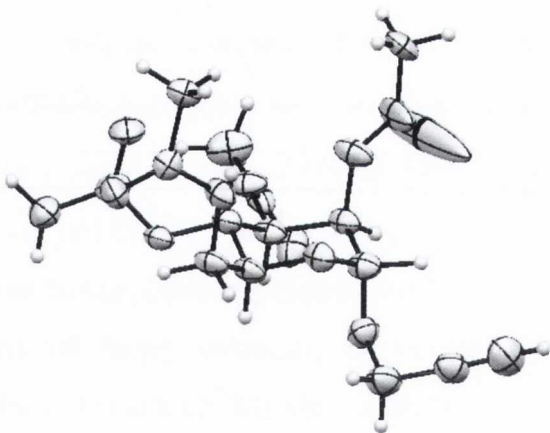
Stock solutions of photosensitizers were prepared at 1×10^{-4} M in EtOH:propylene glycol (6:4 (v/v)), and diluted with phosphate buffer. Both OE21 and Het 1a cell lines were incubated on 96 well plates, with various concentrations of clinically approved Foscan® (3 - 0.5 μM), and selected glycoporphyrins (3 - 0.5 μM) for 24 h at 37 °C, containing 5 % CO_2 in a humidified atmosphere. The GP containing media was removed and replaced with fresh GP free medium. The plates were then separated into two groups, one illuminated for 2 min using a white light LED ($1.7\text{mW}/\text{cm}^2$, clinically relevant dose²⁸⁰), and one kept in the dark. The cells were left to recover for four hours in the dark. The plates were separated again into two groups, (one illuminated and one non-illuminated) for toxicity testing by MTT (Promega, USA) assay according to manufacturer's instructions and another (one illuminated and one non-illuminated) for fluorescent evaluation by high content imaging and analysis. This last group of plates were fixed with pre-heated 4 % paraformaldehyde (Acros Organics, USA) and were stained with FITC labelled phalloidin (Sigma, USA) and Hoechst (Invitrogen, USA) before analysis on the InCell 1000 (GE Healthcare, USA).

6.5.3 General procedure for DPBF photo-degradation relative $^1\text{O}_2$ production

An air saturated stock solution of 1,3-diphenylisobenzofuran (DPBF) (5×10^{-5} M) was prepared. 25 mL of solution was stirred in a tin foil covered Duran flask placed in a RT water bath as a heat sink. One fibre optic probe from a dual probe Olympus KL1500 LCD light source (150 W halogen bulb, output filter settings set at C, and a power output of 1,

red light filter removed) was placed into the Duran flask. Aliquots (2 mL) were removed from the solution at 2 min intervals and UV/Vis absorptions at 411nm were performed. The sample was aerated and returned to Duran. Analogous experiments were performed with DPBF (5×10^{-5} M) and in the presence of a photosensitizer (1×10^{-6} M) in dimethylformamide, or DMF:H₂O (1:1 (v/v)).

7.1 Appendix of crystal data



7.1.1 Propargyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside, 64

Table 1. Crystal data and structure refinement for m1.

Identification code	shelxl	
Empirical formula	C60 H72 O36	
Formula weight	1369.18	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 9.6000(19) Å	α = 90°.
	b = 10.369(2) Å	β = 90°.
	c = 19.780(4) Å	γ = 90°.
Volume	1968.9(7) Å ³	
Z	1	
Density (calculated)	1.155 Mg/m ³	
Absorption coefficient	0.097 mm ⁻¹	
F(000)	720	
Crystal size	0.4 x 0.2 x 0.1 mm ³	
Theta range for data collection	2.85 to 25.00°.	
Index ranges	-11<=h<=11, -12<=k<=7, -23<=l<=16	
Reflections collected	8967	
Independent reflections	3452 [R(int) = 0.0661]	
Completeness to theta = 25.00°	99.7 %	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	3452 / 0 / 260	
Goodness-of-fit on F ²	1.042	
Final R indices [I>2sigma(I)]	R1 = 0.0825, wR2 = 0.1710	
	264	

R indices (all data)	R1 = 0.1157, wR2 = 0.1932
Absolute structure parameter	0(3)
Largest diff. peak and hole	0.818 and -0.837 e.Å ⁻³

Table 2. Atomic coordinates (x 10⁴) and equivalent isotropic displacement parameters (Å²x 10³) for m1. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	5548(4)	761(4)	888(2)	39(1)
O(3)	3683(4)	1223(4)	1591(2)	42(1)
O(4)	7759(4)	-668(4)	420(2)	40(1)
O(5)	8888(4)	-2544(4)	353(2)	47(1)
O(6)	7444(4)	-1045(4)	2174(2)	37(1)
O(7)	9705(4)	-698(5)	1940(2)	54(1)
O(8)	7415(4)	1413(4)	2739(2)	43(1)
O(9)	5750(4)	2027(4)	3464(2)	48(1)
C(1)	4879(6)	1706(6)	1292(3)	40(2)
C(2)	6006(6)	-364(5)	1264(3)	35(1)
C(3)	7060(6)	72(5)	1787(3)	36(1)
C(4)	6381(6)	1027(5)	2269(3)	34(1)
C(5)	5821(6)	2159(5)	1856(3)	37(1)
C(6)	2658(7)	794(6)	1111(3)	49(2)
C(7)	2211(7)	1830(7)	668(3)	47(2)
C(9)	6591(6)	-1294(6)	750(3)	39(2)
C(10)	8843(6)	-1403(6)	259(3)	33(1)
C(11)	9980(6)	-611(6)	-50(3)	38(1)
C(13)	8818(6)	-1327(6)	2214(3)	40(2)
C(14)	9052(6)	-2506(6)	2623(3)	47(2)
C(15)	6970(6)	1865(5)	3340(3)	37(1)
C(16)	8146(7)	2121(7)	3800(3)	57(2)
C(35)	1891(9)	2664(9)	312(4)	63(2)
O(25)	6976(4)	2804(4)	1546(2)	46(1)
C(27)	7188(8)	4004(6)	1645(5)	76(3)
C(28)	8401(7)	4540(6)	1290(4)	56(2)
O(29)	6495(12)	4538(6)	1994(8)	330(11)

Table 3. Bond lengths [Å] and angles [°] for m1.

O(1)-C(1)	1.419(6)
O(1)-C(2)	1.452(7)
O(3)-C(1)	1.385(6)
O(3)-C(6)	1.437(7)
O(4)-C(10)	1.328(6)
O(4)-C(9)	1.451(6)
O(5)-C(10)	1.199(7)
O(6)-C(13)	1.353(7)
O(6)-C(3)	1.436(6)
O(7)-C(13)	1.203(7)
O(8)-C(15)	1.347(6)
O(8)-C(4)	1.418(6)
O(9)-C(15)	1.209(7)
C(1)-C(5)	1.511(8)
C(1)-H(30)	1.20(6)
C(2)-C(9)	1.510(7)
C(2)-C(3)	1.515(8)
C(2)-H(20)	0.9800
C(3)-C(4)	1.521(7)
C(3)-H(21)	0.9800
C(4)-C(5)	1.527(8)
C(4)-H(22)	0.9800
C(5)-O(25)	1.432(6)
C(5)-H(31)	0.91(5)
C(6)-C(7)	1.451(9)
C(6)-H(6A)	0.9700
C(6)-H(6B)	0.9700
C(7)-C(35)	1.157(10)
C(9)-H(9A)	0.9700
C(9)-H(9B)	0.9700
C(10)-C(11)	1.496(8)
C(11)-H(11A)	0.9600
C(11)-H(11B)	0.9600
C(11)-H(11C)	0.9600
C(13)-C(14)	1.483(8)
C(14)-H(14A)	0.9600
C(14)-H(14B)	0.9600
C(14)-H(14C)	0.9600
C(15)-C(16)	1.474(8)

C(16)-H(16A)	0.9600
C(16)-H(16B)	0.9600
C(16)-H(16C)	0.9600
C(35)-H(27)	0.81(8)
C(35)-H(27)	0.81(8)
O(25)-C(27)	1.275(7)
C(27)-O(29)	1.107(9)
C(27)-C(28)	1.469(9)
C(28)-H(28A)	0.9600
C(28)-H(28B)	0.9600
C(28)-H(28C)	0.9600
C(1)-O(1)-C(2)	113.8(4)
C(1)-O(3)-C(6)	113.5(4)
C(10)-O(4)-C(9)	117.2(4)
C(13)-O(6)-C(3)	117.1(4)
C(15)-O(8)-C(4)	117.1(4)
O(3)-C(1)-O(1)	111.4(5)
O(3)-C(1)-C(5)	107.1(5)
O(1)-C(1)-C(5)	111.1(5)
O(3)-C(1)-H(30)	100(3)
O(1)-C(1)-H(30)	108(3)
C(5)-C(1)-H(30)	119(3)
O(1)-C(2)-C(9)	106.3(4)
O(1)-C(2)-C(3)	108.2(4)
C(9)-C(2)-C(3)	113.7(5)
O(1)-C(2)-H(20)	109.5
C(9)-C(2)-H(20)	109.5
C(3)-C(2)-H(20)	109.5
O(6)-C(3)-C(2)	107.1(4)
O(6)-C(3)-C(4)	107.6(4)
C(2)-C(3)-C(4)	109.6(5)
O(6)-C(3)-H(21)	110.8
C(2)-C(3)-H(21)	110.8
C(4)-C(3)-H(21)	110.8
O(8)-C(4)-C(3)	107.1(4)
O(8)-C(4)-C(5)	112.3(5)
C(3)-C(4)-C(5)	108.4(4)
O(8)-C(4)-H(22)	109.6
C(3)-C(4)-H(22)	109.6

C(5)-C(4)-H(22)	109.6
O(25)-C(5)-C(1)	107.0(5)
O(25)-C(5)-C(4)	108.4(5)
C(1)-C(5)-C(4)	111.5(5)
O(25)-C(5)-H(31)	112(3)
C(1)-C(5)-H(31)	102(3)
C(4)-C(5)-H(31)	116(3)
O(3)-C(6)-C(7)	111.9(5)
O(3)-C(6)-H(6A)	109.2
C(7)-C(6)-H(6A)	109.2
O(3)-C(6)-H(6B)	109.2
C(7)-C(6)-H(6B)	109.2
H(6A)-C(6)-H(6B)	107.9
C(35)-C(7)-C(6)	178.2(8)
O(4)-C(9)-C(2)	107.8(4)
O(4)-C(9)-H(9A)	110.2
C(2)-C(9)-H(9A)	110.2
O(4)-C(9)-H(9B)	110.2
C(2)-C(9)-H(9B)	110.2
H(9A)-C(9)-H(9B)	108.5
O(5)-C(10)-O(4)	123.9(5)
O(5)-C(10)-C(11)	125.3(5)
O(4)-C(10)-C(11)	110.8(5)
C(10)-C(11)-H(11A)	109.5
C(10)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5
C(10)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5
O(7)-C(13)-O(6)	123.1(6)
O(7)-C(13)-C(14)	125.9(6)
O(6)-C(13)-C(14)	111.0(5)
C(13)-C(14)-H(14A)	109.5
C(13)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	109.5
C(13)-C(14)-H(14C)	109.5
H(14A)-C(14)-H(14C)	109.5
H(14B)-C(14)-H(14C)	109.5
O(9)-C(15)-O(8)	122.3(6)
O(9)-C(15)-C(16)	126.3(5)

O(8)-C(15)-C(16)	111.4(5)
C(15)-C(16)-H(16A)	109.5
C(15)-C(16)-H(16B)	109.5
H(16A)-C(16)-H(16B)	109.5
C(15)-C(16)-H(16C)	109.5
H(16A)-C(16)-H(16C)	109.5
H(16B)-C(16)-H(16C)	109.5
H(27)-C(35)-C(7)	179(7)
H(27)-C(35)-H(27)	0(10)
C(7)-C(35)-H(27)	179(7)
C(27)-O(25)-C(5)	120.9(5)
O(29)-C(27)-O(25)	119.2(7)
O(29)-C(27)-C(28)	125.8(7)
O(25)-C(27)-C(28)	114.9(6)
C(27)-C(28)-H(28A)	109.5
C(27)-C(28)-H(28B)	109.5
H(28A)-C(28)-H(28B)	109.5
C(27)-C(28)-H(28C)	109.5
H(28A)-C(28)-H(28C)	109.5
H(28B)-C(28)-H(28C)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for m1. The anisotropic displacement factor exponent takes the form: $-2\pi^2 [h^2 a^{*2} U^{11} + \dots + 2 h k a^* b^* U^{12}]$

	U ¹¹	U ²²	U ³³	U ²³	U ¹³	U ¹²
O(1)	41(2)	32(2)	44(2)	-9(2)	10(2)	-2(2)
O(3)	36(2)	43(2)	46(2)	-5(2)	-2(2)	0(2)
O(4)	47(2)	29(2)	44(2)	-4(2)	16(2)	-2(2)
O(5)	48(3)	34(3)	58(3)	12(2)	4(2)	2(2)
O(6)	33(2)	31(2)	46(2)	-1(2)	9(2)	-9(2)
O(7)	34(2)	58(3)	71(3)	13(3)	17(2)	-3(2)
O(8)	33(2)	50(3)	45(2)	-14(2)	14(2)	-9(2)
O(9)	50(3)	59(3)	37(2)	1(2)	14(2)	18(2)
C(1)	36(3)	38(4)	45(3)	-9(3)	10(3)	-4(3)
C(2)	38(3)	24(3)	44(3)	-10(3)	9(3)	-9(3)
C(3)	33(3)	31(3)	43(3)	-3(3)	8(3)	-7(3)
C(4)	34(3)	36(3)	31(3)	-5(3)	10(2)	-5(3)
C(5)	35(3)	26(3)	48(4)	-12(3)	15(3)	-5(3)
C(6)	44(4)	46(4)	57(4)	-6(3)	-1(3)	-10(3)
C(7)	47(4)	47(4)	48(4)	-3(3)	-6(3)	-10(3)
C(9)	42(3)	30(3)	47(3)	-12(3)	8(3)	-12(3)
C(10)	37(3)	38(3)	24(3)	-6(3)	-2(2)	2(3)
C(11)	42(3)	38(3)	34(3)	-1(3)	3(3)	-10(3)
C(13)	32(3)	43(4)	45(3)	-18(3)	8(3)	-3(3)
C(14)	40(4)	46(4)	56(4)	-1(3)	7(3)	3(3)
C(15)	49(4)	31(3)	32(3)	4(3)	17(3)	2(3)
C(16)	56(4)	77(5)	39(3)	-22(4)	3(3)	-13(4)
C(35)	72(5)	61(5)	54(4)	4(4)	-20(4)	-8(4)
O(25)	55(3)	34(2)	49(2)	-12(2)	17(2)	-15(2)
C(27)	63(5)	21(3)	144(8)	-2(4)	55(5)	5(3)
C(28)	55(4)	31(4)	81(5)	18(4)	6(4)	-13(3)
O(29)	286(12)	44(4)	660(30)	-79(9)	383(17)	-43(6)

Table 5. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^{-3}$) for m1.

	x	y	z	U(eq)
H(20)	5206	-759	1492	42
H(21)	7878	455	1569	43
H(22)	5612	606	2509	40
H(6A)	1858	456	1352	59
H(6B)	3047	101	841	59
H(9A)	6897	-2079	971	47
H(9B)	5883	-1516	420	47
H(11A)	10863	-1016	34	57
H(11B)	9976	235	147	57
H(11C)	9832	-545	-529	57
H(14A)	9965	-2841	2531	71
H(14B)	8366	-3143	2507	71
H(14C)	8977	-2299	3094	71
H(16A)	7800	2460	4219	86
H(16B)	8763	2737	3596	86
H(16C)	8642	1333	3884	86
H(28A)	8137	5320	1063	83
H(28B)	8733	3926	965	83
H(28C)	9125	4723	1611	83
H(30)	4380(60)	2490(60)	920(30)	47(16)
H(31)	5260(50)	2720(50)	2080(20)	23(13)
H(27)	1660(90)	3240(90)	60(40)	110(40)

Table 6. Torsion angles [°] for m1.

C(6)-O(3)-C(1)-O(1)	-60.5(6)
C(6)-O(3)-C(1)-C(5)	177.9(5)
C(2)-O(1)-C(1)-O(3)	-61.6(6)
C(2)-O(1)-C(1)-C(5)	57.7(6)
C(1)-O(1)-C(2)-C(9)	176.0(4)
C(1)-O(1)-C(2)-C(3)	-61.5(6)
C(13)-O(6)-C(3)-C(2)	125.4(5)
C(13)-O(6)-C(3)-C(4)	-116.8(5)
O(1)-C(2)-C(3)-O(6)	177.2(4)
C(9)-C(2)-C(3)-O(6)	-65.0(6)
O(1)-C(2)-C(3)-C(4)	60.8(6)
C(9)-C(2)-C(3)-C(4)	178.6(5)
C(15)-O(8)-C(4)-C(3)	-156.6(5)
C(15)-O(8)-C(4)-C(5)	84.4(6)
O(6)-C(3)-C(4)-O(8)	64.7(5)
C(2)-C(3)-C(4)-O(8)	-179.3(4)
O(6)-C(3)-C(4)-C(5)	-173.9(4)
C(2)-C(3)-C(4)-C(5)	-57.8(6)
O(3)-C(1)-C(5)-O(25)	-172.9(5)
O(1)-C(1)-C(5)-O(25)	65.3(6)
O(3)-C(1)-C(5)-C(4)	68.8(6)
O(1)-C(1)-C(5)-C(4)	-53.1(6)
O(8)-C(4)-C(5)-O(25)	54.2(5)
C(3)-C(4)-C(5)-O(25)	-64.0(6)
O(8)-C(4)-C(5)-C(1)	171.8(4)
C(3)-C(4)-C(5)-C(1)	53.6(6)
C(1)-O(3)-C(6)-C(7)	-59.8(7)
O(3)-C(6)-C(7)-C(35)	48(24)
C(10)-O(4)-C(9)-C(2)	143.3(5)
O(1)-C(2)-C(9)-O(4)	61.1(6)
C(3)-C(2)-C(9)-O(4)	-57.8(6)
C(9)-O(4)-C(10)-O(5)	2.3(8)
C(9)-O(4)-C(10)-C(11)	-177.6(4)
C(3)-O(6)-C(13)-O(7)	-0.7(8)
C(3)-O(6)-C(13)-C(14)	-179.5(4)
C(4)-O(8)-C(15)-O(9)	-5.2(8)
C(4)-O(8)-C(15)-C(16)	175.0(5)
C(6)-C(7)-C(35)-H(27)	127(100)

C(1)-C(5)-O(25)-C(27)	116.2(7)
C(4)-C(5)-O(25)-C(27)	-123.4(7)
C(5)-O(25)-C(27)-O(29)	2.7(17)
C(5)-O(25)-C(27)-C(28)	-179.4(6)

Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen bonds for m1 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)

7.1.2 Ethyl-2,3,4-tri-*O*-*tert*-butyldimethylsilyl-1-thio- α -L-fucopyranoside, 132

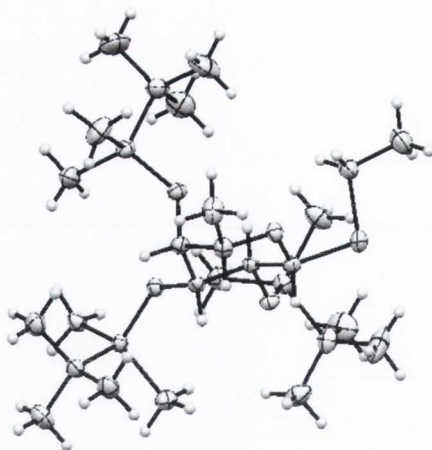


Table 1. Crystal data and structure refinement for m1.

Identification code	shelxl	
Empirical formula	C _{20.80} H _{46.40} O _{3.20} S _{0.80} Si _{2.40}	
Formula weight	440.84	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 10.811(2) Å	$\alpha = 90^\circ$.
	b = 14.427(3) Å	$\beta = 90^\circ$.
	c = 21.917(4) Å	$\gamma = 90^\circ$.
Volume	3418.3(12) Å ³	
Z	5	
Density (calculated)	1.071 Mg/m ³	
Absorption coefficient	0.226 mm ⁻¹	
F(000)	1216	
Crystal size	0.70 x 0.30 x 0.30 mm ³	
Theta range for data collection	1.69 to 25.00°.	
Index ranges	-9<= <i>h</i> <=12, -17<= <i>k</i> <=11, -25<= <i>l</i> <=26	
Reflections collected	14454	
Independent reflections	5951 [R(int) = 0.0311]	
Completeness to theta = 25.00°	99.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.0000 and 0.8521	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	5951 / 0 / 324	
Goodness-of-fit on F ²	1.072	

Final R indices [I>2sigma(I)]	R1 = 0.0343, wR2 = 0.0821
R indices (all data)	R1 = 0.0356, wR2 = 0.0834
Absolute structure parameter	0.04(7)
Largest diff. peak and hole	0.232 and -0.213 e.Å ⁻³

Table 2. Atomic coordinates (x 10⁴) and equivalent isotropic displacement parameters (Å²x 10³) for m1. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
S(1)	2180(1)	4786(1)	10780(1)	27(1)
Si(2)	5077(1)	5570(1)	9821(1)	23(1)
Si(1)	5058(1)	3307(1)	8315(1)	20(1)
Si(3)	689(1)	4637(1)	8045(1)	24(1)
O(2)	4355(1)	4550(1)	9830(1)	22(1)
O(4)	1254(1)	4316(1)	8712(1)	23(1)
O(3)	1130(1)	3763(1)	9949(1)	23(1)
O(1)	3877(1)	3920(1)	8592(1)	21(1)
C(3)	3139(2)	4394(2)	9606(1)	19(1)
C(2)	3140(2)	3650(1)	9103(1)	20(1)
C(4)	2350(2)	4010(2)	10137(1)	21(1)
C(1)	1800(2)	3472(2)	8909(1)	21(1)
C(21)	5672(2)	4066(2)	7696(1)	27(1)
C(6)	6380(2)	5459(2)	10392(1)	31(1)
C(18)	4591(2)	2138(2)	7990(1)	26(1)
C(20)	6241(2)	3103(2)	8922(1)	29(1)
C(5)	1097(2)	3098(2)	9463(1)	24(1)
C(10)	1044(2)	5599(2)	10481(1)	31(1)
C(25)	1870(2)	4562(2)	7431(1)	39(1)
C(24)	-257(2)	2904(2)	9345(1)	34(1)
C(13)	197(2)	5873(2)	8180(1)	34(1)
C(16)	3618(2)	2233(2)	7483(1)	34(1)
C(23)	4031(2)	6545(2)	10035(1)	38(1)
C(17)	5759(2)	1693(2)	7713(1)	38(1)
C(22)	5670(2)	5815(2)	9037(1)	32(1)
C(7)	7296(2)	4698(2)	10202(1)	38(1)
C(8)	7090(3)	6383(2)	10422(2)	54(1)
C(14)	1324(3)	6460(2)	8371(1)	49(1)
C(11)	803(3)	6334(2)	10969(1)	46(1)

C(12)	-353(3)	6278(2)	7593(1)	60(1)
C(19)	4090(2)	1471(2)	8483(1)	33(1)
C(15)	-764(3)	5917(2)	8692(1)	59(1)
C(26)	-663(2)	3900(2)	7830(1)	45(1)
C(9)	5860(3)	5219(3)	11023(1)	63(1)

Table 3. Bond lengths [Å] and angles [°] for m1.

S(1)-C(4)	1.809(2)
S(1)-C(10)	1.820(2)
Si(2)-O(2)	1.6659(15)
Si(2)-C(23)	1.864(2)
Si(2)-C(22)	1.868(2)
Si(2)-C(6)	1.892(2)
Si(1)-O(1)	1.6682(15)
Si(1)-C(21)	1.865(2)
Si(1)-C(20)	1.868(2)
Si(1)-C(18)	1.898(2)
Si(3)-O(4)	1.6518(14)
Si(3)-C(25)	1.858(2)
Si(3)-C(26)	1.868(3)
Si(3)-C(13)	1.884(3)
O(2)-C(3)	1.421(2)
O(4)-C(1)	1.420(3)
O(3)-C(4)	1.428(2)
O(3)-C(5)	1.435(2)
O(1)-C(2)	1.428(2)
C(3)-C(2)	1.539(3)
C(3)-C(4)	1.546(3)
C(2)-C(1)	1.531(3)
C(1)-C(5)	1.531(3)
C(6)-C(9)	1.533(3)
C(6)-C(7)	1.536(4)
C(6)-C(8)	1.540(4)
C(18)-C(16)	1.535(3)
C(18)-C(17)	1.541(3)
C(18)-C(19)	1.544(3)
C(5)-C(24)	1.512(3)

C(10)-C(11)	1.529(3)
C(13)-C(15)	1.530(3)
C(13)-C(12)	1.533(3)
C(13)-C(14)	1.541(4)
C(4)-S(1)-C(10)	100.79(10)
O(2)-Si(2)-C(23)	112.32(10)
O(2)-Si(2)-C(22)	109.79(9)
C(23)-Si(2)-C(22)	107.28(11)
O(2)-Si(2)-C(6)	105.46(9)
C(23)-Si(2)-C(6)	110.40(12)
C(22)-Si(2)-C(6)	111.66(11)
O(1)-Si(1)-C(21)	103.04(9)
O(1)-Si(1)-C(20)	110.38(9)
C(21)-Si(1)-C(20)	111.51(10)
O(1)-Si(1)-C(18)	113.80(9)
C(21)-Si(1)-C(18)	110.08(10)
C(20)-Si(1)-C(18)	108.02(10)
O(4)-Si(3)-C(25)	111.72(9)
O(4)-Si(3)-C(26)	110.64(10)
C(25)-Si(3)-C(26)	108.82(12)
O(4)-Si(3)-C(13)	103.32(9)
C(25)-Si(3)-C(13)	111.31(11)
C(26)-Si(3)-C(13)	110.98(13)
C(3)-O(2)-Si(2)	124.67(13)
C(1)-O(4)-Si(3)	131.52(13)
C(4)-O(3)-C(5)	113.84(15)
C(2)-O(1)-Si(1)	124.59(13)
O(2)-C(3)-C(2)	110.95(16)
O(2)-C(3)-C(4)	107.86(15)
C(2)-C(3)-C(4)	106.84(16)
O(1)-C(2)-C(1)	110.84(15)
O(1)-C(2)-C(3)	111.80(16)
C(1)-C(2)-C(3)	108.38(16)
O(3)-C(4)-C(3)	112.42(15)
O(3)-C(4)-S(1)	106.59(13)
C(3)-C(4)-S(1)	114.90(15)
O(4)-C(1)-C(5)	109.67(16)
O(4)-C(1)-C(2)	109.50(16)
C(5)-C(1)-C(2)	107.97(16)

C(9)-C(6)-C(7)	108.6(2)
C(9)-C(6)-C(8)	109.9(2)
C(7)-C(6)-C(8)	108.0(2)
C(9)-C(6)-Si(2)	110.05(17)
C(7)-C(6)-Si(2)	111.14(15)
C(8)-C(6)-Si(2)	109.06(18)
C(16)-C(18)-C(17)	108.25(18)
C(16)-C(18)-C(19)	108.69(19)
C(17)-C(18)-C(19)	107.6(2)
C(16)-C(18)-Si(1)	112.01(16)
C(17)-C(18)-Si(1)	107.46(16)
C(19)-C(18)-Si(1)	112.61(15)
O(3)-C(5)-C(24)	105.90(18)
O(3)-C(5)-C(1)	109.90(17)
C(24)-C(5)-C(1)	114.24(17)
C(11)-C(10)-S(1)	108.09(17)
C(15)-C(13)-C(12)	109.7(2)
C(15)-C(13)-C(14)	108.4(2)
C(12)-C(13)-C(14)	108.9(2)
C(15)-C(13)-Si(3)	110.24(19)
C(12)-C(13)-Si(3)	109.75(19)
C(14)-C(13)-Si(3)	109.83(17)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for m1. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2 a^{*2}U^{11} + \dots + 2 h k a^* b^* U^{12}]$

	U ¹¹	U ²²	U ³³	U ²³	U ¹³	U ¹²
S(1)	31(1)	30(1)	19(1)	-2(1)	2(1)	0(1)
Si(2)	25(1)	21(1)	25(1)	-1(1)	2(1)	-3(1)
Si(1)	22(1)	20(1)	19(1)	1(1)	2(1)	1(1)
Si(3)	23(1)	27(1)	22(1)	4(1)	-1(1)	1(1)
O(2)	19(1)	21(1)	25(1)	1(1)	-2(1)	-2(1)
O(4)	24(1)	22(1)	22(1)	3(1)	-1(1)	1(1)
O(3)	22(1)	25(1)	22(1)	-1(1)	3(1)	-1(1)
O(1)	22(1)	22(1)	19(1)	1(1)	3(1)	1(1)
C(3)	20(1)	16(1)	22(1)	0(1)	0(1)	1(1)
C(2)	23(1)	16(1)	20(1)	3(1)	3(1)	2(1)
C(4)	24(1)	19(1)	20(1)	2(1)	0(1)	1(1)
C(1)	24(1)	17(1)	22(1)	2(1)	0(1)	-1(1)
C(21)	29(1)	26(1)	26(1)	1(1)	7(1)	2(1)
C(6)	32(1)	32(1)	29(1)	-3(1)	-2(1)	-11(1)
C(18)	29(1)	24(1)	25(1)	-2(1)	3(1)	3(1)
C(20)	26(1)	30(1)	30(1)	0(1)	-2(1)	3(1)
C(5)	28(1)	20(1)	24(1)	0(1)	1(1)	-5(1)
C(10)	32(1)	30(1)	32(1)	-4(1)	4(1)	5(1)
C(25)	45(1)	43(2)	28(1)	7(1)	6(1)	13(1)
C(24)	34(1)	37(2)	30(1)	-2(1)	3(1)	-14(1)
C(13)	33(1)	33(1)	35(1)	6(1)	2(1)	8(1)
C(16)	44(1)	27(1)	31(1)	-5(1)	-4(1)	0(1)
C(23)	40(1)	22(1)	53(2)	-4(1)	10(1)	-3(1)
C(17)	41(1)	29(1)	43(1)	-8(1)	9(1)	4(1)
C(22)	39(1)	26(1)	31(1)	3(1)	3(1)	-3(1)
C(7)	38(1)	32(2)	45(1)	6(1)	-15(1)	-5(1)
C(8)	47(2)	40(2)	76(2)	-16(1)	-15(2)	-10(2)
C(14)	58(2)	30(2)	60(2)	-1(1)	1(1)	-3(1)
C(11)	53(2)	42(2)	43(1)	-10(1)	7(1)	8(1)
C(12)	74(2)	47(2)	58(2)	12(1)	-17(2)	22(2)
C(19)	42(1)	22(1)	34(1)	0(1)	4(1)	2(1)
C(15)	53(2)	52(2)	73(2)	1(2)	29(2)	20(2)
C(26)	47(2)	48(2)	39(1)	7(1)	-15(1)	-12(1)
C(9)	45(2)	119(3)	25(1)	5(2)	-5(1)	-17(2)

Table 5. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^{-3}$) for m1.

	x	y	z	U(eq)
H(3)	2783	4973	9452	23
H(2)	3480	3075	9272	23
H(4)	2754	3445	10285	25
H(1)	1779	3016	8577	25
H(21A)	5015	4222	7420	40
H(21B)	6311	3743	7478	40
H(21C)	6006	4622	7872	40
H(20A)	6520	3687	9079	43
H(20B)	6929	2771	8752	43
H(20C)	5880	2746	9246	43
H(5)	1497	2525	9601	29
H(10A)	1355	5889	10112	38
H(10B)	282	5276	10383	38
H(25A)	2623	4849	7567	58
H(25B)	1573	4875	7073	58
H(25C)	2026	3923	7336	58
H(24A)	-640	2688	9714	50
H(24B)	-333	2438	9034	50
H(24C)	-658	3462	9212	50
H(16A)	3434	1631	7319	51
H(16B)	3935	2624	7165	51
H(16C)	2879	2501	7649	51
H(23A)	3387	6602	9736	57
H(23B)	4497	7111	10052	57
H(23C)	3672	6424	10428	57
H(17A)	5547	1106	7534	56
H(17B)	6364	1600	8028	56
H(17C)	6092	2094	7405	56
H(22A)	6231	5333	8916	48
H(22B)	6093	6399	9035	48

H(22C)	4989	5838	8756	48
H(7A)	7936	4646	10503	57
H(7B)	7656	4854	9815	57
H(7C)	6867	4117	10169	57
H(8A)	6541	6867	10554	81
H(8B)	7410	6531	10025	81
H(8C)	7762	6328	10706	81
H(14A)	1071	7091	8430	74
H(14B)	1941	6433	8056	74
H(14C)	1661	6220	8744	74
H(11A)	519	6039	11336	69
H(11B)	184	6759	10826	69
H(11C)	1554	6666	11052	69
H(12A)	-1060	5919	7473	89
H(12B)	257	6261	7275	89
H(12C)	-600	6908	7664	89
H(19A)	3327	1710	8643	49
H(19B)	4683	1417	8807	49
H(19C)	3948	872	8305	49
H(15A)	-410	5679	9062	89
H(15B)	-1471	5550	8582	89
H(15C)	-1014	6548	8754	89
H(26A)	-420	3260	7831	67
H(26B)	-946	4070	7430	67
H(26C)	-1318	3991	8120	67
H(9A)	5384	4658	10997	95
H(9B)	5341	5715	11163	95
H(9C)	6530	5132	11305	95

Table 6. Torsion angles [°] for m1.

C(23)-Si(2)-O(2)-C(3)	41.45(17)
C(22)-Si(2)-O(2)-C(3)	-77.82(16)
C(6)-Si(2)-O(2)-C(3)	161.75(14)
C(25)-Si(3)-O(4)-C(1)	-58.7(2)
C(26)-Si(3)-O(4)-C(1)	62.8(2)
C(13)-Si(3)-O(4)-C(1)	-178.41(17)
C(21)-Si(1)-O(1)-C(2)	-176.47(15)

C(20)-Si(1)-O(1)-C(2)	-57.29(17)
C(18)-Si(1)-O(1)-C(2)	64.35(17)
Si(2)-O(2)-C(3)-C(2)	121.77(15)
Si(2)-O(2)-C(3)-C(4)	-121.53(15)
Si(1)-O(1)-C(2)-C(1)	-115.60(16)
Si(1)-O(1)-C(2)-C(3)	123.36(16)
O(2)-C(3)-C(2)-O(1)	-61.0(2)
C(4)-C(3)-C(2)-O(1)	-178.28(15)
O(2)-C(3)-C(2)-C(1)	176.59(15)
C(4)-C(3)-C(2)-C(1)	59.3(2)
C(5)-O(3)-C(4)-C(3)	57.1(2)
C(5)-O(3)-C(4)-S(1)	-176.20(13)
O(2)-C(3)-C(4)-O(3)	-175.64(16)
C(2)-C(3)-C(4)-O(3)	-56.3(2)
O(2)-C(3)-C(4)-S(1)	62.22(19)
C(2)-C(3)-C(4)-S(1)	-178.43(13)
C(10)-S(1)-C(4)-O(3)	-48.76(15)
C(10)-S(1)-C(4)-C(3)	76.47(16)
Si(3)-O(4)-C(1)-C(5)	-124.02(16)
Si(3)-O(4)-C(1)-C(2)	117.68(16)
O(1)-C(2)-C(1)-O(4)	-66.0(2)
C(3)-C(2)-C(1)-O(4)	57.0(2)
O(1)-C(2)-C(1)-C(5)	174.66(17)
C(3)-C(2)-C(1)-C(5)	-62.3(2)
O(2)-Si(2)-C(6)-C(9)	-58.4(2)
C(23)-Si(2)-C(6)-C(9)	63.1(2)
C(22)-Si(2)-C(6)-C(9)	-177.6(2)
O(2)-Si(2)-C(6)-C(7)	61.92(17)
C(23)-Si(2)-C(6)-C(7)	-176.53(16)
C(22)-Si(2)-C(6)-C(7)	-57.3(2)
O(2)-Si(2)-C(6)-C(8)	-179.08(17)
C(23)-Si(2)-C(6)-C(8)	-57.5(2)
C(22)-Si(2)-C(6)-C(8)	61.7(2)
O(1)-Si(1)-C(18)-C(16)	57.20(17)
C(21)-Si(1)-C(18)-C(16)	-57.89(17)
C(20)-Si(1)-C(18)-C(16)	-179.86(15)
O(1)-Si(1)-C(18)-C(17)	175.97(14)
C(21)-Si(1)-C(18)-C(17)	60.88(17)
C(20)-Si(1)-C(18)-C(17)	-61.09(17)
O(1)-Si(1)-C(18)-C(19)	-65.67(17)

C(21)-Si(1)-C(18)-C(19)	179.24(15)
C(20)-Si(1)-C(18)-C(19)	57.27(18)
C(4)-O(3)-C(5)-C(24)	178.07(17)
C(4)-O(3)-C(5)-C(1)	-58.1(2)
O(4)-C(1)-C(5)-O(3)	-59.2(2)
C(2)-C(1)-C(5)-O(3)	60.1(2)
O(4)-C(1)-C(5)-C(24)	59.7(2)
C(2)-C(1)-C(5)-C(24)	178.91(19)
C(4)-S(1)-C(10)-C(11)	-178.77(18)
O(4)-Si(3)-C(13)-C(15)	-59.3(2)
C(25)-Si(3)-C(13)-C(15)	-179.33(19)
C(26)-Si(3)-C(13)-C(15)	59.3(2)
O(4)-Si(3)-C(13)-C(12)	179.82(19)
C(25)-Si(3)-C(13)-C(12)	59.8(2)
C(26)-Si(3)-C(13)-C(12)	-61.6(2)
O(4)-Si(3)-C(13)-C(14)	60.09(18)
C(25)-Si(3)-C(13)-C(14)	-59.95(19)
C(26)-Si(3)-C(13)-C(14)	178.69(17)

Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen bonds for m1 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
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