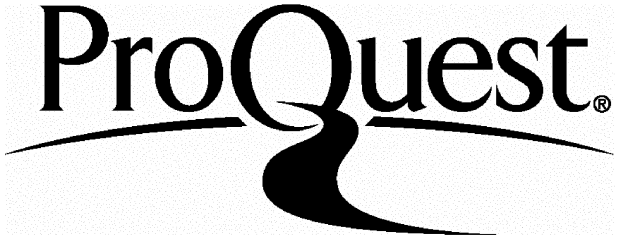
DESIGN AND SYNTHESIS OF GLYCOSIDES AS POTENTIAL ANTICANCER AGENTS

GEORGIA TSOUKALA

A thesis submitted for the degree of Doctor of Philosophy of the University of London

The School of Pharmacy
University of London

February 2009



This thesis describes research conducted in the School of Pharmacy, University of London between November 2004 and October 2008 under the supervision of Dr. John P. Malkinson. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

GTrask 13/03/09

Signature Date

Acknowledgements

I would like to express my gratitude to my supervisors Dr. John P. Malkinson, Dr. Mark Searcey and Dr. Robert A. Falconer for their considerable expertise, advice, encouragement and enthusiasm. I truly thank them for the enormous effort in guiding me through the difficult phase of writing up this thesis.

I would also like to thank the School of Pharmacy for funding and for the use of their facilities and analytical services.

Finally, I would like to thank my colleagues in the lab for their friendship and help and my friends and family for their support and patience.

Abstract

Despite carbohydrates being abundant in Nature, there are surprisingly few carbohydrate-based compounds used in medicine. This thesis describes two investigations into carbohydrate derivatives with potential in the treatment of cancer.

The ipomoeassins, which were isolated from the leaves of *Ipomoea squamosa*, a member of the morning glory family, exhibit highly potent cytotoxic activity against human ovarian cancer cell lines (IC_{50} 35 nM) and thus constitute an interesting target for further investigation. They are polyacylated macrocyclic glycoresins, each incorporating a β-D-glucopyranosyl-(1→2)-β-D-fucopyranose glycoside and cyclised via a macrolactone with the glucose primary hydroxyl.
Our initial synthetic approach involved the successful formation of the protected disaccharide using a glucosyl trichloroacetimidate donor and a suitably-derivatised fucose glycoside. However, all attempts at macrolactonisation proved fruitless, although several different routes to the precursors were identified. The potential of the glycolipid assembly was demonstrated by the synthesis of a novel non-macrocyclic glycoresin **212**, similar to those found in several *Ipomoea* species.
An alternative synthesis involved formation of the macrolactone ring via ring closing metathesis (RCM). For that purpose a glucose thioglycoside donor bearing a hept-6-enoate ester and a suitable hex-5-enyl fucoside acceptor were successfully synthesised. Unfortunately, assembly of the disaccharide was hampered by unpredictable side-reactions and unexpected protecting group migration.
Glycoresins **212** and **227** are currently being evaluated for anticancer and anti-bacterial activity and for their potential as efflux pump inhibitors.

Polysialic acid (PSA), a homopolymer of sialic acid, plays an important role during development and neural regeneration, but by adulthood is essentially non-existent. Significantly, it has been identified on the surface of a growing number of tumours and is associated with cancer progression and metastasis.
PSA is synthesised by two polysialyltransferases, PST and STX, which act synergistically. The polysialyltransferases are exciting potential novel targets for cancer therapy since their inhibition will result in prevention of PSA synthesis and might therefore reduce metastatic potential. The aim of this project was the design and synthesis of a library of molecule inhibitors of PST and STX. A series of L-rhamnose-based compounds was successfully synthesised and characterised.

Table of Contents

List of Figures

List of Schemes

Abbreviations

Ac	Acetyl
ADDP	1,1′-(Azodicarbonyl)dipiperidine
All	Allyl
Ar	Aromatic
BDA	Butane-2,3-diacetal
bis-DHP	6,6′-Bis(3,4-dihydro-2*H*-pyran)
Bn	Benzyl
br	broad
Bz	Benzoyl
CAN	Ceric ammonium nitrate
Cbz	Benzyloxy carbonyl
CDA	Cyclohexane-1,2-diacetal
CMP	Cytidine monophosphate
COSY	Correlation spectroscopy
CTP	Cytidine triphosphate
CSA	(±)-Camphor-10-sulfonic acid
DAST	Diethylaminosulfur trifluoride
DCC	*N,N'*-Dicyclohexylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyanobenzoquinone
DIBAL	Diisobutylaluminum hydride
DIC	Diisopropylcarbodiimide
DISPOKE	1,8,13,16-Tetraoxadispiro[5.0.5.4] hexadecane
DMAP	4-Dimethylaminopyridene
DMDO	Dimethyldioxirane
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMTST	Dimethyl(methylthio)sulfonium triflate
DTBMP	2,6-Di-*tert*-butyl-4-methylpyridine
EDCI	*N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride
EI	Electron ionisation mass spectrometry
ES-MS	Electrospray mass spectrometry
FAB	Fast atom bombardment
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HR-MS	High resolution mass spectrometry
IAD	Intramolecular aglycon delivery
IDCP	Iodonium dicollidine perchlorate
KAPA	Potassium 3-aminopropylamide
KDN	Deaminoneuraminic acid
Lev	Levulinate (4-oxopentanoate)
LSI-MS	Liquid secondary ion mass spectrometry
ManBut	*N*-Butanoylmannosamine
ManLev	*N*-Levulinoylmannosamine
ManNAc	*N*-Acetylmannosamine
ManPent	*N*-Pentanoylmannosamine
ManProp	*N*-Propranoylmannosamine

mCPBA	3-Chloroperbenzoic acid
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
Ms	Mesyl
NBS	*N*-Bromosuccinimide
NCAM	Neural cell adhesion molecule
NeuNAc	*N*-Acetylneuraminic acid
NeuNGc	*N*-Glycolylneuraminic acid
NIS	*N*-Iodosuccinimide
NMR	Nuclear magnetic resonance
NSCLC	Non-small cell lung carcinoma
PCK	Protein kinase C
PEP	Phosphoenolpyruvate
Ph	Phenyl
Piv	Pivaloate
PMB	*p*-Methoxybenzyl
PPTS	Pyridinium *p*-toluenesulfonate
PSA	Polysialic acid
PST	Polysialyltransferase (ST8Sia IV)
PTSA	*p*-Toluenesulfonic acid
RCM	Ring closing metathesis
rt	Room temperature
SCLC	Small cell lung carcinoma
ST	Sialyltransferases
STX	Polysialyltransferase (ST8Sia II)
TBAB	Tetrabutylammonium bromide
TBAF	Tetrabutylammonium fluoride
TBAHS	Tetrabutylammonium hydrogen sulfate
TBDPS	*tert*-Butyldiphenylsilyl
TBS/TBDMS	*tert*-Butyldimethylsilyl
Tf/ Triflate	Trifluoromethanesulfonate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TMEDA	*N,N,N',N'*-Tetramethylethylenediamine
TMS	Trimethylsilyl
Tr/Trityl	Triphenylmethyl
UDP	Uridine diphosphate
UDP-GlcNAc	UDP-*N*-acetylglucosamine

I

TOTAL SYNTHESIS OF IPOMOEASSIN ANALOGUES

1. INTRODUCTION TO GLYCORESINS

1.1. Introduction

Carbohydrates are the most abundant and diverse molecules encountered in Nature. Until recently they were considered to be only useful for energy storage and as skeletal components. It is now clear that carbohydrates play important roles in many crucial biological processes. They are present on the cell surface, where they regulate cell-cell interactions and initiate fertilisation, cell growth and immune responses, and are also involved in protein folding and transport. On the other hand, alterations in cell surface carbohydrates have been associated with inflammation, cancer progression and metastasis. Moreover, pathogens bind to specific glycan structures of the host cell in order to invade the body.[1]

Despite the abundance of carbohydrates in Nature and their involvement in so many biological systems, there are surprisingly few carbohydrate-based compounds used in medicine. Due to the lack of tools for studying glycobiology, the poor understanding of the mechanisms of action of sugars in biological processes and the lack of practical synthetic and analytical methods, progress in this field has been rather slow. Furthermore, the physical properties of sugars often make them poor drug candidates. Solubility, absorption and clearance issues due to their high polarity, inadequate binding to the target receptors and rapid degradation by glycosidases have further hindered the development of carbohydrate-based therapeutics. Some examples of carbohydrate-based drugs are heparin[2] for the treatment of thrombosis, acarbose[3] for diabetes, aminoglycosides,[4] e.g. streptomycin, used as antibiotics and Tamiflu (oseltamivir),[5,6] a monosaccharide-based drug for the treatment of influenza[7-9] (Figure 1.1).

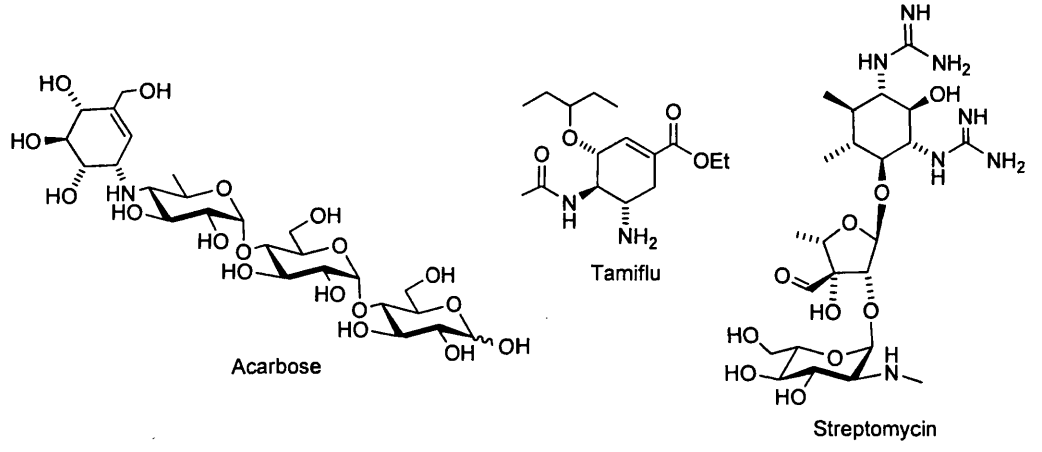


Figure 1.1: Examples of carbohydrate-based therapeutics

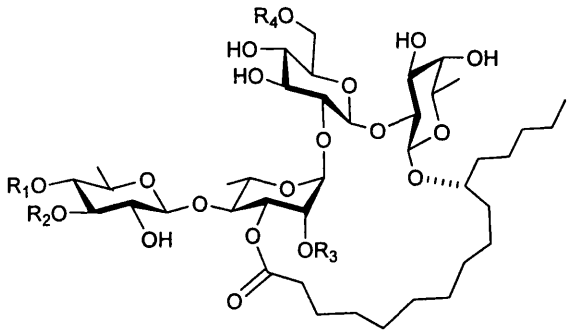
Cancer is a leading cause of human death and there is a need for novel and more efficient drugs. Natural products are a valuable source of compounds with a wide variety of chemical structures and biological activities that enable the discovery of new classes of therapeutic agents. More than 60% of approved anticancer drugs are derived from natural compounds.[10] Taxol (paclitaxel), derived from the Pacific yew tree (*Taxus brevifolia*), and its semi-synthetic analogue docetaxel are used for the treatment of breast, ovarian and non-small cell lung cancer.[11] Vincristine and vinblastine, both isolated from the periwinkle plant of Madagascar, are used against several types of cancer including Hodgkin's lymphoma.[12] Doxorubicin and daunorubicin are anthracycline anticancer antibiotics isolated from the fungus *Streptomyces peucetius* and are effective against a wide range of cancers including several types of leukaemia.[13]

In 2005, a new family of carbohydrate-based compounds with anti-tumour activity, termed the ipomoeassins, was disclosed by Cao and co-workers and involved extraction from *Ipomoea squamosa*, a member of the morning glory family. The morning glories (Convolvulaceae family), and the genus *Ipomoea* in particular, are examples of plant species that have been used for nutritional and medicinal purposes in Mexico for centuries. They are usually vines or small trees with funnel-shaped flowers that open in the morning and last for a single day. The most characteristic feature of these species is the presence of cells that secrete resin glycosides in the foliar tissues and roots. The common chemical feature of these resin glycosides is that they consist of two or more sugar units and at least one long fatty acid chain, which usually forms a characteristic macrolactone ring with the sugar units. The sugar units commonly found in glycoresins are D-glucose, D-fucose, D-quinovose and L-rhamnose.[14-16] The therapeutic properties of the morning glories have been attributed to the presence of these glycoresins and there is a growing interest in their potential as pharmacological agents.

1.2. Orizabins

Ipomoea orizabensis, also known as Mexican scammony, has been mainly used for its purgative properties but also to treat abdominal fever, dysentery, epilepsy, meningitis and tumours.[17,18] Although chemical investigation of its constituent resin glycosides was initiated as early as 1840, it was not until 1987 when the first

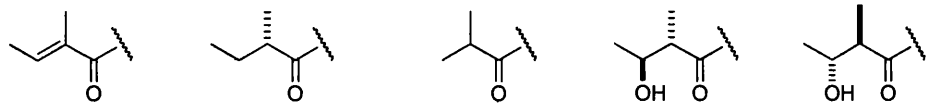
glycoresins, namely orizabins I, II, III and IV, (**1**, **2**, **3** and **4** respectively, Figure 1.2), were isolated from the ether-soluble fraction of *Ipomoea orizabensis*.[17]



1: R_1 = Mba, R_2 = H, R_3 =Tga, R_4 = (+)-Nla
2: R_1 = Iba, R_2 = H, R_3 = Tga, R_4 = (+)-Nla
3: R_1 = (+)-Nla, R_2 = H, R_3 = Tga, R_4 = (+)-Nla
4: R_1 = H, R_2 = Iba, R_3 = Tga, R_4 = (+)-Nla

Figure 1.2: Orizabins I-IV

Chromatographic and degradative studies in combination with NMR and FAB-MS were used to characterise compounds **1-4**. Saponification yielded several organic acids and the glycosidic acid **5** (Figure 1.4). The organic acids were identified as tiglic, nilic and (*S*)-2-methylbutyric acids for compound **1**; tiglic, nilic and isobutyric acids for compounds **2** and **4**; and tiglic and nilic acids for compound **3** (Figure 1.3). Acidic hydrolysis of the glycosidic acid **5**, which was found to be identical for all four compounds, gave 11-hydroxyhexadecanoic acid (jalapinolic acid) and a sugar mixture consisting of D-fucose, D-glucose, L-rhamnose and D-quinovose. The absolute configuration of C-11 in jalapinolic acid was determined as *R*, which is rather unusual since jalapinolic acid is normally found in the *S* configuration in glycoresins. Further analysis of the ^{1}H NMR and COSY spectra showed two unequal signals for the α-CH_2 of jalapinolic acid, which suggested a macrocyclic lactone-type structure for compounds **1-4** and orizabins I-IV were assigned the structures shown in Figure 1.2.[17]



Tga (tigloyl) | **Mba** ((*S*)-2-methylbutanoyl) | **Iba** (isobutanoyl) | **(+)-Nla** ((2*S*,3*S*)-niloyl) | **(-)-Nla** ((2*R*,3*R*)-niloyl)

Figure 1.3: Common acyl substituents found in glycoresins

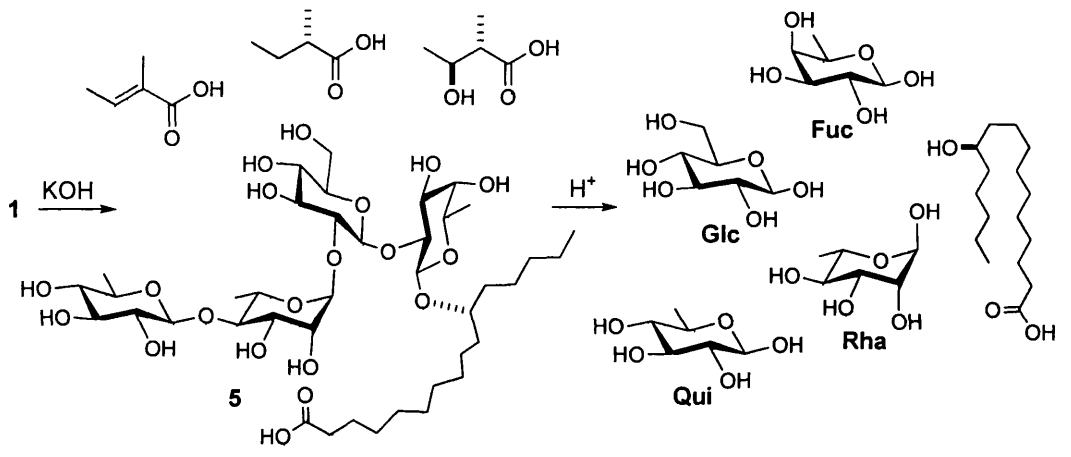
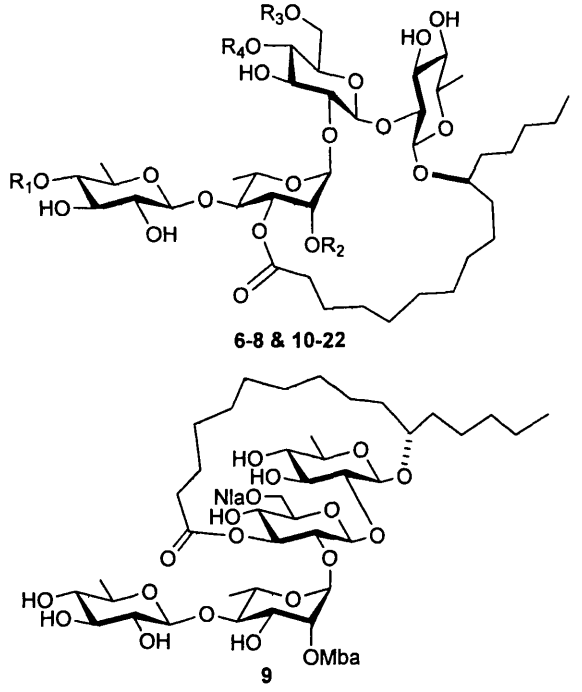


Figure 1.4: Basic and acidic hydrolysis of orizabin I

Further analysis of the *Ipomoea orizabensis* extracts afforded a major polar fraction containing four new glycoresins, orizabins V-VIII (**6-9**)[18] and a non-polar fraction containing thirteen new glycoresins, orizabins IX-XXI (**10-22**)[19] (Figure 1.5).



	R_1	R_2	R_3	R_4
6	(-)-Nla	Mba	H	H
7	H	(-)-Nla	Mba	H
8	H	Mba	(-)-Nla	H
10	Tga	Mba	H	(-)-Nla
11	Tga	(-)-Nla	Iba	H
12	Tga	(+)-Nla	Iba	H
13	Tga	Iba	(-)-Nla	H
14	Tga	Iba	(+)-Nla	H
15	Tga	(-)-Nla	Mba	H
16	Tga	(+)-Nla	Mba	H
17	Tga	Mba	(-)-Nla	H
18	Tga	Mba	(+)-Nla	H
19	Mba	(-)-Nla	Mba	H
20	Mba	(+)-Nla	Mba	H
21	Mba	Mba	(-)-Nla	H
22	Mba	Mba	(+)-Nla	H

Figure 1.5: Orizabins V-XXI

The cytotoxicity of compounds **6-22** was evaluated using four human cancer cultured cell lines. All compounds showed weak cytotoxity against colon carcinoma, squamous cell cervix carcinoma and ovarian cancer cell lines (ED_{50} > 15 μg/mL for **6-9** and ED_{50} 4-20 μg/mL for **10-22**) but a stronger activity against oral epidermoid carcinoma with ED_{50} values of 7-10 μg/mL for compounds **6-9** and 1-5 μg/mL for compounds **10-22**. It was observed that glycoresins **10-22**,

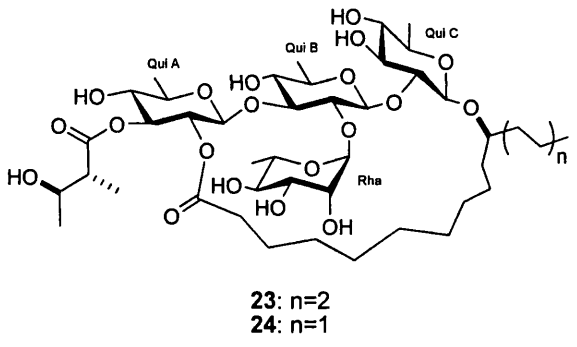
which consist of the same basic tetrasaccharide as **6-9** but contain three rather than two short-chain fatty acids, have a higher potency than glycoresins **6-9**.[18,19]

Orizabins IX-XXI were also evaluated for anti-microbial activity against four *Staphylococcus aureus* strains, i.e. the effluxing strains SA-1199B and XU-212, an epidermic methicillin-resistant strain (EMRSA-15) and a standard *S. aureus* strain (ATCC 25923). Orizabins X, XI, XIII and XVII were the most active compounds against SA-1199B, EMRSA-15 and XU-212 with MIC values of 4 μg/mL, 4-8 μg/mL and 32 μg/mL, respectively, while orizabins XIII, XVI, XVII and XXI exhibited the highest inhibitory activity against ATCC 25923 with MIC values of 8 μg/mL. It was observed that, while substitution of (2*S*,3*S*)-nilic acid by its enantiomer (2*R*,3*R*)-nilic acid caused a 4-fold reduction in inhibition in most cases, interchange of the other ester moieties (tiglate, methylbutyrate and isobutyrate) did not have any significant effect on inhibition.[20]

1.3. Calonyctins

Calonyctin, which was isolated from the moonlight flower (*Calonyction aculeatum* L. House), a plant of the Convolvulaceae family, is a growth regulator that promotes the yield of various crops. It consists of two homologous glycoresins, calonyctin A1 (**23**) and A2 (**24**)[21] (Figure 1.6).

Dried leaves of the moonlight flower, collected in Tong'An, China, were extracted with ethanol and the residue thus obtained chromatographed on an aluminium hydroxide column and subsequently on a silica gel column. Further HPLC purification yielded two compounds designated as DC-2b and DC-2d (calonyctin A1 and A2 respectively).[21]



23: n=2
24: n=1

Figure 1.6: Calonyctins A1 (**23**) and A2 (**24**)

Initial LSI-MS indicated that the two compounds differed by the mass of an ethyl unit. From their ^{1}H and ^{13}C NMR spectra the presence of four 6-deoxy sugars

could be determined. The sugars were identified as α-L-rhamnose and β-quinovose whose configuration was provisionally assigned as D, since quinovose in natural glycoresins is usually found in this configuration. The tetrasaccharide was assigned the following structure: D-Quiβ(1→3)[L-Rhaα(1→2)]-D-Quiβ(1→2) D-Qui.[21]

Acidic methanolysis afforded the aglycon products 11-hydroxyhexadecanoic acid and 11-hydroxytetradecanoic acid for DC-2b and DC-2d respectively, and 3-hydroxy-2-methylbutanoic acid, which was common to both glycoresins. The absolute configuration of the acyl substituents was not reported.

Further NMR analysis indicated that the compounds are glycoresins, in which the hydroxyl group of the aglycon is glycosidically linked to quinovose C (Figure 1.6). The non-equivalent shifts of the two protons of the α-CH_2 of the aglycon moiety suggested that the carbonyl group of the aglycon is intramolecularly linked with a hydroxyl of the tetrasaccharide and, from the downfield shifts of H-2 and H-3 of quinovose A, the sites of esterification could be assigned as shown in Figure 1.6. No biological activity data was reported for these compounds.

1.4. Tricolorins

Ipomoea tricolor has been used in traditional Mexican agriculture as a cover crop for the protection of sugar cane because it can inhibit the growth of invasive weeds. The seeds of *Ipomoea tricolor* have also been used in religious rituals because of their hallucinogenic properties.[15,22]

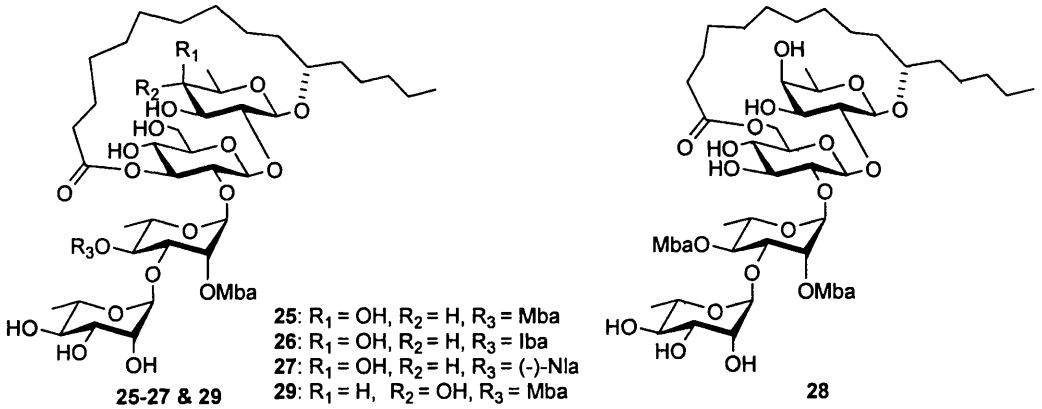


Figure 1.7: Tricolorins A-E (**25-29**)

A total of 10 glycoresins, tricolorins A-J, have been isolated from *Ipomoea tricolor* by recycling HPLC and their structures elucidated using extensive NMR and FAB-MS studies.[22-24]

Tricolorin A (**25**) (Figure 1.7), the main constituent, is assembled from the tetrasaccharide *α*-L-rhamnopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-fucopyranoside, glycosidically linked to the hydroxyl of (*S*)-11-hydroxyhexadecanoic acid. The site of lactonisation was found to be on the glucose C-3 hydroxyl and the presence of two (*S*)-2-methylbutanoyl groups on both the C-2 and C-4 hydroxyl of rhamnose was confirmed, as shown in Figure 1.7.[22,23]

Tricolorins B (**26**) and C (**27**) differ from tricolorin A in the type of acyl substituents present on the rhamnose C-4 hydroxyl; an isobutyrate group is present in tricolorin B (**26**) and a nilate group in tricolorin C (**27**), as shown in Figure 1.7. In tricolorin D (**28**) the site of lactonisation is on glucose C-6 and tricolorin E (**29**) contains D-quinovose instead of D-fucose.[23]

Tricolorins F (**30**) and G (**31**) (Figure 1.8) consist of trisaccharides of fucose, glucose, quinovose and fucose, glucose, rhamnose, respectively, that form macrolactones with (*S*)-11-hydroxyhexadecanoic acid. Tricolorins H (**32**), I (**33**) and J (**34**) were found to be ester-type dimers of either tricolorins F or G and tricoloric acid C, as shown in Figure 1.9.[24]

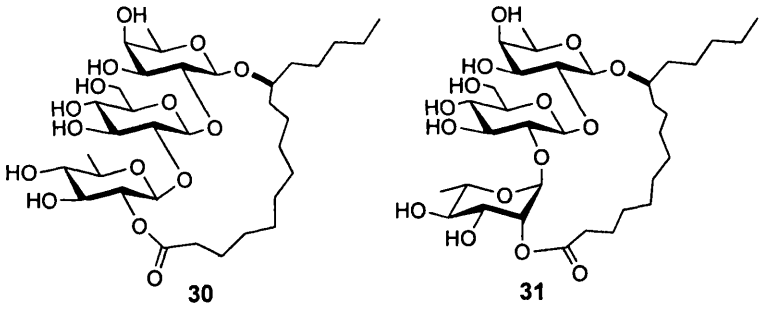


Figure 1.8: Tricolorins F (**30**) and G (**31**)

Tricolorin A was found to exhibit significant cytotoxic activity (ED_{50} 2.2 *μ*g/ml) against murine lymphocytic leukaemia and human breast cancer cells. It was also shown to antagonise phorbol ester binding with protein kinase C (PKC). Since phorbol esters can act as tumour promoters through activation of PKC, antagonists of phorbol binding activity may be considered as potential anti-tumour agents.[15,22]

Tricolorins A-E also exhibited antimicrobial activity against four *Staphylococcus aureus* strains with MIC values of 4-32 *μ*g/mL[20] and anti-mycobacterial activity against *Mycobacterium tuberculosis* with MIC values of 16-32 *μ*g/mL.[25] Tricolorins F and J on the other hand did not show any antimicrobial activity at the concentrations tested.[20]

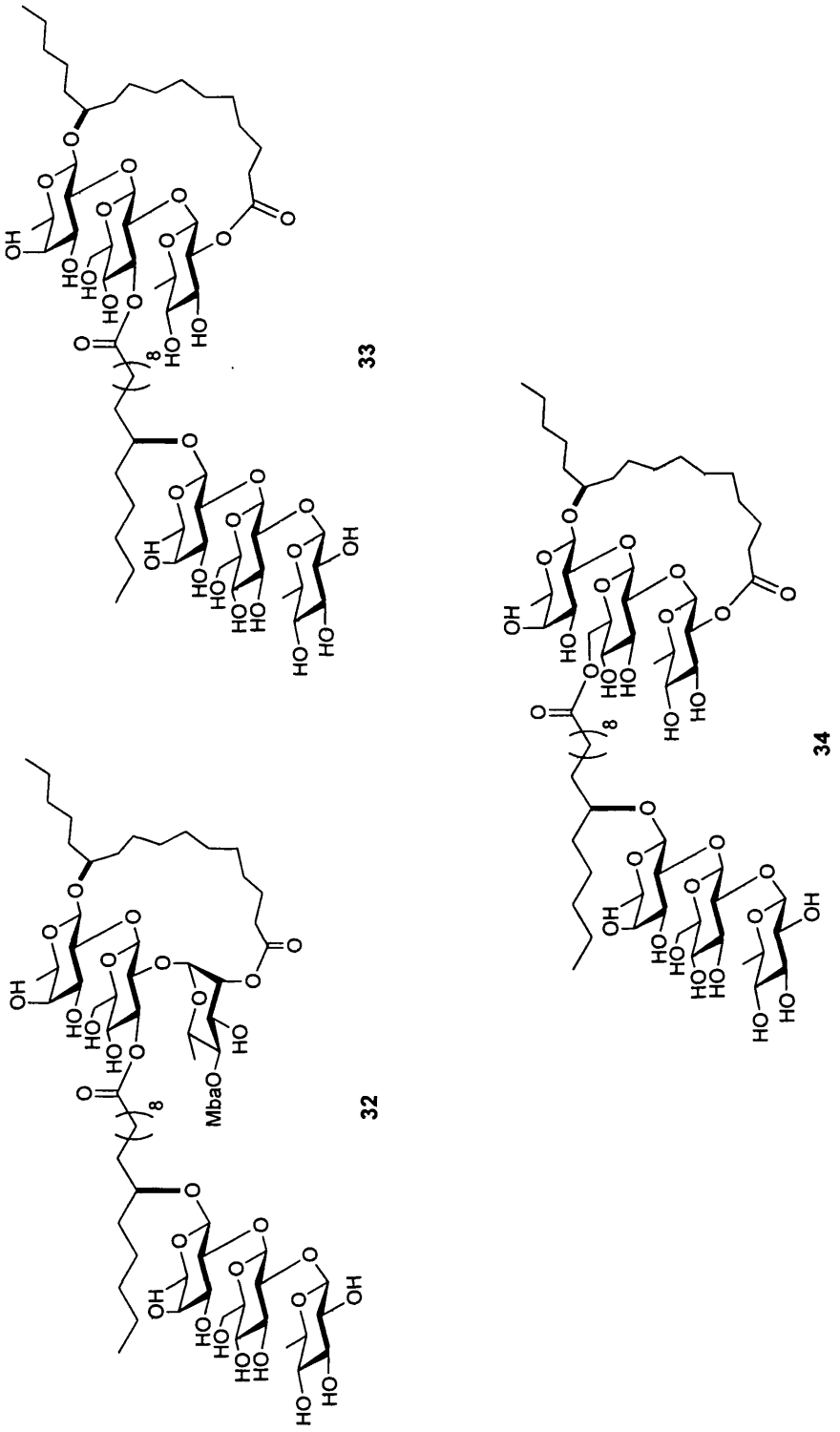


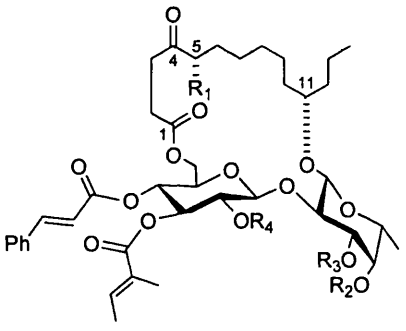
Figure 1.9: Tricolorins H-J (**32-34**)

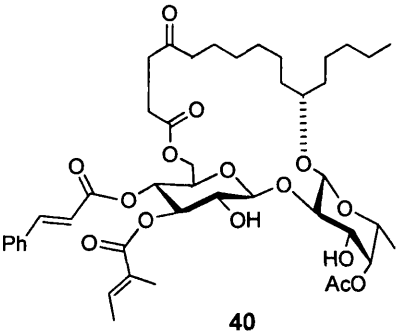
1.5. Ipomoeassins

Bristol Myers Squibb Pharmaceutical Research Institute (BMS) obtained a compound (BMS-247181) from *Ipomoea squamosa*, which was identified as a glycoresin and showed potent activity against several lung cancer cell lines. Research was discontinued, however, since preliminary *in vivo* experiments indicated that it had a very narrow therapeutic index.[16]

Cao and co-workers decided to re-investigate the constituents of *Ipomoea squamosa* as they might contain further, more potent glycoresins. They analysed several extracts by LC-MS, including one (E940631) that had been extracted from leaves of *Ipomoea squamosa* collected in the Suriname rainforest and was a re-collection of the plant that had yielded BMS-247181. This extract was chosen for further investigation since its chromatogram was similar to BMS-247181 and it had an IC_{50} value of 8.0 μg/mL against human ovarian cancer cell lines.[16]

The crude extract of E940631 was separated by flash chromatography into five fractions. The third fraction was the most active and was further purified by HPLC to yield ipomoeassins A-E (**35-39**)[16] (Figure 1.10). Further analysis of this fraction yielded another compound, ipomoeassin F (**40**).[26] Extensive spectroscopic studies identified the ipomoeassins as macrocyclic glycoresins containing D-glucose and D-fucose.





40

35: $R_1 = R_3 = R_4 = H$, $R_2 = Ac$
36: $R_1 = R_2 = R_3 = R_4 = H$
37: $R_1 = OH$, $R_2 = Ac$, $R_3 = R_4 = H$
38: $R_1 = OAc$, $R_2 = Ac$, $R_3 = R_4 = H$
39: $R_1 = OAc$, $R_2 = R_3 = R_4 = H$
41: $R_1 = H$, $R_2 = R_3 = R_4 = Ac$
42: $R_1 = OAc$, $R_2 = R_3 = R_4 = Ac$

Figure 1.10: Ipomoeassins A-F (**35-40**)

Ipomoeassin A (**35**) was identified as β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside glycosidically linked to the hydroxyl of (*S*)-11-hydroxy-4-

oxotetradecanoic acid and cyclised via a macrolactone with the glucose primary hydroxyl. It was also found to contain the esters of *trans*-cinnamic, tiglic and acetic acids, at the C-4 and C-3 hydroxyls of glucose and the C-4 hydroxyl of fucose, respectively.[16]

Ipomoeassin B (**36**) is almost identical to ipomoeassin A. Their only difference is the presence of a free hydroxyl at fucose C-4 in place of the acetate ester in **35**. This structure was also confirmed by acetylation of both **35** and **36** to yield the same acetylated derivative **41**.

Ipomoeassin C (**37**) is also closely related to **35** but contains an additional hydroxyl group at C-5 of the long fatty acid side-chain. The absolute stereochemistry of this carbon was determined as *S*. In ipomoeassin D (**38**) this hydroxyl group at C-5 is acetylated; ipomoeassin E (**39**) has the same structure as **38** except that the C-4 hydroxyl of fucose is not acetylated. Acetylation of **37**, **38** and **39** resulted in the same acetylated derivative **42**.[16]

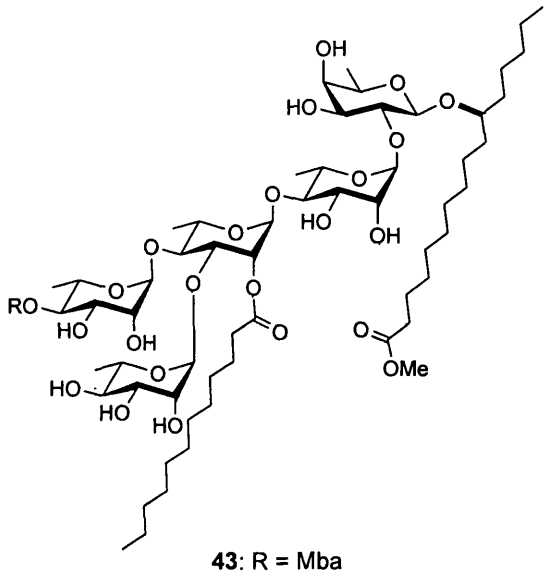
Ipomoeassin F (**40**) was found to contain two additional methyl groups compared to ipomoeassin A and it was determined that it incorporates an (*S*)-11-hydroxy-4-oxohexadecanoic acid residue rather than the C14 hydroxy-acid residue that is present in all the other ipomoeassins.[26]

All ipomoeassins were found to exhibit potent cytotoxic activity against human ovarian cancer cell lines, with IC_{50} values of 0.035 and 0.036 μM for ipomoeassins D (**38**) and F (**40**), respectively and 0.4-3.3 μM for ipomoeassins A-C (**35-37**) and E (**39**). The acetylated derivatives **41** and **42** were significantly less active with IC_{50} values of 15.8 and 19.1 μM, respectively.[16,26] The observation that slight structural modifications can result in such significant differences in cytotoxicity makes these compounds interesting targets for further investigation.

1.6. Pescapreins

Ipomoea pes-caprae is another plant that has been used in folk medicine for the treatment of several conditions such as wounds and skin infections, arthritis, rheumatism and kidney infections. A total of eleven new glycoresins were isolated from *Ipomoea pes-caprae*, namely pescaprosides A (**43**) and B (**44**) and pescapreins I-IX. All compounds are pentasaccharides of jalapinolic acid with the exception of pescapreins V and VI, which are tetrasaccharides. Interestingly, pescaprosides A (**43**) and B (**44**) were found to be non-macrocyclic glycoresins

and are rare examples of naturally-occurring acylated glycosidic acids in the Convolvulaceae family.[27]

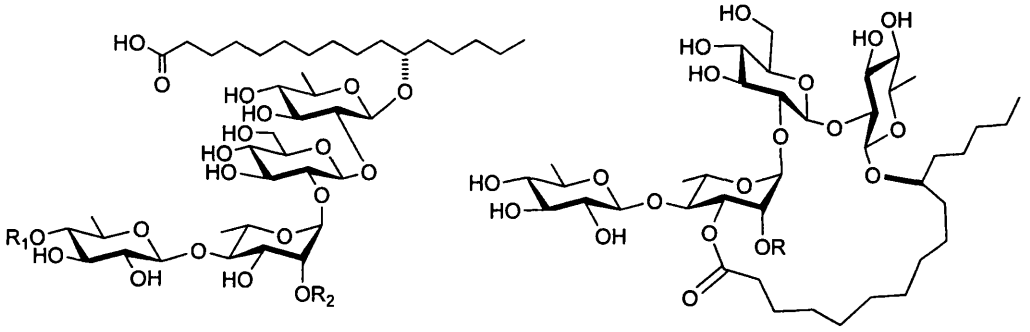


43: R = Mba
44: R = H

Figure 1.11: Pescaprosides A (**43**) and B (**44**)

1.7. Tyrianthins

Recently eleven new partially acylated tetrasaccharides of (*S*)-11-hydroxyhexadecanoic acid, i.e. tyrianthins 1-9[28] and tyrianthinic acids I (**45**) and II (**46**)[29] (Figure 1.12), were isolated from *Ipomoea tyrianthina*. Tyrianthins 1-9, which are closely related to the orizabins, are macrocyclic glycoresins, while tyrianthinic acids I and II are other examples of naturally acylated non-macrocyclic glycosidic acids.



45: R_1 = Nla, R_2 = Mba
46: R_1 = Tga, R_2 = Mba

47: R = Tga
48: R = Propanoyl

Figure 1.12: Tyrianthinic acids I (**45**) and II (**46**) and tyrianthins 8 (**47**) and 9 (**48**)

Tyrianthins 8 (**47**) and 9 (**48**) and tyrianthinic acids I (**45**) and II (**46**) (Figure 1.12) were evaluated for cytotoxic and anti-mycobacterial activity. These compounds showed moderate activity against *Mycobacterium tuberculosis* with MIC values of 25 μg/mL. The cytotoxicity of compounds **45-48** was evaluated using four cancer cell lines, i.e. nasopharyngeal carcinoma (KB), colon carcinoma (HCT-15), cervical carcinoma (UISO-SQC-1) and ovarian carcinoma (OVCAR-5) cells. All four compounds showed significant cytotoxic activity against nasopharyngeal carcinoma with ED_{50} values of 2.6, 2.8, 2.2 and 2.5 μg/mL for compounds **45-48**, respectively, but were inactive against the other cancer cell lines (ED_{50} > 5 μg/mL).[28,29]

1.8. Summary

The diverse structures and biological activity of resin glycosides, as detailed above, have made them attractive targets for synthetic chemistry and for further biological investigation. The ipomoeassins, with their disaccharide structure and potent cytotoxic activity, are the synthetic targets of the first part of this thesis. Chapter 3 details previous efforts towards the synthesis of these natural products, following an introduction to carbohydrate chemistry in Chapter 2.

2. CARBOHYDRATE CHEMISTRY

2.1. Introduction

Carbohydrates are complex molecules that possess several stereocentres and a large number of functional groups, mainly hydroxyl groups but also amino and carboxylic acid groups. Consequently, carbohydrate chemistry and oligosaccharide synthesis has to deal with two important challenges:

- regioselective protecting group strategies
- regio- and stereoselective formation of glycosidic linkages

Despite the development of a large number of protecting groups and glycosylation strategies and agents, carbohydrate synthesis still often remains a challenging task.[30,31]

2.2. Protecting Groups

Protecting groups are important components in organic synthesis and particularly in carbohydrate chemistry, due to the presence of a large number of chemically similar hydroxyl groups. When designing an oligosaccharide synthesis, the protecting group strategy has to be chosen carefully and several factors have to be taken into consideration. The ideal protecting groups should be easy to introduce, completely stable to the desired reaction conditions and easy to remove selectively under conditions that do not affect other protecting groups.

Carbohydrate hydroxyls differ in reactivity depending on whether they are anomeric, primary or secondary and this difference in reactivity can sometimes be utilised in order to achieve a desired protection pattern. Protecting groups, however, can increase or decrease the reactivity of neighbouring hydroxyl groups and lead to unexpected outcomes that may require alternative strategies. Protecting groups also influence the stereochemical outcome of glycosylation reactions and can be chosen as neighbouring participating or non-participating groups.

Although a large number of protecting groups have been developed, in practice the majority of carbohydrate syntheses are built around just a few basic and well-proven groups. These can be categorised according to their chemical nature, namely esters, ethers and acetals.[30,32,33]

2.2.1. Esters

Esters are widely used as protecting groups in carbohydrate chemistry. They are easily introduced and are stable to acidic and mildly basic reaction conditions. Standard conditions for their introduction are the appropriate acyl chloride or anhydride in pyridine. Sometimes, if acylation is slow, a catalytic amount of 4-dimethylaminopyridine (DMAP) can be added to accelerate the reaction.[34,35]

Esters are quite acid-stable but are readily cleaved under appropriate basic conditions. Besides saponification, standard conditions for their removal are catalytic amounts of sodium methoxide in methanol (Zemplen procedure) or ammonia in methanol.[36,37]

Some of the most common ester protecting groups used in carbohydrate synthesis are acetates, benzoates, chloroacetates, levulinates and pivaloates.

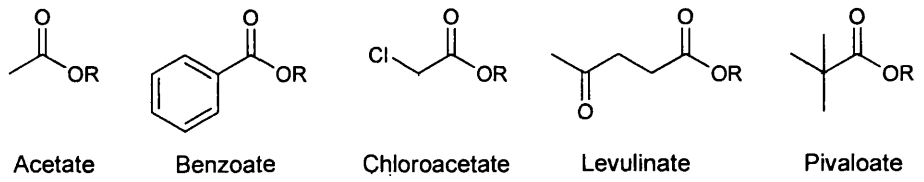
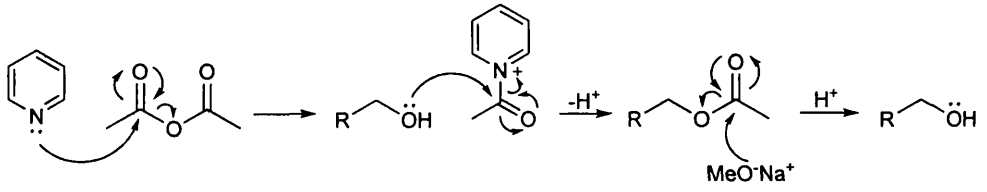


Figure 2.1: Common ester protecting groups

Acetate esters (Ac)

Acetate esters are the most common ester protecting groups in carbohydrate synthesis. They can be introduced and removed under mild conditions commonly in quantitative yields. Often the per-*O*-acetylated sugar is formed, which can be further converted into glycosyl donors such as glycosyl bromides and thio-glycosides or act itself as glycosyl donor for the preparation of simple glycosides (Scheme 2.2).

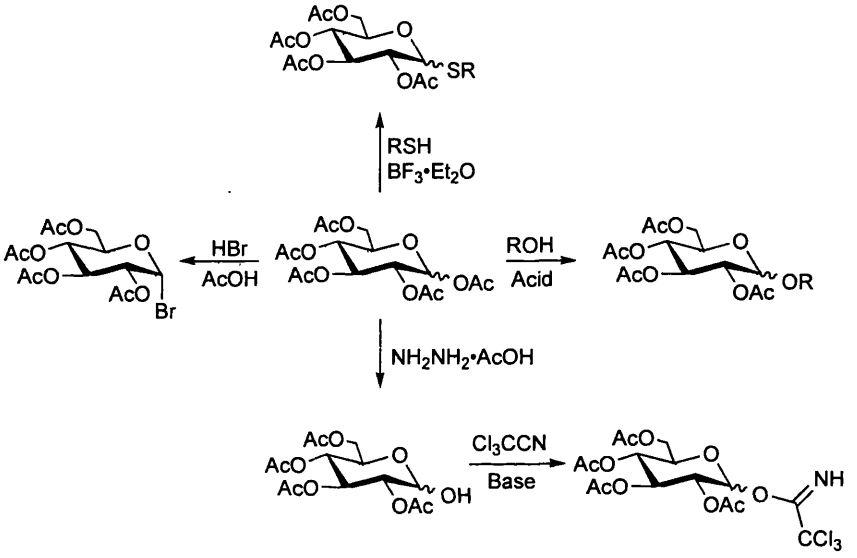


Scheme 2.1: Proposed mechanism for acetylation and deacetylation of alcohols

The anomeric hydroxyl group is more reactive and therefore selective deacetylation at the anomeric position can be achieved under specific conditions such as hydrazine acetate ($NH_2NH_2 \cdot AcOH$)[38] or benzylamine ($BnNH_2$).[39] The

resulting sugars are important intermediates especially for the formation of glycosyl trichloroacetimidates, one of the most widely used glycosyl donors.

One major drawback of acetates is that they tend to migrate under both basic and acidic conditions, preferentially from an axial to an equatorial position in *cis*-hydroxyls and from a secondary (usually O-4) to the primary O-6 hydroxyl.[40]



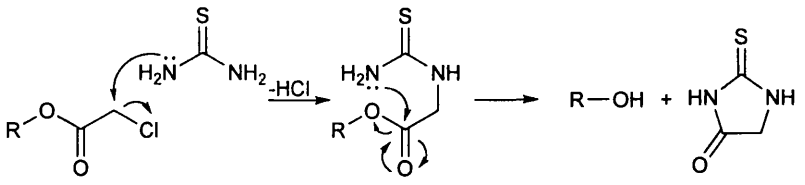
Scheme 2.2: Reactions of per-*O*-acetylated sugars

Benzoate esters (Bz)

Benzoate esters (Figure 2.1) are usually introduced by reaction of the desired alcohol with benzoyl chloride or anhydride in the presence of pyridine. Benzoate esters are more robust protecting groups and thus less prone to migration than acetates. Benzoate hydrolysis requires a longer reaction time and therefore acetates can be removed selectively in their presence, e.g. by treatment with magnesium methoxide.[41,42]

Chloroacetate esters

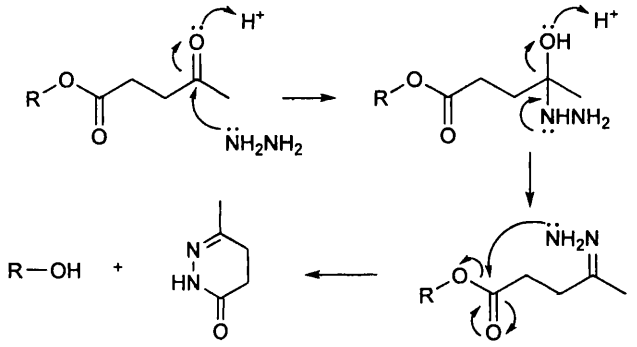
Chloroacetate esters have often been employed orthogonally to acetates and benzoates since they can be cleaved under very mild conditions, such as treatment with thiourea or hydrazine dithiocarbonate, that leave other acyl groups intact.[43-45] Dichloro- and trichloroacetates hydrolyse even faster but have only been used occasionally because they are too labile to survive even simple synthetic manipulations.



Scheme 2.3: Proposed mechanism for the chloroacetate deprotection of alcohols

Levulinate esters (Lev)

The levulinate (4-oxopentanoate) ester is another group that can be used orthogonally to acetates and benzoates. They are usually introduced by reaction of the alcohol with levulinic anhydride in the presence of pyridine and removed by hydrazine acetate under conditions that leave even anomeric acetates intact (Scheme 2.4). A further advantage is that they are less prone to migration than acetates or benzoates.[46,47]



Scheme 2.4: Proposed mechanism for the levulinate deprotection of alcohols

Pivaloate esters (Piv)

Pivaloate esters (Figure 2.1) are useful protecting groups in carbohydrate chemistry since they can be introduced selectively on primary alcohols due to their steric bulk.[48] Sometimes however, they show low reactivity towards standard conditions that deprotect esters and harsher conditions such as the use of diisobutylaluminium hydride (DIBAL) reduction are needed for their successful removal.[49,50]

2.2.2. Alkyl Ethers

Alkyl ethers are reliable protecting groups since they are stable to a wide range of conditions and can be removed under rather specific conditions. In fact, methyl ethers are so stable that they have found limited application as protecting groups in carbohydrate chemistry. More commonly used ethers are benzyl, *p*-

methoxybenzyl, allyl and triphenylmethyl (trityl) ethers. They are usually introduced under strongly basic conditions e.g. the alkyl bromide and sodium hydride (Williamson ether synthesis[51]), with the exception of trityl ethers where milder bases can be employed e.g. pyridine.[52] Alternatively, the alkyl trichloroacetimidate can be used with an acid catalyst allowing their introduction in a manner compatible with the presence of acetates or other base labile groups.[53]

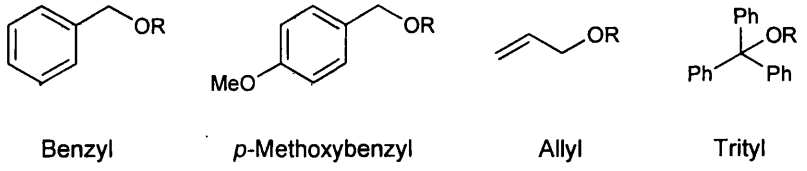
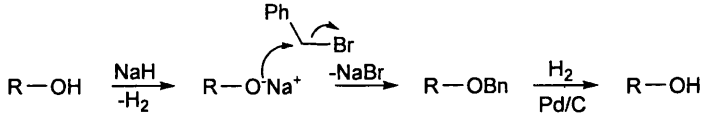


Figure 2.2: Common ether protecting groups

Benzyl ethers (Bn)

Benzyl ethers are robust protecting groups that can withstand a great variety of reaction conditions. Catalytic hydrogenation in the presence of palladium on carbon or palladium hydroxide is the most common method for their removal (Scheme 2.5) and yields are often quantitative. Sometimes however, problems might be encountered especially in the presence of amines or thiols that can "poison" the catalyst, i.e. they are adsorbed on the catalyst, thus blocking its active sites and inhibiting deprotection.[54] Transfer hydrogenation, which is less susceptible to poisoning, is an alternative, where formic acid,[55] ammonium formate[56] or 1,4-cyclohexadiene[57] can be used as the source of hydrogen in the presence of palladium on carbon.



Scheme 2.5: Benzyl protection and deprotection of alcohols

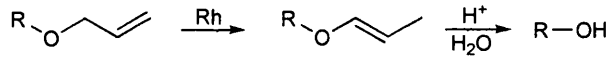
p-Methoxybenzyl ethers (PMB)

p-Methoxybenzyl ethers have found increasing application over recent years. They are more acid-labile than benzyl ethers and can be cleaved under relatively mild acidic conditions, in addition to the same conditions used for benzyl ether removal.[58] Their major advantage however, is that they can be cleaved under mild oxidative conditions with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)[59] or ceric ammonium nitrate (CAN),[60] methods that are compatible with a wide range of

other protecting groups. The use of PMB ethers is thus an important alternative in orthogonal protection strategies.

Allyl ethers (All)

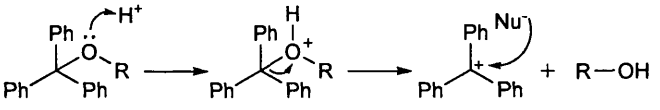
The allyl protecting group was originally developed for the protection of hydroxyl groups in carbohydrates.[61] It is a robust protecting group, compatible with a wide range of reaction conditions used in carbohydrate chemistry. Deprotection of allyl ethers takes place in two stages. Initially, rearrangement of the double bond takes place using strong base or metal-complex catalysts. The resulting vinyl ether can then be hydrolysed by a number of methods and under acidic, basic or neutral conditions[62,63] (Scheme 2.6).



Scheme 2.6: Two stage allyl deprotection of alcohols

Trityl ethers (Tr)

Trityl (triphenylmethyl) ethers have been widely used for the selective protection of primary alcohols in the presence of secondary alcohols. Secondary alcohols react more slowly and under more forcing conditions due to the steric bulk of the trityl group.[52] Trityl ethers are stable to base and nucleophiles but are readily cleaved under very mild protic or Lewis acidic conditions owing to the stability of the triphenylmethyl carbocation (Scheme 2.7). Other acid sensitive protecting groups remain intact under these conditions.[64] Catalytic hydrogenation can be unreliable and is usually avoided. Trityl ethers are not stable enough to withstand standard glycosylation conditions and their importance has diminished in recent years by the introduction of various silyl ethers.[65]



Scheme 2.7: Proposed mechanism for trityl deprotection of alcohols

2.2.3. Silyl Ethers

Silyl ethers have become some of the most widely used protecting groups in organic synthesis. They comprise a large family of compounds providing a wide spectrum of chemical stability and steric demand (Figure 2.3). The most common method for their introduction is treatment of the alcohol with the appropriate

chlorosilane in the presence of a base, usually imidazole, in dimethylformamide (DMF).[66] Alternatively, the silyl triflates in the presence of base can be used for sterically hindered hydroxyls.[67]

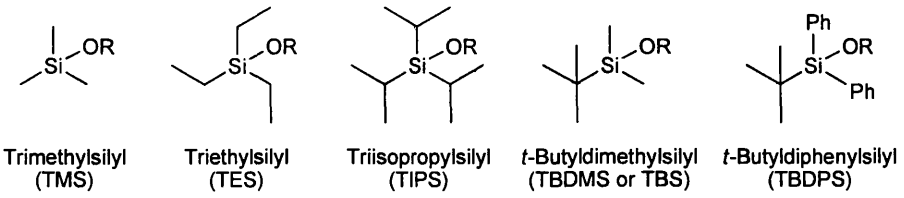
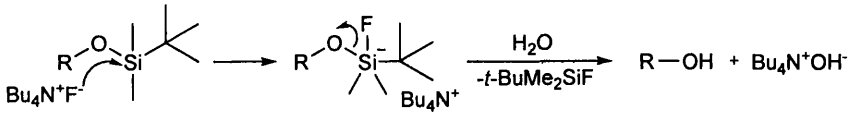


Figure 2.3: Common silyl protecting groups

Silyl ethers can be cleaved under both acidic and basic conditions. In general, stability towards acidic and basic hydrolysis increases with bulkier substituents on silicon; electronic effects, however, also play an important role. Thus, replacement of a methyl group with the phenyl group on silicon decreases sensitivity to acidic hydrolysis but has the opposite effect under basic conditions. Stability towards acid for some of the more common silyl ethers increases in the following order: TMS (1) < TES (64) < TBDMS (20,000) < TIPS (700,000) < TBDPS (5,000,000) while stability towards base increases as follows: TMS (1) < TES (10-100) < TBDPS ~ TBDPS (20,000) < TIPS (100,000).[32,33,68] Silicon has a high affinity for fluorine and as a result the most widely applied method for deprotection of silyl ethers is by nucleophilic attack by fluoride ions, often from tetra-*n*-butylammonium fluoride (TBAF)[66] (Scheme 2.8). Complexes of HF with various amines can be used as an alternative.[69]



Scheme 2.8: Proposed mechanism for silyl deprotection of alcohols with TBAF

Trimethylsilyl ethers (TMS)

TMS ethers have often been used in the past for the poly-protection of carbohydrates. The resulting per-*O*-silylated compounds are more volatile and are more suitable for investigation by gas-chromatography and mass spectrometry. TMS ethers however, are too sensitive to acidic and basic hydrolysis and even to aqueous conditions and are readily cleaved on silica gel; therefore their

application as protecting groups in carbohydrate chemistry has been rather limited. This led to the introduction of analogues having more hindered silicon centres.[70]

Triethylsilyl ethers (TES)

TES ethers are sufficiently stable to withstand column chromatography and various chemical transformations such as hydrogenation, oxidation and reduction. On the other hand, they can be selectively cleaved under relatively mild acidic conditions and are often used in combination with other more stable silyl protecting groups such as TBDPS and TIPS.[50]

tert-Butyldimethylsilyl ethers (TBDMS or TBS)

Since its discovery by Corey[66] in 1972, the TBS group has become one of the most useful protecting groups in carbohydrate chemistry. It is easily introduced, fairly stable to a wide range of organic reaction conditions and can be removed under conditions that do not affect other protecting groups.

One major problem often encountered with TBDMS ethers is their tendency to migrate to neighbouring free hydroxyl groups. Migration is favoured under basic conditions and in protic solvents but can also occur under acidic conditions.[71]

tert-Butyldiphenylsilyl ethers (TBDPS)

The large steric bulk of the TBDPS group enables the selective protection of primary hydroxyls in the presence of secondary ones. This, and the fact that it is fairly stable to acid hydrolysis, has made it a convenient alternative to the trityl protecting group. The TBDPS group is less prone to migration than the TBDMS group under acidic conditions, but may migrate under basic conditions.[72]

Triisopropylsilyl ethers (TIPS)

The bulkiness of the TIPS group makes it more stable to basic hydrolysis than both the TBDMS and TBDPS groups. It can withstand the cleavage conditions for a methyl ester and is inert towards powerful bases such as *t*-butyllithium. TBDPS however, is more stable to acidic hydrolysis. As with TBDPS, the TIPS ether can be easily introduced with high selectivity onto primary over secondary hydroxyls.[68]

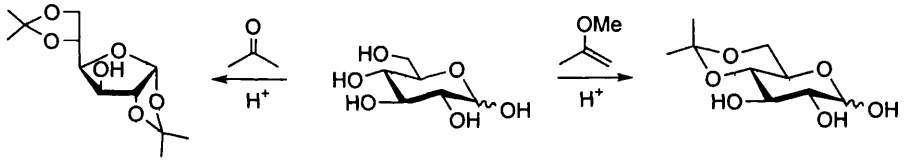
2.2.4. Diol Protecting Groups

Since carbohydrates possess several hydroxyl groups, the need to protect diols simultaneously arises. Cyclic acetals proved the most suitable compounds to serve this purpose and are present in almost every carbohydrate synthesis. Of these, isopropylidene and benzylidene acetals are by far the most widely used protecting groups.

Isopropylidene ketals

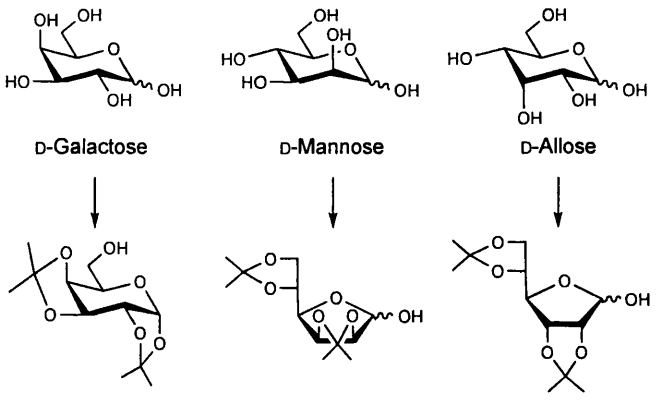
The isopropylidene ketal was the first one to be synthesised by Fischer in 1895,[73] by reaction of various sugars with acetone in the presence of acid. This general method remains one of the most important for the simultaneous protection of diols.

As an alternative to the use of acetone, isopropylidene ketals can also be formed by treatment of a diol with 2,2-dimethoxypropane via a transacetalisation reaction, in the presence of an acid catalyst such as *p*-toluenesulfonic acid (PTSA) or camphorsulfonic acid (CSA).[74] Additionally, 2-methoxypropene may be used with a milder acid e.g. pyridinium *p*-toluenesulfonate (PPTS)[75] (Scheme 2.9).



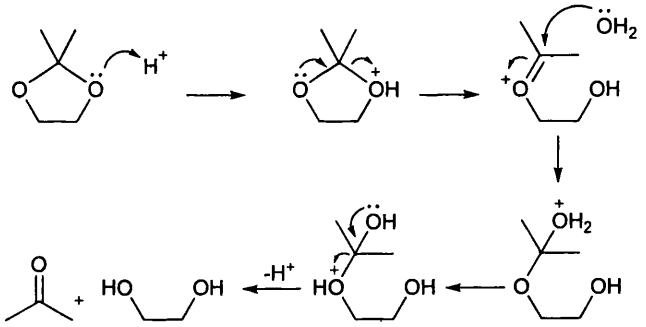
Scheme 2.9: Isopropylidenation of D-glucose. Reaction with acetone causes the pyranose isomer to rearrange in order to form the thermodynamically more stable 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, while reaction with 2-methoxypropene favours the kinetic product 4,6-*O*-isopropylidene-D-glucopyranose

Isopropylidene ketals show preference for the protection of *cis*-positioned 1,2-diols where the more stable five-membered 1,3-dioxolane ring is formed (Scheme 2.10). The six-membered 1,3-dioxane ring is less favoured because one methyl group adopts an axial orientation and thus destabilises the ring by diaxial interactions. However, when the more reactive 2-methoxypropene is used, the reaction proceeds under kinetic control and the six-membered 1,3-dioxane ring is formed. Thus, isopropylidenation of D-glucose results in two different products, depending on the conditions, as shown in Scheme 2.9.[76,77]



Scheme 2.10: Examples of isopropylidenation of various sugars with acetone

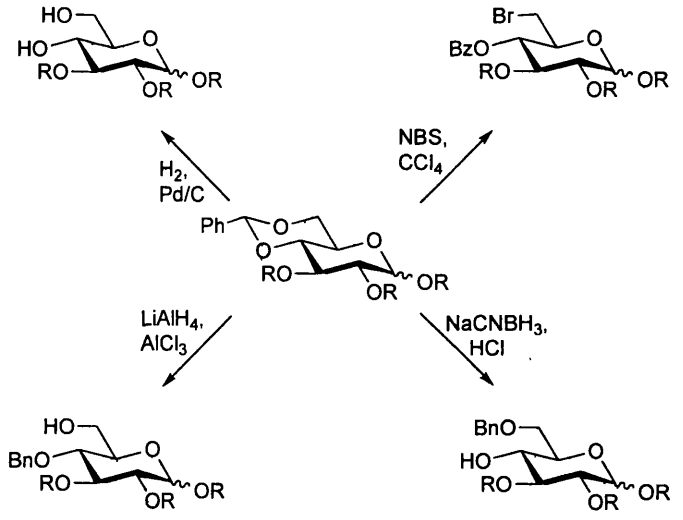
Isopropylidene ketals are stable to most reaction conditions except for protic and Lewis acids. Deprotection is usually effected under acid-catalysed hydrolysis (Scheme 2.11) but acid strength and reaction times vary widely as not all isopropylidene ketals are of equal stability.[78]



Scheme 2.11: Proposed mechanism for isopropylidene deprotection of diols

Benzylidene acetals

Benzylidene acetals are either introduced by reaction of the diol with benzaldehyde and a Lewis acid, usually zinc chloride, or with benzyldehyde dimethyl acetal in the presence of PTSA or CSA. Unlike isopropylidene ketals, they are more stable as the six-membered 1,3-dioxane rings, where the phenyl group can adopt an equatorial conformation. Hence they are most frequently used for the 4,6-*O*-protection of hexapyranoses.[79,80]



Scheme 2.12: Reactions of 4,6-*O*-benzylidene-glucopyranose

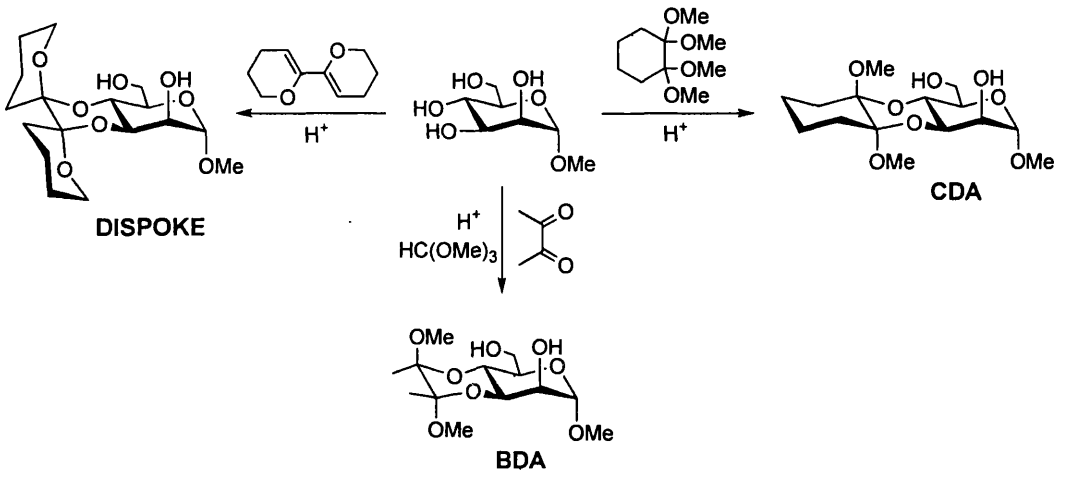
Benzylidene acetals are stable to bases, mild acids and mild oxidants. They can be removed under strongly acidic conditions or by catalytic hydrogenation in the presence of palladium or platinum. Their major advantage, however, is that they can be regioselectively ring opened to yield a benzyl ether and an alcohol, which can be further manipulated. In the case of 4,6-*O*-benzylidene pyranoses, treatment with aluminium chloride and lithium aluminium hydride results in 4-*O*-benzyl ethers while treatment with sodium cyanoborohydride in the presence of anhydrous hydrochloric acid (HCl) or triflic acid yields the 6-*O*-benzyl ether. Another useful transformation involves treatment of 4,6-*O*-benzylidene pyranoses with *N*-bromosuccinimide (NBS) to give 6-bromo-4-*O*-benzoyl derivatives (Scheme 2.12).[81]

1,2-Diacetals

Isopropylidene ketals and benzylidene acetals are the most commonly used diol protecting groups. However, they are not suitable for the protection of *trans*-diequatorial vicinal diols. This problem was solved with the development of 1,2-diacetals primarily by Ley *et al.*[82-84]

The dispiroacetal DISPOKE was the first 1,2-diacetal protecting group and is formed by reaction of the appropriate diol with 6,6′-bis(3,4-dihydro-2*H*-pyran) (bis-DHP) and an acid catalyst.[82,83] The synthetic utility of this protecting group, however, has been limited by various factors, such as high cost of the reagent, short shelf life, low yields and rather harsh conditions for its removal. These limitations led to the introduction of the simpler cyclohexane-1,2-diacetals (CDA)

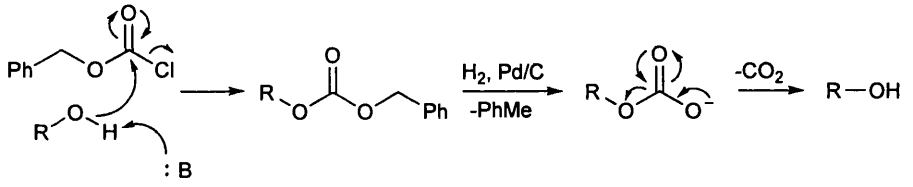
prepared from 1,1,2,2-tetramethoxycyclohexane under acidic conditions.[85] This protecting group offers the same selectivity as DISPOKE but at a lower cost. Finally, the best protecting group for diequatorial diol protection in terms of yields and selectivity proved to be butane-2,3-diacetal (BDA), formed from butane-2,3-dione[84,86,87] (Scheme 2.13).



Scheme 2.13: 1,2-Diacetal protecting groups

2.2.5. Carbonates

Carbonates provide another orthogonal set of protecting groups. Although the synthesis of pentacarbomethoxy- and pentacarboethoxy glucose was reported by Zemplén and László[88] as early as 1915, their use in carbohydrate chemistry has been rather limited. However, they present a useful alternative to ethers and esters since they can be cleaved under highly specific conditions (Scheme 2.14).



Scheme 2.14: Introduction and removal of the Cbz group

The benzyloxy carbonyl group (Cbz) is widely used for the protection of the amino group in amino sugars and peptides and has recently attracted attention as a hydroxyl protecting group in carbohydrate chemistry. Benzyl carbonates are more stable than esters towards basic hydrolysis due to the reduced electrophilicity of the carbonyl group caused by the two oxygens. Moreover, they have the advantage that they can be readily removed by catalytic hydrogenation without affecting other ester substituents. Their introduction is achieved under mild basic

conditions by reacting the desired alcohol with benzyl chloroformate in the presence of DMAP or *N,N,N′,N′*-tetramethylethylenediamine (TMEDA). Benzyl carbonates are therefore a useful alternative to benzyl ethers, which are introduced by much harsher conditions that are not compatible with other functional groups e.g. esters.[89-92]

2.2.6. Regioselective Protection

The protection methods discussed above, although very useful, usually result in the poly-protection of sugars. Since efficient regioselective glycosylations are difficult to achieve, regioselective protection of sugars to obtain derivatives with one hydroxyl free, is an important aspect of carbohydrate chemistry. Some examples of strategies concerning the regioselective protection of glucose will be discussed below.

Anomeric OH free

A free anomeric hydroxyl group is readily obtained by selective anomeric deprotection of per-*O*-acylated sugars with $NH_2NH_2 \cdot AcOH$ or $BnNH_2$ as discussed in section 2.1.1.

6-OH free

The 6-OH of glucose is the most reactive since it is the only primary hydroxyl group. It can therefore be selectively protected with bulky substituents such as trityl or TBDPS. Subsequent protection of the remaining hydroxyls and selective removal of trityl or TBDPS yields the 6-OH glucose. Alternately, regioselective opening of 4,6-*O*-benzylidene acetals of the fully-protected glucose with $AlCl_3/LiAlH_4$ results in the 4-OBn and 6-OH glucose (Section 2.1.4., Scheme 2.12).

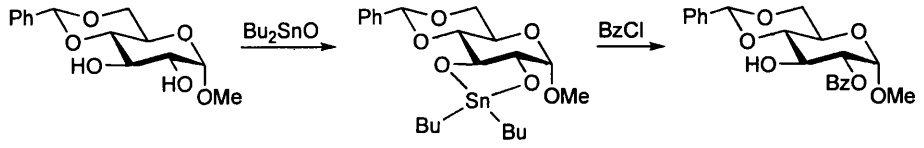
4-OH free

Protection of the free hydroxyls of 4,6-*O*-benzylidene glucopyranose and subsequent regioselective opening of the 4,6-*O*-benzylidene acetal with $NaCNBH_3/HCl$ yields the 6-OBn, 4-OH glucose (Section 2.1.4., Scheme 2.12).

3-OH free

Regioselective protection of glucose to obtain the free 2-OH or 3-OH are the most difficult cases, since both hydroxyls are equatorial and therefore of similar

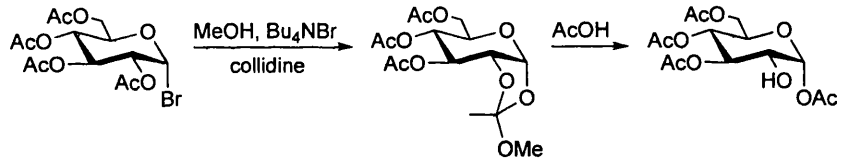
reactivity. Selective acylation and alkylation of the 2-OH of methyl α-D-glucopyranoside or methyl 4,6-*O*-benzylidene-α-D-glucopyranoside has been achieved by stannyl activation. Reaction of the sugar with dibutyltin oxide forms a dibutylstannylene ketal intermediate, which is then treated with acyl or alkyl halides to yield the respective esters or ethers, usually in high yields and good regioselectivity (Scheme 2.15).[93-95]



Scheme 2.15: Stannyl activated regioselective protection

2-OH free

Glucose derivatives with the 2-OH free can be obtained by selective opening of 1,2-orthoesters. 1,2-Orthoesters are formed by reaction of the corresponding 1-bromo-2-ester derivative (e.g. tetra-*O*-acetyl-α-D-glucopyranosyl bromide) with an alcohol in the presence of *sym*-collidine and tetra-*n*-butylammonium bromide (TBAB). Mild acidic hydrolysis of the orthoester results in the anomeric ester derivative exposing the 2-OH (Scheme 2.16).[96-98]



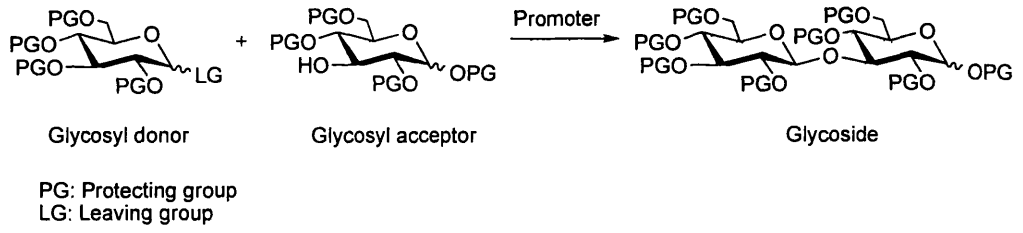
Scheme 2.16: Formation and regioselective opening of 1,2-orthoesters

2.3. Glycosylation Methods

Most carbohydrates encountered in Nature do not exist in a free form but are glycosidically linked to each other or to other compounds. Thus an essential part of carbohydrate chemistry deals with the assembly of oligo- and polysaccharides by linking the monomers with glycosidic bonds.

In Nature, glycosylation is achieved by enzymes, namely the glycosyltransferases. These enzymes are highly specific regarding their donor and acceptor substrates and form glycosides in a highly regio- and stereoselective manner. Unfortunately, chemical synthesis of glycosidic bonds is far more complicated. It involves activation of a sugar into a suitable glycosyl donor that bears a leaving group at

the anomeric position and subsequent reaction with a glycosyl acceptor, in the presence of a promoter (Scheme 2.17). The glycosyl donor and acceptor have to be appropriately protected in order to achieve regioselectivity.[30,31]



Scheme 2.17: Chemical synthesis of glycosides

2.3.1. Control of Stereoselectivity

The anomeric effect

In general equatorial substituents are, for steric reasons, more favoured than axial ones since the latter have a destabilising effect on the sugar ring. At the anomeric position, however, this rule is not followed completely due to the anomeric effect,[99] according to which, electronegative substituents prefer to occupy an axial rather than an equatorial position. Several explanations exist for the origin of the anomeric effect, the most accepted being (a) the dipole-dipole interaction and (b) the stereoelectronic effect.[99-101]

The non-bonding electron pairs on the endocyclic oxygen form a dipole and another arises from the polarised bond between the anomeric carbon and the heteroatom attached to it. Thus, the axial configuration, where the two dipoles partially neutralise each other, is favoured over the equatorial, where the dipoles are almost parallel and point to the same direction[100] (Figure 2.4).

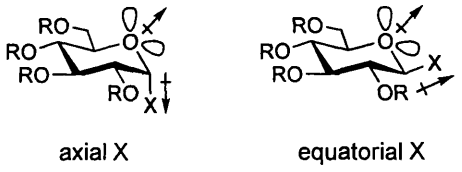


Figure 2.4: Dipole-dipole interaction

According to the stereoelectronic effect, the non-bonding pair of electrons on the oxygen is syn-periplanar to the unoccupied anti-bonding molecular orbital (σ^*) of the C1-X bond when X is axial. Consequently they can overlap and this interaction results in delocalisation of electron density, slight shortening of the

C1-O bond and lengthening of the C1-X bond. This interaction is not possible when the X substituent is equatorial (Figure 2.5).[99,101]

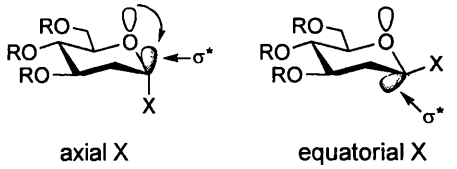
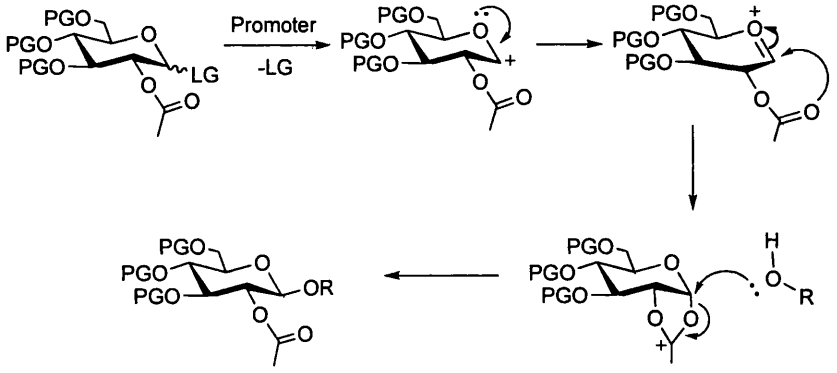


Figure 2.5: The stereoelectronic effect

The anomeric effect is influenced by several factors. It increases with increasing electronegativity of the anomeric substituent and decreases with increasing solvent polarity. Moreover, an axial substituent at C-2 enhances the anomeric effect, as in mannose, while an equatorial one (e.g. glucose) weakens it. The anomeric effect has important implications in carbohydrate chemistry and glycoside synthesis.[99,101]

Neighbouring group participation

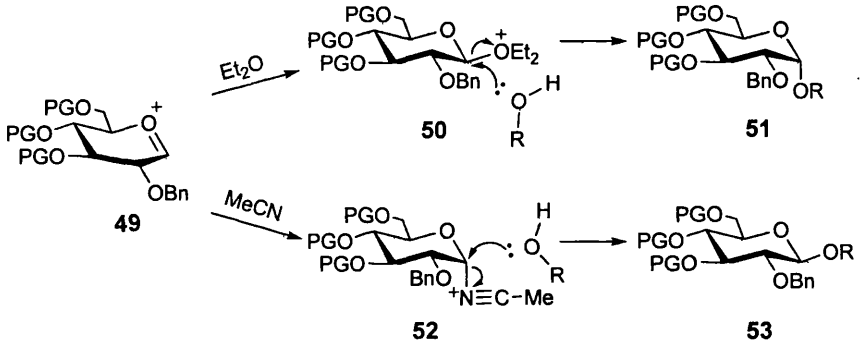
Stereoselectivity is one of the most challenging aspects in oligosaccharide synthesis. Glycosylations, in which the glycosyl donor bears an ester group at C-2 (e.g. acetate, benzoate or pivaloate), proceed via neighbouring group participation and the 1,2-*trans*-glycoside is usually formed exclusively (Scheme 2.18). If a non-participating group (e.g. benzyl ether) is present at C-2, glycosylation will result in the formation of both 1,2-*trans*- and 1,2-*cis*-glycosides. There is no general method for the exclusive synthesis of 1,2-*cis*-glycosides, but several potential solutions exist.[102]



Scheme 2.18: Synthesis of 1,2-*trans*-glycosides by neighbouring group participation

Solvent participation in glycoside synthesis

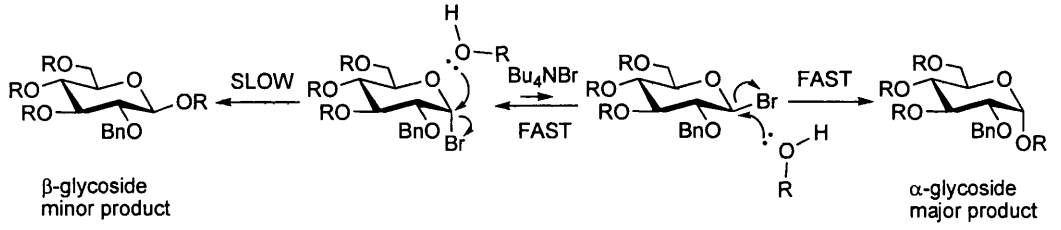
The choice of solvent can have an effect on the stereochemical outcome of the glycosylation reaction in the absence of neighbouring group participation. Ethers such as diethyl ether and THF favour 1,2-*cis*-glycoside formation while acetonitrile promotes 1,2-*trans*-glycoside formation. Diethyl ether forms an equatorial oxonium cation (**50**) from the oxocarbenium ion **49**, which subsequently reacts with the glycosyl acceptor to yield the axial disaccharide **51,** as shown in Scheme 2.19. Acetonitrile on the other hand, favours the formation of the axial nitrilium cation (**52**) and results in the equatorial glycoside **53** as the main product.[103-106]



Scheme 2.19: Solvent effects on glycosylation

In situ anomerisation

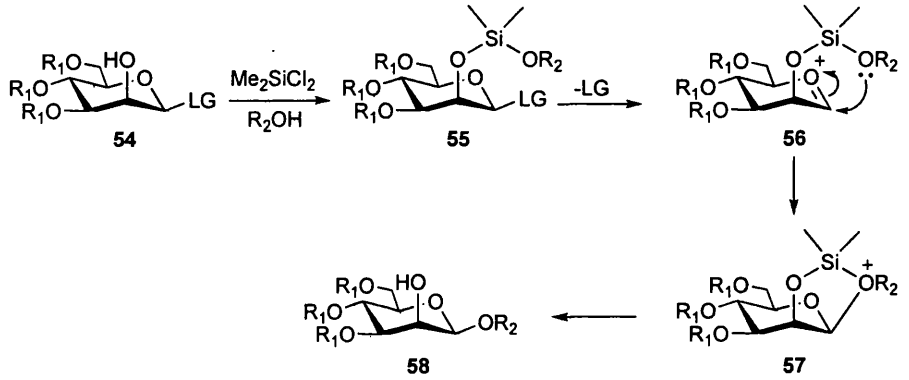
In situ anomerisation, introduced by Lemieux and co-workers, was the first successful method for 1,2-*cis*-glycoside synthesis. They found that addition of tetraalkylammonium bromide to readily-formed α-pyranosyl bromides results in equilibrium between α- and β-pyranosyl bromides. The highly reactive β-pyranosyl bromides will then rapidly react with glycosyl acceptors in a kinetically controlled S_N2 reaction to produce *cis*-glycosides (Scheme 2.20). It is important that anomerisation occurs faster than glycosylation. This method has been useful for the construction of α-galactosides and α-fucosides but is less effective for α-glucosides and β-mannosides.[107]



Scheme 2.20: *In situ* anomerisation

Intramolecular aglycon delivery (IAD)

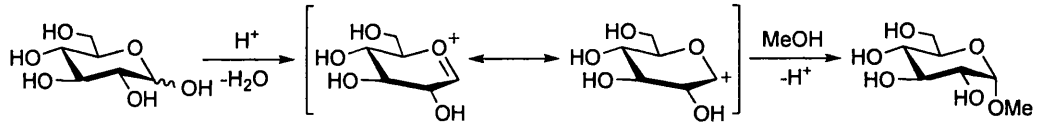
Intramolecular aglycon delivery (IAD) has been successfully applied for the synthesis of 1,2-*cis*-*β*-mannosides, one the most difficult linkages to form. In this method, the glycosyl acceptor is temporarily tied to the 2-hydroxyl of the glycosyl donor (**55**) via an acetal or silicon tether. Activation of the glycosyl donor results in stereocontrolled intramolecular delivery of the acceptor to give intermediates e.g. **57** and subsequent hydrolysis yields the *β*-mannosides e.g. **58**[108-110] (Scheme 2.21).



Scheme 2.21: Intramolecular aglycon delivery

2.3.2. Fischer Glycosylation

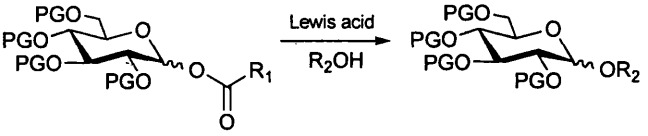
The Fischer glycosylation proceeds via direct conversion of the hemiacetal hydroxyl group to a glycoside and is one of the earliest methods for glycoside formation. This procedure involves the acid-catalysed reaction of a reducing sugar with an alcohol and is useful for the synthesis of simple glycosides such as methyl, benzyl and allyl glycosides. Fischer glycosylations yield a mixture of products; under kinetic control, furanosides can be obtained in good yields while axial pyranosides, which are favoured by the anomeric effect, are the thermodynamically more stable products[111] (Scheme 2.22).



Scheme 2.22: Fischer glycosylation of D-glucose with methanol

2.3.3. Glycosyl Esters

The ease of preparation of glycosyl esters makes them useful glycosyl donors, especially for the synthesis of simple glycosides. Glycosyl esters, however, are not as good leaving groups as e.g. glycosyl bromides or trichloroacetimidates and therefore harsher activation conditions are required. Glycosyl acetates are the most frequently used acyl glycosyl donors and their activation can be achieved by various Lewis acids, such as $BF_3{\cdot}Et_2O$,[112] $SnCl_4$[113] and $FeCl_3$[114] (Scheme 2.23).



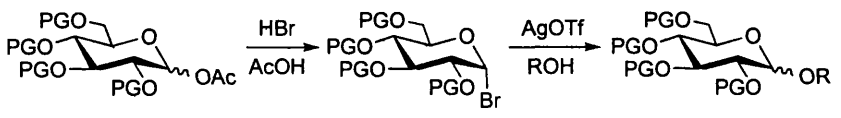
Scheme 2.23: Glycosylation with glycosyl esters

2.3.4. Glycosyl Halides

Koenigs-Knorr method

Glycosyl bromides and chlorides, introduced by Koenigs and Knorr in 1901,[115] are the oldest and still widely used glycosyl donors for oligosaccharide synthesis. They are easily formed by treatment of anomeric acetates with hydrogen bromide or chloride in acetic acid. Under these conditions, the thermodynamically favoured axial anomer is formed (due to the anomeric effect) in high yields.

Activation of glycosyl bromides and chlorides occurs in the presence of heavy metal salts. Silver oxide (Ag_2O) or carbonate (Ag_2CO_3) were originally used as promoters but have been largely replaced by soluble mercury salts (e.g. $HgBr_2$ and $Hg(CN)_2$) or the more efficient silver perchlorate ($AgClO_4$) and silver triflate (AgOTf) (Scheme 2.24). Several other activators have been reported besides heavy metal salts, including Lewis and protic acids. The stability and reactivity of glycosyl halides is influenced by the protecting groups on the sugar ring. Ester protecting groups decrease while ethers increase reactivity.[115-117]



Scheme 2.24: Formation and activation of glycosyl bromides

Glycosyl halides are not particularly stable and will not survive most synthetic manipulations. They are usually prepared and then used directly for glycosylation. This, and the fact that they require activation by toxic and expensive heavy metal salts, has somewhat limited their application.

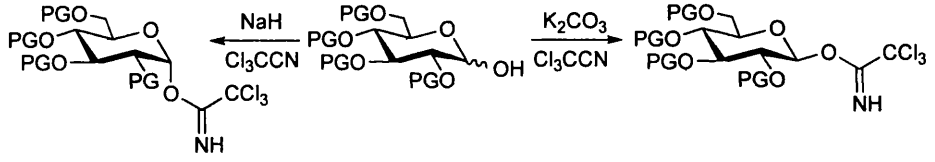
Glycosyl fluorides

Glycosyl fluorides were for a long time considered too unreactive to be used as glycosyl donors until Mukaiyama and co-workers[118] demonstrated that they could be activated with a mixture of silver perchlorate ($AgClO_4$) and tin(II) chloride ($SnCl_2$). Several other catalysts, including $BF_3{\cdot}Et_2O$ and TMSOTf, were also found to be effective.

Glycosyl fluorides offer certain advantages over other glycosyl halides. They are considerably more stable and can even undergo a limited number of synthetic manipulations. Glycosyl fluorides are usually formed from thioglycosides or the free anomeric hydroxyl group by treatment with diethylaminosulfur trifluoride (DAST).[119]

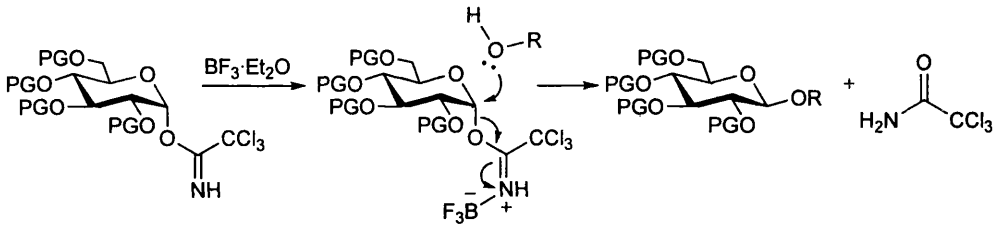
2.3.5. Trichloroacetimidate Method

Reaction of reducing sugars with trichloroacetonitrile in the presence of base gives anomeric trichloroacetimidates, converting the anomeric hydroxyl into a good leaving group. When a weak base such as potassium carbonate (K_2CO_3) is used the β-anomer is predominantly formed, while with a stronger base, e.g. sodium hydride (NaH), the α-anomer is the major product (Scheme 2.25).[120,121]



Scheme 2.25 Synthesis of glycosyl trichloroacetimidates

Activation of trichloroacetimidates occurs under acidic conditions. In the presence of a neighbouring participating group at C-2 the *trans*-glycoside is typically exclusively formed. With non-participating groups, treatment of the imidate with boron trifluoride etherate ($BF_3{\cdot}Et_2O$) at low temperature furnishes the β-glycoside in an S_N2 type reaction (Scheme 2.26). Stronger acids such as trimethylsilyl triflate (TMSOTf) favour the formation of the thermodynamically more stable α-glycoside.[122-124]



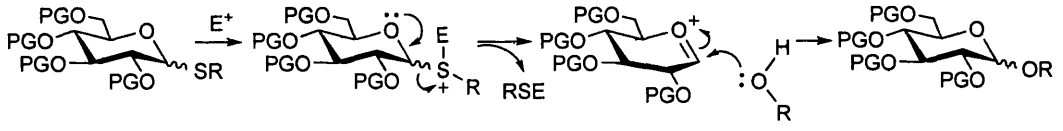
Scheme 2.26: Stereoselective glycosylation with trichloroacetimidates

Glycosyl trichloroacetimidates are more stable than glycosyl bromides and can be activated under mild conditions. The trichloroacetimidate method is therefore a powerful glycosylation method and has found wide application in the synthesis of complex oligosaccharides.

2.3.6. Thioglycosides

Thioglycosides are conveniently synthesised by nucleophilic substitution at the anomeric centre. The most common method involves treatment of the anomeric acetates with an alkyl or aryl thiol in the presence of a Lewis acid, usually $BF_3{\cdot}Et_2O$.[125] Other glycosyl donors such as glycosyl halides and trichloroacetimidates can also be used. An alternative method is reaction of a glycosyl bromide with thiourea to form a pseudothiouronium salt, which, upon hydrolysis, yields a thiosugar. This in turn can be alkylated to form the desired thioglycoside.[126]

The major advantage of thioglycosides over trichloroacetimidates and glycosyl bromides is that they are remarkably stable and can tolerate diverse synthetic manipulations. They are usually introduced early in a synthetic sequence where they function as anomeric protecting groups until they are activated for glycosylation under very specific reaction conditions.

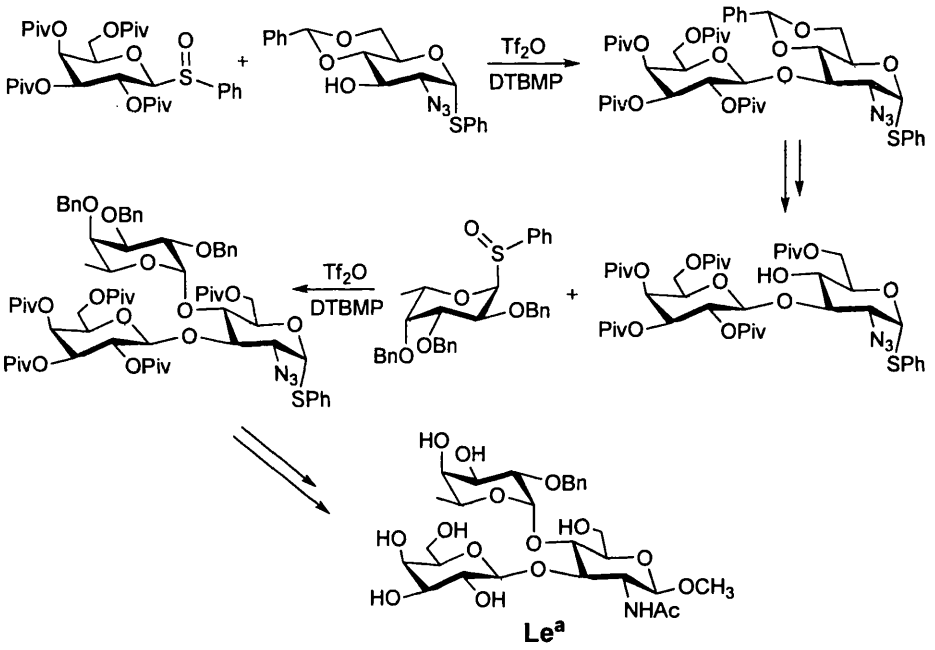


Scheme 2.27: Activation of thioglycosides by electrophilic reagents

Activation of thioglycosides is achieved by soft electrophilic reagents under mild conditions (Scheme 2.27). *N*-Iodosuccinimide (NIS) in the presence of a catalytic amount of triflic acid (TfOH)[127] is probably the most common promoter, while other reagents such as methyl triflate (MeOTf)[128] and dimethyl(methylthio)sulfonium triflate (DMTST)[129] are also frequently used. Thioglycosides can

also be converted to other glycosyl donors such as glycosyl fluorides and glycosyl sulfoxides.

Glycosyl sulfoxides are amongst the most reactive glycosyl donors and enable glycosylations with very unreactive glycosyl acceptors.[130] They are readily formed by oxidation of thioglycosides with a peracid such as *m*-chloroperbenzoic acid (mCPBA) and activated by triflic anhydride in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP). These activation conditions do not affect thioglycosides and enable orthogonal glycosylations.[130,131] This strategy has been successfully applied in the synthesis of the Lewis blood group antigen Le^a as shown in Scheme 2.28.[131]



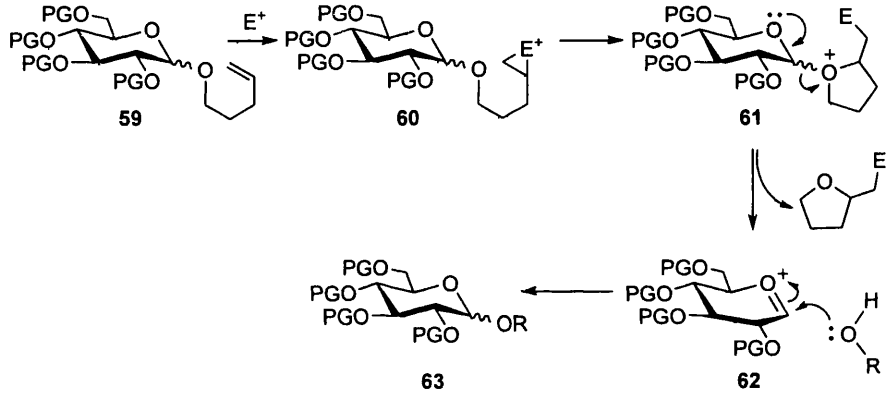
Scheme 2.28: Application of glycosyl sulfoxides in the synthesis of Le^a

2.3.7. Selenoglycosides and Telluroglycosides

Seleno- and telluroglycosides are closely related in behaviour to thioglycosides. They can be prepared in a similar manner to thioglycosides and are activated by the same reagents. Their major advantage, however, is that, being more reactive, they can be activated under very mild conditions (AgOTf, K_2CO_3) that do not affect thioglycosides, making them important tools in orthogonal glycoside synthesis. Moreover, telluroglycosides are even more reactive and can be activated selectively over selenoglycosides.[132,133]

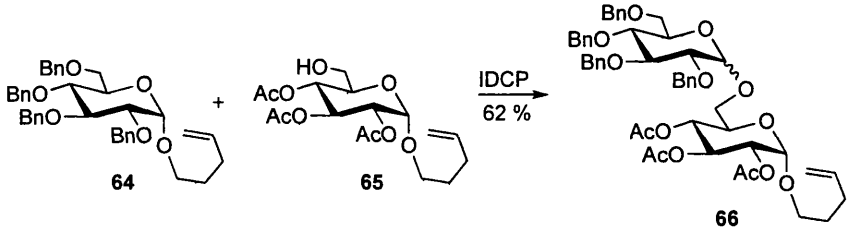
2.3.8. *n*-Pentenyl Glycosides

n-Pentenyl glycosides, which were introduced by Reid and co-workers in 1988,[134] can be readily synthesised by Fischer or Koenigs-Knorr glycosylations with 4-pentenol. Their easy installation, high stability to diverse synthetic manipulations and highly specific activation has made *n*-pentenyl glycosides popular glycosyl donors. Activation occurs by electrophilic addition of a halonium ion to the double bond (**60**), which results in rearrangement (**61**) and eventual release of the aglycon, thus providing the active oxonium species (**62**) (Scheme 2.29). The halonium ion is usually provided from *N*-bromosuccinimide (NBS) or NIS in the presence of a protic or Lewis acid, e.g. triflic acid or triethylsilyl triflate (TESOTf).[134]



Scheme 2.29: Glycosylation with *n*-pentenyl glycosides

During glycosylation studies with *n*-pentenyl glycosides it was observed that benzyl protected *n*-pentenyl glycosides were more reactive than the respective acetylated derivatives. This observation led to the introduction of the armed-disarmed glycosylation strategy, according to which an armed (alkylated) *n*-pentenyl glycoside (**64**) can be activated in the presence of a disarmed (acylated) *n*-pentenyl glycoside (**65**) and a single product (**66**) is obtained (Scheme 2.30). The armed-disarmed concept is a general phenomenon that can be performed with other glycosyl donors such as thioglycosides and glycosyl fluorides.[135,136]

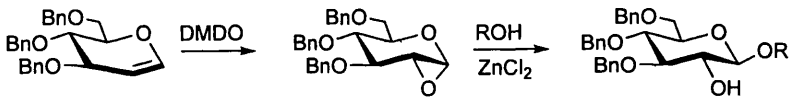


Scheme 2.30: The armed-disarmed glycosylation strategy

2.3.9. Glycals

Glycals have, over recent years, become another useful starting point for the stereoselective synthesis of glycosides. They are usually obtained by zinc-catalysed reductive elimination of per-*O*-acetylated glycosyl bromides to form the triacetyl glycals.[137]

Glycals react smoothly with, for example, 2,2-dimethyldioxirane (DMDO) to yield 1,2-anhydro sugars, usually in high stereoselectivity. The stereoselectivity of the epoxidation depends on the type of protecting groups and the steric hindrance of the substituents. Further reaction of the epoxide with a glycosyl acceptor in the presence of $ZnCl_2$ will form the desired glycoside[138] (Scheme 2.31). Alternatively, 1,2-anhydro sugars can be converted to other glycosyl donors such as thioglycosides, glycosyl fluorides and *n*-pentenyl glycosides.[139]



Scheme 2.31: Glycosylation with glycals as glycosyl donors

2.4. Summary

In this chapter, several methods and strategies for the regioselective protection and the regio- and stereoselective formation of glycosidic linkages have been introduced. The following chapter will describe the application of these methods to the synthesis of glycoresins.

3. SYNTHESIS OF GLYCORESINS

3.1. Introduction

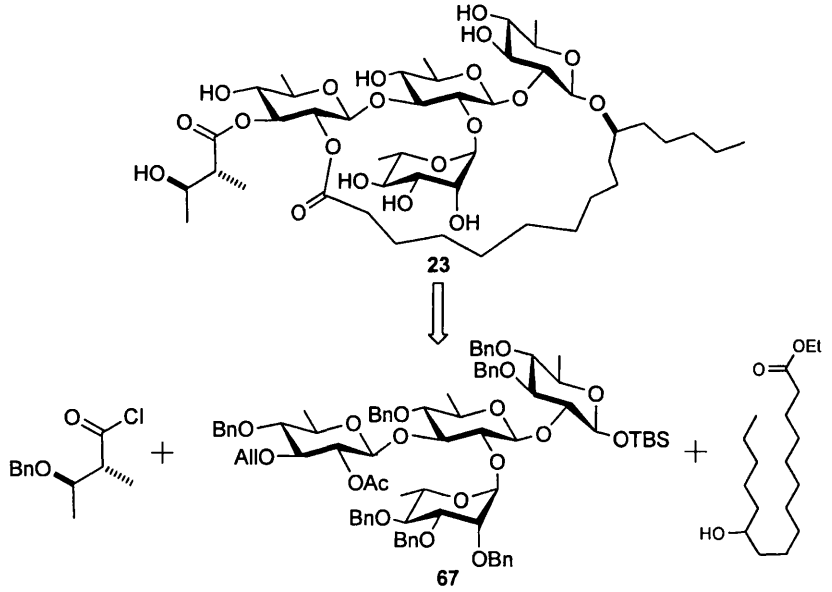
Glycoresins, usually isolated from plants belonging to the morning glory family, have been found to exhibit interesting biological activities including cytotoxicity and anti-bacterial activity. However, little is known about their mechanism of action.

The intricate structure of glycoresins and the lack of a systematic survey of their biological properties have made these compounds interesting synthetic targets. Despite advances in carbohydrate chemistry, their synthesis poses many difficulties. The major challenge lies in the stereoselective formation of the macrolactone ring, which can be achieved by several intramolecular esterification methods and by ring closing metathesis.

3.2. Calonyctins

3.2.1. Calonyctin A1

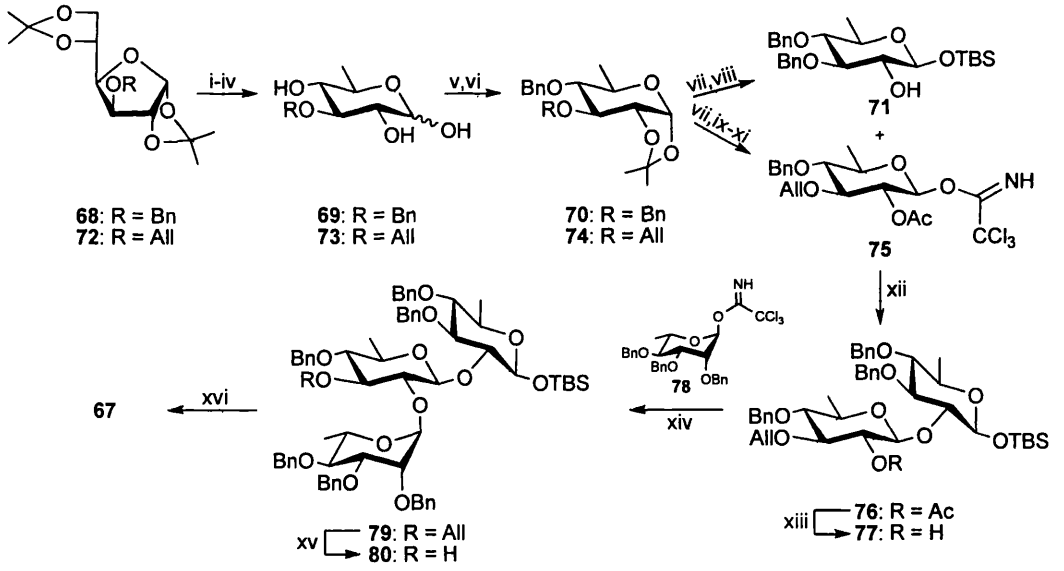
The first total synthesis of resin glycosides was reported by Schmidt and co-workers[140] in 1995, namely the synthesis of calonyctin A1 (**23**) (Scheme 3.1). Calonyctin A1 comprises a 22-membered macrocycle containing the tetrasaccharide D-Quiβ(1→3)[L-Rhaα(1→2)]-D-Quiβ(1→2)D-Qui and the ester side-chains nilic acid and 11-hydroxyhexadecanoic acid, whose configurations had not been assigned.[140]



Scheme 3.1: Retrosynthetic analysis of calonyctin A1

Their synthetic approach involved construction of the tetrasaccharide and subsequent incorporation of 11-hydroxyhexadecanoic acid in order to form the macrolactone (Scheme 3.1).[140,141]

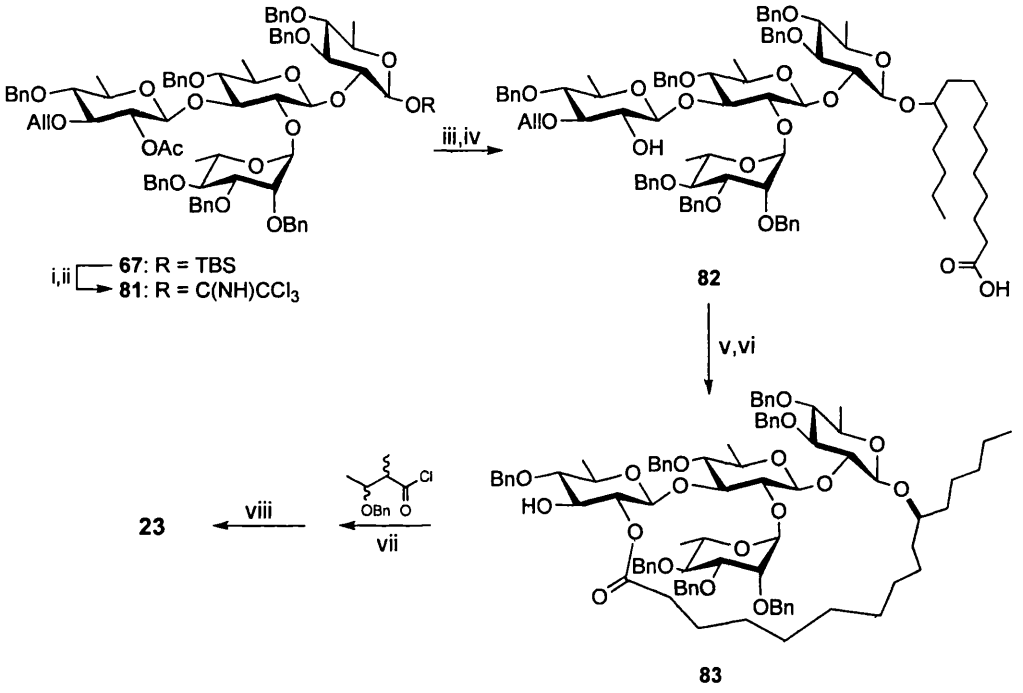
D-Quinovose was prepared from di-*O*-isopropylidene-D-glucose by benzylation of the free hydroxyl to give **68**, followed by selective hydrolysis of the 5,6-isopropylidene ketal. Elimination of the 6-position hydroxyl was achieved by tosylation and subsequent reduction with $LiAlH_4$, with final hydrolysis of the remaining isopropylidene ketal to yield the 6-deoxy sugar **69** (Scheme 3.2). Subsequent isopropylidene re-protection and benzylation resulted in **70** which was converted to glycosyl acceptor **71** by isopropylidene removal and selective silylation of the anomeric hydroxyl group. Similarly intermediate **74** was obtained from **72** by the same sequence of steps and converted to glycosyl donor **75**. Glycosylation of **71** with **75** yielded disaccharide **76**, which was deacetylated and reacted with glycosyl donor **78** to form trisaccharide **79**. Allyl deprotection and glycosylation with **75** gave the desired tetrasaccharide **67**.[140,141]



Scheme 3.2: Synthesis of the tetrasaccharide unit of calonyctin A1

Reagents and conditions: (i) H_2SO_4, MeOH, rt, 20 h, 95%; (ii) TsCl, DMAP, pyridine, CH_2Cl_2, -18 °C → rt, 4 days, 89%; (iii) $LiAlH_4$, THF, 0 °C → rt, 8 h, 86%; (iv) AcOH, H_2O, 105 °C, 4 h, 88%; (v) $(CH_3)_2CH(OCH_3)_2$, CSA, THF, 40h, 74%; (vi) BnBr, NaH, DMF, 5 h, 94%; (vii) AcOH, H_2O, 105 °C, 4 h, 92%; (viii) TBSCl, DMAP, Et_3N, CH_2Cl_2, 0 °C → rt, 36 h, 77%; (ix) Ac_2O, pyridine, 15 h, 96%; (x) $NH_2NH_2 \cdot AcOH$, DMF, 50 °C, 35 min, 87%; (xi) CCl_3CN, DBU, CH_2Cl_2, 5 h, 89%; (xii) TMSOTf, CH_2Cl_2, 40 min, 90%; (xiii) (a) NaOMe, MeOH, 4 h; (b) Amberlite IRC 76 (H^+ form) ion exchange resin, 92%; (xiv) TMSOTf, CH_2Cl_2, 30 min, 76%; (xv) (a) $(Ph_3P)_3RhCl$, EtOH/H_2O, reflux, 8 h; (b) $HgCl_2$, HgO, acetone/H_2O, 15 h, 78%; (xvi) **75**, TMSOTf, CH_2Cl_2, 40 min, 85%.

With tetrasaccharide **67** in hand, deprotection of the silyl group and reaction with trichloroacetonitrile furnished the glycosyl donor **81**, which was utilised for glycosylation of racemic ethyl-11-hydroxyhexadecanoate to yield *β*-glycoside **82** as a 1:1 mixture of two diastereoisomers (Scheme 3.3). Saponification of the esters and purification enabled the separation and characterisation of the diastereoisomers. Macrolactonisation, using Corey-Nicolaou lactonisation conditions[142] (2-pyridyl disulfide, triphenylphosphine), was performed with the *S*-isomer, since this configuration is usually found in glycoresins. Subsequent allyl deprotection gave intermediate **83**.[140]



Scheme 3.3: Total synthesis of calonyctin A1

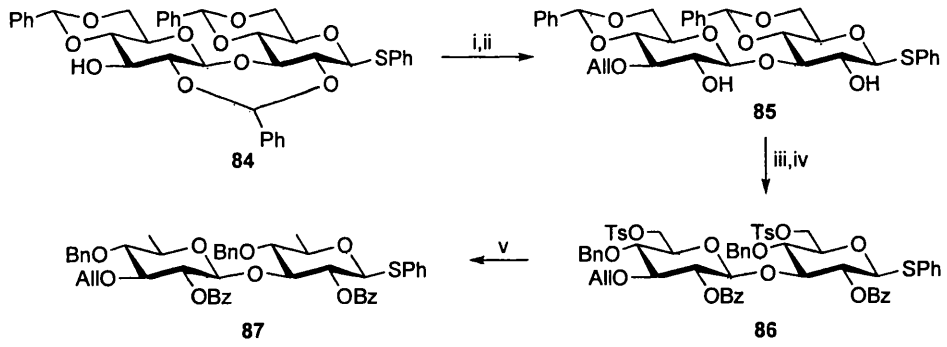
Reagents and conditions: (i) TBAF, THF, 94%; (ii) CCl_3CN, DBU, CH_2Cl_2, 70%; (iii) ethyl-11-hydroxyhexadecanoate, $BF_3{\cdot}Et_2O$, CH_2Cl_2, -8 °C, 80% (1:1); (iv) KOH, MeOH, H_2O, 90%; (v) 2-pyridyl disulfide, PPh_3, toluene, reflux, 80%; (vi) $(Ph_3P)_3RhCl$, $HgCl_2$, HgO, acetone/H_2O, 81%; (vii) pyridine, 70%; (viii) Pd/C, H_2, MeOH, quant.

The benzyl protected niloyl chloride was prepared in both configurations (*R,R*) and (*S,S*) and used to esterify the free hydroxyl of intermediate **83** (Scheme 3.3). Removal of the benzyl protecting groups yielded calonyctin A1 (**23**) and its diastereoisomer. Comparison of optical rotations and NMR data of the two diastereoisomers with those of the natural product confirmed the structures of the side-chains as (*S*)-11-hydroxyhexadecanoic acid and (*R,R*)-nilic acid.[140]

3.2.2. Calonyctin A2

Calonyctin A2 differs from calonyctin A1 only in the length of the hydroxy fatty acid side-chain, i.e. (*S*)-11-hydroxytetradecanoic acid instead of (*S*)-11-hydroxy-hexadecanoic acid. Furukawa and co-workers[143] explored an alternative approach for the total synthesis of calonyctin A2.

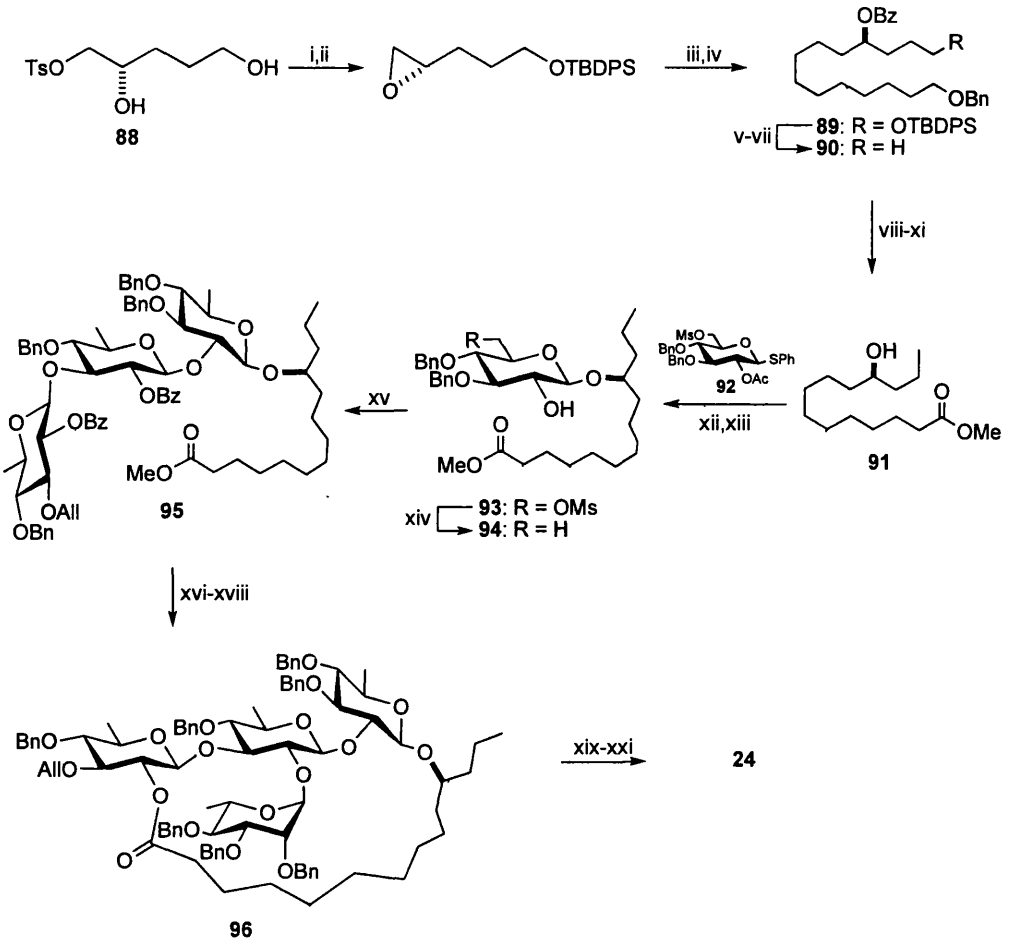
Starting from commercially available $\beta(1\rightarrow3)$-linked laminaribiose, the tribenzylidene derivative of 1-thio-β-laminaribioside (**84**) was obtained. The free hydroxyl group was then allylated and the most labile 2,2′-benzylidene group could be selectively cleaved with PPTS to yield **85** (Scheme 3.4). Regioselective cleavage of the two remaining benzylidene groups proved difficult but could finally be achieved with dimethylamine borane ($BH_3 \cdot Me_2NH$) in the presence of $BF_3 \cdot Et_2O$ to give **86**. One-pot tosylation of both primary hydroxyls and benzoyl protection of the secondary hydroxyls, followed by reduction with sodium borohydride ($NaBH_4$) resulted in the dideoxy derivative **87**.[143]



Scheme 3.4: Synthesis of calonyctin A2 building block

Reagents and conditions: (i) AllBr, NaH, DMF; (ii) PPTS, MeOH/$CHCl_3$, 79%; (iii) $BF_3 \cdot Et_2O$, $Me_2NH \cdot BH_3$, CH_2Cl_2, 77%; (iv) (a) TsCl, pyridine; (b) BzCl; (v) $NaBH_4$, DMF, 70 °C, 47% over 2 steps.

For the synthesis of (*S*)-11-hydroxytetradecanoic acid, tosylated triol **88**, derived from L-glutamic acid through a series of transformations, was converted to the methyl ester **91** in nine steps as shown in Scheme 3.5.



Scheme 3.5: Total synthesis of calonyctin A2

Reagents and conditions: (i) TBDPSCl, pyridine, CH_2Cl_2; (ii) *t*-BuOK, THF; (iii) 9-(benzyloxy)nonyl magnesium bromide, CuI, THF, 0 °C; (iv) BzCl, pyridine, 72% over 2 steps; (v) TBAF, THF; (vi) CBr_4, PPh_3, DMF; (vii) Bu_3SnH, AIBN, toluene, 80 °C; (viii) NaOMe, MeOH, 70 °C; (ix) liq. NH_3, Na, -80 °C; (x) (a) TEMPO, TBACl, NCS, CH_2Cl_2/H_2O, pH 8.6; (b) $NaClO_2$, NaH_2PO_4, *t*-BuOH, 2-methyl-2-butene/H_2O; (xi) CH_2N_2, Et_2O; (xii) thioglycoside **92**, NIS, TfOH, CH_2Cl_2, -20 °C; (xiii) NaOMe, MeOH; (xiv) $NaBH_4$, DMF, 70 °C; (xv) **87**, NIS, TfOH, CH_2Cl_2, -20 °C, 71%; (xvi) KOH, MeOH, 40 °C; (xvii) $Cl_3C_6H_2COCl$, Et_3N, DMAP, toluene, 60%; (xviii) 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside, NIS, TfOH, CH_2Cl_2, -20 °C, 15%; (xix) (a) $RhCl(PPh_3)_3$, diazabicyclo[2,2,2]octane, EtOH, reflux; (b) 2 M HCl, 45 °C; (xx) (2*R*,3*R*)-3-benzyloxy-2-methylbutyric acid, DMAP, carbodiimide, CH_2Cl_2, 62%; (xxi) Pd/C, H_2, MeOH.

Methyl (*S*)-11-hydroxytetradecanoate **91** underwent glycosylation with thioglycoside donor **92** (obtained from readily available phenyl 4,6-*O*-benzylidene-3-*O*-benzyl-1-thio-β-D-glucopyranoside) to yield glycoside **93** after de-*O*-acetylation (Scheme 3.5). Glycoside **93** was then converted into the 6-deoxy glycosyl acceptor **94** by reduction of the 6-position mesyl group. Acceptor **94** was glycosylated with glycosyl donor **87** in the presence of NIS-TfOH to form the trisaccharide **95**. Saponificaton of the ester groups and macrolactonisation by a

mixed anhydride procedure[144] (2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP) gave the desired protected product. Finally, glycosylation with phenyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside resulted in the tetrasaccharide intermediate **96**, and subsequent allyl deprotection, esterification with (*R,R*)-nilic acid and removal of the benzyl groups yielded calonyctin A2 (**24**).[143]

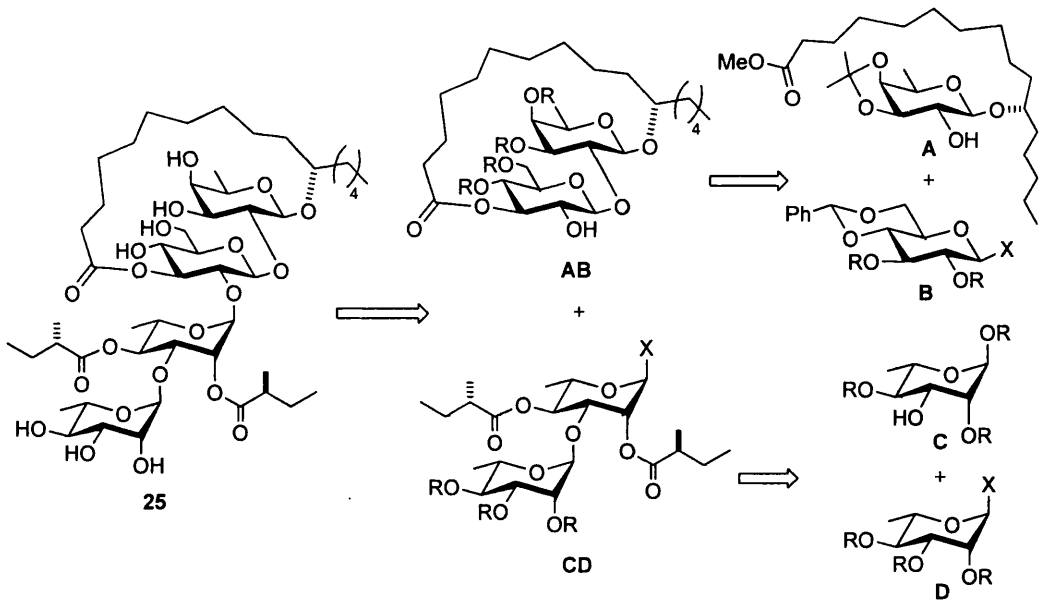
3.2.3. Summary

Schmidt's approach for the assembly of calonyctin A1 has the advantage that the late introduction of the side-chains enables the parallel synthesis of analogues with different ring sizes by varying the fatty acid side-chain. This parallel synthesis approach also allowed the convenient synthesis of both diastereoisomers of 11-hydroxyundecanoic acid as a racemic mixture, which could subsequently be separated in order to elucidate the configuration of 11-hydroxyundecanoic acid in the natural product. This, however, meant that each diastereoisomer would be obtained at best in 50% yield in a late stage of the synthesis.

Furukawa and co-workers' approach, on the contrary, hardly allows the convenient synthesis of analogues, since each compound would require an independent multistep synthesis. Another disadvantage in their approach was that the final glycosylation of the macrolactone trisaccharide with the thiorhamnoside in such a late stage resulted in the formation of the tetrasaccharide in very low yield (15%) probably due to steric hindrance in the glycosyl acceptor.

3.3. Tricolorins

The synthesis of tricolorin A was reported independently by two groups in 1997.[145,146] The key step in each synthesis was the formation of the macrolactone ring, which was achieved by condensation of the fatty acid carboxyl with a free hydroxyl on the appropriate sugar. More recently, an alternative synthesis of tricolorin A[147] and the synthesis of tricolorin G[148] were described where the macrolactone was formed by ring closing metathesis (RCM). In addition the synthesis of tricolorin F has also recently been reported.[149]



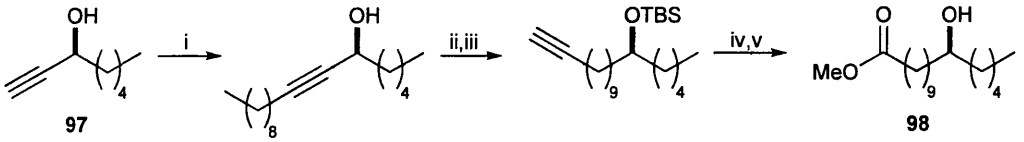
Scheme 3.6: Retrosynthetic analysis of tricolorin A

3.3.1. Tricolorin A

Synthesis of tricolorin A by Larson and Heathcock

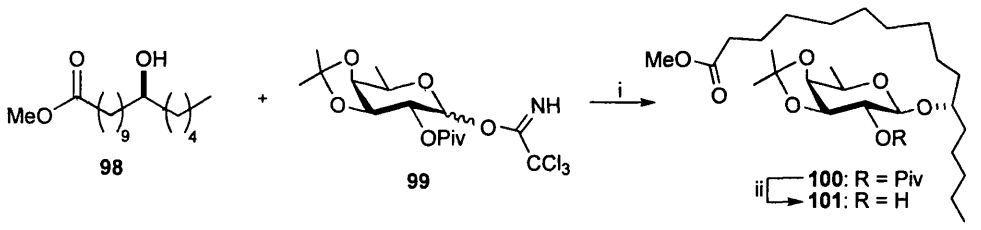
Retrosynthetic analysis of tricolorin A (Scheme 3.6) indicated a key disconnection in the glycosidic linkage between glucose and rhamnose to yield disaccharides **AB** and **CD**. Further disconnection of the disaccharides suggested the monosaccharide building blocks **A**, **B**, **C** and **D**.[145]

The synthesis of methyl (*S*)-11-hydroxyhexadecanoate (**98**) was achieved from (*S*)-propargylic alcohol **97** in five steps as shown in Scheme 3.8. The readily available glycosyl donor **99** was obtained from benzyl fucose by isopropylidenation of the *cis*-hydroxyls, followed by pivaloyl protection of the remaining hydroxyl. Subsequent anomeric deprotection and formation of the trichloroacetimidate yielded glycosyl donor **99**. Glycosylation of alcohol **98** with glycosyl donor **99** yielded glycoside **100** (Scheme 3.8). The pivaloate ester was then removed to yield glycosyl acceptor **101** (building block **A**).



Scheme 3.7: Synthesis of methyl (*S*)-11-hydroxyhexadecanoate (**98**)

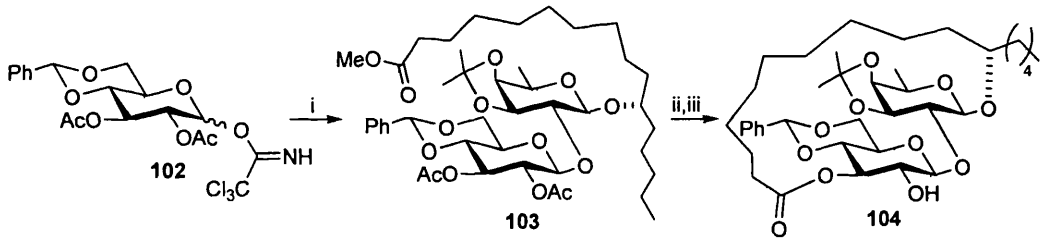
Reagents and conditions: (i) (a) $LiNH_2$, NH_3, -33 °C; (b) $C_9H_{19}I$, THF, -33 °C → rt, 94%; (ii) KAPA, THF, 79%; (iii) TBSCl, imidazole, DMF, 98%; (iv) $KMnO_4$, AcOH, H_2O, pentane; (v) MeOH, H_2SO_4, 75%.



Scheme 3.8: Synthesis of building block **A** of tricolorin A

Reagents and conditions: (i) TMSOTf, CH_2Cl_2, 79%; (ii) NaOMe, MeOH, MeOAc, 96%.

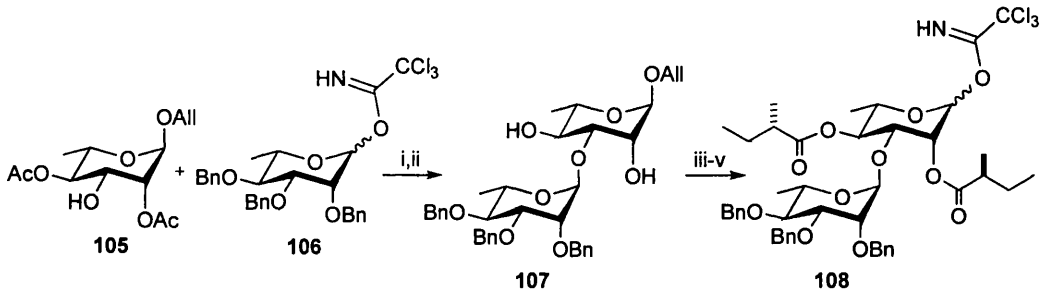
Glycosyl donor **B** (**102**) was prepared by acetylation of 4,6-*O*-benzylidene glucose, selective anomeric deprotection and reaction with trichloroacetonitrile in the presence of cesium carbonate. Donor **B** was then utilised for glycosylation of acceptor **101** (Scheme 3.9). The resulting disaccharide **103** was saponified and then subjected to macrolactonisation using the mixed anhydride procedure,[144] which took place at the desired C-3 hydroxyl with a high degree of selectivity over the C-2 hydroxyl, yielding macrolactone **104** (building block **AB**).



Scheme 3.9: Synthesis of building block **AB** of tricolorin A

Reagents and conditions: (i) **101**, AgOTf, CH_2Cl_2, 84%; (ii) LiOH, THF, H_2O, 74%; (iii) $Cl_3C_6H_2COCl$, Et_3N, DMAP, benzene, 71%.

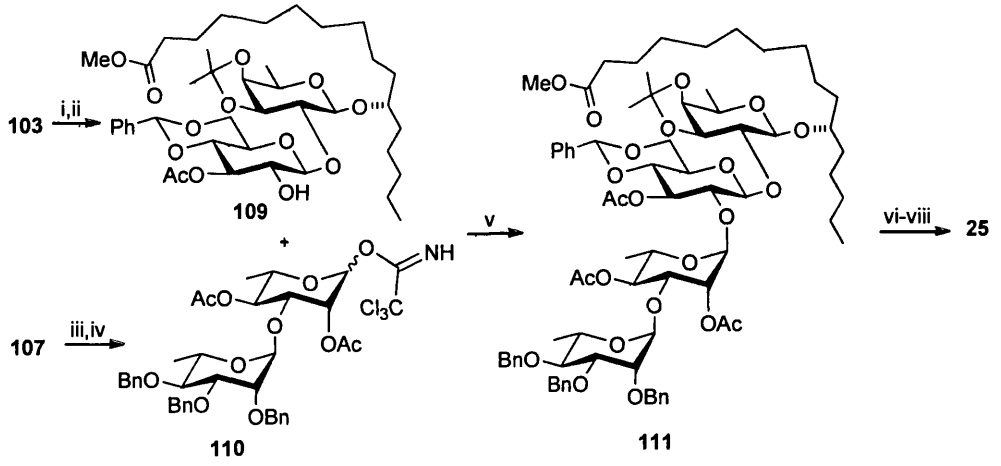
Building block **C** (**105**) was prepared from allyl rhamnoside via orthoester formation and selective opening to yield the free 3-position hydroxyl. Glycosylation of **105** was attempted with glycosyl trichloroacetimidate **106** also derived from allyl rhamnoside (Scheme 3.10). This glycosylation, however, proved problematic and could only be achieved by "inverse glycosylation" i.e. the glycosyl donor is added slowly to a mixture of the glycosyl acceptor and the activator.[150] Subsequent deacetylation of **107**, installation of the side-chains and conversion to the glycosyl trichloroacetimidate yielded intermediate **108**.[145]



Scheme 3.10: Synthesis of building block **CD** of tricolorin A

Reagents and conditions: (i) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 93%; (ii) NaOMe, MeOH, 87%; (iii) (*S*)-2-methylbutyric acid, DCC, DMAP, CH_2Cl_2, 92%; (iv) (a) $(Ph_3P)_3RhCl$, EtOH, H_2O, reflux; (b) $HgCl_2$, HgO, acetone, H_2O, 84%; (v) CCl_3CN, Cs_2CO_3, CH_2Cl_2.

Attempts to form the tetrasaccharide from glycosyl donor **108** and the lactone disaccharide acceptor **104** were unsuccessful, probably due to steric hindrance of the bulky substituents at the sites adjacent to the reaction site. Thus, the synthetic approach towards tricolorin A was modified. Intermediate **103** was converted to glycosyl acceptor **109** by de-*O*-acetylation and regioselective acetylation, and disaccharide **107** to glycosyl donor **110** by anomeric de-*O*-allylation and trichloroacetimidate formation. The tetrasaccharide **111** could then be formed from these building blocks in good yield (Scheme 3.11). Saponification of the esters and macrolactonisation resulted in the desired macrolactone. Installation of the acyl side-chains and removal of the remaining protecting groups yielded tricolorin A (**25**).[145]



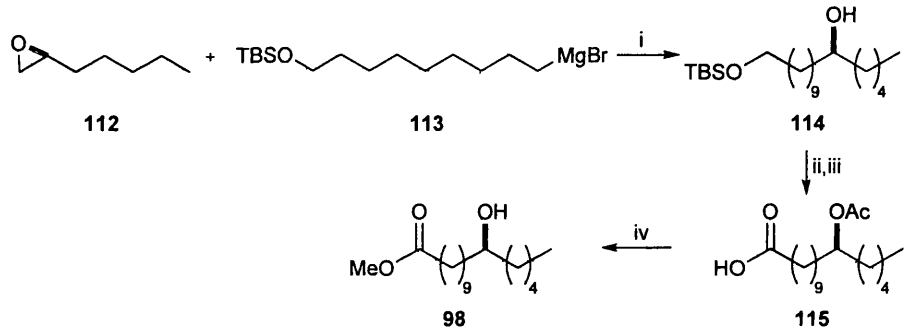
Scheme 3.11: Total synthesis of tricolorin A (**25**)

Reagents and conditions: (i) NaOMe, MeOH, MeOAc 84%; (ii) Ac_2O (1 equiv), Et_3N, DMAP, CH_2Cl_2, 80%; (iii) (a) $(Ph_3P)_3RhCl$, *n*-BuLi, THF, reflux; (b) $HgCl_2$, HgO, acetone, H_2O, 95%; (iv) CCl_3CN, Cs_2CO_3, CH_2Cl_2; (v) TMSOTf, CH_2Cl_2, 75%; (vi) LiOH, THF, H_2O, 72%; (vii) (a) $Cl_3C_6H_2COCl$, Et_3N, DMAP, benzene; (b) (*S*)-2-methylbutyric acid, 61%; (viii) $Pd(OH)_2$, H_2, HCl, MeOH, 77%.

Synthesis of tricolorin A by Lu and co-workers

Lu and co-workers[146] used a similar strategy to the above for the synthesis of tricolorin A. A significant difference is the utilisation of thioglycosides as glycosyl donors. Also the synthesis of (*S*)-11-hydroxyhexadecanoic acid was achieved by a chiral pool procedure, starting from D-mannitol.

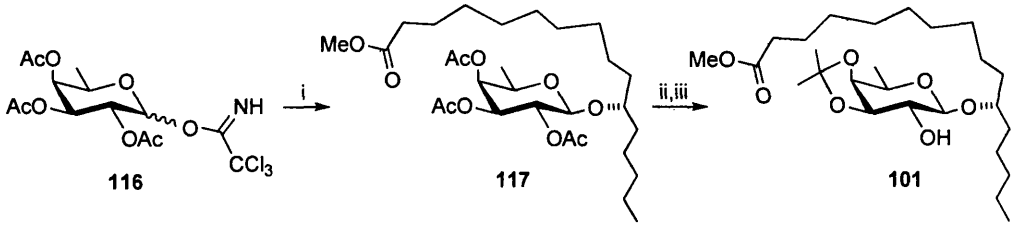
Epoxide **112** obtained from D-mannitol by a known procedure[151] was reacted with Grignard reagent **113**, which was obtained from undecylenic acid in seven steps, to yield alcohol **114**, as shown in Scheme 3.12. Subsequent acetylation of the alcohol, followed by Jones oxidation, formed acid **115**. Treatment of acid **115** with $BF_3{\cdot}Et_2O$ in MeOH resulted in simultaneous de-acetylation and protection of the acid as the methyl ester to yield methyl (*S*)-11-hydroxyhexadecanoate (**98**).



Scheme 3.12: Synthesis of methyl (*S*)-11-hydroxyhexadecanoate (**98**)

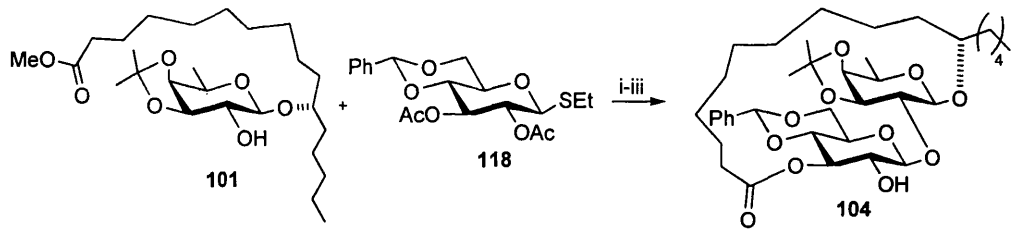
Reagents and conditions: (i) CuI, THF, -78 °C → rt, 66%; (ii) Ac_2O, pyridine, 91%; (iii) Jones reagent, acetone, 89%; (iv) $BF_3{\cdot}Et_2O$, MeOH, 97%.

The synthesis of glycoside **101** representing synthon **A** (Scheme 3.6) was achieved by glycosylation of methyl (*S*)-11-hydroxyhexadecanoate (**98**) with readily available glycosyl donor **116** (Scheme 3.13). Glycoside **117** was subjected to deacetylation and isopropylidenation to yield glycosyl acceptor **101**. Glycosylation of acceptor **101** with thioglycoside **118** representing synthon **B** (Scheme 3.14) resulted in disaccharide **103**, which was converted to the desired macrolactone **104** using the Corey-Nicolaou protocol.[142]



Scheme 3.13: Synthesis of building block **A** of tricolorin A

Reagents and conditions: (i) **98**, $BF_3{\cdot}Et_2O$, CH_2Cl_2, -15 °C, 66%; (ii) NaOMe, MeOH, -15 °C; (iii) $(CH_3)_2CH(OCH_3)_2$, PTSA, acetone, 82%.

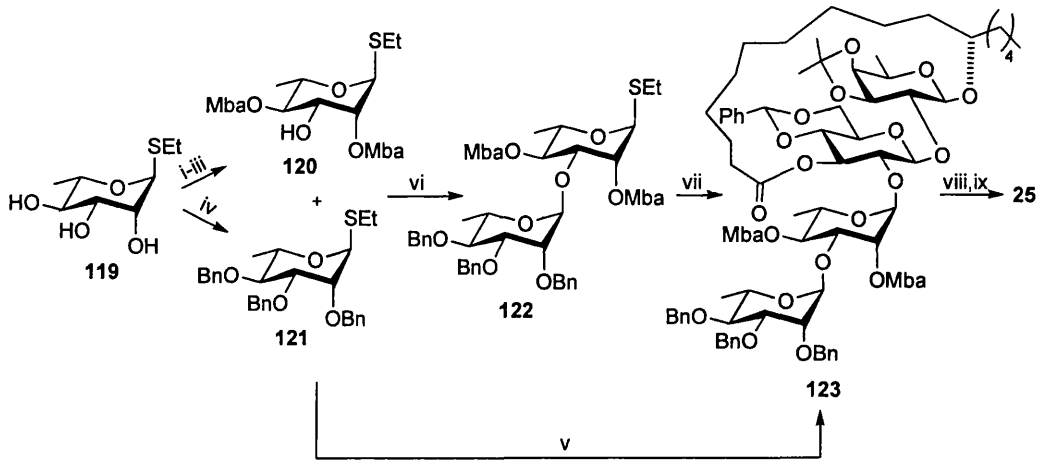


Scheme 3.14: Synthesis of building block **AB** of tricolorin A

Reagents and conditions: (i) NIS, AgOTf, CH_2Cl_2, -15 °C, 76%; (ii) KOH, MeOH, H_2O, reflux, 91%; (iii) $(PyS)_2$, PPh_3, toluene, reflux, 69%.

Building blocks **C** and **D** (Scheme 3.6) were both prepared from ethyl-1-thio-α-L-rhamnopyranoside **119**. Selective PMB protection of the 3-OH, installation of the chiral side-chains and removal of the PMB group gave thioglycoside **120**, while benzylation of ethyl-1-thio-α-L-rhamnopyranoside yielded glycosyl donor **121** (Scheme 3.15).

Construction of the tetrasaccharide was successfully accomplished by an one-pot, two step glycosylation with glycosyl acceptor **104** and glycosyl donors **120** and **121**. Alternatively, the disaccharide **122** was first formed and isolated, starting from **120** and **121**, exploiting the armed-disarmed glycosylation strategy. Subsequently, donor **122** was coupled with glycosyl acceptor **104** to yield the tetrasaccharide **123** over two steps (Scheme 3.15). Removal of the remaining protecting groups yielded tricolorin A (**25**).[146]

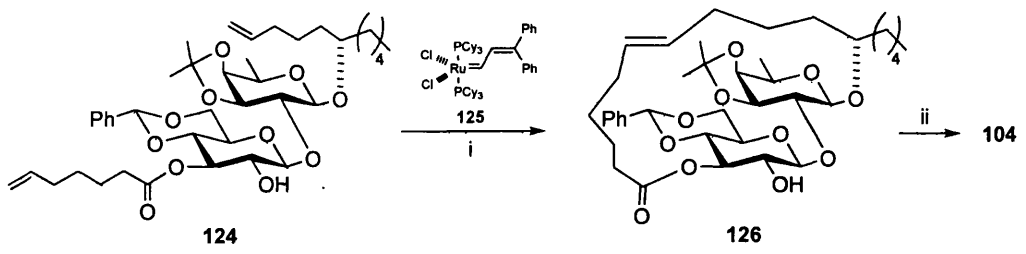


Scheme 3.15: Total synthesis of tricolorin A (**25**)

Reagents and conditions: (i) (a) Bu_2SnO, toluene, reflux; (b) PMBCl, CsF, Bu_4NI, DMF, 79%; (ii) (*S*)-2-methylbutyric anhydride, DMAP, pyridine, 70%; (iii) CAN, MeCN, H_2O, 97%; (iv) BnBr, NaH, DMSO, 0 °C, 52%; (v) (a) NIS, TfOH, Et_2O, 1,2-dichloroethane, -15 °C, 15 min; (b) **104**, NIS, TfOH, rt, 43%; (vi) IDCP, CH_2Cl_2, 98%; (vii) NIS, AgOTf, CH_2Cl_2, 86%; (viii) DDQ, MeCN, H_2O, reflux, 80%; (ix) H_2, Pd/C, EtOH, 60 °C, 88%.

Synthesis of tricolorin A by RCM

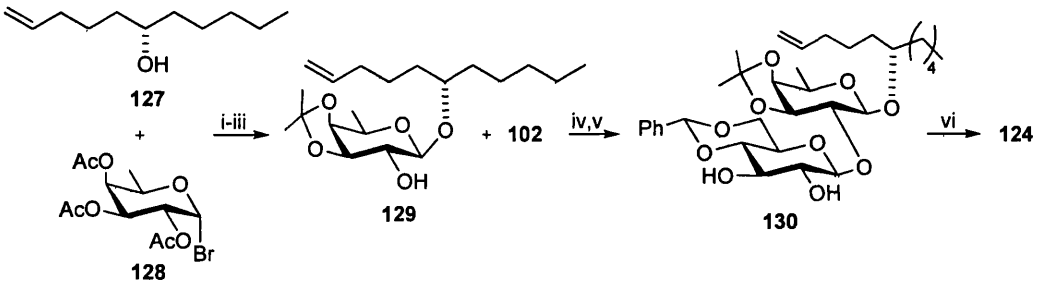
In 1998, Fürstner and Müller[147] reported an alternative strategy to tricolorin A employing RCM for the construction of the macrolactone. RCM enables the formation of a cyclic alkene by reaction of two terminal alkenes in the presence of a ruthenium catalyst[152] (Scheme 3.16).



Scheme 3.16: Synthesis of the tricolorin A subunit (**104**) by RCM

Reagents and conditions: (i) CH_2Cl_2, reflux, high dilution; (ii) H_2, Pd/C, EtOH, 77% over two steps.

Since tricolorin A had been previously synthesised, they focused only on the preparation of the macrolactone subunit **104** (building block **AB**, Scheme 3.6), which could be obtained from diene **124** by RCM (Scheme 3.16). The synthesis of the disaccharide is shown in Scheme 3.17. The required (*S*)-undec-1-en-6-ol **127** could be obtained by enantioselective ligand-controlled addition of dipentylzinc to hex-5-enal. Glycosylation with fucosyl donor **128**, deacetylation and conversion to the isopropylidene ketal yielded glycosyl acceptor **129**. Subsequent glycosylation with glycosyl donor **102** and deacetylation of the resulting compound gave disaccharide **130**.



Scheme 3.17: Synthesis of the RCM precursor

Reagents and conditions: (i) $AgNO_3$ on silica/alumina, CH_2Cl_2, -10 °C, 69%; (ii) KOMe, MeOH; (iii) $(CH_3)_2CH(OCH_3)_2$, PTSA, acetone, 91% over two steps; (iv) $BF_3{\cdot}Et_2O$, CH_2Cl_2, *n*-hexane, -20 °C, 82%; (v) KOMe, MeOH, 71%; (vi) hept-6-enoic acid, DCC, DMAP, CH_2Cl_2, 71%.

Regioselective acylation with hept-6-enoic acid afforded ester **124** as the only product. This high regioselectivity had also been observed during macrolactonisation in the previous two syntheses of tricolorin A and is probably due to the bulky anomeric substituent, which renders the 2-OH much less reactive than the 3-OH. Diene **124** was then subjected to RCM in the presence of Grubbs catalyst **125**. Hydrogenation of the resulting alkene yielded macrolactone **104** (Scheme 3.16).[147]

Summary

The total synthesis of tricolorin was achieved by two groups independently. Larson's and Heathcock's approach required a total of 39 steps. The longest linear sequence consisted of 14 steps with an overall yield for this sequence of 6%. The macrolactone was formed by intramolecular esterification using the mixed anhydride procedure. Glycosylations were performed using the trichloroacetimidate method. However, the final glycosylation, involving coupling of the macrolactone disaccharide **104** with the disaccharide trichloroacetimidate **108**, was unsuccessful. A slight modification in their approach involving the formation of the tetrasaccharide prior to macrolactonisation finally furnished tricolorin A.

Lu's and co-workers synthesis of tricolorin A was achieved in 45 steps with the longest linear sequence being 20 steps and an overall yield of 0.65% for this sequence. Glycosylations were performed using thioglycosides and macrolactonisation was achieved using the Corey-Nicolaou protocol. Interestingly, by employing thioglycosides, they succeeded in performing the glycosylation, which was unsuccessful in the hands of Larson and Heathcock using the trichloroacetimidate method.

Alternatively the synthesis of the tricolorin subunit **104** was achieved by Fürstner and Müller employing RCM for the construction of the macrolactone. This approach has the advantage that it enables the parallel synthesis of analogues with different ring sizes by modifying the alkene acyl substituent. Furthermore, the two alkene building blocks required for the RCM, were readily available compared to methyl (*S*)-11-hydroxyhexadecanoate, which required a multistep synthesis. However, this approach required three steps for the construction of the macrolactone **104** (i.e esterification, RCM, catalytic reduction) with an overall

yield of 55% from the macrolactone precursor **130**, compared to the previous two approaches, where the macrolactone **104** was obtained from precursor **103** in one intramolecular esterification step and in 71% or 69% yield.

3.3.2. Total Synthesis of Tricolorin G

Intermediate **130** (Scheme 3.15) prepared by Fürstner and Müller for the synthesis of tricolorin A served also as precursor for the synthesis of tricolorin G (**31**) (Figure 3.1) in which (*S*)-11-hydroxyhexadecanoic acid spans a trisaccharide core.[148]

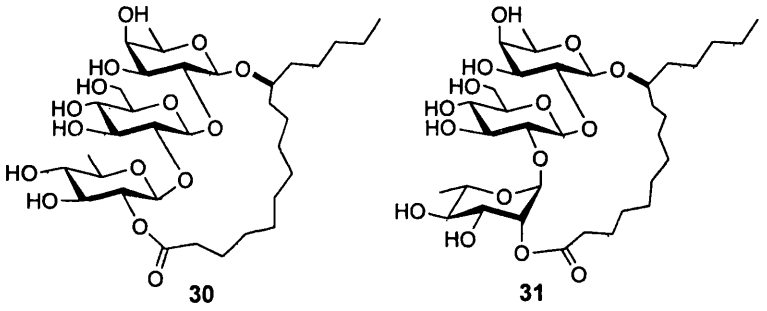
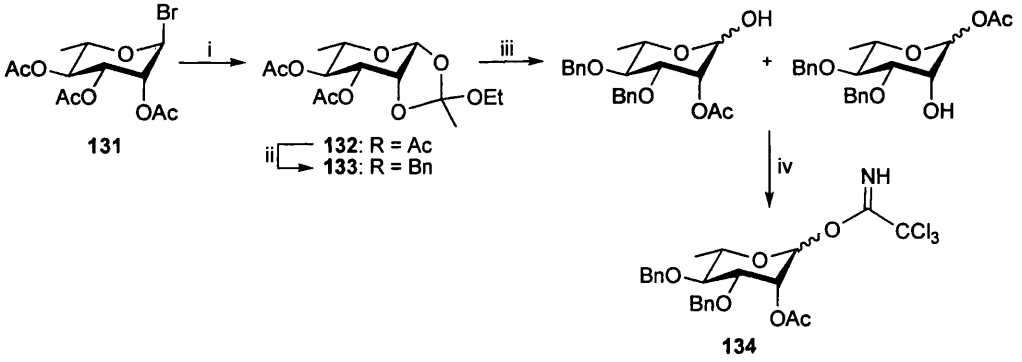


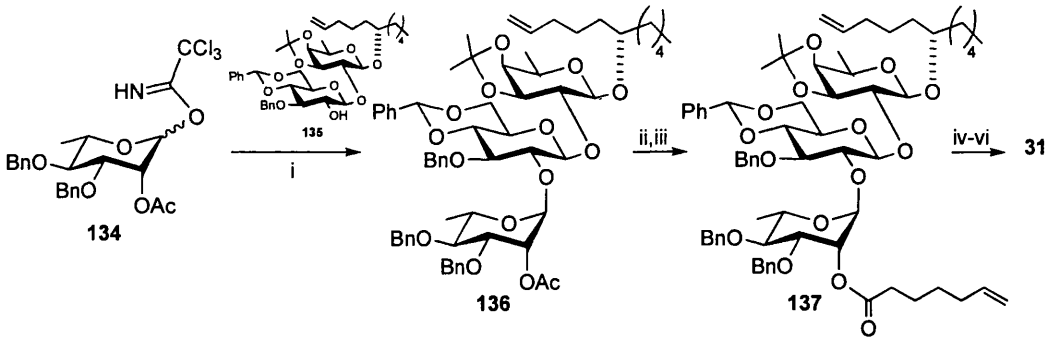
Figure 3.1: Tricolorins F (**30**) and G (**31**)

The glycosyl donor required for the construction of the trisaccharide was prepared from 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide **131** by formation of the orthoester **132**, replacement of the acetates by benzyl groups (**133**) and hydrolysis of the orthoester, which gave a mixture of the 1-OAc and 2-OAc derivatives (Scheme 3.18). Treatment of this mixture with trichloroacetonitrile in the presence of cesium carbonate yielded a single product, namely glycosyl donor **134**.[148]



Scheme 3.18: Synthesis of the tricolorin G building block **134**

Reagents and conditions: (i) *sym*-Collidine, EtOH, Bu_4NI, 80 °C, 66%; (ii) BnBr, KOH, THF, reflux, 78%; (iii) AcOH, H_2O; (iv) CCl_3CN, Cs_2CO_3, CH_2Cl_2, 46%.



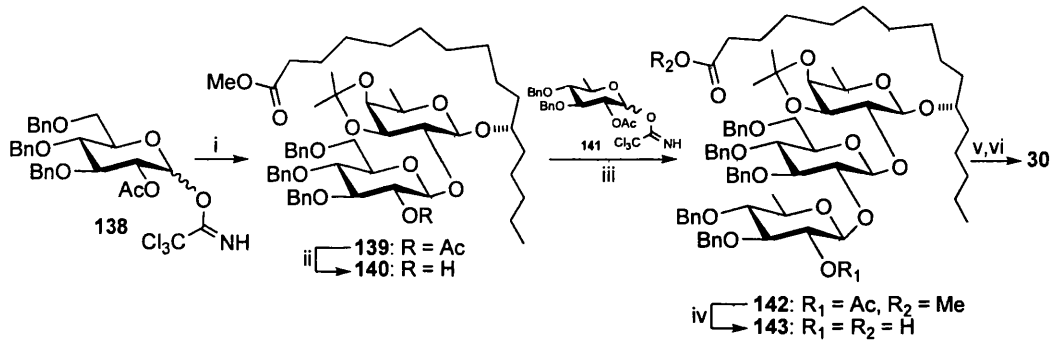
Scheme 3.19: Synthesis of tricolorin G (**31**)

Reagents and conditions: (i) $BF_3{\cdot}Et_2O$, CH_2Cl_2, *n*-hexane -20 °C, 53%; (ii) KOMe, MeOH; (iii) hept-6-enoic acid, DCC, DMAP, CH_2Cl_2, 84% over two steps; (iv) **125**, CH_2Cl_2, reflux; (v) H_2, $RhCl(PPh_3)_3$, EtOH, 93% over two steps; (vi) (a) TFA, CH_2Cl_2; (b) H_2, Pd/C, MeOH, TFA, 49%.

Since the 3-OH of **130** is more reactive than the 2-OH, benzyl protection was required prior to glycosylation. Interestingly, the selectivity observed during acylation did not apply during benzylation and a 3.2:1 mixture of the 3-OBn (**135**) and 2-OBn was obtained, which could be separated by conventional flash chromatography. Trisaccharide **136** was obtained from **134** and **135** and subsequent deacetylation and acylation with 6-heptenoic acid afforded diene **137** (Scheme 3.19). Exposure of the diene to catalytic amounts of Grubbs catalyst **125**, hydrogenation of the resulting alkene in the presence of Wilkinson's catalyst and removal of the remaining protecting groups yielded tricolorin G (**31**).[148]

3.3.3. Total Synthesis of Tricolorin F

In 2004 Brito-Arias[149] and co-workers achieved the synthesis of tricolorin F employing an intramolecular esterification step for the formation of the macrolactone. The known glycosyl acceptor **101** and glycosyl donor **138** (prepared as described above for compound **134**) were coupled to form disaccharide **139** (Scheme 3.20). Deacetylation afforded alcohol **140** which was glycosylated with imidate **141** to form trisaccharide **142**. Saponification of the esters resulted in the intermediate **143**, which underwent macrolactonisation using the mixed anhydride procedure.[144] Subsequent removal of the remaining protecting groups completed the synthesis of tricolorin F (**30**).[149]



Scheme 3.20: Total synthesis of tricolorin F (**30**)

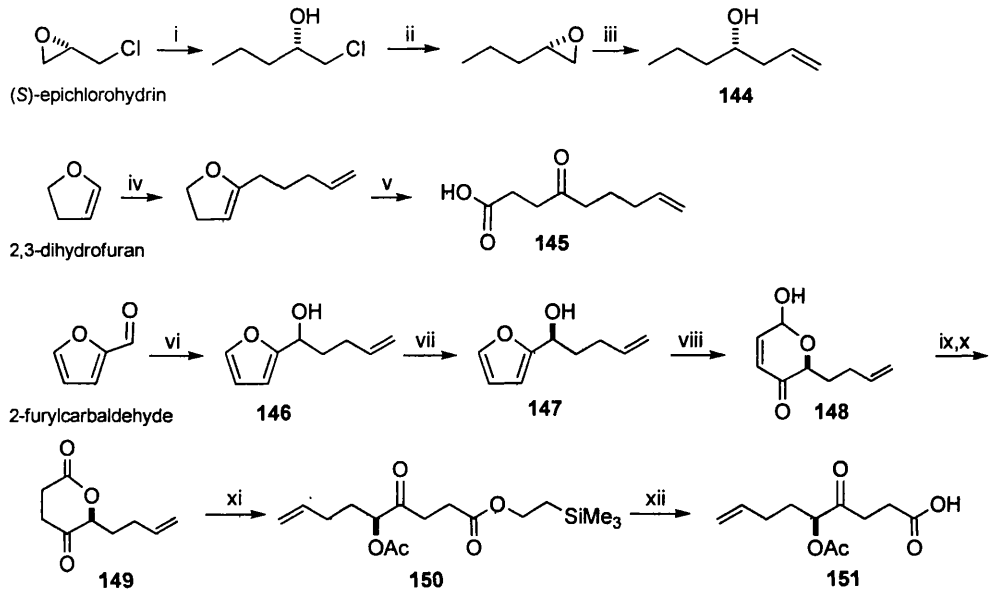
Reagents and conditions: (i) **101**, $BF_3 \cdot Et_2O$, CH_2Cl_2, -20 °C, 70%; (ii) NaOMe, MeOH, 95%; (iii) $BF_3 \cdot Et_2O$, CH_2Cl_2, -20 °C, 60%; (iv) KOH, MeOH, H_2O, reflux, 80%; (v) $Cl_3C_6H_2COCl$, Et_3N, DMAP, benzene, 60%; (vi) H_2, $Pd(OH)_2/C$, 10% HCl-MeOH, MeOH, 80%.

3.4. Ipomoeassins

In 2007* Fürstner and Nagano reported the total syntheses of ipomoeassins B (**36**) and E (**39**).[153] RCM was the method of choice for the formation of the macrolactone since it had served well in previous syntheses. As this approach requires hydrogenation of the resulting alkene, it was planned to install the cinnamoyl ester after the reduction of the alkene. The tiglate ester should not be affected due to its trisubstituted double bond, if reduction was carried out in the presence of Wilkinson's catalyst ($RhCl(PPh_3)_3$).

The syntheses of the required RCM precursors are shown in Scheme 3.21. (*S*)-hept-1-en-4-ol **144** could be obtained from (*S*)-epichlorohydrin in three steps. 4-Oxonon-8-enoic acid **145**, required for the synthesis of ipomoeassin B, was prepared by alkylation of lithiated 2,3-dihydrofuran with 5-iodo-1-pentene and subsequent hydrolysis/oxidation with Jones' reagent. Acid **151**, required for the synthesis of ipomoeassin E, was synthesised from 2-furylcarbaldehyde, which was alkylated to yield racemic **146**. The desired (*S*)-alcohol **147** could be obtained by a Sharpless-type kinetic resolution of the racemic alcohol. Subsequent oxidative rearrangement (**148**), oxidation of the resulting alcohol and conjugate reduction yielded **149**, which was converted to acid **151** by treatment with trimethylsilylethanol (yielding **150**), acetylation and cleavage of the ester.

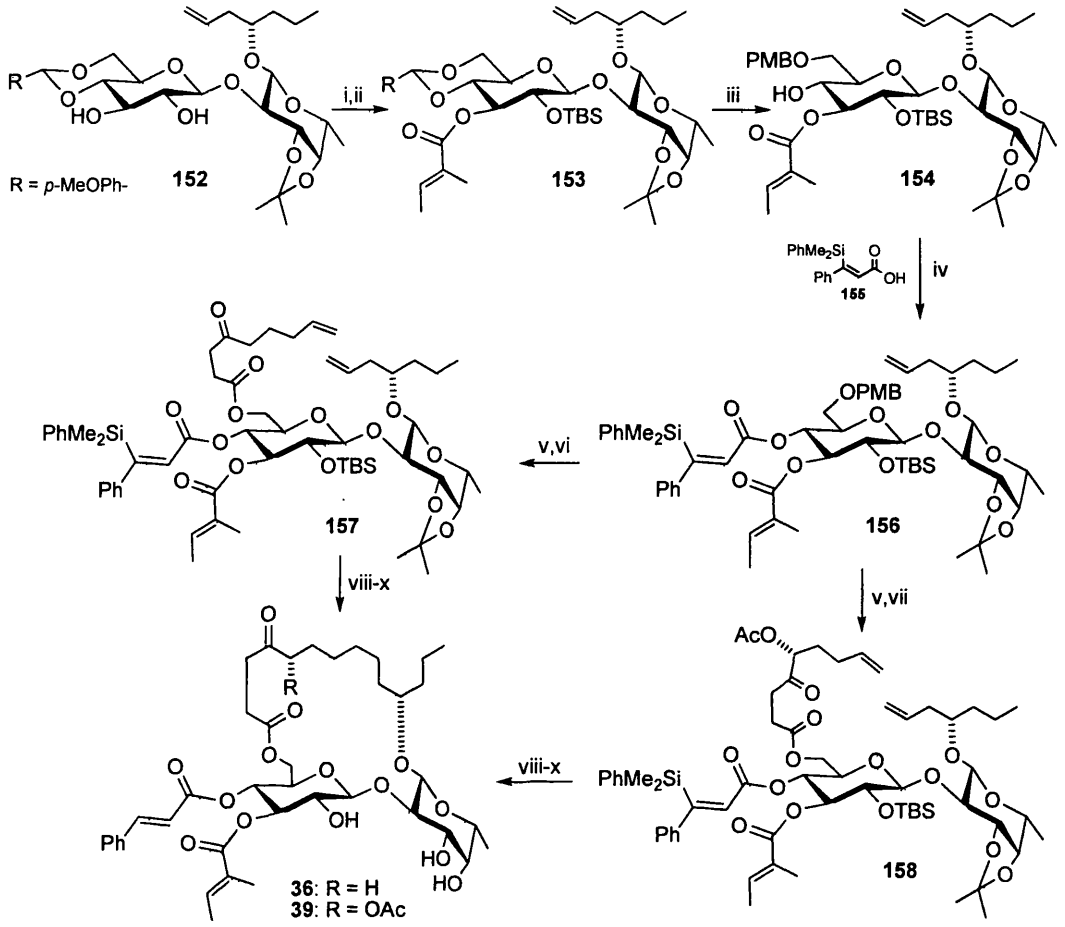
* The total syntheses of ipomoeassins B and E were disclosed by Fürstner and Nagano approximately 14 months after commencement of the research project discussed in this PhD thesis.



Scheme 3.21: Synthesis of the RCM precursors

Reagents and conditions: (i) EtMgCl, CuCN, THF, -78 → -20 °C; (ii) NaOH, Et_2O; (iii) CH2=CHMgBr, CuCN, THF, -78 → -20 °C, 77% over three steps; (iv) (a) *t*-BuLi, THF, -78 °C → 0 °C, 30 min; (b) 5-iodopent-1-ene, THF, -78 °C → rt; (v) Jones' reagent (H_2CrO_4), THF, 0 °C → rt; (vi) 1-bromo-3-pentene, Mg, THF, then 2-furylcarbaldehyde, 82%; (vii) Ti(O*i*Pr)$_4$, DIPT, *t*-BuOOH, CH_2Cl_2, −20°C, 47%, > 99 ee; (viii) *t*-BuOOH, VO(acac)$_2$, CH_2Cl_2, 71%; (ix) Jones' reagent (H_2CrO_4), acetone, 0°C; (x) Zn, AcOH, CH_2Cl_2, 78% (over two steps); (xi) (a) $HO(CH_2)_2SiMe_3$, PTSA, CH_2Cl_2; (b) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 93%, 97% ee (over two steps); (xii) TASF, DMF, 68%.

Disaccharide **152** (prepared as described above for compound **130**) was acylated with tiglic acid at the 3-OH, and the 2-OH was protected as the TBS ether (**153**) (Scheme 3.22). Attempts to selectively open the substituted benzylidene acetal, in order to obtain the 4-*O*-PMB, failed. Surprisingly, reduction with sodium cyanoborohydride and TMSCl yielded the 6-*O*-PMB (**154**) rather than the expected 4-*O*-PMB. At this stage a slight modification in the synthetic approach was required. Therefore, the C-silylated derivate **155** was prepared, which contains a trisubstituted double bond and would remain intact during reduction in the presence of Wilkinson's catalyst. Installation of **155** at the 4-OH, removal of the PMB group and esterification with either **145** or **151** yielded RCM precursors **157** and **158** respectively. These were treated with Grubbs catalyst to form the respective cyclic alkenes. Hydrogenation over Wilkinson's catalyst proceeded smoothly without affecting the unsaturated esters. Subsequent removal of the remaining protecting groups yielded ipomoeassins B (**36**) and E (**39**).[153]



Scheme 3.22: Total synthesis of ipomoeassins B (**36**) and E (**39**)

Reagents and conditions: (i) Tiglic acid, DCC, DMAP, CH_2Cl_2, 55%; (ii) TBSCl, 2,6-lutidine, CH_2Cl_2, 96%; (iii) $NaBH_3CN$, TMSCl, MeCN; (iv) $Cl_3C_6H_2COCl$, Et_3N, DMAP, toluene, 79% over two steps; (v) DDQ, CH_2Cl_2, H_2O; (vi) **145**, $Cl_3C_6H_2COCl$, Et_3N, DMAP, toluene, 78% over two steps; (vii) **151**, $Cl_3C_6H_2COCl$, Et_3N, DMAP, toluene, 87%; (viii) Grubbs catalyst, CH_2Cl_2, reflux, 71% and 85%; (ix) H_2, $RhCl(PPh_3)_3$, EtOH, 81% and 83%; (x) (a) TASF, MeCN; (b) TFA, CH_2Cl_2, 45% for **36** and 63% for **39** over both steps.

3.5. Summary

In this chapter, previous efforts towards the synthesis of macrocyclic glycoresins have been discussed. Several protecting group strategies and glycosylation methods have been employed in order to achieve the desired regio- and stereoselectivity. In addition, two general methods for the formation of the macrolactone have been utilised, i.e. either intramolecular esterification or RCM. Chapter 4 will describe several attempts made to synthesise ipomoeassin B analogues employing similar strategies to the ones discussed above.

4. RESULTS AND DISCUSSION

4.1. Synthesis of Ipomoeassin B Analogues

The ipomoeassins, isolated in 2005,[16] were found to exhibit highly potent cytotoxic activity against human ovarian cancer cell lines (IC_{50} 35 nM). However, little is known about their mode of action and thus a synthesis-driven evaluation is required. Therefore, the ipomoeassins, and ipomoeassin B in particular, which is the simplest and one of the most potent of the ipomoeassins, were chosen as synthetic target. The generation of analogues would enable the probing of their diverse biological activities and exploration of structure-activity relationships.

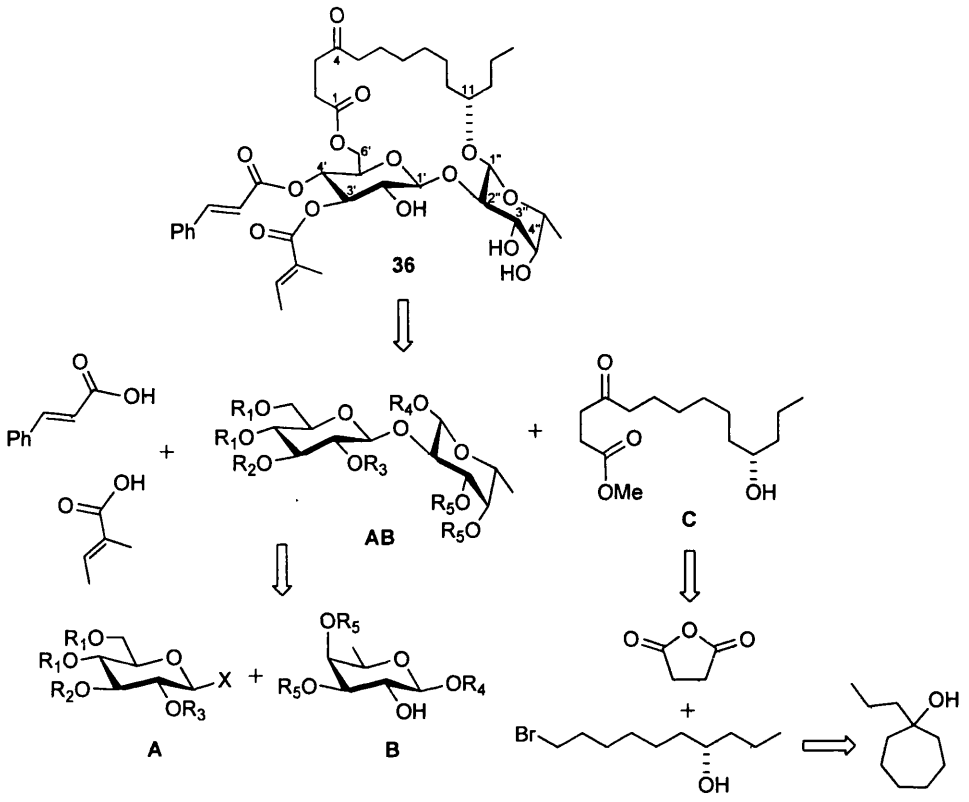
4.2. First Approach

The major challenge towards the synthesis of ipomoeassin analogues lies in the formation of the macrolactone ring. This has been achieved either by intramolecular esterification methods or by ring closing metathesis in previous syntheses of glycoresins.[145-147,149,153] Other challenges involve two stereoselective glycosylations and the regioselective installation of the acyl side-chains.

It has been suggested that the presence of the macrolactone ring is essential for the activity of glycoresins.[154] However, since glycoresins also occur as non-macrocyclic glycosidic acids in the Convolvulaceae family (e.g. pescapreins, Section 1.6 and tyrianthins Section 1.7) we decided to follow a synthetic approach which would enable the generation of macrocyclic and non-macrocyclic analogues. This could be best achieved by macrolactonisation via an intramolecular ester-forming step.

4.2.1. Retrosynthetic Analysis

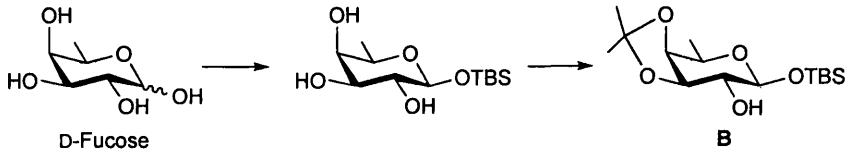
Retrosynthetic analysis of the target molecule (Scheme 4.1) suggested that the introduction of the acyl substituents at C-3′ and C-4′ of glucose should be postponed to the final steps of the synthesis. This parallel synthesis approach would enable the formation of a late stage intermediate, which could subsequently be regioselectively acylated with various acyl side-chains in order to produce several analogues. It would therefore be necessary to block these positions with two orthogonal protecting groups. Since the removal of these protecting groups would take place after the formation of the macrolactone, esters were not suitable and thus ether protecting groups were chosen for this purpose.



Scheme 4.1: Retrosynthetic analysis of ipomoeassin B

Retrosynthetic cleavage of O-C1 and O-C1′′ results in the protected disaccharide intermediate **AB** and the hydroxy acid building block **C**. Further cleavage of O-C1′ of disaccharide **AB** furnishes the glucose and fucose building blocks **A** and **B** respectively.

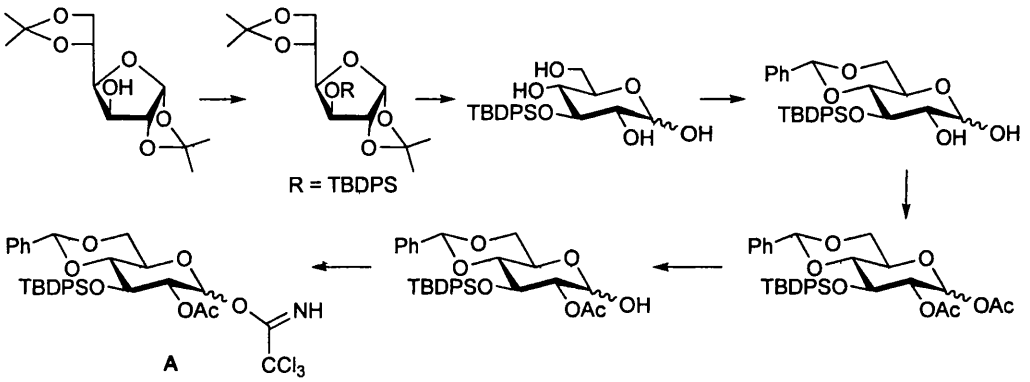
In the synthetic direction, the fucose building block **B** with the 2-OH free would be readily obtained by selective protection of the anomeric hydroxyl group with e.g. an allyl[155] or TBS ether.[141] Subsequent simultaneous protection of the 3-OH and 4-OH as the isopropylidene ketal[145] would yield the desired glycosyl acceptor representing building block **B**, as shown in Scheme 4.2.



Scheme 4.2: Suggested route for the synthesis of the glycosyl acceptor

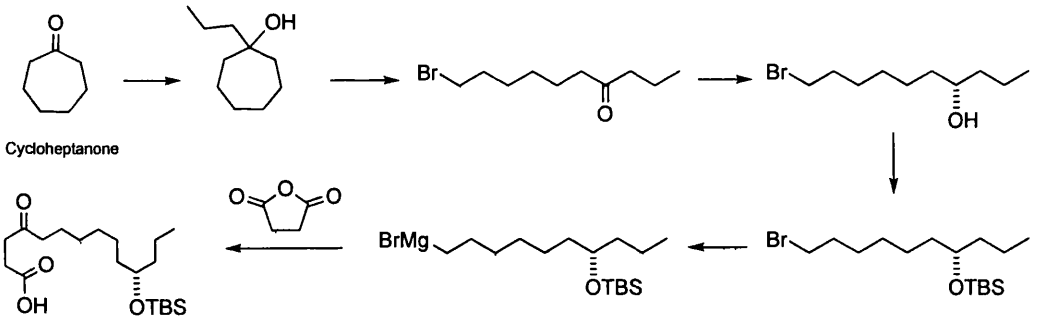
The glycosyl donor representing glucose building block **A** could be obtained from the commercially available 1,2:5,6-di-*O*-isopropylidene-*α*-D-glucofuranose. Protection of the free hydroxyl as the TBDPS ether and subsequent hydrolysis of the isopropylidene ketals would result in the 3-*O*-TBDPS-glucopyranose.[156]

Protection of the 4-OH and 6-OH as the benzylidene acetal[80] would be followed by acetylation of the remaining hydroxyl groups. Selective anomeric deprotection would unmask the anomeric hydroxyl group, which could then be converted to the glucosyl trichloroacetimidate representing building block **A** (Scheme 4.3).



Scheme 4.3: Suggested route for the synthesis of the glycosyl donor

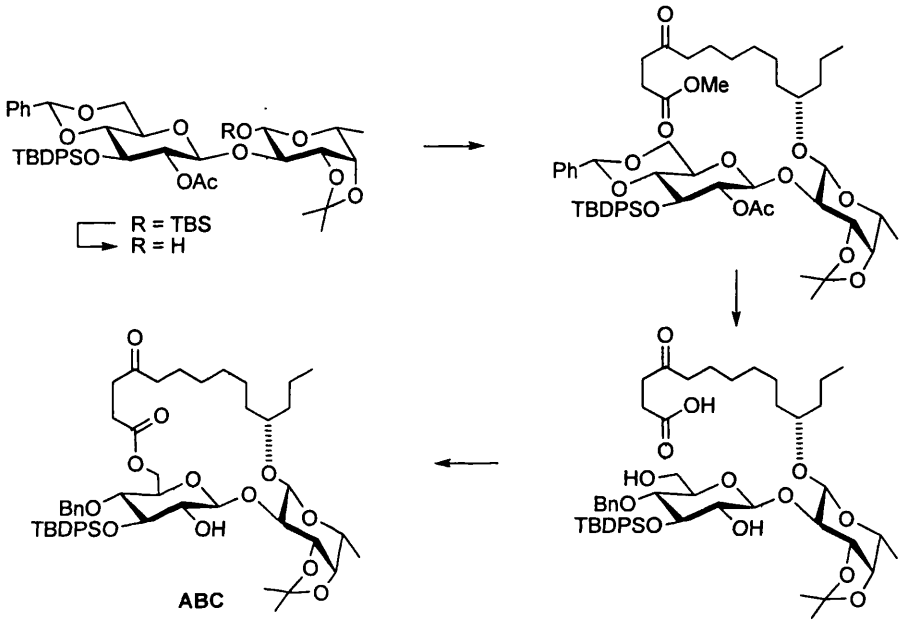
Building block **C** (Scheme 4.1) could be assembled from cycloheptanone via a Grignard reaction, followed by retro-Barbier fragmentation[157] to yield 10-bromodecan-4-one. Stereoselective reduction of the ketone,[158,159] TBS protection of the resulting hydroxyl and subsequent Grignard reaction with succinic anhydride[160,161] would give the acid intermediate as shown in Scheme 4.4. Protection of the acid as the methyl ester and TBS deprotection would furnish building block **C**.



Scheme 4.4: Suggested route for the synthesis of the building block **C**

Disaccharide **AB** would be obtained by glycosylation of glycosyl acceptor **B** with glycosyl donor **A**. The presence of the acetate ester at C-2 of glucose would ensure the stereoselective formation of the desired *β*-glycoside. Selective deprotection of the anomeric hydroxyl group of fucose and subsequent glycosylation with methyl (*S*)-11-hydroxy-4-oxotetradecanoate would yield the

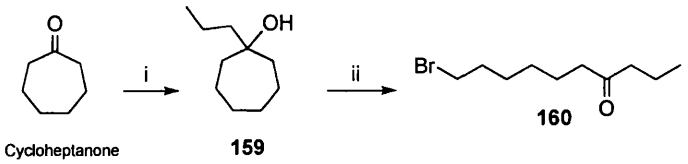
glycoside intermediate[140] shown in Scheme 4.5. Regioselective cleavage of the 4,6-*O*-benzylidene acetal would unmask the 6-OH required for the macrolactonisation step. Saponification of the ester protecting groups and subsequent macrolactonisation gives the macrolactone intermediate **ABC** as shown in Scheme 4.5. Removal of the protecting groups at C-3 and C-4 of glucose intermediate **ABC** and installation of various acyl side-chains results in a small library of ipomoeassin B analogues.



Scheme 4.5: Suggested route for the synthesis of the building block **ABC**

4.2.2. Synthesis of (*S*)-11-Hydroxy-4-oxotetradecanoic Acid

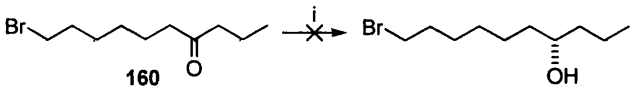
The proposed synthesis of (*S*)-11-hydroxy-4-oxotetradecanoic acid required the preparation of the (*S*)-10-bromodecan-4-ol precursor, which would be obtained by stereoselective reduction of 10-bromodecan-4-one. Commercially available cycloheptanone was subjected to Grignard reaction with propylmagnesium chloride to form intermediate **159** in high yield. Subsequent retro-Barbier fragmentation[157] resulted in ketone **160** as shown in Scheme 4.6.



Scheme 4.6: Synthesis of the ketone intermediate **160**

Reagents and conditions: (i) Propylmagnesium chloride, Et_2O, 0 °C, 15 min, 81%; (ii) Br_2, K_2CO_3, $CHCl_3$, 0 °C, 5 h, 50%.

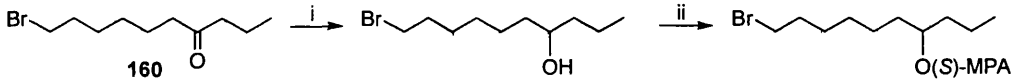
The stereoselective reduction of the ketone to the desired (*S*)-alcohol was attempted by two methods. The first method involved the yeast-catalysed reduction of **160** in the presence of sucrose (Scheme 4.7) according to a published procedure.[158] However, no product was obtained even after extended reaction time (7 days).



Scheme 4.7: Attempted yeast catalysed stereoselective reduction

Reagents and conditions: (i) Baker's yeast, sucrose, H_2O, 30 °C, 7 days.

The alternative approach involved borane-mediated enantioselective CBS (Corey-Bakshi-Shibata) reduction of **160**, which successfully yielded the alcohol (Scheme 4.8). This was then converted to the Mosher ester by reaction with the chiral derivatising agent (*S*)-methoxyphenylacetic acid ((*S*)-MPA) in the presence of EDCI and DMAP. Unfortunately, NMR analysis showed the presence of two methyl triplets at 0.71 ppm and 0.88 ppm, indicating that both stereoisomers had formed (~ 1:1).

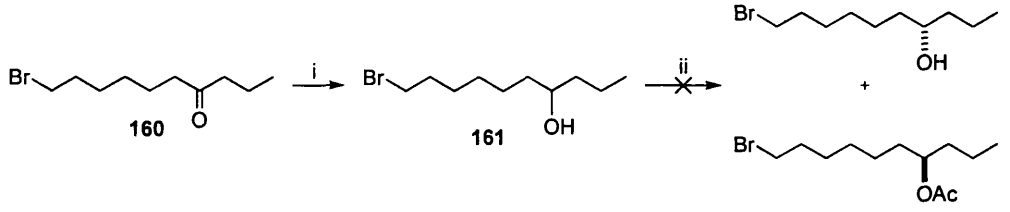


Scheme 4.8: Attempted enantioselective CBS reduction

Reagents and conditions: (i) CBS, catecholborane, toluene, -78 °C, 4 h, 56%; (ii) (*S*)-MPA, EDCI, DMAP, CH_2Cl_2, rt, 18 h.

Since the stereoselective reduction of the ketone was unsuccessful, we decided to reduce it non-selectively and attempt to resolve the resulting enantiomeric mixture. Therefore, the reduction was carried out with $NaBH_4$ to form alcohol **161** (Scheme 4.9), which proceeded smoothly and in good yield. Attempts to separate the stereoisomers by Novozym 435 catalysed kinetic resolution of the racemic mixture with vinyl acetate did not yield satisfactory results. This enzyme selectively acetylates the (*R*)-alcohol forming the (*R*)-acetate, which can then be separated from the (*S*)-alcohol by chromatography. Although Novozym 435 has been reported to work on a wide range of substrates[162] it might not be compatible with halogenated alcohols such as compound **161**. Therefore we decided to continue our synthesis with the racemic mixture and attempt to separate the

stereoisomers after glycosylation with the disaccharide, like Schmidt and co-workers did in their synthesis of calonyctin A1.[140]



Scheme 4.9: Non-stereoselective reduction of the ketone

Reagents and conditions: (i) $NaBH_4$, ethanol, 0 °C, 3 h, 68%; (ii) Novozym 435, vinyl acetate, 40 °C, 48 h.

Protection of the hydroxyl group of **161** was achieved by reaction of the alcohol with TBSCl in the presence of imidazole. Interestingly, NMR analysis of the product indicated the presence of a second minor product (**162**:**163** = 1:0.6), the only difference in the ^{1}H NMR spectrum being the shifts of the methylene protons next to the bromine atom (Table 4.1).

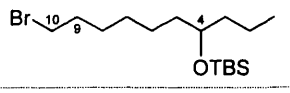
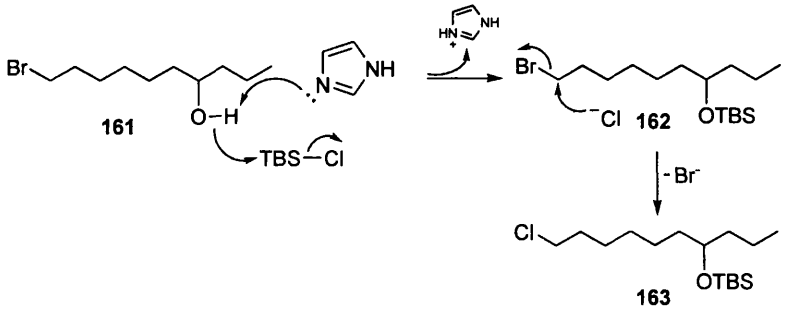
	^{1}H NMR shifts in ppm	
Compound	**162**	**163**
CH_2-10	3.40	3.53
CH_2-9	1.89-1.82	1.80-1.73

Table 4.1: ^{1}H NMR shifts of compounds **162** and **163**

These shifts suggested the presence of a compound in which the bromine atom had been replaced by a chlorine atom (**163**). A possible mechanism is shown in Scheme 4.10, in which the bromoalkane undergoes displacement by the chloride anion via an S_N2 route.



Scheme 4.10: Proposed mechanism of TBS protection and side-product formation

This assumption was confirmed by EI-MS analysis, which showed fragments at m/z 263/265 and 307/309 with the characteristic isotope-patterns for chlorine- and

bromine-substituted compounds, as shown in Figure 4.1, representing compounds **162** and **163** with loss of a propyl radical.

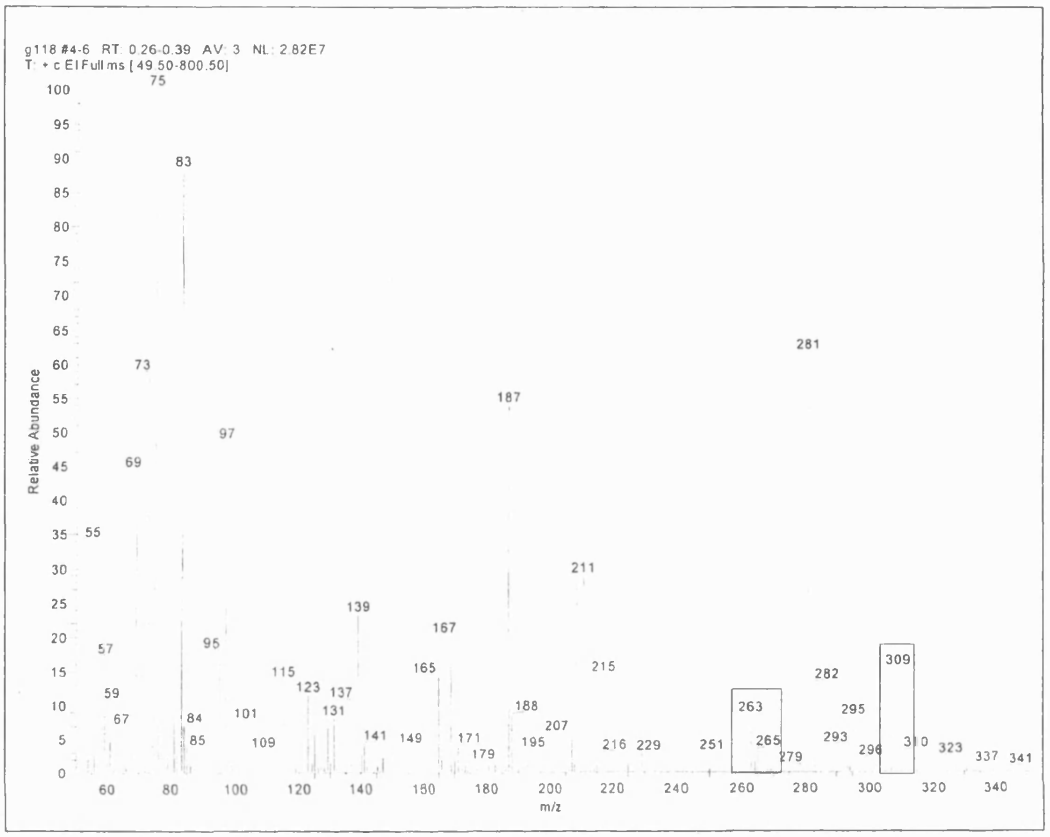
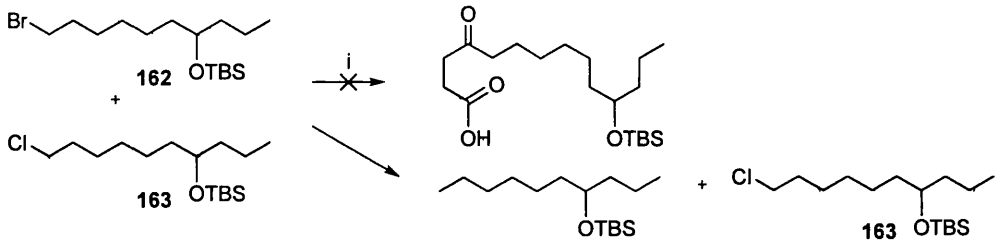


Figure 4.1: Mass spectrum of **162/163**

The next step towards building block **C** was the coupling of intermediate **162/163** with succinic anhydride. Since Grignard reagents can equally well be formed form bromo- and chloroalkanes it was not anticipated that the presence of chloroalkane **163** would pose a problem. The mixture **162/163** was therefore converted to the corresponding magnesium halide Grignard reagents by reaction with magnesium turnings, which had been activated by addition of a small amount of 1,2-dibromoethane.[153] The reaction mixture became milky white, implying the successful formation of the Grignard reagent. A solution of succinic anhydride in THF was added dropwise to the solution of the Grignard reagent (Scheme 4.11). Unfortunately, this reaction was unsuccessful. From the ^{1}H NMR spectra of the products it could be concluded that, while bromoalkane **162** was successfully converted to the Grignard reagent it was not sufficiently reactive to react with succinic anhydride and instead hydrolysed to the alkane after aqueous work-up.

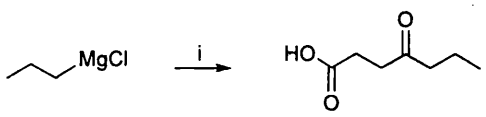
Chloroalkane **163** on the other hand failed to form the Grignard reagent and was recovered after aqueous work-up and purification by flash chromatography.



Scheme 4.11: Attempted Grignard reaction with succinic anhydride

Reagents and conditions: (i) (a) Mg, 1,2-dibromoethane (cat.), Et_2O, rt, 3 h; (b) succinic anhydride, THF, -30 °C, 1 h.

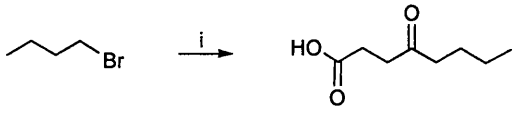
To test this theory two model reactions were performed. The first one involved reaction of the commercially-available propylmagnesium chloride with succinic anhydride (Scheme 4.12). This resulted in the successful formation of the desired product as judged by TLC and NMR. It was observed that this reaction was successful only when the Grignard reagent was added dropwise to succinic anhydride and not the other way around.



Scheme 4.12: Model reaction I

Reagents and conditions: (i) Succinic anhydride, THF, -78 °C, 3 h.

The second test reaction involved formation of the Grignard reagent by addition of bromobutane to magnesium turnings and subsequent reaction with succinic anhydride (Scheme 4.13). TLC and NMR analysis of the crude reaction mixture also indicated the presence of the desired product. However, when the reaction was repeated with compounds **162/163** employing identical conditions as in the test reactions, the outcome was unsuccessful as before.

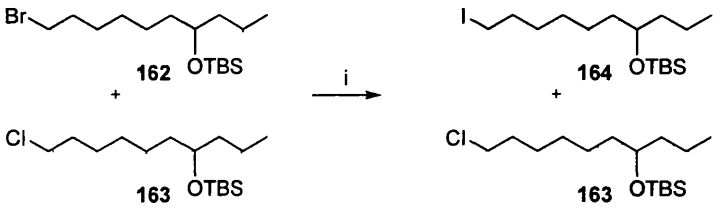


Scheme 4.13: Model reaction II

Reagents and conditions: (i) (a) Mg, Et_2O, rt, 2 h; (b) succinic anhydride, THF, -78 °C, 2 h.

Next, we attempted to convert the bromo- and chloroalkane mixture **162/163** to the iodoalkane, since iodo derivatives are known to be more reactive towards

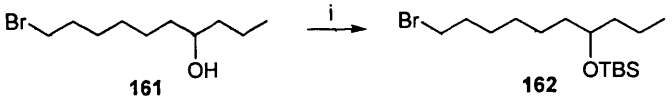
Grignard reactions. Surprisingly, reaction with NaI resulted in another mixture, namely of the iodo- and chloroalkane (1:0.6) (Scheme 4.14), according to analysis of the ^{1}H NMR spectrum, which showed a tripled at 3.19 ppm corresponding to the methylene protons next to the iodine atom and another triplet at 3.53 ppm corresponding to the methylene protons next to the chlorine atom.



Scheme 4.14: Attempted conversion of mixture **162/163** to the iodoalkane

Reagents and conditions: (i) NaI, acetone, reflux, 1 h.

In order to overcome this problem, protection of the alcohol **161** was carried out with TBSOTf instead of TBSCl (Scheme 4.15) and a single product (**162**) was obtained, which further confirms the aforementioned assumption that displacement of the bromine by the chloride anion took place.



Scheme 4.15: TBS protection of alcohol **161**

Reagents and conditions: (i) TBSOTf, imidazole, DMF, rt, 18 h, 94%.

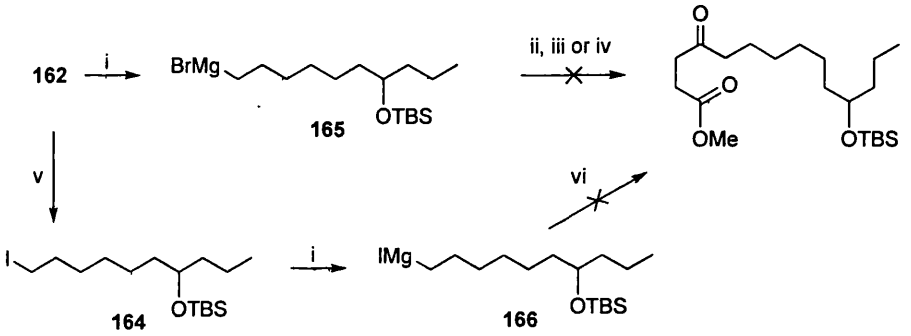
Grignard reaction of the pure bromoalkane **162** with succinic anhydride was then attempted one more time (Scheme 4.16); however, no product could be obtained and a small amount of starting material was recovered. Succinic anhydride was then replaced by the more reactive methyl succinyl chloride. Several reaction conditions and procedures were investigated.

Compound **162** was converted to the more reactive iodo derivative **164** by reaction with NaI and reacted with methyl succinyl chloride after formation of the Grignard reagent **166** (Scheme 4.16). However, after work-up of the reaction mixture only recovered starting material and a compound, which appeared to be the hydrolysed Grignard reagent, was obtained.

Compound **165** was also subjected to reaction with methyl succinyl chloride in the presence of catalytic amounts of iron(III) acetylacetonate ($Fe(acac)_3$) according to

an established method (Scheme 4.16).[163] These reaction conditions resulted in loss of the TBS ether. Further characterisation of the product, however, was not possible.

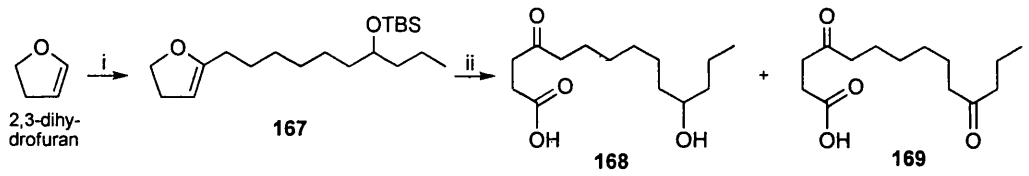
Alternatively, methyl succinyl chloride was converted to the aldehyde by reaction with triethylsilane (Et_3SiH) in the presence of Pd/C and reacted with Grignard reagent **165** (Scheme 4.16). Unfortunately only the hydrolysis product of the Grignard reagent was obtained.



Scheme 4.16: Attempts towards the synthesis of building block **C**

Reagents and conditions: (i) Mg, 1,2-dibromoethane (cat.), Et_2O, rt, 2 h; (ii) succinic anhydride, THF, -78 °C, 2 h; (iii) methyl succinyl chloride, $Fe(acac)_3$, THF, -78 °C 15 min; (iv) methyl succinaldehyde, Et_2O, 0 °C → rt, 18 h; (v) NaI, acetone, reflux, 3 h, 83%; (vi) methyl succinyl chloride, Et_2O, -78 °C, 2 h.

Since the approach involving the Grignard reagent was not successful an alternative method was employed. This involved the reaction of 2,3-dihydrofuran with *t*-BuLi and subsequent addition of the iodo-derivative **164** to the resulting solution (bromide derivatives have been reported to be much less reactive).[164,165] After stirring for 1 h, TLC indicated the formation of a new product (**167**). The reaction was quenched and after aqueous work-up the resulting compound was subjected to Jones oxidation and hydrolysis without further purification (Scheme 4.17). Unfortunately the silyl group was insufficiently stable to withstand the relatively harsh oxidation conditions, resulting in oxidation of the alcohol **168** to ketone **169**. The ketone **169**, which would be impossible to regioselectively reduce to the alcohol **168**, was obtained as the major product together with a small amount of the desired 11-hydroxy-4-oxotetradecanoic acid (**168**) (Scheme 4.17). Although this approach gave poor yields of the desired acid due to the incompatibility of the TBS protecting group, the results were encouraging, since varying the alcohol protecting group could potentially afford the desired product in improved yields.



Scheme 4.17: Synthesis of 11-hydroxy-4-oxotetradecanoic acid

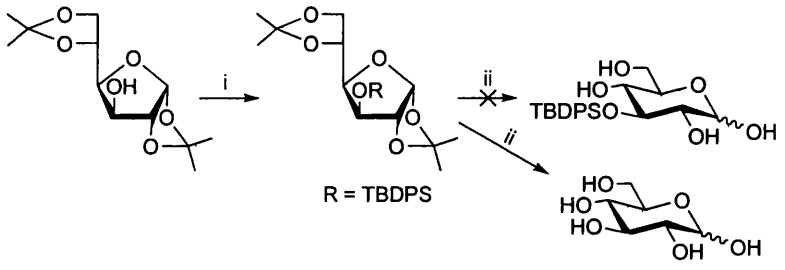
Reagents and conditions: (i) (a) *t*-BuLi, THF, -78 °C → 0 °C, 30 min; (b) **164**, THF, -78 °C → rt, 1 h; (ii) Jones' reagent (H_2CrO_4), THF, 0 °C → rt, 18 h, 11% for **168**, 20% for **169**.

In parallel to these synthetic efforts, which culminated in the synthesis of **168**, albeit in low yields, the synthesis of the disaccharide was also under investigation. At this point, it was decided to substitute 11-hydroxy-4-oxotetradecanoic acid with the simpler and commercially available 11-hydroxyundecanoic acid and return to the study of alternative protecting strategies should time permit.

4.2.3. Synthesis of the Disaccharide

Synthesis of the glycosyl donor (Glucose building block A)

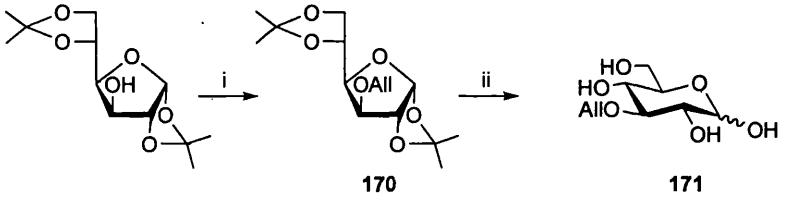
Our synthetic approach started with commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, which allowed the protection of the remaining free 3-OH. After removal of the isopropylidene groups the sugar reverts to the pyranose conformation with only the 3-OH protected.[156] The first choice of protecting group for the hydroxyl group at C-3 was the TBDPS ether. However, deprotection of the isopropylidene groups proved quite difficult. Although the 5,6-isopropylidene group could be readily removed under mild acidic conditions, the 1,2-isopropylidene group was found to be more stable and much harsher conditions (refluxing in acid) were needed for its removal; conditions that led to the concomitant removal of the TBDPS ether (Scheme 4.18). Therefore, the TBDPS protecting group was replaced with the more acid-stable allyl group.



Scheme 4.18: Attempted synthesis of the glycosyl donor

Reagents and conditions: (i) TBDPSCl, imidazole, DMF, rt, 93%; (ii) Amberlite IR 120 (H^+ form) ion exchange resin, H_2O:dioxane 1:1, reflux.

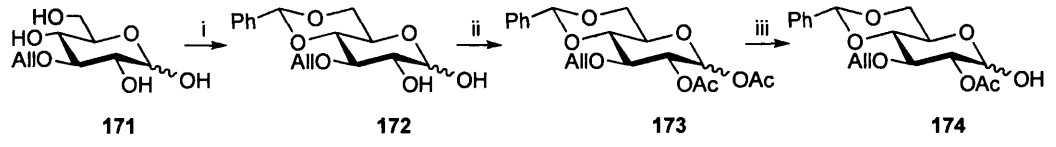
Allyl protection of 1,2:5,6-di-*O*-isopropylidene-*α*-D-glucofuranose proceeded smoothly and in very good yield. Several conditions were investigated for the successful removal of the isopropylidene groups including 50% trifluoroacetic acid (TFA) in $CHCl_3$, refluxing in a 1:1 (*v/v*) mixture of 1-10% aqueous HCl and dioxane, refluxing in 5% aqueous H_2SO_4/dioxane 1:1 (*v/v*) and refluxing in dioxane/H_2O in the presence of H^+ ion exchange resin. The latter conditions were found to be the most efficient and high yielding, forming the 3-*O*-allyl glucopyranose derivative in 76% yield (Scheme 4.19).



Scheme 4.19: Synthesis of the glycosyl donor precursor I

Reagents and conditions: (i) NaH, AllBr, DMF, rt, 1 h, 94%; (ii) Amberlite IR 120 (H^+ form) ion exchange resin, H_2O/dioxane 1:1, 100 °C, 18 h, 76%.

Simultaneous protection of the 4-OH and 6-OH of intermediate **171** was achieved by reaction with benzaldehyde dimethyl acetal in the presence of PTSA forming the 3-*O*-allyl-4,6-*O*-benzylidene glucopyranose **172**. Subsequent acetylation of the remaining hydroxyl groups with acetic anhydride in pyridine resulted in diacetate **173**. Selective anomeric deprotection with NH_2NH_2·AcOH failed to proceed to completion, but longer reaction times resulted in the formation of a more polar by-product, most likely the diol, as could be judged by TLC, resulting in very poor yields (26%) of **174**. The yield could be significantly improved when deprotection was carried out with $BnNH_2$ (Scheme 4.20).

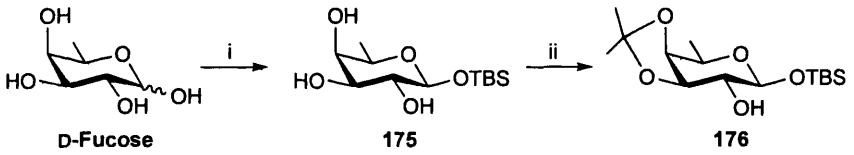


Scheme 4.20: Synthesis of the glycosyl donor precursor II

Reagents and conditions: (i) $C_6H_5CH(OCH_3)_2$, PTSA, DMF, 70 °C, 18 h, 67%; (ii) Ac_2O, pyridine, rt, 18 h, 97%; (iii) (a) $BnNH_2$, THF; rt, 18 h; (b) 1 M HCl, 61%.

Synthesis of the glycosyl acceptor (Fucose building block B)

The synthesis of the glycosyl acceptor **176** was achieved in two steps from D-fucose, as shown in Scheme 4.21. The first step involved the selective protection of the anomeric position as the *O*-TBS ether. Although the anomeric hydroxyl group is more reactive compared to the other hydroxyls, the undesired formation of multiply-protected by-products was unavoidable resulting in lower yields of **175**. Several conditions were investigated including lowering the temperature (-10 °C), varying the solvent (DMF or CH_2Cl_2) and using different bases (imidazole or Et_3N/DMAP). The best results were obtained by reaction of D-fucose with TBSCl in CH_2Cl_2 in the presence of imidazole at room temperature (Scheme 4.21). Interestingly, only the β-anomer (J = 6.8 Hz) was obtained, as judged by the ^1H NMR spectrum, probably because the bulky TBS group prefers the more stable equatorial position.



D-Fucose 175 176

Scheme 4.21: Synthesis of the glycosyl acceptor (building block **B**)

Reagents and conditions: (i) TBSCl, imidazole, CH_2Cl_2, rt, 18 h, 43%; (ii) $(CH_3)_2CH(OCH_3)_2$, CSA, acetone, rt, 18 h, 88%.

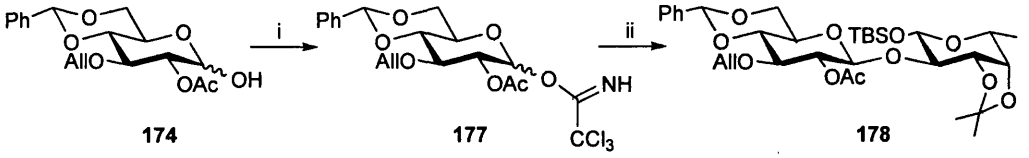
Reaction of the silylated fucose derivative **175** with 2,2-dimethoxypropane in acetone in the presence of CSA resulted in the simultaneous protection of the *cis*-oriented 3-OH and 4-OH as the isopropylidene ketal, yielding glycosyl acceptor **176** in high yield (Scheme 4.21).

Synthesis of the disaccharide (Building block AB)

With the glycosyl donor precursor **174** and glycosyl acceptor **176** in hand the next task was to form the disaccharide. The glucosyl intermediate **174** was first converted to the trichloroacetimidate **177** by reaction with trichloroacetonitrile in the presence of Cs_2CO_3 (Scheme 4.22). Attempted purification by flash chromatography led to significant loss of product, due to hydrolysis of the trichloroacetimidate to the alcohol. Purification was therefore limited to filtration through a short silica pad, yielding glycosyl donor **177**.

Treatment of alcohol **176** with trichloroacetimidate **177** in the presence of TMSOTf furnished the desired disaccharide **178** in 82% yield (Scheme 4.22). Due

to the presence of the acetate ester at the C-2 hydroxyl of glucose (neighbouring group participation) the β-glycoside (J = 7.5 Hz) was obtained exclusively, according to the ^{1}H NMR spectrum, in which only one compound could be detected.

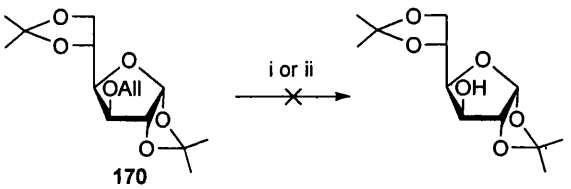


Scheme 4.22: Synthesis of the disaccharide **178** (Building block **AB**)

Reagents and conditions: (i) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 18 h; (ii) **176**, TMSOTf, CH_2Cl_2, -20 °C, 1 h, 82%.

Before continuing the synthesis towards the target compound we decided to investigate the optimal conditions for the allyl deprotection. Allyl protecting groups are notorious in terms of the unpredictability of their removal, often not proceeding to completion under a variety of conditions in a highly substrate-specific manner.[166,167] Since the removal of the allyl group would be one of the last steps of the synthesis we wanted to ensure that it could be achieved under mild conditions, with minimal effect on the remainder of the molecule. Therefore a series of small-scale investigations were carried out, first using allyl ether **170** as a model substrate.

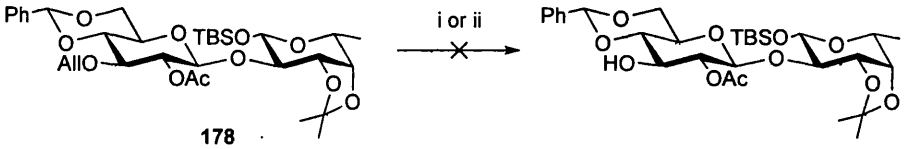
The first conditions examined involved treatment of allyl ether **170** with Pd/C in methanol in the presence of catalytic amounts of PPTS at reflux (Scheme 4.23). This protocol has been reported to give better results than the two step (isomerisation-cleavage) deprotection and has been successfully employed in allyl deprotections in the presence of benzyl ethers.[168,169] Unfortunately, in our case, these deprotection conditions resulted in decomposition of the starting material.



Scheme 4.23: Attempted allyl deprotection I

Reagents and conditions: (i) Pd/C, PPTS cat., MeOH, reflux, 24 h; (ii) $Ti(O\text{-}i\text{-}Pr)_4$, $cC_6H_{11}MgCl$, THF, rt, 1 h.

Next, the two-step deprotection approach was employed, involving isomerisation of the allyl ether of disaccharide **178** to the vinyl ether with *t*-BuOK and subsequent cleavage with $HgO/HgCl_2$ (Scheme 4.24). However, these harsh conditions (heating at 100 °C in DMSO in the presence of *t*-BuOK) resulted in decomposition of the starting material according to TLC analysis, to give several uncharacterised by-products.



Scheme 4.24: Attempted allyl deprotection II

Reagents and conditions: (i) (a) *t*-BuOK, DMSO, 100 °C, 7 h; (b) HgO, $HgCl_2$, acetone/H_2O (10:1), rt, 18 h; (ii) Ti(O-*i*-Pr)$_4$, $cC_6H_{11}MgCl$, THF, rt, 1 h.

Finally, another one-step deprotection involving titanium(IV) isopropoxide Ti(O-*i*-Pr)$_4$ in the presence of cyclohexylmagnesium chloride[170] was carried out with allyl ether **170** and disaccharide **178** (Scheme 4.23 and Scheme 4.24 respectively). These mild reaction conditions are reportedly compatible with a wide range of protecting groups including benzylidene acetals, acetates and silyl ethers.[170] However, no reaction occurred and only starting material was recovered. In view of this outcome we decided to modify our synthetic approach, thereby limiting the potential for highly undesirable late-stage failure in the synthesis of the natural product.

4.2.4. Summary

Racemic 11-hydroxy-4-oxotetradecanoic acid was successfully synthesised from cycloheptanone in seven steps and 2.4% overall yield. The low yield is due to the incompatibility of the TBS protecting group with the oxidation conditions employed, which resulted in loss of the TBS ether and oxidation of the alcohol to the ketone, which was obtained as the main product. Using alternative protecting groups could potentially produce the desired fatty acid in improved yields.

The synthesis of disaccharide subunit **178** has been achieved by glycosylation of glycosyl donor **177** and glycosyl acceptor **176** using the trichloroacetimidate method in 82% yield. Glycosyl donor precursor **174** was assembled from commercially available 1,2:5,6-di-*O*-isopropylidene-*α*-D-glucofuranose in five steps and 28% overall yield. Glycosyl acceptor **176** was obtained from D-fucose in

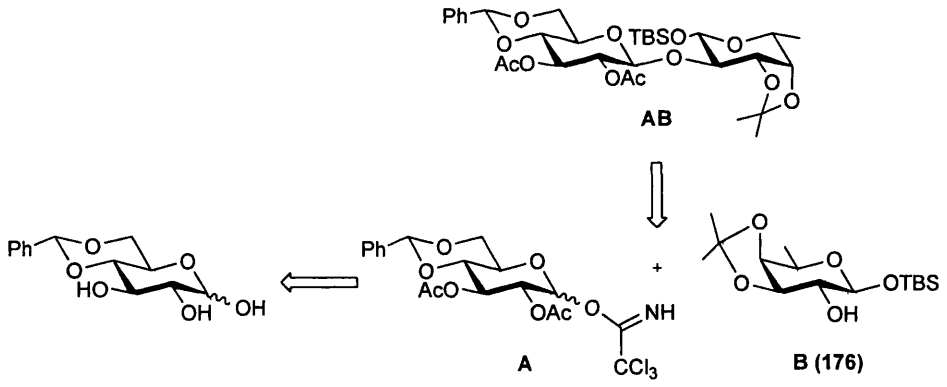
two steps and 38% overall yield. However, due to difficulties encountered in the allyl deprotection our synthetic approach had to be modified as a precaution against subsequent late-stage failure.

4.3. Second Approach

During the synthesis of tricolorins (Section 3.2) it had been observed that the 3-OH in glucose in the glucose-fucose disaccharide was more reactive than the 2-OH and selective acylation could be achieved with high regioselectivity.[145,146] Encouraged by this observation we decided to modify our synthetic approach by replacing the allyl protecting group with an acetate group, which would result in disaccharide building block **AB** as shown in Scheme 4.25. This approach has the advantage over the previous one in that fewer protecting groups are used, which in turn means fewer steps and therefore a potentially higher overall yield.

4.3.1. Retrosynthetic Analysis

Retrosynthetic analysis demonstrated that disaccharide **AB** could be obtained from building block **A** and glycosyl acceptor **176** which had been synthesised in the previous approach. The glycosyl donor **A** could be readily obtained from commercially available 4,6-*O*-benzylidene-D-glucose in three steps (Scheme 4.25). Disaccharide **AB** could then be formed by the trichloroacetimidate glycosylation method since this method had worked well in the assembly of disaccharide **178**.

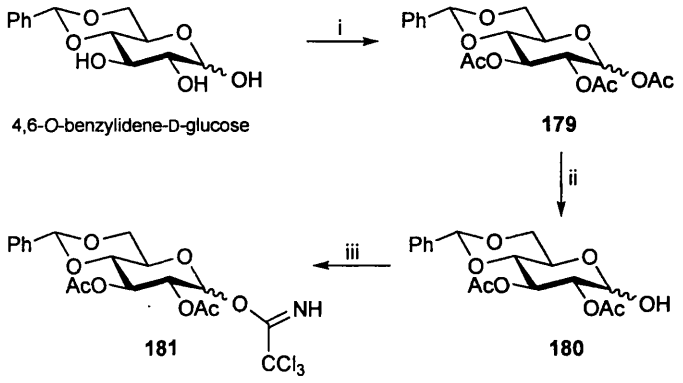


Scheme 4.25: Retrosynthetic analysis of disaccharide **AB**

4.3.2. Synthesis of the Glycosyl Donor (Building Block A)

Building block **A** was synthesised as described previously in the synthesis of tricolorin A (Section 3.2.1).[145] The commercially available 4,6-*O*-benzylidene-D-glucopyranose was acetylated with acetic anhydride in pyridine. Subsequent anomeric deprotection with $BnNH_2$ yielded intermediate **180** in 72% over two steps. Activation of compound **180** was achieved by treatment with trichloro-

acetonitrile in the presence of Cs_2CO_3 (Scheme 4.26) and glycosyl donor **181** (building block **A**) was obtained after filtration through a short pad of silica and could be used for glycosylation without further purification.

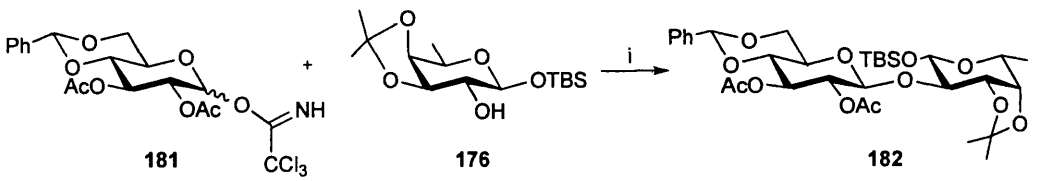


Scheme 4.26: Synthesis of the glycosyl donor (building block **A**)

Reagents and conditions: (i) Ac_2O, pyridine, rt, 1 h; (ii) (a) $BnNH_2$, THF, rt, 18 h; (b) 1 M HCl, rt, 72% over two steps; (iii) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 18 h.

4.3.3. Synthesis of the Disaccharide (Building Block AB)

With the crude trichloroacetimidate **181** and glycosyl acceptor **176** in hand, glycosylation was carried out in the presence of TMSOTf and disaccharide **182** obtained in 60% yield (Scheme 4.27). However, no product could be obtained when the reaction was repeated on a multigram scale, and a significant amount of imidate **181** was recovered after purification by flash chromatography. It seemed likely that some residual impurity present in the crude imidate was reacting with the Lewis acid catalyst thereby hindering formation of the glycoside. Subsequent use of the recovered (pure) imidate **181** resulted in successful glycosylation.



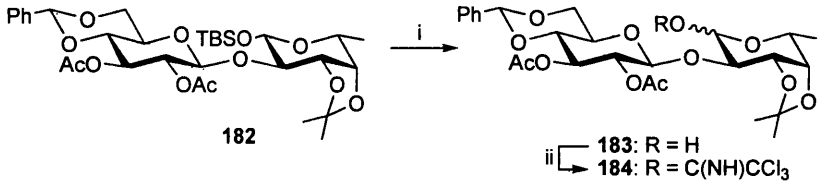
Scheme 4.27: Synthesis of the disaccharide **182** (Building block **AB**)

Reagents and conditions: (i) TMSOTf, CH_2Cl_2, -20 °C, 1 h, 60%.

4.3.4. Attempts Towards the Synthesis of the Macrolactone

After successful synthesis of disaccharide **182**, the next task towards the synthesis of the macrolactone was the incorporation of the long fatty acid side-chain. For this purpose, the anomeric hydroxyl group of fucose was unmasked by treatment

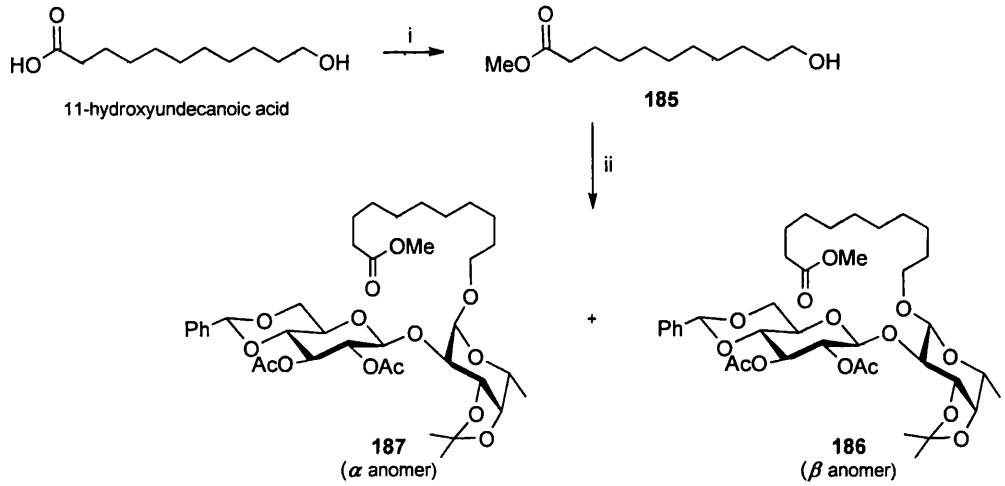
with TBAF in the presence of acetic acid, yielding disaccharide **183** as a mixture of anomers. Subsequent activation by reaction with trichloroacetonitrile in the presence of Cs_2CO_3 yielded glycosyl donor **184** (Scheme 4.28), which was filtered through a short pad of silica and used without further purification.



Scheme 4.28: Synthesis of glycosyl donor **184**

Reagents and conditions: (i) TBAF, AcOH, THF, rt, 2 h, 64%, (α:β = 60:40)
(ii) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 18 h.

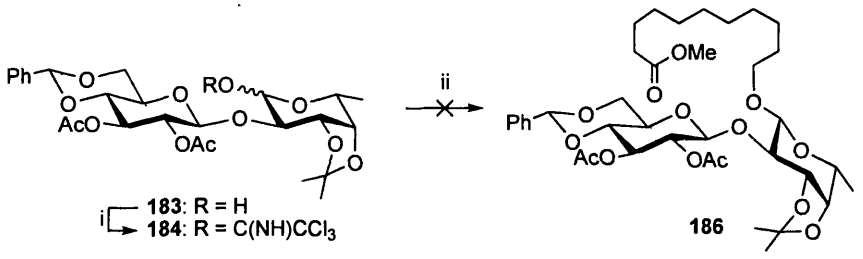
The simpler, commercially available 11-hydroxyundecanoic acid, which was used in place of 11-hydroxy-4-oxotetradecanoic acid in order to explore the feasibility of this approach, was protected as the methyl ester (**185**) by treatment with H^+ ion exchange resin in methanol. It then underwent glycosylation with the crude glycosyl donor **184** to yield glycosides **186** ($J_{H1'\text{-}H2'}$ = 8.1 Hz) and **187** ($J_{H1'\text{-}H2'}$ = 3.3 Hz) as a mixture of anomers (α:β = 2:1) (Scheme 4.29), which could be separated by conventional flash chromatography. Unfortunately, the desired β-glycoside was obtained as the minor product, since there was no neighbouring participating group at C-2 of fucose to drive the reaction into the formation of the desired β-glycoside.



Scheme 4.29: Synthesis of glycosides **186** and **187**

Reagents and conditions: (i) Amberlite IR 120 (H^+ form) ion exchange resin, MeOH, rt, 48 h, 97%; (ii) **184**, TMSOTf, CH_2Cl_2, -20 °C, 1 h, 59% of α anomer, 27% of β anomer (α:β = 2:1).

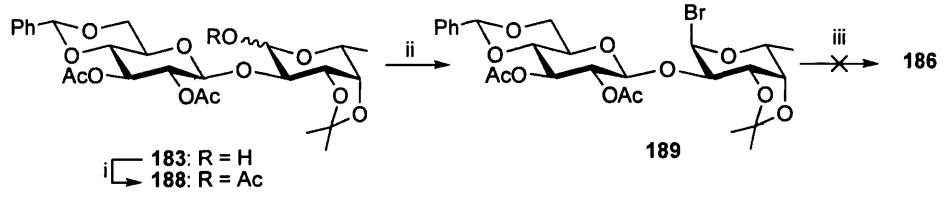
Since the yield of the desired β-glycoside was not satisfactory an attempt was made to improve it by modifying the trichloroacetimidate formation and glycosylation conditions. Therefore, trichloroacetimidate **184** was formed by reaction of **183** with trichloroacetonitrile in the presence of NaH, which favours the formation of the α-anomer. The crude trichloroacetimidate was then subjected to glycosylation with **185** in the presence of $BF_3 \cdot Et_2O$. Glycosylation under these conditions proceeds via S_N2 mechanism and thus the β-glycoside is usually formed from the α-trichloroacetimidate. However, no product was obtained under these glycosylation conditions (Scheme 4.30).



Scheme 4.30: Attempted synthesis of the β-glycoside **186** I

Reagents and conditions: (i) CCl_3CN, NaH, CH_2Cl_2, 0 °C → rt, 18 h; (ii) **185**, $BF_3 \cdot Et_2O$, CH_2Cl_2, 0 °C → rt, 30 min.

As an alternative, glycosylation by the Koenigs-Knorr method was also attempted. For this purpose, disaccharide **183** was acetylated with acetic anhydride in pyridine and then converted to the glycosyl bromide **189** by reaction with HBr in acetic anhydride. Glycosylation of alcohol **185** with the glycosyl bromide was carried out in the presence of AgOTf and *sym*-collidine, the latter acting as an acid scavenger (Scheme 4.31). Unfortunately, no product could be isolated after purification by flash chromatography and only alcohol **185** was recovered.



Scheme 4.31: Attempted synthesis of the β-glycoside **186** II

Reagents and conditions: (i) Ac_2O, pyridine, rt, 1 h; (ii) HBr (45% in AcOH), Ac_2O, CH_2Cl_2, rt, 1 h; (iii) **185**, AgOTf, *sym*-collidine, CH_2Cl_2, rt, 18 h.

It is possible that the anomeric position of fucose is sterically congested due to the bulky glycosyl substituent at the 2-OH. This makes the β-glycosylation less favourable and therefore favours formation of the α-glycoside. Although the desired β-glycoside could be obtained, the yield was not satisfactory, since the

major compound formed was the α-glycoside. Thus, the synthetic approach was modified in order to allow earlier stage incorporation of the acid side-chain **185** in a stereoselective manner.

4.3.5. Summary

In the second approach, the difficult-to-remove allyl ether at the 3-OH of glucose was replaced by a more readily-cleaved acetate ester. This had also the advantage that the number of steps required for the synthesis of the glycosyl donor **181** was reduced from six in the first approach to three in the second, with an accompanying improvement in the overall yield to 72%. Glycosylation with glycosyl acceptor **176** proceeded smoothly yielding disaccharide **182**, which was converted to glycosyl donor **184** by silyl deprotection and reaction with trichloroacetonitrile.

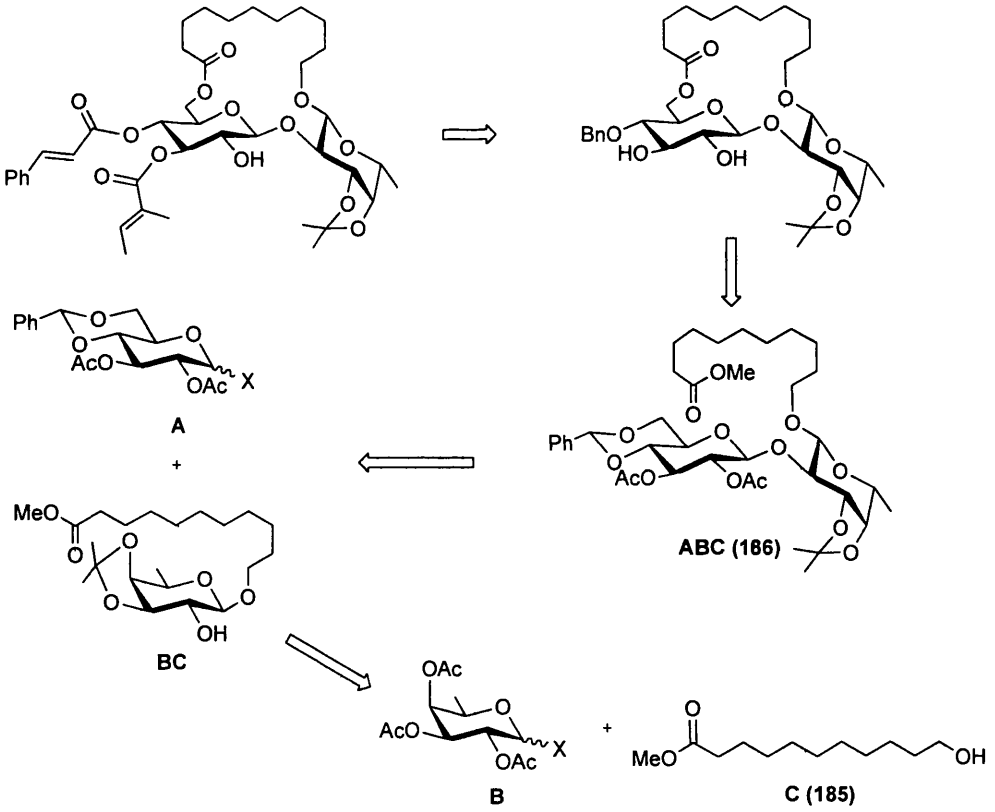
The commercially-available 11-hydroxyundecanoic acid was protected as the methyl ester and subjected to glycosylation with trichloroacetimidate **184** yielding glycosides **186** and **187** as a mixture of anomers (α:β = 2:1). Unfortunately, the desired β-glycoside was obtained as the minor product. Despite the fact that the anomers could be separated by conventional flash chromatography, the yield was not satisfactory. Therefore an alternative approach was sought, where the protected fatty acid side-chain could be introduced stereoselectively.

4.4. Third Approach

The second approach failed to produce the desired β-glycoside in acceptable yield. Therefore, another modification was required that would result in the stereoselective formation of the β-glycoside. As discussed in Section 2.2.1, the best way to achieve β-stereoselectivity is by the presence of a neighbouring participating group at the C-2 hydroxyl of fucose. This means that the acid side-chain must be installed prior to formation of the disaccharide. This approach, however, has the disadvantage that the early installation of the side-chain hardly allows for the convenient synthesis of analogues with differing ring sizes, since each analogue would require an independent multistep synthesis.

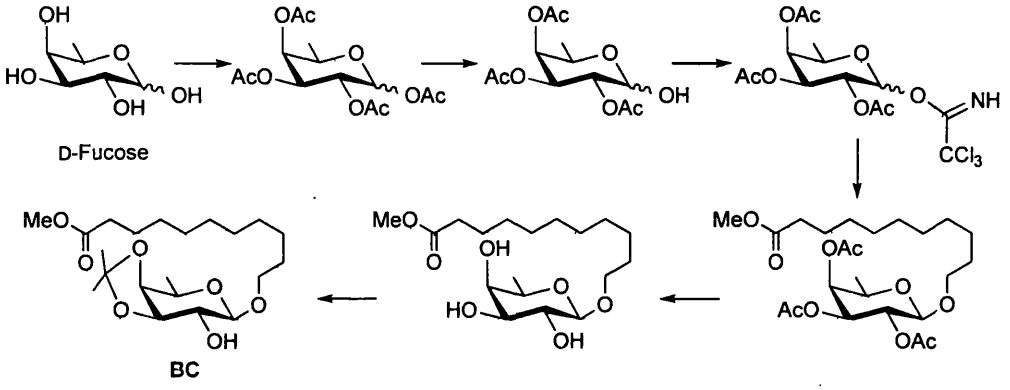
4.4.1. Retrosynthetic Analysis

Retrosynthetic analysis of glycoside **186** (building block **ABC**) demonstrated that it could be obtained from glycosyl donor **181** (**A**: X = $OC(NH)CCl_3$) and building block **BC**. Further retrosynthetic cleavage at the anomeric position of building block **BC** resulted in glycosyl donor **B** and the ω-hydroxy ester synthon **C** (Scheme 4.32).



Scheme 4.32: Retrosynthetic analysis of glycoside **ABC**

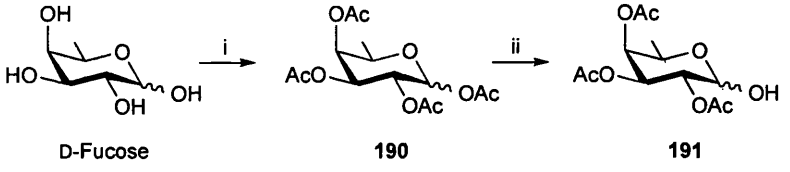
In the synthetic direction, the readily accessible building block **B** would undergo stereoselective glycosylation with alcohol **185** to form the β-glycoside, due to the presence of an acetate group at C-2 of fucose. The resulting glycoside could then be converted to synthon **BC** after a few simple protecting group manipulations (Scheme 4.33).



Scheme 4.33: Suggested route for the synthesis of the glycosyl acceptor

4.4.2. Synthesis of Building Block BC

The synthesis of building block **BC** was achieved in six steps from D-fucose. The first step involved the conversion of D-fucose to the per-*O*-acetylated derivative **190** by reaction with acetic anhydride in pyridine. Subsequent anomeric deprotection with $BnNH_2$ yielded glycosyl donor precursor **191** as shown in Scheme 4.34.

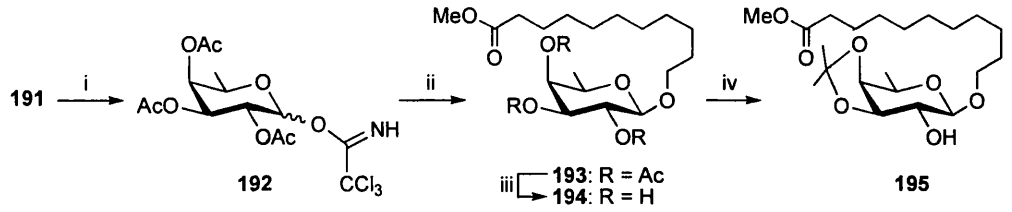


Scheme 4.34: Synthesis of the glycosyl donor precursor **191**

Reagents and conditions: (i) Ac_2O, pyridine, rt, 18 h; (ii) (a) $BnNH_2$, THF, rt, 18 h; (b) 1 M HCl, 76% over two steps.

Reaction of the glycosyl donor precursor **191** with trichloroacetonitrile in the presence of Cs_2CO_3 yielded trichloroacetimidate **192** which was used directly in the next step. Glycosylation of alcohol **185** with glycosyl donor **192** in the presence of TMSOTf proceeded smoothly and yielded the desired β-glycoside **193** (J = 7.9 Hz) as the only product in 81% yield. No signals corresponding to the α-glycoside were present in the ^{1}H NMR spectrum. Subsequent acetate removal with NaOMe and protection of the 3-OH and 4-OH of fucose as the

isopropylidene ketal resulted in intermediate **195**, representing synthon **BC** (Scheme 4.35).

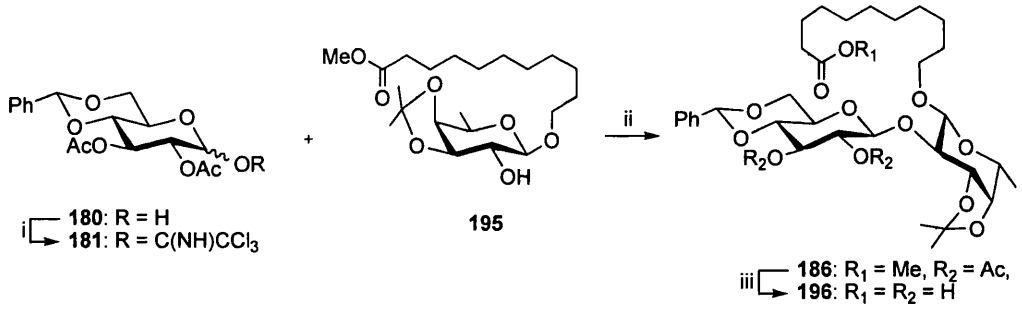


Scheme 4.35: Synthesis of glycosyl acceptor **195** (building block **BC**)

Reagents and conditions: (i) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 18 h; (ii) **185**, TMSOTf, CH_2Cl_2, -20 °C, 1 h, 81%; (iii) (a) NaOMe, MeOH, rt, 15 min; (b) Amberlite IR 120 (H^+ form) ion exchange resin; (iv) $(CH_3)_2CH(OCH_3)_2$, CSA, acetone, rt, 18 h, 85% over two steps.

4.4.3. Synthesis of Building Block ABC

The next task towards the synthesis of the target compound was the formation of disaccharide **186**. For this purpose the previously synthesised glycosyl donor **181**, freshly prepared from glucose derivative **180**, was used for the glycosylation of alcohol **195** in the presence of TMSOTf. The desired β-disaccharide **186** (J = 7.7 Hz) was obtained as the only product in 77% yield, as could be concluded from the absence of signals corresponding to the α-disaccharide in the ^{1}H NMR spectrum. Subsequent saponification of the esters with LiOH furnished intermediate **196** (Scheme 4.36).



Scheme 4.36: Synthesis of the disaccharide **196**

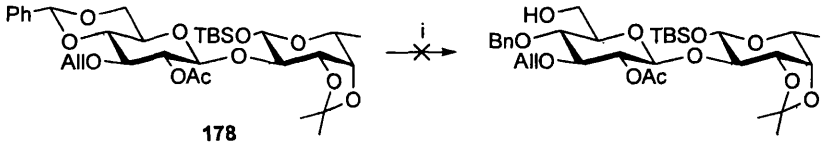
Reagents and conditions: (i) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 18 h; (ii) TMSOTf, CH_2Cl_2, -20 °C, 2 h, 81%; (iii) LiOH, THF, MeOH, rt, 15 h, 98%.

4.4.4. Attempts Towards the Synthesis of the Macrolactone

With disaccharide **196** in hand, we sought to construct the macrolactone by intramolecular esterification of the primary hydroxyl at C-6 of glucose with the carboxylic acid group of the fatty acyl substituent. Since macrolactonisations had been achieved regioselectively before (Section 3.2),[145,146] we thought that it might

be possible to form the macrolactone in the presence of the unprotected secondary hydroxyls at C-2 and C-3 of glucose. This approach conveniently eliminates the need for another set of protecting groups at the 2-OH and 3-OH, which would necessitate a much more protracted synthesis of the target molecule.

In order to unmask the primary hydroxyl group required for the macrolactonisation step, regioselective reduction of the benzylidene acetal was attempted. For this purpose two model reactions were performed to investigate the feasibility of this approach. In the first method, Et_3SiH was used as the reducing agent in the presence of dichlorophenylborane ($PhBCl_2$) as the Lewis acid, according to an established procedure.[171] These conditions have been reported to regioselectively reduce 4,6-benzylidene acetals, yielding the 4-OBn and 6-OH in good yield. However, when the previously synthesised disaccharide **178** was subjected to these reaction conditions, as shown in Scheme 4.37, several uncharacterised by-products were obtained, according to TLC analysis.

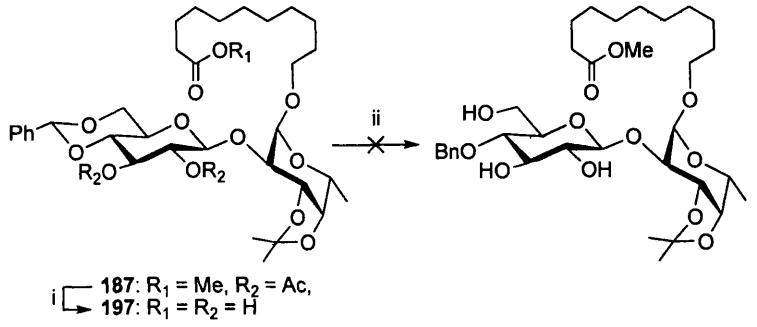


178

Scheme 4.37: Attempted regioselective 4,6-benzylidene reduction

Reagents and conditions: (i) Et_3SiH, $PhBCl_2$, CH_2Cl_2, -78 °C, 2 h.

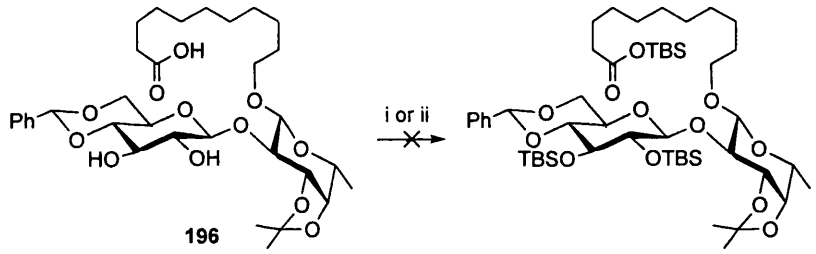
The second model reaction involved the employment of DIBAL as the reducing agent.[172] Disaccharide **187** was used as the model compound, because this is more similar to the desired substrate, differing only in stereochemistry. Disaccharide **187** underwent saponification with LiOH prior to treatment with DIBAL, due to incompatibility of the esters with these conditions (Scheme 4.38). Unfortunately, no reaction occurred and only starting material was recovered.



Scheme 4.38: Attempted regioselective 4,6-benzylidene reduction

Reagents and conditions: (i) LiOH, THF, MeOH, rt, 18 h, 72%; (ii) DIBAL, CH_2Cl_2, -78 °C, 6 h.

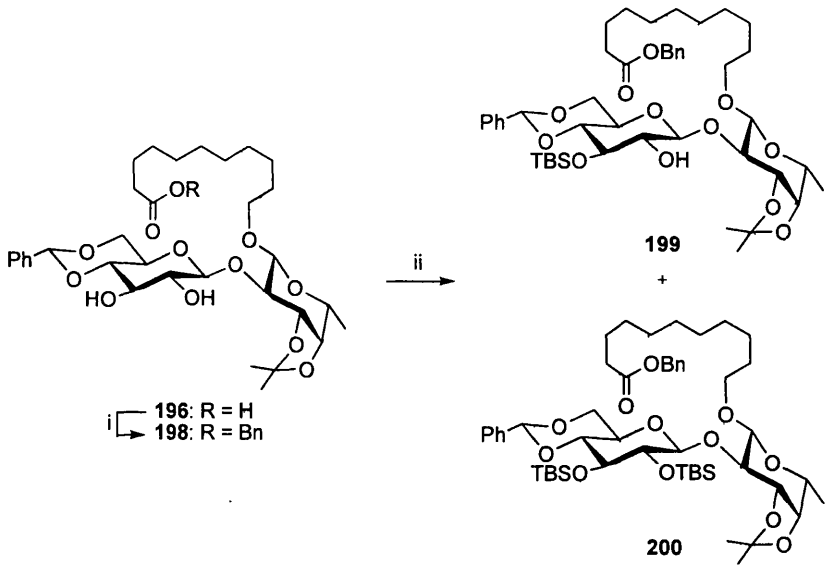
Disappointed by the failure of this promising approach, an alternative method was sought. The best solution (using the already synthesised glycosidic acid **196**) seemed to be the complete removal of the benzylidene acetal e.g. by catalytic hydrogenation. In order to minimise side-reactions during the subsequent formation of the macrolactone, it appeared sensible to protect the hydroxyl groups at C-2 and C-3 of glucose, prior to removal of the benzylidene acetal. Disaccharide **196** was therefore treated with TBSCl in the presence of imidazole. Under these conditions, silyl ester protection of the acid would also occur. Silyl esters, though, are more acid labile than the respective ethers and selective hydrolysis is possible. This reaction, however, was unsuccessful, probably due to steric hindrance and low reactivity of the hydroxyl groups (Scheme 4.39).



Scheme 4.39: Attempted TBS protection of **196**

Reagents and conditions: (i) TBSCl, imidazole, DMF, rt, 18 h; (ii) TBSOTf, pyridine, MeCN, rt, 18 h.

The silyl chloride reagent was thus replaced with the more reactive silyl triflate reagent. Unfortunately, reaction of disaccharide **196** with TBSOTf in the presence of pyridine resulted in multiple uncharacterised side-products (Scheme 4.39). It was suspected that the presence of the free acid might be hindering the formation of the desired product and so we decided to protect it as the benzyl ester. This protecting group was chosen because it would be readily removed simultaneously during the catalytic hydrogenation of the benzylidene acetal to form the desired macrolactone precursor. Treatment of disaccharide **196** with Cs_2CO_3 produced the caesium salt, which was then reacted with benzyl bromide to yield the benzyl ester **198** (Scheme 4.40).



Scheme 4.40: Conversion of **186** to the benzyl ester and TBS ethers

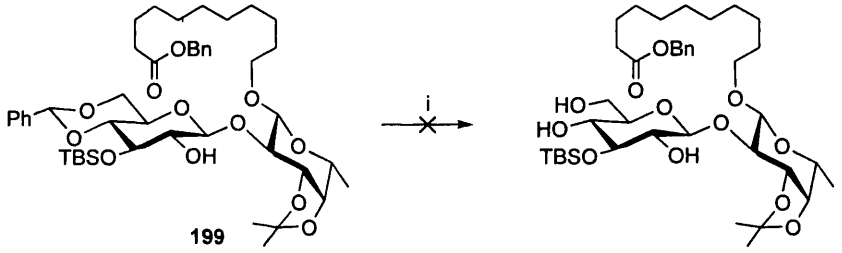
Reagents and conditions: (i) (a) Cs_2CO_3, MeOH, rt, 1 h; (b) BnBr, DMF, rt, 18 h, 67%; (ii) TBSOTf, pyridine, CH_2Cl_2, rt, 18 h, 59% of **199**, 38% of **200**.

With benzyl ester **198** in hand, protection of the hydroxyls was attempted again. Reaction with TBSOTf in the presence of pyridine resulted in a new product, as judged by TLC analysis, which was identified as the mono-TBS ether **199** after purification by flash chromatography and extensive ^1H NMR, COSY and HMQC/HMBC analysis. This is consistent with previous observations, where the 3-OH of glucose was found to be more reactive and could undergo selective acylation (Sections 3.2 and 3.3). Further attempts to achieve protection of the 2-OH by using excesses of reagents and longer reaction times resulted in at best a 1.2:1 mixture of the mono- and bis-TBS ether (Scheme 4.40). It appears that the more reactive 3-OH reacts faster than the 2-OH forming the 3-OTBS ether thus rendering the 2-OH less accessible due to the bulkiness of the adjacent substituents.

The next step towards the synthesis of the macrolactone was the removal of the benzylidene acetal. This can be achieved either by catalytic hydrogenation or under acidic conditions. Since acidic hydrolysis was not compatible with the acid-labile isopropylidene ketal and TBS ether, catalytic hydrogenation was the method of choice.

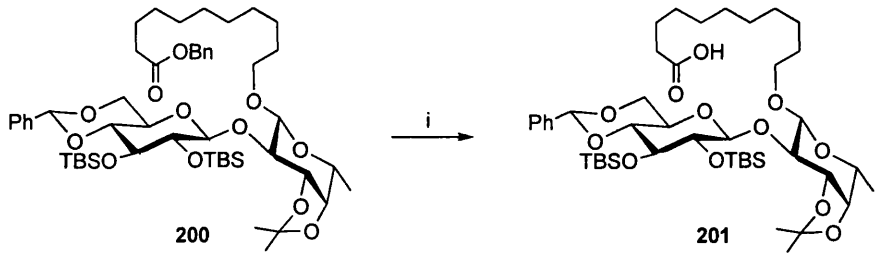
Disaccharide **199** was thus subjected to reaction with H_2 in the presence of catalytic amounts of Pd/C in THF (Scheme 4.41). TLC analysis of the reaction mixture after 24 h indicated the presence of several products, which were thought

to be intermediate products during the benzylidene acetal and benzyl ester cleavage. After replenishing the H_2 atmosphere, the reaction was allowed to proceed for another 24 h and subsequently filtered through a short celite pad. ^1H NMR analysis of the crude product indicated the presence of at least two compounds. However, no peaks corresponding to the isopropylidene ketal or the silyl ether could be identified and the mixture was therefore not subjected to further purification and analysis. Cleavage of TBS ethers by catalytic hydrogenation is known to occur in methanol but should be much less efficient in THF.[173] It seemed however, that the prolonged reaction time resulted in the complete cleavage of the TBS ether. Cleavage of isopropylidene ketals by catalytic hydrogenation is less common but has also been reported to occur.[174]



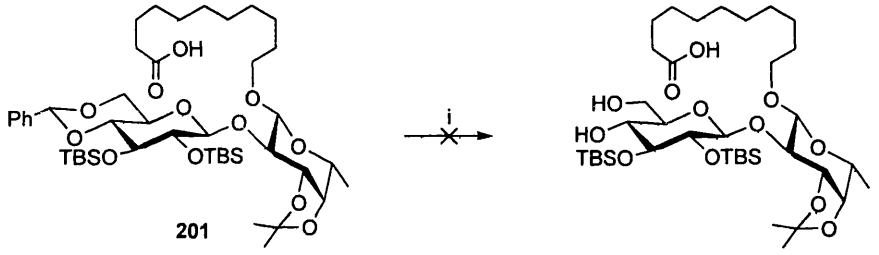
Scheme 4.41: Attempted benzylidene deprotection by catalytic hydrogenation I
Reagents and conditions: (i) H_2, Pd/C, THF, rt, 48 h.

The reaction was repeated with disaccharide **200** under slightly modified conditions. A few drops of AcOH[156] were added to the reaction mixture in addition to H_2 and Pd/C, in order to accelerate the reaction. After 18 h, two new products could be identified by TLC. Unfortunately, after purification by flash chromatography only one compound could be isolated. NMR analysis identified the product as acid **201** (Scheme 4.42). It is possible, given the seeming unexpected lability of these protected derivatives (as indicated by the results discussed above), that the other identified product was insufficiently stable in the presence of silica during column chromatography to allow its isolation.



Scheme 4.42: Attempted benzylidene deprotection by catalytic hydrogenation II
Reagents and conditions: (i) H_2, Pd/C, THF, AcOH, rt, 18 h, 31%.

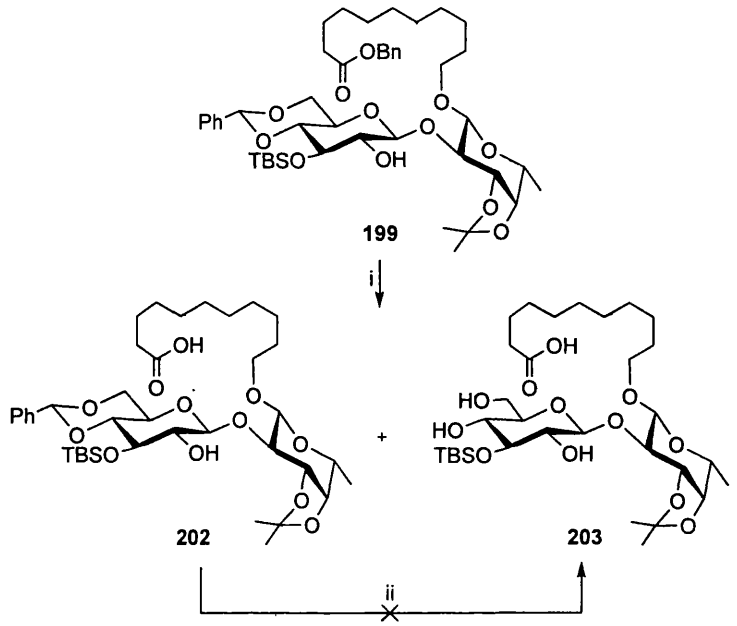
In a further attempt to remove the benzylidene acetal, acid **201** was treated with DDQ in MeCN/H_2O 9:1 (Scheme 4.43), according to a published procedure.[175,176] This is a mild and convenient method for the removal of benzylidene acetals and is compatible with various ether and ester protecting groups. TLC analysis of the reaction mixture, however, indicated the presence of several by-products. These conditions have been reported to cleave isopropylidene ketals in addition to benzylidene acetals, although at a much lower rate.[175] It is therefore possible that partial cleavage of the isopropylidene ketal occurred, contributing to the by-products, which were not further isolated or characterised.



Scheme 4.43: Attempted benzylidene deprotection by DDQ
Reagents and conditions: (i) DDQ, MeCN:H_2O = 9:1, rt, 18 h.

Since the hydrogenation in the presence of catalytic amounts of AcOH had shown some promising results, it was repeated with mono-silyl ether **199** (Scheme 4.44). TLC analysis after 24 h indicated the presence of two new products, which were likely to be acid **202** and the desired diol **203**. The reaction was left to proceed for another 16 h but no change occurred according to TLC analysis. The solution was thus filtered through a celite pad and subjected to normal aqueous work-up. NMR analysis of the crude product indicated that it was a 2:1 mixture of acid **202** and the desired diol **203**. Isolation by flash chromatography, however, seemed inadvisable since previous attempts had resulted in loss of the desired product. Instead, the mixture was subjected to hydrogenation in the presence of a 1:1 (*w/w*) mixture of Pd/C and $Pd(OH)_2$/C. This combination of catalysts had been reported to be more efficient for the cleavage of a benzyl ether, when either of the catalysts alone had failed to produce the desired alcohol.[177] Since benzylidene acetals and benzyl ethers are cleaved under similar conditions we thought that it would be worth exploring the potential of this method for cleavage of the benzylidene acetal. However, no reaction occurred after 7 h as judged by TLC analysis and

prolonged reaction time (48 h) resulted in apparent decomposition according to NMR analysis of the crude product.



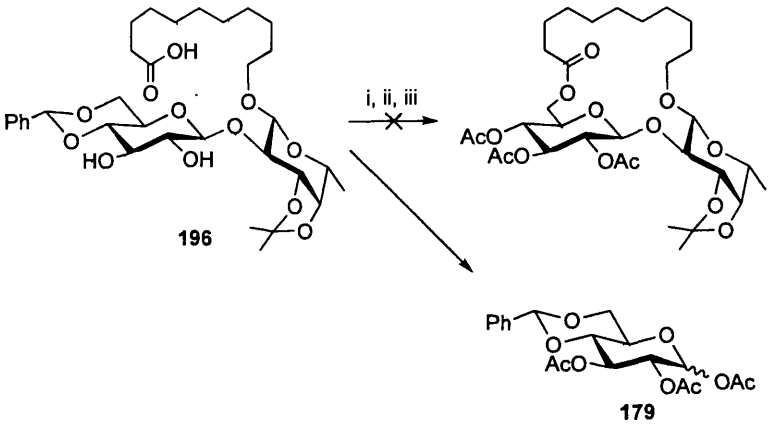
Scheme 4.44: Attempted benzylidene deprotection by catalytic hydrogenation III

Reagents and conditions: (i) H_2, Pd/C, THF, AcOH, rt, 24 h, **202**:**203** = 2:1; (ii) H_2, Pd/C:$Pd(OH)_2$ = 1:1 (*w/w*), THF, rt, 48 h.

One last attempt to remove the benzylidene acetal by catalytic hydrogenation was carried out. This time AcOH was used as the solvent.[178] Since it was unlikely that the TBS ethers would withstand these acidic conditions; acid **196** was used for this purpose (Scheme 4.45). After three hours, TLC analysis indicated the formation of a new product and only a very small amount of starting material was detectable. The reaction mixture was then filtered through a celite pad, the solvent was removed and the residue was subjected to NMR analysis. Although the crude ^{1}H NMR spectrum was not very clear, some conclusions could be drawn: (a) two signals corresponding to the anomeric protons of glucose and fucose were present, each integrating for one proton, suggesting the presence of the disaccharide, (b) there seemed to be no clearly defined aromatic signals, which implied that the benzylidene acetal had been removed, and (c) two methyl signals, characteristic to the isopropylidene ketal, were present, which suggested that the reaction conditions had not affected the isopropylidene ketal.

Even though we could not be certain about the identity of the compound we decided to attempt the macrolactonisation. The crude compound was thus treated with *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and

DMAP and stirred at room temperature overnight. TLC analysis indicated the presence of several products and, after purification by preparative TLC, one major compound was isolated. NMR analysis of this compound, however, suggested the presence of more than a single compound and characterisation was not possible. The product was therefore acetylated and purified again by flash chromatography. Surprisingly, the major compound thus obtained turned out to be the acetylated 4,6-*O*-benzylidene glucopyranose **179** (Scheme 4.45).



Scheme 4.45: Attempted benzylidene deprotection and macrolactonisation
Reagents and conditions: (i) H_2, Pd/C, AcOH, rt, 3 h; (ii) EDCI, DMAP, CH_2Cl_2, DMF, rt, 18 h; (iii) Ac_2O, pyridine, rt, 18 h.

It could be possible that the catalytic hydrogenation in combination with the acidic conditions may have resulted in hydrolysis of the disaccharide. The crude product, that was then subjected to NMR analysis, may have been an 1:1 mixture of the two monosaccharides, which would explain the presence of the two anomeric signals in the ^1H NMR, each integrating for one proton. However, in this case, one sugar would bear an anomeric OH, which would result in a mixture of anomers of the glucose moiety. Hence, it is more probable that the disaccharide hydrolysis occurred at a later stage of the reaction sequence. Furthermore, the presence of by-products could have caused suppression of the aromatic signals and therefore it was erroneously assumed that the benzylidene acetal had been removed.

Although our expectations for the success of the macrolactonisation step were not high, the outcome of the reaction was still surprising. The efficient removal of the benzylidene acetal proved impossible in our hands and therefore alternative

protecting groups were required for the protection of the 4-OH and 6-OH of glucose. Consequently, this approach had to be abandoned.

4.4.5. Summary

The problem encountered in the second approach involving the stereoselective introduction of the fatty acid side-chain could be overcome in the third approach by glycosylation of the acid side-chain with fucose prior to the formation of the glucose-fucose disaccharide. This approach exploited the presence of an acetate group at C-2 of fucose, which resulted exclusively in the formation of the desired β-fucoside due to neighbouring group participation. Subsequent formation of the disaccharide and saponification of the ester bonds proceeded smoothly, yielding the acid intermediate **196**.

The next task towards the synthesis of the target compound was to unmask the primary hydroxyl group of glucose in order to form the macrolactone precursor. Numerous methods and reaction conditions were investigated for the regioselective opening or the complete removal of the benzylidene acetal. However, none of these yielded the desired product and therefore another alternative had to be sought.

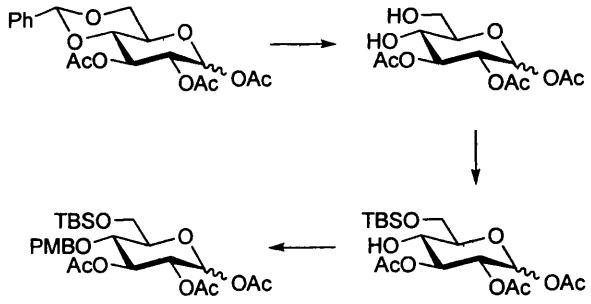
4.5. Fourth Approach

Since the regioselective opening or the complete removal of the benzylidene acetal proved difficult, alternative protecting groups for the hydroxyl groups at C-4 and C-6 of the glucose building block were required. Several routes could be envisaged for the preparation of an appropriately protected glycosyl donor.

4.5.1. Synthesis of the Glucose Building Block I

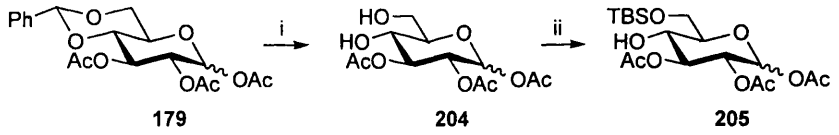
The first route involved the employment of the previously synthesised acetylated 4,6-benzylidene glucopyranose **180**. We theorised that the presence of the acid side-chain might, in some way, have been hindering the removal of the benzylidene acetal in the previous approach. We thus decided to replace the benzylidene acetal prior to the synthesis of the disaccharide. This approach would also minimise the requirement for complex protecting group manipulations once the disaccharide was formed.

Removal of the benzylidene acetal of the glucose derivative **179**, would yield the 4,6-diol, which would then be selectively silylated at the primary hydroxyl. The remaining hydroxyl group would subsequently be protected with an orthogonal protecting group. The PMB ether was chosen for this purpose, since it can be removed under various conditions, including catalytic hydrogenation, mild acidic or oxidative (e.g. DDQ) conditions (Scheme 4.46).



Scheme 4.46: Route for the synthesis of the glucose building block I

The benzylidene acetal of the glucose derivative **179** was readily removed by catalytic hydrogenation in the presence of Pd/C providing the diol **204** in 68% yield after purification by flash chromatography. The primary alcohol was then protected as the TBS ether by treatment of diol **204** with TBSCl in the presence of Et_3N and DMAP in CH_2Cl_2 as shown in Scheme 4.47.



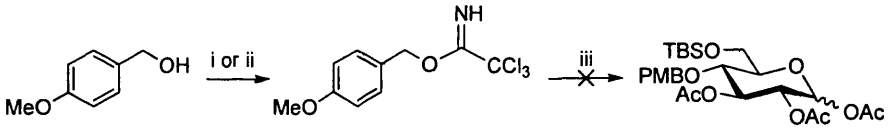
179 204 205

Scheme 4.47 Synthesis of the glucose intermediate **205**

Reagents and conditions: (i) H_2, Pd/C, THF, rt, 48 h, 65%; (ii) TBSCl, Et_3N, DMAP, CH_2Cl_2, DMF, rt, 18 h, 74%.

With alcohol **205** in hand the next step was the protection of the remaining hydroxyl group as the PMB ether. Since the usual conditions for ether synthesis (NaH, PMBBr) are not compatible with the presence of *O*-acetyl protecting groups, an alternative method, involving the PMB trichloroacetimidate and an acid catalyst, had to be employed.

p-Methoxybenzyl alcohol (PMB-OH) was first converted to the trichloroacetimidate by reaction with trichloroacetonitrile in the presence of 50% $KOH_{(aq)}$ and tetrabutylammonium hydrogen sulfate (TBAHS).[179] Alternatively, the reaction was performed in the presence Cs_2CO_3. Both methods yielded a new, less polar product, as judged by TLC (Scheme 4.48). The PMB trichloroacetimidate has been reported to be very unstable[180] and was thus not isolated, but used in the next step after filtration through a short silica pad.



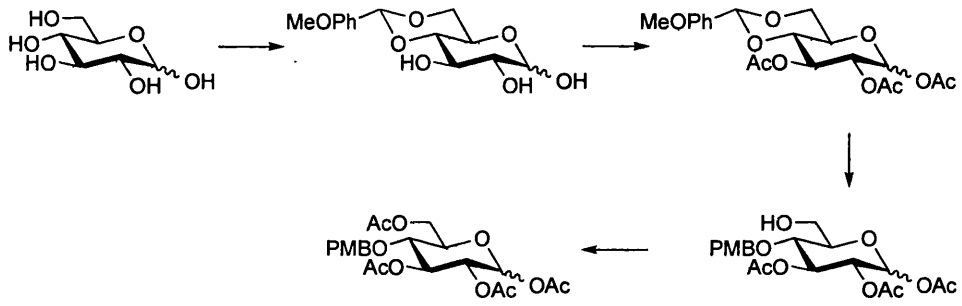
Scheme 4.48: Attempted synthesis of the PMB ether

Reagents and conditions: (i) CCl_3CN, 50% $KOH_{(aq)}$, TBAHS, CH_2Cl_2, -15 °C → rt, 1.5 h; (ii) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 18 h; (iii) **205**, TMSOTf, CH_2Cl_2, 0 °C, 1 h.

Alcohol **205** was treated with the crude PMB trichloroacetimidate in the presence of catalytic amounts of TMSOTf[181] (Scheme 4.48). However, the desired compound could not be obtained from the resulting complex mixture of several products. The failure of this reaction is likely to be due to the low reactivity of the hydroxyl, which is probably deactivated due to presence of the acetate groups. Moreover, the bulky TBS ether at C-6 makes the hydroxyl group even less accessible. Therefore, it seems that decomposition of the unstable PMB trichloroacetimidate occurred before it could react with the alcohol.

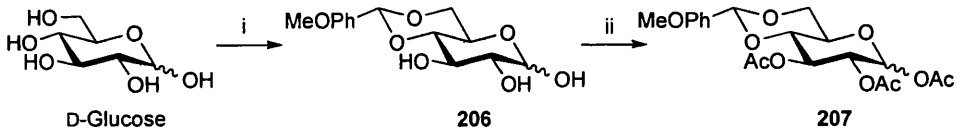
4.5.2. Synthesis of the Glucose Building Block II

An alternative route towards a suitably protected glucose building block involved the regioselective opening of the *p*-methoxybenzylidene acetal as shown in Scheme 4.49. The *p*-methoxybenzylidene acetal was chosen for this purpose instead of the benzylidene acetal, because, after successful cleavage, it would give the 4-OPMB ether, which is more readily removed than the Bn ether. The 6-OH could then be protected as the acetate ester.



Scheme 4.49: Route for the synthesis of the glucose building block II

The *p*-methoxybenzylidene acetal could be obtained by reaction of D-glucose with *p*-methoxybenzaldehyde dimethyl acetal in the presence of PTSA. The reaction, however, had to be quenched before proceeding to completion because a less polar by-product, presumably the 1,2-4,6 di-acetal, had started to form. Therefore the product was obtained in 42% yield after purification by flash chromatography. Acetylation of **206** with acetic anhydride in pyridine proceeded smoothly, yielding the glucose derivative **207** in 92% yield (Scheme 4.50).

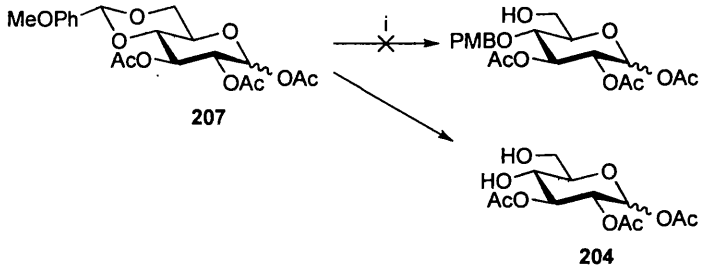


Scheme 4.50: Synthesis of the glucose intermediate **207**

Reagents and conditions: (i) *p*-$MeOPhCH(OCH_3)_2$, PTSA, DMF, 60 °C, 30 min, 42%; (ii) Ac_2O, pyridine, rt, 18 h, 92%.

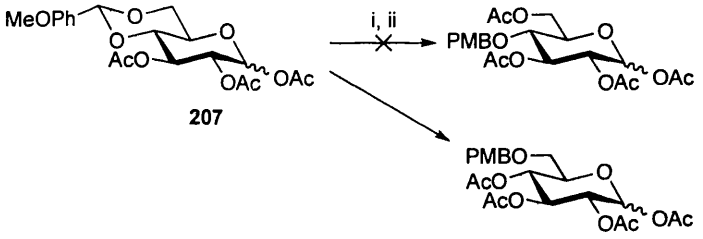
With glucose derivative **207** in hand, the next step was the regioselective opening of the acetal. Procedures involving reducing agents such as DIBAL or $LiAlH_4$ could not be employed since these would result in the unwanted reduction of the acetate esters. Therefore Et_3SiH was chosen for this purpose.[171] Acetal **207** was treated with Et_3SiH and $PhBCl_2$ in CH_2Cl_2 as shown in Scheme 4.51. TLC

analysis of the reaction mixture indicated the presence of several products and after purification by flash chromatography three compounds could be isolated. The least polar product appeared to be a *p*-methoxybenzyl derivative, since only aromatic signals and a singlet at 3.8 ppm, corresponding to the methoxy group, could be detected by NMR analysis. The major and most polar compound was found to be diol **204** by NMR analysis. Only a small amount of the third compound could be obtained and characterisation was not possible.



Scheme 4.51: Attempted regioselective *p*-methoxybenzylidene cleavage I
Reagents and conditions: (i) Et_3SiH, $PhBCl_2$, CH_2Cl_2, -78 °C, 2 h.

Alternatively, the regioselective reductive opening of the acetal was attempted using the conditions of Johansson and Samuelsson.[182] Acetal **207** was treated with $NaBH_3CN$ and TMSCl in MeCN, as shown in Scheme 4.52. After purification by flash chromatography two compounds could be isolated (~ 1:1). The more polar product appeared to be a mixture of diols by NMR analysis, suggesting that migration of an acetate ester had occurred. NMR analysis of the second compound indicated the presence of a PMB ether, however, the site of *p*-methoxybenzylation could not be determined due to overlapping of signals corresponding to the protons at C-4 and C-6. The compound was therefore acetylated by reaction with Ac_2O in pyridine and subjected to extensive ^1H and ^{13}C NMR, along with COSY, HMQC and HMBC analysis to assist structure elucidation. The downfield signal at 5.1-5.2 ppm corresponding to the proton at C-4 indicated the presence of an acetate ester at C-4, which could be further confirmed by its HMBC correlation with the carbonyl at 170.5 ppm. It was therefore concluded that the isolated compound was, in contrast to the literature,[182] the undesired 6-OPMB ether (Scheme 4.52). The same outcome was also observed by Fürstner and Nagano in their synthesis of the ipomoeassins.[153]

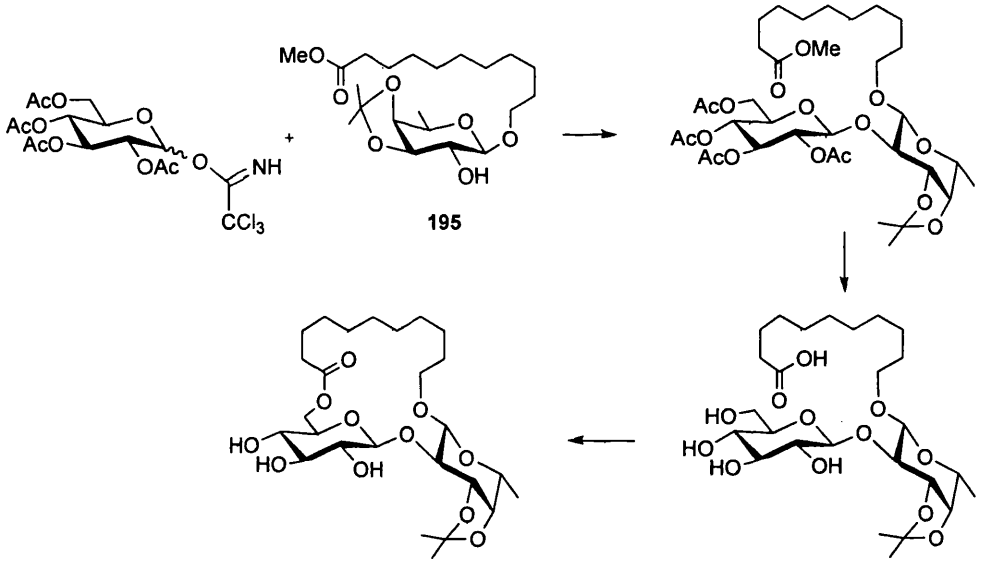


Scheme 4.52: Attempted regioselective *p*-methoxybenzylidene cleavage II

Reagents and conditions: (i) $NaBH_3CN$, TMSCl, MeCN, 0 °C → rt, 18 h; (ii) Ac_2O, pyridine, rt, 18 h.

4.5.3. Synthesis of the Macrolactone Precursor

Since the desired glucose building block could not be obtained by reaction with the PMB trichloroacetimidate or by regioselective opening of the *p*-methoxybenzylidene acetal, we decided to proceed with the per-*O*-acetylated glucose and attempt to make use of the slightly different reactivities of the hydroxyl groups, as shown in Scheme 4.53.

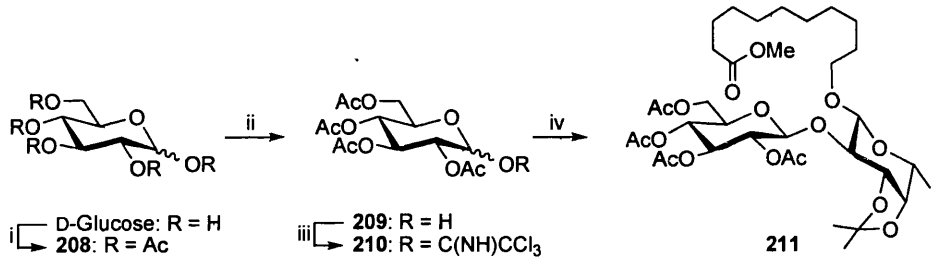


Scheme 4.53: Suggested route for the synthesis of the macrolactone

Glycosyl acceptor **195** would undergo glycosylation with the readily available glucose trichloroacetimidate. Upon saponification of the ester bonds of the disaccharide, an intermediate with four hydroxyl groups would be obtained. The primary hydroxyl group of glucose should be significantly more reactive than the secondary hydroxyls and selective acylations can be achieved under appropriate conditions. Therefore macrolactonisation would be attempted and, although we expected that formation of regioisomeric by-products would be inevitable, we were hoping that the desired macrolactone would be obtained as the major

product. Furthermore, we were interested in the outcome of this approach, as this could represent a significantly simplified and improved access to this class of compounds if successful.

D-Glucose was acetylated by reaction with Ac_2O in pyridine and selectively deprotected at the anomeric position with $BnNH_2$ to yield alcohol **209**. Reaction with trichloroacetonitrile in the presence of Cs_2CO_3 furnished glucose trichloroacetimidate, which was subjected to glycosylation with the previously synthesised glycosyl acceptor **195** to furnish disaccharide **211** in 90% yield, as shown in Scheme 4.54.



Scheme 4.54: Synthesis of glycolipid **209**

Reagents and conditions: (i) Ac_2O, pyridine, rt, 1 h, 92%; (ii) (a) $BnNH_2$, THF, rt, 18 h; (b) 1 M HCl, 93%; (iii) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 18 h; (iv) **195**, TMSOTf, CH_2Cl_2, -20 °C, 1 h, 90%.

4.5.4. Synthesis of the Non-Macrocyclic Glycoresin

Non-macrocyclic glycoresins have been isolated from plants belonging to the morning glory family and therefore we were also interested in the synthesis of non-macrocyclic ipomoeassin analogues. Since the most potent ipomoeassins (D and F) contained acetate esters we thought that glycolipid **212** (Figure 4.2), containing four acetate esters might be an interesting target compound.

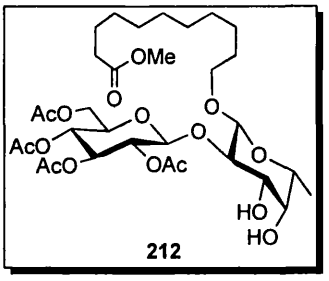
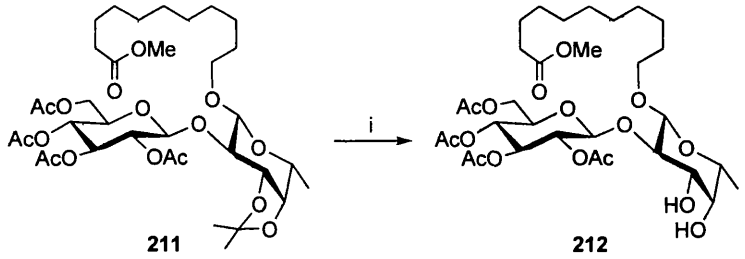


Figure 4.2: Glycoresin **212**

Glycolipid **211** was treated with TFA:H_2O (4:1) for 15 minutes in order to remove the isopropylidene ketal. After purification by flash chromatography, glycoresin **212** was obtained as a colourless oil in 62% yield (Scheme 4.55).



211 212

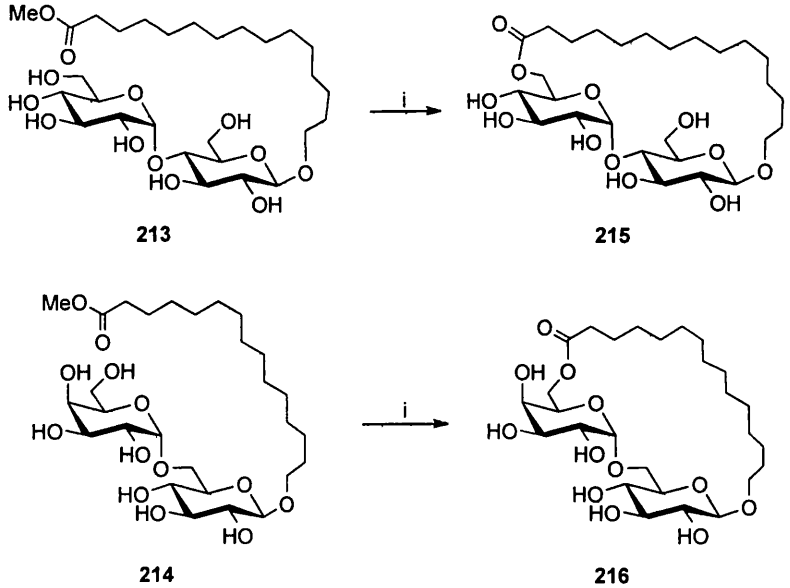
Scheme 4.55: Synthesis of glycoresin **212**

Reagents and conditions: (i) TFA:H_2O, rt, 15 min, 62%.

4.5.5. Attempts Towards the Synthesis of the Macrolactone

Lipase catalysed macrolactonisation

In the first attempt to form the macrolactone, a lipase-catalysed macrolactonisation, as described by Bisht and co-workers,[183] was employed. Bisht and co-workers succeeded in synthesising macrolactones **215** and **216** by treating glycolipids **213** and **214** with the lipase from *Candida antarctica* (Novozym 435), as shown in Scheme 4.56. The regioselectivity of the enzyme is remarkable since, although six other hydroxyl groups were present, including two primary hydroxyls in glycolipid **213**, only the desired macrolactones **215** and **216** were obtained in 75% and 76% yield, respectively.



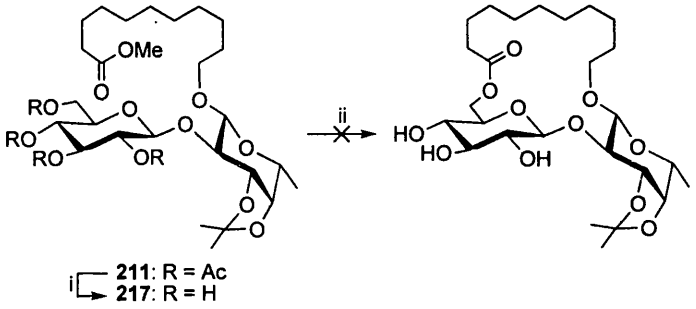
213 215

214 216

Scheme 4.56: Synthesis of macrolactones **215** and **216** by Bisht and co-workers[183]

Reagents and conditions: (i) Novozym 435, THF, 30 °C, 90 h, 75% for **215** and 76% for **216**.

Encouraged by the reported success of this method in the syntheses of macrolactones **215** and **216** we decided to employ the same conditions in our synthesis. Glycolipid **211** was therefore subjected to acetate cleavage by sodium methoxide in MeOH, providing the macrolactone precursor **217** in 93% yield. Glycolipid **217** was then treated with Novozym 435 in THF and stirred for five days at 30 °C (Scheme 4.57). However, after purification by flash chromatography, only an unidentified by-product and recovered starting material could be obtained.



Scheme 4.57: Attempted enzyme catalysed macrolactonisation

Reagents and conditions: (i) (a) NaOMe, MeOH, rt, 15 min; (b) Amberlite IR 120 (H^+ form) ion exchange resin, 93%; (ii) Novozym 435, THF, 30 °C, 5 days.

Enzymes are typically highly substrate-specific, and will only catalyse reactions on molecules with the correct shape to fit tightly into their active site. There are some significant differences between glycolipids **217** and **213/214** that may have affected the outcome of the reaction. Glycolipid **217** contains the Glcβ(1→2)Fuc disaccaride, which has a very different conformation to either the Glcα(1→4)Glc or Galα(1→6)Glc disaccharide present in glycolipids **213** and **214**, respectively. Moreover, there is an isopropylidene group present in glycolipid **217** and the acid side-chain is slightly shorter (11 carbons vs. 15). These factors may have prevented glycolipid **217** from fitting into the active site of the enzyme, which was hence unable to catalyse the formation of the macrolactone.

Macrolactonisation by the carbodiimide method

Next, macrolactonisation was attempted by the employment of carbodiimides. This reaction involves treatment of an acid with a carbodiimide[184,185] (e.g. DIC, DCC or EDCI, Figure 4.3) in the presence of DMAP. It proceeds via an *O*-acylisourea intermediate, converting the acid into a good leaving group.

Subsequent reaction with an alcohol results in the formation of an ester, as shown in Scheme 4.58.

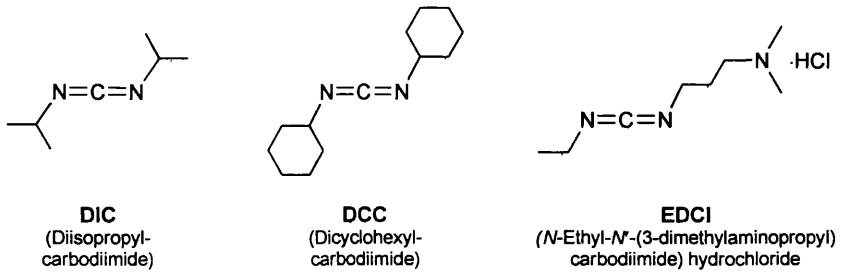
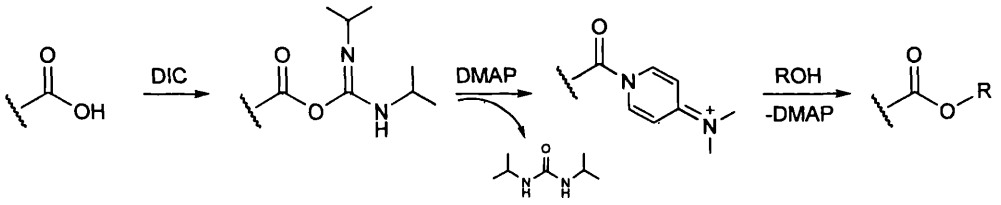


Figure 4.3: Examples of carbodiimide reagents

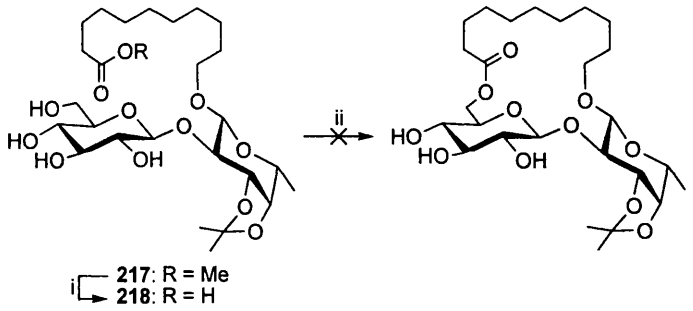
A by-product in this reaction is the corresponding urea derivative (Scheme 4.58). In the case of DCC, dicyclohexylurea is formed, which is insoluble and can usually be removed by filtration. However traces often remain, which are difficult to separate from the ester product. Therefore, EDCI is preferred, whose urea by-product is water soluble and can be removed by aqueous work-up.



Scheme 4.58: Ester formation by DIC and DMAP

The acid, required for the esterification step, was unmasked by saponification of the methyl ester of glycolipid **217**. The product could be obtained by precipitation with hexanes/EtOAc to furnish pure acid **218** in 79% yield. This was subsequently treated with EDCI (2 equiv) and DMAP (0.1 equiv) under very dilute conditions (1 mM) in order to avoid competing intermolecular by-product formation (Scheme 4.59). This reaction is usually performed in CH_2Cl_2; however, due to low solubility of acid **218** in CH_2Cl_2, THF:DMF (9:1) was used instead. After 18 hours, TLC analysis indicated the presence of a significant amount of starting material and therefore another equivalent of EDCI was added. The reaction mixture was allowed to stir for additional 48 h after which it was quenched. Purification by flash chromatography provided several products but none appeared to be the desired macrolactone. Only one of the products showed

characteristic sugar signals in the ^{1}H NMR; however, the amount obtained after purification was very small and full characterisation was not possible.



Scheme 4.59: Attempted macrolactonisation by EDCI and DMAP

Reagents and conditions: (i) LiOH, THF, MeOH, rt, 18 h, 79%; (ii) EDCI, DMAP, THF:DMF (9:1), rt, 3 days.

Alternatively, macrolactonisation was attempted with DIC. Acid **218** was dissolved in THF and DMF, cooled to 0 °C and DIC was added. The reaction mixture was stirred for 1 hour and TLC analysis indicated the formation of a less polar product, presumably the *O*-acylisourea intermediate. DMAP and Et_3N were added and the reaction was allowed to stir overnight. After aqueous work-up and purification by flash chromatography two compounds could be isolated.

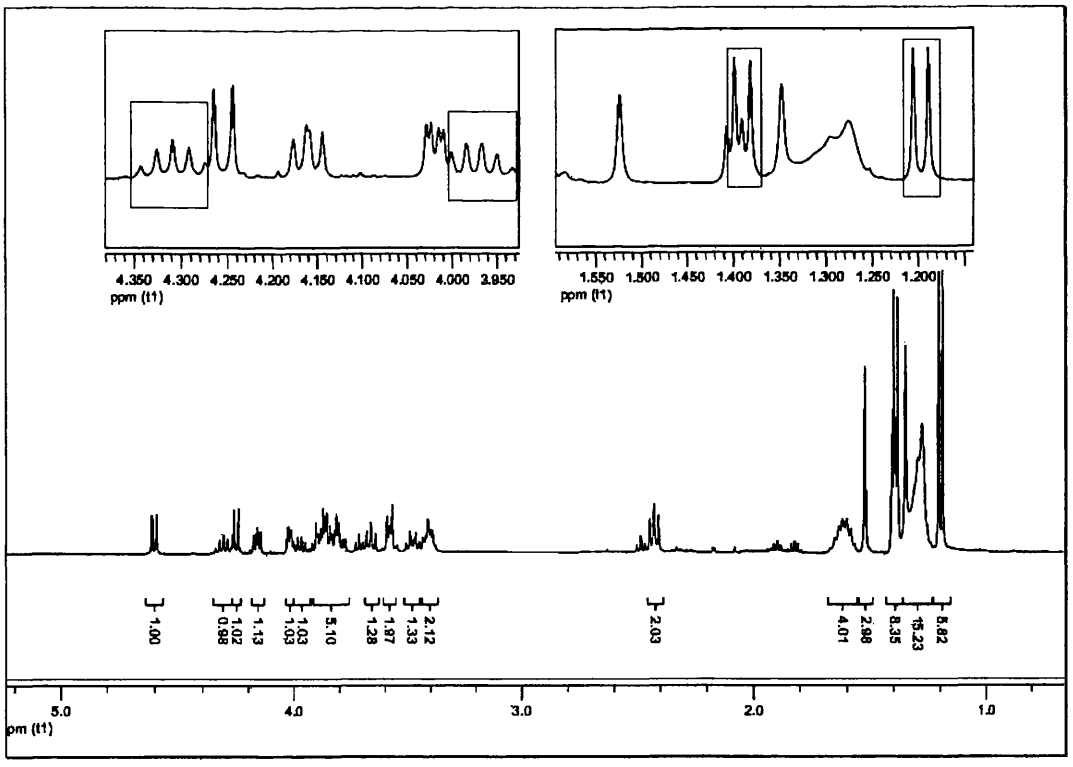
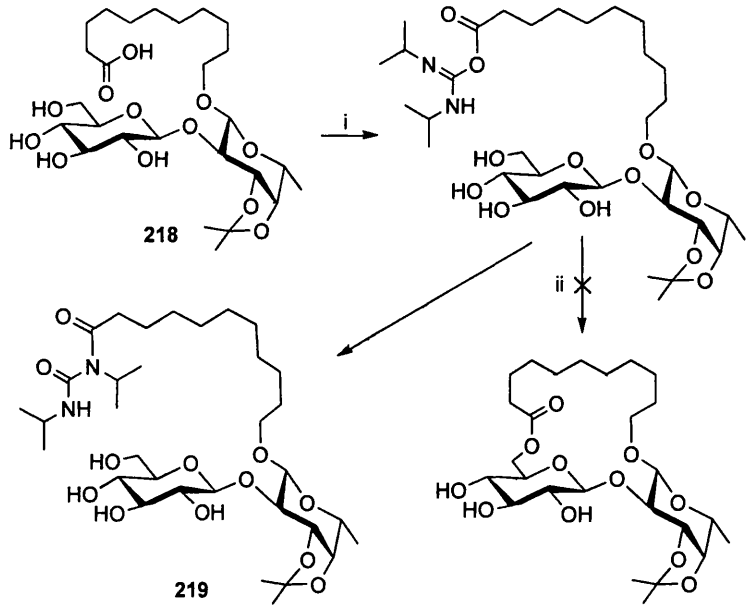


Figure 4.4: ^{1}H NMR spectrum of **219**

The more polar product turned out to be recovered starting material. NMR analysis of the second compound showed the presence of two doublets at 1.39 and 1.20 ppm, which integrated for six protons each, and the presence of two multiplets at 4.31 and 3.97 ppm, corresponding to one proton each (Figure 4.4). In addition, one of the protons on the tertiary carbon of the isopropyl group showed an HMBC correlation with the carbonyl carbon of the acid side-chain, which only occurs in the case of an *N*-acylurea. From this data it could be concluded that isomerisation of the *O*-acylisourea to the stable *N*-acylurea had occurred and that the isolated compound was compound **219**, as shown in Scheme 4.60.

O-acylisourea to *N*-acylurea rearrangement is frequently encountered in peptide synthesis[186-188] and solvents such as THF and DMF have been found to enhance the formation of this unwanted side-product.[189] We also considered that the delayed addition of DMAP may have favoured the formation of the *N*-acylurea. The reaction was therefore repeated. This time, however, DIC, DMAP and Et_3N were added simultaneously at 0 °C and the reaction was allowed to stir overnight. Unfortunately, TLC analysis again indicated the formation of the undesired side-product **219**, whereas no product corresponding to the desired macrolactone could be detected.

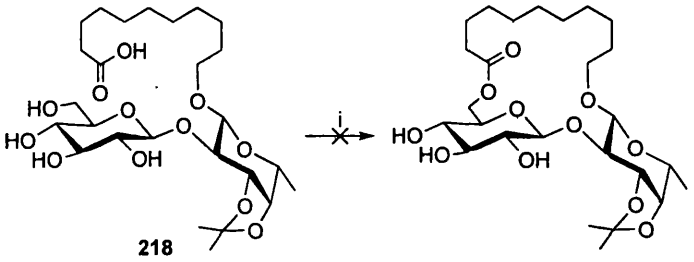


Scheme 4.60: Attempted macrolactonisation by DIC and DMAP/Et_3N

Reagents and conditions: (i) DIC, THF, DMF, 0 °C, 1 h; (ii) DMAP, Et_3N, 0 °C → rt, 18 h.

Macrolactonisation by the mixed anhydride method

Since the attempted esterification by the carbodiimide method failed to produce the desired macrolactone, the mixed anhydride procedure was investigated. This method, which was introduced by Yamaguchi[189] and further optimised by Yonemitsu[144] and co-workers, involves the reaction of an acid with 2,4,6-trichlorobenzoyl chloride in order to form a very reactive mixed anhydride, which then reacts with an alcohol to yield the desired ester.



Scheme 4.61: Attempted macrolactonisation by the mixed anhydride method

Reagents and conditions: (i) (a) Et_3N, 2,4,6-$Cl_3C_6H_2COCl$, THF, DMF, 0 °C, 2 h; (b) DMAP, toluene, 0 °C → rt, 18 h.

Acid **218** was dissolved in THF/DMF and treated with Et_3N and 2,4,6-trichlorobenzoyl chloride for 2 hours at 0 °C before DMAP was added. The reaction was allowed to warm to room temperature and stirring was continued overnight (Scheme 4.61). TLC analysis indicated the presence of several compounds, however no product could be isolated after aqueous work-up and purification by flash chromatography.

It is likely that the low solubility of acid **218** may have hindered the formation of the macrolactone. In addition, the high dilution conditions, required in order to avoid intermolecular esterification, and the possible interference of the hydroxyl groups could have made activation less efficient. Furthermore, the fatty acid side-chain has a great deal of conformational freedom, and it may be unlikely that the activated carboxyl group and the primary hydroxyl of glucose approach each other sufficiently closely in space to allow efficient reaction. We, therefore, decided to modify our synthetic approach one more time in order to decrease the polarity of the glucose building block, thus making the macrolactone precursor more soluble in solvents such as CH_2Cl_2 or toluene.

4.5.6. Summary

In the fourth approach the synthesis of a glucose derivative incorporating a PMB ether at C-4 was attempted via two alternate routes. The first one involved reaction of the protected glucose intermediate **205** with PMB trichloroacetimidate, while in the second route the regioselective opening of the *p*-methoxybenzylidene acetal was attempted. However, none of the approaches yielded the desired glucose building block. Therefore, we decided to proceed with the per-*O*-acetylated glucose. Disaccharide **211** was formed in 90% yield. Cleavage of the isopropylidene ketal furnished a non-macrocyclic target compound, namely glycoresin **212**.

Saponification of the esters yielded the macrolactone precursor **218**, which contained four hydroxyl groups. Macrolactonisation was attempted in the hope that the more reactive primary alcohol would react faster with the acid than the secondary alcohols to form the desired macrolactone as the main product. Several esterification methods were investigated, including a lipase-catalysed macrolactonisation, ester formation using carbodiimides and a mixed anhydride procedure. However, none of these methods yielded the desired macrolactone. These reactions proceed best in non-polar solvents such as CH_2Cl_2 and toluene. Since acid **218** was not sufficiently soluble in these solvents, the reactions had to be performed in THF/DMF. In the carbodiimide method, this might have caused the isomerisation of the *O*-acylisourea intermediate to the stable *N*-acylurea, which, once formed, cannot undergo esterification. Also the low solubility of acid **218** might have had a negative effect on the esterification by the mixed anhydride method. Therefore an alternative approach was sought, which would involve the employment of a macrolactone precursor with decreased polarity.

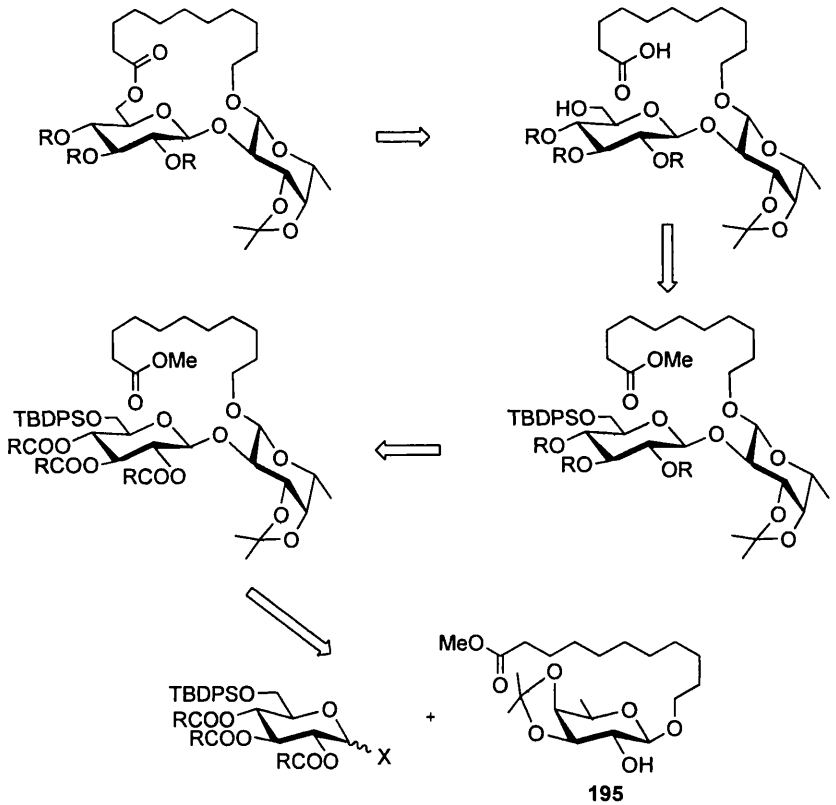
4.6. Fifth Approach

It is possible that one of the reasons for the failure of the macrolactone formation in the previous approach was the low solubility of glycolipid **218** in low polarity solvents. Therefore, we attempted to modify the macrolactone precursor by protecting the secondary hydroxyl groups of glucose, thus decreasing its polarity and increasing its solubility in solvents such as CH_2Cl_2 and toluene.

4.6.1. Retrosynthetic Analysis

During the design of the new route towards the target macrolactone the following factors had to be taken into consideration:

(a) Ester protecting groups were not suitable for the protection of the secondary hydroxyl groups of glucose, since their removal would take place after the formation of the macrolactone and would therefore result in macrolactone hydrolysis. Moreover, ester protecting groups would undergo cleavage during saponification of the methyl ester in order to unmask the acid required for the macrolactone formation. Thus, ether protecting groups had to be employed (Scheme 4.62).

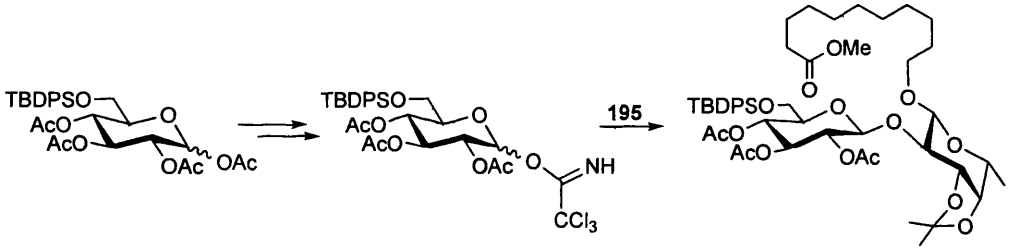


Scheme 4.62: Retrosynthetic analysis of the macrolactone

(b) Ether protecting groups, however, do not act as neighbouring participating groups and glycosylation of the glucose building block with glycosyl acceptor **195** would result in the formation of the disaccharide as a mixture of anomers. Therefore, we decided to start with an ester protected glucose derivative and then replace the ester protecting groups with ethers prior to the formation of the macrolactone as shown in Scheme 4.62.

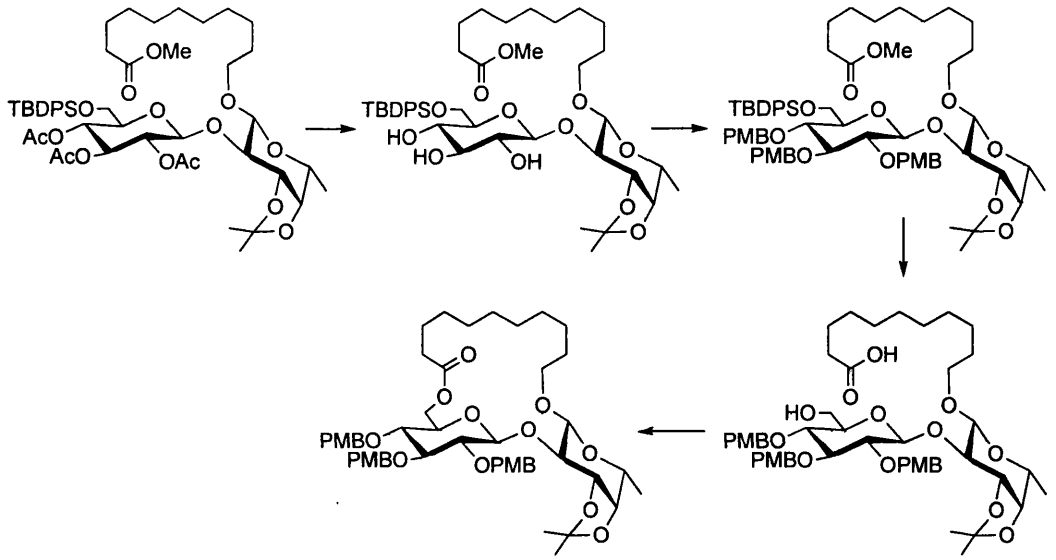
(c) For the primary hydroxyl group of glucose a protecting group was require that could be introduced selectively and would withstand glycosylation, cleavage of the ester protecting groups and ether protection of the secondary alcohols of glucose. The obvious choice was a TBDPS ether (Scheme 4.62).

In the forward synthesis, the glucose building block would be synthesised by selective TBDPS protection of the primary hydroxyl group and subsequent acetylation of the secondary hydroxyls. Since the trichloroacetimidate method had worked well so far it was chosen for the formation of the disaccharide. Selective anomeric deprotection and formation of the glucosyl trichloroacetimidate would yield the glycosyl donor, which would undergo glycosylation with alcohol **195** to furnish the desired disaccharide as shown in Scheme 4.63.



Scheme 4.63: Suggested route for the formation of the disaccharide

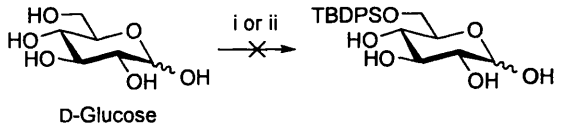
At this stage the acetate esters would be replaced by ether protecting groups. Silyl ethers were not an option, since the disaccharide would already bear a TBDPS ether at its primary hydroxyl group. The most suitable alternatives, which could withstand TBDPS deprotection and methyl ester saponification, were benzyl or PMB ethers. Furthermore, their removal could be achieved without affecting the macrolactone.[145,146] After successful benzylation, TBDPS removal would take place followed by methyl ester cleavage to yield the desired glycolipid. With the macrolactone precursor in hand esterification would be attempted as shown in Scheme 4.64.



Scheme 4.64: Suggested route for the formation of the macrolactone

4.6.2. Synthesis of the Glucose Building Block

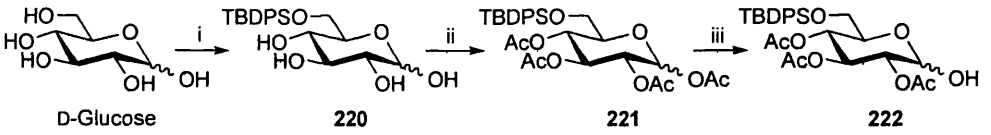
The first step towards the synthesis of the glucose building block involved the selective protection of the primary hydroxyl group of glucose as the TBDPS ether. This was attempted by reaction of D-glucose with TBDPSCl in the presence of DMAP and Et_3N in DMF or CH_2Cl_2/DMF (2:1). These reaction conditions have been reported to be effective for the selective protection of primary over secondary alcohols.[190] However, no reaction occurred by this method (Scheme 4.65).



Scheme 4.65: Attempted selective TBDPS protection of D-glucose

Reagents and conditions: (i) TBDPSCl, DMAP, Et_3N, CH_2Cl_2,:DMF (2:1), rt, 18 h; (ii) TBDPSCl, DMAP, Et_3N, DMF, rt, 18 h.

When the silyl protection was repeated under the usual reaction conditions, i.e. TBDPSCl in the presence of imidazole in DMF, the desired silyl ether could be obtained in 71% yield. Subsequent acetylation by reaction of TBDPS ether **220** with acetic anhydride in pyridine yielded the protected glucose intermediate **221**, which was subjected to selective anomeric deprotection with $BnNH_2$ to furnish alcohol **222** in 69% over two steps (Scheme 4.66).

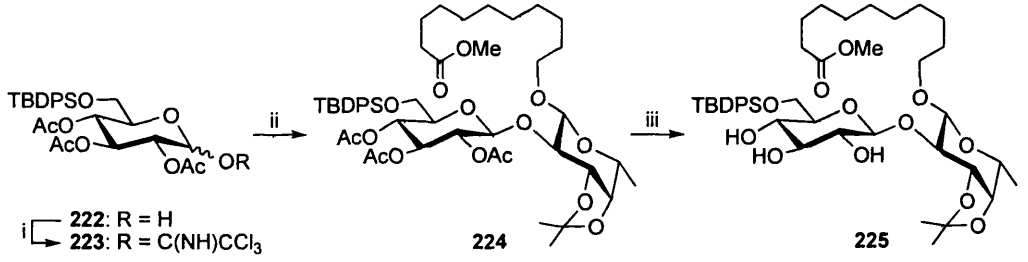


Scheme 4.66: Synthesis of the glucose building block **222**

Reagents and conditions: (i) TBDPSCl, imidazole, DMF, rt, 1 h, 71%; (ii) Ac_2O, pyridine, rt, 1 h; (ii) (a) $BnNH_2$, THF, rt, 18 h; (b) 1 M HCl, 69% over 2 steps.

4.6.3. Synthesis of the Disaccharide

With glucose building block **222** in hand the next step was the formation of the disaccharide. Alcohol **222** was therefore treated with trichloroacetonitrile in the presence of Cs_2CO_3 for 18 h. After filtration through a short pad of silica the crude glucosyl trichloroacetimidate was utilised for glycosylation of the previously synthesised fucosyl acceptor **195** in the presence of catalytic amounts of TMSOTf to yield glycoside **224** in 83% yield (Scheme 4.67).



Scheme 4.67: Synthesis of the disaccharide **225**

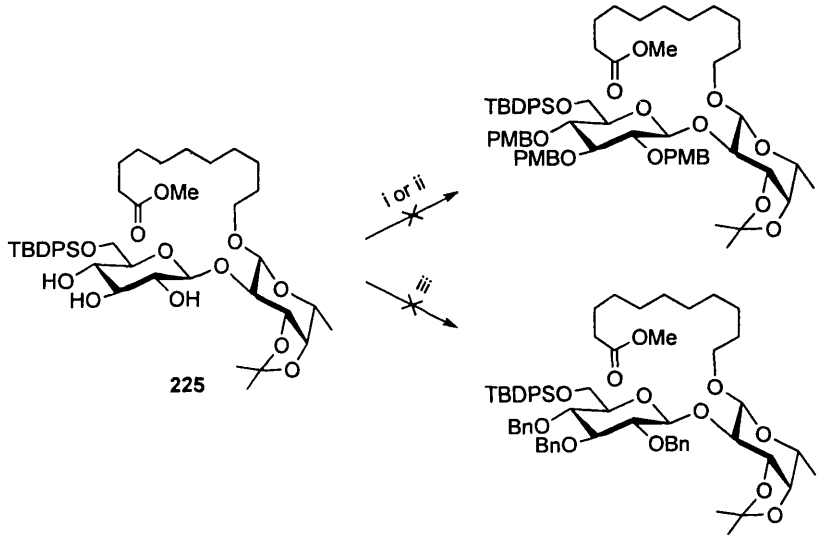
Reagents and conditions: (i) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 18 h; (ii) **195**, TMSOTf, CH_2Cl_2, -20 °C, 1 h, 83%; (iii) (a) NaOMe, MeOH, rt, 30 min; (b) Amberlite IR 120 (H^+ form) ion exchange resin, 97%.

During the removal of the acetate esters with NaOMe in methanol we were concerned that silyl migration might take place, which is known to be favoured under basic conditions and in protic solvents.[71] Fortunately, this problem did not occur and the triol **225** could be obtained in 97% yield (Scheme 4.67).

The next step in our synthetic approach was the benzylation of the hydroxyls. The PMB protecting group was chosen over the benzyl ether, since it can be removed more readily. Triol **225** was treated with NaH for 30 minutes before PMBCl was added at 0 °C and the reaction was continued overnight. TLC analysis indicated the presence of several compounds; however, no product could be isolated after purification by flash chromatography (Scheme 4.68).

The reaction was then repeated under slightly modified conditions. A catalytic amount of tetra-*n*-butylammonium iodide (TBAI) was added to the reaction

mixture prior to the introduction of PMBCl. TBAI is known to increase reactivity of the PMB reagent by the *in situ* generation of PMBI.[191] The reaction was allowed to proceed for five hours at room temperature (Scheme 4.68). After aqueous work-up and purification by flash chromatography two compounds could be isolated. The less polar compound appeared to be a PMB derivative, by NMR analysis, since only two singlets at 4.37 and 3.60, corresponding to the methylene and methoxy groups respectively, and aromatic signals integrating for four protons, could be detected. The second product showed signals for the TBDPS group and the PMB group, but no signals corresponding to carbohydrate protons could be identified in the ^{1}H NMR.



Scheme 4.68: Attempted benzylation of triol **225**

Reagents and conditions: (i) (a) NaH, DMF, rt, 30 min; (b) PMBCl, 0 °C → rt, 18 h; (ii) (a) NaH, THF, rt, 30 min; (b) TBAI, PMBCl, rt, 5 h; (iii) (a) NaH, DMF, rt, 10 min; (b) BnBr, rt, 18 h.

One more attempt was carried out, this time with BnBr instead of PMBCl (Scheme 4.68). Unfortunately, the outcome of the reaction was the same. It seems that the harsh conditions required for ether formation were resulting in decomposition of the disaccharide. Moreover, it was noticed by TLC analysis that disaccharide **225** had partially decomposed upon standing at room temperature. After purification by flash chromatography two compounds could be isolated, namely silyl derivative **226** and glycoresin **227** (28% and 19% respectively) (Figure 4.5).

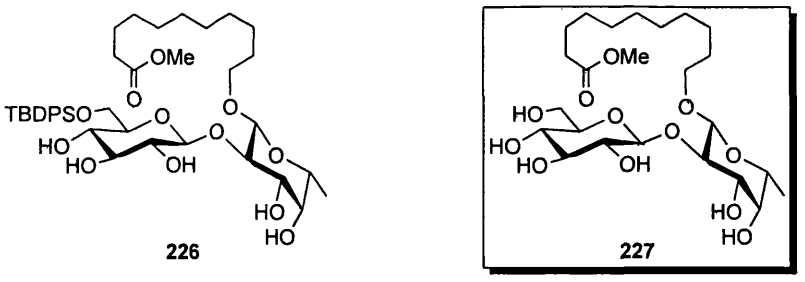


Figure 4.5: TBDPS ether **226** and glycoresin **227**

4.6.4. Summary

In the fifth approach the synthesis of a macrolactone precursor was attempted, which would bear a TBDPS protecting group at the primary hydroxyl group and benzyl or PMB protecting groups at the secondary hydroxyls. Selective silylation of D-glucose was achieved in 71% yield and the disaccharide could be obtained after a few protecting group manipulations, formation of the glucosyl trichloro-acetimidate and subsequent glycosylation with the previously synthesised glycosyl acceptor **195** in 83% yield.

Removal of the acetate esters proceeded smoothly, yielding triol **225** in 97% yield. Several reaction conditions were investigated for the PMB or benzyl protection of the triol; however, all methods failed to produce the desired compound. In addition, partial hydrolysis of the triol **225**, upon standing at room temperature, resulted in TBDPS ether **226** and in another non-macrocyclic compound, namely glycoresin **227**.

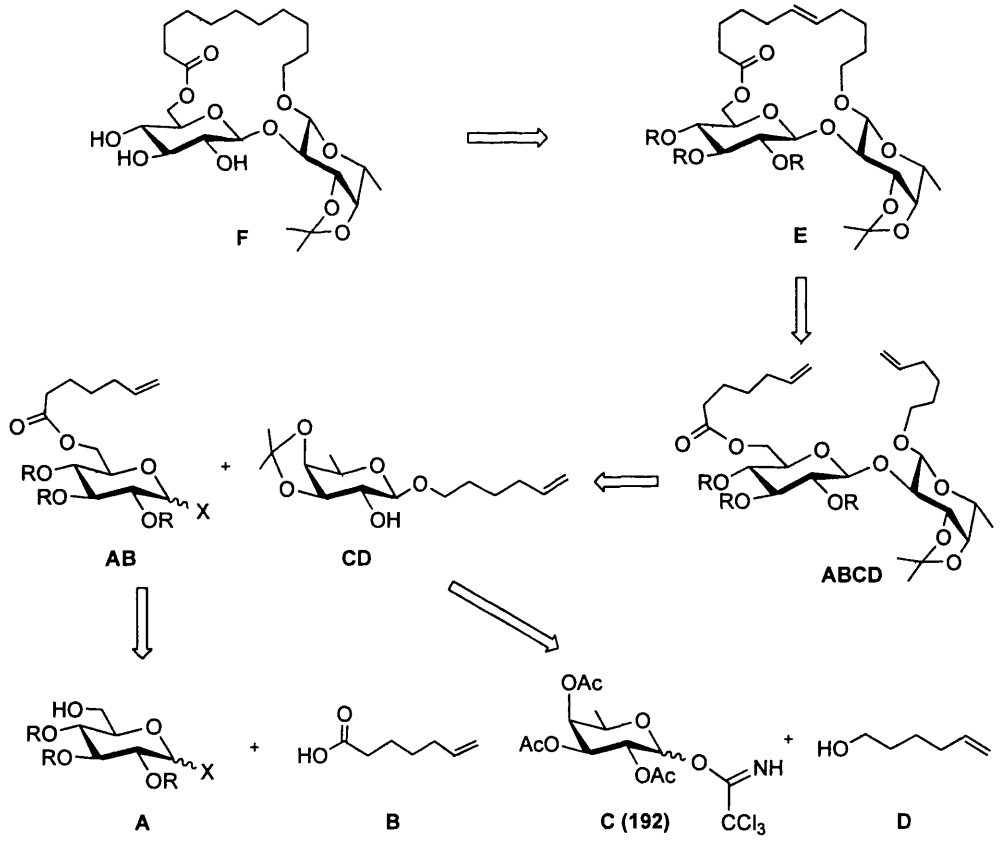
After the failure of the fifth approach to produce the desired macrocyclic compound we decided to abandon the route involving the formation of the macrolactone by esterification of the acid with the primary alcohol. Instead we considered an alternative route, where macrolactonisation would be attempted by ring closing metathesis.

4.7. Sixth Approach

In the sixth approach we decided to investigate an alternative route, involving macrolactonisation by ring closing metathesis, since the formation of the macrolactone by esterification proved difficult. Given that we had already synthesised two non-macrocyclic glycoresins of 11-hydroxyundecanoic acid, we decided to attempt the formation of macrolactone **F** (Scheme 4.69) containing an 11-hydroxyundecanoic acid residue. Moreover the alkene building blocks required for formation of this macrolactone, namely hept-6-enoic acid and hex-5-en-1-ol, were commercially available.

4.7.1. Retrosynthetic Analysis

Retrosynthetic analysis of the target macrolactone **F** is shown in Scheme 4.69.



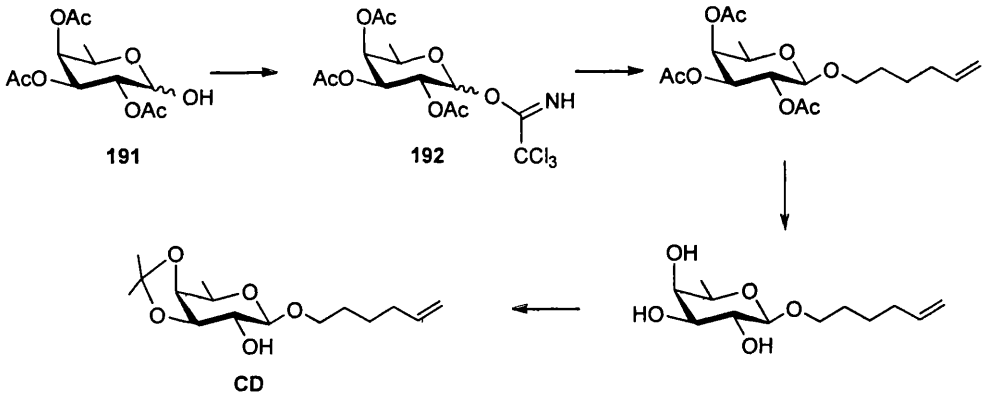
Scheme 4.69: Retrosynthetic analysis of macrolactone **F**

RCM of intermediate **ABCD** would result in the formation of the alkene **E**, which subsequently would require reduction by catalytic hydrogenation. Therefore we

decided to choose the protecting groups for the secondary hydroxyls of glucose such that they could be removed simultaneously by catalytic hydrogenation. Moreover, they should be able to function as neighbouring participating groups during glycosylation, in order to provide the desired β-glycoside **ABCD**. The most suitable protecting group to meet these conditions appeared to be the Cbz carbonate.

Glycoside **ABCD** could be obtained by glycosylation of glycosyl acceptor **CD** with an appropriately protected glycosyl donor **AB**, bearing a leaving group such as trichloroacetimidate or thioglycoside. Glucose building block **AB** could be synthesised by esterification of the primary hydroxyl of monosaccharide **A** with hept-6-enoic acid (**B**), while building block **CD** could be obtained by glycosylation of hex-5-en-1-ol (**D**) with fucosyl trichloroacetimidate **C** and a few simple protecting group manipulations.

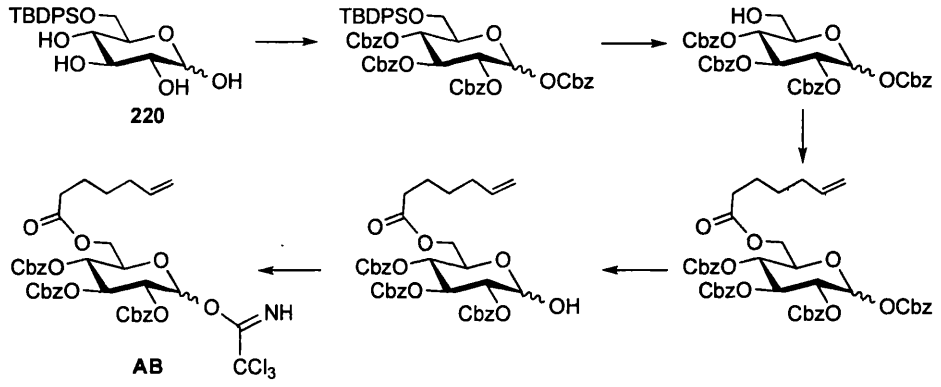
In the synthetic route, the glycosyl acceptor corresponding to building block **CD** could be obtained in a similar manner to the previously synthesised methyl ester fucoside **195**, as shown in Scheme 4.70. Alcohol **191** would be converted to the fucosyl trichloroacetimidate **192**, which would be utilised for glycosylation of hex-5-en-1-ol. Subsequent deacetylation followed by simultaneous protection of the hydroxyl groups at C-3 and C-4 with the isopropylidene ketal would result in glycosyl acceptor **CD**.



Scheme 4.70: Suggested route for the synthesis of the glycosyl acceptor

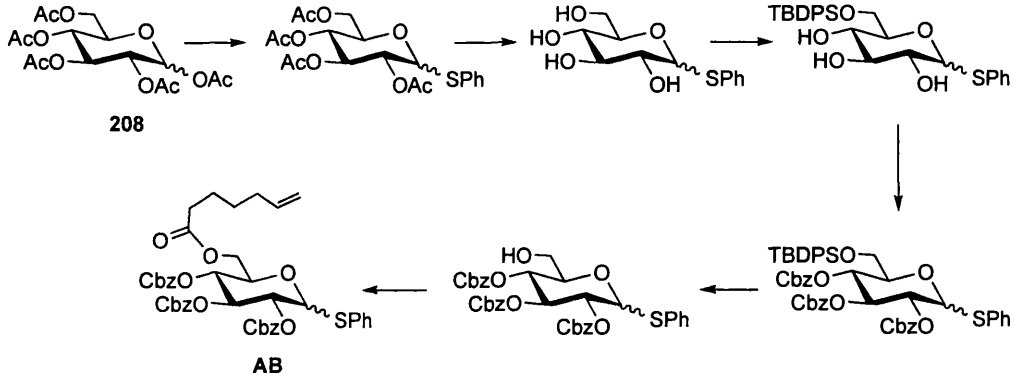
For the assembly of the glucose building block **A** two routes could be envisaged. The first one involved the synthesis of the glycosyl trichloroacetimidate, as shown in Scheme 4.71. The previously synthesised silyl ether **220** would firstly be per-*O*-Cbz protected. Subsequent replacement of the TBDPS ether with a hept-6-enoate

ester would yield the appropriately acylated glucose intermediate. Anomeric deprotection with $NH_2NH_2 \cdot AcOH$ according to a published procedure[92] would then yield the alcohol, which could be converted to the glycosyl trichloroacetimidate representing building block **AB**.



Scheme 4.71: Suggested route for the synthesis of the glycosyl donor I

The alternative proposed route involved the synthesis of the thioglucoside **AB** as shown in Scheme 4.72. Starting with the previously synthesised per-*O*-acetylated glucose **208**, it would be simply converted to the phenyl thioglycoside. Subsequent deacetylation and selective TBDPS protection of the primary alcohol would form the 6-*O*-silyl intermediate. Cbz protection of the secondary alcohols followed by replacement of the TBDPS ether with the hept-6-enoate ester would yield the alternative thioglycosyl donor corresponding to building block **AB**.

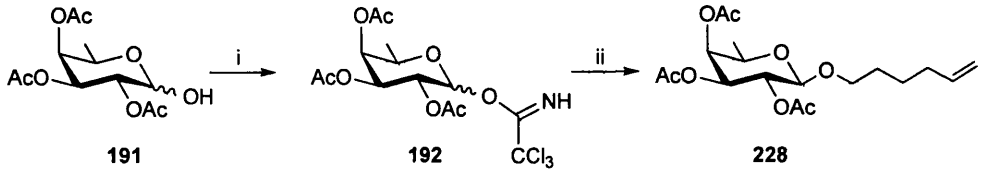


Scheme 4.72: Suggested route for the synthesis of the glycosyl donor II

4.7.2. Synthesis of the Fucose Building Block

The glycosyl acceptor representing building block **AB** was obtained from the previously synthesised alcohol **191**. The tri-*O*-acetyl-fucopyranose **191** was

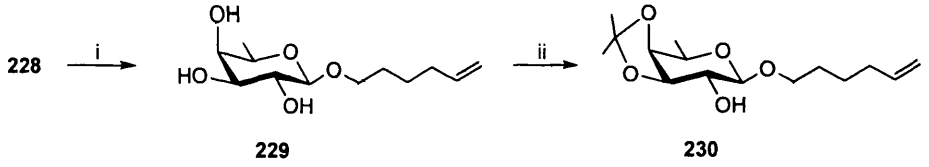
converted to the glycosyl trichloroacetimidate **192** by reaction with trichloroacetonitrile in the presence of Cs_2CO_3 and subsequently used for glycosylation of the commercially available hex-5-en-1-ol in the presence of catalytic amounts of TMSOTf, yielding exclusively the β-glycoside **228** as shown in Scheme 4.73.



Scheme 4.73: Synthesis of glycoside **228**

Reagents and conditions: (i) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 18 h; (ii) hex-5-en-1-ol, TMSOTf, CH_2Cl_2, -20 °C, 1 h, 89%.

Glycoside **228** was deacetylated by reaction with NaOMe in methanol and the resulting triol **229** was treated with 2,2-dimethoxypropane in the presence of PTSA in order to simultaneously protect the hydroxyl groups at C-3 at C-4 as the isopropylidene ketal. The fucoside **230** representing building block **CD** was obtained in 79% yield as shown in Scheme 4.74.



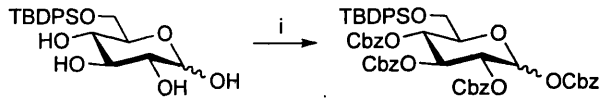
Scheme 4.74: Synthesis of glycosyl acceptor **230** (building block **CD**)

Reagents and conditions: (i) (a) NaOMe, MeOH, rt, 15 min; (b) Amberlite IR 120 (H^+ form) ion exchange resin; (ii) $(CH_3)_2CH(OCH_3)_2$, CSA, acetone, rt, 18 h, 79% over two steps.

4.7.3. Synthesis of the Glucose Building Block

Route I

For the synthesis of the glycosyl donor we decided to follow the first route, which would result in the formation of the glucosyl trichloroacetimidate, since glycosylations involving trichloroacetimidates had worked well so far. For this purpose, the previously synthesised TBDPS ether **220** was treated with CbzCl in the presence of DMAP[92] to yield the tetra-*O*-Cbz carbonate **231** in 62% yield (Scheme 4.75).

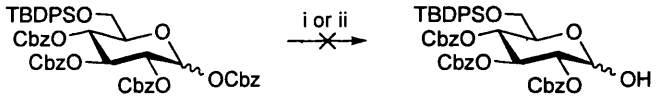


220 231

Scheme 4.75: Cbz protection of TBDPS ether **220**

Reagents and conditions: (i) CbzCl, DMAP, DMF, rt, 1 h, 62%.

Before continuing to the next step of the proposed route, i.e. the removal of the TBDPS ether in order to form the hept-6-enoate ester, we decided to investigate reaction conditions for the selective anomeric deprotection of the Cbz carbonate. The glucose derivative **231** was therefore treated with $NH_2NH_2 \cdot AcOH$ and refluxed for 1 h in MeCN (Scheme 4.76) according to a published procedure.[92] However, only recovered starting material and several uncharacterised by-products were obtained.



231

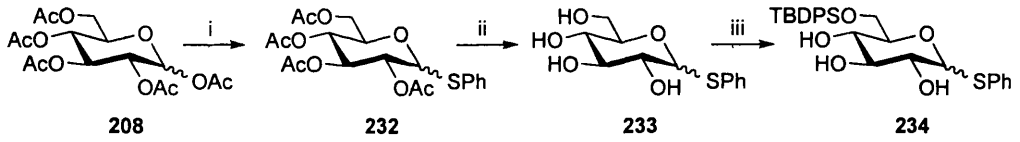
Scheme 4.76: Attempted selective anomeric Cbz deprotection

Reagents and conditions: (i) $NH_2NH_2 \cdot AcOH$, MeCN, reflux, 1 h; (ii) $BnNH_2$, THF, rt, 18 h.

The anomeric deprotection was then attempted with $BnNH_2$. Unfortunately no reaction occurred after stirring the reaction mixture overnight and only starting material could be recovered (Scheme 4.76). Since the selective anomeric Cbz deprotection was not successful, we decided to abandon this route and follow the alternative one, involving the synthesis of the thioglucoside.

Route II

The first step towards the synthesis of the glycosyl donor, corresponding to building block **AB**, via route II, involved the reaction of the per-*O*-acetylated glucose **208** with thiophenol in the presence of $BF_3 \cdot Et_2O$ in order to form thioglycoside **232**. This was subsequently deacetylated with NaOMe to yield the deprotected thiosugar **233**. Selective protection of the primary hydroxyl was achieved by treatment of intermediate **233** with TBDPSCl in the presence of imidazole, resulting in triol **234** as shown in Scheme 4.77.

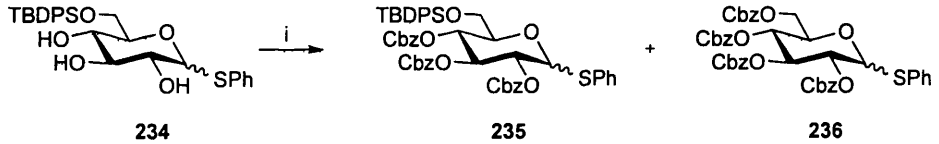


208 232 233 234

Scheme 4.77: Synthesis of thioglycoside triol **234**

Reagents and conditions: (i) PhSH, $BF_3 \cdot Et_2O$, CH_2Cl_2, rt, 18 h, 72%; (ii) (a) NaOMe, MeOH, rt, 1 h; (b) Amberlite IR 120 (H^+ form) ion exchange resin; (iii) TBDPSCl, imidazole, DMF, rt, 1 h, 73% over two steps.

With triol **234** in hand the next step was the protection of the secondary alcohols as the Cbz carbonates. Triol **234** was therefore treated with CbzCl in the presence of DMAP. These conditions, however, resulted in partial hydrolysis of the TBDPS ether, giving the tetra-*O*-Cbz derivative **236** as the main product together with a small amount of the desired compound **235** (Scheme 4.78).

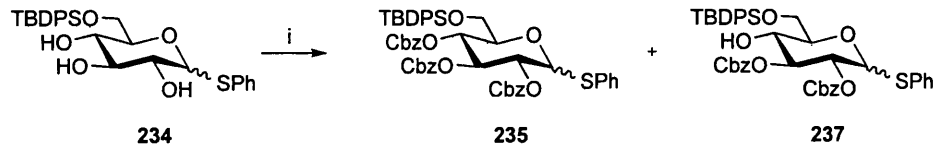


234 235 236

Scheme 4.78: Cbz carbonate protection using DMAP

Reagents and conditions: (i) CbzCl, DMAP, DMF, rt, 15 min, 4% for **235** and 8% for **237**.

This problem could be overcome by employing an alternative method, i.e. CbzCl in the presence of TMEDA.[90] Interestingly, besides the desired compound **235** a small amount of the di-substituted Cbz carbonate derivate **237** could be obtained under these reaction conditions (Scheme 4.79), and was confirmed by extensive NMR analysis. In the ^1H NMR of alcohol **237** only two signals corresponding to the Cbz groups were present. Furthermore, an upfield shift of ~1.2 ppm was observed for the H-4 proton of the di-substituted derivative **237** when compared to tri-substituted derivative **235**. The HMBC spectrum showed cross-couplings between the carbonyl carbons of the Cbz carbonates and the H-2 and H-3 protons, whereas no such coupling was observed between the proton at C-4 and any carbonyl carbon.

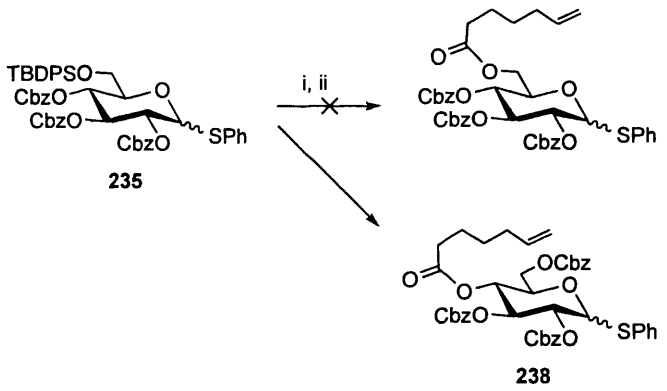


234 235 237

Scheme 4.79: Cbz carbonate protection using TMEDA

Reagents and conditions: (i) CbzCl, TMEDA, CH_2Cl_2, 0 °C, 45 min, 61% for **235** and 19% for **237**.

The next step towards the synthesis of the glucose building block was the removal of the TBDPS ether. This could be achieved by treatment of intermediate **235** with TBAF in the presence of AcOH. It has been reported that Cbz carbonates have a tendency to migrate during purification by column chromatography (neutral Al_2O_3 or SiO_2).[192] Therefore, purification was avoided in order to prevent undesired migrations. The crude alcohol and DMAP were added to a solution of hept-6-enoic acid, Et_3N and 2,4,6-trichlorobenzoyl chloride and the reaction mixture was allowed to stir at room temperature for one hour. TLC analysis indicated the completion of the reaction and after purification by flash chromatography, a compound could be isolated. Surprisingly, thorough characterisation by NMR indicated that the isolated compound was the 4-*O*-hept-6-enoate ester **238** instead of the desired 6-*O*-hept-6-enoate ester (Scheme 4.80). While the protons at H-2 (4.79 ppm), H-3 (5.17 ppm) and H-6 (4.32-4.18 ppm) showed HMBC correlations with the carbonyl groups of the Cbz carbonates (at 154.7, 154.3 and 154.0 ppm), the H-4 proton (5.05 ppm) showed an HMBC correlation with the carbonyl of the hept-6-enoate group (at 172.0 ppm) (Figure 4.6).



Scheme 4.80: Attempted synthesis of the glucose building block

Reagents and conditions: (i) TBAF, AcOH, THF, rt, 5 h; (ii) (a) hept-6-enoic acid, Et_3N, 2,4,6-$Cl_3C_6H_2COCl$, toluene, rt, 2 h; (b) DMAP, rt, 1 h, 61% over two steps.

It appears that migration of the 4-*O*-Cbz carbonate to the 6-OH must have occurred during the removal of the TBDPS ether. Although disappointed by this outcome, we decided to continue with our suggested synthesis in order to investigate the conditions for the formation of the disaccharide and the subsequent RCM.

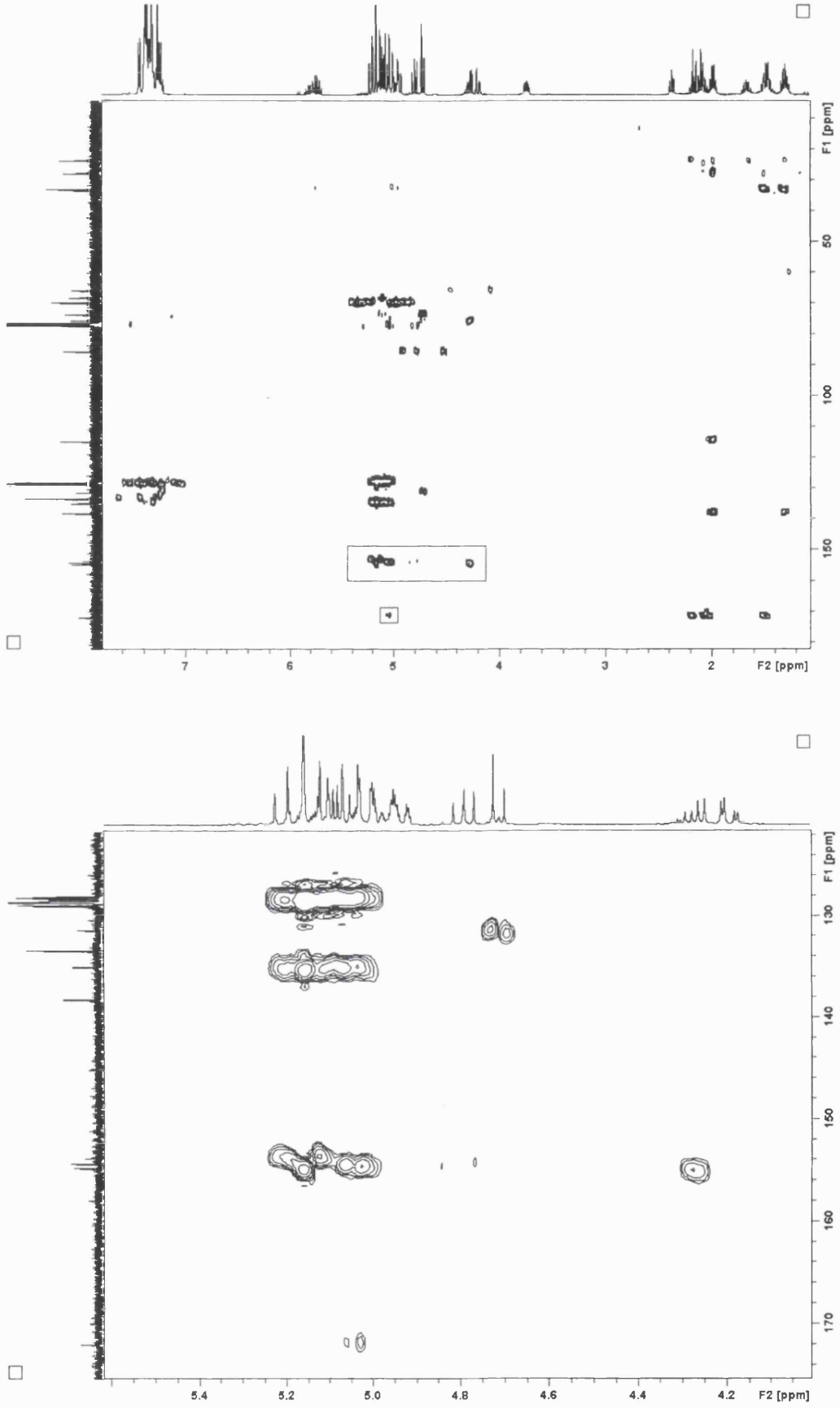
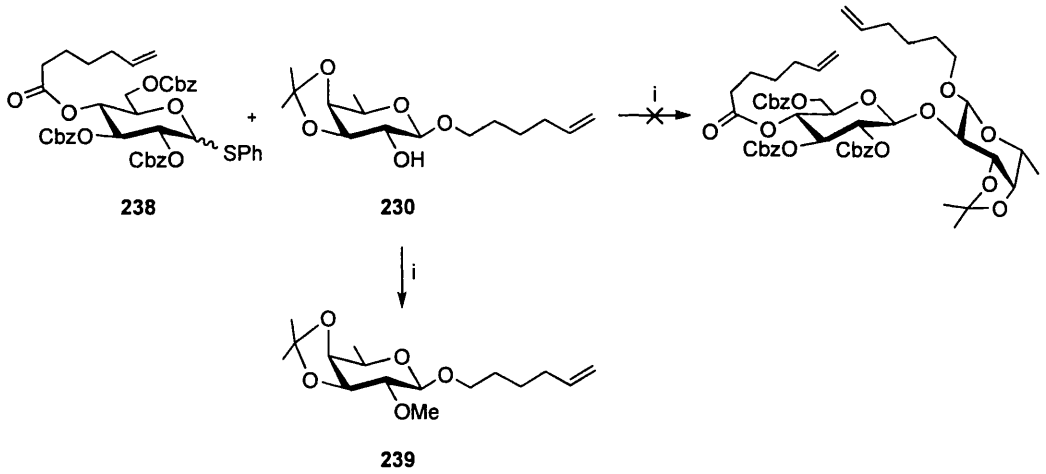


Figure 4.6: HMBC spectrum of **238**

4.7.4. Attempted Synthesis of the Disaccharide

With glycosyl donor **238** and glycosyl acceptor **230** in hand the next step was the formation of the disaccharide. MeOTf instead of NIS was chosen as the thioglycoside activator in order to avoid undesired electrophilic addition of I^+ to the alkenes present on both the glycosyl donor and acceptor. Acceptor **230** and thioglycoside donor **238** were therefore treated with MeOTf for 18 hours. After aqueous work-up and purification by flash chromatography one major product could be obtained, which was subjected to thorough NMR analysis.

From the ^1H NMR spectrum the following observations could be made: all peaks expected from the disaccharide were present; however, the signals corresponding to protons of the glucose moiety were almost identical to those in the ^1H NMR spectrum of the starting material (thioglycoside **238**); in addition a singlet corresponding to three protons was present at 3.59 ppm, suggesting the presence of a methyl ether. Furthermore, the HMBC spectrum showed a cross-coupling between the methyl singlet and the C-2 carbon of fucose. From this data it was suspected that no glycosylation had taken place and instead methylation of fucose by MeOTf had occurred as shown in Scheme 4.81. The isolated product was determined to be a 1:1 mixture of thioglycoside **238** and methyl ether **239**.



Scheme 4.81: Attempted glycosylation of thioglycoside **238** with alcohol **230**
Reagents and conditions: (i) MeOTf, CH_2Cl_2, rt, 18 h.

This assumption was confirmed by ES-MS analysis, which showed peaks at m/z 323 and 807 corresponding to methyl ether **239** $[M+Na]^+$ and thioglycoside **238** $[M+Na]^+$, respectively. Additionally a small peak at m/z 984 was present, indicating that a small amount of the desired disaccharide had formed. However,

it was not possible to isolate this compound by conventional flash chromatography.

4.7.5. Summary

In the sixth approach the synthesis of the target compound was attempted via an alternative route, which would involve an RCM step for the formation of the macrolactone. This approach required the re-design of the disaccharide synthesis and the protecting group strategy. Cbz carbonates were chosen to protect the secondary hydroxyl groups of glucose, since they could function as neighbouring participating groups in order to obtain the desired β-glycoside. In addition, catalytic hydrogenation, which would be employed for the reduction of the alkene (obtained by RCM), would simultaneously cleave the Cbz carbonates.

The glycosyl acceptor was readily synthesised in four steps from alcohol **191**. The synthesis of the glycosyl donor proved more complicated due to the unexpected Cbz migration from the 4-OH to the 6-OH, which in turn resulted in the formation of the 4-*O*-hept-6-enoate ester **238** instead of the desired 6-*O*-hept-6-enoate ester. We decided, however, to carry on the synthesis of the disaccharide in order to investigate the feasibility of this approach. Thioglycoside **238** was therefore subjected to glycosylation with alcohol **230** in the presence of MeOTf. Unfortunately, glycosylation was not successful and, instead, methylation at the C-2 hydroxyl group of fucose by MeOTf occurred.

5. CONCLUSIONS AND FUTURE WORK

5.1. Conclusions

The ipomoeassins, which were isolated from the leaves of *Ipomoea squamosa*, a member of the morning glory family, exhibit highly potent cytotoxic activity against human ovarian cancer cell lines (IC_{50} 35 nM) and thus constitute interesting targets for further investigation. The aim of this project was therefore the synthesis of ipomoeassin B and analogues as potential anticancer agents. Challenges in the synthesis of ipomoeassin analogues involve the formation of the macrolactone ring, two stereoselective glycosylations and the regioselective installation of the acyl side-chains.

Our first approach towards the synthesis of the ipomoeassins involved the formation of the disaccharide and subsequent incorporation of the fatty acid side-chain. The regioselective protection of the glycosyl donor precursor was achieved from commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose in five steps and 28% overall yield. The suitably protected glycosyl acceptor was obtained from D-fucose in two steps and 38% overall yield. The desired β-glycoside was successfully formed as the only product, using the trichloroacetimidate method. However, allyl deprotection, employing several established methods, proved difficult and therefore we decided to modify our synthetic approach by using alternative protecting groups.

The synthesis of 11-hydroxy-4-oxotetradecanoic acid was achieved in seven steps from cycloheptanone and in 2.4% overall yield. Key steps involved the conversion of cycloheptanone into the required hydroxybromoalkane via a retro-Barbier fragmentation, and the alkylation of 2,3-dihydrofuran and subsequent hydrolysis to yield the target acid. Interestingly, the required protection of 10-bromodecan-4-ol as the TBS ether, using TBSCl, resulted in partial nucleophilic substitution of the bromine atom by the chloride anion, yielding a mixture of the TBS protected bromo- and chloroalkanes. This problem was overcome by performing the TBS protection with TBSOTf and the desired bromoalkane was obtained as the only product and in high yield.

The target acid was obtained in low yield (2.4%) due to the incompatibility of the TBS protecting group with the oxidation conditions employed in the final step. These conditions resulted in loss of the TBS ether and oxidation of the resulting alcohol to the ketone, which was obtained as the major product. At this point we

decided to substitute 11-hydroxy-4-oxotetradecanoic acid with the simpler and commercially available 11-hydroxyundecanoic acid in order to examine the feasibility of our approach towards the synthesis of ipomoeassin analogues.

In the second approach, the allyl ether was replaced with a more readily cleavable acetate ester. This approach had also the advantage over the previous one that the desired disaccharide was obtained in fewer steps and higher overall yield. Subsequent glycosylation of methyl 11-hydroxyundecanoate with the fucosyl trichloroacetimidate gave a mixture of the α- and β-fucoside (α:β = 2:1). Attempts to drive the glycosylation towards the formation of the desired β-fucoside as the major product were unsuccessful due to the absence of a neighbouring participating group at C-2 of fucose. Despite the fact that the anomers could be separated by conventional flash chromatography, the yield was not satisfactory. Therefore an alternative approach was sought, where the protected fatty acid side-chain could be introduced stereoselectively.

In order to achieve the desired stereoselectivity, methyl 11-hydroxyundecanoate was introduced prior to the formation of the disaccharide. This way, the desired β-fucoside was formed exclusively due to the presence of the neighbouring participating acetate group at C-2 of fucose. The fucoside acceptor was obtained in a total of six steps and 52% overall yield. Subsequent glycosylation with the glucose trichloroacetimidate donor furnished the β-disaccharide as the only product in 81% yield. Further problems, though, were encountered during the attempted regioselective opening or complete removal of the benzylidene acetal and therefore alternative protecting groups had to be sought for the 4- and 6-OH of glucose.

In the fourth approach we decided to attempt the regioselective opening of the *p*-methoxybenzylidene acetal to the 4-OPMB ether prior to the formation of the disaccharide. This approach would also minimise the requirement for complex protecting group manipulations once the disaccharide was formed. However, the reported conditions for forming the 4-OPMB ether yielded, in contrast to the literature, the undesired 6-OPMB ether.

Alternatively, the per-*O*-acetylated glucosyl trichloroacetimidate was utilised for the formation of the disaccharide. Glycosylation of the previously synthesised fucoside acceptor formed the desired β-disaccharide in 90% yield. Saponification of the esters yielded the macrolactone precursor bearing four hydroxyl groups.

This intermediate was subjected to macrolactonisation in the hope that the more reactive primary hydroxyl would undergo esterification with the acid faster than the secondary hydroxyl groups. Several esterification methods were investigated, including a previously reported lipase-catalysed macrolactonisation, but no macrocyclic product was obtained. Activation of the acid with DIC resulted in isomerisation of the *O*-acylisourea intermediate to the stable *N*-acylurea, which, once formed, cannot undergo esterification.

It is possible that the increased polarity and low solubility of the glycosidic acid might have had a negative effect on the macrolactonisation. In addition, the high dilution conditions, required in order to avoid intermolecular esterification, and the possible interference of the hydroxyl groups could have made activation less efficient. Furthermore, the conformational freedom of the fatty acid side-chain might not allow the activated carboxyl group and the primary hydroxyl of glucose to approach each other sufficiently closely in space to allow efficient reaction. This synthetic route, however, yielded a non-macrocyclic glycoresin (**212**) of potential biological interest.

In an attempt to decrease the polarity of the macrolactone precursor, the acetylated 6-OTBDPS glucosyl donor precursor was prepared in three steps and 49% overall yield. After glycosylation, the acetate esters would be replaced by benzyl or PMB ethers. Glycosylation of the fucoside acceptor furnished the desired β-disaccharide in 83% yield, which was subsequently subjected to acetate deprotection in order to unmask the hydroxyl groups. However, installation of the ether protecting groups was not successful. In addition, partial hydrolysis of the TBDPS and isopropylidene protecting groups occurred, resulting in another non-macrocyclic glycoresin **227**.

In the final approach we decided to investigate macrolactonisation by RCM. This approach required the re-design of the disaccharide synthesis and the protecting group strategy. The desired fucoside acceptor was successfully prepared from the previously synthesised 2,3,4-tri-*O*-acetyl glucopyranose and hex-5-en-1-ol in four steps and 70% overall yield.

The Cbz group has found limited use in carbohydrate chemistry and its application in the synthesis of glycoresins has not been reported before. However, it was the only protecting group that met our requirements (i.e. acting as neighbouring participating group and cleavable by catalytic hydrogenation) and

was therefore chosen for the protection of the secondary hydroxyl groups of glucose. The primary hydroxyl group was masked as the TBDPS ether and the remaining hydroxyls were protected as the Cbz carbonates. Selective anomeric deprotection (necessary prior to conversion to the desired trichloroacetimidate donor), however, using either $NH_2NH_2 \cdot AcOH$, according to an established procedure, or $BnNH_2$ was not successful.

The phenyl thioglucoside was then chosen as the glycosyl donor, which was formed from the per-*O*-acetylated glucose. The acetate groups were replaced by the TBDPS ether for the primary hydroxyl and by Cbz carbonates for the secondary hydroxyls. This approach, however, was unsuccessful due to the unexpected Cbz migration from the 4-OH to the 6-OH during the cleavage of the TBDPS ether, resulting in the formation of 4-*O*-hept-6-enoate ester instead of the desired 6-*O*-hept-6-enoate ester. In addition, glycosylation in the presence of MeOTf resulted in methylation of fucose instead of the formation of the desired disaccharide.

5.2. Summary and Future Work

The stereoselective introduction of the fatty-acid side-chain and disaccharide formation were successfully accomplished. Furthermore, two non-macrocyclic resin glycosides were successfully synthesised. However, the synthesis of macrocyclic analogues was hampered by unanticipated failure of protecting group strategies, unexpected protecting group migrations and unpredictable side-reactions. In many cases, reactions that have been reported to be successful in the literature failed on the particular substrate investigated.

An alternative approach towards the synthesis of the macrolactone could involve the selective formation of the tosylate at the primary hydroxyl group of the glucose moiety[193] and subsequent reaction with the caesium salt of the acid.[194] This method could be employed either for intramolecular macrolactonisation or for the introduction of hept-6-enoic acid and subsequent macrolactonisation via RCM.

In addition, the employment of ether protecting groups (e.g. benzyl or PMB ethers) for the glucose moiety could be investigated. The drawback of this approach, though, is that glycosylation of the fucosyl acceptor with a benzyl protected glucosyl donor would result in a mixture of α- and β-anomers, due to the

absence of a neighbouring participating group at C-2 of glucose. On the other hand this would enable the parallel synthesis of α- and β-analogues of the ipomoeassins.

Furthermore, the employment of a glucal[195] in combination with ether protecting groups could be explored. Glycosylations with glucals via the epoxide usually favour the formation of the β-glycoside. Selective deprotection of the primary hydroxyl group would give the macrolactone precursor. Macrolactonisation could then be attempted either by intramolecular esterification or via RCM after the installation of the required alkene.

After successful formation of the macrolactone, the synthesis of the ipomoeassin B analogue would be completed by installation of the appropriate acyl substituents. Moreover, once the synthetic route towards the formation of the macrolactone is established, it can be employed for the synthesis of ipomoeassin B containing 11-hydroxy-4-oxotetradecanoic acid. Finally, a small library of analogues would be generated by varying the acyl substituents, increasing or decreasing the macrolactone ring size, and changing the stereochemistry and/or the sugar moieties. This methodology, once optimised, would also provide access to other ipomoeassins and potentially to other resin glycosides.

Finally, the cytotoxicity of these compounds will be assessed against several cancer cell lines. Antibacterial activity will be explored as well along with their potential to act as efflux pump inhibitors, since glycoresins have been also found to be potent antibacterial agents. The biological evaluation of these compounds will also enable the investigation of structure-activity relationships to better understand their mechanism(s) of action. Favourable structural features from the most active compounds could be retained and combined to produce more potent second-generation analogues with a view to developing lead compounds of potential clinical interest.

II

POLYSIALYLTRANSFERASES AS A THERAPEUTIC TARGET IN CANCER

6. DESIGN AND SYNTHESIS OF POTENTIAL CARBOHYDRATE-BASED POLYSIALYL-TRANSFERASE INHIBITORS

6.1. Introduction

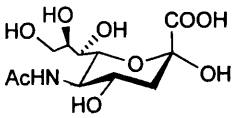
Carbohydrates are the most complex and diverse biopolymers found in nature. Their chain length may vary from monosaccharides to oligosaccharides of 30 building blocks and complex polysaccharides of many thousand building blocks.[196] They are present in the form of glycoconjugates (glycoproteins and glycolipids) on the cell surface and in the extracellular matrix and are involved in many biological processes such as cell-cell recognition and interaction, signal transduction, neural development and function and immune recognition.[197,198]

Glycoconjugates are formed by glycosyltransferases (i.e. enzymes that form glycosidic bonds) and glycosidases (i.e. enzymes that cleave glycosidic bonds). Carbohydrates are commonly attached to glycoproteins either through the amide nitrogen of an asparagine residue (*N*-linked glycoproteins) or through the oxygen of serine or threonine residues (*O*-linked glycoproteins). Glycolipids contain a lipid carrier such as a sphingolipid.[198,199]

Alterations in the glycosylation of glycoproteins and glycolipids have been linked to many pathological conditions such as malignant transformation, inflammation and tuberculosis. These changes involve over- and underexpression of naturally occurring carbohydrates as well as neoexpression of glycans normally restricted to embryonic tissues. Moreover, carbohydrates on the cell surface enable microorganisms such as viruses and bacteria to evade the body's defences. Therefore, synthetic carbohydrates and glycoconjugates have become important tools in biology and pharmaceutical research.[196-201]

6.1.1. Sialic Acid

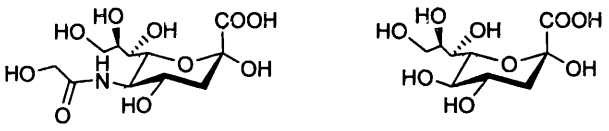
The sialic acids are a family of 9-carbon monosaccharides comprising about 50 members, which play a variety of roles in nature. Most of them are derivatives of *N*-acetylneuraminic acid (Neu5Ac) and deaminoneuraminic acid (KDN) that are modified by various substitutions at the 4, 5, 7, 8 and 9-carbon positions[197,202,203] (Figure 6.1). These substitutions include acetates, phosphates, sulfates and methyl ethers.[204] All sialic acids have a carboxylate at the 2-carbon position, which is ionized at physiological pH.[202] The amino group is normally acetylated or glycolylated.[197]



Neu5Ac

Figure 6.1: Neu5Ac or 'sialic acid'

The three most common sialic acid derivatives are Neu5Ac (Figure 6.1), *N*-glycolylneuraminic acid (Neu5Gc) and KDN (Figure 6.2). Neu5Ac, which is often referred as 'sialic acid', is ubiquitous in all living systems; Neu5Gc is widely distributed in mammalians, fungi and protozoa but not in healthy human tissues and KDN mostly occurs in aquatic organisms and bacteria.[197,203]



Neu5Gc KDN

Figure 6.2: Common sialic acids

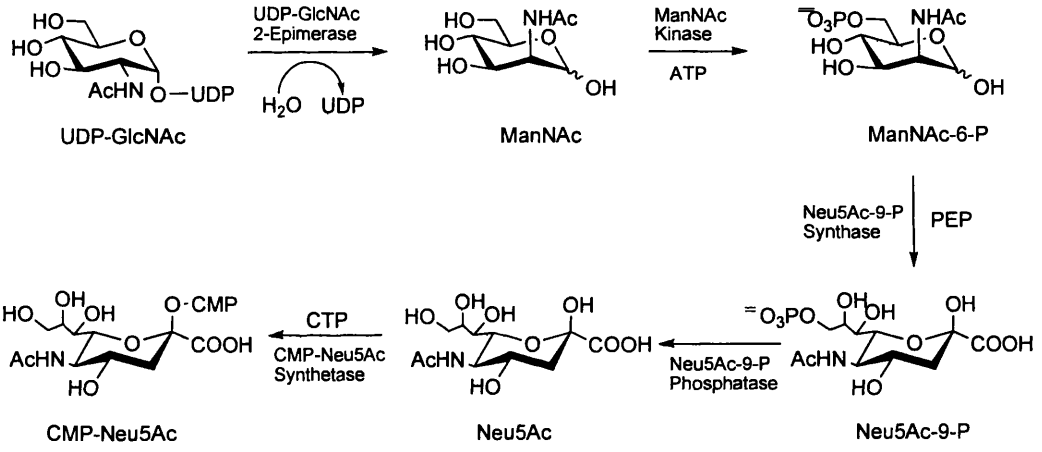
Biological role of sialic acid

Sialic acids are usually located at terminal positions of glycan chains of glycolipids and glycoproteins and are α-glycosidically linked via C-2 to position 3 or 6 of the acceptor sugar. Rarely, they are linked to each other via α2,8-linkages to form the homopolymer polysialic acid.[205,206]

The structural diversity and wide occurrence of sialic acids suggests their involvement in a variety of biological functions. The negative charge causes repulsions and attractions between cells and molecules and allows the sialic acids to bind on and transfer cationic compounds. Moreover, sialic acids influence the conformation of macromolecules and protect them from proteolytic cleavage. The immune system distinguishes between self and non-self structures due to the presence of sialic acids. However, it is the presence of sialic acids that enables viruses, bacteria and other microorganisms to invade the body and cause infection.[197,205,207]

Biosynthesis and activation of sialic acid

The biosynthesis of the most common sialic acid, Neu5Ac, in mammals begins in the cytosol with the epimerization of UDP-*N*-acetylglucosamine (UDP-GlcNAc) to *N*-acetylmannosamine (ManNAc) by the enzyme UDP-GlcNAc 2-epimerase. This enzyme is bifunctional, since it not only catalyses the inversion of stereochemistry at C-2 but also the hydrolysis of the phosphate bond to yield ManNAc and UDP. ManNAc is then phosphorylated by *N*-acetylmannosamine kinase to give N-acetyl-mannosamine-6-phosphate (ManNAc-6P).[204,208,209] *N*-Acetylneuraminic acid synthase subsequently catalyses the formation of *N*-acetylneuraminic acid (Neu5Ac)-9-phosphate by condensation of ManNAc-6P with phosphoenolpyruvate (PEP). Finally, (Neu5Ac)-9-phosphate is dephosphorylated to give Neu5Ac (Scheme 6.1).[204,209]



Scheme 6.1: Biosynthesis and activation of sialic acid in mammals[204]

The activation of Neu5Ac takes place in the nucleus. Cytidine monophosphate (CMP) is transferred from cytidine triphosphate (CTP) to Neu5Ac by CMP-Neu5Ac synthetase to form CMP-sialic acid. The activated CMP-sialic acid is then translocated to the Golgi apparatus or the endoplasmic reticulum where it is added by sialyltransferases onto glycolipids and glycoproteins.[204,205]

6.1.2. Sialyltransferases

Sialyltransferases (ST) are a family of over 20 distinct type II transmembrane glycoproteins, which normally reside within the Golgi apparatus. They catalyse the general reaction:

$$\text{CMP-sialic acid} + \text{acceptor} \rightarrow \text{CMP} + \text{sialyl-acceptor}$$

All sialyltransferases contain a short 3-11 amino acid NH_2–terminal cytosolic domain not required for catalytic activity, a 16-20 amino acid transmembrane domain, a stem region varying from 20-200 amino acids in length, followed by an extensive 300-350 residue COOH-terminal catalytic domain residing in the lumen.[197,210] However, there is little sequence homology among the sialyltransferases except of four conserved peptide regions in their catalytic domain, referred to as sialylmotifs S, L, VL and VS.[211] The L-sialylmotif participates in the binding of the donor substrate while the S-motif contributes to binding of both, donor and acceptor substrates.[197,210,211]

The sialyltransferases are divided into three broad categories according to the manner of sialic acid binding to the acceptor substrate:

- The α2,3-sialyltransferases that catalyse the addition of sialic acid to the 3rd hydroxyl position of the acceptor sugar. Six distinct α2-3 ST (ST3Gal I, ST3Gal II, ST3Gal III, ST3Gal IV, ST3Gal V) have been cloned from human cells or tissues.[210]
- The α2,6-sialyltransferases, which add sialic acid to 6th hydroxyl position of the acceptor sugar. So far cloning and characterization of human ST6Gal I and II[212] and ST6GalNAc I, II and IV have been reported, but six ST6GalNAC are known to exist in mouse.[210]
- The α2,8-sialyltransferases that add sialic acid to 8th hydroxyl position of the acceptor sugar. The cloned and characterized human α2-8ST are ST8Sia I, II, III, IV, V[210] and recently ST8Sia VI.[213]

The mammalian sialyltransferase family and the structures they form are summarized in Table 6.1:

Sialyltransferases	***Structures formed***
ST6Gal I	**Neu5Acα2-6**Galβ1-4GlcNAcβ-
ST6GalNAc I	(Neu5Acα2-3)$_{0-1}$(Galβ1-3)$_{0-1}$GalNAc-Ser / **Neu5Acα2-6**
ST6GalNAc II	(Neu5Acα2-3)$_{0-1}$Galβ1-3GalNAc-Ser / **Neu5Acα2-6**
ST6GalNAc III	Neu5Acα2-3Galβ1-3GalNAc-R / **Neu5Acα2-6**
ST6GalNAc IV	Neu5Acα2-3Galβ1-3GalNAc-R / **Neu5Acα2-6**
ST6GalNAc V	$G_{D1\alpha}$
ST6GalNAc VI	$G_{D1\alpha}$, ($G_{T1\alpha}$)
ST3Gal I	**Neu5Acα2-3**Galβ1-3GalNAc-
ST3Gal II	**Neu5Acα2-3**Galβ1-3GalNAc
ST3Gal III	**Neu5Acα2-3**Galβ1-3/4GlcNAcβ-
ST3Gal IV	**Neu5Acα2-3**Galβ1-4GlcNAc **Neu5Acα2-3**Galβ1-3GalNAc
ST3Gal V	**Neu5Acα2-3**Galβ1-4Glc-Cer
ST3Gal VI	**Neu5Acα2-3**Galβ1-4GlcNAcβ-
ST8Sia I found in foetal brain and lung.	**Neu5Acα2-8**Neu5Acα2-3Galβ1-4Glc-Cer
ST8Sia II (STX) found in foetal brain and kidney and in adult heart, thymus and brain.	**Neu5Acα2-8**Neu5Acα2-3Galβ1-4GlcNAc
ST8Sia III found in foetal and adult brain and liver.	**Neu5Acα2-8**Neu5Acα2-3Galβ1-4GlcNAc
ST8Sia IV (PST) found in foetal brain, kidney and lung and in adult spleen, thymus, heart and leukocyte.	**Neu5Acα2-8**(Neu5Acα2-8)$_n$Neu5Acα2-3Galβ1-R
ST8Sia V found in foetal brain and in adult brain, heart and skeletal muscle.	G_{D1c}, G_{T1a}, G_{Q1b}, G_{T3}

Table 6.1: The mammalian sialyltransferase family

6.1.3. Polysialic Acid

Polysialic acid (PSA) is a linear homopolymer of up to 200 sialic acid residues joined by α2,8-glycosidic bonds (Figure 6.3). It is attached to the neural cell adhesion molecule (NCAM or CD56), a member of the immunoglobulin family of adhesion molecules. Polysialylated NCAM plays an important role during development and neural regeneration and is involved in learning and memory.[214]

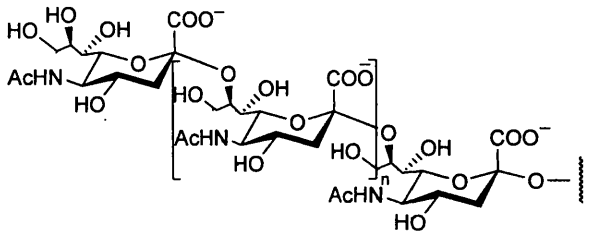


Figure 6.3: Polysialic acid

6.1.4. Polysialyltransferases

PSA is synthesised in the Golgi apparatus, on the fifth immunoglobulin-like (Ig5) domain of NCAM by two sialyltransferases, ST8Sia II and ST8Sia IV (also known as STX and PST, respectively). The two enzymes belong to the family of type II transmembrane proteins and share 59% identity at the amino acid level. The genes for PST and STX are located on chromosomes 5 and 15, respectively. Expression of PSA is always correlated with the presence of either or both enzymes that act synergistically. The amount of PSA produced by cooperative action of PST and STX is larger than that produced by either alone.[215] Some studies have indicated that PST forms longer PSA chains on NCAM than STX. Furthermore, PST can add sialic acid on NCAM that already contains polysialic acid more efficiently than STX. However, highly polysialylated NCAM is a poor acceptor for either enzyme.[216]

It has been shown that in order to be able to synthesise polysialic acid, STX and PST require an α2,3- or α2,6- linked sialic acid attached on *N*-glycans in NCAM. It seems though, that α2,3-linked sialic acid is a better acceptor than α-2,6- linked sialic acid.[214] Although PSA is synthesised on Ig domain 5, adjacent domains such as the 4th Ig and the 1st fibronectin (FN) domain also play a role.[214,217] NCAM's Ig5 domain contains six potential *N*-glycosylation sites.[215] PSA is added on the 5th

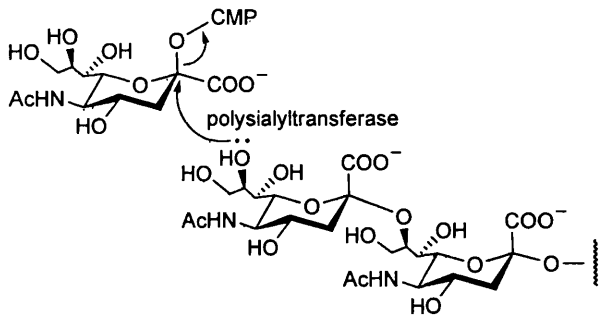
and 6th glycosylation sites, with PST showing a preference for the 6th over the 5th glycosylation site whereas STX does not show this preference.[216]

Besides forming polysialic acid on NCAM, PST and STX are able to polysialylate themselves (autopolysialylation). PSA chains are synthesised on *N*-glycans of the enzymes. In contrast to NCAM polysialylation however, the presence of an α2,3- or α2,6-linked sialic acid attached on the *N*-glycans is not required for autopolysialylation. Although the mechanism of autopolysialylation is not yet clear, it has been shown that PSA synthesised on the enzymes is not transferred to NCAM-PSA. It had been suggested that autopolysialylation is required for the efficient function of the polysialyltransferases.[218,219] However, further studies showed that non-autopolysialylated enzymes can still synthesise polysialic acid on NCAM, although to a lesser degree.[220]

The mechanism by which the sialyltransferases catalyse polysialic acid synthesis still remains unknown. Both enzymes are capable of adding sialic acid to NCAM but polymerisation is higher when they act cooperatively. It has been suggested that they exhibit distinct roles on PSA synthesis. Furthermore, STX is thought to be more abundant in embryonic tissues, while PST is constantly expressed but to a lesser degree. The major question that arises is why two distinct sialyltransferases with the same function exist in the vertebrate system.[216,221]

Biosynthesis of polysialic acid

PSA is synthesised in the Golgi apparatus, on the fifth immunoglobulin-like (Ig5) domain of NCAM. NCAM's Ig5 domain contains three asparagine residues at positions 404, 430 and 459, which could act as PSA acceptors. However, only 430 and 459 have been shown to be associated with PSA.[222] Sialic acid is added to NCAM in its activated CMP-sialic acid form. The CMP-moiety acts as a high energy leaving group and facilitates the addition of sialic acid to PSA by the action of polysialyltransferases PST and STX (Scheme 6.2). The synthesis of PSA begins with the addition of sialic acid via α2,8-linkage to sialyl *N*-acetyllactosamine (NeuNAcα2-3(or 6)Galβ1-4GlcNAcβ1-R) on NCAM. The subsequent sialic acids are then attached to form a long α2,8-linked polysialic acid chain.[216]



Scheme 6.2: Biosynthesis of Polysialic acid

The expression of polysialic acid on NCAM is regulated at the transcriptional level by the activity of the sialyltransferases PST and STX as well as by non-transcriptional ways.[215,222] Developmental and physiological events may result in localised and rapid changes of PSA expression. For example, nerve-muscle interactions alter the pattern of PSA expression. Contact of axons with muscle cells downregulates PSA expression in neurons, while blockage of synaptic activity results in up- and downregulation of PSA expression in neurons and muscle cells, respectively.[223,224]

6.1.5. Role of Polysialic Acid

Polysialic acid attached to NCAM is abundantly expressed in embryonic tissues and during development. Its large volume and multiple negative charges enable it to regulate interaction of NCAM with other molecules on the same membrane (*cis*-interaction) or on other cell membranes (*trans*-interaction). PSA is also involved in cell migration by regulating adhesion and de-adhesion of the cells, which is required for cell motility. Furthermore, PSA plays an important role in axon guidance. It allows axons to defasciculate in order to form muscle-specific nerves. This process is hindered in the absence of PSA.[221,224]

It was shown that NCAM-deficient mice or mice treated with endoneuraminidase-N (endo-N) (which specifically removes PSA on NCAM) developed a smaller olfactory bulb and brain because the cells failed to migrate from the subventricular zone (SVZ) to the olfactory bulb.[225] Moreover, it was found that the cells had undergone premature differentiation, since the morphology of these cells had changed from the characteristic chains of cells into a mass of cell bodies and long neurites.[226] PSA-deficiency also resulted in impairment of spatial memory and prevented the induction of long term potentiation (LTP) and long

term depression (LTD) i.e. the strengthening and weakening of synapses, respectively. These results indicate that synapse formation, through which the nervous system transmit signals to other systems of the body, is regulated by PSA.[227,228]

Significantly, PSA is almost non-existent in adult tissues, except for some regions of the brain such as the hippocampus, hypothalamus and olfactory bulb, where neural regeneration and plasticity persists.[221,229,230]

6.1.6. Polysialic Acid in Cancer

Polysialylated NCAM is virtually non-existent in adult tissues, limited to some restricted areas of the brain. Significantly, however, re-expression of PSA-NCAM has been demonstrated on the cell surface of a number of malignant tumours principally of neural crest origin, such as Wilms tumours,[231] neuroblastomas,[232] small and non-small cell lung carcinomas (SCLC and NSCLC, respectively)[233-236] (Table 6.2) and has been associated with poor prognosis and metastatic spread.

Tumours expressing PSA
Neuroblastomas
Wilms tumour
Medulloblastomas
Phaeochromocytomas
Medullary carcinomas of the thyroid
Small cell lung cancer
Non-small cell lung cancer
Pituitary adenomas
Rhabdomyosarcoma
Multiple myeloma
Pancreatic carcinoma
Gliomas

Table 6.2: Tumours that express polysialic acid

The metastatic cascade is a multistep process involving the detachment of cells from the primary tumour, invasion to the surrounding tissues and migration to distant sites. PSA-NCAM has been associated with these events, and our

hypothesis is that synthesis of PSA on NCAM signals this change in cell phenotype. The negative charge of PSA attenuates cell-cell contact, leading to reduced cell-cell adhesion, thereby allowing detachment of cells from the primary tumour. This subsequently results in invasion and metastasis.[235,237,238]

Daniel and co-workers developed a metastatic model in nude mice by using the human rhabdomyosarcoma cell line TE 671, which strongly expresses PSA-NCAM, in order to investigate if PSA-NCAM is involved in the formation of metastases. Injection of this cell line to mice gave rise to lung metastases. Subsequent enzymatic removal of PSA on primary tumours, using Endo-N, resulted in reduction of the number of lung metastases when compared to untreated mice. Therefore, it was concluded that there was a correlation between PSA expression on primary tumours and metastasis.[239]

Pituitary adenomas are usually benign neuroendocrine tumours; however, some behave aggressively and metastasise. In a study it was shown that benign tumours with low growth rate never expressed PSA-NCAM, while benign tumours with a high growth rate expressed a low amount of PSA-NCAM. The highest PSA-NCAM expression, however, was observed in tumours that invaded the surrounding tissues and metastasised. PSA-NCAM expression correlated also with cell proliferation, although to a lesser degree.[240]

Neuroblastoma, a childhood neoplasm, is the second most common solid tumour found in children. It belongs to the highly malignant and metastatic tumours and has consistently been found to express PSA-NCAM. A number of genetic and biological features have been identified in recent years that correlate with disease progression, the most significant of which is the N-MYC oncogene, which is linked with aggressive, invasive behaviour. Studies have demonstrated that there is a correlation between PSA expression and N-MYC amplification. PSA expression may also be a marker for undifferentiated neuroblastoma, correlating with aggressive disease. In addition, current evidence suggests that modulation of NCAM in neuroblastoma is associated with cells spontaneously adapting and changing to chemoresistant forms.[241-244]

SCLC, a particularly aggressive neoplasm, is fatal despite initial sensitivity to chemotherapy. PSA expression has consistently been observed in SCLC. Studies also correlate PSA expression with aggressive disease, poor prognosis, and high metastatic potential. In addition, current evidence suggests, like in neuroblastoma,

that NCAM modulation in SCLC is associated with cells spontaneously adapting and changing to chemoresistant forms.[245-247]

PSA expression has also been correlated with tumour progression of NSCLC. It was demonstrated that PSA was expressed only in 20.8% of pathological stage I cases, whereas it was expressed in most stage IV cases (76.8%). Moreover, it was found that the percentage of patients with PSA expression was significantly higher in cases with lymph node metastases (69.6% vs. 35.3% in node-negative cases) or distant metastases (78.6% vs. 39.5% in cases without distant metastasis). No correlation was observed between the local extent of the primary tumour and PSA expression.[235]

Tanaka and co-workers suggested that PSA synthesis in NSCLC depends mainly on STX. They showed that PSA is expressed in NSCLC even on NCAM-negative cells, supporting the idea that PSA can be attached to other molecules as well, especially in malignant transformation. Moreover, they found that PST was expressed in normal and cancer tissues, while STX was expressed only in advanced stage cancer tissues. However, PSA was present on NCAM-negative cells only when STX was present whereas PSA on NCAM-positive cells could be expressed with or without STX, suggesting that PST can synthesise PSA only on NCAM in NSCLC.[235]

On the other hand, PST was shown to play a predominant role in polysialic acid synthesis in neuroblastoma[237] and glioma tumours.[248] The amount of PSA as well as the size of PSA-NCAM were found to be correlated with the mRNA level of PST in neuroblastoma cells, although the mRNA level of STX was significantly higher.[237] Furthermore, it was demonstrated that the invasive nature of glioma cells was mainly associated with PSA synthesised by PST. Gliomas, the most common type of brain tumours, normally do not metastasise to other organs but are invasive to surrounding tissues and the presence of PSA was found to increase invasion.[248]

Schreiber and co-workers[249] recently demonstrated the importance of PSA in the interaction of NCAM with E-cadherin, which resulted in decreased cell aggregation and increased cell migration. E-cadherin is an epithelial calcium-dependant cell-cell adhesion protein, which mediates adhesion through homotypic binding. E-cadherin is known to be a tumour suppressor protein, while loss of its function has been associated with increased invasiveness and cancer metastasis.[250]

90% of pancreatic adenocarcinomas harbour K-Ras mutations. It was shown that the K-Ras oncogene induced the expression of the polysialyltransferases PST and STX and of PSA-NCAM in pancreatic tumour cells. This in turn led to PSA-NCAM binding to E-cadherin, thereby blocking cell-cell adhesion and initiating cancer metastasis. Importantly, enzymatic removal of PSA from NCAM or inhibition of expression of NCAM and/or the polysialyltransferases by siRNA, diminished the NCAM-E-cadherin interaction and significantly reduced the enhanced migration of K-Ras-expressing pancreatic carcinoma cells.[249]

From all these studies it can be concluded that PSA plays an important role in cancer progression and metastasis. The presence of PSA-NCAM on tumour cells has been associated with poor prognosis, aggressive disease, invasion to surrounding tissues and high metastatic potential. PSA is synthesised on NCAM by two sialyltransferases, i.e. PST and STX, both of which are able to synthesise PSA independently. Therefore, PST and STX are potential targets for cancer therapy since their inhibition might result in prevention of PSA synthesis and thus reduction or even prevention of cancer progression and metastasis.

6.1.7. Inhibition of PSA Synthesis

It has been shown that the unnatural sialic acids *N*-levulinoylmannosamine (ManLev),[251] *N*-propranoylmannosamine (ManProp) and *N*-butanoylmannosamine (ManBut)[252,253] (Figure 6.4) can be incorporated in the polysialic acid biosynthetic pathway. The presence of ManLev or ManProp on NT2 neurons did not impede PSA synthesis and neurite outgrowth, although polymerisation was reduced, whereas ManBut, which contains one more methylene group than ManProp, did.[251,253] Moreover, incubation of ManBut with several cancer cells resulted in reversible inhibition of polysialic acid synthesis while ManProp did not have such effect on the biosynthetic process.[252] These results indicate that mannosamine derivatives with at least two additional methylene groups at the *N*-acyl domain can efficiently inhibit PSA synthesis. On the other hand the presence of a ketone at the *N*-acyl domain (ManLev) does not affect PSA biosynthesis.[251,253]

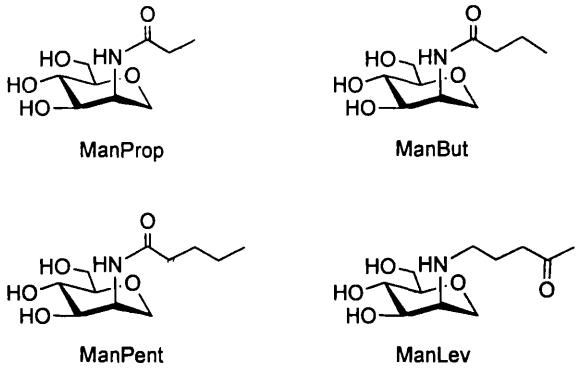


Figure 6.4: Unnatural sialic acids

The study was further extended to explore if there was any selective inhibition of the polysialyltransferases. Cells that expressed either STX or PST were treated with ManProp, ManBut and *N*-pentanoylmannosamine (ManPent) (Figure 6.4). All three molecules were able to significantly reduce (ManProp) or block (ManBut and ManPent) polysialic acid synthesis on cells that expressed STX. On the contrary PST was able to synthesise polysialic acid in the presence of either of the unnatural sialic acids. These results indicate that it is in fact the transformed CMP-sialic acid derivative of ManBut/ManPent that acts as an inhibitor of STX, but not PST, since all the other enzymes in the biosynthetic pathway are identical. Furthermore, this demonstrates the potential that PST and STX may possess distinct functions.[254]

6.1.8. Hypothesis

Inhibition of PST and STX by small molecules will diminish the opportunity for cancer cells to transform to a phenotype which exhibits reduced cell-cell adhesion, increased cell invasion and migration and thus metastatic potential.

6.2. Strategies for Design and Synthesis of Sialyltransferase Inhibitors

The aim of this project is the design and synthesis of potential carbohydrate-based inhibitors of PST and STX.

Sialic acid is transferred by the polysialyltransferases to the acceptor sugar in its activated CMP-sialic acid form (Figure 6.5), which is subsequently hydrolysed to yield polysialic acid. The sialic acid moiety is linked to cytidine via a phosphate group in CMP-sialic acid. Replacing the phosphate group with a more stable sulfur or carbon linkage will lead to a non-hydrolysable compound as such is a potential inhibitor. Moreover, modifying the type of sugar and the cytidine moiety will result in simpler compounds that retain the structural features of CMP-sialic acid and potentially are able to bind irreversibly on the polysialyltransferases.

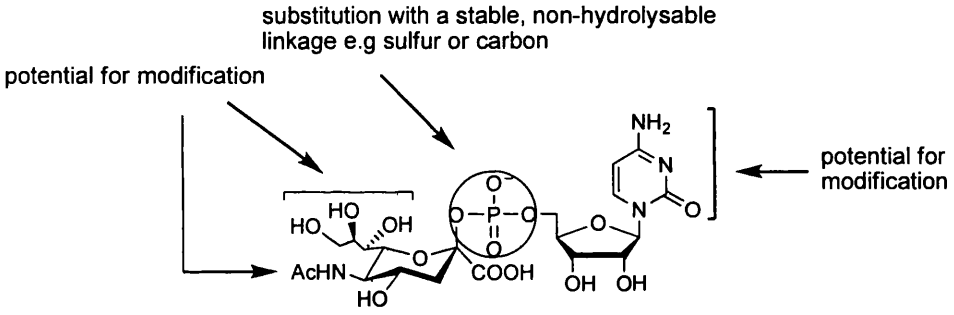


Figure 6.5: CMP-sialic acid and potential modifications

To date few potent sialyltransferase inhibitors have been synthesised (Figure 6.6). These include compounds where the phosphate linkage has been replaced by a non-hydrolysable sulfur linkage[255,256] (**240**), *C*-glycosides[257] (**241**), CMP-quinic acid[258] (**242**), peptide mimetics of CMP-sialic acid[259] (**243**) and sialyl mimetic nucleosides where the entire sialic acid moiety has been replaced by a non-sugar moiety[260] (**244**).

It has been suggested that the sialic acid moiety of the substrate may not be essential for tight binding with the active site of the enzyme. Moreover the phosphate linkage may not be necessary since mimetic **241** was found to exhibit significant sialyltransferase inhibiting activity.[257]

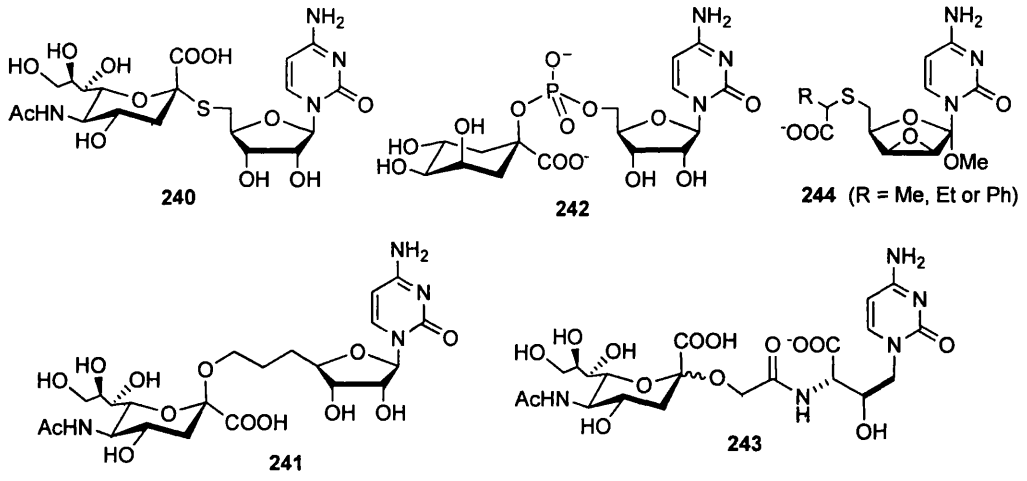


Figure 6.6: Examples of previously synthesised CMP-sialic acid mimetics

The initial aim of the project was to investigate the importance of various regions of the CMP-sialic acid structure in terms of potential binding to the enzyme active site. Initially, the sialic acid component was replaced by a simpler sugar mimic, and for this purpose L-rhamnose was selected (general structure Figure 6.7). L-Rhamnose has a similar conformation to sialic acid but is much simpler. It does not bear the carboxylic acid group at C-1, the *N*-acyl domain at C-5 and the long poly-hydroxyl group side-chain at C-6, which will help to explore the importance of these domains when binding to the polysialyltransferases. This is the starting point for a detailed structure-activity relationship, which will eventually lead to sialic acid analogues. The thioether-linked aglycon R would initially include variously substituted benzyl groups, as simple CMP substitutes and build up to full cytidine analogues.

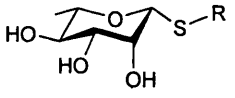


Figure 6.7: Thio-linked rhamnose derivatives

The second part of the project aimed at synthesising the CMP-sialic acid mimetic shown in Figure 6.8. In this compound the sialic acid moiety would be replaced by a rhamnose derivative and the phosphate group by a non-hydrolysable sulfur linkage, which would mimic the distance between CMP and the sugar moiety in CMP-sialic acid.

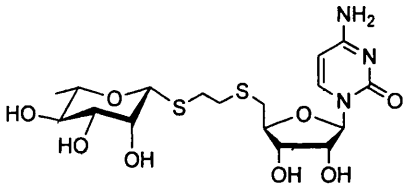
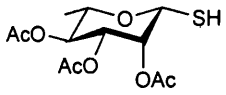


Figure 6.8: CMP-sialic acid mimetic

Further modifications can be introduced at the 7′, 8′ and 9′ hydroxyls of the sialic acid side-chain as well as at the amine group as shown in Figure 6.5. Future work will also involve biological screening of these compounds in order to evaluate their effectiveness as polysialyltransferase inhibitors and to help synthesise more potent second-generation analogues.

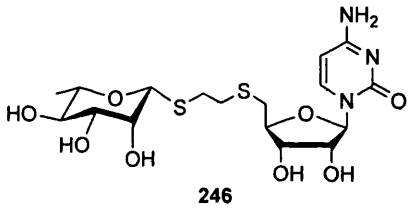
6.3. Results and Discussion

The initial aim of the project was to synthesise a small library of L-rhamnose-based compounds with the potential to act as polysialyltransferase inhibitors. Thio-linked rhamnose **245** (2,3,4-tri-*O*-acetyl-1-thio-*α*/*β*-L-rhamnopyranoside) was the key intermediate, which could be subsequently coupled with variously substituted benzyl groups to yield the target compounds.



245

The next aim was to synthesise a rhamnose-based compound **246** that would mimic sialic acid in PSA biosynthesis but would not promote addition to the PSA chain due to the presence of the non-hydrolysable sulfur linkage.

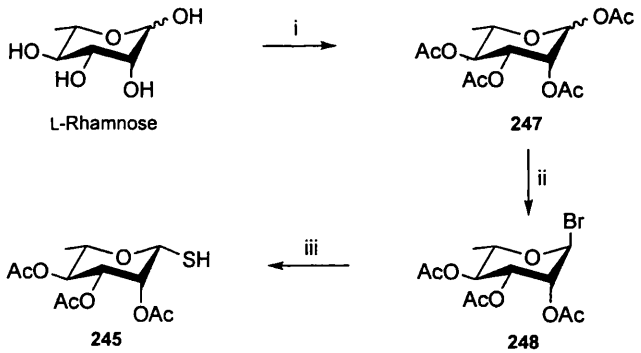


246

6.3.1. Synthesis of Thio-Linked Rhamnose Compounds

Synthesis of the thio-rhamnoside

The important intermediate **245** was synthesised from L-rhamnose in three steps as shown in Scheme 6.3:



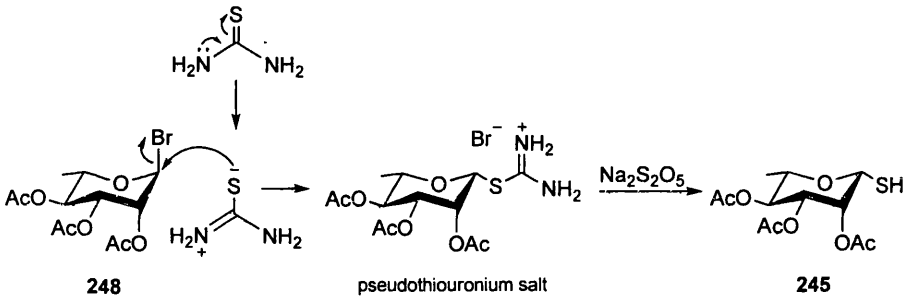
L-Rhamnose **247** **248** **245**

Scheme 6.3: Synthesis of the thioglycoside **245**

Reagents and conditions: (i) Ac_2O, pyridine, rt, 2 h, 97%; (ii) HBr (45% in AcOH), Ac_2O, CH_2Cl_2, rt, 2 h, 81%; (iii) (a) thiourea, acetone, reflux, 12 h; (b) $Na_2S_2O_5$, 1,2-dichloroethane, H_2O, reflux, 15 min, 58%.

The first step involved the protection of the hydroxyl groups in L-rhamnose via acetylation. This was achieved by stirring L-rhamnose with acetic anhydride in the presence of pyridine at room temperature. This reaction gave a mixture of α- and β-anomers. The protected sugar was then treated with a solution of HBr in acetic acid to yield the rhamnosyl bromide **248**.

In the final step 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide was reacted with thiourea in acetone to give a pseudothiouronium salt. This reaction is likely to proceed via an S_N2 mechanism as shown in Scheme 6.4.

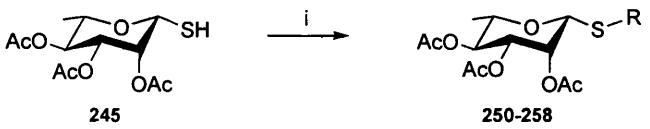


Scheme 6.4: Proposed mechanism for thioglycoside formation

The resulting pseudothiouronium salt was then hydrolysed with aqueous sodium metabisulfite ($Na_2S_2O_5$) to yield 2,3,4-tri-*O*-acetyl-1-thio-α/β-L-rhamnopyranoside (β:α=77:23). The obtained mixture of α- and β-anomers of **245** indicates the presence of a small amount of β-rhamnosyl bromide in the starting material, since the thioglycoside formation proceeds via an S_N2 mechanism.

Coupling and deprotection of rhamnose derivatives

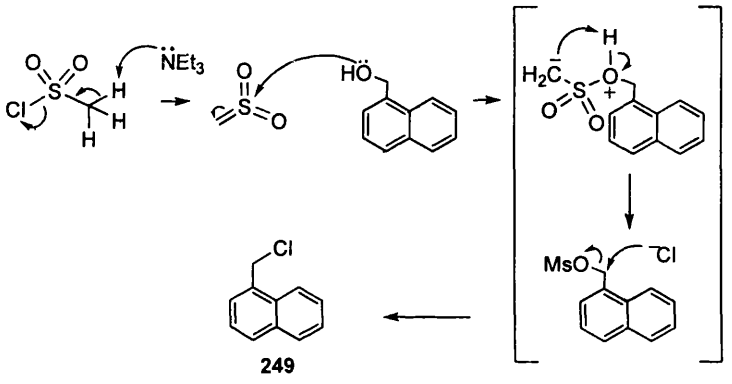
In three simple steps we accomplished the synthesis of the important intermediate thio-rhamnose **245**, which was subsequently coupled with a series of substituted benzyl halides (Table 6.3) in dimethylformamide (DMF) in the presence of diethylamine (Et_2NH).[256] The general reaction outline is shown in Scheme 6.5. The products and their respective yields are summarised in Table 6.3.



Scheme 6.5: General coupling reaction outline

Reagents and conditions: (i) RBr or RCl, Et_2NH, DMF, 1 h, rt.

All the benzyl halides used were commercially available, with the exception of 1-naphtalene-methyl chloride **249** (Table 6.3), which had to be synthesised from 1-naphtalene-methanol (Scheme 6.6). Since alcohols are poor leaving groups and will not undergo substitution reactions easily it was necessary to first convert the OH group into a good leaving group. The mesyl group (Ms) was thus introduced. Surprisingly, reaction with the chloride anion did not result in the expected mesylate but gave the methyl chloride instead. It is likely that the mesylate had been formed initially but, due to the prolonged reaction time (12 hours), it was substituted by the chloride atom eventually. The possible mechanism is shown in Scheme 6.6:



Scheme 6.6: Proposed mechanism of the formation of 1-naphtalenemethyl chloride

In the synthesis of compounds **250** and **251**, the reaction mixture was subjected to aqueous worked-up by washing with 10% HCl before purification by column chromatography. However, it was suspected that this resulted in considerable loss of product. Hence, the washing step was eliminated in the synthesis of compounds **252-258** and the yield increased dramatically (Table 6.3).

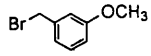
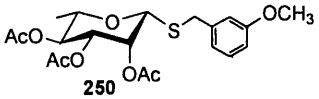
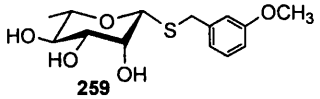
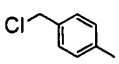
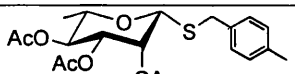
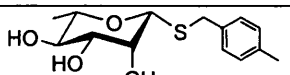
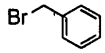
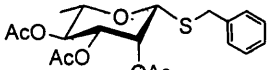
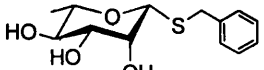
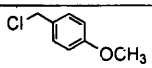
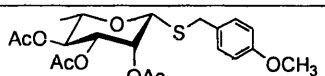
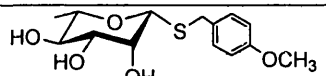
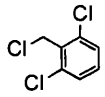
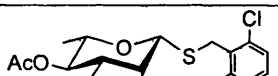
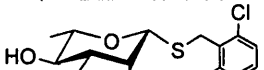
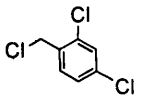
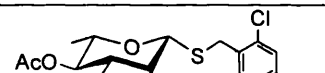
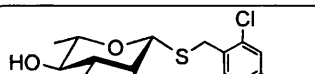
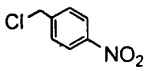
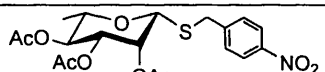
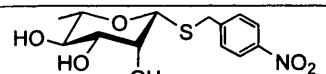
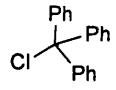
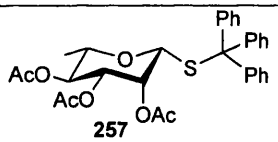
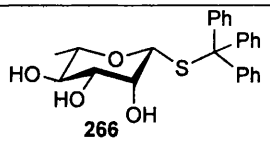
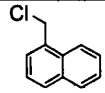
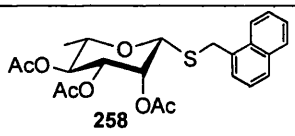
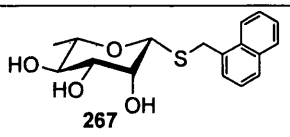
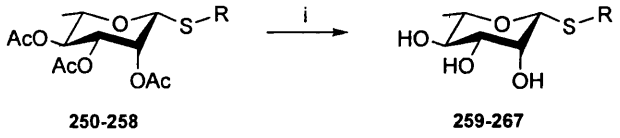
R-X	Acetylated product	Yield (%)	Target product	Yield (%)
	 **250**	13%	 **259**	78%
	 **251**	16%	 **260**	37%
	 **252**	69%	 **261**	66%
	 **253**	79%	 **262**	59%
	 **254**	81%	 **263**	65%
	 **255**	64%	 **264**	59%
	 **256**	93%	 **265**	70%
	 **257**	59%	 **266**	60%
 **249**	 **258**	77%	 **267**	28%

Table 6.3: Coupling and deacetylation products

Finally, products **250-258** were deprotected to give the target products **259-267**. Deacetylation was achieved by the Zemplen procedure (Scheme 6.7) i.e. treatment of the sugars with sodium methoxide in methanol. After completion of the

reaction ion exchange resin was added in order to neutralize the solution. The yields of the products are summarised in Table 6.3.

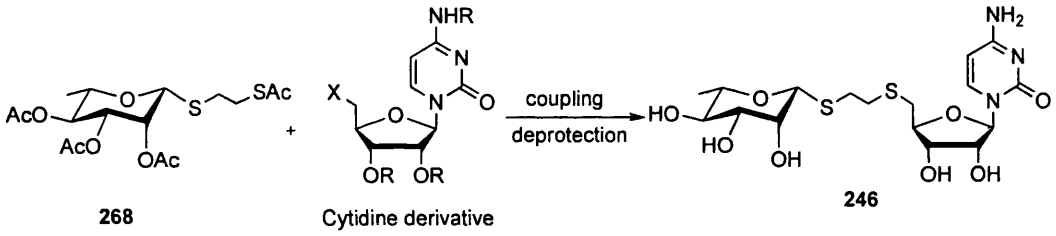


Scheme 6.7: Synthesis of the target compounds

Reagents and conditions: (i) (a) NaOMe, MeOH, rt, 1 h;
(b) Amberlite IR 120 (H^+ form) ion exchange resin.

6.3.2. Synthesis of a CMP-Sialic Acid Mimetic

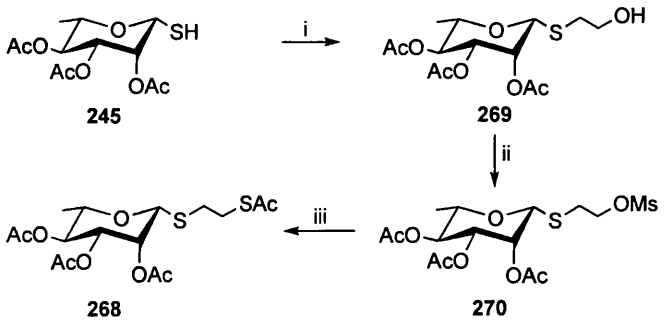
The second part of this project aimed at synthesising the CMP-sialic acid mimetic **246**. A reasonable synthesis would be to couple compound **268** with a cytidine derivative and subsequently deprotect, as shown in Scheme 6.8:



Scheme 6.8: Suggested synthesis of the CMP-sialic acid mimetic

Synthesis of the rhamnose moiety

Since (*S*-ethyl thioacetyl)-2,3,4-tri-*O*-acetyl-1-thio-*β*-L-rhamnopyranoside (**268**) is not commercially available the first step was to synthesise this compound. This was easily achieved in three steps from **245**, as shown in Scheme 6.9:



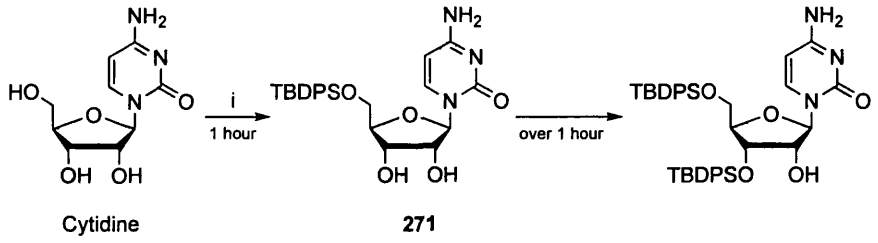
Scheme 6.9: Synthesis of the thioglycoside **268**

Reagents and conditions: (i) 2-chloroethanol, Et_2NH, DMF, 12 h, rt, 70%; (ii) MsCl, Et_3N, CH_2Cl_2, 0 °C → rt, 12 h, 85%; (iii) KSAc, DMF, 80 °C, 12 h, 71%.

The thio-rhamnoside **245** was first coupled with 2-chloroethanol in DMF using Et_2NH as catalyst to yield thioglycoside **269**. The hydroxyl group of **269** was then converted to a good leaving group by mesylation. Treatment of the mesylated **270** with potassium thioacetate gave **268** ((1-*S*-ethyl thioacetyl)-2,3,4-tri-*O*-acetyl-1-thio-*β*-L-rhamnopyranoside). This reaction was initially conducted in CH_2Cl_2 at room temperature as described by Hasegawa et al[261] but after two days the reaction had not proceeded to completion. Changing the solvent to DMF and heating at 80 °C drove the reaction completely towards formation of the desired product **268**.

Synthesis of the cytidine derivative and coupling

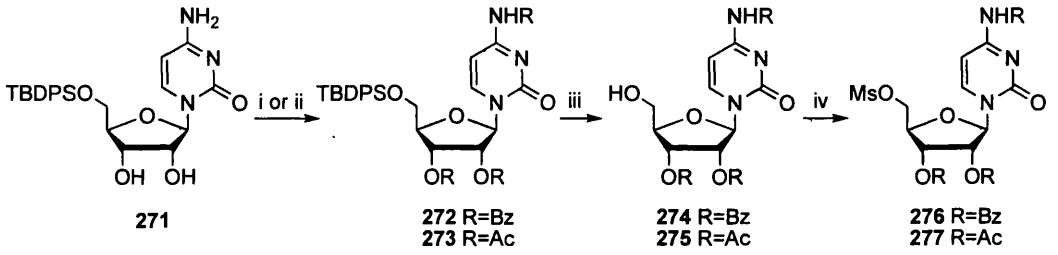
In order to couple the hydroxyl group at C-5 of cytidine with the rhamnose moiety it had to be converted into a good leaving group. Before activation, however, cytidine required to be selectively protected i.e. the NH_2 and the secondary OH groups, to prevent unwanted substitutions. To achieve this, the 5-OH was first selectively protected as the TBDPS ether by reaction with TBDPSCl in the presence of imidazole[262] (Scheme 6.10). It was observed that a longer reaction time afforded the di-substituted product and therefore the reaction had to be quenched after an hour.



Scheme 6.10: TBDPS-cytidine and sideproduct
(i) TBDPSCl, imidazole, DMF, rt, 1 h, 94%.

After successful protection of the 5-OH the next step was to protect the amine and the remaining hydroxyl groups. This was easily achieved by stirring the TBDPS ether **271** with benzoic or acetic anhydride in pyridine. It was observed that the acetylated cytidine **273** gave higher yields in this and subsequent reactions than the benzoylated cytidine **272** (Scheme 6.11), probably because the bulky benzoates are sterically more hindered and thus less reactive than acetates. The drawback of using acetates, however, is that their removal from amines is more

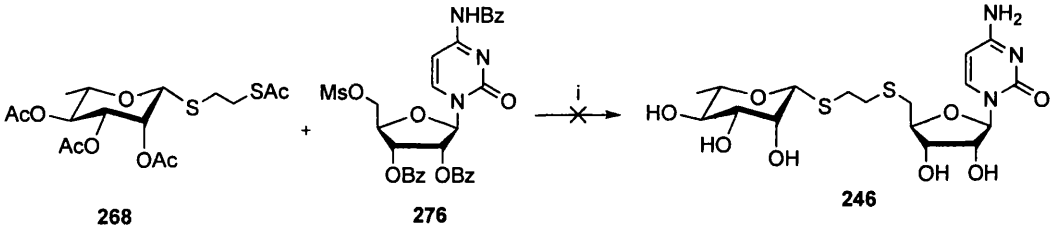
complicated compared to benzoates. The hydroxyl group at C-5 was then unmasked by treatment of **272** or **273** with TBAF in the presence of acetic acid. The 5-OH of the cytidine derivatives was subsequently converted into a good leaving group. This was achieved by treatment of compounds **274** and **275** with MsCl in the presence of Et_3N to give the mesylated derivatives **276** and **277** (Scheme 6.11).



Scheme 6.11: Synthesis of cytidine intermediates **276** and **277**

Reagents and conditions: (i) Bz_2O, pyridine, rt, 2 days, 55%; (ii) Ac_2O, pyridine, rt, 18 h, 71%; (iii) TBAF, AcOH, THF, rt, 18 h, 75% for **274**, quant. for **275**; (iv) MsCl, Et_3N, DMF, 0 °C → rt, 12 h, 27% for **276**, 77% for **277**.

With the required building blocks in hand the next step towards the synthesis of the target compound was the coupling of thioglycoside **268** first with the benzoylated cytidine derivative **276**. For this purpose, precursors **268** and **276** were treated with NaOMe in MeOH, in an one-pot deprotection-coupling attempt. Unfortunately, no coupling occurred and only the deprotected starting materials were obtained (Scheme 6.12).

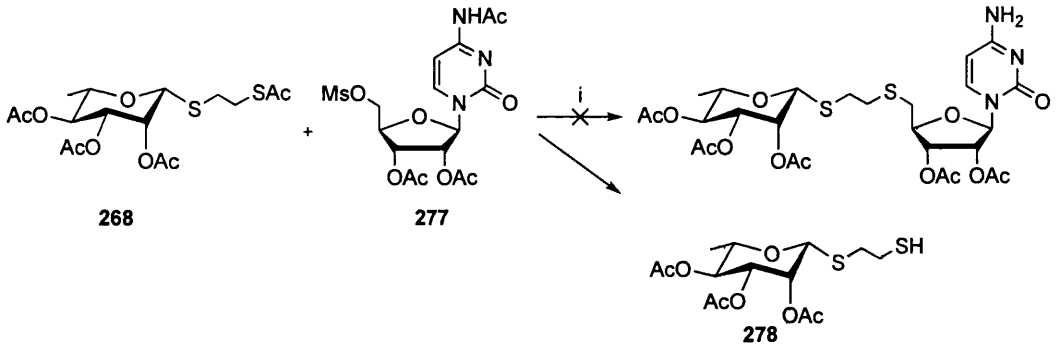


Scheme 6.12: Attempted coupling I

Reagents and conditions: (i) (a) NaOMe, MeOH, rt, 18 h; (b) Amberlite IR 120 (H^+ form) ion exchange resin.

Alternatively, coupling was attempted using Et_2NH as the base. These conditions had been employed in a similar coupling[256] and would result in hydrolysis of the thioacetate, followed by nucleophilic attack on the mesylated cytidine. This time, the acetylated cytidine derivative **277** was used, since it appeared to be more reactive than the benzoylated derivative. However, treatment of precursors **268**

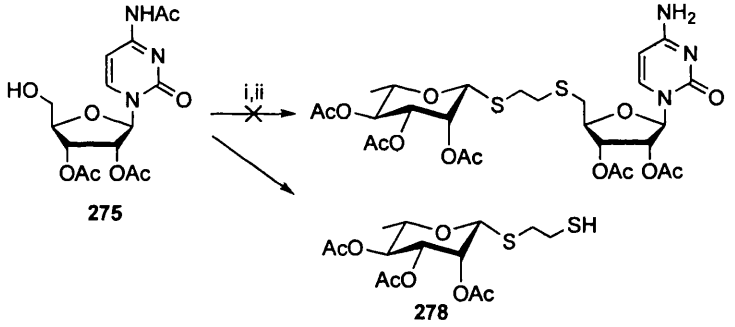
and **277** with Et_2NH only resulted in de-acetylation of the thioacetate (**278**) and no coupling product was obtained even after prolonged reaction time (Scheme 6.13).



Scheme 6.13: Attempted coupling II

Reagents and conditions: (i) Et_2NH, DMF, rt, 48 h.

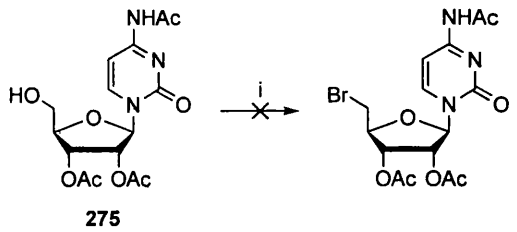
It was assumed that the mesyl derivative was not sufficiently reactive; hence, acetyl cytidine **275** was activated with the far more reactive trifluromethanesulfonate (OTf) (Scheme 6.14). Since triflates are very unstable, this compound was not isolated but used directly in the next step. However, the reaction did not proceed as expected, possibly due to decomposition of the triflate, and again the hydrolysed thioacetate was obtained.



Scheme 6.14: Attempted coupling III

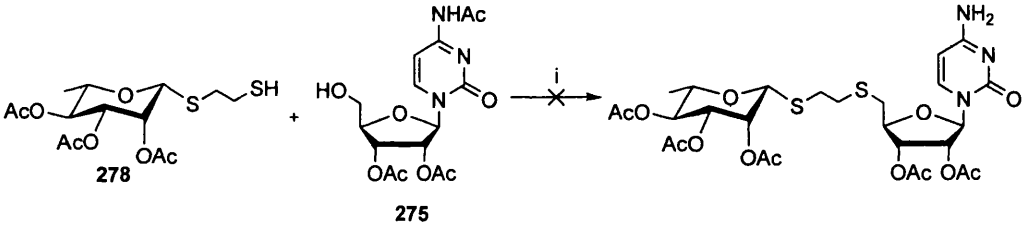
Reagents and conditions: (i) Tf_2O, pyridine, 0 °C, 1 h; (ii) **268**, Et_2NH, DMF, 80 °C, 48 h.

An alternative to activate **275** was by converting it to the bromide derivative by reaction with the brominating agent dibromotriphenylphosphorane and then carry out the coupling reaction.[256] However, treatment of cytidine derivative **275** with this reagent resulted in multiple products, according to TLC analysis, and therefore no further characterisation was attempted.



Scheme 6.15: Attempted synthesis of the bromo-cytidine derivative
Reagents and conditions: (i) Ph_3PBr_2, THF, -10 → 65 °C, 18 h.

Since the attempts to couple the activated cytidine derivatives with thiorhamnoside **268** were not successful, we decided to investigate coupling via a Mitsunobu condensation.[263] This method enables an alcohol to react directly with a thiosugar, without initial activation. Thiol **278** and cytidine derivative **275** were therefore treated with a mixture of trimethylphosphine and azodicarboxylic acid dipiperidine (ADDP) in THF. Unfortunately, no reaction occurred under these reaction conditions and only starting material was recovered.



Scheme 6.16: Attempted coupling by Mitsunobu condensation
Reagents and conditions: (i) Me_3P, ADDP, THF, 0 °C → rt, 2 h.

6.4. Conclusions and Future Work

The expression of PSA-NCAM has been demonstrated on the cell surface of a number of malignant tumours and has been associated with poor prognosis and cancer metastasis. PSA is synthesised on NCAM by two polysialyltransferases, PST and STX. These enzymes transfer sialic acid to the acceptor sugar in its activated CMP-sialic acid form. Therefore inhibition of the polysialyltransferases by small molecules might result in prevention of PSA synthesis and consequently in reduction or even prevention of cancer metastasis.

For this purpose a small library of CMP-sialic acid analogues was successfully synthesised with the potential to act as polysialyltransferase inhibitors. In these compounds the sialic acid moiety was replaced by the simpler L-rhamnose, the phosphate linkage by a non-hydrolysable sulfur linkage and the cytidine moiety was replaced by variously substituted benzyl groups. This is the starting point for

a detailed structure-activity relationship, which will eventually lead to sialic acid analogues.

The second part of this project aimed at the synthesis of a CMP-sialic acid mimetic (**246**) bearing a non-hydrolysable sulfur linkage. Again the sialic acid moiety was initially replaced by L-rhamnose. This compound might have the potential to mimic sialic acid in PSA biosynthesis but would not promote addition to the PSA chain due to the presence of the non-hydrolysable sulfur linkage. The coupling precursors were successfully synthesised from L-rhamnose and cytidine. However, although several coupling conditions were investigated, the coupling of the thioacetate rhamnoside with a suitably protected cytidine derivative proved difficult.

Further work will involve the completion of the synthesis of the CMP-sialic acid mimetic. This could include the synthesis of a suitably activated for coupling rhamnosyl moiety and an *S*-ethyl thioacetyl cytidine derivative and subsequent coupling to form the target compound. Once the synthetic route towards the target compound is established, synthesis of analogues incorporating sialic acid will be attempted.

Future work will also involve synthesis of compounds in which the phosphate group has been replaced by a more stable carbon linkage. In addition, modifications can be introduced on the hydroxyl groups of the sialic acid side-chain. These compounds will be able to be incorporated in the PSA chain; however, since sialic acids are joint via α2,8-glycosidic bonds in PSA, addition of the next sialic acid residue will be blocked resulting in PSA chain termination. Furthermore, biological screening of the compounds synthesised so far will evaluate their effectiveness as polysialyltransferase inhibitors and help synthesise more potent second-generation analogues.

EXPERIMENTAL

General Methods

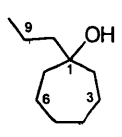
All moisture sensitive reactions were carried out under a nitrogen or argon atmosphere using oven-dried glassware. THF and Et_2O were dried over sodium/benzophenone and freshly distilled prior to use. CH_2Cl_2 was dried and distilled over CaH_2. All commercially available compounds were reagent grade and were used as received.

Chromatographic separations were performed on silica gel for flash chromatography (particle size 40-63 μm) (BDH Laboratory Supplies, Poole, Dorset) using the specified mobile phases. TLC was performed on Merck precoated silica gel 60 F_{254} TLC plates. TLC plates were visualised under UV light and also dipped into a solution of either phosphomolybdic acid in ethanol (5% solution) or sulfuric acid in ethanol (10%), followed by heating.

Proton and carbon NMR spectra were recorded at 400 MHz and 100MHz, respectively on a Bruker Avance spectrometer using the specified deuterated solvent. Chemical shifts are given in ppm relative to the solvent used ($CDCl_3$: 7.26 for ^1H, 77.0 for ^{13}C; MeOD: 3.35 for ^1H, 49.0 for ^{13}C). Two-dimensional COSY, HMQC, HMBC, TOCSY and NOESY experiments were recorded to assist with spectral assignment. Mass spectra were recorded using electrospray analysis in positive or negative modes on a Finnegan Navigator Single Quadrapole mass spectrometer. Elemental analysis was performed using a Carbo Erbar CHN1108 elemental analyser. Infrared spectra were recorded using a Nicolet Smart Golden Gate spectrometer (Avatar 360 FT-IR E.S.P).

Synthesised Compounds – Part I

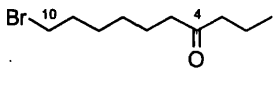
1-Propylcycloheptanol (159)



6.0 mL (50.9 mmol) of cycloheptanone in 60 mL Et_2O were added dropwise over 20 min to 50.5 mL (0.10 mol, 2 equiv) of a 2 M propylmagnesium chloride solution in Et_2O at 0 °C and stirring continued for another 15 min. The reaction mixture was then quenched by slow addition of sat. NaCl (50 ml) and 5% HCl was added until the solution became clear. The layers were separated and the aqueous phase extracted with Et_2O (2 x 50 mL). The organic extracts were dried over $MgSO_4$, concentrated and purified by flash chromatography (100% hexanes to hexanes/EtOAc 9:1) to yield 6.48 g

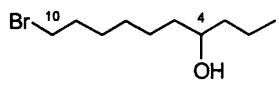
(81%) of a colourless oil. R_f 0.38 (hexanes/EtOAc 4:1). ^{1}H NMR ($CDCl_3$): δ 1.62-1.33 (m, 16H, 8 x CH_2), 0.90 (t, 3H, J = 6.9 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 74.5 (C-1), 45.1 (C-8), 40.2 (C-2, C-7), 28.9 (C-4, C-5), 21.5 (C-3, C-6), 15.6 (C-9), 13.7 (C-10). ES-MS ($C_{10}H_{20}O$) 156.27, *m/z* (%) 157.3 (23) $[M+H]^+$.

10-Bromodecan-4-one (160)



To a solution of **159** (4.34 g, 27.8 mmol) in 30 mL of $CHCl_3$ was added K_2CO_2 (23.0 g, 0.17 mol, 6 equiv). The reaction mixture was stirred at 0 °C for 10 min and bromine (7.1 mL, 0.14 mol, 5 equiv) was then added. After stirring at 0 °C for 5 h the reaction mixture was washed with sat. $Na_2S_2O_3$ (100 mL) and H_2O (100 mL). The organic extracts were dried over $MgSO_4$, concentrated and purified by flash chromatography (100% hexanes to hexanes/EtOAc 9:1) to yield 3.27 g (50%) of a light yellow oil. R_f 0.51 (hexanes/EtOAc 4:1). ^{1}H NMR ($CDCl_3$): δ 3.34 (t, 2H, J = 6.7 Hz, CH_2-10), 2.35-2.29 (m, 4H, CH_2-5, CH_2-3), 1.83-1.76 (m, 2H, CH_2-9), 1.58-1.49 (m, 4H, CH_2-6, CH_2-2), 1.42-1.35 (m, 2H, CH_2-8), 1.29-1.21 (m, 2H, CH_2-7), 0.85 (t, 3H, J = 7.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 211.0 (C=O), 44.7 (C-5), 42.5 (C-3), 33.7 (C-10), 32.5 (C-9), 28.3 (C-7), 27.9 (C-8), 23.5 (C-6), 17.3 (C-2), 13.7 (C-1). ES-MS ($C_{10}H_{19}BrO$) 235.16, *m/z* (%) 235.2 (35), 237.2 (32) $[M+H]^+$, 257.2 (100), 259.2 (98) $[M+Na]^+$. HR-MS calcd for $C_{10}H_{19}BrO$ $[M+H]^+$ 235.0692, 237.0672, found 235.0684, 237.0677, calcd for $C_{10}H_{19}BrO$ $[M+Na]^+$ 257.0512, 259.0492, found 257.0506, 259.0489.

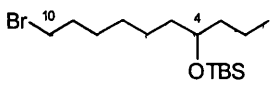
10-Bromodecan-4-ol (161)



$NaBH_4$ (1.52 g, 38.4 mmol, 3.4 equiv) was added in small portions to a solution of **160** (2.77 g, 11.8 mmol) in EtOH (30 mL) at 0 °C. After stirring at 0 °C for 3 h, the solvents were evaporated and the mixture taken up in Et_2O (50 mL). The organic layer was washed with H_2O (2 x 50 mL), dried over $MgSO_4$, concentrated and purified by flash chromatography (100% hexanes to hexanes/EtOAc 9:1) to yield 1.90 g (68%) of **161** as a colourless oil. R_f 0.37 (hexanes/EtOAc 4:1). ^{1}H NMR ($CDCl_3$): δ 3.61-3.58 (m, 1H, CH), 3.40 (t, 2H, J = 6.8 Hz, CH_2-10), 1.89-1.82 (m, 2H, CH_2-9), 1.48-1.29 (m, 12H, 6 x CH_2), 0.92 (t, 3H, J = 7.0 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 71.6 (C-

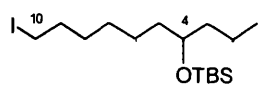
4), 39.7 (C-5), 37.3 (C-3), 33.9 (C-10), 32.7 (C-9), 28.8 (C-6), 28.1 (C-8), 25.4 (C-7), 18.8 (C-2), 14.1 (C-1).

10-Bromo-4-*O-tert*-butyldimethylsilyl-decan-4-ol (162)



158 mg (0.68 mmol) of **161** were dissolved in 1 mL DMF, treated with 0.2 mL (0.80 mmol, 1.2 equiv) TBSOTf and 114 mg (1.67 mmol, 2.5 equiv) imidazole and stirred at room temperature overnight. The reaction mixture was then concentrated and purified by flash chromatography (100% hexanes) to yield 219 mg (94%) of colourless oil. R_f 0.65 (100% hexanes). ^{1}H NMR ($CDCl_3$): δ 3.66-3.60 (m, 1H, CH), 3.40 (t, 2H, J = 6.8 Hz, CH_2-10), 1.89-1.82 (m, 2H, CH_2-9), 1.43-1.24 (m, 12H, 6 x CH_2), 0.89 (t, 3H, J = 7.3 Hz, CH_3), 0.89 (s, 9H, *t*Bu, TBS), 0.04 (s, 6H, 2 x CH_3 TBS). ^{13}C NMR ($CDCl_3$): δ 72.1 (C-4), 39.5 (C-5), 37.0 (C-3), 33.9 (C-10), 32.8 (C-9), 29.0 (C-6), 28.2 (C-8), 25.9 (*t*Bu TBS), 25.1 (C-7), 18.6 (C-2), 18.1 (C_{quart} TBS), 14.3 (C-1), -4.4 (2 x CH_3 TBS).

10-Iodo-4-*O-tert*-butyldimethylsilyl-decan-4-ol (164)



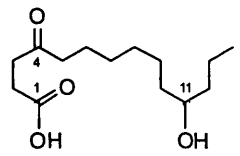
1.12 g (3.20 mmol) of **162** were dissolved in 30 mL acetone and 0.62 g (4.15 mmol, 1.3 equiv) NaI was added. After stirring at reflux for 3 h the solvent was evaporated and the mixture taken up in CH_2Cl_2 (30 mL). The organic layer was washed with H_2O (30 mL) and 10% $Na_2S_2O_3$ (30 mL), dried over $MgSO_4$ and concentrated to yield 1.06 g (83%) of pure **164** as a colourless oil. R_f 0.67 (100% hexanes). ^{1}H NMR ($CDCl_3$): δ 3.66-3.60 (m, 1H, CH), 3.19 (t, 2H, J = 7.0 Hz, CH_2-10), 1.86-1.79 (m, 2H, CH_2-9), 1.41-1.26 (m, 12H, 6 x CH_2), 0.89 (t, 3H, J = 7.3 Hz, CH_3), 0.89 (s, 9H, *t*Bu, TBS), 0.04 (s, 6H, 2 x CH_3 TBS). ^{13}C NMR ($CDCl_3$): δ 72.0 (C-4), 39.5 (C-5), 37.0 (C-3), 33.5 (C-9), 30.5 (C-8), 28.8 (C-6), 25.9 (*t*Bu TBS), 25.1 (C-7), 18.6 (C-2), 18.1 (C_{quart} TBS), 14.3 (C-1), 7.1 (C-10), -4.4 (2 x CH_3 TBS).

11-Hydroxy-4-oxotetradecanoic acid (168) & 4,11-di-oxotetradecanoic acid (169)

A solution of 2,3-dihydrofuran (0.18 mL, 2.37 mmol) in 10 mL THF was cooled to -78 °C and *t*-BuLi (1.8 mL of 1.7 M soln in pentane, 3.08 mmol, 1.3 equiv) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C for 30 min

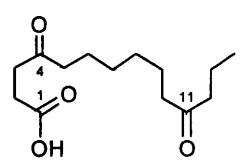
and then re-cooled to -78 °C. A solution of **164** (0.94 g, 2.37 mmol) in 1 mL THF was added dropwise and the resulting solution was allowed to stir for 1 h at room temperature. The reaction was quenched with slow addition of sat. NH_4Cl_{aq} (5 ml) and the aqueous layer extracted with pentane/Et_2O (1:1, 3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under redussed pressure. The crude product was dissolved in THF (13 mL), cooled to 0 °C and Jones' reagent (1.9 mL of 2.7 M aq. soln, 5.04 mmol) was added dropwise over 10 min. The resulting mixture was allowed to stir at room temperature for 18 h, diluted with Et_2O (5 mL) and H_2O (5 mL) and stirred vigorously for another 30 min. The layers were separated and the aqueous phase extracted with Et_2O (4 x 5 mL) the combined organic layers washed with H_2O (3 x 5 mL) and extracted with 10% $NaOH_{(aq)}$ (3 x 5 mL). The combined basic extracts were acidified with conc. HCl to pH 1, extracted with CH_2Cl_2 (4 x 5 mL) and the organic extracts dried over $MgSO_4$ concentrated and purified by flash chromatography (hexanes/EtOAc 2:1 to hexanes/EtOAc 1:3) to yield 0.12 g (20%) of the diketone **169** and 0.07 g (11%) of the alcohol **168**.

Analytical and spectroscopic data of **168**: R_f 0.12 (hexanes/EtOAc 1:3). ^1H NMR



($CDCl_3$): δ 3.61-3.59 (m, 1H, C<u>H</u>OH), 2.71 (t, 2H, J = 6.7 Hz, CH_2-3), 2.62 (t, 2H, J = 6.7 Hz, CH_2-2), 2.45 (t, 2H, J = 7.4 Hz, CH_2-5), 1.61-1.58 (m, 2H, CH_2-6), 1.46-1.39 (m, 4H, CH_2-10, CH_2-12), 1.35-1.25 (m, 8H, CH_2-7, CH_2-8, CH_2-9, CH_2-13), 0.92 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 209.2 (C-4), 177.4 (C-1), 70.7 (C-11), 41.6 (C-5), 38.6 (C-12), 36.2 (C-10), 35.8 (C-2), 28.3 (C-8), 28.0, (C-7), 26.7 (C-3), 24.3 (C-9), 22.6, (C-6), 17.8 (C-13), 13.1 (C-14). ES-MS ($C_{14}H_{26}O_4$) 258.35, *m/z* (%) 281.1 (79) $[M+Na]^+$. HR-MS calcd for $C_{14}H_{26}O_4$ $[M+Na]^+$ 281.1729, found 281.1742.

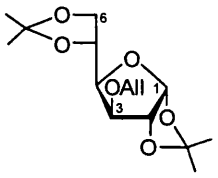
Analytical and spectroscopic data of **169**: R_f 0.24 (hexanes/EtOAc 1:3). ^1H NMR



($CDCl_3$): δ 2.72-2.69 (m, 2H, CH_2-3), 2.62-2.61 (m, 2H, CH_2-2), 2.43 (t, 2H, J = 7.4 Hz, CH_2-5), 2.39-2.35 (m, 4H, CH_2-10, CH_2-12), 1.62-1.54 (m, 6H, CH_2-6, CH_2-9, CH_2-13), 1.30-1.26 (m, 4H, CH_2-7, CH_2-8), 0.91 (t, 3H, J = 7.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 211.5 (C-11), 208.8 (C-4), 177.8 (C-1), 44.7 (C-12), 42.6 (C-5), 42.6 (C-10), 36.8 (C-2), 29.0, 28.9 (C-7, C-8), 27.7 (C-3), 23.6, 23.5 (C-6, C-9), 17.3 (C-13), 13.8

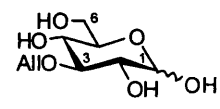
(C-14). ES-MS ($C_{14}H_{24}O_4$) 256.34, *m/z* (%) 279.1 (100) $[M+Na]^+$. HR-MS calcd for $C_{14}H_{24}O_4$ $[M+Na]^+$ 279.1567, found 279.1569.

3-*O*-Allyl-1,2:5,6-di-*O*-isopropylidene-*α*-D-glucofuranose (170)



10.1 g (38.8 mmol) of 1,2:5,6-di-*O*-isopropylidene-*α*-D-glucofuranose was dissolved in 70 mL DMF and 4.7 g (116.4 mmol, 3 equiv) NaH added. The mixture was stirred at room temperature for 30 min before 10.1 mL (116.4 mmol, 3 equiv) AllBr was added dropwise. After the reaction mixture was stirred for an additional 1 h it was evaporated to dryness, re-dissolved in $CHCl_3$ and the insoluble precipitates were removed by filtration. The solution was concentrated and purified by flash chromatography (100% hexanes to hexanes/EtOAc 5:1) to give 10.9 g (94%) of a pale yellow oil. R_f 0.54 (hexanes/EtOAc 1:1). ^1H NMR ($CDCl_3$): δ 5.93-5.84 (m, 2H, H-1, CH_2=C<u>H</u>CH_2), 5.30 (dq, 1H, J = 17.2, 4.6, 1.6 Hz, C<u>H</u>$_2$=CHCH_2 *trans*), 5.19 (dq, 1H, J = 10.4, 4.6, 1.6 Hz, C<u>H</u>$_2$=CHCH_2 *cis*), 4.54 (d, 1H, J = 3.4 Hz, H-2), 4.33-4.28 (m, 1H, H-5), 4.14-4.07 (m, 4H, H-4, CH_2-6, CH_2=CHC<u>H</u>$_2$), 4.00 (dd, 1H, J = 8.6, 5.8 Hz, CH_2-6), 3.94 (d, 1H, J = 3.4 Hz, H-3), 1.49, 1.42, 1.35, 1.31 (s, 12H, 4 x CH_3 isopropylidene). ^{13}C NMR ($CDCl_3$): δ 134.2 (CH_2=<u>C</u>HCH_2), 117.3 (<u>C</u>H$_2$=CHCH_2), 111.8, 109.0 (2 x C_{quart} isopropylidene), 105.3 (C-1), 82.8 (C-2), 81.4 (C-3), 81.2 (C-4), 72.5 (C-5), 71.4 (CH_2=CH<u>C</u>H$_2$), 67.3 (C-6), 26.9, 26.8, 26.3, 25.4 (4 x CH_3 isopropylidene). ES-MS ($C_{15}H_{24}O_6$) 300.35, *m/z* (%) 322.9 (27) $[M+Na]^+$. HR-MS calcd for $C_{15}H_{24}O_6$ $[M+H]^+$ 301.1646, found 301.1649.

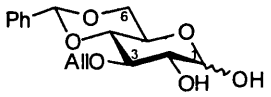
3-*O*-Allyl-*α/β*-D-glucopyranose (171)



10.9 g (36.3 mmol) of **170** and Amberlite IR 120 (H^+ form) ion exchange resin (5 g) in 100 mL H_2O/dioxane (1:1) were stirred at 100 °C for 18 h. The resin was filtered off, solvents removed under reduced pressure and the residue was purified by flash chromatography (100% $CHCl_3$ to $CHCl_3$/MeOH 1:1) to yield 6.07 g (76%, *α*:*β* = 51:49) of a white foam. R_f 0.20 ($CHCl_3$/MeOH 10:2). ^1H NMR (MeOD): δ 6.08-5.98 (m, 2H, CH_2=C<u>H</u>CH_2 *α,β*), 5.30 (dquin, 2H, J = 17.4, 3.5, 1.7 Hz, C<u>H</u>$_2$=CHCH_2 *trans α,β*), 5.17-5.16 (dm, 2H, C<u>H</u>$_2$=CHCH_2 *cis α,β*), 5.14 (d, 1H, J = 3.6 Hz, H-1*α*), 4.52 (d, 1H, J = 7.2 Hz, H-1*β*), 4.41-4.38 (m, 4H, CH_2=CHC<u>H</u>$_2$ *α,β*), 3.90-3.80 (m, 3H, H-5*α*, CH_2-6*α*,

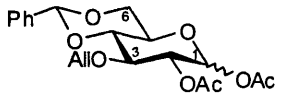
CH_2-6β), 3.73- 3.69 (m, 2H, CH_2-6α, CH_2-6β), 3.58 (t, 1H, J = 9.2 Hz, H-3α), 3.47 (dd, 1H, J = 9.2, 3.6 Hz, H-2α), (dd, 2H, H-4β, J = 9.2, 2.4 Hz, H-4α), 3.33-3.30 (m, 1H, H-5β), 3.30-3.21 (m, 1H, H-2β, H-3β). ^{13}C NMR (MeOD): δ 137.2, 137.1 (CH_2=$\underline{C}HCH_2$ α,β), 116.7, 116.6 ($\underline{C}H_2$=$CHCH_2$ α,β), 98.3 (C-1β), 94.1 (C-1α), 86.0 (C-3β), 83.1 (C-3α), 78.0 (C-5β), 76.3 (C-2β), 75.2, 75.0 (CH_2=$CH\underline{C}H_2$ α,β), 73.9 (C-2α), 73.0 (C-5α), 71.6, 71.5 (C-4α, C-4β), 62.9, 62.7 (C-6α, C-6β). ES-MS ($C_9H_{16}O_6$) 220.20, *m/z* (%) 242.9 (18) $[M+Na]^+$, 259.9 (100) $[M+K]^+$. HR-MS calcd for $C_9H_{16}O_6$ $[M+Na]^+$ 243.0845, found 243.0844.

3-*O*-Allyl-4,6-*O*-benzylidene-α/β-D-glucopyranose (172)



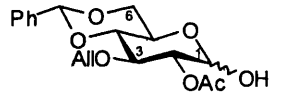
5.51 g (25.0 mmol) of **171** were dissolved in 30 mL anhydrous DMF and benzaldehyde dimethyl acetal (5.7 mL, 37.8 mmol, 1.5 equiv) and PTSA (136 mg) were added. The reaction mixture was stirred at 70 °C for 18 h, quenched with Et_3N, concentrated and purified by flash chromatography (hexanes/EtOAc 3:1 to 100% EtOAc) to yield 5.16 g (67%, $\alpha:\beta$ = 60:40) of a white amorphous solid. R_f 0.47 ($CHCl_3$/MeOH 10:2). ^{1}H NMR (MeOD): δ 7.53-7.50 (m, 10H, Ar α,β), 6.02-5.94 (m, 2H, CH_2=$C\underline{H}CH_2$ α,β), 5.62 (s, 1H, C$\underline{H}$-Phα), 5.61 (s, 1H, C$\underline{H}$-Phβ), 5.30 (dquin, 2H, J = 17.2, 3.2 Hz, 1.6, CH_2=$CHCH_2$ *trans* α,β), 5.17 (d, 1H, J = 3.8 Hz, H-1α), 5.14 (dquin, 2H, J = 10.4, 3.2, 1.6 Hz, CH_2=$CHCH_2$ *cis* α,β), 4.64 (d, 1H, J = 7.6 Hz, H-1β), 4.42-4.30 (m, 4H, CH_2=$CHC\underline{H}_2$ α,β), 4.30 (dd, 1H, J = 10.0, 4.8 Hz, CH_2-6β), 4.22 (dd, 1H, J = 10.0, 4.8 Hz, CH_2-6α), 4.05-3.99 (m, 1H, H-5α), 3.82-3.74 (m, 3H, H-3α, CH_2-6α, CH_2-6β), 3.65-3.53 (m, 4H, H-2α, H-3β, H-4α, H-4β), 3.53-3.46 (m, 1H, H-5β), 3.39-3.33 (m, 1H, H-2β). ^{13}C NMR (MeOD): δ 139.3, 139.2 (C_{quart} Ar α,β), 136.8, 136.8 (CH_2=$\underline{C}HCH_2$ α,β), 129.9, 129.9, 129.1, 127.3, 127.3 (Ar α,β), 116.9, 116.8 ($\underline{C}H_2$=$CHCH_2$ α,β), 102.7, 102.6 ($\underline{C}$H-Ph α,β), 99.0 (C-1α), 94.8 (C-1β), 83.5 (C-4β), 82.9 (C-4α), 82.1 (C-3β), 79.5 (C-3α), 76.6 (C-2β), 74.8, 74.6 (CH_2=$CH\underline{C}H_2$ α,β), 73.8 (C-2α), 70.3 (C-6β), 69.8 (C-6α), 67.6 (C-5β), 63.5 (C-5α). ES-MS ($C_{16}H_{20}O_6$) 308.33, *m/z* (%) 353.0 (100) $[M+HCOOH]^-$. HR-MS calcd for $C_{16}H_{20}O_6$ $[M+Na]^+$ 309.1333, found 309.1317.

1,2-Di-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-*α/β*-D-glucopyranose (173)



Acetic anhydride (14 mL) was added to a solution of **172** (5.15 g, 16.7 mmol) in pyridine (21 mL) and the mixture was allowed to stir at room temperature overnight. After completion of the reaction the mixture was co-evaporated with toluene to dryness to yield pure **173** as a white amorphous solid (6.36 g, 97%, *α:β* = 55:45). R_f 0.48 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): *δ* 7.50-7.46 (m, 10H, Ar *α,β*), 6.27 (d, 1H, *J* = 3.8 Hz, H-1*α*), 5.95-5.79 (m, 2H, CH_2=C<u>H</u>CH_2 *α,β*), 5.72 (d, 1H, *J* = 8.2 Hz, H-1*β*), 5.58 (s, 1H, C<u>H</u>-Ph*α*), 5.56 (s, 1H, C<u>H</u>-Ph*β*), 5.29-5.21 (m, 2H, C<u>H$_2$</u>=CHCH_2 *trans α,β*), 5.17-5.08 (m, 3H, H-2*β*, C<u>H$_2$</u>=CHCH_2 *cis α,β*), 5.04 (dd, 1H, *J* = 9.7, 3.8 Hz, H-2*α*), 4.40-4.33 (m, 3H, CH_2-6*β*, CH_2=CHC<u>H$_2$</u> *α,β*), 4.31 (dd, 1H, *J* = 10.4, 4.8 Hz, CH_2-6*α*), 4.21-4.10 (m, 2H, CH_2=CHC<u>H$_2$</u> *α,β*), 3.98-3.90 (m, 2H, H-3*α*, H-5*α*), 3.79-3.69 (m, 5H, H-3*β*, H-4*α*, H-4*β*, CH_2-6*α*, CH_2-6*β*), 3.60-3.54 (m, 1H, H-5*β*), 2.16, 2.07 (s, 6H, 2 x CH_3*α*, Ac), 2.10, 2.09 (s, 3H, 2 x CH_3*β*, Ac). ^{13}C NMR ($CDCl_3$): *δ* 169.8, 169.3, 169.2, 169.1 (4 x C=O *α,β*, Ac), 137.1 (C_{quart} *α,β*, Ar), 134.8, 134.5 (CH_2=<u>C</u>HCH_2 *α,β*), 129.1, 129.1, 126.0 (Ar *α,β*), 116.9, 116.6 (<u>C</u>H_2=CHCH_2 *α,β*), 101.4, 101.3 (<u>C</u>H-Ph *α,β*), 92.5 (C-1*β*), 90.0 (C-1*α*), 81.5 (C-4*α*), 81.0 (C-4*β*), 78.5 (C-3*β*), 75.5 (C-3*α*), 73. 6, 73.3 (CH_2=CH<u>C</u>H_2 *α,β*), 71.8 (C-2*β*), 71.1 (C-2*α*), 68.6, 68.4 (C-6*α*, C-6*β*), 66.9 (C-5*β*), 64.9 (C-5*α*), 20.9, 20.8, 20.8, 20.6 (4 x CH_3 *α,β*, Ac). ES-MS ($C_{20}H_{24}O_8$) 392.40, *m/z* (%) 415.0 (100) $[M+Na]^+$. HR-MS calcd for $C_{20}H_{24}O_8$ $[M+H]^+$ 393.1544, found 393.1549, calcd for $C_{20}H_{24}O_8$ $[M+Na]^+$ 415.1364, found 415.1361.

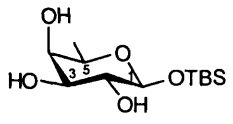
2-*O*-Acetyl-3-*O*-allyl-4,6-*O*-benzylidene-*α/β*-D-glucopyranose (174)



A solution of **173** (4.43 g, 11.3 mmol) and $BnNH_2$ (1.9 mL, 16.9 mmol, 1.5 equiv) in 22 mL THF was stirred at room temperature for 18 h. The reaction mixture was then diluted with 100 mL CH_2Cl_2 and 50 mL of 1 M HCl, the layers separated and the aqueous phase extracted with CH_2Cl_2 (3x 100 mL). The combined organic layers were dried over $MgSO_4$, concentrated and purified by flash chromatography (hexanes/EtOAc 5:1 to hexanes/EtOAc 2:1) to yield 2.42 g (61%, *α:β* = 70:30) of a white foam. R_f 0.28 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): *δ* 7.50-7.46 (m, 4H, Ar *α,β*), 7.38-7.36 (m, 6H, Ar *α,β*), 5.93-5.81 (m, 2H, CH_2=C<u>H</u>CH_2 *α,β*), 5.57 (s, 1H, C<u>H</u>-Ph*α*), 5.56 (s, 1H, C<u>H</u>-Ph*β*), 5.41 (d, 1H, *J* = 3.6 Hz, H-1*α*), 5.29-5.23 (m, 2H, C<u>H$_2$</u>=CHCH_2

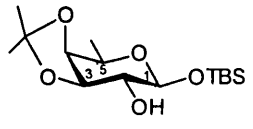
α,β), 5.17-5.13 (m, 2H, $C\underline{H}_2$=CHCH$_2$ α,β), 4.86 (dd, 1H, J = 9.6, 3.4 Hz, H-2α), 4.81 (t, 1H, J = 8.2 Hz, H-2β), 4.69 (d, 1H, J = 8.2 Hz, H-1β), 4.39-4.34 (m, 2H, CH$_2$=CHC$\underline{H}_2$ α,β), 4.37 (dd, 1H, J = 10.2, 5.0 Hz, CH$_2$-6β), 4.27 (dd, 1H, J = 10.2, 5.0 Hz, CH$_2$-6α), 4.21-4.06 (m, 3H, H-5α, CH$_2$=CHC$\underline{H}_2$ α,β), 3.98 (t, 1H, J = 9.6 Hz, H-3α), 3.82-3.63 (m, 5H, H-3β, H-4α, H-4β, CH$_2$-6α, CH$_2$-6β), 3.50-3.44 (m, 1H, H-5β), 2.15 (s, 3H, CH$_3$β, Ac), 2.14 (s, 3H, CH$_3$α, Ac). ^{13}C NMR (CDCl$_3$): δ 170.3 (C=O α,β, Ac), 137.3 (C_{quart} α,β, Ar), 134.9, 134.7 (CH$_2$=$\underline{C}$HCH$_2$ α,β), 129.0, 129.0, 128.3, 128.2, 126.0, 126.0 (Ar α,β), 116.9, 116.7 ($\underline{C}$H$_2$=CHCH$_2$ α,β), 101.4, 101.3 ($\underline{C}$H-Ph α,β), 96.4 (C-1β), 91.2 (C-1α), 82.0 (C-4α), 81.5 (C-4β), 78.0 (C-2β), 75.7 (C-3β), 75.3 (C-3α), 73.7, 73.5 (CH$_2$=CH$\underline{C}$H$_2$ α,β), 73.1 (C-2α), 68.9 (C-6α), 68.6 (C-6β), 66.6 (C-5β), 62.5 (C-5α), 20.9 (CH$_3$ α,β, Ac). ES-MS ($C_{18}H_{22}O_7$) 350.36, *m/z* (%) 350.9 (88) [M+H]$^+$, 372.9 (68) [M+Na]$^+$. HR-MS calcd for $C_{18}H_{22}O_7$ [M+H]$^+$ 351.1438, found 351.1421.

tert-Butyldimethylsilyl-β-D-fucopyranose (175)



6.0 g (36.7 mmol) of D-fucose were dissolved in 35 mL CH_2Cl_2, treated with 6.6 g (44.0 mmol, 1.2 equiv) TBSCl and 5.0 g (73.4 mmol, 2 equiv) imidazole and stirred at room temperature for 18 h. The reaction mixture was then concentrated and purified by flash chromatography (100% $CHCl_3$ to $CHCl_3$/MeOH 10:1) to yield 4.4 g (43%) of a viscous colourless oil. R_f 0.46 ($CHCl_3$/MeOH 10:2). ^{1}H NMR (MeOD): δ 4.48 (d, 1H, J = 6.8 Hz, H-1), 3.67-3.63 (m, 2H, H-4, H-5), 3.49-3.41 (m, 2H, H-2, H-3), 1.29 (d, 3H, J = 6.6 Hz, CH$_3$-6), 0.98 (s, 9H, *t*Bu TBS), 0.17 (s, 6H, 2 x CH$_3$ TBS). ^{13}C NMR (MeOD): δ 99.6 (C-1), 75.1 (C-2), 74.3 (C-3), 73.2 (C-4), 71.9 (C-5), 26.4 (*t*Bu TBS), 19.1 (C_{quart} TBS), 16.9 (C-6), -4.2, -4.7 (2 x CH$_3$ TBS). ES-MS ($C_{12}H_{26}O_5Si$) 278.42, *m/z* (%) 277.0 (24) [M-H]$^-$, 323.1 (100) [M+HCOOH]$^-$. HR-MS calcd for $C_{12}H_{26}O_5Si$ [M-H]$^-$ 277.1477, found 277.1472.

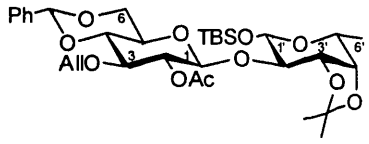
tert-Butyldimethylsilyl-3,4-*O*-isopropylidene-β-D-fucopyranose (176)



2.20 g (7.90 mmol) of **175** were dissolved in 100 mL acetone. 50 mL 2,2-dimethoxypropane and 150 mg CSA were added and the mixture was stirred at room temperature overnight. The reaction was then quenched with NH_4OH and the solvents

removed under reduced pressure. Purification by flash chromatography (100% hexanes to hexanes/EtOAc 5:1) gave 2.21 g (88%) of compound **176** as a pale yellow viscous oil. R_f 0.57 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 4.36 (d, 1H, J = 8.0 Hz, H-1), 4.02-3.97 (m, 2H, H-3, H-4), 3.84 (dddd, 1H, J = 6.8, 2.0 Hz, H-5), 3.46-3.44 (m, 1H, H-2), 1.53 (s, 3H, CH_3 isopropylidene), 1.38 (d, 3H, J = 6.8 Hz, CH_3-6), 1.34 (s, 3H, CH_3 isopropylidene), 0.91 (s, 9H, *t*Bu TBS), 0.13, 0.12 (s, 6H, 2 x CH_3 TBS). ^{13}C NMR ($CDCl_3$): δ 110.2 (C_{quart} isopropylidene), 97.1 (C-1), 78.7 (C-3), 76.5 (C-4), 75.4 (C-2), 69.3 (C-5), 28.7, 26.8 (2 x CH_3 isopropylidene), 26.3 (*t*Bu TBS), 18.6 (C_{quart} TBS), 17.1 (C-6), -3.7, -4.6 (2 x CH_3 TBS). ES-MS ($C_{15}H_{30}O_5Si$) 318.48 *m/z* (%) 341.0 (100) $[M+Na]^+$. HR-MS calcd for $C_{15}H_{30}O_5Si$ $[M+Na]^+$ 341.1755, found 341.1753.

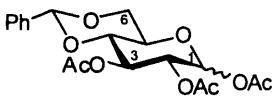
***tert*-Butyldimethylsilyl (2-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-*β*-D-glucopyranosyl)-(1→2)-3,4-*O*-isopropylidene-*β*-D-fucopyranoside (178)**



A solution of **174** (1.02 g, 2.91 mmol), trichloroacetonitrile (0.9 mL, 8.73 mmol, 3 equiv) and Cs_2CO_3 (0.57 g, 1.74 mmol, 0.6 equiv) in 20 mL CH_2Cl_2 were stirred at room temperature overnight. The reaction mixture was filtered through a short pad of silica which was rinsed with hexanes/EtOAc 2:1. The filtrate was concentrated to give 1.4 g of crude product (**177**) which was used without further purification. The crude trichloroacetimidate (1.4 g) and alcohol **176** (0.62 g, 1.94 mmol) were dissolved in 15 mL CH_2Cl_2 and cooled to -20 °C. TMSOTf (35 μL, 0.19 mmol, 0.1 equiv) was added to the solution and stirred at this temperature for 1 h. The reaction was quenched with Et_3N, concentrated and purified by flash chromatography (100% hexanes to hexanes/EtOAc 9:1) to yield 1.1 g (82%) of disaccharide **178** as a white foam. R_f 0.51 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 7.49-7.46 (m, 2H, Ar), 7.38-7.34 (m, 3H, Ar), 5.87-5.77 (m, 1H, CH_2=C<u>H</u>CH_2), 5.55 (s, 1H, C<u>H</u>-Ph), 5.17 (dq, 1H, J = 17.3, 1.6, 1.5 Hz, C<u>H$_2$</u>=CHCH_2 *trans*), 5.13 (dq, 1H, J = 10.6, 1.6, 1.3 Hz, C<u>H$_2$</u>=CHCH_2 *cis*), 5.05 (d, 1H, J = 7.5 Hz, H-1), 5.05 (dd, 2H, J = 12.6, 7.5 Hz, H-2), 4.46 (d, 1H, J = 8.0 Hz, H-1′), 4.35-4.27 (m, 2H, CH_2-6, CH_2=CHC<u>H$_2$</u>), 4.10 (m, 1H, CH_2=CHC<u>H$_2$</u>), 4.02 (dd, 1H, J = 7.5, 5.3 Hz, H-3′), 3.96 (dd, 1H, J = 5.4, 2.1 Hz, H-4′), 3.88 (t, 1H, J = 10.3 Hz, CH_2-6), 3.84-3.76 (m, 2H, H-4, H-5′), 3.65 (t, 1H, J = 7.8 Hz, H-2′), 3.61-3.56 (m, 1H, H-3), 3.43-3.37 (m, 1H, H-5), 2.07 (s, 3H, CH_3 Ac),

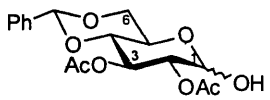
1.53, 1.34 (s, 6H, 2 x CH_3 isopropylidene), 1.36 (d, 3H, J = 6.4 Hz, CH_3-6′), 0.94 (s, 9H, *t*Bu TBS), 0.15, 0.13 (s, 6H, 2 x CH_3 TBS). ^{13}C NMR ($CDCl_3$): δ 169.3 (C=O Ac), 137.3 (C_{quart} Ar), 134.7 (CH_2=$\underline{C}HCH_2$), 128.9, 128.2, 126.0 (Ar), 116.8 ($\underline{C}H_2$=$CHCH_2$), 109.7 (C_{quart} isopropylidene), 101.1 ($\underline{C}$H-Ph), 100.1 (C-1), 97.1 (C-1′), 81.1 (C-4), 79.0 (C-3), 78.6 (C-2′), 77.5 (C-3′), 76.1 (C-4′), 73.0 (CH_2=$CH\underline{C}H_2$), 72.9 (C-2), 68.9 (C-5′), 68.5 (C-6), 66.4 (C-5), 27.9, 26.3 (2 x CH_3 isopropylidene), 25.9 (*t*Bu TBS), 21.0 (CH_3 Ac), 18.0 (C_{quart} TBS), 16.6 (C-6′), -3.8, -5.1 (2 x CH_3 TBS). ES-MS ($C_{33}H_{50}O_{11}Si$) 650.83, *m/z* (%) 673.2 (94) $[M+Na]^+$. HR-MS calcd for $C_{33}H_{50}O_{11}Si$ $[M+Na]^+$ 673.3020, found 673.3046.

1,2,3-Tri-*O*-acetyl-4,6-*O*-benzylidene-*α/β*-D-glucopyranose (179)



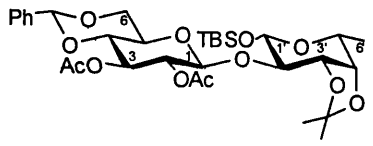
Acetic anhydride (20 mL) was added to a solution of 4,6-*O*-benzylidene-D-glucopyranose (7.64 g, 28.5 mmol) in pyridine (30 mL) and the mixture was allowed to stir at room temperature for 1 h. After completion of the reaction the mixture was co-evaporated with toluene to dryness to yield an off white amorphous solid (α:β = 45:55, R_f 0.32 hexanes/EtOAc 1:1), which was used in the following step without further purification. ^{1}H NMR ($CDCl_3$): δ 7.46-7.42 (m, 4H, Ar α,β), 7.37-7.35 (m, 6H, Ar α,β), 6.31 (t, 1H, J = 3.8 Hz, H-1α), 5.79 (d, 1H, J = 8.4 Hz, H-1β), 5.59 (t, 1H, J = 9.8 Hz, H-3α), 5.52 (s, 1H, C$\underline{H}$-Phα), 5.50 (s, 1H, C$\underline{H}$-Phβ), 5.37 (t, 1H, J = 9.2 Hz, H-3β), 5.13 (dd, 1H, J = 9.2, 8.4 Hz, H-2β), 5.11 (dd, 1H, J = 9.8, 3.8 Hz, H-2α), 4.38 (dd, 1H, J = 10.3, 4.6 Hz, CH_2-6β), 4.32 (dd, 1H, J = 10.3, 4.8 Hz, CH_2-6α), 4.04 (td, 1H, J = 10.0, 4.8 Hz, H-5α), 3.80-3.64 (m, 5H, H-4α, H-4β, H-5β, CH_2-6α, CH_2-6β), 2.19, 2.07, 2.03 (s, 6H, 3 x $CH_3\alpha$, Ac), 2.11, 2.06, 2.05 (s, 6H, 3 x $CH_3\beta$, Ac). ^{13}C NMR ($CDCl_3$): δ 169.9, 169.9, 169.8, 169.5, 169.1, 168.9 (3 x C=O α,β, Ac), 136.7, 136.6, 129.2, 129.1, 128.3, 126.2, 126.1 (Ar α,β), 101.7 ($\underline{C}$H-Ph α,β), 92.2 (C-1β), 89.7 (C-1α), 78.7 (C-4α), 78.0 (C-4β), 71.8 (C-3β), 71.3 (C-2α), 69.9 (C-2β), 68.8 (C-3α), 68.6 (C-6β), 68.3 (C-6α), 67.0 (C-5β), 65.0 (C-5α), 20.9, 20.8, 20.7, 20.6, 20.5 (3 x CH_3 α,β, Ac). ES-MS ($C_{19}H_{22}O_9$) 394.37, *m/z* (%) 395.1 (32) $[M+H]^+$, 417.1 (63) $[M+Na]^+$. HR-MS calcd for $C_{19}H_{22}O_9$ $[M+H]^+$ 395.1337, found 395.1336.

2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-*α/β*-D-glucopyranose (180)



The crude **179** and $BnNH_2$ (4.7 mL, 42.8 mmol, 1.5 equiv) in 50 mL THF were stirred at room temperature overnight. 14 mL of 1 M HCl were added and the reaction mixture was stirred for another 1 h. It was then diluted with 50 mL CH_2Cl_2 and 50 mL of 1 M HCl, the layers separated and the aqueous phase extracted with CH_2Cl_2 (2 x 50 mL). The combined organic layers were dried over $MgSO_4$, concentrated and purified by flash chromatography (hexanes/EtOAc 5:1 to hexanes/EtOAc 3:1) to yield 7.23 g (72% over 2 steps, $\alpha:\beta$ = 55:45) of white foam. R_f 0.28 (hexanes/EtOAc 1:1). ^1H NMR ($CDCl_3$): δ 7.46-7.41 (m, 4H, Ar α,β), 7.37-7.34 (m, 6H, Ar α,β), 5.63 (t, 1H, J = 9.8 Hz, H-3α), 5.51 (s, 1H, C<u>H</u>-Phα), 5.50 (s, 1H, C<u>H</u>-Phβ), 5.44 (br.s, 1H, H-1α), 5.37 (t, 1H, J = 9.6 Hz, H-3β), 4.94 (dd, 1H, J = 9.9, 3.6 Hz, H-2α), 4.89 (dd, 1H, J = 9.2, 7.9 Hz, H-2β), 4.82 (t, 1H, J = 7.8 Hz, H-1β), 4.38 (dd, 1H, J = 10.5, 4.9 Hz, CH_2-6β), 4.30 (dd, 1H, J = 10.3, 4.9 Hz, CH_2-6α), 4.22-4.16 (m, 1H, H-5α), 3.80 (t, 1H, J = 10.2, CH_2-6α), 3.75 (t, 1H, J = 10.2 Hz, CH_2-6β), 3.70 (t, 1H, J = 9.8 Hz, H-4α), 3.66 (t, 1H, J = 9.6 Hz, H-4β), 3.60-3.54 (m, 1H, H-5β), 3.08 (br s, 1H, OHβ), 2.11, 2.06 (s, 6H, 2 x $CH_3\beta$, Ac), 2.10, 2.06 (s, 6H, 2 x $CH_3\alpha$, Ac). ^{13}C NMR ($CDCl_3$): δ 170.3, 169.9 (2 x C=O α,β, Ac), 136.9, 136.7, 129.2, 129.1, 128.3, 128.2, 126.2, 126.1 (Ar α,β), 101.6 (<u>C</u>H-Ph α,β), 96.1 (C-1β), 91.1 (C-1α), 79.2 (C-4α), 78.6 (C-4β), 74.3 (C-2β), 71.9 (C-2α), 71.1 (C-3β), 68.9 (C-6α), 68.8 (C-3α), 68.5 (C-6β), 66.7 (C-5β), 62.5 (C-5α), 20.8, 20.7, (2 x CH_3 α,β, Ac). ES-MS ($C_{17}H_{20}O_8$) 352.34, *m/z* (%) 353.0 (24) $[M+H]^+$, 375.0 (100) $[M+Na]^+$. HR-MS calcd for $C_{17}H_{20}O_8$ $[M+H]^+$ 353.1231, found 353.1223.

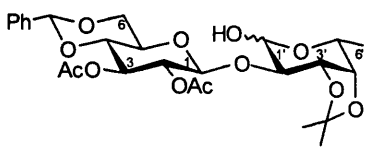
***tert*-Butyldimethylsilyl (2,3-di-*O*-acetyl-4,6-*O*-benzylidene-*β*-D-glucopyranosyl)-(1→2)-3,4-*O*-isopropylidene-*β*-D-fucopyranoside (182)**



A solution of **180** (1.07 g, 3.05 mmol), trichloroacetonitrile (0.9 mL, 9.15 mmol, 3 equiv) and Cs_2CO_3 (0.6 g, 1.83 mmol, 0.6 equiv) in 20 mL CH_2Cl_2 were stirred at room temperature for 18 h. The reaction mixture was filtered through a short pad of silica which was rinsed with hexanes/EtOAc 3:1. The filtrate was concentrated to give 1.2 g of crude product (**181**) which was used without further purification. The crude trichloroacetimidate (1.2 g) and alcohol **176** (0.66 g, 2.08 mmol) were dissolved in 15 mL CH_2Cl_2 and cooled to -20 °C.

TMSOTf (45 μL, 0.25 mmol, 0.1 equiv) was added to the solution and stirred at this temperature for 1 h. The reaction was quenched with Et_3N, concentrated and purified by flash chromatography (100% hexanes to hexanes/EtOAc 9:2) to yield 0.81 g (60%) of disaccharide **182** as a white foam. R_f 0.28 (hexanes/EtOAc 2:1). ^1H NMR ($CDCl_3$): δ 7.43-7.42 (m, 2H, Ar), 7.35-7.33 (m, 3H, Ar), 5.48 (s, 1H, CH-Ph), 5.25 (dd, 1H, J = 9.5, 8.5 Hz, H-3), 5.14-5.07 (m, 2H, H-1, H-2), 4.45 (d, 1H, J = 7.8 Hz, H-1′), 4.30 (dd, 1H, J = 10.4, 5.2 Hz, CH_2-6), 4.03 (dd, 1H, J = 7.8, 5.3 Hz, H-3′), 3.96 (dd, 1H, J = 5.3, 2.1 Hz, H-4′), 3.86 (t, 1H, J = 10.4 Hz, CH_2-6), 3.80 (t, 1H, J = 9.5 Hz, H-4), 3.80-3.77 (m, 1H, H-5′), 3.67 (t, 1H, J = 7.8 Hz, H-2′), 3.52-3.46 (m, 1H, H-5), 2.03, 2.02 (s, 6H, 2 x CH_3 Ac), 1.53, 1.34 (s, 6H, 2 x CH_3 isopropylidene), 1.36 (d, 3H, J = 6.6 Hz, CH_3-6′), 0.95 (s, 9H, *t*Bu TBS), 0.14, 0.13 (s, 6H, 2 x CH_3 TBS). ^{13}C NMR ($CDCl_3$): δ 170.1, 169.4 (2 x C=O Ac), 136.9 (C_{quart} Ar), 129.0, 128.3, 126.1 (Ar), 109.7 (C_{quart} isopropylidene), 101.4 (CH-Ph), 99.8 (C-1), 97.2 (C-1′), 78.9 (C-2′), 78.0 (C-4), 77.6 (C-3′), 76.5 (C-4′), 72.6 (C-2), 72.3 (C-3), 68.8 (C-5′), 68.5 (C-6), 66.4 (C-5), 27.9, 26.3 (2 x CH_3 isopropylidene), 25.9 (*t*Bu TBS), 20.9, 20.8 (2 x CH_3 Ac), 18.0 (C_{quart} TBS), 16.6 (C-6′), -3.9, -4.9 (2 x CH_3 TBS). ES-MS ($C_{32}H_{48}O_{12}Si$) 652.80, *m/z* (%) 675.3 (100) $[M+Na]^+$. HR-MS calcd for $C_{32}H_{48}O_{12}Si$ $[M+Na]^+$ 675.2813, found 675.2811.

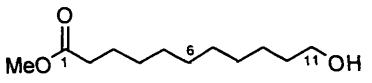
(2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-*β*-D-glucopyranosyl)-(1→2)-3,4-*O*-isopropylidene-*α/β*-D-fucopyranoside (183)



182 (0.42 g, 0.64 mmol) was dissolved in THF (2 mL). Acetic acid (55 μL, 0.96 mmol, 1.5 equiv) and TBAF (1.9 mL, 1 M soln in THF, 3 equiv) were added and the reaction mixture was stirred at room temperature for 2 h. The solvents were evaporated and the crude compound was taken up in CH_2Cl_2 (25 mL) and washed with sat. $NaHCO_3$ (20 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 20mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, concentrated and purified by flash chromatography (hexanes/EtOAc 2:1 to hexanes/EtOAc 1:1) to yield 0.22 g (64%, α:β = 60:40) of white foam. R_f 0.51 (hexanes/EtOAc 1:3). ^1H NMR ($CDCl_3$): δ 7.46-7.44 (m, 4H, Ar α,β), 7.38-7.36 (m, 6H, Ar α,β), 5.52 (s, 1H, CH-Phα), 5.51 (s, 1H, CH-Phβ), 5.37 (t, 2H, J = 9.3 Hz, H-3α, H-3β), 5.21 (dd, 1H, J = 6.8, 3.4 Hz, H-1′α), 5.05

(dd, 2H, J = 9.3, 7.8 Hz, H-2α, H-2β), 4.95 (d, 1H, J = 7.8 Hz, H-1β), 4.79 (d, 1H, J = 7.8 Hz, H-1α), 4.68 (t, 1H, J = 6.5 Hz, H-1'β), 4.45 (dd, 1H, J = 6.5, 6.3 Hz, H-3'α), 4.39-4.33 (m, 2H, CH_2-6α, CH_2-6β), 4.24-4.17 (m, 2H, H-3'β, H-5'β), 4.08 (dd, 1H, J = 6.5, 2.0 Hz, H-4'α), 4.04 (dd, 1H, J = 6.0, 2.0 Hz, H-4'β), 3.93-3.89 (m, 2H, H-2'α, H-5'α), 3.84 (t, 2H, J = 10.1 Hz, CH_2-6α, CH_2-6β), 3.75 (t, 2H, J = 9.7 Hz, H-4α, H-4β), 3.70 (t, 1H, J = 6.5 Hz, H-2'β), 3.59-3.52 (m, 2H, H-5α, H-5β), 3.39 (d, 1H, J = 6.5 Hz, OHβ), 3.11 (d, 1H, J = 6.8 Hz, OHα), 2.10, 2.07 (s, 6H, 2 x $CH_3\beta$, Ac), 2.08, 2.07 (s, 6H, 2 x $CH_3\alpha$, Ac), 1.55, 1.37 (s, 6H, 2 x $CH_3\beta$, isopropylidene), 1.51, 1.37 (s, 6H, 2 x $CH_3\alpha$, isopropylidene), 1.37 (d, 3H, J = 5.6 Hz, CH_3-6β), 1.30 (d, 3H, J = 6.5 Hz, CH_3-6α). ^{13}C NMR ($CDCl_3$): δ 170.1, 169.8 (2 x C=O, α,β, Ac), 136.8, 136.7 (C_{quart} α,β, Ar), 129.2, 129.1, 128.3, 128.2, 126.1 (Ar α,β), 109.8, 109.5 (C_{quart} α,β, isopropylidene), 101.7, 101.6 (CH-Ph α,β), 101.5 (C-1α), 101.2 (C-1β), 95.5 (C-1'β), 89.9 (C-1'α), 79.7 (C-2'β), 78.2 (C-4β), 78.1 (C-4α), 76.8 (C-2'α), 76.6 (C-3'β), 76.1 (C-4'β), 75.3 (C-4'α), 73.6 (C-3'α), 72.6 (C-2α), 72.6 (C-2β), 71.9 (C-3β), 71.7 (C-3α), 68.4 (C-6α), 68.3 (C-6β), 68.3 (C-5'α), 66.6 (C-5α), 66.5 (C-5β), 65.1 (C-5'β), 27.5, 25.8 (2 x $CH_3\beta$, isopropylidene), 27.2, 25.6 (2 x $CH_3\alpha$, isopropylidene), 20.8, 20.7 (2 x CH_3 α,β, Ac), 16.6,16.5 (C-6'α, C-6'β). ES-MS ($C_{26}H_{34}O_{12}$) 538.54, *m/z* (%) 561.3 (72) $[M+Na]^+$. HR-MS calcd for $C_{26}H_{34}O_{12}$ $[M+Na]^+$ 561.1943, found 561.1913.

Methyl 11-hydroxyundecanoate (185)



To a solution of 11-hydroxyundecanoic acid (1.03 g, 5.10 mmol) in 20 mL MeOH was added 2 g of Amberlite IR 120 (H^+ form) ion exchange resin and the reaction mixture was stirred for 2 days at room temperature. The resin was then filtered off, washed with MeOH and the filtrate evaporated to give 1.07 g (97%) of a light yellow oil. R_f 0.59 ($CHCl_3$/MeOH 5:1). ^{1}H NMR ($CDCl_3$): δ 3.65 (s, 3H, OMe), 3.62 (t, 2H, J = 6.6 Hz, CH_2-11), 2.29 (t, 2H, J = 7.6 Hz, CH_2-2), 1.62-1.51 (m, 4H, CH_2-3, CH_2-10), 1.27 (br s, 12H, 6 x CH_2). ^{13}C NMR ($CDCl_3$): δ 173.0 (C-1), 63.0 (C-11), 51.5 (OMe), 34.1 (C-2), 32.8 (C-10), 29.5, 29.4, 29.3, 29.2, 29.1, 25.7 (C-4 – C-9), 24.9 (C-3). ES-MS ($C_{12}H_{24}O_3$) 216.32, *m/z* (%) 216.3 (100) $[M]^+$. HR-MS calcd for $C_{12}H_{24}O_3$ $[M+H]^+$ 217.1798, found 217.1806.

Methyl [11-[(2,3-di-*O*-acetyl-4,6-*O*-benzylidene-*α/β*-D-glucopyranosyl)-(1→2)-3,4-*O*-isopropylidene-*α/β*-D-fucopyranosyl]oxy]undecanoate (186 & 187)

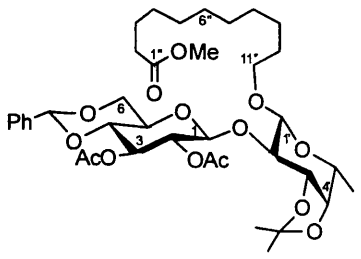
Method A:

A solution of **183** (0.31 g, 0.58 mmol), trichloroacetonitrile (0.2 mL, 1.74 mmol, 3 equiv) and Cs_2CO_3 (0.11 g, 0.35 mmol, 0.6 equiv) in 6 mL CH_2Cl_2 were stirred at room temperature overnight. The reaction mixture was filtered through a short pad of silica which was rinsed with hexanes/EtOAc 3:1. The filtrate was concentrated to give 0.3 g of crude product which was used without further purification. The crude trichloroacetimidate and alcohol **185** (84.8 mg, 0.39 mmol) were dissolved in 10 mL CH_2Cl_2 and cooled to -20 °C. TMSOTf (7 μL, 0.04 mmol, 0.1 equiv) was added to the solution and stirred at this temperature for 1 h. The reaction was quenched with Et_3N, concentrated and purified by flash chromatography (hexanes/EtOAc 4:1 to hexanes/EtOAc 3:1) to yield 169 mg (59%) of the α anomer and 81 mg (27%) of the β anomer (α:β = 2:1).

Method B:

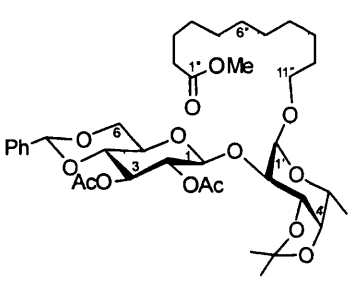
A solution of **180** (1.34 g, 3.82 mmol), trichloroacetonitrile (0.9 mL, 9.15 mmol, 3 equiv) and Cs_2CO_3 (0.75 g, 2.29 mmol, 0.6 equiv) in 25 mL CH_2Cl_2 were stirred at room temperature for 18 h. The reaction mixture was filtered through a short pad of silica which was rinsed with hexanes/EtOAc 3:1. The filtrate was concentrated to give 1.5 g of crude product (**181**) which was used without further purification. The crude trichloroacetimidate (1.5 g) and alcohol **195** (1.01 g, 2.50 mmol) were dissolved in 25 mL CH_2Cl_2 and cooled to -20 °C. TMSOTf (50 μL, 0.25 mmol, 0.1 equiv) was added to the solution and stirred at this temperature for 2 h. The reaction was quenched with Et_3N, concentrated and purified by flash chromatography (hexanes/EtOAc 4:1) to yield 1.42 g (77%) of disaccharide **186** as a colourless viscous oil.

Analytical and spectroscopic data of **186** (β anomer): R_f 0.49 (hexanes/EtOAc 1:2). ^{1}H NMR ($CDCl_3$): δ 7.45-7.41 (m, 2H, Ar), 7.36-7.33 (m, 3H, Ar), 5.50 (s, 1H, C<u>H</u>-Ph), 5.32 (t, 1H, J = 9.1 Hz, H-3), 5.00 (dd, 1H, J = 9.1, 7.7 Hz, H-2), 4.90 (d, 1H, J = 7.7 Hz, H-1), 4.31 (dd, 1H, J = 10.5, 5.0 Hz, CH_2-6), 4.20 (d, 1H, J = 8.1 Hz, H-1′), 4.02 (t, 1H, J = 6.2 Hz, H-3′), 3.95 (dd, 1H, J = 6.2, 2.1 Hz, H-4′), 3.84-3.72 (m, 4H, CH_2-11′′, CH_2-6, H-5′, H-4), 3.66 (s, 3H, OMe), 3.56 (dd, 1H, J = 8.1,

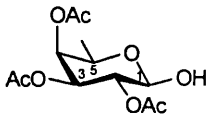


6.2 Hz, H-2′), 3.53-3.44 (m, 2H, H-5, CH_2-11′′), 2.28 (t, 2H, J = 7.5 Hz, CH_2-2′′), 2.08, 2.05 (s, 6H, 2 x CH_3 Ac), 1.64-1.57 (m, 4H, CH_2-3′′, CH_2-10′′), 1.50, 1.32 (s, 6H, 2 x CH_3 isopropylidene), 1.38 (d, 3H, J = 6.6 Hz, CH_3-6′), 1.27 (br s, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′). ^{13}C NMR ($CDCl_3$): δ 174.3 (C-1′′), 170.1, 169.8 (2 x C=O Ac), 136.9 (C_{quart} Ar), 129.1, 128.2, 126.2 (Ar), 109.7 (C_{quart} isopropylidene), 101.6 (CH-Ph), 101.5 (C-1), 101.4 (C-1′), 81.9 (C-2′), 78.8 (C-3′), 78.4 (C-4), 76.3 (C-4′), 72.9 (C-2), 71.9 (C-3), 69.9 (C-11′′), 68.7 (C-6), 68.6 (C-5′), 66.4 (C-5), 51.4 (OMe), 34.1 (C-2′′), 29.7, 29.5, 29.5, 29.4, 29.2, 29.1, 25.9, 25.0 (C-3′′ – C-10′′), 28.0, 26.3 (2 x CH_3 isopropylidene), 20.9, 20.8 (2 x CH_3 Ac), 16.6 (C-6′). ES-MS ($C_{38}H_{56}O_{14}$) 736.84, *m/z* (%) 759.4 (100) $[M+Na]^+$. HR-MS calcd for $C_{38}H_{56}O_{14}$ $[M+Na]^+$ 759.3563, found 759.3564.

Analytical and spectroscopic data of **187** (α anomer): R_f 0.57 (hexanes/EtOAc 1:2). ^{1}H NMR ($CDCl_3$): δ 7.43-7.40 (m, 2H, Ar), 7.35-7.33 (m, 3H, Ar), 5.48 (s, 1H, CH-Ph), 5.30 (t, 1H, J = 9.4 Hz, H-3), 5.02 (dd, 1H, J = 9.4 Hz, 7.9, H-2), 4.79 (d, 1H, J = 3.3 Hz, H-1′), 4.75 (d, 1H, J = 7.9 Hz, H-1), 4.31-4.24 (m, 2H, CH_2-6, H-3′), 4.10 (m, 2H, H-4′, H-5′), 3.87-3.81 (m, 2H, CH_2-6, H-2′), 3.75 (t, 1H, J = 9.4 Hz, H-4), 3.68-3.60 (m, 1H, CH_2-11′′), 3.65 (s, 3H, OMe), 3.52-3.46 (m, 1H, H-5), 3.40-3.35 (m, 1H, CH_2-11′′), 2.29 (t, 2H, J = 7.6 Hz, CH_2-2′′), 2.04, 2.03 (s, 6H, 2 x CH_3 Ac), 1.62-1.53 (m, 4H, CH_2-3′′, CH_2-10′′), 1.50, 1.33 (s, 6H, 2 x CH_3 isopropylidene), 1.31 (d, 3H, J = 6.6 Hz, CH_3-6′), 1.27 (br s, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′). ^{13}C NMR ($CDCl_3$): δ 174.4 (C-1′′), 170.3, 169.4 (2 x C=O Ac), 136.8 (C_{quart} Ar), 129.1, 128.2, 126.1 (Ar), 109.0 (C_{quart} isopropylidene), 101.5 (CH-Ph), 99.8 (C-1), 96.2 (C-1′), 78.2 (C-4), 76.1 (C-4′), 76.1 (C-2′), 74.0 (C-3′), 72.5 (C-3), 71.9 (C-2), 68.6 (C-11′′), 68.4 (C-6), 66.5 (C-5), 63.7 (C-5′), 51.4 (OMe), 34.1 (C-2′′), 29.6, 29.5, 29.4, 29.4, 29.2, 29.1, 26.1, 24.9 (C-3′′ – C-10′′), 27.9, 26.2 (2 x CH_3 isopropylidene), 20.8, 20.7 (2 x CH_3 Ac), 16.3 (C-6′). ES-MS ($C_{38}H_{56}O_{14}$) 736.84, *m/z* (%) 759.3 (100) $[M+Na]^+$. HR-MS calcd for $C_{38}H_{56}O_{14}$ $[M+Na]^+$ 759.3563, found 759.3563.

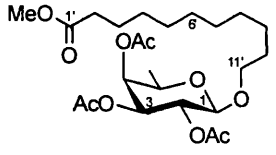


2,3,4-Tri-*O*-acetyl-*α/β*-D-fucopyranoside (191)



Acetic anhydride (30 mL) was added to a solution of D-fucose (5.25 g, 32.0 mmol) in pyridine (45 mL) and the mixture was allowed to stir at room temperature overnight. After completion of the reaction the mixture was co-evaporated with toluene to dryness to yield a yellow viscous oil (**190**) (R_f 0.57, hexanes/EtOAc 1:3), which was used in the following step without further purification.The crude per-*O*-acetylated D-fucose and $BnNH_2$ (5.2 mL, 48.0 mmol, 1.5 equiv) in 70 mL THF were stirred at room temperature overnight. The reaction mixture was then diluted with 140 mL CH_2Cl_2 and washed with ice-cold 1 M HCl (2 x 100 mL), the aqueous phase extracted with CH_2Cl_2 (100 mL) and the combined organic layers washed with brine (100 mL). The extracts were dried over $MgSO_4$, concentrated and purified by flash chromatography (hexanes/EtOAc 2:1 to hexanes/EtOAc 1:1) to yield 7.06 g (76% over 2 steps, α:β = 77:23) of a yellow viscous oil. R_f 0.42 (hexanes/EtOAc 1:2). ^{1}H NMR ($CDCl_3$): δ 5.47 (t, 1H, J = 3.4 Hz, H-1α), 5.41 (dd, 1H, J = 10.8, 3.2 Hz, H-3α), 5.31 (dd, 1H, J = 3.2, 0.6 Hz, H-4α), 5.23 (dd, 1H, J = 2.7, 0.1 Hz, H-4β), 5.16 (dd, 1H, J = 10.8, 3.4 Hz, H-2α), 5.06-5.04 (m, 2H, H-2β, H-3β), 4.66 (t, 1H, J = 8.5 Hz, H-1β), 4.44-4.39 (m, 1H, H-5α), 3.87-3.82 (m, 1H, H-5β), 3.50 (d, 1H, J = 8.5 Hz, OHβ), 2.97 (d, 1H, J = 2.7 Hz, OHα), 2.17, 2.10, 1.99 (s, 9H, 3 x $CH_3\beta$, Ac), 2.16, 2.09, 1.99 (s, 9H, 3 x $CH_3\alpha$, Ac), 1.23 (d, 3H, J = 6.4 Hz, CH_3-6β), 1.14 (d, 3H, J = 6.4 Hz, CH_3-6α). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.4, 170.1 (3 x C=O α,β, Ac), 95.9 (C-1β), 90.7 (C-1α), 71.3 (C-3β), 71.2 (C-4α), 70.7 (C-2β), 70.3 (C-4β), 69.5 (C-5β), 68.4 (C-2α), 67.7 (C-3α), 64.5 (C-5α), 20.8, 20.7, 20.6 (3 x CH_3 α,β, Ac), 16.1 (C-6β), 15.9 (C-6α). ES-MS ($C_{12}H_{18}O_8$) 290.27, *m/z* (%) 313.1 (100) [M+Na] $^+$. HR-MS calcd for $C_{12}H_{18}O_8$ $[M+Na]^+$ 313.0894, found 313.0882.

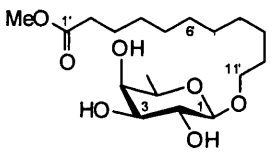
Methyl [11-(2,3,4-tri-*O*-acetyl-*β*-D-fucopyranosyl)oxy]undecanoate (193)



A solution of **191** (3.35 g, 11.6 mmol), trichloroacetonitrile (3.5 mL, 34.6 mmol, 3 equiv) and Cs_2CO_3 (2.45 g, 6.93 mmol, 0.6 equiv) in 35 mL CH_2Cl_2 were stirred at room temperature overnight. The reaction mixture was filtered through a short pad of silica which was rinsed with hexanes/EtOAc 1:1. The filtrate was concentrated to give 4.49 g of crude product (R_f 0.49 hexanes/EtOAc 1:2), which was used

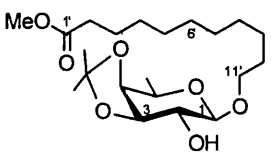
without further purification. The crude trichloroacetimidate and alcohol **185** (1.10 g, 5.09 mmol) were dissolved in 45 mL CH_2Cl_2 and cooled to -20 °C. TMSOTf (90 μL, 0.51 mmol, 0.1 equiv) was added to the solution and stirred at this temperature for 1 h. The reaction was quenched with Et_3N, concentrated and purified by flash chromatography (hexanes/EtOAc 4:1) to yield 2.02 g (81%) of glycoside **193** as a white oily solid. R_f 0.17 (hexanes/EtOAc 4:1). ^1H NMR ($CDCl_3$): δ 5.22 (dd, 1H, J = 3.5, 0.9 Hz, H-4), 5.17 (dd, 1H, J = 10.5, 7.9 Hz, H-2), 5.01 (dd, 1H, J = 10.5, 3.5 Hz, H-3), 4.41 (d, 1H, J = 7.9 Hz, H-1), 3.91-3.85 (m, 1H, CH_2-11′), 3.79 (qd, 1H, J = 6.5, 0.9 Hz, H-5), 3.66 (s, 3H, OMe), 3.47-3.41 (m, 1H, H-11′), 2.29 (t, 2H, J = 7.9 Hz, CH_2-2′), 2.16, 2.04, 1.98 (s, 9H, 3 x CH_3 Ac), 1.65-1.51 (m, 4H, CH_2-3′, CH_2-10′), 1.26 (br s, 12H, 6 x CH_2, CH_2-4′ - CH_2-9′), 1.22 (d, 3H, J = 6.5 Hz, CH_3-6). ^{13}C NMR ($CDCl_3$): δ 174.3 (C-1′), 170.7, 170.3, 169.4 (3 x C=O Ac), 101.2 (C-1), 71.4 (C-3), 70.4 (C-4), 70.1 (C-11′), 69.1 (C-2, C-5), 51.4 (OMe), 34.1 (C-2′), 29.5, 29.4, 29.4, 29.3, 29.2, 29.1, 25.8, 24.9 (C-3′ – C-10′), 20.8, 20.7, 20.6 (3 x CH_3, Ac), 16.1 (C-6). ES-MS ($C_{24}H_{40}O_{10}$) 488.57, *m/z* (%) 510.4 (38) $[M+Na]^+$. HR-MS calcd for $C_{24}H_{40}O_{10}$ $[M+Na]^+$ 511.2514, found 511.2534.

Methyl [11-(β-D-fucopyranosyl)oxy]undecanoate (194)



193 (2.02 g, 4.13 mmol) was dissolved in MeOH (30 mL) and NaOMe (4.1 mL of 0.5 M soln in MeOH, 2.06 mmol, 0.5 equiv) was added. The reaction was stirred at room temperature for 15 min. Amberlite IR 120 (H^+ form) ion exchange resin was added for neutralisation and the mixture was stirred for a few minutes. The resin was then filtered off, washed with MeOH and the filtrate evaporated to give a white solid (R_f 0.53, $CHCl_3$/MeOH 4:1) which was used in the following step without further purification. ES-MS ($C_{18}H_{34}O_7$) 362.46, *m/z* (%) 384.2 (100) $[M+Na]^+$. HR-MS calcd for $C_{18}H_{34}O_7$ $[M+Na]^+$ 385.2202, found 385.2191.

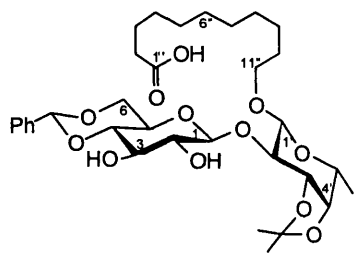
Methyl [11-(3,4-*O*-isopropylidene-β-D-fucopyranosyl)oxy]undecanoate (195)



The crude **194** was dissolved in 140 mL acetone. 80 mL 2,2-dimethoxypropane and 200 mg CSA were added and the mixture was stirred at room temperature overnight. The reaction was then quenched with NH_4OH and the solvents removed under reduced

pressure. Purification by flash chromatography (hexanes/EtOAc 4:1 to hexanes/EtOAc 1:1) gave 1.38 g (85% over 2 steps) of compound **195** as a colourless oil. R_f 0.38 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 4.12 (d, 1H, J = 7.8 Hz, H-1), 4.03 (dd, 1H, J = 7.8, 5.4 Hz, H-3), 4.00 (dd, 1H, J = 5.4, 2.3 Hz, H-4), 3.93-3.83 (m, 2H, CH_2-11′, H-5), 3.66 (s, 3H, OMe), 3.52 (dt, 1H, J = 7.8, 2.1 Hz, H-2), 3.49-3.43 (m, 1H, H-11′), 2.32 (d, 1H, J = 2.1 Hz, OH), 2.30 (t, 2H, J = 7.6 Hz, CH_2-2′),1.65-1.57 (m, 4H, CH_2-3′, CH_2-10′), 1.53, 1.35 (s, 6H, 2 x CH_3 isopropylidene), 1.42 (d, 3H, J = 6.6 Hz, CH_3-6), 1.28 (br s, 12H, 6 x CH_2, CH_2-4′ - CH_2-9′). ^{13}C NMR ($CDCl_3$): δ 174.3 (C-1′), 109.8 (C_{quart} isopropylidene), 102.2 (C-1), 78.7 (C-3), 76.4 (C-4), 73.7 (C-2), 69.9 (C-11′), 69.2 (C-5), 51.4 (OMe), 34.1 (C-2′), 29.6, 29.4, 29.4, 29.3, 29.2, 29.1, 26.0, 24.9 (C-3′ – C-10′), 28.2, 26.3 (2 x CH_3 isopropylidene), 16.6 (C-6). ES-MS ($C_{21}H_{38}O_7$) 402.5, *m/z* (%) 402.5 (33) $[M+H]^+$, 424.6 (86) $[M+Na]^+$. HR-MS calcd for $C_{21}H_{38}O_7$ $[M+H]^+$ 403.2690, found 403.2704.

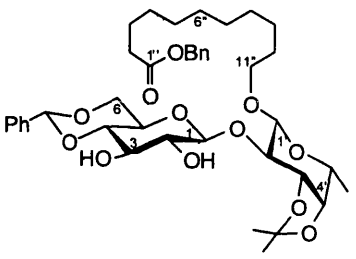
[11-[(4,6-*O*-Benzylidene-*β*-D-glucopyranosyl)-(1→2)-3,4-*O*-isopropylidene-*β*-D-fucopyranosyl]oxy]undecanoic acid (196)



1.40 g (1.90 mmol) of **186** were dissolved in 50 ml THF/MeOH (3:2), 12.5 mL (25.0 mmol, 13 equiv) of 2 M LiOH solution were added and the mixture was stirred at room temperature for 15 h. The solvents were removed and the residue dissolved in Et_2O (30 mL) and extracted with H_2O (30 mL). The aqueous layer was acidified with conc. HCl to pH 4 and extracted with EtOAc (2 x 30 mL). The extracts were dried over $MgSO_4$ and concentrated to yield 1.19 g (98%) of pure **196** as a colourless viscous oil. R_f 0.45 ($CHCl_3$/MeOH 4:1). ^{1}H NMR ($CDCl_3$): δ 7.50-7.48 (m, 2H, Ar), 7.38-7.35 (m, 3H, Ar), 5.53 (s, 1H, C<u>H</u>-Ph), 4.65 (d, 1H, J = 7.8 Hz, H-1), 4.32 (dd, 1H, J = 10.3, 4.8 Hz, CH_2-6), 4.25 (d, 1H, J = 7.8 Hz, H-1′), 4.16 (dd, 1H, J = 7.8, 5.4 Hz, H-3′), 4.02 (dd, 1H, J = 5.4, 2.1 Hz, H-4′), 3.93-3.82 (m, 2H, CH_2-11′′, H-5′), 3.85 (t, 1H, J = 9.1 Hz, H-4), 3.79 (t, 1H, J = 10.3 Hz, CH_2-6), 3.67 (dd, 1H, J = 7.8 Hz, H-2′), 3.58-3.53 (m, 2H, H-2, H-3), 3.51-3.45 (m, 2H, H-5, CH_2-11′′), 2.31 (t, 2H, J = 7.4 Hz, CH_2-2′′), 1.64-1.57 (m, 4H, CH_2-3′′, CH_2-10′′), 1.54, 1.36 (s, 6H, 2 x CH_3 isopropylidene), 1.41 (d, 3H, J = 6.6 Hz, CH_3-6′), 1.29 (br s, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′). ^{13}C NMR ($CDCl_3$): δ 178.4

(C-1′′), 137.0 (C_{quart} Ar), 129.2, 128.3, 126.3 (Ar), 110.4 (C_{quart} isopropylidene), 104.6 (C-1), 102.0 (CH-Ph), 101.3 (C-1′), 81.4 (C-2′), 80.6 (C-2), 78.4 (C-3′), 76.5 (C-4′), 75.8 (C-3), 72.7 (C-4), 69.9 (C-11′′), 68.8 (C-6), 68.7 (C-5′), 67.0 (C-5), 33.8 (C-2′′), 29.6, 29.3, 29.2, 29.1, 28.9, 25.8, 24.6 (C-3′′ – C-10′′), 27.9, 26.2 (2 x CH_3 isopropylidene), 16.5 (C-6′). ES-MS ($C_{33}H_{50}O_{12}$) 638.74, *m/z* (%) 637.7 (100) $[M-H]^-$, 661.4 (100) $[M+Na]^+$. HR-MS calcd for $C_{33}H_{50}O_{12}$ $[M+Na]^+$ 661.3200, found 661.3199.

Benzyl 11-[[(4,6-*O*-benzylidene-*β*-D-glucopyranosyl)-(1→2)-3,4-*O*-isopropylidene -*β*-D-fucopyranosyl]oxy]undecanoate (198)

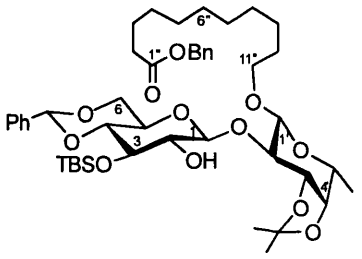


Cs_2CO_3 (0.15, 0.47 mmol, 0.6 equiv) was added to a solution of **196** (0.50g, 0.78 mmol) in MeOH (10 mL) and the reaction mixture was allowed to stir for 1 h at room temperature. The solvent was then evaporated to dryness and the residue taken up in DMF (20 mL). BnBr (0.12 mL, 1.01 mmol, 1.3 equiv) was added and the reaction was left to stir for 18 h at room temperature. The solvents were removed under reduced pressure and purification by flash chromatography (hexanes/EtOAc 3:1 to hexanes/EtOAc 1:3) gave 0.38 g (67%) of compound **198** as a colourless viscous oil. R_f 0.36 (hexanes/EtOAc 1:6). ^1H NMR ($CDCl_3$): δ 7.49-7.47 (m, 2H, Ar), 7.37-7.31 (m, 8H, Ar), 5.53 (s, 1H, CH-Ph), 5.10 (s, 2H, CH_2-Ph), 4.64 (d, 1H, J = 7.7 Hz, H-1), 4.32 (dd, 1H, J = 10.5, 4.8 Hz, CH_2-6), 4.25 (d, 1H, J = 7.8 Hz, H-1′), 4.16 (dd, 1H, J = 7.8, 5.5 Hz, H-3′), 4.03 (dd, 1H, J = 5.5, 2.1 Hz, H-4′), 3.93-3.74 (m, 4H, H-4, CH_2-6, CH_2-11′′, H-5′), 3.66 (dd, 1H, J = 7.8 Hz, H-2′), 3.58-3.47 (m, 4H, H-2, H-3, H-5, CH_2-11′′), 2.33 (t, 2H, J = 7.6 Hz, CH_2-2′′), 1.63-1.58 (m, 4H, CH_2-3′′, CH_2-10′′), 1.55, 1.36 (s, 6H, 2 x CH_3 isopropylidene), 1.41 (d, 3H, J = 6.6 Hz, CH_3-6′), 1.27 (br s, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′). ^{13}C NMR ($CDCl_3$): δ 171.5 (C-1′′), 137.0, 136.1 (C_{quart} Ar), 129.2, 128.5, 128.3, 128.1, 126.3 (Ar), 110.3 (C_{quart} isopropylidene), 104.8 (C-1), 102.0 (CH-Ph), 101.3 (C-1′), 81.6 (C-2′), 80.7 (C-2), 78.3 (C-3′), 76.5 (C-4′), 75.9 (C-3), 72.7 (C-4), 70.0 (C-11′′), 68.7 (C-6), 68.7 (C-5′), 67.0 (C-5), 66.0 (CH_2-Ph), 34.3 (C-2′′), 29.6, 29.5, 29.4, 29.4, 29.2, 29.1, 25.9, 24.9 (C-3′′ – C-10′′), 27.9, 26.2 (2 x CH_3 isopropylidene), 16.4 (C-6′). ES-MS ($C_{40}H_{56}O_{12}$) 728.87, *m/z* (%) 751.5 (100) $[M+Na]^+$.

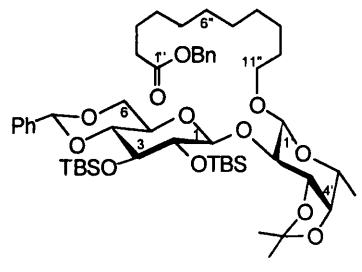
Benzyl [11-[(3-*O*-TBS-4,6-*O*-benzylidene-*β*-D-glucopyranosyl)-(1→2)-3,4-*O*-isopropylidene-*β*-D-fucopyranosyl]oxy]undecanoate (199) & benzyl [11-[(2,3-di-*O*-TBS-4,6-*O*-benzylidene-*β*-D-glucopyranosyl)-(1→2)-3,4-*O*-isopropylidene-*β*-D-fucopyranosyl]oxy]undecanoate (200)

52.6 mg (0.07 mmol) of **198** were dissolved in 1.5 mL CH_2Cl_2. Pyridene (40 μL, 0.50 mmol, 7 equiv) was then added, followed by TBSOTf (40 μL, 0.17 mmol, 2.4 equiv) and the reaction was stirred for 18 h at room temperature. The solvents were removed under reduced pressure and purification by flash chromatography (hexanes/EtOAc 95:5 to hexanes/EtOAc 1:1) gave 35.8 mg (59%) of compound **199** and 26.2 (38%) of compound **200** as colourless oils.

Analytical and spectroscopic data of **199**: R_f 0.37 (hexanes/EtOAc 2:1). ^{1}H NMR



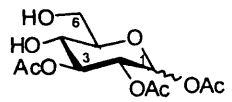
($CDCl_3$): δ 7.49-7.47 (m, 2H, Ar), 7.36-7.32 (m, 8H, Ar), 5.51 (s, 1H, C<u>H</u>-Ph), 5.11 (s, 2H, C<u>H</u>$_2$-Ph), 4.64 (d, 1H, J = 7.6 Hz, H-1), 4.30 (dd, 1H, J = 10.2, 4.5 Hz, CH_2-6), 4.25 (d, 1H, J = 7.6 Hz, H-1′), 4.14 (dd, 1H, J = 7.6, 5.4 Hz, H-3′), 4.01 (dd, 1H, J = 5.4, 2.3 Hz, H-4′), 3.91-3.85 (m, 1H, CH_2-11′′), 3.85-3.80 (m, 1H, H-5′), 3.78-3.74 (m, 1H, CH_2-6), 3.76 (t, 1H, J = 8.6 Hz, H-3), 3.69 (t, 1H, J = 7.8 Hz, H-2′), 3.50-3.39 (m, 4H, H-2, H-4, H-5, CH_2-11′′), 2.33 (t, 2H, J = 7.6 Hz, CH_2-2′′), 1.64-1.58 (m, 4H, CH_2-3′′, CH_2-10′′), 1.53, 1.36 (s, 6H, 2 x CH_3 isopropylidene), 1.40 (d, 3H, J = 6.6 Hz, CH_3-6′), 1.27 (br s, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′), 0.88 (s, 9H, *t*Bu, TBS), 0.11, 0.05 (s, 6H, 2 x CH_3 TBS). ^{13}C NMR ($CDCl_3$): δ 173.7 (C-1′′), 137.4, 136.2 (C_{quart} Ar), 128.8, 128.5, 128.2, 128.1, 126.1 (Ar), 110.1 (C_{quart} isopropylidene), 104.3 (C-1), 101.6 (<u>C</u>H-Ph), 101.5 (C-1′), 81.6 (C-2), 80.5 (C-2′), 78.5 (C-3′), 76.5 (C-4′), 76.2 (C-4), 74.1 (C-3), 69.9 (C-11′′), 68.8 (C-6), 68.7 (C-5′), 66.9 (C-5), 66.0 (<u>C</u>H_2-Ph), 34.3 (C-2′′), 29.6, 29.5, 29.4, 29.2, 29.1, 25.9, 25.0 (C-3′′ – C-10′′), 27.7, 26.3 (2 x CH_3 isopropylidene), 25.9 (*t*Bu, TBS), 18.4 (C_{quart} TBS), 16.5 (C-6′) -4.5, -4.6 (2 x CH_3 TBS). ES-MS ($C_{46}H_{70}O_{12}$) 843.13, *m/z* (%) 866.5 (78) $[M+Na]^+$.



Analytical and spectroscopic data of **200**: R_f 0.51 (hexanes/EtOAc 2:1). ^{1}H NMR ($CDCl_3$): δ 7.47-7.45 (m, 2H, Ar), 7.37-7.31 (m, 8H, Ar), 5.42 (s, 1H, C<u>H</u>-Ph), 5.12 (s, 2H, C<u>H</u>$_2$-Ph), 4.94 (d, 1H, J = 6.4 Hz,

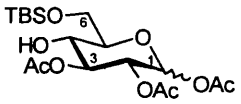
H-1), 4.24 (d, 1H, J = 8.1 Hz, H-1′), 4.22 (dd, 1H, J = 10.4, 4.9 Hz, CH_2-6), 4.17 (dd, 1H, J = 6.8, 5.7 Hz, H-3′), 3.98 (dd, 1H, J = 5.7, 2.1 Hz, H-4′), 3.88 (dd, 1H, J = 8.1, 6.8 Hz, H-2′), 3.86-3.72 (m, 4H, H-4, CH_2-6, H-5′, CH_2-11′′), 3.64 (dd, 1H, J = 9.6, 6.4 Hz, H-3), 3.56 (t, 2H, J = 6.4 Hz, H-2), 3.54-3.45 (m, 2H, H-5, CH_2-11′′), 2.35 (t, 2H, J = 7.5 Hz, CH_2-2′′), 1.67-1.60 (m, 4H, CH_2-3′′, CH_2-10′′), 1.50, 1.34 (s, 6H, 2 x CH_3 isopropylidene), 1.40 (d, 3H, J = 6.6 Hz, CH_3-6′), 1.26 (br s, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′), 0.92, 0.88 (s, 18H, *t*Bu, 2 x TBS), 0.12, 0.11, 0.02, -0.01 (s, 6H, 4x CH_3 2 x TBS). ^{13}C NMR ($CDCl_3$): δ 173.7 (C-1′′), 137.5, 136.2 (C_{quart} Ar), 129.0, 128.6, 128.2, 128.1, 126.5 (Ar), 109.7 (C_{quart} isopropylidene), 102.2 (CH-Ph), 101.2 (C-1), 99.6 (C-1′), 81.8 (C-3), 79.6 (C-3′), 76.6 (C-4′), 76.4 (C-2), 75.9 (C-4), 75.2 (C-2′), 69.7 (C-11′′), 69.2 (C-6), 68.7 (C-5′), 66.0 (CH_2-Ph), 65.7 (C-5), 34.3 (C-2′′), 29.7, 29.5, 29.5, 29.4, 29.3, 29.1, 26.0, 25.0 (C-3′′ – C-10′′), 27.8, 26.5 (2 x CH_3 isopropylidene), 26.2, 26.1 (*t*Bu, 2 x TBS), 18.3, 18.2 (C_{quart} 2 x TBS), 16.6 (C-6′) -3.1, -3.4, -3.7, -3.8 (4 x CH_3 2 x TBS). ES-MS ($C_{52}H_{84}O_{12}Si_2$) 957.39, *m/z* (%) 980.8 (83) $[M+Na]^+$.

1,2,3-Tri-*O*-acetyl-*α/β*-D-glucopyranose (204)



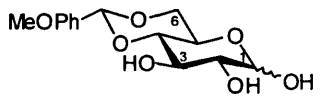
To a solution of **180** (9.13 g, 0.02 mol) in THF (50 mL) was added 0.9 g of Pd/C (10% *w/w*) and the solution was stirred for two days under an atmosphere of H_2. The reaction mixture was then filtere through a celite pad, the solvents were evaporated and the residue purified by flash chromatography (hexanes/EtOAc 1:1 to hexanes/EtOAc 1:3) to give 4.55 g (65%, α:β = 46:54) of compound **204** as a white sticky foam. R_f 0.13 (hexanes/EtOAc 1:3). ^{1}H NMR ($CDCl_3$): δ 6.28 (d, 1H, J = 3.5 Hz, H-1α), 5.71 (d, 1H, J = 7.8 Hz, H-1β), 5.31 (t, 1H, J = 9.9 Hz, H-3α), 5.11-4.99 (m, 3H, H-2α, H-2β, H-3β), 3.92 (dd, 1H, J = 12.1, 3.2 Hz, CH_2-6α), 3.89-3.78 (m, 6H, H-4α, H-4β, H-5α, CH_2-6α, CH_2-6β), 3.59-3.54 (m, 1H, H-5β), 2.16, 2.10 (s, 9H, 3 x $CH_3\beta$, Ac), 2.11, 2.03, 2.02 (s, 9H, 3 x $CH_3\alpha$, Ac). ^{13}C NMR ($CDCl_3$): δ 171.7, 171.5, 169.9, 169.5, 169.2, 169.1 (6 x C=O α,β, Ac), 91.9 (C-1β), 89.3 (C-1α), 76.4 (C-5β), 75.9 (C-3β), 73.9 (C-5α), 72.9 (C-3α), 70.3 (C-2β), 69.3 (C-2α), 69.0 (C-4α), 68.9 (C-4β), 61.7 (C-6β), 61.6 (C-6α), 20.9, 20.9, 20.8, 20.6, 20.5 (CH_3, 6 x Ac α,β). ES-MS ($C_{12}H_{18}O_9$) 306.27, *m/z* (%) 329.3 (54) $[M+Na]^+$. HR-MS calcd for $C_{12}H_{18}O_9$ $[M+Na]^+$ 329.0848, found 329.0829.

1,2,3-Tri-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-*α/β*-D-glucopyranose (205)



0.46 g (1.52 mmol) of **204** were dissolved in 2 mL CH_2Cl_2, treated with 0.25 g (1.67 mmol, 1.1 equiv) TBSCl, 0.25 mL (1.82 mmol, 1.2 equiv) Et_3N and 7.4 mg (0.06 mmol, 0.04 equiv) imidazole and stirred at room temperature overnight. The reaction mixture was then concentrated and purified by flash chromatography (hexanes/EtOAc 2:1) to yield 0.47 g (74%, *α:β* = 46:54) of a white foam. R_f 0.44 (hexanes/EtOAc 1:3). ^{1}H NMR ($CDCl_3$): *δ* 6.26 (d, 1H, *J* = 3.7 Hz, H-1*α*), 5.68 (d, 1H, *J* = 8.3 Hz, H-1*β*), 5.36 (t, 1H, *J* = 10.3 Hz, H-3*α*), 5.13 (t, 1H, *J* = 9.5 Hz, H-3*β*), 5.02 (dd, 1H, *J* = 9.5, 8.3 Hz, H-2*β*), 4.98 (dd, 1H, *J* = 10.3, 3.7 Hz, H-2*α*), 3.99-3.92 (m, 2H, CH_2-6*β*), 3.83-3.75 (m, 5H, H-4*α*, H-4*β*, H-5*α*, CH_2-6*α*), 3.58-3.53 (m, 1H, H-5*β*), 3.32 (d, 1H, *J* = 2.2 Hz, OH*β*), 3.19 (br s, 1H, OH*α*), 2.11, 2.03, 2.01 (s, 9H, 3 x CH_3*α*, Ac) 2.09, 2.08, 2.02 (s, 9H, 3 x CH_3*β*, Ac), 0.89 (s, 9H, *t*Bu*α*, TBS), 0.89 (s, 9H, *t*Bu*β*, TBS), 0.08, 0.07 (s, 12H, 2 x CH_3 *α,β*, TBS). ^{13}C NMR ($CDCl_3$): *δ* 171.1, 169.9, 169.0 (3 x C=O*α*, Ac), 170.8, 169.6, 169.1 (3 x C=O*β*, Ac), 91.8 (C-1*β*), 89.4 (C-1*α*), 75.3 (C-3*β*), 74.7 (C-5*β*), 72.6, (C-5*α*), 72.2 (C-3*α*), 71.3 (C-4*β*), 71.1 (C-4*α*), 70.4 (C-2*β*), 69.3 (C-2*α*), 64.2 (C-6*β*), 63.9 (C-6*α*), 25.8 (*t*Bu *α,β*, TBS), 18.3 (C_{quart} *α,β*, TBS), 21.0, 20.9, 20.8, 20.8, 20.6, 20.5 (3 x CH_3 *α,β*, Ac), -5.5 (2 x CH_3 *α,β*, TBS). ES-MS ($C_{18}H_{32}O_9Si$) 420.53, *m/z* (%) 443.3 (94) $[M+Na]^+$.

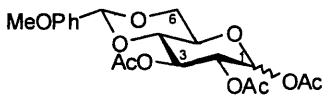
4,6-*O*-*p*-Methoxybenzylidene-*α/β*-D-glucopyranose (206)



To a solution of D-glucose (15.1 g, 0.08 mol) in DMF (60 mL) were added p-methoxybenzaldehyde dimethyl acetal (21.4 mL, 0.13 mol, 1.5 equiv) and PTSA (333 mg) and the reaction was heated at 60 °C for 30 min. The reaction was then quenched with Et_3N and the solvents removed under reduced pressure. Purification by flash chromatography (100% $CHCl_3$ to $CHCl_3$/MeOH 10:1) gave 10.0 g (42%, *α:β* = 46:54) of compound **206** as a white solid. R_f 0.38 (hexanes/EtOAc 1:1). ^{1}H NMR (MeOD): *δ* 7.46-7.42 (m, 4H, Ar *α,β*), 6.90-6.88 (m, 4H, Ar *α,β*), 5.52 (s, 1H, C<u>H</u>-Ph*β*), 5.51 (s, 1H, C<u>H</u>-Ph*α*), 5.18 (t, 1H, *J* = 3.5 Hz, H-1*α*), 4.62 (d, 1H, *J* = 8.0 Hz, H-1*β*), 4.27 (dd, 1H, *J* = 10.2, 4.6 Hz, CH_2-6*β*), 4.20 (dd, 1H, *J* = 10.2, 4.9 Hz, CH_2-6*α*), 4.02 (td, 1H, *J* = 9.6, 4.9 Hz, H-5*α*), 3.91 (t, 1H, *J* = 9.6 Hz, H-3*α*), 3.81 (s, 3H, OMe *α,β*), 3.78-3.72 (m, 2H, CH_2-6*α*, CH_2-6*β*), 3.67 (t, 1H, *J* = 8.8 Hz, H-3*β*), 3.53-3.43 (m, 3H, H-2*α*, H-4*β*, H-5*β*), 3.44 (t, 1H, *J* = 9.6 Hz, H-4*α*), 3.30

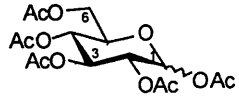
(dd, 1H, J = 8.8, 8.0 Hz, H-2β). ^{13}C NMR ($CDCl_3$): δ 161.5, 131.4, 128.9, 114.7 (Ar α,β), 103.1 (CH-Ph α,β), 98.9 (C-1β), 94.7 (C-1α), 83.0 (C-4α), 82.4 (C-4β), 77.2 (C-2β), 74.7 (C-3β), 74.5 (C-2α), 71.9 (C-3α), 70.4 (C-6α), 70.0 (C-6β), 67.8 (C-5β), 63.5 (C-5α), 56.2 (OMe α,β). ES-MS ($C_{20}H_{24}O_{10}$) 298.29, *m/z* (%) 321.7 (64) $[M+Na]^+$.

1,2,3-Tri-*O*-acetyl-4,6-*O*-*p*-methoxybenzylidene-α/β-D-glucopyranose (207)



Acetic anhydride (10 mL) was added to a solution of **206** (4.28 g, 14.4 mmol) in pyridine (15 mL) and the mixture was allowed to stir at room temperature for 18 h. After completion of the reaction the mixture was co-evaporated with toluene to dryness to yield a white amorphous solid (α:β = 44:56, R_f 0.31 hexanes/EtOAc 1:1), which was used in the following step without further purification. ^{1}H NMR ($CDCl_3$): δ 7.38-7.34 (m, 4H, Ar α,β), 6.89-6.87 (m, 4H, Ar α,β), 6.30 (t, 1H, J = 3.7 Hz, H-1α), 5.79 (d, 1H, J = 8.5 Hz, H-1β), 5.57 (t, 1H, J = 10.0 Hz, H-3α), 5.47 (s, 1H, CH-Phα), 5.46 (s, 1H, CH-Phβ), 5.35 (t, 1H, J = 9.2 Hz, H-3β), 5.14-5.09 (m, 2H, H-2α, H-2β), 4.36 (dd, 1H, J = 10.3, 4.6 Hz, CH_2-6β), 4.29 (dd, 1H, J = 10.3, 4.9 Hz, CH_2-6α), 4.02 (td, 1H, J = 9.9, 4.8 Hz, H-5α), 3.80 (s, 3H, OMeα), 3.79 (s, 3H, OMeβ), 3.75-3.61 (m, 5H, H-4α, H-4β, H-5β, CH_2-6α, CH_2-6β), 2.18, 2.06, 2.03 (s, 6H, 3 x $CH_3\alpha$, Ac), 2.11, 2.05 (s, 6H, 3 x $CH_3\beta$, Ac). ^{13}C NMR ($CDCl_3$): δ 169.9, 169.9, 169.8, 169.5, 169.1, 168.9 (3 x C=O, α,β, Ac), 160.2, 132.0, 129.2, 129.1, 127.5, 127.5, 113.6 (Ar α,β), 101.6 (CH-Ph α,β), 92.2 (C-1β), 89.7 (C-1α), 78.6 (C-4α), 78.0 (C-4β), 71.8 (C-3β), 71.3 (C-2α), 69.9 (C-2β), 68.8 (C-3α), 68.5 (C-6α), 68.3 (C-6β), 67.1 (C-5β), 65.0 (C-5α), 55.3 (OMe α,β), 20.9, 20.8, 20.7, 20.6, 20.5 (6 x CH_3 α,β, Ac). ES-MS ($C_{20}H_{24}O_{10}$) 424.40, *m/z* (%) 447.1 (82) $[M+Na]^+$.

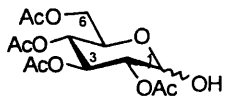
1,2,3,4,6-Penta-*O*-acetyl-α/β-D-glucopyranose (208)



Acetic anhydride (20 mL) was added to a solution of D-glucose (2.98 g, 16.5 mmol) in pyridine (30 mL) and the mixture was allowed to stir at room temperature for 1 h. After completion of the reaction the mixture was co-evaporated with toluene to dryness. The compound was dissolved in CH_2Cl_2 (50 mL) and washed 5% HCl (2 x 50 mL), the aqueous phase extracted with CH_2Cl_2 (50 mL) and the combined organic layers washed with brine (50 mL). The extracts were dried over $MgSO_4$ and concentrated to

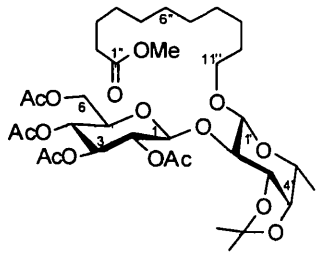
yield 5.93 g of pure per-*O*-acetylated glucose (92%, α:β = 88:12). R_f 0.23 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 6.33 (d, 1H, J = 3.7 Hz, H-1α), 5.72 (d, 1H, J = 8.1 Hz, H-1β), 5.47 (t, 1H, J = 9.9 Hz, H-3α), 5.25 (t, 1H, J = 9.4 Hz, H-3β), 5.16-5.08 (m, 2H, H-2β, H-4β), 5.14 (t, 1H, J = 9.9 Hz, H-4α), 5.10 (dd, 1H, J = 9.9, 3.7 Hz, H-2α), 4.31-4.24 (m, 1H, CH_2-6β), 4.27 (dd, 1H, J = 12.6, 4.2 Hz, CH_2-6α), 4.14-4.08 (m, 3H, H-5α, CH_2-6α, CH_2-6β), 3.86-3.81 (m, 1H, H-5β), 2.18, 2.09, 2.04, 2.02, 2.01 (s, 15H, 5 x $CH_3\alpha$, Ac), 2.16, 2.11, 2.08, 2.03, 2.01 (s, 15H, 5 x $CH_3\beta$, Ac). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.2, 169.6, 169.4, 168.7 (5 x C=O α,β, Ac), 91.4 (C-1β), 89.1 (C-1α), 72.8 (C-3β), 69.8 (C-3α, C-5α), 69.2 (C-2α), 67.9 (C-4α), 61.5 (C-6α), 20.9, 20.7, 20.6, 20.5, 20.4 (5 x CH_3 α,β, Ac). ES-MS ($C_{16}H_{22}O_{11}$) 390.34, *m/z* (%) 412.9 (100) $[M+Na]^+$. HR-MS calcd for $C_{16}H_{22}O_{11}$ $[M+Na]^+$ 413.1055, found 413.1044.

2,3,4,6-Tetra-*O*-acetyl-α/β-D-glucopyranose (209)



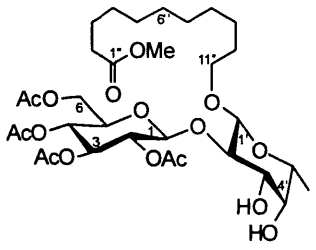
2.86 g (7.33 mmol) of per-*O*-acetylated glucose **208** and $BnNH_2$ (1.2 mL, 10.9 mmol, 1.5 equiv) in 15 mL THF were stirred at room temperature overnight. The reaction mixture was then diluted with 50 mL CH_2Cl_2 and washed with ice-cold 1 M HCl (2 x 35 mL), the aqueous phase extracted with CH_2Cl_2 (35 mL) and the combined organic layers washed with brine (35 mL). The extracts were dried over $MgSO_4$, concentrated and purified by flash chromatography (hexanes/EtOAc 4:1 to hexanes/EtOAc 1:1) to yield 2.36 g (93%, α:β = 80:20) of yellow viscous oil. R_f 0.29 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 5.54 (t, 1H, J = 9.7 Hz, H-3α), 5.46 (d, 1H, J = 3.5 Hz, H-1α), 5.25 (t, 1H, J = 9.7 Hz, H-3β), 5.08 (t, 2H, J = 9.7 Hz, H-4α, H-4β), 4.90 (dd, 1H, J = 9.7, 3.5 Hz, H-2α), 4.89-4.86 (m, 1H, H-2β), 4.74 (d, 1H, J = 8.1 Hz, H-1β), 4.29-4.21 (m, 3H, H-5α, CH_2-6α, CH_2-6β), 4.16-4.09 (m, 2H, CH_2-6α, CH_2-6β), 3.76-3.72 (m, 1H, H-5β), 2.08, 2.07, 2.03, 2.01 (s, 24H, 4 x CH_3 α,β Ac). ^{13}C NMR ($CDCl_3$): δ 170.8, 170.1, 170.1, 169.6 (4 x C=Oα, Ac), 170.7, 170.1, 169.5, (4 x C=Oβ, Ac), 95.6 (C-1β), 90.2 (C-1α), 73.3 (C-2β), 72.3 (C-3β), 72.1 (C-5β), 71.1 (C-2α), 69.9 (C-3α), 68.5 (C-4α, C-4β), 67.2 (C-5α), 62.0 (C-6α, C-6β), 20.7, 20.7, 20.7, 20.6 (4 x $CH_3\alpha$, Ac), 20.7, 20.7, 20.6, 20.6 (4 x $CH_3\beta$, Ac). ES-MS ($C_{14}H_{20}O_{10}$) 348.30, *m/z* (%) 393.2 (54) $[M+HCOOH]^-$. HR-MS calcd for $C_{14}H_{20}O_{10}$ $[M+Na]^+$ 371.0949, found 371.0941.

Methyl [11-[(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-(1→2)-3,4-*O*-isopropylidene-*β*-D-fucopyranosyl]oxy]undecanoate (211)



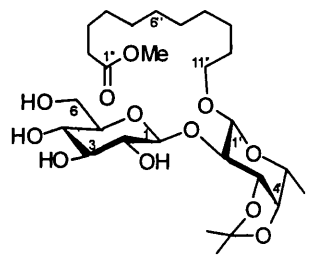
A solution of **209** (0.58 g, 1.67 mmol), trichloroacetonitrile (0.5 mL, 5.0 mmol, 3 equiv) and Cs_2CO_3 (0.35 g, 1.0 mmol, 0.6 equiv) in 5 mL CH_2Cl_2 were stirred at room temperature overnight. The reaction mixture was filtered through a short pad of silica which was rinsed with hexanes/EtOAc 3:1. The filtrate was concentrated to give 0.5 g of crude product (**210**) which was used without further purification. (R_f 0.4 hexanes/EtOAc 1:1). The crude trichloroacetimidate (0.5 g) and alcohol **195** (207 mg, 0.51 mmol) were dissolved in 6 mL CH_2Cl_2 and cooled to -20 °C. TMSOTf (10 μL, 0.05 mmol, 0.1 equiv) was added to the solution and stirred at this temperature for 1 h. The reaction was quenched with Et_3N, concentrated and purified by flash chromatography (hexanes/EtOAc 3:1 to hexanes/EtOAc 1:1) to yield 329 mg (90%) of disaccharide **211** as a yellow oil. R_f 0.43 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 5.21 (t, 1H, J = 9.5 Hz, H-3), 5.10 (t, 1H, J = 9.5 Hz, H-4), 4.98 (dd, 1H, J = 9.5, 8.0 Hz, H-2), 4.84 (d, 1H, J = 8.0 Hz, H-1), 4.26 (dd, 1H, J = 12.2, 4.1 Hz, CH_2-6), 4.23 (d, 1H, J = 7.8 Hz, H-1′), 4.13-4.08 (m, 2H, CH_2-6), 4.03 (t, 1H, J = 6.1 Hz, H-3′), 3.95 (dd, 1H, J = 6.1, 1.9 Hz, H-4′), 3.84-3.74 (m, 2H, CH_2-11″, H-5′), 3.70-3.66 (m, 1H, H-5), 3.66 (s, 3H, OMe), 3.56 (dd, 1H, J = 7.8, 6.1 Hz, H-2′), 3.53-3.45 (m, 1H, CH_2-11″), 2.29 (t, 2H, J = 7.6 Hz, CH_2-2″), 2.06, 2.05, 2.01, 2.00 (s, 12H, 4 x CH_3 Ac), 1.63-1.55 (m, 4H, CH_2-3″, CH_2-10″), 1.49, 1.31 (s, 6H, 2 x CH_3 isopropylidene), 1.37 (d, 3H, J = 6.6 Hz, CH_3-6′), 1.27 (br s, 12H, 6 x CH_2, CH_2-4″ - CH_2-9″). ^{13}C NMR ($CDCl_3$): δ 174.3 (C-1″), 170.6, 170.3, 169.5, 169.4 (4 x C=O Ac), 109.7 (C_{quart} isopropylidene), 101.4 (C-1′), 100.9 (C-1), 81.6 (C-2′), 78.8 (C-3′), 76.3 (C-4′), 72.9 (C-3), 71.9 (C-2), 71.8 (C-5), 69.8 (C-11″), 68.5 (C-4), 68.4 (C-5′), 62.1 (C-6), 51.4 (OMe), 34.1 (C-2″), 29.8, 29.5, 29.4, 29.4, 29.2, 29.1, 25.9, 25.0 (C-3″ – C-10″), 28.1, 26.3 (2 x CH_3 isopropylidene), 20.8, 20.7, 20.6, 20.6 (4 x CH_3 Ac), 16.6 (C-6′). ES-MS ($C_{35}H_{56}O_{16}$) 732.81, *m/z* (%) 755.3 (40) $[M+Na]^+$. HR-MS calcd for $C_{35}H_{56}O_{16}$ $[M+K]^+$ 771.3200, found 771.3171.

Methyl [11-[(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-(1→2)-*β*-D-fucopyranosyl]oxy]undecanoate (212)



211 (45.1 mg, 0.06 mmol) was dissolved in 1 mL TFA/H_2O (4:1) and stirred for 15 min at room temperature. The solvents were removed and the crude compound purified by flash chromatography (hexanes/EtOAc 2:1 to hexanes/EtOAc 1:2) to yield 26.5 mg (62%) of **212** as a colourless oil. R_f 0.21 (hexanes/EtOAc 1:3). ^1H NMR ($CDCl_3$): δ 5.22 (t, 1H, J = 9.7 Hz, H-3), 5.10 (t, 1H, J = 9.7 Hz, H-4), 5.00 (dd, 1H, J = 9.7 Hz, 8.0, H-2), 4.90 (d, 1H, J = 8.0 Hz, H-1), 4.32 (d, 1H, J = 7.4 Hz, H-1′), 4.29 (dd, 1H, J = 12.3, 4.2 Hz, CH_2-6), 4.10 (dd, 1H, J = 12.3, 2.1 Hz, CH_2-6), 3.87-3.83 (m, 1H, CH_2-11′′), 3.71-3.68 (m, 2H, H-3′, H-5), 3.66 (s, 3H, OMe), 3.63-3.54 (m, 3H, H-2′, H-4′, H-5′), 3.52-3.48 (m, 1H, CH_2-11′′), 2.30 (t, 2H, J = 7.6 Hz, CH_2-2′′), 2.07, 2.02, 2.01 (s, 12H, 4 x CH_3 Ac), 1.63-1.55 (m, 4H, CH_2-3′′, CH_2-10′′), 1.32 (d, 3H, J = 6.5 Hz, CH_3-6′), 1.28 (br s, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′). ^{13}C NMR ($CDCl_3$): δ 174.3 (C-1′′), 170.8, 170.6, 170.2, 169.4 (4 x C=O Ac), 102.0 (C-1′), 101.2 (C-1), 80.9 (C-2′), 72.8 (C-4′), 72.5 (C-3), 72.4 (C-2), 72.0 (C-5), 71.3 (C-3′), 70.1 (C-5′), 70.0 (C-11′′), 68.1 (C-4), 61.9 (C-6), 51.5 (OMe), 34.1 (C-2′′), 30.0 (C-10′′), 29.5, 29.5, 29.4, 29.3, 29.1, (C-4′′ – C-8′′), 26.0 (C-9′′), 25.0 (C-3′′), 20.9, 20.7, 20.6, 20.6 (4 x CH_3 Ac), 16.2 (C-6′). ES-MS ($C_{32}H_{52}O_{16}$) 692.75, *m/z* (%) 715.4 (100) $[M+Na]^+$. HR-MS calcd for $C_{32}H_{52}O_{16}$ $[M+H]^+$ 693.3329, found 693.3323.

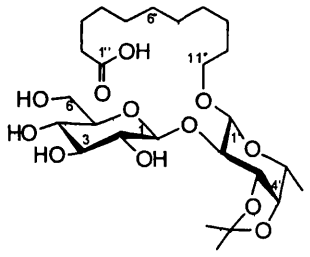
Methyl [11-[*β*-D-glucopyranosyl-(1→2)-3,4-*O*-isopropylidene-*β*-D-fucopyranosyl]oxy]undecanoate (217)



211 (361 mg, 0.49 mmol) was dissolved in MeOH (5 mL) and NaOMe (0.5 mL of 0.5 M soln in MeOH, 0.25 mmol, 0.5 equiv) was added. The reaction was stirred at room temperature for 15 min. Amberlite IR 120 (H^+ form) ion exchange resin was added for neutralisation and the mixture was allowed to stir for a few minutes. The resin was then filtered off, washed with MeOH and the filtrate evaporated to give 256 mg (93%) of pure **217** as a white solid. R_f 0.29 ($CHCl_3$/MeOH 9:1). ^1H NMR ($CDCl_3$): δ 4.59 (d, 1H, J = 7.7 Hz, H-1), 4.21 (d, 1H, J = 8.1 Hz, H-1′), 4.13 (dd, 1H, J = 6.8, 5.5

Hz, H-3′), 3.96 (d, 1H, J = 6.8 Hz, H-4′), 3.83-3.76 (m, 3H, H-5′, CH_2-6, CH_2-11′′), 3.70-3.61 (m, 2H, H-2′, CH_2-6), 3.61 (s, 3H, OMe), 3.48-3.37 (m, 3H, H-3, H-4, CH_2-11′′), 3.33-3.25 (m, 2H, H-2, H-5), 2.25 (t, 2H, J = 7.4 Hz, CH_2-2′′), 1.55 (br s, 4H, CH_2-3′′, CH_2-10′′), 1.47, 1.30 (s, 6H, 2 x CH_3 isopropylidene), 1.34 (d, 3H, J = 6.4 Hz, CH_3-6′), 1.22 (br s, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′). ^{13}C NMR ($CDCl_3$): δ 175.1 (C-1′′), 110.1 (C_{quart} isopropylidene), 102.1 (C-1), 101.1 (C-1′), 78.9 (C-3′), 78.8 (C-2′), 76.4 (C-4′), 76.0 (C-3), 75.9 (C-2), 73.6 (C-5), 70.0 (C-4), 69.9 (C-11′′), 68.6 (C-5′), 61.8 (C-6), 51.4 (OMe), 34.1 (C-2′′), 29.4, 29.4, 29.3, 29.1, 29.0 (C-4′′ – C-8′′, C-10′′), 27.7, 26.1 (2 x CH_3 isopropylidene), 25.7 (C-9′′), 24.9 (C-3′′), 16.3 (C-6′). ES-MS ($C_{27}H_{48}O_{12}$) 564.66, *m/z* (%) 587.3 (25) $[M+Na]^+$. HR-MS calcd for $C_{27}H_{48}O_{12}$ $[M+Na]^+$ 587.3038, found 587.3029.

[11-(β-D-Glucopyranosyl-(1→2)-3,4-*O*-isopropylidene-β-D-fucopyranosyl) oxy]undecanoate (218)



256 mg (0.45 mmol) of **217** were dissolved in 10 ml THF/MeOH (3:2), 2.3 mL (4.5 mmol, 10 equiv) of 2 M LiOH solution were added and the mixture was stirred at room temperature for 18 h. The solvents were removed and the residue dissolved in Et_2O (10 mL) and extracted with H_2O (10 mL). The aqueous layer was acidified with conc. HCl to pH 4 and extracted with EtOAc (2 x 10 mL). The extracts were dried over $MgSO_4$ and filtered. On addition of hexanes, the compound was observed to precipitate from this solution to yield 162 mg (79%) of pure **218** as a white solid. R_f 0.1 ($CHCl_3$/MeOH 8:1). ^{1}H NMR (MeOD): δ 4.78 (d, 1H, J = 7.8 Hz, H-1), 4.39 (d, 1H, J = 7.8 Hz, H-1′), 4.31 (dd, 1H, J = 6.5, 5.7 Hz, H-3′), 4.11 (dd, 1H, J = 5.7, 1.9 Hz, H-4′), 3.96 (qd, 1H, J = 6.5, 1.0 Hz, H-5′), 3.90-3.82 (m, 3H, CH_2-11′′, H-2′, CH_2-6), 3.71 (dd, 1H, J = 12.0, 5.6 Hz, CH_2-6), 3.61-3.56 (m, 1H, CH_2-11′′), 3.42-3.32 (m, 2H, H-3, H-4), 3.27-3.21 (m, 2H, H-2, H-5), 2.32 (t, 2H, J = 7.4 Hz, CH_2-2′′), 1.67-1.60 (m, 4H, CH_2-3′′, CH_2-10′′), 1.54, 1.37 (s, 6H, 2 x CH_3 isopropylidene), 1.36 (d, 3H, J = 6.6 Hz, CH_3-6′), 1.29 (br s, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′). ^{13}C NMR (MeOD): δ 113.4 (C_{quart} isopropylidene), 105.0 (C-1′), 104.7 (C-1), 83.3 (C-3′), 81.0 (C-2′), 80.7 (C-3), 80.6 (C-5), 80.4 (C-4′), 77.9 (C-2), 74.3 (C-4), 73.4 (C-11′′), 72.4 (C-5′), 65.4 (C-6), 37.6 (C-2′′), 33.2, 33.2,

33.1, 33.0, 32.9, 32.8, 29.6, 28.7, (C-3′′ – C-10′′), 30.8, 29.1 (2 x CH_3 isopropylidene), 19.4 (C-6′). ES-MS ($C_{26}H_{46}O_{12}$) 550.64, *m/z* (%) 573.3 (100) $[M+Na]^+$. HR-MS calcd for $C_{26}H_{46}O_{12}$ $[M+Na]^+$ 573.2881, found 573.2881.

Macrolactonisation by the carbodiimide method

Method A:

Acid **218** (20.6 mg, 0.04 mmol) was dissolved in THF (10 mL) and DMF (0.5 mL), cooled to 0 °C and DIC (7.5 μL, 0.05 mmol, 1.3 equiv) was added. The reaction mixture was allowed to stir for 1 h at 0 °C prior to the addition of DMAP (2.3 mg, 0.02 mmol, 0.05 equiv) and Et_3N (5μL, 0.04 mmol, 1 equiv). After stirring at room temperature for another 18 h the solvents were evaporated and the crude compound purified by flash chromatography (100% $CHCl_3$ to $CHCl_3$/MeOH 4:1). The compounds thus obtained were identified as recovered starting material (10 mg) and the *N*-acylurea **219** (10 mg).

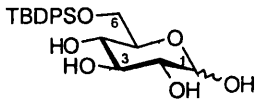
Method B:

Acid **218** (17.9 mg, 0.03 mmol) was dissolved in THF (10 mL) and DMF (0.5 mL), cooled to 0 °C and DIC (6.5 μL, 0.04 mmol, 1.3 equiv), DMAP (4.0 mg, 0.03 mmol, 1 equiv) and Et_3N (4.5 μL, 0.03 mmol, 1 equiv) were added simultaneously. The reaction mixture was allowed to stir at room temperature overnight. TLC analysis of the product indicated again the presence of starting material and the *N*-acylurea **219**.

Macrolactonisation by the mixed anhydride method

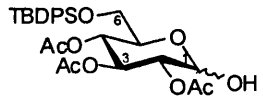
Acid **218** (44.5 mg, 0.08 mmol) was dissolved in THF (4 mL) and DMF (1 mL) and treated with Et_3N (0.05 mL, 0.36 mmol, 4.4 equiv). The solution was cooled to 0 °C and 2,4,6-trichlorobenzoyl chloride (0.03 mL, 0.16 mmol, 2 equiv) was added dropwise. The reaction mixture was allowed to stir for 2 h at 0 °C before a solution of DMAP (9.9 mg, 0.08 mmol, 1 equiv) in 7 mL toluene was introduced. The reaction was allowed to warm to room temperature and stirring was continued overnight. TLC analysis indicated the presence of several compounds but no product could be isolated after aqueous work-up (30 mL of 5% HCl and 2 x 30 mL EtOAc) and purification by flash chromatography (100% $CHCl_3$ to $CHCl_3$/MeOH 4:1).

6-*O-tert*-Butyldiphenylsilyl-*α/β*-D-glucopyranoside (220)



3.31 g (12.7 mmol) of D-glucose were dissolved in 30 mL DMF, treated with 5.3 mL (0.02 mmol, 1.1 equiv) TBDPSCl and 3.13 g (3.1 mmol, 0.05 mol, 2.5 equiv) imidazole and stirred at room temperature for 1 h. The reaction mixture was then quenched with MeOH, concentrated and purified by flash chromatography (100% $CHCl_3$ to $CHCl_3$/MeOH 4:1) to yield 5.35 g (71%, α:β = 1:1) of white foam. R_f 0.22 ($CHCl_3$/MeOH 9:1). ^{1}H NMR (MeOD): 7.78-7.75 (m, 8H, Ar α,β TBDPS), 7.45-7.40 (m, 12H, Ar α,β TBDPS), 5.18 (d, 1H, J = 3.4 Hz, H-1α), 4.53 (d, 1H, J = 7.9 Hz, H-1β), 4.03-3.87 (m, 5H, H-5α, CH_2-6α, CH_2-6β), 3.73 (t, 1H, J = 9.6 Hz, H-3α), 3.55 (t, 1H, J = 9.6 Hz, H-4α), 3.47-3.36 (m, 4H, H-2α, H-3β, H-4β, H-5β), 3.19 (t, 1H, J = 7.9 Hz, H-2β), 1.07 (s, 18H, *t*Bu α,β, TBDPS). ^{13}C NMR (MeOD): δ 135.4, 135.3, 134.9, 133.3, 129.3, 129.2, 127.2, 127.2, 127.2 (Ar α,β, TBDPS), 96.8 (C-1β), 92.6 (C-1α), 76.9 (C-2α), 76.8 (C-3β), 74.9 (C-2β), 73.6 (C-3α), 72.5 (C-4α), 71.8 (C-5α), 70.2 (C-5β), 70.1 (C-4β), 63.5 (C-6α), 63.3 (C-6β), 25.9 (*t*Bu α,β, TBDPS), 18.7 (C_{quart} α,β, TBDPS). ES-MS ($C_{22}H_{30}O_6Si$) 418.56, *m/z* (%) 417.3 (78) [M-H]$^-$. HR-MS calcd for $C_{22}H_{30}O_6Si$ [M+Na]$^+$ 441.1704, found 441.1697.

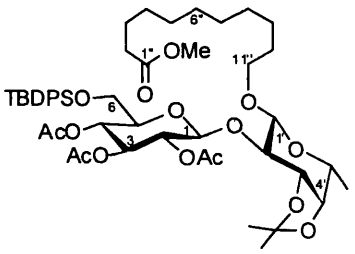
2,3,4,-Tri-*O*-acetyl-6-*O-tert*-butyldiphenylsilyl-*α/β*-D-glucopyranoside (222)



Acetic anhydride (20 mL) was added to a solution of **220** (5.06 g, 12.1 mmol) in pyridine (30 mL) and the mixture was allowed to stir at room temperature for 1 h. After completion of the reaction the mixture was co-evaporated with toluene to dryness to yield a yellow oil (**221**) (R_f 0.67, $CHCl_3$/MeOH 9:1), which was used in the following step without further purification.The crude **221** and $BnNH_2$ (2.8 mL, 25.5 mmol, 1.5 equiv) in 50 mL THF were stirred at room temperature overnight. The reaction mixture was then diluted with 100 mL CH_2Cl_2 and washed with ice-cold 1 M HCl (2 x 70 mL), the aqueous phase extracted with CH_2Cl_2 (70 mL) and the combined organic layers washed with brine (70 mL). The extracts were dried over $MgSO_4$, concentrated and purified by flash chromatography (hexanes/EtOAc 9:1 to hexanes/EtOAc 1:1) to yield 4.55 g (69% over 2 steps, α:β = 72:28) of a yellow viscous oil. R_f 0.20 (hexanes/EtOAc 3:1). ^{1}H NMR ($CDCl_3$): δ 7.70-7.64 (m, 8H, Ar α,β TBDPS), 7.45-7.35 (m, 12H, Ar α,β TBDPS), 5.50 (t, 1H, J = 10.0 Hz, H-3α), 5.42 (t, 1H, J

= 3.3 Hz, H-1α), 5.25-5.12 (m, 3H, H-3β, H-4α, H-4β), 4.87 (dd, 1H, J = 10.0, 3.3 Hz, H-2α), 4.84 (dd, 1H, J = 9.4, 8.4 Hz, H-2β), 4.63 (t, 1H, J = 8.4 Hz, H-1β), 4.11-4.07 (m, 1H, H-5α), 3.79-3.69 (m, 4H, CH_2-6α, CH_2-6β), 3.60-3.56 (m, 1H, H-5β), 3.10 (d, 1H, J = 8.4 Hz, OHβ), 2.68 (d, 1H, J = 3.3 Hz, OHα), 2.08, 2.00, 1.91, 1.90 (s, 18H, 3 x CH_3 α,β, Ac), 1.06 (s, 9H, *t*Buβ, TBDPS), 1.05 (s, 9H, *t*Buα, TBDPS). ^{13}C NMR ($CDCl_3$): δ 169.7, 169.5, 169.3, 169.1, 168.4, 168.3 (3 x C=O α,β, Ac), 135.8, 135.7, 135.6, 129.7, 129.7, 129.6 (Ar α,β, TBDPS), 95.4 (C-1β), 90.1 (C-1α), 74.8 (C-5β), 73.5 (C-2β), 72.7 (C-3β), 71.2 (C-2α), 70.3 (C-3α), 69.9 (C-5α), 68.7 (C-4α, C-4β), 62.7 (C-6β), 62.6 (C-6α),), 26.8 (*t*Bu α,β, TBDPS), 20.7, 20.6, 20.6, 20.5 (3 x CH_3 α,β, Ac), 18.6 (C_{quart} α,β, TBDPS). ES-MS ($C_{28}H_{36}O_9Si$) 544.67, *m/z* (%) 567.2 (72) $[M+Na]^+$. HR-MS calcd for $C_{28}H_{36}O_9Si$ $[M+Na]^+$ 567.2010, found 567.2039.

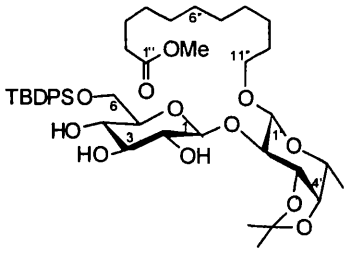
Methyl [11-[(2,3,4-tri-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-(1→2)-3,4-*O*-isopropylidene-β-D-fucopyranosyl]oxy]undecanoate (224)



A solution of **222** (3.81 g, 7.0 mmol), trichloroacetonitrile (2.1 mL, 21.0 mmol, 3 equiv) and Cs_2CO_3 (1.48 g, 4.2 mmol, 0.6 equiv) in 50 mL CH_2Cl_2 were stirred at room temperature overnight. The reaction mixture was filtered through a short pad of silica which was rinsed with hexanes/EtOAc 3:1. The filtrate was concentrated to give 3.5 g of crude product (R_f 0.40 hexanes/EtOAc 1:1) which was used without further purification. The crude trichloroacetimidate (1.0 g) and alcohol **195** (0.45 g, 1.12 mmol) were dissolved in 15 mL CH_2Cl_2 and cooled to -20 °C. TMSOTf (20 μL, 0.11 mmol, 0.1 equiv) was added to the solution and stirred at this temperature for 1 h. The reaction was quenched with Et_3N, concentrated and purified by flash chromatography (hexanes/EtOAc 2:1) to yield 0.85 g (83%) of disaccharide **224** as a white oily solid. R_f 0.52 (hexanes/EtOAc 1:2). ^{1}H NMR ($CDCl_3$): δ 7.71-7.61 (m, 4H, Ar TBDPS), 7.43-7.34 (m, 6H, Ar TBDPS), 5.33 (t, 1H, J = 9.6 Hz, H-3), 5.20 (t, 1H, J = 9.6 Hz, H-4), 4.99 (dd, 1H, J = 9.6, 8.0 Hz, H-2), 4.85 (d, 1H, J = 8.0 Hz, H-1), 4.22 (d, 1H, J = 7.8 Hz, H-1′), 4.06 (t, 1H, J = 6.1 Hz, H-3′), 3.96 (dd, 1H, J = 6.1, 2.0 Hz, H-4′), 3.79-3.68 (m, 4H, CH_2-6, CH_2-11′′, H-5′), 3.66 (s, 3H, OMe), 3.64 (dd, 1H, J = 7.8, 6.1 Hz, H-2′), 3.52-3.48 (m, 1H, H-5), 3.40-3.34 (m, 1H, CH_2-11′′), 2.29 (t, 2H, J = 7.6 Hz, CH_2-

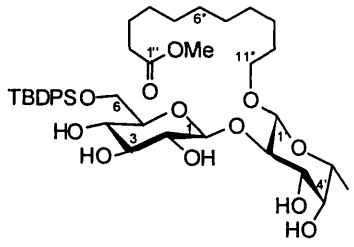
2′′), 2.06, 2.01, 1.91 (s, 9H, 3 x CH_3 Ac), 1.64-1.55 (m, 4H, CH_2-3′′, CH_2-10′′), 1.39 (d, 3H, J = 6.7 Hz, CH_3-6′), 1.39, 1.31 (s, 6H, 2 x CH_3 isopropylidene), 1.15 (br s, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′), 1.04 (s, 9H, *t*Bu TBDPS). ^{13}C NMR ($CDCl_3$): δ 174.6 (C-1′′), 170.6, 169.6, 169.2 (3 x C=O Ac), 135.8, 135.6, 129.6, 127.7 (Ar TBDPS), 109.7 (C_{quart} isopropylidene), 101.5 (C-1′), 100.3 (C-1), 80.4 (C-2′), 79.0 (C-3′), 76.3 (C-4′), 74.3 (C-5), 73.5 (C-3), 72.0 (C-2), 69.7 (C-11′′), 68.6 (C-5′), 68.4 (C-4), 62.3 (C-6), 51.4 (OMe), 34.1 (C-2′′), 29.7, 29.5, 29.4, 29.2, 29.1, 25.8, 25.0 (C-3′′ – C-10′′), 27.0, 26.3 (2 x CH_3 isopropylidene), 25.9 (*t*Bu TBDPS), 21.0 (C_{quart} TBDPS), 20.8, 20.7, 20.6 (3 x CH_3 Ac), 16.7 (C-6′). ES-MS ($C_{49}H_{72}O_{15}Si$) 929.17, *m/z* (%) 951.4 (32) $[M+Na]^+$. HR-MS calcd for $C_{49}H_{72}O_{15}Si$ $[M+Na]^+$ 951.4532, found 951.4573.

Methyl [11-[(6-*O*-*tert*-butyldiphenylsilyl-*β*-D-glucopyranosyl)-(1→2)-3,4-*O*-isopropylidene-*β*-D-fucopyranosyl]oxy]undecanoate (225)



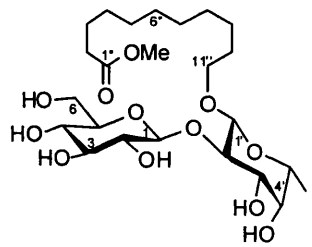
224 (0.49 g, 0.52 mmol) was dissolved in MeOH (5 mL) and NaOMe (1.0 mL of 0.5 M soln in MeOH, 0.52 mmol, 1 equiv) was added. The reaction was stirred at room temperature for 30 min. Amberlite IR 120 (H^+ form) ion exchange resin was added for neutralisation and the mixture was allowed to stir for a few minutes. The resin was then filtered off, washed with MeOH and the filtrate evaporated to give 0.39 g (97%) of pure **225**. R_f 0.21 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 7.70-7.65 (m, 4H, Ar TBDPS), 7.43-7.36 (m, 6H, Ar TBDPS), 4.56 (d, 1H, J = 7.9 Hz, H-1), 4.22 (d, 1H, J = 8.4 Hz, H-1′), 4.14 (dd, 1H, J = 7.3, 5.5 Hz, H-3′), 3.99-3.94 (m, 2H, H-4′, CH_2-6), 3.86-3.72 (m, 4H, H-3, CH_2-6, H-5′, CH_2-11′′), 3.67 (s, 3H, OMe), 3.65-3.61 (m, 2H, H-2′, H-4), 3.48-3.36 (m, 3H, H-2, H-5, CH_2-11′′), 2.30 (t, 2H, J = 7.5 Hz, CH_2-2′′), 1.64-1.56 (m, 4H, CH_2-3′′, CH_2-10′′), 1.44, 1.32 (s, 6H, 2 x CH_3 isopropylidene), 1.38 (d, 3H, J = 6.7 Hz, CH_3-6′), 1.27-1.16 (m, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′), 1.06 (s, 9H, *t*Bu TBDPS). ES-MS ($C_{43}H_{66}O_{12}Si$) 803.06, *m/z* (%) 825.4 (51) $[M+Na]^+$. HR-MS calcd for $C_{43}H_{66}O_{12}Si$ $[M+Na]^+$ 825.4216, found 825.4216.

Methyl [11-[(6-*O*-*tert*-butyldiphenylsilyl-*β*-D-glucopyranosyl)-(1→2)-*β*-D-fucopyranosyl]oxy]undecanoate (226)



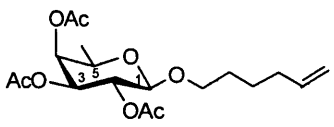
Colourless oil. (R_f 0.40, $CHCl_3$/MeOH 4:1). ^1H NMR ($CDCl_3$): δ 7.69-7.66 (m, 4H, Ar TBDPS), 7.44-7.36 (m, 6H, Ar TBDPS), 4.55 (d, 1H, J = 8.2 Hz, H-1), 4.30 (d, 1H, J = 7.8 Hz, H-1′), 3.93-3.85 (m, 3H, CH_2-6, CH_2-11′′), 3.79 (t, 1H, J = 7.8 Hz, H-2′), 3.74 (br s, 1H, H-4′), 3.68-3.53 (m, 5H, H-2, H-3, H-4, H-3′, H-5′), 3.66 (s, 3H, OMe), 3.51-3.44 (m, 2H, H-5, CH_2-11′′), 3.03 (br s, 1H, 4′-OH), 3.01 (d, 1H, J = 2.4 Hz, 3′-OH), 2.29 (t, 2H, J = 7.6 Hz, CH_2-2′′), 1.63-1.51 (m, 4H, CH_2-3′′, CH_2-10′′), 1.35 (d, 3H, J = 6.5 Hz, CH_3-6′), 1.27-1.20 (m, 12H, 6x CH_2, CH_2-4′′ - CH_2-9′′), 1.05 (s, 9H, *t*Bu TBDPS). ^{13}C NMR ($CDCl_3$): δ 174.6 (C-1′′), 135.6, 129.9, 129.9, 127.9, 127.8 (Ar TBDPS), 101.7 (C-1), 101.1 (C-1′), 76.1 (C-2′), 75.9 (C-5), 75.7 (C-2), 72.7 (C-3′), 70.9 (C-3), 70.8 (C-4), 70.6 (C-4′), 70.4 (C-5′), 70.0 (C-11′′), 63.4 (C-6), 51.5 (OMe), 34.1 (C-2′′), 29.4, 29.3, 29.3, 29.2, 29.1, 25.7, 24.9 (C-3′′ – C-10′′), 26.8 (*t*Bu TBDPS), 19.2 (C_{quart} TBDPS), 16.3 (C-6′). ES-MS ($C_{40}H_{62}O_{12}Si$) 763.00, *m/z* (%) 763.4 (15) $[M+Na]^+$. HR-MS calcd for $C_{40}H_{62}O_{12}Si$ $[M+Na]^+$ 763.4089, found 763.4097.

Methyl [11-(*β*-D-glucopyranosyl-(1→2)-*β*-D-fucopyranosyl)oxy]undecanoate (227)



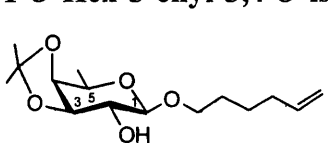
Colourless oil. (R_f 0.20, $CHCl_3$/MeOH 4:1). ^1H NMR (MeOD): δ 4.62 (d, 1H, J = 8.0 Hz, H-1), 4.36 (d, 1H, J = 7.4 Hz, H-1′), 3.90-3.85 (m, 2H, CH_2-6, CH_2-11′′), 3.75-3.65 (m, 5H, H-2′, H-3′, H-4′, H-5′, CH_2-6), 3.68 (s, 3H, OMe), 3.62-3.58 (m, 1H, CH_2-11′′), 3.43-3.37 (m, 2H, H-3, H-4), 3.32-3.25 (m, 2H, H-2, H-5), 2.35 (t, 2H, J = 7.4 Hz, CH_2-2′′), 1.67-1.61 (m, 4H, CH_2-3′′, CH_2-10′′), 1.43-1.35 (m, 12H, 6 x CH_2, CH_2-4′′ - CH_2-9′′), 1.30 (d, 3H, J = 6.4 Hz, CH_3-6′). ^{13}C NMR ($CDCl_3$): δ 174.6 (C-1′′), 103.5 (C-1), 101.7 (C-1′), 79.0 (C-2′), 76.7 (C-3), 76.2 (C-2), 74.3 (C-4′), 73.5 (C-3′), 71.3 (C-5′), 70.2 (C-5), 69.9 (C-4), 69.4 (C-11′′), 61.2 (C-6), 50.5 (OMe), 33.3 (C-2′′), 29.3, 29.2, 29.1, 28.9, 28.7, 25.6, 24.5 (C-3′′ – C-10′′), 15.2 (C-6′). ES-MS ($C_{24}H_{44}O_{12}$) 524.60, *m/z* (%) 547.3 (78) $[M+Na]^+$. HR-MS calcd for $C_{24}H_{44}O_{12}$ $[M+Na]^+$ 547.2726, found 547.2718.

1-*O*-Hex-5-enyl-2,3,4-tri-*O*-acetyl-*β*-D-fucopyranoside (228)



A solution of **191** (3.69 g, 12.7 mmol), trichloroacetonitrile (3.8 mL, 38.2 mmol, 3 equiv) and Cs_2CO_3 (2.69 g, 7.64 mmol, 0.6 equiv) in 40 mL CH_2Cl_2 were stirred at room temperature overnight. The reaction mixture was filtered through a short pad of silica which was rinsed with hexanes/EtOAc 1:1. The filtrate was concentrated to give 4.70 g of crude product which was used without further purification. The crude trichloroacetimidate **192** (4.70 g) and hex-5-en-1-ol (0.87 mL, 7.21 mmol) were dissolved in 65 mL CH_2Cl_2 and cooled to -20 °C. TMSOTf (130 μL, 0.72 mmol, 0.1 equiv) was added to the solution and stirred at this temperature for 1 h. The reaction was quenched with Et_3N, concentrated and purified by flash chromatography (hexanes/EtOAc 9:1 to hexanes/EtOAc 7:1) to yield 2.39 g (89%) of glycoside **228** as a white oily solid. R_f 0.32 (hexanes/EtOAc 3:1). ^{1}H NMR ($CDCl_3$): δ 5.82-5.72 (m, 1H, C<u>H</u>=CH_2), 5.21 (dd, 1H, J = 3.5, 1.0 Hz, H-4), 5.15 (dd, 1H, J = 10.6, 7.8 Hz, H-2), 5.00 (dd, 1H, J = 10.6, 3.5 Hz, H-3), 4.98 (dq, 1H, J = 17.1, 2.0, 1.6 Hz, CH=C<u>H$_2$</u> *trans*), 4.93 (dquin, 1H, J = 10.3, 2.0, 1.1 Hz, CH=C<u>H$_2$</u> *cis*), 4.41 (d, 1H, J = 7.8 Hz, H-1), 3.92-3.86 (m, 1H, CH_2-1′), 3.79 (qd, 1H, J = 6.5, 1.0 Hz, H-5), 3.47-3.42 (m, 1H, CH_2-1′), 2.15, 2.02, 1.96 (s, 9H, 3 x CH_3 Ac), 2.08-2.00 (m, 2H, CH_2-4′), 1.62-1.51 (m, 2H, CH_2-2′), 1.47-1.36 (m, 2H, CH_2-3′), 1.20 (d, 3H, J = 6.5 Hz, CH_3-6). ^{13}C NMR ($CDCl_3$): δ 170.7, 170.3, 169.5 (3 x C=O Ac), 138.5 (C-5′), 114.6 (C-6′), 101.1 (C-1), 71.4 (C-3), 70.4 (C-4), 69.8 (C-1′), 69.1 (C-2, C-5), 33.3 (C-4′), 28.8 (C-3′), 25.8 (C-2′), 20.8, 20.7, 20.6 (3 x CH_3 Ac), 16.1 (C-6). ES-MS ($C_{18}H_{28}O_8$) 372.41, *m/z* (%) 395.1 (100) $[M+Na]^+$. HR-MS calcd for $C_{18}H_{28}O_8$ $[M+Na]^+$ 395.1677, found 395.1673.

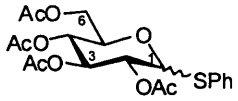
1-*O*-Hex-5-enyl-3,4-*O*-isopropylidene-*β*-D-fucopyranoside (230)



228 (2.39 g, 6.42 mmol) was dissolved in MeOH (40 mL) and NaOMe (6.4 mL of 0.5 M soln in MeOH, 3.21 mmol, 0.5 equiv) was added. The reaction was stirred at room temperature for 30 min. Amberlite IR 120 (H^+ form) ion exchange resin was added for neutralisation and the mixture was allowed to stir for a few minutes. The resin was then filtered off, washed with MeOH and the filtrate evaporated to give 1.58 g of crude **229** which was used without further

purification. The crude **229** was dissolved in 150 mL acetone. 90 mL 2,2-dimethoxypropane and 230 mg CSA were added and the mixture was stirred at room temperature overnight. The reaction was then quenched with NH_4OH and the solvents removed under reduced pressure. Purification by flash chromatography (hexanes/EtOAc 9:1 to hexanes/EtOAc 1:1) gave 1.45 g (79% over 2 steps) of compound **230** as a colourless oil. R_f 0.39 (hexanes/EtOAc 1:1). ^1H NMR ($CDCl_3$): δ 5.84-5.74 (m, 1H, C<u>H</u>=CH_2), 5.02-4.93 (m, 2H, CH=C<u>H</u>$_2$), 4.12 (d, 1H, J = 8.3 Hz, H-1), 4.05-3.98 (m, 2H, H-3, H-4), 3.93-3.83 (m, 2H, H-5, CH_2-1′), 3.56-3.45 (m, 2H, H-2, CH_2-1′), 2.39 (d, 1H, J = 2.0 Hz, OH), 2.09-2.02 (m, 2H, CH_2-4′), 1.67-1.60 (m, 2H, CH_2-2′), 1.52, 1.35 (s, 6H, 2 x CH_3 isopropylidene), 1.49-1.46 (m, 2H, CH_2-3′), 1.41 (d, 3H, J = 6.7 Hz, CH_3-6). ^{13}C NMR ($CDCl_3$): δ 138.5 (C-5′), 114.7 (C-6′), 109.8 (C_{quart} isopropylidene), 101.2 (C-1), 78.7 (C-3), 76.4 (C-4), 73.7 (C-2), 69.6 (C-1′), 69.2 (C-5), 33.4 (C-4′), 29.0 (C-3′), 28.2, 26.3 (2 x CH_3 isopropylidene), 25.3 (C-2′), 16.6 (C-6). ES-MS ($C_{15}H_{26}O_5$) 286.36, *m/z* (%) 309.4 (100) $[M+Na]^+$. HR-MS calcd for $C_{15}H_{26}O_5$ $[M+Na]^+$ 309.1673, found 309.1669.

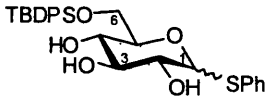
Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-*α*/*β*-D-glucopyranoside (232)



$BF_3.Et_2O$ (7.3 mL, 58.2 mmol, 6 equiv) and thiophenol (2.0 mL, 19.4 mmol, 2 equiv) were added to a solution of per-*O*-acetylated glucose **208** (3.78 g, 9.69 mmol) in CH_2Cl_2 and and were allowed to stir at room temperature for 18 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with sat. $NaHCO_3$ (2 x 100 mL). The organic layer was dried over $MgSO_4$, concentrated and purified by flash chromatography (hexanes/EtOAc 9:1 to hexanes/EtOAc 1:1) to yield 3.07 g (72%, α:β = 12:88) of white crystalline solid. R_f 0.40 (hexanes/EtOAc 1:1). ^1H NMR ($CDCl_3$): 7.50-7.47 (m, 2H, Arβ, SPh), 7.45-7.42 (m, 2H, Arα, SPh), 7.33-7.30 (m, 3H, Arβ, SPh), 7.30-7.28 (m, 3H, Arα, SPh), 5.91 (d, 1H, J = 5.8 Hz, H-1α), 5.44 (dd, 1H, J = 10.0, 9.6 Hz, H-3α), 5.22 (t, 1H, J = 9.5 Hz, H-3β), 5.12-5.05 (m, 2H, H-2α, H-4α), 5.04 (t, 1H, J = 9.5 Hz, H-4β), 4.97 (dd, 1H, J = 10.1, 9.5 Hz, H-2β), 4.70 (d, 1H, J = 10.1 Hz, H-1β), 4.59-4.54 (m, 1H, H-5α), 4.30-4.15 (m, 3H, CH_2-6α, CH_2-6β), 4.03 (dd, 1H, J = 12.3, 2.2 Hz, CH_2-6α), 3.74-3.70 (m, 1H, H-5β), 2.10, 2.05, 2.04, 2.02 (s, 12H, 4 x $CH_3\alpha$, Ac), 2.08, 2.07, 2.01, 1.98 (s, 12H, 4 x $CH_3\beta$, Ac). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.2, 169.4, 169.3 (4 x C=Oβ, Ac), 170.0, 169.9, 168.6, 169.6

(4 x C=Oα, Ac), 133.1, 132.5, 131.9, 131.6, 129.2, 128.9, 128.4, 127.8 (Ar α,β, SPh), 85.7 (C-1β), 85.0 (C-1α), 75.8 (C-5β), 74.0 (C-3β), 70.7 (C-2α), 70.4 (C-3α), 69.9 (C-2β), 68.6 (C-4α), 68.2 (C-4β), 68.2 (C-5α), 62.1 (C-6β), 61.9 (C-6α), 20.8, 20.7, 20.6, 20.6 (4 x CH_3, α,β, Ac). ES-MS ($C_{20}H_{24}O_9S$) 440.46, *m/z* (%) 463.0 (14) $[M+Na]^+$. HR-MS calcd for $C_{20}H_{24}O_9S$ $[M+Na]^+$ 463.1034, found 463.1004.

Phenyl 6-*O*-*tert*-butyldiphenylsilyl-1-thio-α/β-D-glucopyranoside (234)

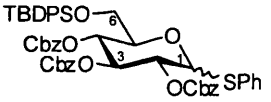


232 (3.07 g, 6.96 mmol) was dissolved in MeOH (35 mL) and NaOMe (7.0 mL of 0.5 M soln in MeOH, 3.48 mmol, 0.5 equiv) was added. The reaction was stirred at room temperature for 30 min. Amberlite IR 120 (H^+ form) ion exchange resin was added for neutralisation and the mixture was allowed to stir for a few minutes. The resin was then filtered off, washed with MeOH and the filtrate evaporated to give 1.96 g of crude **233** which was used without further purification. The crude **233** was dissolved in 10 mL DMF, treated with 2.0 mL (7.66 mmol, 1.1 equiv) TBDPSCl and 1.18 g (17.4 mmol, 2.5 equiv.) imidazole and stirred at room temperature for 1 h. The reaction mixture was then quenched with MeOH, concentrated and purified by flash chromatography (100% $CHCl_3$ to $CHCl_3$/MeOH 4:1) to yield 2.60 g (73%, $\alpha:\beta$ = 12:88) of white foam. R_f 0.54 ($CHCl_3$/MeOH 9:1). ^1H NMR ($CDCl_3$): 7.73-7.67, 7.55-7.52, 7.45-7.37, 7.29-7.22 (m, 30H, Ar α,β, TBDPS, SPh), 5.54 (d, 1H, J = 5.1 Hz, H-1α), 4.52 (d, 1H, J = 9.7 Hz, H-1β), 4.22-4.17 (m, 1H, H-5α), 3.99-3.91 (m, 4H, CH_2-6α, CH_2-6β), 3.84 (br s, 1H, H-2α), 3.66-3.58 (m, 4H, H-3α, H-4α, H-3β, H-4β), 3.48-3.44 (m, 1H, H-5β), 3.36 (t, 1H, J = 9.15 Hz, H-2β), 3.27 (br s, 1H, 3-OHβ), 3.09 (br s, 1H, 4-OHβ), 2.78 (br s, 1H, 2-OHβ), 1.07 (s, 9H, *t*Buβ, TBDPS), 1.06 (s, 9H, *t*Buα, TBDPS). ^{13}C NMR ($CDCl_3$): δ 135.7, 135.6, 132.6, 132.0, 129.9, 129.0, 128.1, 127.8, 127.8 (Arβ, TBDPS, SPh), 90.4 (C-1α), 87.9 (C-1β), 79.0 (C-5β), 77.8 (C-3β), 71.6 (C-2β), 71.2 (C-4β), 64.4 (C-6β), 26.8 (*t*Buβ, TBDPS), 19.2 ($C_{quart}\beta$, TBDPS). ES-MS ($C_{28}H_{34}O_5SSi$) 510.72, *m/z* (%) 533.2 (62) $[M+Na]^+$. HR-MS calcd for $C_{28}H_{34}O_5SSi$ $[M+H]^+$ 511.1974, found 511.1992.

Phenyl 2,3,4-tri-*O*-benzyloxycarbonyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-*α*/*β*-D-glucopyranoside (235) & 2,3-di-*O*-benzyloxycarbonyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-*β*-D-glucopyranoside (237)

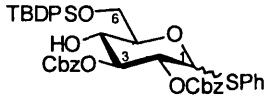
TMEDA (0.9 mL, 6.21 mmol, 1.8 equiv) and benzyl chloroformate (1.6 mL, 11.4 mmol, 3.3 equiv) were added at 0 °C to a solution of **234** (1.76 g, 3.45 mmol) in CH_2Cl_2. After 45 min the mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (2 x 30 mL), dried over $MgSO_4$, concentrated and purified by flash chromatography (hexanes/EtOAc 9:1 to hexanes/EtOAc 1:1) to yield 1.94 g (61%, α:β = 14:86) of **235** and 0.51 g (19%) of **237** as light yellow oils.

Analytical and spectroscopic data of **235**: R_f 0.31 (hexanes/EtOAc 2:1). ^{1}H NMR



($CDCl_3$): 7.72-7.60 (m, 8H, Ar α,β, TBDPS), 7.46-7.17 (m, 52H, Ar α,β, TBDPS, 3 x Cbz, SPh), 5.95 (d, 1H, J = 5.8 Hz, H-1α), 5.32 (dd, 1H, J = 10.0, 9.6 Hz, H-3α), 5.22-4.98 (m, 16H, 3 x Cbz α,β, H-2α, H-4α, H-3β, H-4β), 4.84 (dd, 1H, J = 9.9, 8.9 Hz, H-2β), 4.74 (d, 1H, J = 9.9 Hz, H-1β), 4.48-4.44 (m, 1H, H-5α), 3.85-3.73 (m, 4H, CH_2-6α, CH_2-6β), 3.65-3.61 (m, 1H, H-5β), 1.04 (s, 9H, *t*Buβ, TBDPS), 1.01 (s, 9H, *t*Buα, TBDPS). ^{13}C NMR ($CDCl_3$): δ 154.3, 153.8, 153.8 (3 x C=Oβ, Cbz), 135.7, 135.6, 135.1, 135.0, 134.8, 133.1, 132.9, 131.8, 129.7, 129.0, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.7, 127.6 (Arβ, Cbz, TBDPS, SPh), 85.8 (C-1β), 78.4 (C-3β), 78.2 (C-5β), 73.9 (C-2β), 72.1 (C-4β), 70.1, 70.1. 69.9 (3 x $CH_2\beta$, Cbz), 62.4 (C-6β), 26.7 (*t*Buβ, TBDPS), 19.2 ($C_{quart}\beta$, TBDPS). ES-MS ($C_{52}H_{52}O_{11}S$) 913.11, *m/z* (%) 935.3 (61) $[M+Na]^+$. HR-MS calcd for $C_{52}H_{52}O_{11}S$ $[M+Na]^+$ 935.2897, found 935.2927.

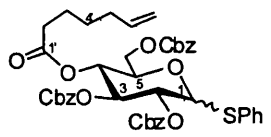
Analytical and spectroscopic data of **237**: R_f 0.29



(hexanes/EtOAc 2:1). ^{1}H NMR ($CDCl_3$): 7.71-7.68 (m, 4H, Ar TBDPS), 7.45-7.18 (m, 21H, Ar TBDPS, 2 x Cbz, SPh), 5.21-5.06 (m, 4H, 2 x Cbz), 5.00- 4.95 (m, 1H, H-3), 4.73-4.72 (m, 2H, H-1, H-2), 3.95 (d, 2H, J = 4.1 Hz, CH_2-6), 3.87 (td, 1H, J = 9.5, 3.9 Hz, H-4), 3.50-3.45 (m, 1H, H-5), 2.80 (dd, 1H, J = 3.9, 2.1 Hz, OH), 1.06 (s, 9H, *t*Bu TBDPS). ^{13}C NMR ($CDCl_3$): δ 155.2, 154.0 (2 x C=O, Cbz), 135.7, 135.6, 135.1, 134.9, 132.9, 132.7, 132.0, 129.9, 128.9, 128.6, 128.2, 127.8 (Ar, Cbz, TBDPS, SPh), 85.7 (C-1), 80.7 (C-3), 79.3 (C-5), 73.8 (C-2), 70.2, 70.1 (2 x CH_2, Cbz), 69.9 (C-4), 63.8 (C-6), 26.8 (*t*Bu TBDPS), 19.2 (C_{quart} TBDPS). ES-MS ($C_{44}H_{46}O_9S$) 778.98, *m/z* (%)

801.0 (55) $[M+Na]^+$. HR-MS calcd for $C_{44}H_{46}O_9S$ $[M+Na]^+$ 801.2529, found 801.2568.

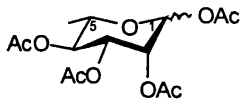
Phenyl 2,3,6,-tri-*O*-benzyloxycarbonyl-4-*O*-hept-6-enoyl-1-thio-*β*-D-glucopyranoside (238)



235 (1.68 g, 1.8 mmol) was dissolved in THF (12 mL). Acetic acid (160 μL, 2.8 mmol, 1.5 equiv) and TBAF (5.5 mL, 1 M soln in THF, 5.5 mmol, 3 equiv) were added and the reaction mixture was stirred at room temperature for 5 h. The solvents were evaporated and the crude compound was taken up in CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$ (100 mL) and brine (100 mL), dried over $MgSO_4$ and concentrated. The crude product was used in the next step without further purification. To a solution of hept-6-enoic acid (0.3 mL, 2.16 mmol, 1.2 equiv) in toluene (23 mL) were added Et_3N (1.1 mL, 7.74 mmol, 4.3 equiv) and 2,4,6-trichlorobenzoyl chloride (0.6 mL, 3.67 mmol, 2 equiv) and stirred for 2 h at room temperature. The crude alcohol (1.8 mmol) and DMAP (0.22 g, 1.8 mmol, 1 equiv) were dissolved in toluene (36 mL) and added to the reaction mixture. After 1 h the reaction was diluted with EtOAc (100 mL), washed with 5% HCl (100 mL), $NaHCO_3$ (100 mL) and sat. NaCl (100 mL), dried over $MgSO_4$, concentrated and purified by flash chromatography (100% hexanes to hexanes/EtOAc 7:1) to yield 0.87 g (61% over two steps) of **238** as a colourless oil. R_f 0.58 (hexanes/EtOAc 2:1). ^1H NMR ($CDCl_3$): 7.44-7.22 (m, 20H, Ar 3 x Cbz, SPh), 5.79-5.69 (m, 1H, C<u>H</u>=CH_2), 5.22-5.00 (m, 8H, 3 x Cbz, H-3, H-4), 4.96-4.92 (m, 2H, CH=C<u>H</u>$_2$), 4.79 (t, 1H, J = 10.0 Hz, H-2), 4.72 (d, 1H, J = 10.0 Hz, H-1), 4.32-4.18 (m, 2H, CH_2-6), 3.76-3.71 (m, 1H, H-5), 2.19-2.06 (m, 2H, CH_2-2′), 2.03-1.96 (m, 2H, CH_2-5′), 1.51-1.40 (m, 2H, CH_2-3′), 1.34-1.24 (m, 2H, CH_2-4′). ^{13}C NMR ($CDCl_3$): δ 172.0 (C-1′), 154.7, 154.3, 154.0 (3 x C=O Cbz), 138.2 (C-6′), 135.0, 134.9, 133.4, 131.4, 128.9, 128.6, 128.5, 128.3, 128.2, 128.0 (Ar Cbz, SPh), 114.8 (C-7′), 85.8 (C-1), 77.6 (C-3), 75.6 (C-5), 73.8 (C-2), 70.2, 70.0, 69.9 (3 x CH_2 Cbz), 68.4 (C-4), 66.1 (C-6), 33.6 (C-2′), 33.2 (C-5′), 28.1 (C-4′), 24.0 (C-3′). ES-MS ($C_{43}H_{44}O_{12}S$) 784.87 *m/z* (%) 807.3 (100) $[M+Na]^+$. HR-MS calcd for $C_{43}H_{44}O_{12}S$ $[M+Na]^+$ 807.2451, found 807.2485.

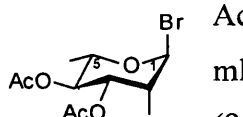
Synthesised Compounds – Part II

1,2,3,4-Tetra-*O*-acetyl-*α/β*-L-rhamnopyranoside 247



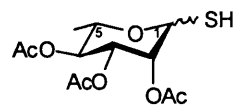
Acetic anhydride (20 ml, 0.21 mol) was added to a solution of L-rhamnose (5.03 g, 27.6 mmol) in pyridine (30 ml, 0.37 mol) and the mixture was allowed to stir at room temperature for 2 hours. After completion of the reaction the mixture was diluted with toluene and co-evaporated to yield **247** as a pale yellow viscous oil (9.42 g, 97%, $\alpha:\beta$ = 25:75). R_f 0.55 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 6.01 (d, 1H, J = 2.0 Hz, H-1β), 5.83 (d, 1H, J = 1.2 Hz, H-1α), 5.47 (dd, 1H, J = 2.8, 1.2 Hz, H-2α), 5.30 (dd, 1H, J = 10.0, 3.6 Hz, H-3β), 5.24 (dd, 1H, J = 3.6, 2.0 Hz, H-2β), 5.11 (t, 1H, J = 10.0 Hz, H-4β), 5.08-5.07 (m, 2H, H-3α, H-4α), 3.97-3.90 (m, 1H, H-5β), 3.70-3.63 (m, 1H, H-5α), 2.21, 2.09, 2.09 1.99 (s, 9H, 4 x $CH_3\alpha$, Ac), 2.16, 2.15, 2.06, 2.00 (s, 9H, 4 x $CH_3\beta$, Ac), 1.28 (d, 3H, J = 6.0 Hz, CH_3-6α), 1.23 (d, 3H, J = 6.2 Hz, CH_3-6β). ES-MS ($C_{14}H_{20}O_9$) 332.30, *m/z* (%) 354.9 (100) $[M+Na]^+$.

2,3,4-Tri-*O*-acetyl-*α*-L-rhamnopyranosyl bromide 248



Acetic anhydride (1 ml) was added to HBr in acetic acid (45%, 10 ml) and allowed to stir for 30 minutes at room temperature. **247** (9.42 g, 26.9 mmol) was then dissolved in CH_2Cl_2, added to the solution and stirred for 2 hours. After completion of the reaction the mixture was diluted with CH_2Cl_2 (ice-cold, 100 ml) and washed with H_2O (3 x 30 ml) and sat. $NaHCO_3$ (30 ml). The organic phase was dried over $MgSO_4$, filtered and evaporated. The product **248** was obtained as a yellow viscous oil (8.11 g, 81 %). R_f 0.66 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 6.25 (d, 1H, J = 1.2 Hz, H-1), 5.65 (dd, 1H, J = 10.2, 3.4 Hz, H-3), 5.43 (dd, 1H, J = 3.4, 1.2 Hz, H-2), 5.14 (t, 1H, J = 10.2 Hz, H-4), 4.13-4.05 (m, 1H, H-5), 2.15, 2.07, 1.99 (s, 9H, 3 x CH_3, Ac), 1.27 (d, 3H, J = 6.3 Hz, CH_3-6). ES-MS ($C_{12}H_{17}O_7Br$) 353.16, *m/z* (%) 351.9 (87) $[M-H]^-$.

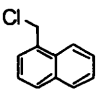
2,3,4-Tri-*O*-acetyl-1-thio-*α/β*-L-rhamnopyranose 245



Compound **248** (8.11 g, 21.8 mmol) and thiourea (6.65 g, 87.3 mmol) were dissolved in acetone (120 mL) and refluxed for 12 hours. The solvent was evaporated and the solid residue suspended in 1,2-

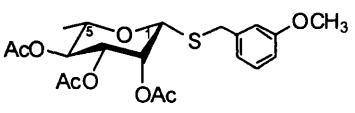
dichloroethane (64 mL). Sodium metabisulfite ($Na_2S_2O_5$) (4.36 g, 22.9 mmol) was dissolved in H_2O (40 mL), which was added to the organic phase and stirred vigorously under reflux for 15 minutes. The organic phase was diluted with CH_2Cl_2 (25 mL) and washed with brine (2 x 30 mL) and sat. $NaHCO_3$ (30 ml). The organic phase was dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography (hexanes/EtOAc 3:1 to hexanes/EtOAc 2:1) gave **245** as an off white viscous oil (3.88 g, 58%, α:β = 23:77). R_f 0.57 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 5.48 (dd, 1H, J = 7.2, 1.6 Hz, H-2β), 5.40 (dd, 1H, J = 3.0, 1.2 Hz, H-2α), 5.32-5.27 (m, 1H, H-3β), 5.31 (d, 1H, J = 1.6 Hz, H-1β), 5.10 (t, 1H, J = 9.6 Hz, H-4β), 4.25-4.18 (m, 1H, H-5β), 5.01-5.03 (m, 2H, H-1α, H-4α), 4.83 (dd, 1H, J = 10.0, 3.0 Hz, H-3α), 3.62-3.55 (m, 1H, H-5α), 2.20, 2.07, 1.99 (s, 9H, 3 x $CH_3\beta$, Ac), 2.05, 2.04, 1.97 (s, 9H, 3 x $CH_3\alpha$, Ac), 1.28-1.24 (m, 3H, CH_3-6α), 1.24 (d, 3H, J = 6.4 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 170.0 (3 x C=O, Ac), 72.3 (C-1), 72.0 (C-2), 71.9 (C-4), 69.9 (C-3), 60.4 (C-5), 20.7 (3 x CH_3, Ac), 17.3 (C-6). ES-MS ($C_{12}H_{18}O_7S$) 306.08, *m/z* (%) 305.3 (77) [M-H]$^-$.

1-Naphtalenemethyl chloride 249



To a cooled (0 °C) CH_2Cl_2 solution (20 mL) of 1-naphtalenemethanol (1.20 g, 7.6 mmol) and Et_3N (1.6 mL, 11.4 mmol) was added MsCl (0.9 mL, 11.4 mmol). The mixture was stirred for 30 minutes at 0 °C and subsequently at room temperature for 12 hours. The organic phase was diluted with CH_2Cl_2 (20 mL) and washed with sat. $NaHCO_3$ (30 ml), 10% HCl (30 mL) and brine (2 x 30 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography (hexanes/EtOAc 9:1) gave **249** as a yellow powder (895 mg, 67%). R_f 0.64 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 8.18 (d, 1H, J = 8.4 Hz, Ar), 7.93-7.87 (m, 2H, Ar), 7.65-7.61 (m, 1H, Ar), 7.58-7.54 (m, 2H, Ar), 7.45 (t, 1H, J = 7.8 Hz, Ar), 5.08 (s, 2H, CH_2Cl). ^{13}C NMR ($CDCl_3$): δ 135.1, 134.4, 132.2, 130.3, 129.4, 128.4, 127.2, 126.8, 126.0, 124.5 (Ar), 44.8 (CH_2Cl).

***S*-(3-Methoxybenzyl) 2,3,4-tri-*O*-acetyl-1-thio-α/β-L-rhamnopyranoside 250**

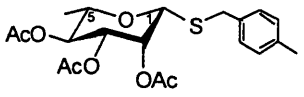


Thioglycoside **245** (245 mg, 0.8 mmol) was dissolved in DMF (2 mL), 3-methoxybenzyl bromide (241 mg, 1.2 mmol) and Et_2NH (50 μL) were added and the mixture was

stirred at room temperature for 1 hour. The solvent was evaporated and the organic phase was then dissolved in CH_2Cl_2, washed with 10% HCl (30 mL), dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography (hexanes/EtOAc 3:1) gave **250** as a colourless oil (44.8 mg, 13%, $\alpha:\beta$ = 27:73). R_f 0.64 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 7.23 (m, 2H, Ar α,β), 6.90-6.79 (m, 6H, Ar α,β), 5.38 (dd, 1H, J = 3.4, 0.8 Hz, H-2α), 5.31 (dd, 1H, J = 3.6, 1.6 Hz, H-2β), 5.23 (dd, 1H, J = 10.0, 3.6 Hz, H-3β), 5.07 (t, 1H, J = 10.0 Hz, H-4α), 5.07 (t, 1H, J = 10.0 Hz, H-4β), 5.05 (d, 1H, J = 1.6 Hz, H-1β), 4.91 (dd, 1H, J = 10.0, 3.4 Hz, H-3α), 4.44 (d, 1H, J = 0.8 Hz, H-1α), 4.24-4.17 (m, 1H, H-5β), 3.81 (s, 6H, OCH_3 α,β), 3.77, 3.71 (d, 4H, J = 13.8 Hz, SCH_2 α,β), 3.44-3.37 (m, 1H, H-5α), 2.18, 2.03, 1.95 (s, 9H, 3 x $CH_3\alpha$, Ac), 2.15, 2.04, 1.96 (s, 9H, 3 x $CH_3\beta$, Ac), 1.30 (d, 3H, J = 6.2 Hz, CH_3-6α), 1.20 (d, 3H, J = 6.0 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 170.0 (3 x C=O, Ac), 159.8 (C-OCH_3, Ar), 138.7, 129.6, 121.3, 114.5, 113.0 (Ar), 81.3 (C-1), 71.3 (C-2), 71.0 (C-4), 69.7 (C-3), 67.1 (C-5), 55.2 (OCH_3), 34.9 (SCH_2), 20.8 (3 x CH_3, Ac), 17.3 (C-6). ES-MS ($C_{20}H_{26}O_8S$) 426.48, *m/z* (%) 449.3 (100) $[M+Na]^+$.

Thioglycosides **251-258** were synthesised from the respective substituted benzyl halides, using the procedure described for **250**.

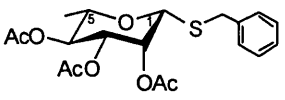
_S_-(4-Methylbenzyl) 2,3,4-tri-_O_-acetyl-1-thio-α/β-L-rhamnopyranoside 251



Yield 16% (white oil, $\alpha:\beta$ = 24:76). R_f 0.66 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 7.20-7.10 (m, 4H, Ar α,β), 5.36 (dd, 1H, J = 3.4, 1.0 Hz, H-2α), 5.30 (dd, 1H, J = 3.2, 1.4 Hz, H-2β), 5.23 (dd, 1H, J = 10.0, 3.2 Hz, H-3β), 5.08 (t, 1H, J = 10.0 Hz, H-4β), 5.07 (t, 1H, J = 10.0 Hz, H-4α), 5.02 (d, 1H, J = 1.4 Hz, H-1β), 4.90 (dd, 1H, J = 10.0, 3.4 Hz, H-3α), 4.39 (d, 1H, J = 1.0 Hz, H-1α), 4.25-4.18 (m, 1H, H-5β), 3.76, 3.70 (d, 4H, J = 13.4 Hz, SCH_2 α,β), 3.40-3.36 (m, 1H, H-5α), 2.34 (s, 6H, CH_3-Ph α,β), 2.16, 2.02, 1.94 (s, 9H, 3 x $CH_3\alpha$, Ac), 2.13, 2.04, 1.95 (s, 9H, 3 x $CH_3\beta$, Ac), 1.30 (d, 3H, J = 6.0 Hz, CH_3-6α), 1.20 (d, 3H, J = 6.4 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 169.9 (3 x C=O, Ac), 137.0, 134.0, 129.4, 129.3, 128.9, 128.9 (Ar), 81.2 (C-1), 71.3 (C-2), 71.0 (C-4), 69.7 (C-3), 67.0 (C-5), 34.6 (SCH_2), 20.9

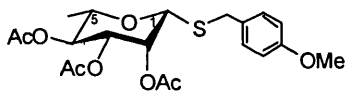
(CH$_3$-Ph), 20.8 (3 x CH$_3$, Ac), 17.4 (C-6). ES-MS ($C_{20}H_{26}O_7S$) 410.48, *m/z* (%) 433.3 (100) $[M+Na]^+$.

S-Benzyl 2,3,4-tri-O-acetyl-1-thio-α/β-L-rhamnopyranoside 252



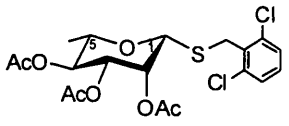
Yield 69% (pale yellow oil, α:β = 21:79). R_f 0.46 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 7.35-7.24 (m, 10H, Ar α,β), 5.39 (dd, 1H, *J* = 3.4, 1.0 Hz, H-2α), 5.30 (dd, 1H, *J* = 3.6, 1.2 Hz, H-2β), 5.23 (dd, 1H, *J* = 10.0, 3.6 Hz, H-3β), 5.08 (t, 1H, *J* = 10.0 Hz, H-4β), 5.07 (t, 1H, *J* = 10.0 Hz, H-4α), 5.07 (d, 1H, *J* = 1.2 Hz, H-1β), 4.91 (dd, 1H, *J* = 10.0, 3.4 Hz, H-3α), 4.42 (d, 1H, *J* = 1.0 Hz, H-1α), 4.23-4.18 (m, 1H, H-5β), 3.81, 3.75 (d, 4H, *J* = 13.6 Hz, SCH$_2$ α,β), 3.42-3.37 (m, 1H, H-5α), 2.17, 2.02, 1.97 (s, 9H, 3 x CH$_3$α, Ac), 2.11, 2.05, 1.96 (s, 9H, 3 x CH$_3$β, Ac), 1.29 (d, 3H, *J* = 6.2 Hz, CH$_3$-6α) 1.18 (d, 3H, *J* = 6.4 Hz, CH$_3$-6β). ^{13}C NMR ($CDCl_3$): δ 169.9 (3 x C=O, Ac), 137.2, 128.9, 128.6, 127.3 (Ar), 81.3 (C-1), 71.3 (C-2), 71.0 (C-4), 69.7 (C-3), 67.1 (C-5), 35.0 (SCH$_2$), 20.6 (3 x CH$_3$, Ac), 17.3 (C-6). ES-MS ($C_{19}H_{24}O_7S$) 396.45, *m/z* (%) 419.3 (100) $[M+Na]^+$.

S-(4-Methoxybenzyl) 2,3,4-tri-O-acetyl-1-thio-α/β-L-rhamnopyranoside 253



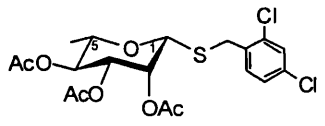
Yield 79% (pale yellow oil, α:β = 24:76). R_f 0.63 ($CHCl_3$/EtOAc 7:3). ^{1}H NMR ($CDCl_3$): δ 7.26- 7.18 (m, 4H, Ar α,β), 6.88-6.83 (m, 4H, Ar α,β), 5.37 (dd, 1H, *J* = 3.6, 0.8 Hz, H-2α), 5.30 (dd, 1H, *J* = 3.6, 1.2 Hz, H-2β), 5.23 (dd, 1H, *J* = 10.0, 3.6 Hz, H-3β), 5.09 (t, 1H, *J* = 10.0 Hz, H-4β), 5.07 (t, 1H, *J* = 10.0 Hz, H-4α), 5.01 (d, 1H, *J* = 1.2 Hz, H-1β), 4.91 (dd, 1H, *J* = 10.0, 3.6 Hz, H-3α), 4.39 (d, 1H, *J* = 0.8 Hz, H-1α), 4.23-4.19 (m, 1H, H-5β), 3.79 (s, 6H, OCH$_3$ α,β), 3.75, 3.70 (d, 4H, *J* = 13.6 Hz, SCH$_2$ α,β), 3.40-3.36 (m, 1H, H-5α), 2.17, 2.03, 1.95 (s, 9H, 3 x CH$_3$α, Ac), 2.11, 2.05, 1.96 (s, 9H, 3 x CH$_3$β, Ac), 1.29 (d, 3H, *J* = 6.0 Hz, CH$_3$-6α), 1.20 (d, 3H, *J* = 6.4 Hz, CH$_3$-6β). ^{13}C NMR ($CDCl_3$): δ 169.9 (3 x C=O, Ac), 158.9 (C-OCH$_3$, Ar), 130.2, 130.1, 129.0, 114.1, 114.1 (Ar), 81.2 (C-1), 71.3 (C-2), 71.1 (C-4), 69.8 (C-3), 67.1 (C-5), 55.3 (OCH$_3$), 34.3 (SCH$_2$), 20.8 (3 x CH$_3$, Ac), 17.4 (C-6). ES-MS ($C_{20}H_{26}O_8S$) 426.48, *m/z* (%) 449.2 (53) $[M+Na]^+$.

S-(2,6-Dichlorobenzyl) 2,3,4-tri-*O*-acetyl-1-thio-α/β-L-rhamnopyranoside 254



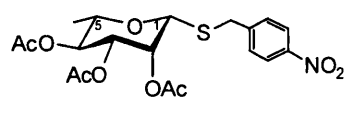
Yield 81% (white oily solid, $\alpha:\beta$ = 21:79). R_f 0.40 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 7.35-7.29 (m, 4H, Ar α,β), 7.19-7.11 (m, 2H, Ar α,β), 5.43 (dd, 1H, J = 3.4, 1.0 Hz, H-2α), 5.42 (d, 1H, J = 1.2 Hz, H-1β), 5.37 (dd, 1H, J = 3.2, 1.2 Hz, H-2β), 5.19 (dd, 1H, J = 10.0, 3.2 Hz, H-3β), 5.10 (t, 1H, J = 10.0 Hz, H-4β), 5.01 (t, 1H, J = 10.0 Hz, H-4α), 4.98 (dd, 1H, J = 10.0, 3.4 Hz, H-3α), 4.85 (d, 1H, J = 1.0 Hz, H-1α), 4.28-4.24 (m, 1H, H-5β), 4.21, 4.14 (d, 4H, J = 12.6 Hz, SCH_2 α,β), 3.59-3.47 (m, 1H, H-5α), 2.18, 2.05, 1.94 (s, 9H, 3 x $CH_3\alpha$, Ac), 2.17, 2.06, 1.97 (s, 9H, 3 x $CH_3\beta$, Ac), 1.25 (d, 3H, J = 6.0 Hz, CH_3-6α), 1.15 (d, 3H, J = 6.0 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 170.0 (3 x C=O, Ac), 135.6, 134.0 (C-Cl, Ar), 129.0, 128.6, 128.4, 128.4 (Ar), 82.6 (C-1), 71.2 (C-2), 71.0 (C-4), 69.5 (C-3), 67.1 (C-5), 30.8 (SCH_2), 20.8 (3 x CH_3, Ac), 17.2 (C-6). ES-MS ($C_{19}H_{22}Cl_2O_7S$) 465.34, *m/z* (%) 487.1 (34), 489.1 (26) $[M+Na]^+$.

S-(2,4-Dichlorobenzyl) 2,3,4-tri-*O*-acetyl-1-thio-α/β-L-rhamnopyranoside 255



Yield 64% (yellow oily solid, $\alpha:\beta$ = 20:80). R_f 0.50 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 7.40 (d, 2H, J = 2.0 Hz, Ar α,β), 7.28 (d, 2H, J = 8.4 Hz, Ar α,β), 7.20 (dd, 2H, J = 8.4, 2.0 Hz, Ar α,β), 5.40 (dd, 1H, J = 3.5, 1.0 Hz, H-2α), 5.30 (dd, 1H, J = 3.6, 1.2 Hz, H-2β) 5.20 (dd, 1H, J = 10.0, 3.6 Hz, H-3β), 5.09 (t, 1H, J = 10.0 Hz, H-4β), 5.09 (d, 1H, J = 1.2 Hz, H-1β), 5.06 (t, 1H, J = 10.0 Hz, H-4α), 4.94 (dd, 1H, J = 10.0, 3.5 Hz, H-3α), 4.54 (d, 1H, J = 1.0 Hz, H-1α), 4.22-4.15 (m, 1H, H-5β), 3.85 (s, 4H, SCH_2 α,β), 3.49-3.45 (m, 1H, H-5α), 2.17, 2.04, 1.95 (s, 9H, 3 x $CH_3\alpha$, Ac), 2.13, 2.05, 1.96 (s, 9H, 3 x $CH_3\beta$, Ac), 1.29 (d, 3H, J = 6.0 Hz, CH_3-6α), 1.19 (d, 3H, J = 6.0 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 169.7 (3 x C=O, Ac), 134.8 (C-Cl, Ar), 133.9 (C-Cl, Ar), 132.5, 129.8, 127.4, 127.2 (Ar), 81.5 (C-1), 71.2 (C-2), 70.9 (C-4), 69.6 (C-3), 67.2 (C-5), 32.0 (SCH_2), 20.6 (3 x CH_3, Ac), 17.3 (C-6). ES-MS ($C_{19}H_{22}Cl_2O_7S$) 465.34, *m/z* (%) 487.1 (100), 489.1 (74) $[M+Na]^+$.

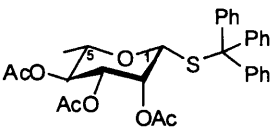
S-(4-Nitrobenzyl) 2,3,4-tri-*O*-acetyl-1-thio-α/β-L-rhamnopyranoside 256



Yield 93%,(pale yellow oily solid, $\alpha:\beta$ = 35:65). R_f

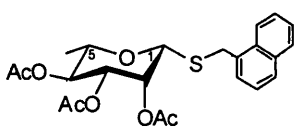
0.37 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 8.25-8.14 (m, 4H, Ar α,β), 7.58-7.46 (m, 4H, Ar α,β), 5.41 (dd, 1H, J = 3.6, 1.0 Hz, H-2α), 5.28 (dd, 1H, J = 3.4, 1.2 Hz, H-2β), 5.20 (dd, 1H, J = 10.0, 3.4 Hz, H-3β), 5.09 (t, 1H, J = 10.0 Hz, H-4β), 5.08 (t, 1H, J = 9.6 Hz, H-4α), 5.00 (d, 1H, J = 1.2 Hz, H-1β), 4.92 (dd, 1H, J = 9.6, 3.6 Hz, H-3α), 4.46 (d, 1H, J = 1.0 Hz, H-1α), 4.15-4.11 (m, 1H, H-5β), 3.87, 3.80 (d, 4H, J = 14.0 Hz, SCH_2 α,β), 3.43-3.39 (m, 1H, H-5α), 2.18, 2.04, 1.96 (s, 9H, 3 x $CH_3\alpha$, Ac), 2.12, 2.05, 1.97 (s, 9H, 3 x $CH_3\beta$, Ac), 1.28 (d, 3H, J = 6.0 Hz, CH_3-6α) 1.16 (d, 3H, J = 6.0 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 169.9 (3 x C=O, Ac), 147.2 (C-NO_2, Ar), 145.0, 129.8, 123.9 (Ar), 81.4 (C-1), 71.0 (C-2), 70.8 (C-4), 69.5 (C-3), 67.4 (C-5), 34.3 (SCH_2), 20.6 (3 x CH_3, Ac), 17.3 (C-6). ES-MS ($C_{19}H_{23}NO_9S$) 441.45, *m/z* (%) 464.2 (40) $[M+Na]^+$.

S-Triphenylmethyl-2,3,4-tri-O-acetyl-1-thio-α/β-L-rhamnopyranoside 257



Yield 59% (colourless oil, $\alpha:\beta$ = 33:67). R_f 0.39 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 7.35-6.98 (m, 30H, Ar α,β), 5.41 (dd, 1H, J = 3.4, 1.0 Hz, H-2α), 5.31 (d, 1H, J = 1.0 Hz, H-1α), 5.21 (dd, 1H, J = 3.4, 1.6 Hz, H-2β), 5.18 (dd, 1H, J = 10.0, 3.4 Hz, H-3α), 5.13 (dd, 1H, J = 10.0, 3.4 Hz, H-3β), 5.06 (t, 1H, J = 10.0 Hz, H-4α), 4.91 (t, 1H, J = 10.0 Hz, H-4β), 4.63 (d, 1H, J = 1.6 Hz, H-1β), 4.30-4.26 (m, 1H, H-5α), 4.11-4.04 (m, 1H, H-5β), 1.97, 1.90, 1.86 (s, 9H, 3 x $CH_3\alpha$, Ac), 1.96, 1.93, 1.90 (s, 9H, 3 x $CH_3\beta$, Ac), 1.16 (d, 3H, J = 6.0 Hz, CH_3-6α), 1.05 (d, 3H, J = 6.0 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 170.0 (3 x C=O, Ac), 144.2 ($SCPh_3$), 129.9, 129.4, 128.4, 128.0, 127.1, 126.5 (Ar), 82.6 (C-1), 72.6 (C-2), 71.2 (C-4), 69.5 (C-3), 68.9 (C-5), 20.7 (3 x CH_3, Ac), 17.3 (C-6). ES-MS ($C_{31}H_{32}O_7S$) 548.65, *m/z* (%) 571.3 (100) $[M+Na]^+$.

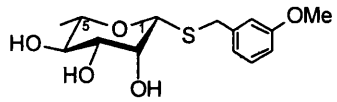
S-(1-Naphtalenemethyl)-2,3,4-tri-O-acetyl-1-thio-α/β-L-rhamnopyranoside 258



Yield 77% (colourless oil, $\alpha:\beta$ = 21:79). R_f 0.42 (hexanes/EtOAc). ^{1}H NMR ($CDCl_3$): δ 8.11-8.07 (m, 2H, Ar α,β), 7.91-7.79 (m, 4H, Ar α,β), 7.60-7.49 (m, 4H, Ar α,β), 7.44-7.37 (m, 4H, Ar α,β), 5.33 (dd, 1H, J = 3.6, 1.0 Hz, H-2α), 5.30 (dd, 1H, J = 3.2, 1.2 Hz, H-2β), 5.21 (dd, 1H, J = 10.0, 3.2 Hz, H-3β), 5.13 (d, 1H, J = 1.2 Hz, H-1β), 5.10 (t, 1H, J = 10.0 Hz, H-4β), 5.00 (t, 1H, J = 10.0 Hz, H-4α),

4.84 (dd, 1H, J = 10.0, 3.6 Hz, H-3α), 4.42 (d, 1H, J = 1.0 Hz, H-1α), 4.27-4.20 (m, 1H, H-5β), 4.15 (d, 4H, SCH_2 α,β), 3.38-3.34 (m, 1H, H-5α), 2.17, 2.03, 1.93 (s, 9H, 3 x $CH_3\alpha$, Ac), 2.12, 2.05, 1.95 (s, 9H, 3 x $CH_3\beta$, Ac), 1.33 (d, 3H, J = 6.2 Hz, CH_3-6α), 1.21 (d, 3H, J = 6.0 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 169.9 (3 x C=O, Ac), 134.1, 132.4, 131.2, 128.9, 128.6, 127.5, 126.4, 126.0, 125.2, 123.8 (Ar), 81.6 (C-1), 71.2 (C-2), 71.0 (C-4), 69.7 (C-3), 67.1 (C-5), 32.6 (SCH_2), 20.8 (3 x CH_3, Ac), 17.4 (C-6). ES-MS ($C_{23}H_{26}O_7S$) 446.51, *m/z* (%) 469.2 (100) $[M+Na]^+$.

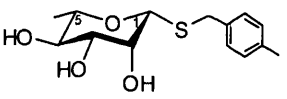
S-(3-Methoxybenzyl) 1-thio-α/β-L-rhamnopyranoside 259



Compound **250** (44.8 mg, 0.11 mmol) was dissolved in MeOH (2 mL) and NaOMe (0.5 M in MeOH, 0.11 mL, 0.05 mmol) was added. The reaction was stirred at room temperature for 1 hour. Amberlite IR 120 (H^+ form) ion exchange resin was added for neutralisation and the mixture was stirred for a few minutes. The resin was then filtered off, washed with MeOH and the filtrate evaporated. Purification by column chromatography ($CHCl_3$/EtOAc 7:3) gave **259** as a colourless oil (24.5 mg, 78%, $\alpha:\beta$ = 23:77). R_f 0.23 ($CHCl_3$/EtOAc 7:3). ^{1}H NMR ($CDCl_3$): δ 7.25-7.19 (m, 2H, Ar α,β), 6.90-6.77 (m, 6H, Ar α,β), 5.09 (d, 1H, J = 0.6 Hz, H-1β), 4.36 (d, 1H, J = 0.4 Hz, H-1α), 4.03-3.99 (m, 1H, H-5β), 3.95 (dd, 1H, J = 3.2, 0.6 Hz, H-2β), 3.86 (s, 1H, H-2α), 3.71 (dd, 1H, J = 9.2, 3.2 Hz, H-3β), 3.79 (s, 6H, OCH_3 α,β), 3.74, 3.67 (d, 4H, J = 13.5 Hz, SCH_2 α,β), 3.47 (t, 1H, J = 9.2 Hz, H-4β), 3.41 (t, 1H, J = 9.2 Hz, H-4α), 3.36 (dd, 1H, J = 9.2, 3.2 Hz, H-3α), 3.21-3.17 (m, 1H, H-5α), 1.37 (d, 3H, J = 6.4 Hz, CH_3-6α), 1.30 (d, 3H, J = 6.4 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 159.7 (C-OCH_3, Ar), 139.2, 129.6, 121.4, 114.7, 112.7 (Ar), 83.1 (C-1), 73.8 (C-3), 72.4 (C-2), 72.2 (C-4), 68.5 (C-5), 55.2 (OCH_3), 34.5 (SCH_3), 17.5 (C-6). IR (neat): υ_{max} 3330 (broad), 2930, 1599, 1584, 1489, 1266, 1058. ES-MS ($C_{14}H_{20}O_5S$) 300.37, *m/z* (%) 345.3 (100) $[M-H+HCOOH]^-$. HR-MS calcd for $C_{14}H_{20}O_5S$ $[M+Na]^+$ 323.0929, found 323.0914.

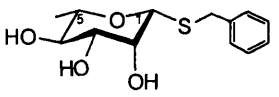
Thioglycosides **260-267** were synthesised using the procedure described for thiosugar **259**.

S-(4-Methylbenzyl) 1-thio-α/β-L-rhamnopyranoside 260



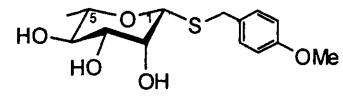
Yield 37% (white oil, α:β = 20:80). R_f 0.11 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 7.20-7.10 (m, 8H, Ar α,β), 5.07 (s, 1H, H-1β), 4.30 (d, 1H, J = 0.6 Hz, H-1α), 4.04-3.97 (m, 1H, H-5β), 3.94 (br s, 1H, H-2β), 3.72 (s, 4H, SCH_2 α,β), 3.67 (m, 1H, H-3β), 3.47 (t, 1H, J = 9.2 Hz, H-4β), 2.33 (s, 6H, $ArCH_3$ α,β), 1.36 (d, 3H, J = 6.0 Hz, CH_3-6α), 1.30 (d, 3H, J = 6.4 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 136.8, 134.5, 129.3, 129.3, 128.9 (Ar), 83.1 (C-1), 73.6 (C-3), 72.4 (C-2), 72.2 (C-4), 68.5 (C-5), 34.2 (SCH_2), 21.1 (CH_3-Ph), 17.5 (C-6). IR (neat): υ_{max} 3355 (broad), 2919, 1513, 1378, 1239, 1135, 1053, 1020, 972, 844, 790. ES-MS ($C_{14}H_{20}O_4S$) 284.37, *m/z* (%) 307.3 (100) $[M+Na]^+$. HR-MS: calcd for $C_{14}H_{20}O_4S$ $[M+Na]^+$ 307.0980, found 307.0985.

S-Benzyl 1-thio-α/β-L-rhamnopyranoside 261



Yield 66% (pale yellow oil, α:β = 19:81). R_f 0.06 (hexanes/EtOAc 1:1). ^{1}H NMR (CD_3OD): δ 7.40-7.20 (m, 10H, Ar α,β), 4.99 (d, 1H, J = 1.2 Hz, H-1β), 4.40 (d, 1H, J = 0.8 Hz, H-1α), 3.94-3.90 (m, 1H, H-5β), 3.81 (dd, 1H, J = 3.6, 1.2 Hz, H-2β), 3.80, 3.73 (d, 4H, J = 13.8 Hz, SCH_2 α,β), 3.58 (dd, 1H, J = 9.4, 3.6 Hz, H-3β), 3.40 (t, 1H, J = 9.4 Hz, H-4β), 3.17-3.12 (m, 1H, H-5α), 1.30 (d, 3H, J = 6.2 Hz, CH_3-6α), 1.24 (d, 3H, J = 6.0 Hz, CH_3-6β). ^{13}C NMR (CD_3OD): δ 139.7, 130.1, 129.5, 128.1 (Ar), 85.2 (C-1), 74.3 (C-3), 73.6 (C-2), 73.2 (C-4), 70.3 (C-5), 35.4 (SCH_2), 18.0 (C-6). IR (neat): υ_{max} 3355 (broad), 2924, 1453, 1061, 975, 844, 766, 701. ES-MS ($C_{13}H_{18}O_4S$) 270.34, *m/z* (%) 315.3 (100) $[M-H+HCOOH]^-$. Anal. calcd for $C_{13}H_{18}O_4S \cdot H_2O$: C 54.15, H 6.99; found: C 54.19, H 7.00.

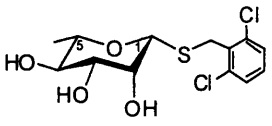
S-(4-Methoxybenzyl) 1-thio-α/β-L-rhamnopyranoside 262



Yield 59% (pale yellow oil, α:β = 18:82). R_f 0.17 ($CHCl_3$/EtOAc 7:3). ^{1}H NMR ($CDCl_3$): δ 7.26-7.18 (m, 4H, Ar α,β), 6.85-6.81 (m, 4H, Ar α,β), 5.05 (br s, 1H, H-1β), 4.31 (s, 1H, H-1α), 4.02-3.98 (m, 1H, H-5β), 3.93 (br s, 1H, H-2β), 3.79 (s, 4H, SCH_2 α,β), 3.78 (s, 6H, OCH_3 α,β), 3.72 (m, 1H, H-3β), 3.47 (t, 1H, J = 9.0 Hz, H-4β), 3.20-3.15 (m, 1H, H-5α), 1.35 (d, 3H, J = 6.0 Hz, CH_3-6α), 1.30 (d, 3H, J = 6.0 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 158.8 (C-OCH_3, Ar), 130.1, 129.5, 114.0 (Ar), 83.0 (C-

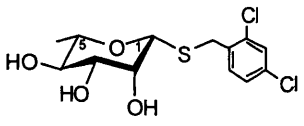
1), 73.7 (C-3), 72.5 (C-2), 72.2 (C-4), 68.5 (C-5), 55.3 (OCH_3), 34.0 (SCH_3), 17.5 (C-6). IR (neat): υ_{max} 3355 (broad), 2929, 1608, 1509, 1240, 1053, 1031, 972, 839, 775, 732. ES-MS ($C_{14}H_{20}O_5S$) 300.37, *m/z* (%) 345.3 (100) $[M-H+HCOOH]^-$. Anal. calcd for $C_{14}H_{20}O_5S \cdot H_2O$: C 52.81, H 6.96; found: C 52.82, H 7.28.

S-(2,6-Dichlorobenzyl) 1-thio-α/β-L-rhamnopyranoside 263



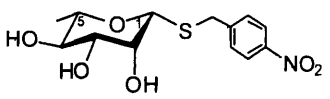
Yield 65%, (white amorphous solid, $\alpha:\beta$ = 21:79). R_f 0.09 (hexanes/EtOAc 1:1). ^1H NMR (CD_3OD): δ 7.34 (d, 4H, J = 8.4 Hz, Ar α,β), 7.21-7.17 (m, 2H, Ar α,β), 5.35 (d, 1H, J = 1.2 Hz, H-1β), 4.74 (d, 1H, J = 1.0 Hz, H-1α), 4.08, 4.03 (d, 4H, J = 12.6 Hz, SCH_2 α,β), 3.96-3.89 (m, 1H, H-5β), 3.86 (dd, 1H, J = 3.2, 1.2 Hz, H-2β), 3.78 (dd, 1H, J = 3.2, 1.0 Hz, H-2α), 3.51 (dd, 1H, J = 9.4, 3.2 Hz, H-3β), 3.37 (t, 1H, J = 9.4 Hz, H-4β), 3.35-3.33 (m, 1H, H-3α), 3.24-3.19 (m, 1H, H-5α), 1.20 (d, 3H, J = 6.0 Hz, $CH_3$6-α), 1.16 (d, 3H, J = 6.0 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 136.7, 136.6, 136.0, 130.2, 130.0, 129.6 (Ar), 86.9 (C-1), 74.3 (C-3), 73.7 (C-2), 73.6 (C-4), 70.4 (C-5), 31.6 (SCH_2), 17.8 (C-6). IR (neat): υ_{max} 3354 (broad), 2904, 1560, 1433, 1057, 969, 776, 761, 691. ES-MS ($C_{13}H_{16}Cl_2O_4S$) 339.23, *m/z* (%) 361.1 (92), 363.1 (57) $[M+Na]^+$. Anal. calcd for $C_{13}H_{16}Cl_2O_4S$: C 46.03, H 4.75; found: C 46.34, H 4.79.

S-(2,4-Dichlorobenzyl) 1-thio-α/β-L-rhamnopyranoside 264



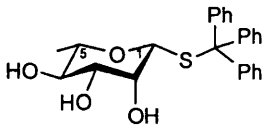
Yield 59% (pale yellow oily solid, $\alpha:\beta$ = 20:80). R_f 0.17 (hexanes/EtOAc 1:1). ^1H NMR (CD_3OD): δ 7.47 (d, 2H, J = 2.4 Hz, Ar α,β), 7.42 (d, 2H, J = 8.0 Hz, Ar α,β), 7.30 (dd, 2H, J = 8.0, 2.4 Hz, Ar α,β), 5.11 (d, 1H, J = 1.2 Hz, H-1β), 4.55 (d, 1H, J = 1.0 Hz, H-1α), 3.90 (s, 4H, SCH_2), 3.93-3.88 (m, 1H, H-5β), 3.89-3.88 (m, 1H, H-3α), 3.86 (dd, 1H, J = 3.4, 1.2 Hz, H-2β), 3.83 (dd, 1H, J = 3.0, 1.0 Hz, H-2α), 3.59 (dd, 1H, J = 9.4, 3.4 Hz, H-3β), 3.42 (t, 1H, J = 9.4 Hz, H-4β), 3.36-3.35 (m, 1H, H-4α), 3.24-3.19 (m, 1H, H-5α), 1.29 (d, 3H, J = 6.0 Hz, CH_3-6α) 1.25 (d, 3H, J = 6.0 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 136.4, 135.9, 134.7, 133.4, 130.4, 128.3 (Ar), 85.8 (C-1), 74.3 (C-3), 73.5 (C-2), 73.1 (C-4), 70.4 (C-5), 32.7 (SCH_2), 17.9 (C-6). IR (neat): υ_{max} 3353 (broad), 2929, 1470, 1093, 1059, 974, 843, 772, 729. ES-MS ($C_{13}H_{16}Cl_2O_4S$) 339.23, *m/z* (%) 360.4 (77), 362.1 (28) $[M+Na]^+$. Anal. calcd for $C_{13}H_{16}Cl_2O_4S$: C 46.03, H 4.75; found: C 46.04, H 4.72.

S-(4-Nitrobenzyl) 1-thio-α/β-L-rhamnopyranoside 265



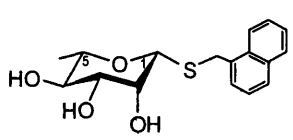
Yield 70% (pale yellow oily solid, α:β = 30:70). R_f 0.09 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 8.20-8.16 (m, 4H, Ar α,β), 7.50-7.47 (m, 4H, Ar α,β), 5.04 (br s, 1H, H-1β), 4.35 (br s, 1H, H-1α), 3.97-3.90 (m, 1H, H-5β), 3.96-3.92 (m, 1H, H-2β), 3.84, 3.75 (d, 4H, *J* = 13.8 Hz, SCH_2), 3.70 (d, 1H, *J* = 9.4 Hz, H-3β), 3.48 (t, 1H, *J* = 9.4 Hz, H-4β), 3.46-3.41 (m, 1H, H-5α), 1.34 (d, 3H, *J* = 6.0 Hz, CH_3-6α), 1.26 (d, 3H, *J* = 6.0 Hz, CH_3-6β). ^{13}C NMR ($CDCl_3$): δ 146.5 (C-NO_2, Ar), 145.6, 129.4, 122.7 (Ar), 83.6 (C-1), 73.5 (C-3), 71.4 (C-5), 71.0 (C-2), 70.7 (C-4), 32.6 (SCH_2), 16.9 (C-6). IR (neat): υ_{max} 3345 (broad), 2926, 1589, 1514, 1342, 1057,1010, 973, 856, 704. ES-MS ($C_{13}H_{17}NO_6S$) 315.34, *m/z* (%) 360.3 (100) $[M-H+HCOOH]^-$. Anal. calcd for $C_{13}H_{17}NO_6S \cdot H_2O$: C 46.84, H 5.74, N 4.20; found: C 46.63, H 5.82, N 4.31.

S-Triphenylmethyl 1-thio-α/β-L-rhamnopyranoside 266



Yield 60% (white foam, α:β = 24:76). R_f 0.07 (hexanes/EtOAc 1:1). ^{1}H NMR (CD_3OD): δ 7.38-720 (m, 30H, Ar α,β), 5.37 (d, 1H, *J* = 1.2 Hz, H-1α), 4.61 (d, 1H, *J* = 1.8 Hz, H-1β), 4.09 (dd, 1H, *J* = 3.2, 1.2 Hz, H-2α), 4.08-4.04 (m, 1H, H-5α), 3.94-3.87 (m, 1H, H-5β), 3.81 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2β), 3.67 (dd, 1H, *J* = 9.4, 3.2 Hz, H-3α), 3.58 (dd, 1H, *J* = 9.2, 3.0 Hz, H-3β), 3.47 (t, 1H, *J* = 9.4 Hz, H-4α), 3.35-3.33 (m, 1H, H-4β), 1.27 (d, 3H, *J* = 6.4 Hz, CH_3-6α) 1.22 (d, 3H, *J* = 6.4 Hz, CH_3-6β). ^{13}C NMR (CD_3OD): δ 146.3 (S-C-Ph_3), 132.8, 131.2, 130.5, 129.4, 128.9, 128.1 (Ar), 86.6 (C-1), 74.0 (C-3), 73.1 (C-2), 72.4 (C-4), 71.0 (C-5), 18.0 (C-6). IR (neat): υ_{max} 3348 (broad), 2904, 1488, 1442, 1053, 738, 696, 672. ES-MS ($C_{25}H_{26}O_4S$) 422.54, *m/z* (%) 445.3 (85) $[M+Na]^+$. Anal. calcd for $C_{25}H_{26}O_4S \cdot H_2O$: C 68.16, H 6.41; found: C 68.50, H 6.58.

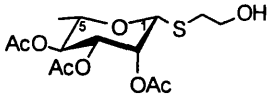
S-(1-Naphtalenemethyl) 1-thio-α/β-L-rhamnopyranoside 267



Yield 28% (off white solid, α:β = 16:84). R_f 0.16 (hexanes/EtOAc 1:1). ^{1}H NMR (CD_3OD): δ 8.13 (d, 2H, *J* = 8.0 Hz, Ar α,β), 7.87 (d, 2H, *J* = 8.0 Hz, Ar α,β), 7.78 (d, 2H, *J* = 8.0 Hz, Ar α,β), 7.55-7.47 (m, 4H, Ar α,β), 7.44-7.37 (m, 4H, Ar α,β), 5.09 (d, 1H, *J* = 1.2 Hz, H-1β), 4.44 (d, 1H, *J* = 0.8 Hz, H-1α), 4.28, 4.21 (d, 4H, *J*

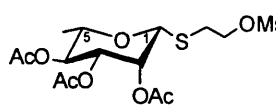
= 13.4 Hz, SCH_2), 3.99-3.92 (m, 1H, H-5β), 3.79 (dd, 1H, J = 3.2, 1.2 Hz, H-2β), 3.72 (dd, 1H, J = 3.2, 0.8 Hz, H-2α), 3.57 (dd, 1H, J = 9.6, 3.2 Hz, H-3β), 3.41 (t, 1H, J = 9.6 Hz, H-4β), 3.36-3.33 (m, 1H, H-4α), 3.25 (dd, 1H, J = 9.6, 3.2 Hz, H-3α), 3.16-3.11 (m, 1H, H-5α), 1.34 (d, 3H, J = 6.2 Hz, CH_3-6α), 1.28 (d, 3H, J = 6.4 Hz, CH_3-6β). ^{13}C NMR (CD_3OD): δ 135.3, 134.4, 132.3, 129.4, 128.8, 128.2, 126.6, 126.4, 125.8, 124.8 (Ar), 85.3 (C-1), 73.9 (C-3), 73.2 (C-2), 72.8 (C-4), 70.0 (C-5), 32.7 (SCH_2), 17.6 (C-6). IR (neat): υ_{max} 3353 (broad), 2924, 1336, 1088, 1066, 1055, 839, 772, 742, 655. ES-MS ($C_{17}H_{20}O_4S$) 320.40, *m/z* (%) 365.3 (72) $[M-H+HCOOH]^-$. Anal. calcd for $C_{17}H_{20}O_4S \cdot H_2O$: C 60.33, H 6.55; found: C 60.45, H 6.19.

S-(2-Hydroxyethyl) 2,3,4-tri-*O*-acetyl-1-thio-β-L-rhamnopyranoside 269



2,3,4-Tri-*O*-acetyl-1-thio-β-L-rhamnopyranose **245** (2.0 g, 6.59 mmol) was dissolved in DMF (8 mL), 2-chloroethanol (796 mg, 9.88 mmol) and Et_2NH (100 μL) were added and the mixture was stirred at room temperature for 12 hours. After completion of the reaction the solvent was evaporated. Purification by column chromatography (hexanes/EtOAc 3:1) gave **269** as a colourless oil (1.62 g, 70%). R_f 0.21 (hexanes/EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 5.39 (dd, 1H, J = 3.2, 1.4 Hz, H-2), 5.24 (d, 1H, J = 1.4 Hz, H-1), 5.23 (dd, 1H, J = 10.0, 3.2 Hz, H-3), 5.12 (t, 1H, J = 10.0 Hz, H-4), 4.28-4.23 (m, 1H, H-5), 3.87-3.76 (m, 2H, CH_2OH), 2.94-2.78 (m, 2H, SCH_2), 2.17, 2.07, 2.0 (s, 9H, 3 x CH_3, Ac), 1.26 (d, 3H, J = 6.4 Hz, CH_3-6). ^{13}C NMR ($CDCl_3$): δ 169.9 (3 x C=O, Ac), 82.8 (C-1), 71.5 (C-2), 71.1 (C-4), 69.3 (C-3), 67.5 (C-5), 61.9 (CH_2OH), 35.2 (SCH_2), 20.8 (3 x CH_3, Ac), 17.3 (C-6). ES-MS ($C_{14}H_{22}O_8S$) 350.38, *m/z* (%) 372.9 (100) $[M+Na]^+$.

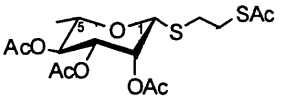
S-(Ethyl methanesulfonyl) 2,3,4-tri-*O*-acetyl-1-thio-β-L-rhamnopyranoside 270



To a cooled (0 °C) CH_2Cl_2 solution (10 mL) of **269** (0.59 g, 1.7 mmol) and Et_3N (0.35 mL, 2.5 mmol) was added MsCl (0.19 mL, 2.5 mmol). The mixture was stirred for 30 minutes at 0 °C and subsequently at room temperature for 12 hours. The solvents were evaporated and the product was purified by column chromatography (hexanes/EtOAc 3:1) to give **270** as a yellow oil (0.61 g, 85%). R_f 0.29 (hexanes/EtOAc 1:2). ^{1}H NMR

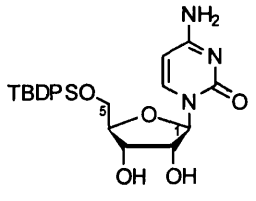
($CDCl_3$): δ 5.33 (dd, 1H, J = 3.4, 1.4 Hz, H-2), 5.24 (d, 1H, J = 1.4 Hz, H-1), 5.19 (dd, 1H, J = 10.0, 3.4 Hz, H-3), 5.11 (t, 1H, J = 10.0 Hz, H-4), 4.42-4.30 (m, 2H, CH_2OMs), 4.21-4.17 (m, 1H, H-5), 3.07 (s, 3H, mes. CH_3), 3.05-2.88 (m, 2H, SCH_2), 2.17, 2.07, 2.0 (s, 9H, 3 x CH_3, Ac), 1.23 (d, 3H, J = 7.2 Hz, CH_3-6). ^{13}C NMR ($CDCl_3$): δ 169.9 (3 x C=O, Ac), 82.8 (C-1), 71.1 (C-2), 71.0 (C-4), 69.2 (C-3), 67.8 (CH_2OMs), 67.5 (C-5), 37.7 (mes.CH_3), 30.3 (SCH_2), 20.8 (3 x CH_3, Ac), 17.3 (C-6). ES-MS ($C_{15}H_{24}O_{10}S_2$) 428.48, *m/z* (%) 451.0 (37) $[M+Na]^+$.

S-(Ethylthioacetyl) 2,3,4-tri-*O*-acetyl-1-thio-β-L-rhamnopyranoside 268



To a solution of **270** (0.61 g, 1.4 mmol) in DMF (6 mL) was added KSAc (0.81 g, 7.1 mmol). The mixture was heated at 80 °C and stirred for 12 hours. The solvent was evaporated and the product was purified by column chromatography (hexanes/EtOAc 3:1 to hexanes/EtOAc 2:1) to give **268** as a yellow oil (0.49 g, 71%). R_f 0.50 (hexanes/EtOAc 1:1). ^1H NMR ($CDCl_3$): δ 5.33 (dd, 1H, J = 3.2, 1.6 Hz, H-2), 5.25 (d, 1H, J = 1.6 Hz, H-1), 5.19 (dd, 1H, J = 10.0, 3.2 Hz, H-3), 5.10 (t, 1H, J = 10.0 Hz, H-4), 4.30-4.18 (m, 1H, H-5), 3.18-3.04 (m, 2H, CH_2SAc), 2.87-2.73 (m, 2H, SCH_2), 2.34 (s, 3H, SAc), 2.16, 2.05, 1.98 (s, 9H, 3 x CH_3, Ac), 1.24 (d, 3H, J = 6.4 Hz, CH_3-6). ^{13}C NMR ($CDCl_3$): δ 195.0 (C=O, SAc), 170.0 (3 x C=O, Ac), 82.7 (C-1), 71.3 (C-2), 71.2 (C-4), 69.4 (C-3), 67.3 (C-5), 31.4 (SCH_2), 30.6 (CH_2SAc), 29.3 (CH_3, SAc), 20.8 (3 x CH_3, Ac), 17.3 (C-6). ES-MS ($C_{16}H_{24}O_8S_2$) 408.49, *m/z* (%) 432.5 (100) $[M+Na]^+$.

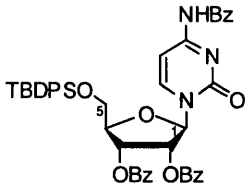
5-*O*-*tert*-Butyldiphenylsilyl cytidine 271



Cytidine (3.0 g, 12.3 mmol) and imidazole (2.1 g, 31.5 mmol) were dissolved in DMF (24 mL). TBDPSCl (3.5 mL, 13.6 mmol) was added dropwise over a period of 10 minutes. The reaction was stirred at room temperature for 1 hour and then quenched with MeOH (5 mL). The solvents were evaporated and the product was purified by column chromatography ($CHCl_3$/MeOH 10:0.5) to give **271** as a white foam (5.59 g, 94%). R_f 0.5 ($CHCl_3$/MeOH 10:2).^1H NMR (CD_3OD): δ 8.02 (d, 1H, J = 7.6 Hz, Ar), 7.76-7.71 (m, 4H, Ar), 7.49-7.42 (m, 6H, Ar), 5.99 (d, 1H, J = 3.0 Hz, H-1), 5.64 (d, 1H, J = 7.6 Hz, Ar), 4.35 (dd, 1H, J = 6.4, 5.2 Hz, H-3), 4.17 (dd, 1H, J = 5.2, 3.0 Hz, H-2), 4.14-4.11 (m, 2H, H-4,

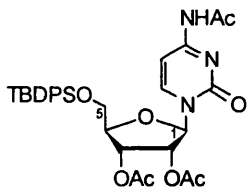
CH_2-5), 3.94 (dd, 1H, J = 12.4, 3.2 Hz, CH_2-5), 1.13 (s, 9H, *t*Bu TBDPS). ES-MS ($C_{25}H_{31}N_3O_5Si$) 481.62, *m/z* (%) 482.2 (60) $[M+H]^+$.

2,3-*O,N*-Tribenzoyl-5-*O*-*tert*-butyldiphenylsilyl cytidine 272



5-*O*-*tert*-Butyldiphenylsilyl cytidine **271** (1.79 g, 3.7 mmol) was dissolved in pyridine (6 mL, 74.3 mmol). Benzoic anhydride (8.41 g, 37.2 mmol) was added and the reaction was stirred at room temperature for 2 days. After completion of the reaction the solvent was evaporated and the product was purified by column chromatography (hexanes/EtOAc 6:1 to 100% EtOAc). The product **272** was obtained as a white foam (1.62 g, 55%). R_f 0.29 (Hexane:EtOAc 1:1). ^{1}H NMR ($CDCl_3$): δ 8.37 (d, 1H, J = 7.6 Hz, Ar), 7.99-7.94 (m, 6H, Ar), 7.76-7.71 (m, 4H, Ar), 7.65-7.36 (m, 16H, Ar), 6.70 (d, 1H, J = 5.6 Hz, H-1), 5.93 (dd, 1H, J = 5.6, 2.0 Hz, H-3), 5.80 (t, 1H, J = 5.6 Hz, H-2), 4.51 (t, 1H, J = 2.0 Hz, H-4), 4.19 (dd, 1H, J = 11.6, 2.0 Hz, CH_2-5), 4.04 (dd, 1H, J = 11.6, 2.0 Hz, CH_2-5), 1.20 (s, 9H, *t*Bu TBDPS). ES-MS ($C_{46}H_{43}N_3O_8Si$) 793.9, *m/z* (%) 794.3 (62) $[M+H]^+$.

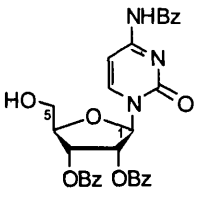
2,3-*O,N*-Triacetyl-5-*O*-*tert*-butyldiphenylsilyl cytidine 273



5-*O*-*tert*-Butyldiphenylsilyl cytidine **271** (5.59 g, 11.6 mmol) was dissolved in pyridine (30 ml, 0.37 mol). Acetic anhydrite (20 ml, 0.21 mol) was added and the reaction was stirred at room temperature for 18 hours. After completion of the reaction the solvent was evaporated and the product was purified by column chromatography (hexanes/EtOAc 3:1 to hexanes/EtOAc 1:2). The product **273** was obtained as a white foam (5.00 g, 71%). R_f 0.59 ($CHCl_3$/MeOH 10:2). ^{1}H NMR ($CDCl_3$): δ 9.31 (s, 1H, NHAc), 8.20 (d, 1H, J = 7.6 Hz, Ar), 7.68-7.64 (m, 4H, Ar), 7.50-7.38 (m, 6H, Ar), 7.20 (d, 1H, J = 7.6 Hz, Ar), 6.32 (d, 1H, J = 4.8 Hz, H-1), 5.50 (t, 1H, J = 4.8 Hz, H-3), 5.45 (t, 1H, J = 4.8 Hz, H-2), 4.23-4.21 (m, 1H, H-4), 4.08 (dd, 1H, J = 12.0, 2.0 Hz, CH_2-5), 3.81 (dd, 1H, J = 12.0, 2.0 Hz, CH_2-5), 2.24 (s, 3H, CH_3, NHAc), 2.09, 2.06 (s, 6H, 2 x CH_3, Ac), 1.12 (s, 9H, *t*Bu TBDPS). ^{13}C NMR ($CDCl_3$): δ 169.6 (2 x C=O, Ac), 169.5 (C=O, NHAc) 162.7 (C=O, Ar), 144.2, 135.7, 132.4, 131.8, 130.2, 128.1, 97.1 (Ar), 87.1 (C-1), 82.7 (C-4), 74.3 (C-3), 69.9 (C-2), 62.7 (C-5), 27.0 (*t*Bu TBDPS), 24.99 (CH_3,

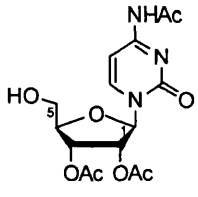
NHAc), 20.60, 20.50 (CH_3, 2 x Ac), 19.24 (C_{quart} TBDPS). ES-MS ($C_{31}H_{37}N_3O_8Si$) 607.73, *m/z* (%) 608.3 (100) $[M+H]^+$.

2,3-*O,N*-Tribenzoyl cytidine 274



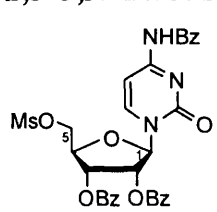
TBDPS-cytidine **272** (1.62 g, 2.0 mmol) was dissolved in THF (7 mL). Acetic acid (0.2 mL, 3.0 mmol) was added, followed by TBAF (1.0 M in THF, 1.8 mL, 6.0 mmol) and the reaction was stirred at room temperature for 18 hours. The solvent was evaporated and the product was purified by column chromatography (hexanes/EtOAc 2:1 to hexanes/EtOAc 1:2) to give **274** as a white foam (0.84 g, 75%). R_f 0.26 (hexanes/EtOAc 1:4). ^1H NMR ($CDCl_3$): δ 8.39 (d, 1H, J = 7.6 Hz, Ar), 7.92 (m, 6H, Ar), 7.61-7.46 (m, 6H, Ar), 7.41-7.33 (m, 4H, Ar), 6.40 (d, H-1, J = 5.2 Hz, 1H), 5.99 (t, 1H, J = 5.2 Hz, H-2), 5.94 (t, 1H, J = 5.2 Hz, H-3), 4.53 (t, 1H, J = 1.8 Hz, H-4), 4.13 (dd, 1H, J = 12.4, 1.8 Hz, CH_2-5), 4.03 (dd, 1H, J = 12.4, 1.8 Hz, CH_2-5). ES-MS ($C_{30}H_{25}N_3O_8$) 555.5, *m/z* (%) 556.1 (100) $[M+H]^+$.

2,3-*O,N*-Triacetyl cytidine 275



TBDPS-cytidine **273** (5.00 g, 8.23 mmol) was dissolved in THF (10 mL). Acetic acid (0.7 mL, 12.3 mmol) was added, followed by TBAF (1.0 M in THF, 7.1 mL, 24.7 mmol) and the reaction was stirred at room temperature for 18 hours. The solvent was evaporated and the product was purified by column chromatography ($CHCl_3$ 100% to $CHCl_3$/MeOH 10:0.4) to give **275** as a white foam (3.04 g, quantitative). R_f 0.42 ($CHCl_3$/MeOH 10:2). ^1H NMR ($CDCl_3$): δ 8.41 (d, 1H, J = 7.6 Hz, Ar), 7.41 (d, 1H, J = 7.6 Hz, Ar), 6.09 (d, 1H, J = 5.2 Hz, H-1), 5.45 (t, 1H, J = 5.2 Hz, H-3), 5.38 (t, 1H, J = 5.2 Hz, H-2), 4.26-4.23 (m, 1H, H-4), 3.86 (dd, 1H, J = 12.4, 2.6 Hz, CH_2-5), 3.74 (dd, 1H, J = 12.4, 2.6 Hz, CH_2-5), 2.14 (s, 3H, CH_3, NHAc), 2.06, 2.03 (s, 6H, 2 x CH_3, Ac). ES-MS ($C_{15}H_{19}N_3O_8$) 369.33, *m/z* (%) 370.1 (34) $[M+H]^+$.

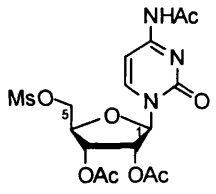
2,3-*O,N*-Tribenzoyl-5-methanesulfonyl cytidine 276



To a cooled (0 °C) CH_2Cl_2 solution (10 mL) of **274** (0.65 g, 1.2 mmol) and Et_3N (0.25 mL, 1.8 mmol) was added MsCl (0.14

mL, 1.8 mmol). The mixture was stirred for 30 minutes at 0 °C and subsequently at room temperature for 12 hours. The solvents were evaporated and the product was purified by column chromatography (hexanes/EtOAc 1:2) to give **276** as a white foam (0.20 g, 27%). R_f 0.30 (hexanes/EtOAc 1:2). ^{1}H NMR ($CDCl_3$): δ 8.73 (s, 1H, NHBz), 8.02 (d, 1H, J = 7.4 Hz, Ar), 7.98-7.94 (m, 5H, Ar), 7.90 (d, 1H, J = 7.4 Hz, Ar), 7.64-7.51 (m, 6H, Ar), 7.41-7.36 (m, 4H, Ar), 6.36 (d, 1H, J = 4.8 Hz, H-1), 5.86 (t, 1H, J = 4.8 Hz, H-3), 5.76 (t, 1H, J = 4.8 Hz, H-2), 4.76 (dd, 1H, J = 12.8, 4.8 Hz, H-4), 4.70-4.63 (m, 2H, CH_2-5), 3.17 (s, 3H, mes. CH_3). ES-MS ($C_{31}H_{27}N_3O_{10}S$) 633.63, *m/z* (%) 634.3 (93) $[M+H]^+$.

2,3-*O,N*-Triacetyl-5-methanesulfonyl cytidine 277



To a cooled (0 °C) CH_2Cl_2 solution (10 mL) of **275** (0.50 g, 1.35 mmol) and Et_3N (0.29 mL, 2.03 mmol) was added MsCl (0.16 mL, 2.03 mmol). The mixture was stirred for 30 minutes at 0 °C and subsequently at room temperature for 12 hours. The solvents were evaporated and the product was purified by column chromatography (hexanes/EtOAc 4:1 to hexanes/EtOAc 1:1) to give **277** as a white foam (0.43 g, 77%). R_f 0.33 ($CHCl_3$/MeOH 10:0.5). ^{1}H NMR ($CDCl_3$): δ 9.25 (s, 1H, NHAc), 7.86 (d, 1H, J = 7.4 Hz, Ar), 7.47 (d, 1H, J = 7.4 Hz, Ar), 6.03 (d, 1H, J = 3.2 Hz, H-1), 5.45-5.41 (m, 2H, H-2, H-3), 4.60 (dd, 1H, J = 11.4, 2.6 Hz, CH_2-5), 4.48 (dd, 1H, J = 11.4, 4.2 Hz, CH_2-5), 4.42-4.40 (m, 1H, H-4), 3.10 (s, 3H, mes. CH_3), 2.54 (s, 3H, CH_3, NHAc), 2,11, 2.08 (s, 6H, 2 x CH_3, Ac).

BIBLIOGRAPHY

Reference List

1. Varki, A. *Glycobiology* **1993,** *3*, 97.

2. Best, C. H. *Circulation* **1959,** *19*, 79.

3. Truscheit, E.; Frommer, W.; Junge, B.; Müller, L.; Schmidt, D. D.; Wingender, W. *Angew. Chem., Int. Ed.* **1981,** *20*, 744.

4. Davies, J. E. *J. Antibiot.* **2006,** *59*, 529.

5. Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997,** *119*, 681.

6. Laver, G. *Future Virology* **2006,** *1*, 577.

7. Osborn, H. M. I.; Evans, P. G.; Gemmell, N.; Osborne, S. D. *J. Pharm. Pharmacol.* **2004,** *56*, 691.

8. Seeberger, P. H.; Werz, D. B. *Nature* **2007,** *446*, 1046.

9. Oppenheimer, S. B.; Alvarez, M.; Nnoli, J. *Acta Histochemica* **2008,** *110*, 6.

10. Cragg, G. M.; Newman, D. J.; Snader, K. M. *J. Nat. Prod.* **1997,** *60*, 52.

11. Crown, J.; O'Leary, M. *The Lancet* **2000,** *355*, 1176.

12. Noble, R. L. *Biochem. Cell Biol.* **1990,** *68*, 1344.

13. da Rocha, A. B.; Lopes, R. M.; Schwartsmann, G. *Curr. Opin. Pharmacol.* **2001,** *1*, 364.

14. Pereda-Miranda, R. In *Phytochemistry of Medicinal Plants*, Plenum Press, New York: 1995; pp 83-112.

15. Pereda-Miranda, R.; Bah, M. *Curr. Top. Med. Chem.* **2003,** *3*, 111.

16. Cao, S.; Guza, R. C.; Wisse, J. H.; Miller, J. S.; Evans, R.; Kingston, D. G. I. *J. Nat. Prod.* **2005,** *68*, 487.

17. Noda, N.; Ono, M.; Miyahara, K.; Kawasaki, T.; Okabe, M. *Tetrahedron* **1987,** *43*, 3889.

18. Hernandez-Carlos, B.; Bye, R.; Pereda-Miranda, R. *J. Nat. Prod.* **1999,** *62*, 1096.

19. Pereda-Miranda, R.; Hernandez-Carlos, B. *Tetrahedron* **2002,** *58*, 3145.

20. Pereda-Miranda, R.; Kaatz, G. W.; Gibbons, S. *J. Nat. Prod.* **2006,** *69*, 406.

21. Fang, Y.; Chai, W.; Chen, S.; He, Y.; Zhao, L.; Peng, J.; Huang, H.; Xin, B. *Carbohydr. Res.* **1993,** *245*, 259.

22. Pereda-Miranda, R.; Mata, R.; Anaya, A. L.; Wickramaratne, D. B. M.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **1993,** *56*, 571.

23. Bah, M.; Pereda-Miranda, R. *Tetrahedron* **1996,** *52*, 13063.

24. Bah, M.; Pereda-Miranda, R. *Tetrahedron* **1997,** *53*, 9007.

25. Rivero-Cruz, I.; Acevedo, L.; Guerrero, J. A.; Martínez, S.; Bye, R.; Pereda-Miranda, R.; Franzblau, S.; Timmermann, B. N.; Mata, R. *J. Pharm. Pharmacol.* **2005,** *57*, 1117.

26. Cao, S.; Norris, A.; Wisse, J. H.; Miller, J. S.; Evans, R.; Kingston, D. G. I. *Nat Prod Res.* **2007,** *21*, 872.

27. Escobedo-Martinez, C.; Pereda-Miranda, R. *J. Nat. Prod.* **2007,** *70*, 974.

28. Miron-Lopez, G.; Herrera-Ruiz, M.; Estrada-Soto, S.; guirre-Crespo, F.; Vazquez-Navarrete, L.; Leon-Rivera, I. *J. Nat. Prod.* **2007,** *70*, 557.

29. Rivera, I.; pez, G.; Molina-Salinas, G. M.; Herrera-Ruiz, M.; Estrada-Soto, S.; rrez, M.; onso-Cortes, D.; Navarrete, V.; os, M.; Said, F. *J. Nat. Prod.* **2008**.

30. Levy, D. E.; Fügedi, P. *The Organic Chemistry of Sugars;* Taylor & Francis Group: 2006.

31. Lindhorst, T. K. *Essentials of Carbohydrate Chemistry and Biochemistry;* 2nd ed.; Wiley-VCH: Weinheim, 2003.

32. Kocienski, P. *Protecting Groups;* 3rd ed.; Georg Thieme Verlag: Stuttgart, 2005.

33. Green, T.; Wuts, P. G. M. *Greene's Protective Groups in Organic Synthesis;* 4th ed.; Wiley: New York, 2006.

34. Einhorn, A.; Hollandt, F. *Justus Liebigs Ann. Chem.* **1898,** *301*, 95.

35. Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem. , Int. Ed.* **1978,** *17*, 569.

36. Zemplén, G. *Ber. Dtsch. Chem. Ges.* **1927,** *60*, 1555.

37. Neilson, T.; Werstiuk, E. S. *Can. J. Chem.* **1971,** *49*, 493.

38. Excoffier, G.; Gagnaire, D.; Utille, J. P. *Carbohydr. Res.* **1975,** *39*, 368.

39. Helferich, B.; Portz, W. *Chem. Ber.* **1953,** *86*, 604.

40. Reinhard, B.; Faillard, H. *Liebigs Ann. Chem.* **1994,** *1994*, 193.

41. Xu, Y. C.; Lebeau, E.; Walker, C. *Tetrahedron Lett.* **1994,** *35*, 6207.

42. Yao-Chang, X.; Bizuneh, A.; Walker, C. *Tetrahedron Lett.* **1996,** *37*, 455.

43. Cook, A. F.; Maichuk, D. T. *J. Org. Chem.* **1970,** *35*, 1940.

44. van Boeckel, C. A. A.; Beetz, T. *Tetrahedron Lett.* **1983,** *24*, 3775.

45. Ziegler, T. *Liebigs Ann. Chem.* **1990,** *1990*, 1125.

46. Koeners, H. J.; Verhoeven, J.; van Boom, J. H. *Tetrahedron Lett.* **1980,** *21*, 381.

47. Rej, R. N.; Glushka, J. N.; Chew, W.; Perlin, A. S. *Carbohydr. Res.* **1989,** *189*, 135.

48. Tomic-Kulenovic, S.; Keglevic, D. *Carbohydr. Res.* **1980,** *85*, 302.

49. Chen, X. T.; Bhattacharya, S. K.; Zhou, B.; Gutteridge, C. E.; Pettus, T. R. R.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999,** *121*, 6563.

50. Boschelli, D.; Takemasa, T.; Nishitani, Y.; Masamune, S. *Tetrahedron Lett.* **1985,** *26*, 5239.

51. Williamson, A. *Philosophical Magazine* **1850,** *37*, 350.

52. Chaudhary, S. K.; Hernandez, O. *Tetrahedron Lett.* **1979,** *20*, 95.

53. Iversen, T.; Bundle, D. R. *J. Chem. Soc. , Chem. Commun.* **1981,** *1981*, 1240.

54. Bashir-Uddin Surfraz, M.; Akhtar, M.; Allemann, R. K. *Tetrahedron Lett.* **2004,** *45*, 1223.

55. El Amin, B.; Anantharamaiah, G. M.; Royer, G. P.; Means, G. E. *J. Org. Chem.* **1979,** *44*, 3442.

56. Bieg, T.; Szeja, W. *Synthesis* **1985,** 76.

57. Felix, A. M.; Heimer, E. P.; Lambros, T. J.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1978,** *43*, 4194.

58. Yan, L.; Kahne, D. *Synlett* **1995,** 523.

59. Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982,** *23*, 885.

60. Classon, B.; Garegg, P. J.; Samuelsson, B. *Acta Chem. Scand.* **1984,** *38B*, 419.

61. Gigg, J.; Gigg, R. *J. Chem. Soc. C* **1966,** 82.

62. Cunningham, J.; Gigg, R.; Warren, C. D. *Tetrahedron Lett.* **1964,** *5*, 1191.

63. Corey, E. J.; Suggs, J. W. *J. Org. Chem.* **1973,** *38*, 3224.

64. Bessodes, M.; Komiotis, D.; Antonakis, K. *Tetrahedron Lett.* **1986,** *27*, 579.

65. du Mortier, C.; Varela, O.; de Lederkremer, R. M. *Carbohydr. Res.* **1989,** *189*, 79.

66. Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972,** *94*, 6190.

67. Corey, E. J.; Cho, H.; Rücker, C.; Hua, D. H. *Tetrahedron Lett.* **1981,** *22*, 3455.

68. Cunico, R. F.; Bedell, L. *J. Org. Chem.* **1980,** *45*, 4797.

69. Newton, R. F.; Reynolds, D. P.; Finch, M. A. W.; Kelly, D. R.; Roberts, S. M. *Tetrahedron Lett.* **1979,** *20*, 3981.

70. Sweeley, C. C.; Bentley, R.; Makita, M.; Wells, W. W. *J. Am. Chem. Soc.* **1963,** *85*, 2497.

71. Olgivie, K. K.; Entwistle, D. W. *Carbohydr. Res.* **1981,** *89*, 203.

72. Hanessian, S.; Lavallee, P. *Can. J. Chem.* **1975,** *53*, 2975.

73. Fischer, E. *Ber. Dtsch. Chem. Ges.* **1895,** *28*, 1145.

74. Hasegawa, A.; Fletcher, H. G. *Carbohydr. Res.* **1973,** *29*, 209.

75. Wolfrom, M. L.; Diwadkar, A. B.; Gelas, J.; Horton, D. *Carbohydr. Res.* **1974,** *35*, 87.

76. Irvine, J. C.; Skinner, A. F. *J. Chem. Soc.* **1926,** *129*, 1089.

77. Fanton, E.; Gelas, J.; Horton, D. *J. Chem. Soc. , Chem. Commun.* **1980,** 21.

78. Ravikumar, K. S.; Farquhar, D. *Tetrahedron Lett.* **2002,** *43*, 1367.

79. Zervas, L. *Ber. Dtsch. Chem. Ges.* **1931,** *64*, 2289.

80. Barili, P. L.; Berti, G.; Catelani, G.; Cini, C.; D'Andrea, F.; Mastrorilli, E. *Carbohydr. Res.* **1995,** *278*, 43.

81. Hanessian, S.; Plessas, N. R. *J. Org. Chem.* **1969,** *34*, 1035.

82. Ley, S. V.; Woods, M.; Zanotti-Gerosa, A. *Synthesis* **1992,** 52.

83. Ley, S. V.; Downham, R.; Edwards, P. J.; Innes, J. E.; Woods, M. *Contemp. Org. Synth.* **1995,** *2*, 365.

84. Ley, S. V.; Baeschlin, D. K.; Dixon, D. J.; Foster, A. C.; Ince, S. J.; Priepke, H. W. M.; Reynolds, D. J. *Chem. Rev.* **2001,** *101*, 53.

85. Ley, S. V.; Priepke, H. W. M.; Warrine, S. L. *Angew. Chem. , Int. Ed.* **1994,** *33*, 2290.

86. Montchamp, J. L.; Tian, F.; Hart, M. E.; Frost, J. W. *J. Org. Chem.* **1996,** *61*, 3897.

87. Hense, A.; Ley, S. V.; Osborn, H. M. I.; Owen, D. R.; Poisson, J. F.; Warriner, S. L.; Wesson, K. E. *J. Chem. Soc. , Perkin Trans. 1* **1997,** 2023.

88. Zemplén, G.; László, E. D. *Ber. Dtsch. Chem. Ges.* **1915,** *48*, 915.

89. Morère, A.; Mouffouk, F.; Chavis, C.; Montero, J. L. *Tetrahedron Lett.* **1997,** *38*, 7519.

90. Adinolfi, M.; Baronė, G.; Guariniello, L.; Iadonisi, A. *Tetrahedron Lett.* **2000,** *41*, 9305.

91. Mouffouk, F.; Morère, A.; Vidal, S.; Leydet, A.; Montero, J. L. *Synth. Commun.* **2005,** *34*, 303.

92. Morère, A.; Mouffouk, F.; Leiris, S.; Leydet, A.; Montero, J. L. *J. Carbohydr. Chem.* **2006,** *25*, 451.

93. Munavu, R. M.; Szmant, H. H. *J. Org. Chem.* **1976,** *41*, 1832.

94. Tsuda, Y.; Haque, M. E.; Yoshimoto, K. *Chem. Pharm. Bull.* **1983,** *31*, 1612.

95. Haque, M. E.; Kikuchi, T.; Yoshimoto, K.; Tsuda, Y. *Chem. Pharm. Bull.* **1985,** *33*, 2243.

96. Lemieux, R. U.; Morgan, A. R. *Can. J. Chem.* **1965,** *43*, 2198.

97. Lemieux, R. U.; Morgan, A. R. *Can. J. Chem.* **1965,** *43*, 2190.

98. Lemieux, R. U.; Driguez, H. *J. Am. Chem. Soc.* **1975,** *97*, 4069.

99. Lemieux, R. U. *Pure Appl. Chem.* **1971,** *25*, 527.

100. Kabayama, M. A.; Patterson, D. *Can. J. Chem.* **1958,** *36*, 563.

101. Juaristi, E.; Cuevas, G. *Tetrahedron* **1992,** *48*, 5019.

102. Schmidt, R. R. *Angew. Chem. , Int. Ed.* **1986,** *25*, 212.

103. Wulff, G.; Schröder, U.; Wilchelhaus, J. *Carbohydr. Res.* **1979,** *72*, 280.

104. Wegmann, B.; Schmidt, R. R. *J. Carbohydr. Chem.* **1987,** *6*, 357.

105. Schmidt, R. R.; Behrendt, M.; Toepfer, A. *Synlett* **1990,** 694.

106. Vankar, Y. D.; Vankar, P. S.; Behrendt, M.; Schmidt, R. R. *Tetrahedron* **1991,** *47*, 9985.

107. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975,** *97*, 4056.

108. Stork, G.; Kim, G. *J. Am. Chem. Soc.* **1992,** *114*, 1087.

109. Stork, G.; La Clair, J. J. *J. Am. Chem. Soc.* **1996,** *118*, 247.

110. Barresi, F.; Hindsgaul, O. *J. Am. Chem. Soc.* **1991,** *113*, 9376.

111. Fischer, E. *Ber. Dtsch. Chem. Ges.* **1893,** *26*, 2400.

112. Elofsson, M.; Walse, B. r.; Kihlberg, J. *Tetrahedron Lett.* **1991,** *32*, 7613.

113. Pathak, A. K.; El-Kattan, Y. A.; Bansal, N.; Maddry, J. A.; Reynolds, R. C. *Tetrahedron Lett.* **1998,** *39*, 1497.

114. Chatterjee, S. K.; Nuhn, P. *Chem. Commun.* **1998,** 1729.

115. Koenigs, W.; Knorr, E. *Ber. Dtsch. Chem. Ges.* **1901,** *34*, 957.

116. Helferich, B.; Weis, K. *Chem. Ber.* **1956,** *89*, 314.

117. Hanessian, S.; Banoub, J. *Carbohydr. Res.* **1977,** *53*, C13-C16.

118. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981,** *10*, 431.

119. Nicolaou, K. C.; Dolle, R. E.; Papahatjis, D. P. *J. Am. Chem. Soc.* **1984,** *106*, 4189.

120. Schmidt, R. R.; Michel, J. *Tetrahedron Lett.* **1984,** *25*, 821.

121. Schmidt, R. R.; Michel, J.; Roos, M. *Liebigs Ann. Chem.* **1984,** *1984*, 1343.

122. Schmidt, R. R.; Grundler, G. *Angew. Chem.* **1982,** *94*, 790.

123. Schmidt, R. R.; Michel, J. *J. Carbohydr. Chem.* **1985,** *4*, 141.

124. Grundler, G.; Schmidt, R. R. *Liebigs Ann. Chem.* **1984,** *1984*, 1826.

125. Ferrier, R.; Furneaux, R. *Methods in Carbohydrate Chemistry* **1980,** *8*, 251.

126. Horton, D. *Methods in Carbohydrate Chemistry* **1963,** *2*, 433.

127. Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990,** *31*, 1331.

128. Lönn, H. *Carbohydr. Res.* **1985,** *139*, 105.

129. Fügedi, P.; Garegg, P. J. *Carbohydr. Res.* **1986,** *149*, C9-C12.

130. Kahne, D.; Walker, S.; Cheng, Y.; Van Engen, D. *J. Am. Chem. Soc.* **1989,** *111*, 6881.

131. Yan, L.; Kahne, D. *J. Am. Chem. Soc.* **1996,** *118*, 9239.

132. Stick, R. V.; Tilbrook, D. M.; Williams, S. J. *Aust. J. Chem.* **1997,** *50*, 237.

133. Stick, R. V.; Tilbrook, D. M.; Williams, S. J. *Aust. J. Chem.* **1997,** *50*, 233.

134. Fraser-Reid, B.; Konradsson, P.; Mootoo, D. R.; Udodong, U. *J. Chem. Soc. , Chem. Commun.* **1988,** 823.

135. Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988,** *110*, 5583.

136. Fraser-Reid, B.; Wu, Z.; Udodong, U. E.; Ottosson, H. *J. Org. Chem.* **1990,** *55*, 6068.

137. Fischer, E.; Zach, K. *Sitzungsber. Kl. Preuss. Akad. Wiss.* **1913,** *27*, 311.

138. Halcomb, R. L.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989,** *111*, 6661.

139. Gordon, D. M.; Danishefsky, S. J. *Carbohydr. Res.* **1990,** *206*, 361.

140. Jiang, Z. H.; Geyer, A.; Schmidt, R. R. *Angew. Chem. , Int. Ed.* **1995,** *34*, 2520.

141. Jiang, Z. H.; Schmidt, R. R. *Liebigs Ann. Chem.* **1994,** *1994*, 645.

142. Corey, E. J.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1974,** *96*, 5614.

143. Furukawa, J.; Kobayashi, S.; Nomizu, M.; Nishi, N.; Sakairi, N. *Tetrahedron Lett.* **2000,** *41*, 3453.

144. Hikota, M.; Sakurai, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1990,** *31*, 6367.

145. Larson, D. P.; Heathcock, C. H. *J. Org. Chem.* **1997,** *62*, 8406.

146. Lu, S. F.; O'yang, Q.; Guo, Z. W.; Yu, B.; Hui, Y. Z. *J. Org. Chem.* **1997,** *62*, 8400.

147. Fürstner, A.; Muller, T. *J. Org. Chem.* **1998,** *63*, 424.

148. Fürstner, A.; Muller, T. *J. Am. Chem. Soc.* **1999,** *121*, 7814.

149. Brito-Arias, M.; Pereda-Miranda, R.; Heathcock, C. H. *J. Org. Chem.* **2004,** *69*, 4567.

150. Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991,** *32*, 3353.

151. Barry, J.; Kagan, H. B. *Synthesis* **1981,** 453.

152. Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995,** *28*, 446.

153. Fürstner, A.; Nagano, T. *J. Am. Chem. Soc.* **2007,** *129*, 1906.

154. Achnine, L.; Pereda-Miranda, R.; Iglesias-Prieto, R.; Moreno-Sanchez, R.; Lotina-Hennsen, B. *Physiol. Plant.* **1999,** *106*, 246.

155. Vermeer, H.; van Dijk, C.; Kamerling, J.; P; Vliegenthart, J. *Eur. J. Org. Chem.* **2001,** *2001*, 193.

156. Ennis, S. C.; Cumpstey, I.; Fairbanks, A. J.; Butters, T. D.; Mackeen, M.; Wormald, M. R. *Tetrahedron* **2002,** *58*, 9403.

157. Zhang, W. C.; Li, C. J. *J. Org. Chem.* **2000,** *65*, 5831.

158. Seebach, D.; Sutter, M. A.; Weber, R. H.; Züger, M. F. *Org. Synth.* **1990,** *Coll. Vol. 7*, 215.

159. Rodríguez, A.; Nomen, M.; Spur, B. W.; Godfroid, J. J. *Eur. J. Org. Chem.* **1999,** *1999*, 2655.

160. Lhommet, G.; Fréville, S.; Thuy, V.; Petit, H.; Célérier, J. P. *Synth. Commun.* **1996,** *26*, 3897.

161. Rahman, M. T.; Nur, H. P. *J. Indian Chem. Soc.* **1994,** *71*, 469.

162. Ghanem, A.; boul-Enein, H. Y. *Tetrahedron: Asymmetry* **2004,** *15*, 3331.

163. Scheiper, B.; Bonnekessel, M.; Krause, H.; Furstner, A. *J. Org. Chem.* **2004,** *69*, 3943.

164. Boeckman, R. K.; Bruza, K. J. *Tetrahedron* **1981,** *37*, 3997.

165. Tschantz, M. A.; Burgess, L. E.; Meyers, A. I. *Org. Synth.* **1998,** *73*, 215.

166. Barbier, M.; Grand, E.; Kovensky, J. *Carbohydr. Res.* **2007,** *342*, 2635.

167. Thomas, R. M.; Mohan, G. H.; Iyengar, D. S. *Tetrahedron Lett.* **1997,** *38*, 4721.

168. Boss, R.; Scheffold, R. *Angew. Chem. , Int. Ed.* **1976,** *15*, 558.

169. Fürstner, A.; Jeanjean, F.; Razon, P.; Wirtz, C.; Mynott, R. *Chem. Eur. J.* **2002,** *9*, 320.

170. Lee, J.; Cha, J. K. *Tetrahedron Lett.* **1996,** *37*, 3663.

171. Sakagami, M.; Hamana, H. *Tetrahedron Lett.* **2000,** *41*, 5547.

172. Mikami, T.; Asano, H.; Mitsunobu, O. *Chem. Lett.* **1987,** *16*, 2033.

173. Ikawa, T.; Hattori, K.; Sajiki, H.; Hirota, K. *Tetrahedron* **2004,** *60*, 6901.

174. Güzlek, H.; Graziani, A.; Kosma, P. *Carbohydr. Res.* **2005,** *340*, 2808.

175. Tanemura, K.; Suzuki, T.; Horaguchi, T. *J. Chem. Soc. , Chem. Commun.* **1992,** 979.

176. García Fernández, J. M.; Ortiz Mellet, C.; Moreno Marín, A.; Fuentes, J. *Carbohydr. Res.* **1995,** *274*, 263.

177. Li, Y.; Manickam, G.; Ghoshal, A.; Subramaniam, P. *Synth. Commun.* **2006,** *36*, 925.

178. Hartung, W. H.; Simonoff, R. In *Organic Reactions*, John Wiley & Sons: 1953; pp 263-326.

179. Patil, V. J. *Tetrahedron Lett.* **1996,** *37*, 1481.

180. Nakajima, N.; Horita, K.; Abe, R.; Yonemitsu, O. *Tetrahedron Lett.* **1988,** *29*, 4139.

181. Karst, N.; Jacquinet, J.-C. *Eur. J. Org. Chem.* **2002,** *2002*, 815.

182. Johansson, R.; Samuelsson, B. *J. Chem. Soc. , Chem. Commun.* **1984,** *1984*, 201.

183. Bisht, K. S.; Bhatt, S.; Muppalla, K. *Tetrahedron Lett.* **2006,** *47*, 8645.

184. Neises, B.; Steglich, W. *Angew. Chem. , Int. Ed.* **1978,** *17*, 522.

185. Jiang, W.; Wanner, J.; Lee, R. J.; Bounaud, P. Y.; Boger, D. L. *J. Am. Chem. Soc.* **2003,** *125*, 1877.

186. DeTar, D. F.; Silverstein, R. *J. Am. Chem. Soc.* **1966,** *88*, 1020.

187. DeTar, D. F.; Silverstein, R. *J. Am. Chem. Soc.* **1966,** *88*, 1013.

188. Iwasawa, T.; Wash, P.; Gibson, C.; Rebek, J. *Tetrahedron* **2007,** *63*, 6506.

189. Hudson, D. *J. Org. Chem.* **1988,** *53*, 617.

190. Chaudhary, S. K.; Hernandez, O. *Tetrahedron Lett.* **1979,** *20*, 99.

191. Czernecki, S.; Georgoulis, C.; Provelenghiou, C. *Tetrahedron Lett.* **1976,** *17*, 3535.

192. Morère, A.; Menut, C.; Vidil, C.; Skaanderup, P.; Thorsen, J.; Roque, J. P.; Montero, J. L. *Carbohydr. Res.* **1997,** *300*, 175.

193. Zottola, M. A.; Alonso, R.; Vite, G. D.; Fraser-Reid, B. *J. Org. Chem.* **1989,** *54*, 6123.

194. Kruizinga, W. H.; Kellogg, R. M. *J. Am. Chem. Soc.* **1981,** *103*, 5183.

195. Fürstner, A.; Jeanjean, F.; Razon, P.; Wirtz, C.; Mynott, R. *Chem. Eur. J.* **2002,** *9*, 307.

196. Werz, D. B.; Seeberger, P. H. *Chem. Eur. J.* **2005,** *11*, 3194.

197. Ernst, B.; Hart, G. W.; Sinay, P. *Carbohydrates in Chemistry and Biology;* Wiley-VCH: 2000; Vol. 4.

198. Davis, B. G.; Fairbanks A.J. In *Carbohydrate Chemistry*, Oxford Science Publications: 2002; pp 78-89.

199. Dube, D. H.; Bertozzi, C. R. *Nat. Rev. Drug Discovery* **2005,** *4*, 477.

200. Dube, D. H.; Bertozzi, C. R. *Curr. Opin. Chem. Biol.* **2003,** *7*, 616.

201. Pratt M.A.; Bertozzi C.R. *Chem. Soc. Rev.* **2005,** *34*, 58.

202. Varki, A. *FASEB J.* **1997,** *11*, 248.

203. Münster-Kühnel, A. K.; Weinhold, B.; Ritz-Sedlacek, V.; Gerardy-Schahn, R.; Tiralongo, J.; Krapp, S.; Jacob, U. *Glycobiology* **2004,** *14*.

204. Tanner, M. E. *Bioorg. Chem.* **2005,** *33*, 216.

205. Traving, C.; Schauer, R. *Cell. Mol. Life Sci.* **1998,** *54*, 1330.

206. Sato, C. *TIGG* **2004,** *16*, 331.

207. Schauer, R. *Trends Biochem. Sci.* **1985,** *10*, 357.

208. Roseman, S.; Jourdian, G. W.; Watson, D.; Rood, R. *PNAS* **1961,** *47*, 958.

209. Kean, E. L.; Münster-Kühnel, A. K.; Gerardy-Schahn, R. *Biochim. Biophys. Acta* **2004,** *1673*, 56.

210. Harduin-Lepers, A.; Vallejo-Ruiz, V.; Krzewinski-Recchi, M. A.; Samyn-Petit, B.; Julien, S.; Delannoy, P. *Biochimie* **2001,** *83*, 727.

211. Jeanneau, C.; Chazalet, V.; Imberty, A.; Breton, C.; Augé, C.; Soumpasis, D. M.; Harduin-Lepers, A.; Delannoy, P. *J. Biol. Chem.* **2004,** *279*, 13461.

212. Takashima, S.; Tsuji, S.; Tsujimoto, M. *J. Biol. Chem.* **2002,** *277*, 45719.

213. Takashima, S.; Ishida, H. k.; Inazu, T.; Ando, T.; Ishida, H.; Kiso, M.; Tsuji, S.; Tsujimoto, M. *J. Biol. Chem.* **2002,** *277*, 24030.

214. Angata, K.; Suzuki, M.; Fukuda, M. *J. Biol. Chem.* **1998,** *273*, 28524.

215. Angata, K.; Fukuda, M. *Biochimie* **2003,** *85*, 195.

216. Angata, K.; Suzuki, M.; Fukuda, M. *J. Biol. Chem.* **2002,** *277*, 36808.

217. Nelson, R. W.; Bates, P. A.; Rutishauser, U. *J. Biol. Chem.* **1995,** *270*, 17171.

218. Mühlenhoff, M.; Eckhardt, M.; Bethe, A.; Frosch, M.; Gerardy-Schahn, R. *EMBO J.* **1996,** *15*, 6943.

219. Close, B. E.; Colley, K. J. *J. Biol. Chem.* **1998,** *273*, 34586.

220. Close, B. E.; Tao, K.; Colley, K. J. *J. Biol. Chem.* **2000,** *275*, 4484.

221. Bruses, J. L.; Rutishauser, U. *Biochimie* **2001,** *83*, 635.

222. Kiss, J. Z.; Rougon, G. *Curr. Opin. Neurobiol.* **1997,** *7*, 640.

223. Rafuse, V. F.; Landmesser, L. *J. Cell Biol.* **1996,** *132*, 969.

224. Rutishauser, U. *Curr. Opin. Cell Biol.* **1996,** *8*, 679.

225. Cremer, H.; Lange, R.; Christoph, A.; Plomann, M.; Vopper, G.; Roes, J.; Brown, R.; Baldwin, S.; Kraemer, P.; Scheff, S.; Barthels, D.; Rajewsky, K.; Wille, W. *Nature* **1994,** *367*, 455.

226. Petridis, A. K.; El Maarouf, A.; Rutishauser, U. *Dev. Dyn.* **2004,** *230*, 675.

227. Becker, C. G.; Becker, T.; Schachner, M.; Welzl, H.; Gerardy-Schahn, R.; Artola, A. *J. Neurosci. Res.* **1996,** *45*, 143.

228. Muller, D.; Toni, N.; Wang, C.; Calaora, V.; Kiss, J. Z.; Skibo, G.; Cremer, H.; Rougon, G. *Neuron* **1996,** *17*, 413.

229. Angata, K.; Long, J. M.; Bukalo, O.; Lee, W.; Dityatev, A.; Wynshaw-Boris, A.; Schachner, M.; Fukuda, M.; Marth, J. D. *J. Biol. Chem.* **2004,** *279*, 32603.

230. Mühlenhoff, M.; Eckhardt, M.; Gerardy-Schahn, R. *Curr. Opin. Struct. Biol.* **1998,** *8*, 558.

231. Roth, J.; Zuber, C.; Wagner, P.; Taatjes, D. J.; Weisgerber, C.; Heitz, P. U.; Goridis, C.; Bitter-Suermann, D. *Proc. Natl. Acad. Sci. U. S. A.* **1988,** *85*, 2999.

232. Livingston, B. D.; Jacobs, J. L.; Glick, M. C.; Troy, F. A. *J. Biol. Chem.* **1988,** *263*, 9443.

233. Komminoth, P.; Roth, J.; Lackie, P. M.; Bitter-Suermann, D.; Heitz, P. U. *Am. J. Pathol.* **1991,** *139*, 297.

234. Scheidegger, E. P.; Lackie, P. M.; Papay, J.; Roth, J. *Lab. Invest.* **1994,** *70*, 95.

235. Tanaka, F.; Otake, Y.; Nakagawa, T.; Kawano, Y.; Miyahara, R.; Li, M.; Yanagihara, K.; Nakayama, J.; Fujimoto, I.; Ikenaka, K.; Wada, H. *Cancer Res.* **2000,** *60*, 3072.

236. Tanaka, F.; Otake, Y.; Nakagawa, T.; Kawano, Y.; Miyahara, R.; Li, M.; Yanagihara, K.; Inui, K.; Oyanagi, H.; Yamada, T.; Nakayama, J.; Fujimoto, I.; Ikenaka, K.; Wada, H. *Cancer Res.* **2001,** *61*, 1666.

237. Seidenfaden, R.; Gerardy-Schahn, R.; Hildebrandt, H. *Eur. J. Cell Biol.* **2000,** *79*, 680.

238. Jimbo, T.; Nakayama, J.; Akahane, K.; Fukuda, M. *Int. J. Cancer* **2001,** *94*, 192.

239. Daniel, L.; Durbec, P.; Gautherot, E.; Rouvier, E.; Rougon, G.; Figarella-Branger, D. *Oncogene* **2001,** *20*, 997.

240. Daniel, L.; Trouillas, J.; Renaud, W.; Chevallier, P.; Gouvernet, J.; Rougon, G.; Figarella-Branger, D. *Cancer Res.* **2000,** *60*, 80.

241. Glüer, S.; Zense, M.; Radtke, E.; Von Schweinitz, D. *Langenbeck's Arch. Surg.* **1998,** *383*, 340.

242. Poongodi, G. L.; Suresh, N.; Gopinath, S. C. B.; Chang, T.; Inoue, S.; Inoue, Y. *J. Biol. Chem.* **2002,** *277*, 28200.

243. Blaheta, R.; Daher, F.; Michaelis, M.; Hasenberg, C.; Weich, E.; Jonas, D.; Kotchetkov, R.; Doerr, H.; Cinatl, J. *BMC Cancer* **2006,** *6*, 294.

244. Seidenfaden R.; Krauter A.; Schertzinger F.; Gerardy-Schahn R.; Hildebrandt H. *Mol. Cell. Biol.* **2003,** *23*, 5908.

245. Lantuejoul, S.; Moro, D.; Michalides, R. J. A. M.; Brambilla, C.; Brambilla, E. *Am. J. Surg. Pathol.* **1998,** *22*, 1267.

246. Michalides, R.; Kwa, B.; Springall, D.; Van, Z. N.; Koopman, J.; Hilkens, J.; Mooi, W. *Int. J. Cancer* **1994,** *57*, 34.

247. Sethi, T.; Rintoul, R. C.; Moore, S. M.; MacKinnon, A. C.; Salter, D.; Choo, C.; Chilvers, E. R.; Dransfield, I.; Donnelly, S. C.; Strieter, R.; Haslett, C. *Nature Med.* **1999,** *5*, 662.

248. Suzuki, M.; Suzuki, M.; Nakayama, J.; Suzuki, A.; Angata, K.; Chen, S.; Sakai, K.; Hagihara, K.; Yamaguchi, Y.; Fukuda, M. *Glycobiology* **2005,** *15*, 887.

249. Schreiber, S. C.; Giehl, K.; Kastilan, C.; Hasel, C.; Mühlenhoff, M.; Adler, G.; Wedlich, D.; Menke, A. *Gastroenterology* **2008,** *134*, 1555.

250. Perl, A. K.; Wilgenbus, P.; Dahl, U.; Semb, H.; Christofori, G. *Nature* **1998,** *392*, 190.

251. Charter, N. W.; Mahal, L. K.; Koshland, D. E., Jr.; Bertozzi, C. R. *Glycobiology* **2000,** *10*, 1049.

252. Mahal L.K.; Charter N.W.; Angata K.; Fukuda M.; Koshland Jr.D.E.; Bertozzi C.R. *Science* **2001,** *294*, 380.

253. Charter N.W.; Mahal Lara K.; Koshland, D. E., Jr.; Bertozzi, C. R. *J. Biol. Chem.* **2002,** *277*, 9255.

254. Horstkorte, R.; Reutter, W.; Nöhring, S.; Zimmermann-Kordmann, M.; Mühlenhoff, M.; Gerardy-Schahn, R. *Exp. Cell Res.* **2004,** *298*, 268.

255. Kanie, O.; Nakamura, J.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1987,** *6*, 105.

256. Smalec, B.; von Itzstein, M. *Carbohydr. Res.* **1995,** *266*, 269.

257. Hatanaka, Y.; Hashimoto, M.; Hidari, K. I. P. I.; Sanai, Y.; Nagai, Y.; Kanaoka, Y. *Heterocycles* **1996,** *43*, 531.

258. Schaub, C.; Muller, B.; Schmidt, R. R. *Glycoconjugate Journal* **1998,** *15*, 345.

259. Tanaka, T.; Ozawa, M.; Miura, T.; Inazu, T.; Tsuji, S.; Kajimoto, T. *Synlett* **2002.**

260. Grice, I. D.; Whelan, C.; Tredwell, G. D.; von Itzstein, M. *Tetrahedron: Asymmetry* **2005,** *16*, 1425.

261. Hasegawa, A.; Nakamura, J.; Kiso, M. *J. Carbohydr. Chem.* **1986,** *5*, 11.

262. Cohen, S. B.; Halcomb, R. L. *J. Org. Chem.* **2000,** *65*, 6145.

263. Falconer, R. A.; Jablonkai, I.; Toth, I. *Tetrahedron Lett.* **1999,** *40*, 8663.