SYNTHESIS OF INHIBITORS FOR ER α-MANNOSIDASE I AND STEREOSELECTIVE GLYCOSYLATIONS USING (R)- OR (S)-(ETHOXYCARBONYL) BENZYL CHIRAL AUXILIARIES AT C-2 OF GLYCOPYRANOSYL DONORS

by

VISHAL KHOT

(Under the Direction of GEERT-JAN BOONS)

ABSTRACT

ER α–mannosidase I plays a pivotal role in the quality control of glycoprotein folding within the endoplasmic reticulum (ER), a process that is commonly referred to as endoplasmic reticulum associated degradation (ERAD). A considerable number of genetic diseases are associated to the proceseses of ERAD. Kifunensine and 1-deoxymannojirimycin are well known inhibitors of this enzyme but they also inhibit Golgi mannosidase I, an enzyme that is essential for the maturation of N-linked glycoproteins to hybrid and complex type glycoproteins. Using these compounds as lead compounds, analogues were synthesized to improve their inhibitory activity and selectivity for ER α-mannosidase I.

The stereoselective introduction of a glycosidic bond is one of the greatest challenges to complex oligosaccharide synthesis. Important developments such as

automated polymer supported oligosaccharide synthesis will not achieve their full potential until this problem is solved. Here, a novel approach for stereoselective glycosylations is described whereby a chiral auxiliary at C-2 of a glycosyl donor controls the anomeric outcome of a glycosylation. It was found that participation of an (*S*) ethoxycarbonylbenzyl auxiliary led to the formation of 1,2-*cis* glycosides, probably through a *trans*-fused dioxolenium ion intermediate. On the other hand, the use of an auxiliary with (*R*) configuration formed 1,2-*trans* glycosides, and this glycosylation proceeds through a *cis*-fused dioxolenium ion intermediate

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DEDICATION

Dedicated to my Parents

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I would like to thank Prof. Geert-Jan Boons for giving me the opportunity to work in his research group, as well as his strong support and very helpful advice during my M.S. study time.

Many thanks to the past and present members of the Boons Group and also the staff of CCRC. Because of their help and kindness, all these years in CCRC is a memorable time in my life.

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LIST OF ABBREVIATIONS

Ac……Acetyl

Ac_2O……..Acetic Anhydride

$BF_3.Et_2O$……..Boron trifluoro diethyl etherate

Bn……Benzyl

Bz……..Benzoyl

C…….Celcius

DBU……1,8-Diazabicyclo[5.4.0]undec-7-ene

DCM……methylene chloride

DMAP…….N,N-Dimethylaminopyridine

DMF……..N,N-Dimethylformamide

Eq……..equivalent

Et……ethyl

Et_2O……...Diethyl ether

EtOAc……Ethyl acetate

EtOH……..Ethanol

Gal……..Galactose

Glc…….Glucose

h…….Hour

Hz……..Hertz

m……..multiplet

m/z……..…Mass to charge ratio

MALDI-TOF……..Mass assisted laser desorption ionization time of flight

Me…….....methyl

MeOH…….Methanol

Min…….Minute

mmol……...millimole

MS……...Molecular sieves

NMR…….Nuclear magnetic resonance

NaN_3…….Sodium azide

NaOMe……Sodium methoxide

Ph……Phenyl

s……...Singlet

t……Triplet

TBAF…….Tetrabutylammonium fluoride

TFA……Trifluoroacetic acid

THF……..Tetrahydrofuran

TMSOTf……..Trimethylsilyl trifluoromethane sulfonate

TLC……Thin layer chromatography

KIF……..Kifunensine

DMJ……1-Deoxymannojirimycin

CHAPTER 1

INTRODUCTION

N-Glycosylation:

1.1. Biosynthesis of *N*-linked oligosaccharides

The biosynthesis of *N*-linked oligosaccharides involves two very distinct processes. The first step gives rise to a lipid-linked oligosaccharide, which is then transferred to the protein chain while the protein is being synthesized on membrane bound ribosomes.[1] In contrast, the second series of reactions involves the alteration of the oligosaccharide chain by the removal of some sugars and the addition of others, to give a large number of different oligosaccharide structures.[2] Figure **1** shows the reactions leading to the production of the final lipid-linked oligosaccharide precursor.

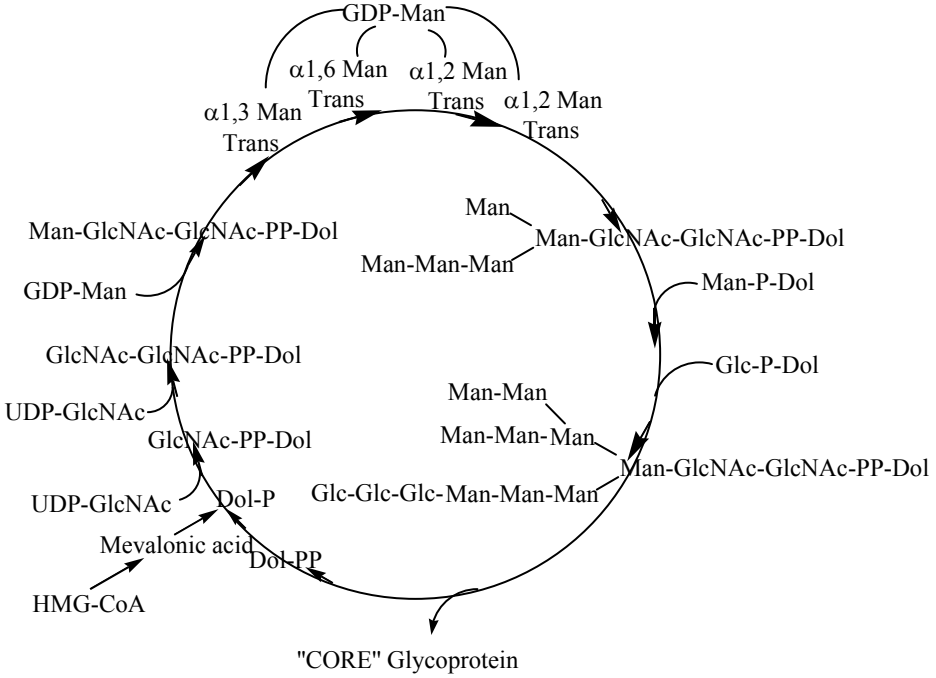


Figure **1**

The assembly of the *N*-linked oligosaccharide chain is initiated in the endoplasmic reticulum (ER) by the transfer of a GlcNAc-1-P from UDP-GlcNAc to dolichyl-P to form the GlNAc-PP-dolichol as shown in the figure.[3] A second GlcNAc is then

added, also from UDP-GlcNAc, to generate GlcNAcβ–1,4GlcNAc-PP-dolichol.[4] Then, five mannose residues are added as α-glycosidic linkages and all arise from GDP-mannose to provide the important intermediate, Man_5-$(GlcNAc)_2$-PP-dolichol.[5] After adding the first seven sugars to give Man_5-$(GlcNAc)_2$-PP-dolichol, the lipid-linked oligosaccharide will undergo a 'flip' in the membrane, so that the oligosaccharide chain becomes inserted into the lumen of the ER.[6] The assembly of the oligosaccharide is finished by the addition of four more mannose residues followed by three glucose units to give a $Glc_3Man_9(GlcNAc)_2$-PP-dolichol.[7] The finishing step in this route is the transfer of the $Glc_3Man_9(GlcNAc)_2$ from its lipid carrier to specific aspargine residues on the ribosome-bound protein, catalyzed by the enzyme oligosaccharyltransferase.[8,9]

1.2. Processing of *N*-linked oligosaccharides

After the oligosaccharide is transferred to the protein, the oligosaccharide begins numerous trimming or processing reactions. The early reactions in the second pathway include the removal of three glucosides and up to six mannosides, while later processing reactions involve the addition of a number of other monosaccharides, principally GlcNAc, galactose and neuraminic acid.[10] Figure **2** outlines the processing pathway.

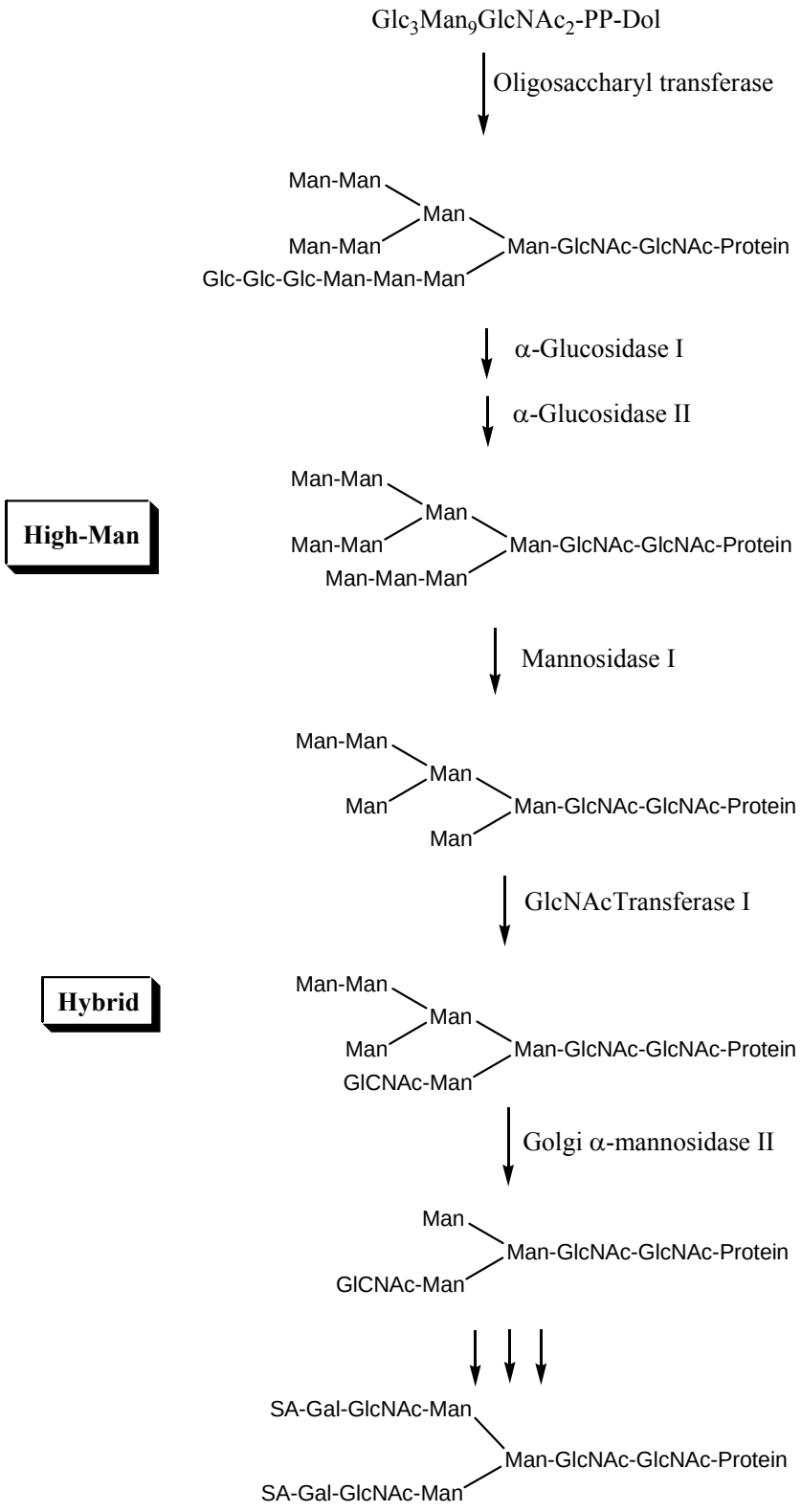


Figure **2** Processing Pathway of N-Linked Oligosaccharides

The initial processing step involves glucosidase I, which removes the outermost α1,2-linked glucoside.[11] Glucosidase II, a second glucosidase positioned in the ER, removes the other two α1,3-linked glucosides to give a $Man_9(GlcNAc)_2$-protein.[12]

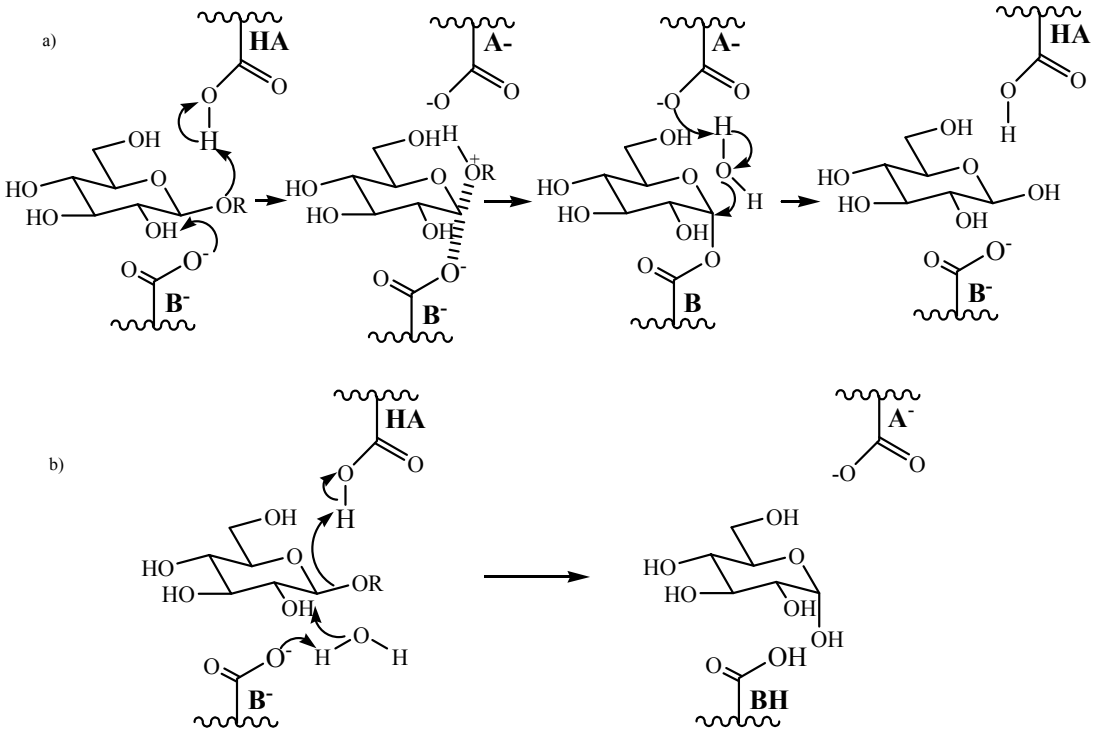
The ER contains the protein calnexin that functions to assist the folding of newly synthesized proteins into their appropriate conformation, a step that is apparently crucial for the transfer of many of these proteins to the Golgi apparatus at the proper rate.[13] The ER also contains a safety mechanism to ensure that improperly folded or unfolded glycoproteins exit from the ER and into the Golgi apparatus. In this regard, the ER contains an unusual glycosyltransferase that functions to transfer a glucose from UDP-glucose to high mannose chains on unfolded or denatured, but not native, glycoproteins.[14] Once this glucoside has been added, calnexin can identify and assist the protein in its proper folding and transfer to the Golgi.[15] As a consequence, a glycoprotein that has had all of its three glucosides removed by glucosidase I and II but has failed to fold into the appropriate conformation can be reglucosylated by this enzyme, and the signal of which then provides the protein another chance to interact with calnexin and fold correctly. This mechanism, involving the removal of glucoses by the glucosidases and reglucosylation by the glucosyl transferase, is postulated to be part of a distinctive 'glycoprotein-specific folding and quality control mechanism' in the ER that allows the organelle to be in charge of and pass properly folded glycoproteins on to the next step in transport and processing. Once the two glucosidases have detached all three glucoses from the *N*-linked oligosaccharide as shown in figure, several α-mannosidases can remove one or more of the four α1,2-linked mannose residues to eventually give a Man_5-$(GlcNAc)_2$-protein.[16] After removal of the four α1,2-linked mannose units , the Man_5-$(GlcNAc)_2$-protein is a

substrate for GlcNAc transferase I, a glycosyl transferase in the medial Golgi stacks, that transfers a GlcNAc from UDP-GlcNAc to the mannoside on the α1,3-branch to give GlcNAc- Man_5-$(GlcNAc)_2$-protein.[17, 18] Once the GlcNAc has been added to the 3-linked mannose, mannosidase II can take away the two mannosides that are linked to the α1,6-linked mannose branch. Following the action of the diverse glycosidases in the trimming part of the pathway, a number of glycosyltransferases act on the $GlcNAcMan_3(GlcNAc)_2$-protein to produce complex types of *N*-linked oligosachharides. Thus, in the trans-Golgi apparatus, there are numerous GlcNAc transferases, galactosyltransferases, fucosyltransferases, and sialyltranferases, that can attach these sugars to the *N*-linked chains to yield a great diversity of complex chains having biantennary, triantennary or tetraantennary structures.

2. Glycosidase Inhibitors

2.1. Glycosidases

Glycosidases play a very important role in various biological processes, such as intestinal digestion, post-translational modification of glycoproteins and the lysosomal catabolism of glycoconjugates.[19] The prospect of modifying or blocking these processes by using glycosidase inhibitors has attracted a lot of attention, since some sugar-mimic alkaloids show potential antidiabetic, antiviral and anticancer effects.[20] The enzymatic cleavage of the glycosidic bond liberates a sugar hemicaetal with either retention or inversion of the substrate. (Scheme I)[21]



Scheme I : a) Catalytic mechanism for configuration retaining glycosidases

b) Catalytic mechanism for configuration inverting glycosidases

2.2 Digestive α–Glucosidase Inhibitors (Antidiabetic agents)

α–Glucosidase inhibitors are oral anti-diabetic drugs used for the treatment of type II *diabetes mellitus* that work by preventing the digestion of carbohydrates such as starch and table sugar. Carbohydrates are normally converted into simple sugars (monosaccharides) which are absorbed by the intestine. Hence, α–glucosidase inhibitors reduce the impact of carbohydrates in blood sugar. In 1966, nojrimycin (**1**) was discovered as the first natural glucose mimic. First described as an antibiotic produced by *Streptomyces roseochromogenes* R-468 and S.*lavendulae* SF-425, nojirimycin was

shown to be a potent inhibitor of α- and β-glucosidases from various sources. However, this iminosugar with a hydroxyl group at C-1 is fairly unstable and can be reduced by sodium borohydride to 1-deoxynojirimycin (**2**). 1-deoxynojirimycin has excellent α-glucosidase inhibitory activity *in vitro*, but its efficacy *in vivo* was only moderate. Hence, a large number of deoxynojirimycin derivatives were prepared to increase *in vivo* activity. Thus, miglitol (**3**) was selected as the most favorable inhibitor. In 1996, miglitol (Glyset) was granted market clearance by the FDA and launched in 1999 as a more potent α-glucosidase inhibitor for the treatment of type II *diabetes mellitus.*

In the 1970s, it was realized that inhibition of all or some of the intestinal disaccharides by inhibitors could control the absorption of carbohydrates and these inhibitors could be used therapeutically for the treatment of non-insulin dependent diabetes. Researchers at Bayer discovered that the *Actinoplanes* strain SE 50 yields a potent sucrase inhibitor, acarbose (**4**) which inhibits pig intestinal sucrase with an IC_{50} value of 0.5 μM. In 1990, acarbose (GLUCOBAY^{TM}) was introduced for the treatment of type-II (non-insulin dependent) diabetes. In 1984, valiolamine (**5**) was reported to be a potent inhibitor of pig intestinal maltase and sucrase with IC_{50} values of 2.2 and 0.049 μM respectively. Various *N*-substituted valiolamine derivatives were synthesized to increase its inhibitory activity *in vitro* and the very simple derivative voglibose (**6**), which was obtained by reductive amination of valiolamine with dihydroxyacetone, was selected as the potential oral diabetic agent. In 1994, voglibose (BASEN^{TM}) was commercially available for the treatment of diabetes in Japan.

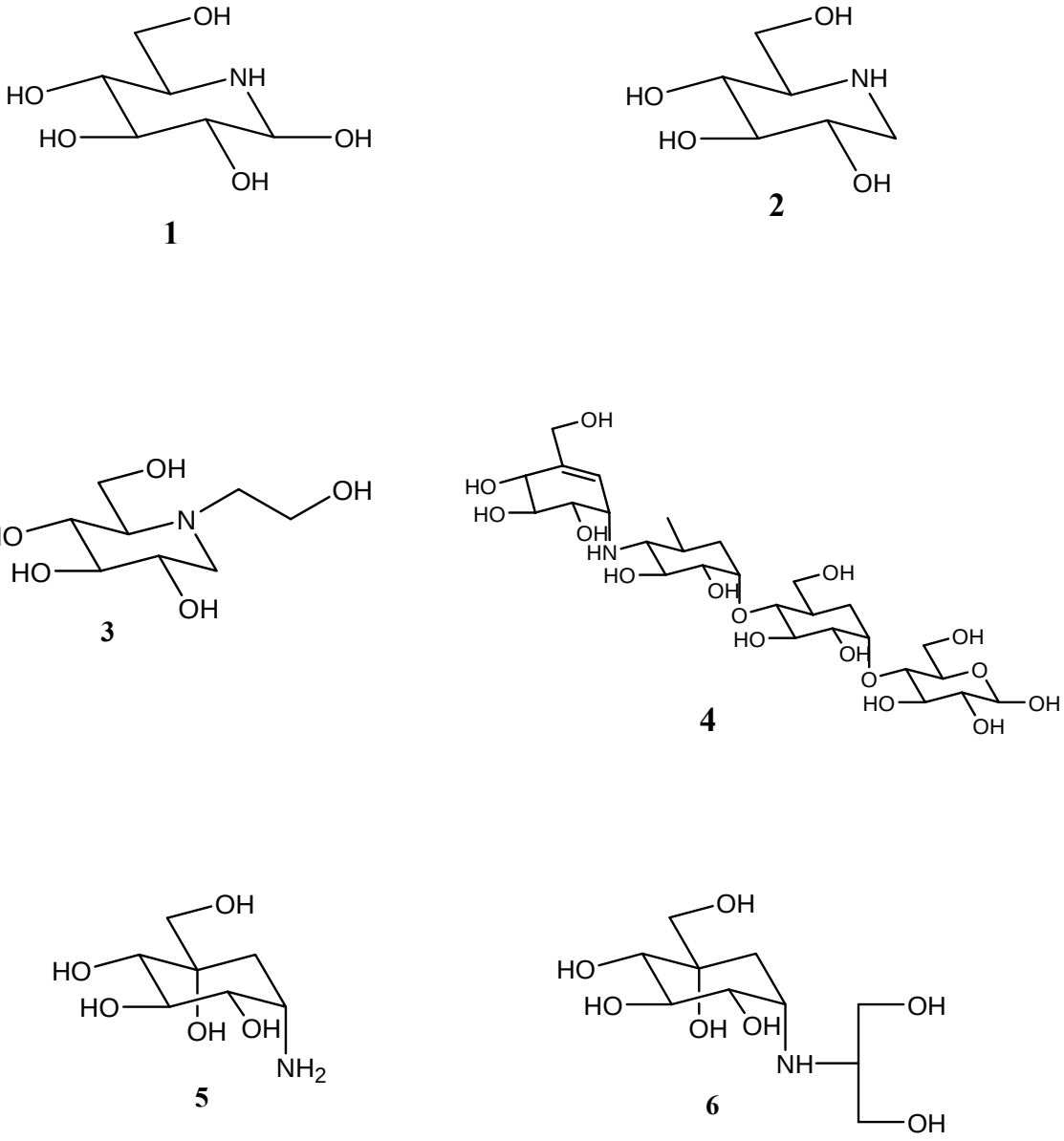
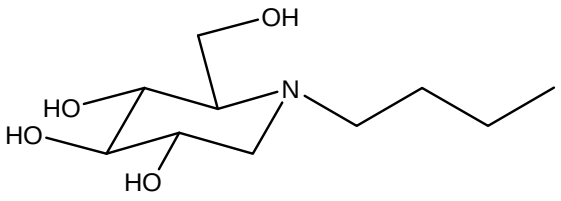


Figure **3** α−glucosidase inhibitors

2.3. Ceramide glycosyl transferase (glucosyl ceramide synthase) inhibitors: substrate deprivation therapy for lysosomal storage diseases.

Ceramide glucosyltransferase (or glucosylceramide synthase) is a glucosyltransferase enzyme involved in the production of glucosylceramide**. Gaucher's disease** is the most common of the lysosomal storage diseases, and is caused by a deficiency of the enzyme glucocerebrosidase, which leads to an accumulation of its substrate, the fatty substance glucocerebroside (also known as glucosylceramide). ZAVESCA® (**7**), an *N*-alkylated imino sugar, a synthetic analogue of D-glucose, is an inhibitor of the enzyme glucosylceramide synthase. In 2002, Zavesca received a positive review recommending approval in the European Union for use in patients with mild to moderate type I Gaucher's disease.[22]



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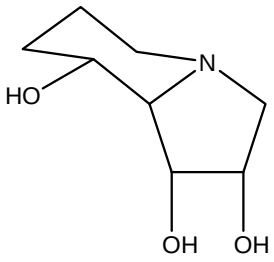
2.4. α–Mannosidase Inhibitors

A number of α-mannosidase inhibitors have been obtained from natural sources or synthesized chemically. The first reported glycoprotein processing α-mannosidase inhibitor was the indolizidine alkaloid swainsonine (**8**). This compound was originally shown to be an inhibitor of the lysosomal α–mannosidase and when administered to

animals caused symptoms similar to those of the lysosomal storage disease α-mannosidosis.

Deoxymannojirimycin (**9**) was first isolated from the seeds of the legume *Lonchocarpus sericeus*. In animal cells, Deoxymannojirimycin inhibited Golgi mannosidase IA/B and caused the accumulation of glycoproteins having a high mannoside sugar. Mannojirimycin (**10**) has been proposed as an intermediate in the biosynthesis of Deoxymannojirimycin.

Kifunensine (**11**) is an alkaloid produced by the actinomycete, *Kitasatsporia kifunese* and corresponds in structure to the cyclic oxamide derivative of 1-amino deoxymannojirimycin. This alkaloid is a potent inhibitor of the Golgi mannosidase I, but like deoxymannojirimycin is a very weak inhibitor of jack bean α-mannosidase. However, Golgi mannosidase I inhibition by kifunensine is 100 times greater than by deoxymannojirimycin.[23]



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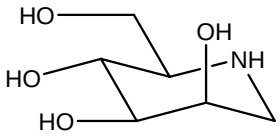

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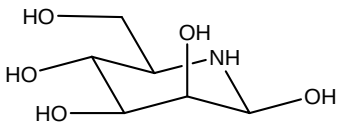

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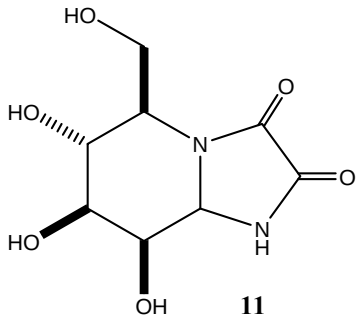

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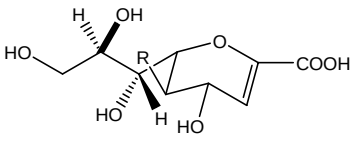
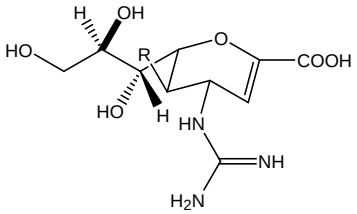
2.5. Neuraminidase inhibitors

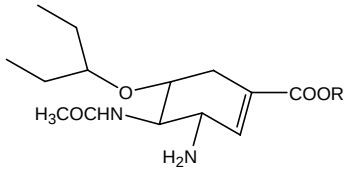
Neuraminidase inhibitors are a class of antiviral drugs targeted at the *influenza* viruses. The mode of action of these inhibitors consists of blocking the function of the viral neuraminidase protein, thus preventing the virus from budding from the host cell (reproducing). The first described substrate-based neuraminidase inhibitor was 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (DANA) (**12**). Introduction of the trifluoroacetamido group in place of the acetamido of DANA to give 2-deoxy-2,3-dihydro-*N*-trifluoroacetylneuraminic acid (FANA) (**13)** resulted in a slight improvement in inhibitory activity towards *influenza* neuraminidase. Using the crystal structure of neuraminidase and DANA as a starting point, the researchers at the Victorian College of Pharmacy, Monash University employed a computer-aided process in an attempt to design a molecule which was a better fit for (and therefore inhibited) the active site of neuraminidase. Zanamivir (**14**), a transition-state analogue inhibitor of neuraminidase, was the result. Oseltamivir carboxylate (**15**), a carbocyclic zanamivir mimetic, inhibits *influenza* neuraminidase with equal potency to zanamivir. and was approved by the FDA in 1999. Oseltamivir carboxylate suffers from low oral bioavailability due to a negatively charged carboxylate moiety. Hence, oseltamivir (Tamiflu) (**16**), the ethyl ester of **15**, was developed to improve oral bioavailability. Oseltamivir is a prodrug metabolized by liver esterases to its active form **15**.[23]



12 R = $NHCOCH_3$
13 R = $NHCOCF_3$



14 R = $NHCOCH_3$



15 R = H
16 R = CH_2CH_3

3. ER QUALITY CONTROL

Kifunensine is a potent naturally occurring class I α-mannosidase inhibitor. Kifunensine inhibits both human ER α-1,2-mannosidase I (ER Man I) and Golgi α-mannosidase IA,IB and IC (Golgi Man IA/IB/IC). ER Man I plays an important role in the quality control of glycoprotein folding within the ER, a process that is commonly referred to as Endoplasmic Reticulum Associated Degradation (ERAD). A large number of genetic diseases are related to the processes of ERAD.

Recently, several studies have verified that kifunensine and deoxymannojirmycin inhibit ER Man I leading to blockage of the degradation of mutant glycoproteins including the T cell receptor subunit CD3-δ, tyrosinase, and α_2-plasmin inhbitor. In contrast, the disposal of misfolded glycoproteins was accelerated by the overexpression of ER Man I. Surprisingly, acceleration of the "disposal clock" by ER Man I overexpression also activated a large disposal of wild-type glycoproteins. Thus, a model was designed in which glycan trimming to $Man_8GlcNAc_2$ moieties in the context of partly folded or misfolded polypeptide structures acts as the rate-determining step in generating the signal for glycoprotein disposal. In this model, ER Man I occupies a unique intersection (Fig. 4) of both catabolic and glycoprotein biosynthetic pathways and defines the binary decision for the fate of nascent glycoproteins toward folding or disposal.

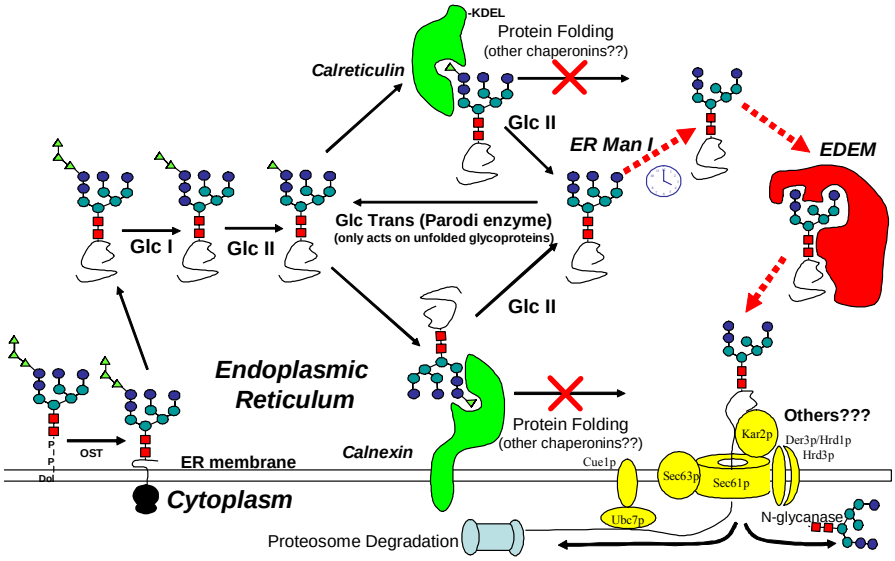


Fig **4** Processed glycans influence the rate and targeting of misfolded proteins for ERAD

These results indicate that inhibition of ER Man I may serve as a possible approach for the treatment of genetic diseases that take place as a direct result of the processes of ERAD. As a result of its potent inhibition of ER Man I, kifunensine and 1-deoxymannojirimycin deliver a superb means for the study and regulation of this important enzyme and its role in ERAD. However, kifunensine also inhibits Golgi Man I, an enzyme that is required for the maturation of *N*-linked glycoproteins into hybrid and complex type glycoproteins. Thus, there is a need to synthesize kifunensine analogues capable of selectively inhibiting ER Man I over Golgi Man I in order to successfully study the mechanism of ER Man I and to help in the development of novel therapeutic agents for the treatment of genetic diseases related to ERAD.[24]

Here, two strategies will be discussed.

1.] **Combinatorial modification of Deoxymannojirimycin and Kifunensine Analogues**

In the crystal structures, the O-2' and O-3' hydroxyls of both inhibitors coordinate with the calcium ion in the catalytic domain, they should interact.[25] The nitrogen atom of deoxymannojirimycin orients toward the outside of the cavity, and there are no interactions with other residues, so this nitrogen atom is a good handle for modification. Reductive amination can be carried out to generate various deoxymannojirimycin analogues and exploit the interactions with the binding site. In case of kifunensine, both the carbonyl groups point away from the catalytic domain. One of these carbonyl groups can be selectively modified to a hydrazone which can be further modified to a hydrazide and serves as an excellent scaffold for combinatorial library synthesis.

2.] **ER Retention Signal**

The carboxy-terminal tetrapeptide KDEL is present in many proteins resident in the lumenal ER. When transplanted into various transporter molecules, it localizes them to the ER, showing that it is both required and enough for this process.[26] So, this signal can be incorporated with DMJ to transport the inhibitor specifically to the ER thereby achieving selectivity. The peptide can be labeled with a fluorescent tag, rhodamine, for the purpose of cellular uptake studies.

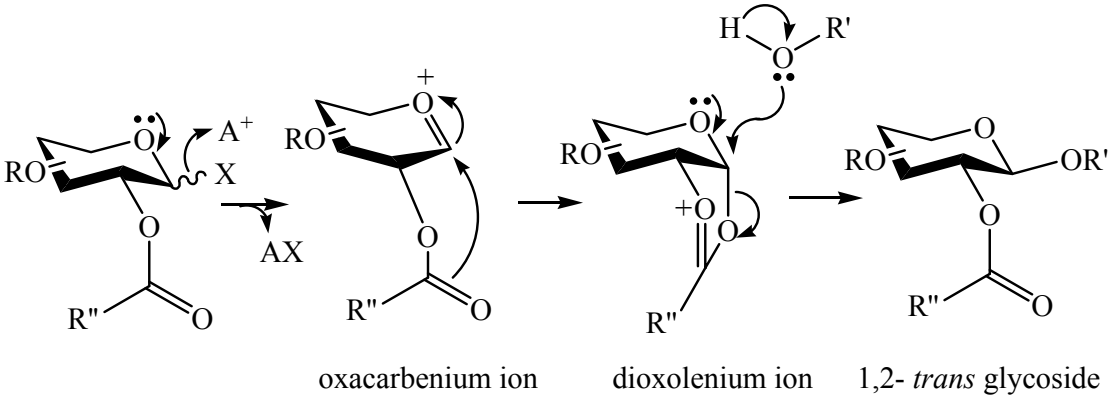
4. STEREOSELECTIVE CONSTRUCTION OF GLYCOSIDIC BONDS

The last two decades have seen dramatic improvements in the methods available for the synthesis of complex oligosaccharides. New anomeric leaving groups such as anomeric fluorides, trichloroacetimidates, and thioglycosides have been introduced which can be prepared under mild conditions, are sufficiently stable for purification, may be stored for a considerable period of time and undergo glycosylations under mild conditions. These beneficial features permit the synthesis of complex oligosaccharides by highly convergent strategies in which most synthetic efforts are directed towards the preparation of monomeric glycosyl acceptors and donors, which can be assembled into complex structures using a minimum number of synthetic steps. The synthesis of complex oligosaccharides has been further streamlined by one-pot multi-step glycosylations[27, 28] and automated polymer-supported syntheses,[29-31] which reduces the need for time-consuming purification protocols.

Despite these important developments, the problem remains that there is no general method for the preparation of complex oligosaccharides of biological importance. One of the main stumbling blocks in complex oligosaccharide synthesis is the formation of mixtures of α/β-anomers during glycosylations. Separation of these anomers needs time-consuming purification protocols resulting in loss of material. The formation of anomeric mixtures also strictly limits the use of one-pot multi-step glycosylations or polymer- supported syntheses.

Currently, the most reliable method for stereoselective glycosylations is based on neighboring-group participation by a 2-*O*-acyl functionality (Scheme I).[32] In these reactions, a promoter activates an anomeric leaving group resulting in its departure and

the formation of an oxacarbenium ion. Subsequent neighboring-group participation of the 2-*O*-acyl protecting group will give a more stable dioxolenium ion. This bicyclic intermediate will only be formed as a 1,2-*cis* isomer because the alternate 1,2-*trans* configuration will experience considerable ring strain. An alcohol can attack the anomeric center of the dioxolenium ion from only one face leading to the stereospecific formation of a 1,2-*trans* glycoside. Thus, in the case of glucosyl-type donors, β-linked products will be formed while mannosyl donors will give α-glycosides.

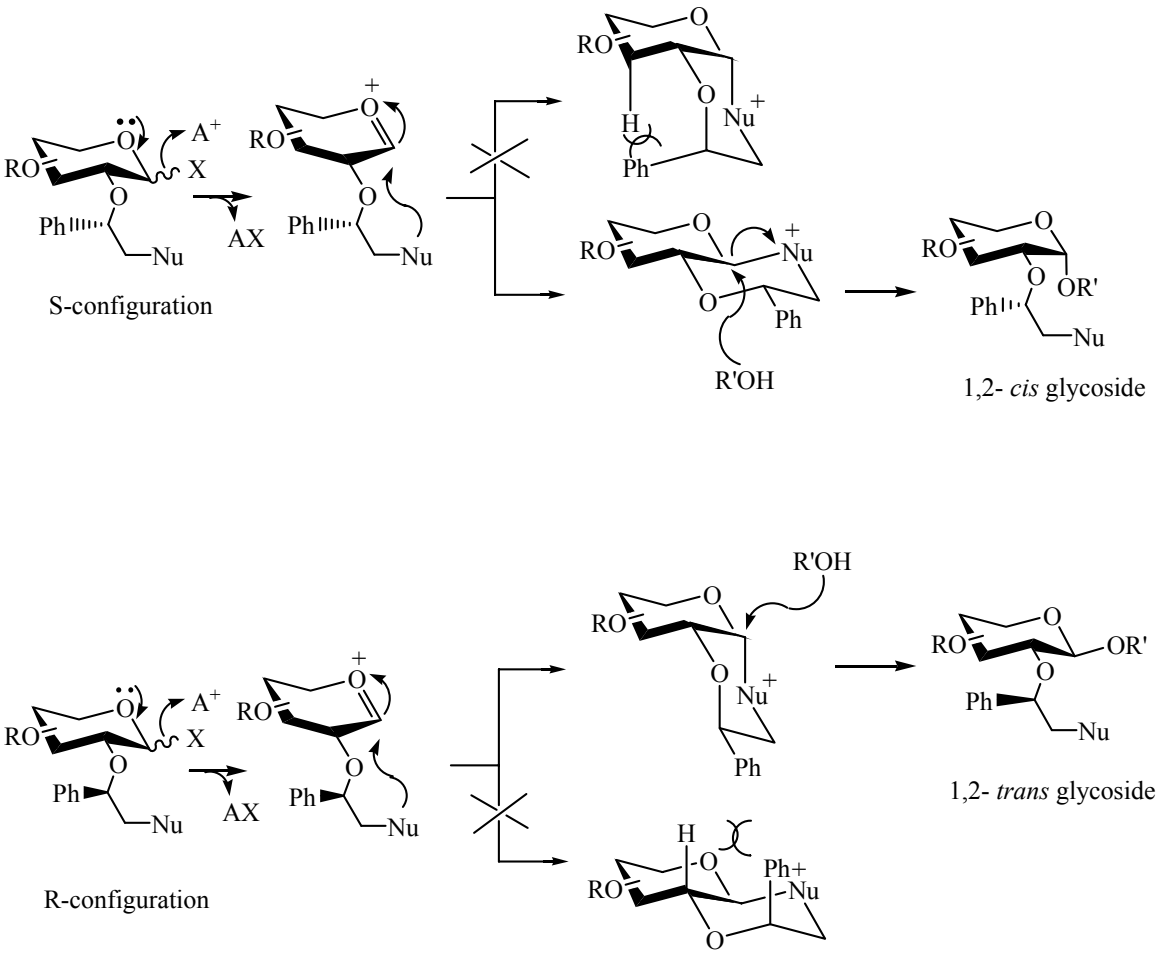


Scheme I Conventional approach for stereoselective glycosylation

Classical neighboring-group participation by C-2 ester leading to 1,2-*trans* glycosides.

The introduction of 1,2-*cis*-glycosidic linkages, such as α-glucosides and α-galactosides, requires glycosyl donors with a non-assisting functionality at C-2. Invariably, the use of these glycosyl donors leads to the formation of mixtures of anomers.[33,34] Thus, the stereoselective formation of 1,2-*cis* glycosides is the principal challenge of complex oligosaccharide synthesis.

Here, we describe a novel strategy for stereoselective glycosylations using a chiral auxiliary at C-2 of a glycosyl donor (Scheme IIb, c). The auxiliary is a C-1 substituted ethyl moiety that contains a nucleophilic group (Nu). Upon formation of an oxacarbenium ion, the nucleophilic moiety of the auxiliary will participate, leading to the formation of either a *trans*- or *cis*-decalin system. It is expected that an auxiliary with (*S*)-stereochemistry will lead only to the formation of *trans*-decalin because the alternate *cis*-fused system will place the phenyl substituent in an axial position inducing unfavorable steric interactions (Scheme II, b). Subsequent displacement of the anomeric moiety of the *trans*-decalin intermediate will lead to the formation of a 1,2- *cis*- glycoside. Alternatively, the use of an auxiliary with (*R*) stereochemistry will lead to the formation of a 1,2-*trans* glycoside because in this case the *trans*-decalin system will experience unfavorable steric interactions. Therefore glycosylation will only take place from the *cis*-decalin intermediate (Scheme II, c)



A = activating group, Nu = nucleophile, X = leaving group

Scheme II : New approach for stereoselective glycosylation.

(a) Neighboring-group participation by an (*S*)-auxiliary at C-2 leading to 1,2-*cis* glycosides. (b) Neighboring-group participation by an (*R*)-auxiliary at C-2 leading to 1,2-*trans* glycosides.

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CHAPTER 2

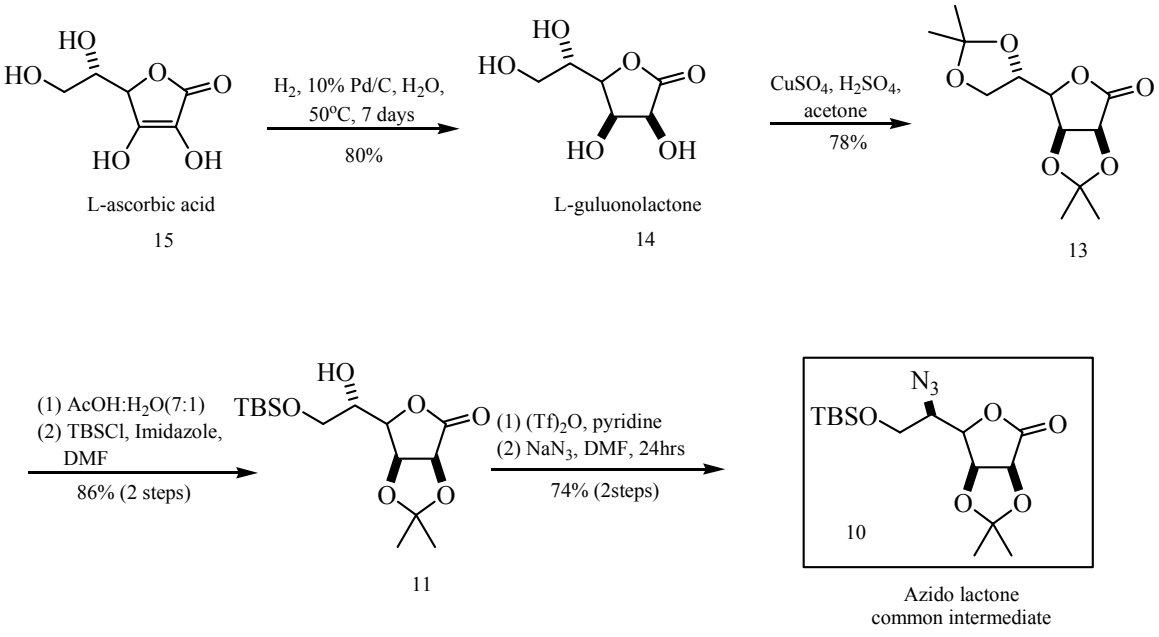
SYNTHESIS OF INHIBITORS FOR ER α-MANNOSIDASE I [1]

[1] Vishal Khot and Geert Jan Boons. To be submitted to *Journal of Organic Chemistry*.

Result and Discussion

1. Synthesis of Azido Lactone :

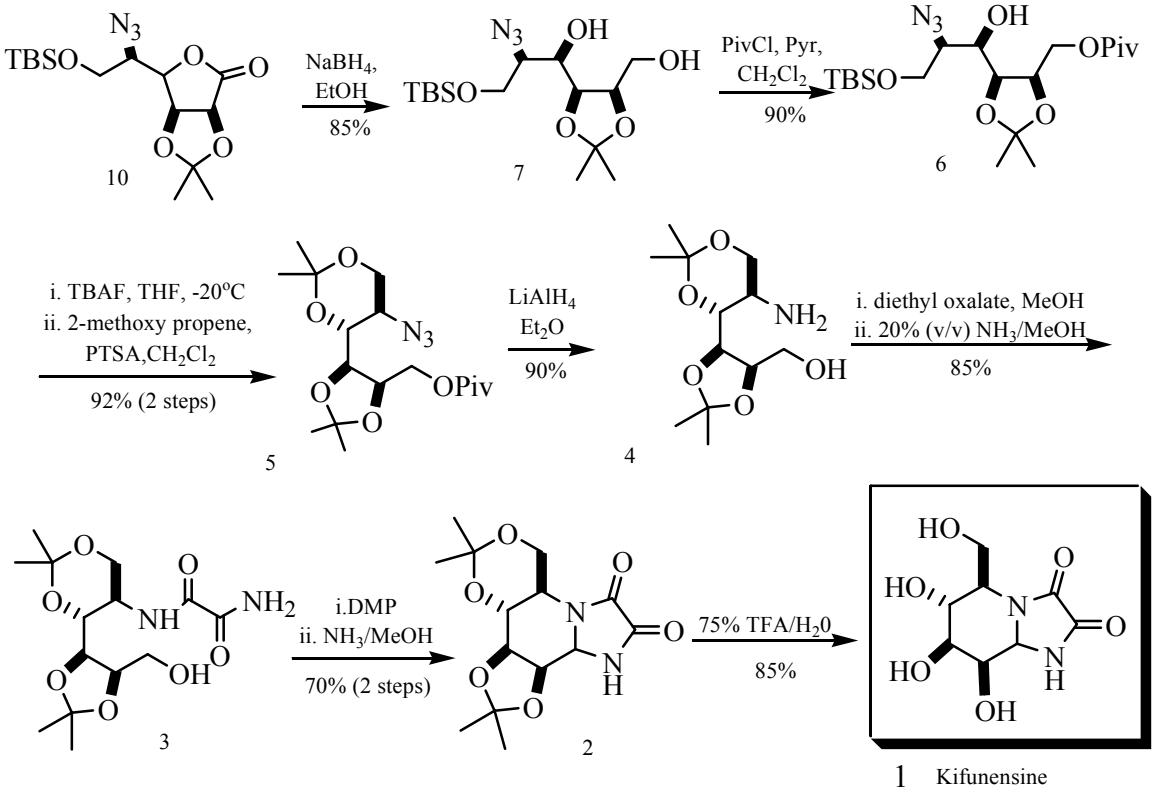
The synthesis of both 1-deoxymannojirimycin **2** and kifunensine **1** began with preparation of azidolactone (**10)** using previously reported methods[1,2] (Scheme 2). Hydrogenation of L-ascorbic acid **15** to L-gulonolactone **14** was accomplished in 80% yield. For the next step, L-gulonolactone **14** was treated with copper sulfate, sulfuric acid, and acetone to deliver the desired bisacetonide **13** in 78% yield. Deprotection of the side chain acetonide of **13** followed by selective protection of the resulting primary alcohol provided silyl-ether **11** in excellent yield. The alcohol **11** was converted to azide **10** first by formation of an triflate and subsequent inversion with sodium azide in 74% yield.



Scheme1: Synthesis of Azido Lactone

Synthesis of Kifunensine :

Sodium borohydride reduction of lactone **10** furnished diol **7** in superb yield. The primary alcohol of **7** was selectively protected as the pivaloate ester **6** in 88% yield by treatment with pivaloyl chloride and pyridine. Treatment with TBAF deprotected the silyl ether, and the resulting diol was readily protected to give the acetonide **5**. Lithium aluminum hydride effected reduction of both the azide and ester moieties of **5**, furnishing key intermediate **4** in 90% yield.



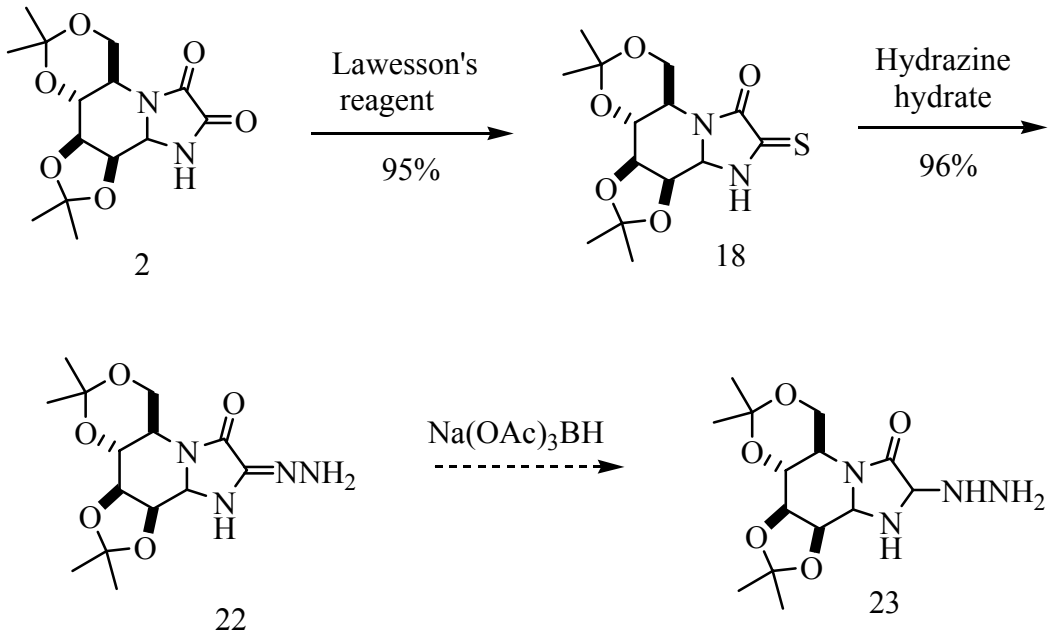
Scheme 2 : Synthesis of Kifunensine

Several attempts were carried out for coupling the amino alcohol **4** with oxamic acid resulting in a very poor yield every time. Various coupling methods like DCC/HOBt, EDC/HOBt, HATU,HOBt,Dipea were tried but resulted in extremely poor yields. Finally, the amino alcohol was refluxed with diethyl oxalate for 5 days to yield an intermediate ester which was subjected to 20%(v/v) ammonia in methanol to yield

oxamylaminoalcohol **3**[3] in 35% yield. Oxamylaminoalcohol **3** was then subjected to Dess-Martin periodinane oxidation followed by treatment with 7.0 N ammonia in methanol to furnish kifunensine diacetonide in 55% yield. It was then subjected to 75% TFA/H_2O to furnish kifunensine **1**.

Synthesis of Kifunensine Analogues:

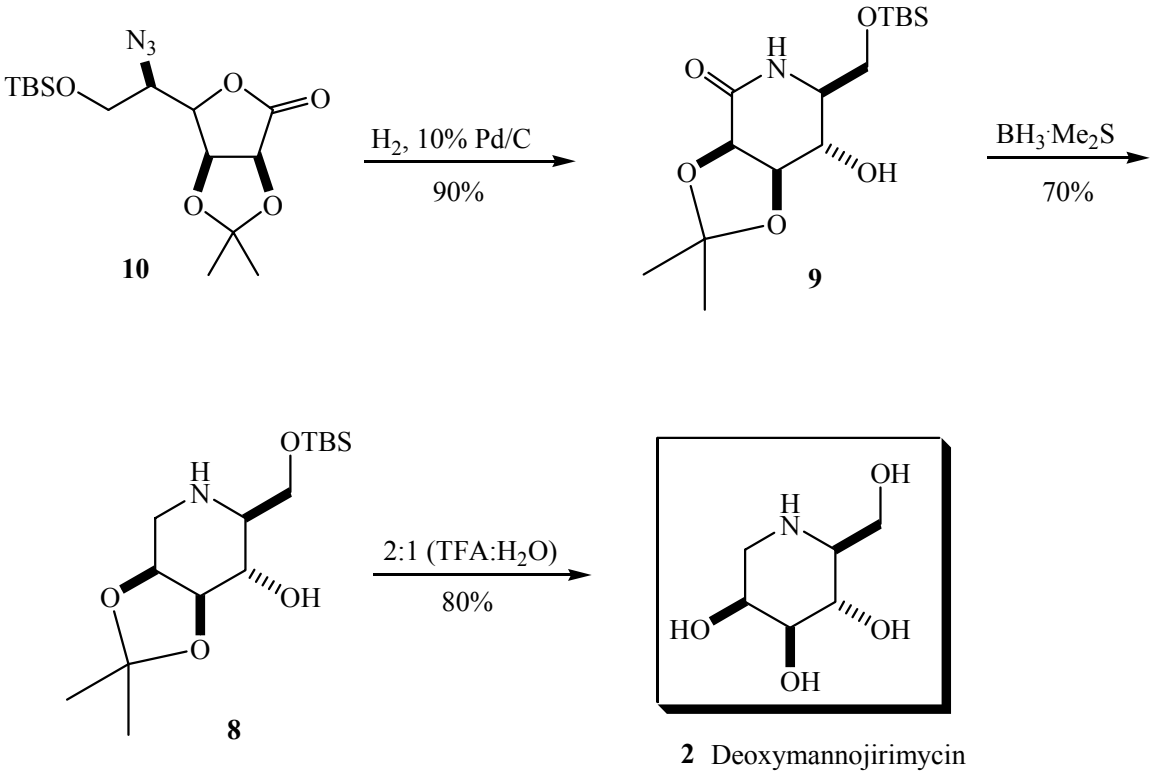
Kifunensine was then subjected to Lawesson's thionation reaction[4] to yield thio analogue **18** in 95% yield. **18** was then converted to the hydrazone **22** almost quantitatively simply by treating with hydrazine hydrate.[5]. **22** can be reduced further with sodium triacetoxy borohydride[6] to a hydrazine. The hydrazine is an excellent substrate for making a combinatorial library of kifunensine analogues.



Scheme 3 : Synthesis of Kifunensine analogues

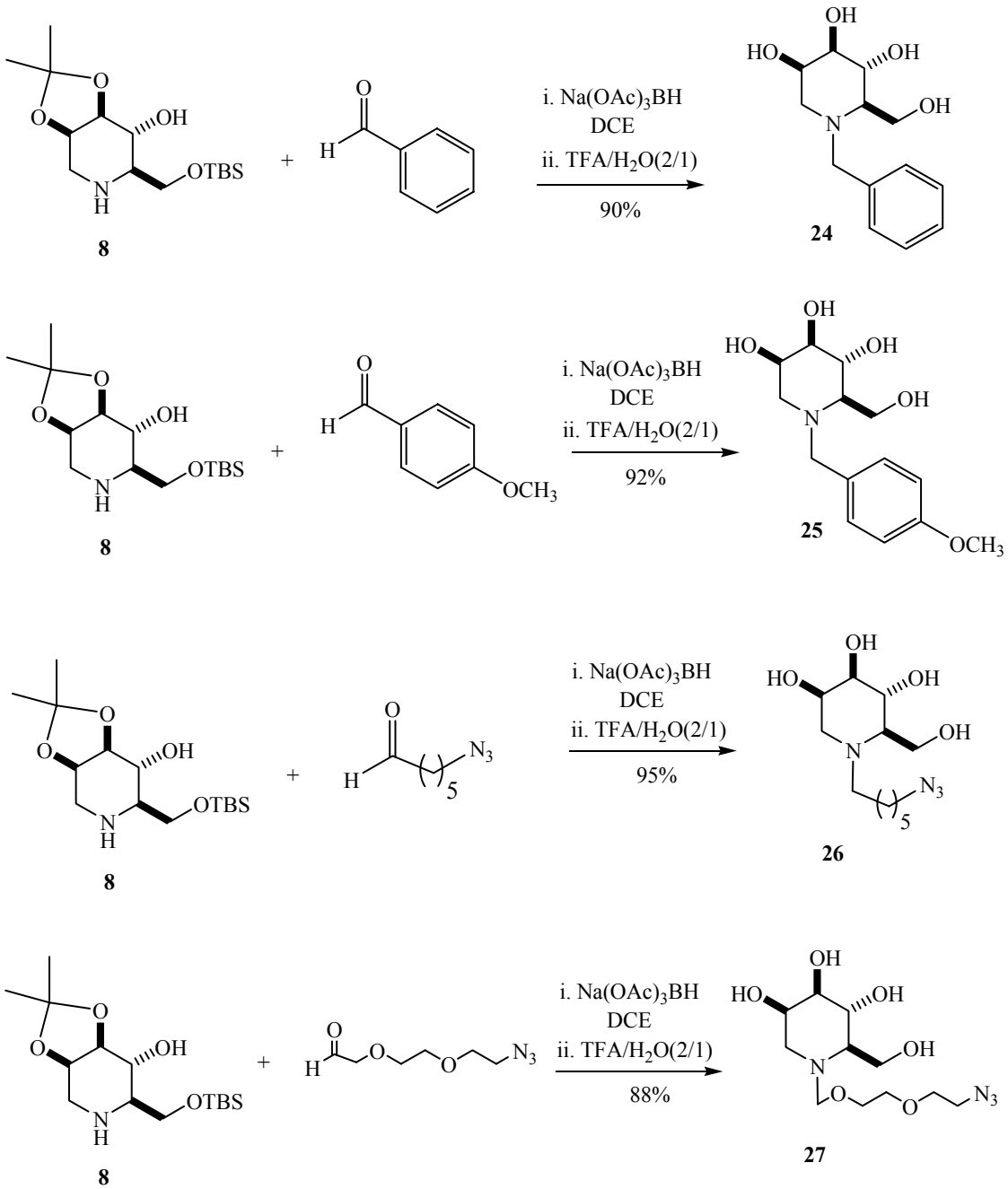
Synthesis of 1-Deoxymannojirimycin :

Azidolactone **10** was subjected to a palladium catalyzed hydrogenation to deliver an intermediate amine which cyclized to furnish the lactam **9** in 90% yield. Subsequent reduction of the lactam with borane methyl sulfide yielded the deoxy compund **8** in 80% yield. The subsequent protecting groups were removed using 2/1 (TFA/H_2O) to give 1-deoxymannojirimycin **2** in excellent yield.



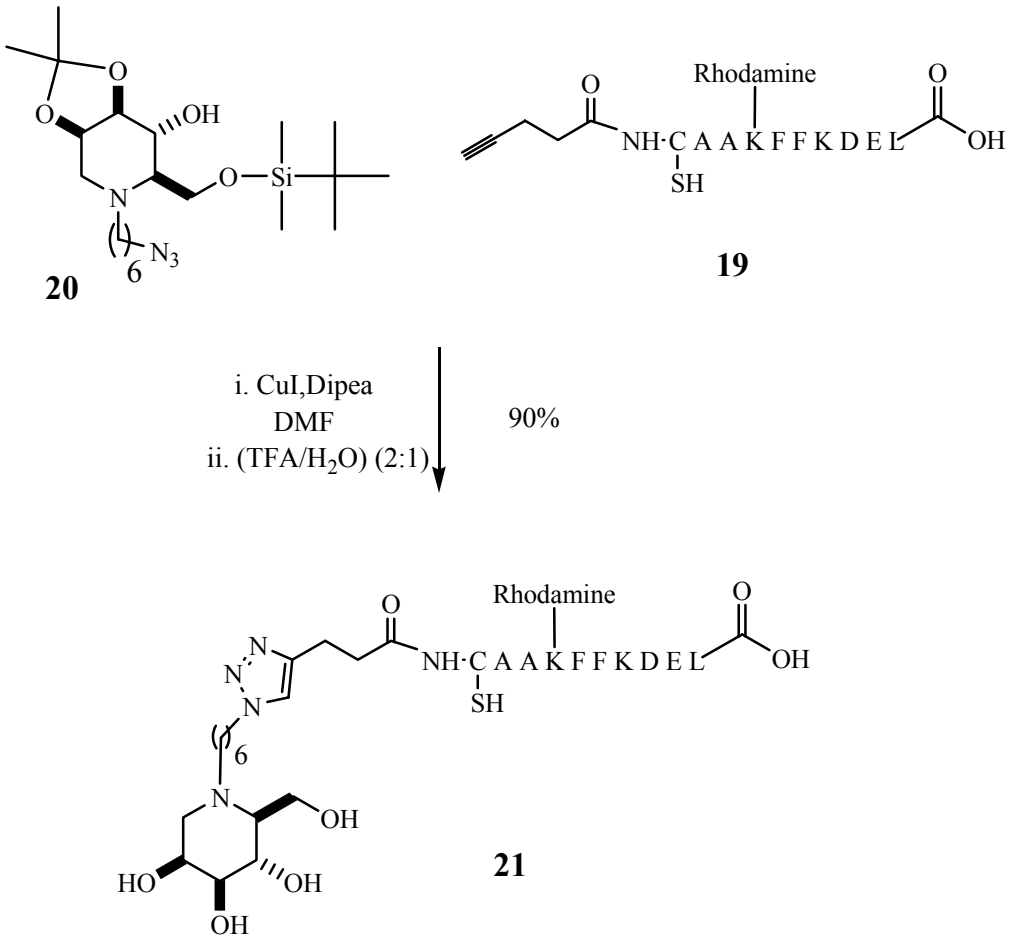
Synthesis of Deoxymannojirimycin analogues :

6-O-tert-butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam **8** was subjected to reductive amination conditions using sodium triacetoxyborohydride to give various deoxymannojirimycin analogues (**24**, **25**, **26**, **27**) in very good yields. The protecting groups were removed using (TFA/H_2O) (2/1).



Assembly of 1-Deoxymannojirimycin and ER Signal peptide

N-Azidohexyl-6-O-tert-butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam **20** was subjected to a click reaction[8] using copper iodide with the ER signal peptide **19** to give triazole in 90% yield . The protecting groups were subsequently removed with 2/1 (TFA/H_2O) to deliver **21** in quantitative yield.



Scheme 4 : Assembly of DMJ and ER Targeting peptide

Conclusion

In conclusion, DMJ analogues were synthesized using reductive amination and a thio analogue of KIF was prepared which was further modified to a hydrazone which serves as an excellent substrate for combinatorial library synthesis. The ER signal peptide was assembled with DMJ by a click reaction sucessfully.

Experimental Section

General Methods and Materials

All reactions were carried out under a positive pressure of argon unless otherwise noted. All chemicals were purchased from commercial suppliers and used without further purification unless otherwise noted. Dichloromethane was distilled from calcium hydride under nitrogen. Toluene was distilled from molten sodium under nitrogen. Dimethylformamide (DMF) was distilled from barium oxide under nitrogen. Column chromatography was performed on silica gel 60 (EM Science, 70–230 mesh). Reactions were monitored by TLC on kieselgel 60 F254 (EM Science), and the compounds were detected by examination under UV light and visualized by dipping the plates in a cerium sulfate/ammonium molybdate solution followed by heating. Organic solutions were concentrated by rotary evaporation below 40 °C under reduced pressure. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Inova 300 spectrometer equipped with Sun workstations. Chemical shifts are reported in parts per million (ppm).Data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, *t* = triplet, dd = double of doublet, m = multiplet and/or multiple resonances), integration, coupling constant in Hertz (Hz). High-resolution mass spectrometry was run in a JMS SX/SX102A tandem mass spectrometer, equipped with FAB source. The matrix used was DHB and the internal standards ultramark 1621 and PEG.

2,3:5,6-Di-O-isopropylidene-L-gulunolactone (13) L-gulunolactone **14** (25g, 0.14mmol) was dissolved in acetone (1000 ml) and $CuSO_4$ (100 g) in presence of catalytic quantity of H_2SO_4 and stirred at r.t. for 36 h. The reaction was quenched with aqueous $NaHCO_3$, filtered and concentrated under reduced pressure. Recrystallisation from ethyl acetate yielded 2,3:5,6-Di-O-isopropylidene-L-gulunolactone 13 (22.0 g, 78%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): 4.81 (t, H-3, 1H), 4.71 (d, H-2, 1H), 4.40 (m, H-4, 1H), 4.20 (m, H-5, 1H), 3.8 (m, H-6, H-7, 2H), 1.44 (s, 2 x CH_3, 6H), 1.37 (s, CH_3, 3H), 1.35 (s, CH_3, 3H) ^{13}CNMR (300 MHz, $CDCl_3$): 173.3 (s, C-1), 114.7 (s), 110.5 (s), 80.9, 76.0, 75.7, 75.3 (4xd, C-2, C-3, C-4, C-5), 65.1 (d, C-6), 26.5 (q), 25.8 (q), 25.0 (q) HR MALDI-TOF MS: *m/z* calcd. for $C_{12}H_{18}O_6$ $[M+Na]^+$ 281.11, found 281.10

2,3-O-Isopropylidene-L-gulunolactone (12) 2,3:5,6-Di-O-isopropylidene-L-gulunolactone **13** (22.00 g, 85.2 mmol) was dissolved in acetic acid/water (7:1, 200 ml) and stirred at r.t. for 24 h when TLC (ethyl acetate/hexane 1:1) revealed no starting material (R_f 0.5) and one major product (R_f 0.1). The solvent was removed under reduced pressure to yield an amorphous yellow solid, which was recrystallised from ethyl acetate to yield 2,3-O-Isopropylidene-L-gulunolactone (12), (12.58 g, 80%) as white crystalline solid.; HR MALDI-TOF MS: *m/z* calcd. for $C_9H_{14}O_6$ $[M+Na]^+$ 241.20 , found 241.25

6-O-tert-butyldimethylsilyl-2,3-O-isopropylidene-L-gulunolactone (11) 2,3-O-Isopropylidene-L-gulunolactone **12** (12.58 g, 57.7 mmol) was stirred in dry

dimethylformamide (100 ml)and the solution cooled to -40^{o}C. Imidazole (6.03 g, 86.55 mmol) and tert-butyldimethylsilyl chloride (9.58 g, 63.50 mmol) were added. The reaction was stirred at -40^{o}C for 2 h when TLC (ethyl acetate/hexane 1:1) revealed no starting material (R_f 0.1) and one major product (R_f 0.8). The solvent was removed under reduced pressure and the residue was dissolved in brine and extracted with dichloromethane. The organic extract was dried (magnesium sulfate) and the solvent removed under educed pressure to give a colourless oil. Purification by flash column chromatography (ethyl acetate/hexane 1:3) gave 6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-L-gulunolactone (11), (13.48 g, 70%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): 4.85 (m, 2H), 4.58 (dd, 1H), 4.07 (m, 1H), 3.83 (dd, 2H), 2.71(d, OH, 1H), 1.50(s, CH_3, 3H), 1.41 (s, CH_3, 3H), 0.92 (s, 9H), 0.11 (s, 6H) HR MALDI-TOF MS: *m/z* calcd. for $C_{15}H_{28}O_6Si$ $[M+Na]^+$ 355.46, found 355.48

5-Azido-6-O-tert-butyldimethylsilyl-5-deoxy-2,3-O-isopropylidene-D-mannonolactone (10) 6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-L-gulunolactone **11** (13.48 g, 40.51 mmmol) was dissolved in dry dichloromethane (100ml) and pyridine (9.76 ml, 121.2 mmol) was added. The reaction was cooled to -30^{o}C. Trifluoromethanesulfonic anhydride (14.05 ml, 83.63 mmol) was added and the reaction was stirred for 1 h when TLC (ethyl acetate/hexane 1:1) revealed no starting material (R_f 0.8) and one product (R_f 0.9). The reaction mixture was then diluted with dichloromethane and washed with aqueous hydrochloric acid, water and saturated sodium bicarbonate. The organic layer was dried (magnesium sulfate) and the solvent removed under reduced pressure to give the crude triflate which was dissolved in dry

dimethylformamide (75 ml) and stirred with sodium azide (7.86 g, 121.6 mmol). After 3 h TLC (ethyl acetate/hexane 1:3) showed no starting material (R_f 0.4) and one product (R_f 0.5). The solvent was removed under reduced pressure and the residue dissolved in brine. This was extracted with dichloromethane. The organic extract was dried (magnesium sulfate) and the solvent removed under reduced pressure to give a crude yellow oil. Elution through a silica plug (ethyl acetate/hexane 1:3) gave a colourless oil which crystallized on standing. Recrystallisation from hexane yielded 5-Azido-6-O-tert-butyldimethylsilyl-5-deoxy-2,3-O-isopropylidene-D-mannonolactone (10), (11.00 g, 76%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.8 (2H, m, H-2 and H-3, J = 5.18 Hz), 4.40 (1H, dd, H-4, J = 3.25 Hz), 4.06 (1H, dd, H-6' J = 10.81 Hz and 5.43 Hz), 3.87 (1H, dd, H-6), 3.75 (1H, ddd, H-5), 1.50 (3H, s, CH_3), 1.45 (3H, s, CH_3), 0.93 (9H, s), 0.12 (6H, s). HR MALDI-TOF MS: *m/z* calcd. for $C_{15}H_{27}N_3O_5Si$ $[M+Na]^+$ 380.17, found 380.17

6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-D-mannono-δ-lactam (9) 5-Azido-6-O-tert-butyldimethylsilyl-5-deoxy-2,3-O-isopropylidene-D-mannonolactone **10** (1.3 g, 4.74 mmol) was dissolved in methanol (50 ml) and stirred under hydrogen with a catalytic amount of 10% palladium on carbon for 14 h when TLC (ethyl acetate/hexane 1:3) revealed baseline material only and one major product. The reaction mixture was filtered through a celite plug which was washed with methanol. Solvents were removed under removed pressure to give a colourless oil. Purification by flash column chromatography (ethyl acetate) yielded 6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-D-mannono-δ-lactam(9), (1.1 g, 90%) as a white crystalline solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.18 (br, NH), 4.63 (1H, d, H-2), 4.30 (1H,dd, H-3), 4.00

(1H, dd, H-6'), 3.59 (2H, m , H-6 and H-4), 3.37 (1H. m, H-5), 1.52 (3H, s, CH3), 1.41 (3H, s, CH3), 0.90 (9H, s), 0.09 (6H, s) MALDI-TOF MS: *m/z* calcd. for $C_{15}H_{27}N_3O_5Si$ $[M+Na]^+$, found

6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam (8) 6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-D-mannono-δ-lactam (9) (1.1 g, 3.29 mmol) was dissolved in dry tetrahydrofuran (15 ml) and Borane/dimethyl sulfide complex (10M, 0.99 ml) was added and the reaction was stirred for 2 h when TLC (ethyl acetate) showed no starting material (R_f 0.5) and one product (R_f 0.9). The reaction was quenched by cautious addition of methanol until effervescence had ceased. Solvents were removed under reduced pressure and methanol (3 x 50 ml) was ditilled from the residue. The residue was purified by silica gel flash column chromatography (2/1Hexanes/EtOAc) to yield 6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam (**8,** 70%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.15 (m,1H), 3.90 (m,1H), 3.70 (dd, 1H), 3.65 (dd, 1H), 3.40 (d, 1H), 3.00 (dd, 1H), 2.41 (m, 1H), 1.51 (s, CH_3, 3H), 1.38 (s, CH_3, 3H), 0.88 (s, 9H), 0.11 (s, 6H) HR MALDI-TOF MS: *m/z* calcd. for $C_{15}H_{31}NO_4Si$ $[M+H]^+$ 318.20 , found 318.18

Deoxymannojirimycin (2) 6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-D-mannono-δ-lactam **9** (1.1 g, 3.29 mmol) was dissolved in dry tetrahydrofuran (15 ml) and Borane/dimethyl sulfide complex (10M, 0.99 ml) was added and the reaction was stirred for 2 h when TLC (ethyl acetate) showed no starting material (R_f 0.5) and one product (R_f 0.9). The reaction was quenched by cautious addition of methanol until effervescence had ceased. Solvents were removed under reduced pressure and methanol

(3 x 50 ml) was ditilled from the residue. The residue was dissolved in Trifluoroacetic acid/water (2:1, 15 ml). The reaction was left to stand for 2 h when TLC (ethyl acetate) revealed baseline material only Solvents were removed under reduced pressure and toluene (3 x 20ml) was distilled from the reaction mixture. The residue was purified by iatro bead column chromatography to yield deoxymannojirimycin **2** which was converted to its hydrochloride salt by treatment with hydrochloric acid (0.5 M) ^{1}H NMR (300 MHz, D_2O): δ 4.10 (1H, ddd, H-2), 3.72 (1H, dd, H-4), 3.69 (1H, dd, H-6), 3.54 (1H, dd, H-3), 3.27 (1H, dd, H-1'), 3.10 (1H, dd, H-1), 3.01 (1H, ddd, H-5). HR MALDI-TOF MS: *m/z* calcd. for $C_6H_{13}NO_4$ $[M+H]^+$ 164.09, found 164.05

(2R,3R,4R,5R)-5-Azido-6-tert-butyldimethylsilanyloxy-2,3-isopropylidene-dioxy-1,4-hexanediol (7) Sodium borohydride (1.11 g, 29.72 mmol) was added to a solution of azide-containing γ-lactone **10** (5.30 g, 14.86 mmol) in ethanol (200 ml) at r.t. and stirred for 4 h. The reaction was quenched with the addition of an excess of ammonium chloride with effervescence, filtered and concentrated to yield an oily white solid. Flash column chromatography (30%EtOAc/hexanes) afforded 4.75 g (88%) of the title compound as a clear oil. R_f = 0.03 (30% EtOAc/hexanes) ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.41 (1H, dd, *J* = 1.4, 7.3 Hz), 4.30 (1H, dt, *J* = 4.4, 7.3 Hz), 4.09 (1H, dd, *J* = 3.1, 10.6 Hz), 3.90-3.85 (m, 2H), 3.80 (1H, dd, *J* = 4.4, 12.4 Hz), 3.62 (1H, dd, *J* = 1.4, 9.1 Hz), 3.48 (1H, ddd, *J* = 3.1, 6.3, 9.1 Hz), 1.53 (3H, s), 1.41 (3H, s), 0.92 (9H, s), 0.11 (6H, d, *J* = 2.2 Hz) HR MALDI-TOF MS: *m/z* calcd. for $C_{15}H_{32}N_3O_5Si$ $[M+H]^+$ 362.21, found 362.21

(2R,3R,4R,5R)-2-Azido-1-tert-butyldimethylsilanyloxy-4,5-O-isopropylidene-dioxy-6-pivaloyl-3-hexanol (6) Pyridine (2.66 ml, 32.85 mmol) and trimethylacetyl chloride (1.94 ml, 15.77 mmol) were added to a solution of diol **7** (4.75 g, 13.12 mmol) in CH_2Cl_2 (60 ml) at room temperature and stirred for 20 h. The reaction was then diluted with CH_2Cl_2, washed with water, dried ($MgSO_4$) and concentrated to yield an oily white solid. Chromatography (5% EtOAc/hexanes) afforded (4.63 g, 80%) of the title compound as a clear oil. R_f = 0.80 (30% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.45 – 4.28 (m, 4H), 4.07 (dd, *J* = 3.2, 10.6 Hz, 1H), 3.86 (dd, *J* = 6.3, 10.6 Hz, 1H), 3.39 (ddd, *J* = 3.2, 6.3, 9.4 Hz, 1H), 2.38 (br, s, 1H), 1.50 (s, 3H), 1.39 (s, 3H), 1.20 (s, 9H), 0.91 (s, 9H), 0.09 (d, *J* = 2.6 Hz, 6H) HR MALDI-TOF MS: *m/z* calcd. for $C_{20}H_{39}N_3O_6Si$ $[M+Na]^+$ 468.25, found 468.249

(2R,3R,4R,5R)-2-Azido-1,3:4,5-diisopropylidenedioxy-6-pivaloylhexane (5) Tetra-*n*-butylammonium fluoride (12.78 ml,1.0 M solution in THF, 12.82 mmol) was added to a solution of TBS-ether **6** (4.75 g, 10.63 mmol) in THF (140 ml) at -20 ^{o}C and stirred for 1 h. The reaction was then warmed to room temperature, poured in to brine, extracted with EtOAc, dried ($MgSO_4$) and concentrated to yield a pale yellow oil. Chromatography (50% EtOAc/hexanes) afforded (3.45 g 98%) of the intermediate diol as a clear oil. R_f = 0.20 (30% EtOAc, Hexanes); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.35 (m, 4H), 4.03 (ddd, *J* = 4.1, 5.5, 11.7 Hz, 1H), 3.90 (ddd, *J* = 5.2, 6.8, 12.0 Hz, 1H), 3.70 (t, *J* = 9.0 Hz, 1H), 3.50 (ddd, *J* = 4.0, 5.2, 9.1 Hz, 1H), 2.45 (m, 2H), 1.51 (s, 3H), 1.40 (s, 3H), 1.21 (s, 9H); HR MALDI-TOF MS: *m/z* calcd. for $C_{14}H_{25}N_3O_6$ $[M+Na]^+$ 354.1641, found 354.1631. 2-methoxy propene (1.97 ml, 20.7 mmol) and PTSA (329 mg, 1.75 mmol) were added to a

solution of intermediate diol (3.45 g, 10.4 mmol) in CH_2Cl_2 (50 ml) at rrom temperature and stirred for 30 minutes. The reaction was quenched with water and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated to yield 3.74 g (97%) of the title compound as a yellow oil. The crude material was pure enough for characterization. R_f = 0.80 (30% EtOAc/hexanes); ^{1}H NMR (C_6D_6, 300 MHz) δ 4.46 (dd, *J* = 6.6, 10.6 Hz, 1H), 4.32 (td, *J* = 4.8, 6.6 Hz, 1H), 4.27 (dd, *J* = 4.8, 10.6 Hz, 1H), 4.18, (dd, *J* = 1.1, 6.6 Hz, 1H),3.66 (td, *J* = 5.5, 9.5 Hz, 1H), 3.59 (dd, *J* = 5.5, 11.2 Hz, 1H), 3,38 (dd, *J* = 1.1, 9.5 Hz, 1H), 3.35 (dd, *J* = 9.5, 11.2, Hz, 1H), 1.54 (s, 3H), 1.25 (s, 3H), 1.24 (s, 3H), 1.16 (s, 9H),1.12 (s, 3H) HR MALDI-TOF MS: *m/z* calcd. for $C_{17}H_{29}N_3O_6$ $[M+Na]^+$ 394.195, found 394.195

(2R,3R,4R,5R)-5-Amino 2,3:4,6 Diisopropylidenedioxy-hexanol (4) Lithium aluminium hydride (1.14 g, 30.3 mmol) was added to a solution of azido ester **5** (3.74 g,10.1 mmol) in diethyl ether (200 ml) at room temperature and stirred 2 h. The reaction was quenched with successive addition of water and sodium hydroxide. The ether was poured off and the white solid was extracted with ethyl acetate. The combined organic phases were dried ($MgSO_4$) and concentrated to give an oily yellow solid. Silica gel flash column chromatography (10% MeOH/CH_2Cl_2) yielded (2.30 g, 90%) of the title compound as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.50 (dd, *J* = 2.3, 6.7 Hz, 1H), 4.29 (dt, *J* = 4.8, 6.7 Hz, 1H), 3.89 (dd, *J* = 5.3, 11.5 Hz, 1H), 3.80, (dd, *J* = 4.8, 12.0 Hz, 1H),3.76 (dd, *J* = 4.8, 12.0 Hz, 1H), 3.53 (dd, *J* = 2.3, 9.3 Hz, 1H), 3.50 (dd, *J* = 9.3, 11.5 Hz, 1H), 3.11 (td, *J* = 5.3, 9.3 Hz, 1H), 1.85 (br s, 3H), 1.51 (s, 3H), 1.46 (s, 3H), 1.41 (s, 3H), 1.37(s,3H); 13C NMR ($CDCl_3$, 100 MHz) δ 108.99, 99.21, 78.00,

74.98, 74.1, 66.45, 61.6, 46.2, 28.6, 26.9, 25.9, 19.5; HR MALDI-TOF MS: *m/z* calcd. for $C_{17}H_{29}N_3O_6$ $[M+Na]^+$ 284.20, found 284.225

(2R,3R,4R,5R)- 2,3:4,6 Diisopropylidene-5-oxamoylamino alcohol (3) Alcohol **4** (2.30 g, 0.008 mmol) was refluxed with diethyl oxalate (3.5 ml) in MeOH (300 ml) for 5 days to give an intermediate ester. The solvent was reomoved under reduced pressure and the crude intermediate was dissolved in 20%v/v (NH_3/MeOH) (250 ml) to yield a white solid. Silica gel flash column chromatography (10/1 EtOAc/Hexanes) yielded (900 mg ,36%) of the title compound as a white solid. ^{1}H NMR (CD_3OH, 300 MHz) δ 8.64 (d,1H), 8.07 (s, 1H), 7.79 (s, 1H), 4.76 (t, 1H), 4.18 (m, 1H), 3.96 (m, 2H), 3.62 (m,1H), 1.42 (s, CH_3, 3H), 1.39 (s, CH_3, 3H), 1.26 (s, CH_3, 3H), 1.22 (s, CH_3, 3H) 13C NMR ($CDCl_3$, 100 MHz) δ 162.49, 161.08, 108.75, 99.31, 78.47, 74.36, 69.34, 61.00, 60.29, 42.01, 29.55, 27.10, 26.51, 19.61 HR MALDI-TOF MS: *m/z* calcd. for $C_{14}H_{24}N_2O_7$ $[M+Na]^+$ 355.164, found 355.1646

(3aS, 3bS, 6bR, 10aR, 10bS)-2,2,9,9-Tetramethyl-1,3,8,10-tetraoxacyclohexa-[e]cyclopenta[g]-5,6-dione-4-azaidolizidine (2) Dess-Martin periodinane (775 mg ,0.0018 mmol) was added to a solution of alcohol **3** (400 mg, 0.001 mmol) in CH_2Cl_2 (200 ml) at r.t. and stirred for 4 h. The reaction was then diluted with ether (15 ml), filtered through celite and concentrated to yield the intermediate aldehyde as a whie solid. The crude intermediate aldehyde was dissolved in 7.0 N NH_3-MeOH (20 ml) and stirred at r.t. for 24 h. The reaction was then concentrated to yield a yellow oil. Chromatography (80% EtOAc/Hexanes) afforded (108 mg, 35 %) of the title compound as white solid.

R_f = 0.2 (75% EtOAc/Hexanes) ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.13 (s, NH, 1H), 4.82 (d, 1H), 4.69 (dd, 1H), 4.34 (t, 1H), 4.14 (m, 1H), 3.97 (t, 1H), 3.77 (t, 1H), 3.57 (ddd, 1H), 1.53 (s, 2 x CH_3, 6H), 1.47 (s, CH_3, 3H), 1.35, (s, CH_3, 3H); HR MALDI-TOF MS: *m/z* calcd. for $C_{14}H_{20}N_2O_6$ $[M+Na]^+$ 335.22, found 335.21

Kifunensine analogues:

Thio analogue of Kifunensine diacetonide (18) :(3aS, 3bS, 6bR, 10aR, 10bS)-2,2,9,9-Tetramethyl-1,3,8,10-tetraoxacyclohexa-[e]cyclopenta[g]-5,6-dione-4-azaindolizidine **16** (6 mg, 0.000019 mmol) was dissolved in toluene and Lawesson's reagent (3mg, 0.0000096 mmol) was added and stirred at 80^{o}C for 1 h. The solvent was removed under reduced pressure and concentrated to give a crude yellow oil. Silica gel flash column chromatography (2/1 Hexanes/EtOAc) yielded (**18**, 90%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.81 (d, 1H), 4.73 (dd, 1H), 4.36 (t, 1H), 4.17 (dd, 1H), 3.99 (t, 1H), 3.77 (t, 1H), 3.6 (ddd, 1H) 13C NMR ; HR MALDI-TOF MS: *m/z* calcd. for $C_{14}H_{20}N_2O_5S$ $[M+Na]^+$ 351.11, found 351.11

Hydrazone analogue of Kifunensine diacetonide (22) : 18 (0.002 g, mol) was dissolved in ethanol (0.6 ml) and hydrazine hydrate (0.2 μl, mol) and stirred at 45^0 C for 25 min. Starting material dissapeared and product was formed. HR MALDI-TOF MS: *m/z* calcd. for $C_{14}H_{22}N_4O_5$ $[M+H]^+$ 326.3, found 326.3 , $[M+Na]^+$ 349.3, found 349.3 $[M+K]^+$ 365.3, found 365.3

Deoxymannojirimycin Analogues :

1-Benzyl-2-hydroxymethyl-piperidine-3,4,5-triol (**24**) : 6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam **(8)** (0.010 g, 0.031 mmol) and benzaldehyde (0.0032 g, 0.031 mmol) were dissolved in DCE and $NaBH(OAc)_3$ (0.007 g ,0.034 mmol) was added and stiired for 1 h. The reaction was quenched by addition of aqueous $NaHCO_3$ and then diluted with DCM. The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated in vacuo. The residue was purified by flash silica gel column chromatography to afford N-Benzyl-6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam in R_f = 0.6 (EtOAc/hexanes) ^1H NMR ($CDCl_3$ 300 MHz) : δ 7.28 (5H, m, Ar), 4.30 (1H, dd), 4.12 (1H, t), 3.97 (2H, m), 3.78 (3H, m), 2.77 (3H, m), 1.49 (1H, s), 1.32 (1H, s), 0.87 (9H, s), 0.037(3H, s), 0.031 (3H, s) HR MALDI-TOF MS: *m/z* calcd. for $C_{22}H_{37}NO_4Si$ $[M+Na]^+$ 430.62, found 430.62 The protecting groups were then removed by treating with 2/1 (TFA/H_2O) for 1 h and purified by iatro beads column chromatography CH_3CN/H_2O (2/0.2) to give **1**-Benzyl-2-hydroxymethyl-piperidine-3,4,5-triol **24** HR MALDI-TOF MS: *m/z* calcd. for $C_3H_{19}NO_4$ $[M+Na]^+$ 276.29, found 276.28

2-Hydroxymethyl-1-(4-methoxy-benzyl)-piperidine-3,4,5-triol (**25**) : 6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam **(8)** (0.010 g, 0.031 mmol) and p-anisaldehyde (0.0042 g, 0.031 mmol) were dissolved in DCE and $NaBH(OAc)_3$ (0.007 g, 0.034 mmol) was added and stiired for 1 h. The reaction was quenched by addition of aqueous $NaHCO_3$ and then diluted with DCM. The organic

phase was dried ($MgSO_4$), filtered and the filtrate was concentrated in vacuo. The residue was purified by flash silica gel column chromatography to afford N-Benzyl-6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam in R_f = 0.6 (EtOAc/hexanes) HR MALDI-TOF MS: *m/z* calcd. for $C_{23}H_{39}NO_5Si$ $[M+H]^+$ 438.64, found 438.64. The protecting groups were then removed by treating with 2/1 (TFA/H_2O) for 1 h and purified by iatro beads column chromatography CH_3CN/H_2O (2/0.2) to give 2-Hydroxymethyl-1-(4-methoxy-benzyl)-piperidine-3,4,5-triol **25** HR MALDI-TOF MS: *m/z* calcd. for $C_{12}H_{24}N_4O_4$ $[M+Na]^+$ 283.32, found 283.31

1-(6-Azido-hexyl)-2-hydroxymethyl-piperidine-3,4,5-triol (**26**) : 6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam **(8)** (0.010 g, 0.031 mmol) and 6-azido hexanal (0.004 g, 0.031 mmol) were dissolved in DCE and $NaBH(OAc)_3$ (0.007 g, 0.0034 mmol) was added and stiired for 1 h. The reaction was quenched by addition of aqueous $NaHCO_3$ and then diluted with DCM. The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated in vacuo. The residue was purified by flash silica gel column chromatography to afford N-Azidohexyl-6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam (**20**) R_f = 0.5 (EtOAc/hexanes) ^1H NMR ($CDCl_3$ 300 MHz): δ 4.08 (1H, dt), 3.80 (1H, t), 3.65 (2H, m), 3.45 (1H, dd), 2.99 (1H, t), 2.55 (1H, t), 2.35 (1H, t), 1.20 (12H, m), 0.64 (9H), 0.18 (6H) HR MALDI-TOF MS: *m/z* calcd. for $C_{21}H_{42}N_4O_4Si$ $[M+Na]^+$ 443.24, found 443.23. The protecting groups were then removed by treating with 2/1 (TFA/H_2O) for 1 h and purified by iatro beads column chromatography CH_3CN/H_2O (2/0.2) to give 1-(6-

Azido-hexyl)-2-hydroxymethyl-piperidine-3,4,5-triol **26** HR MALDI-TOF MS: *m/z* calcd. for $C_{12}H_{24}N_4O_4$ $[M+Na]^+$ 311.34, found 311.34

1-{2-[2-(2-Azido-ethoxy)-ethoxy]-ethyl}-2-hydroxymethyl-piperidine-3,4,5-triol (27) 6-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam **(8)** (0.010 g, 0.031 mmol) and [2-(2-Azido-ethoxy)-ethoxy]-acetaldehyde (0.006 g, 0.031 mmol) were dissolved in DCE and $NaBH(OAc)_3$ (0.007 g, 0.0034 mmol) was added and stiired for 1 h. The reaction was quenched by addition of aqueous $NaHCO_3$ and then diluted with DCM. The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated in vacuo. The residue was purified by flash silica gel column chromatography to afford 5-{2-[2-(2-Azido-ethoxy)-ethoxy]-ethyl}-6-(tert-butyl-dimethyl-silanyloxymethyl)-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-c]pyridin-7-ol R_f = 0.55 HR MALDI-TOF MS: *m/z* calcd. for $C_{21}H_{42}N_4O_6Si$ $[M+H]^+$ 475.67, found 475.67. The protecting groups were then removed by treating with 2/1 (TFA/H_2O) for 1 h and purified by iatro beads column chromatography CH_3CN/H_2O (2/0.2) to give 1-{2-[2-(2-Azido-ethoxy)-ethoxy]-ethyl}-2-hydroxymethyl-piperidine-3,4,5-triol **27**. HR MALDI-TOF MS: *m/z* calcd. for $C_{12}H_{24}N_4O_6$ $[M+H]^+$ 321.34, found 321.34

ER Targeting peptide (**19**) : The peptide was synthesized by SPOS on Wang resin using Fmoc based strategy. Fmoc-Leucine (1.76 g, 5mmol) was dissolved in CH_2Cl_2 (20 ml) at 0^0C and DIPC (0.315 g, 2.5 mmol) was added. The solution was warmed to r.t. and the solvent was removed. The activated amino acid was dissolved in DMF and added to wang resin (0.42 g, 0.5 mmol) along with DMAP (0.006g, 0.05 mmol) on the manual

peptide bubbler for 10 hrs. It was then washed with DMF and the Fmoc was removed with 20% piperidine in DMF. The deprotection was monitored by Kaiser test. It was washed again with DMF to clear of remaining piperidine. The subsequent amino acids Glutamic acid, Aspartic acid, Lysine, Phenyl alanine (2 times), Lysine, Alanine (2 times) and pentynoic acid were then coupled using the following general procedure. Amino acid (3 eq.), PyBOP (3 eq.), HOBt (3 eq.), DIPEA (6 eq.) were added to bubbler and kept for 3 h. Orthogonal protecting groups were used for the two lysines (Mtt and Boc). Mtt was removed with 2% TFA in CH_2Cl_2 and the fluorescent dye (Rhodamine) was installed. The peptide was finally cleaved from the resin using 95%TFA, 2.5%H_2O, 2.5%TIS. HR MALDI-TOF MS: *m/z* calcd. $[M+Na]^+$ 1809.53, found 1809.54

Triazole (21) : N-Azidohexyl-6-O-tert-butyldimethylsilyl-2,3-O-isopropylidene-1-deoxy-D-mannono-δ-lactam **20** (0.010 g, 0.0000238 mol) was mixed with ER targeting peptide **19** (0.04 g, 0.00000238 mol) in the presence of copper iodide (0.002 g, 0.000010 mol) and DIPEA (0.04 ml) in DMF (1 ml) and stirred at r.t. for 48 h. The reaction was monitored by HPLC and M.S. After confirming from M.S. the protecting groups were removed using 2/1 (TFA/H_2O) to give the triazole **21** in 90% yield. 21 was finally purified by reverse pahse HPLC using CH_3CN/H_2O gradient system. MALDI-TOF MS: *m/z* calcd. $[M+Na]^+$ 1935.9338, found 1935.9339.

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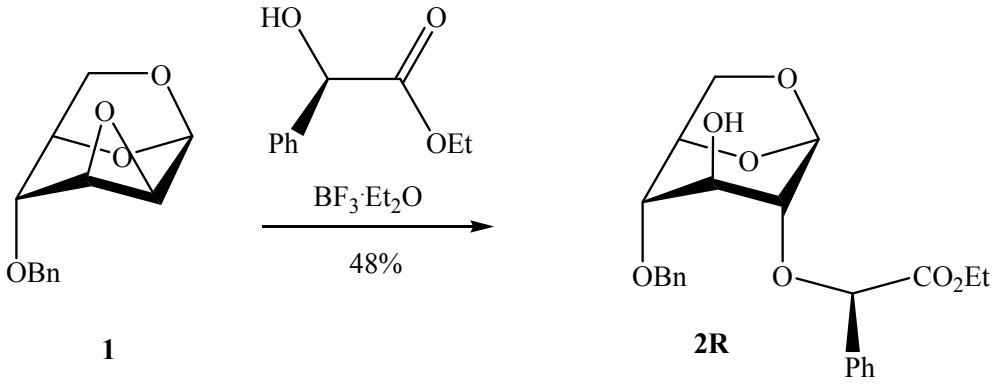
CHAPTER 3

STEREOSLECTIVE GLYCOSYLATIONS USING (R)- OR (S)-(ETHOXYCARBONYL) BENZYL CHIRAL AUXILIARIES AT C-2 OF GLYCOPYRANOSYL DONORS [1]

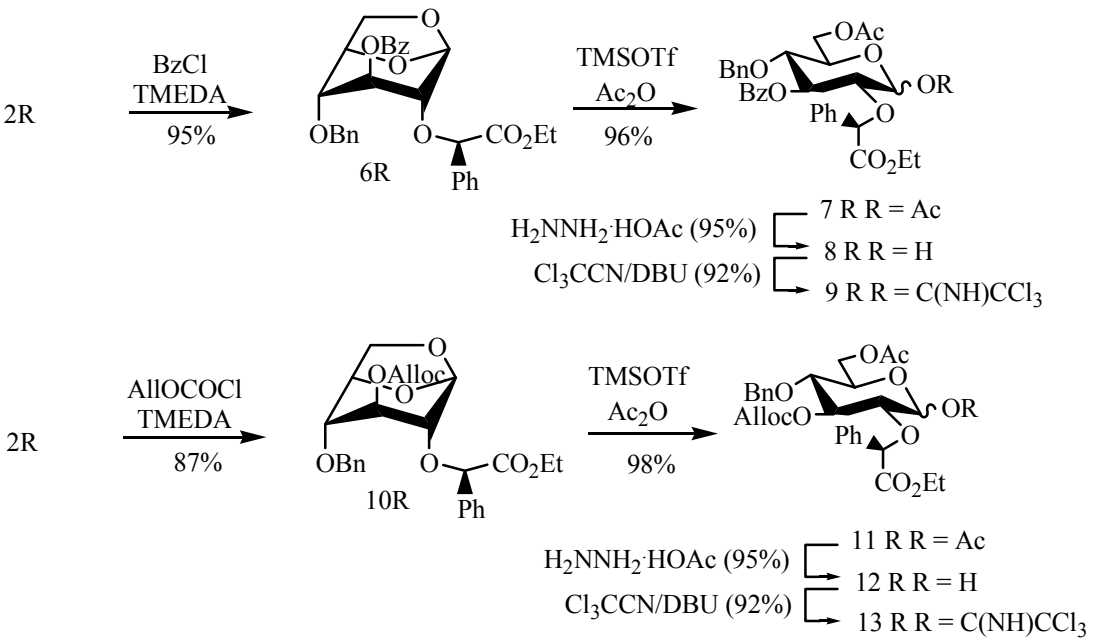
[1] Vishal Khot and Geert Jan Boons. To be submitted to *European Journal of Organic Chemistry*.

Results and Discussion

Ethyl mandelate was used as a first-generation chiral auxiliary because both enantiomers of this compound are readily available. Furthermore, esters are well-established as appropriate participating functionalities in glycosylations. Glucosyl donor **9R** containing a (R)-ethoxy carbonyl benzyl moiety, could be prepared from the readily available epoxide **1**.[1] Thus, reaction of 1 with ethyl (R)-mandelate in the presence of $BF_3{\cdot}Et_2O$ resulted in a trans diaxial opening of the epoxide to give **2R** in 48% yield. Glucosyl donor **9S** can be prepared in a similar fashion using ethyl (S)-mandelate.[2]

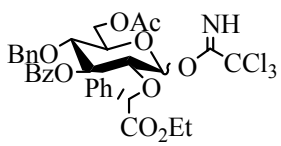
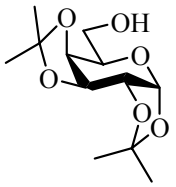
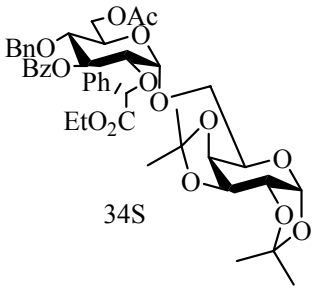
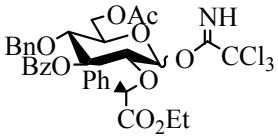
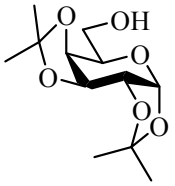
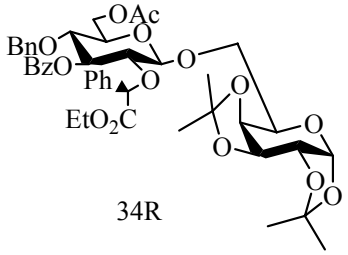
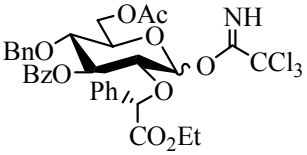
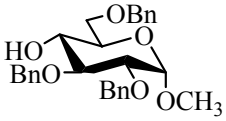
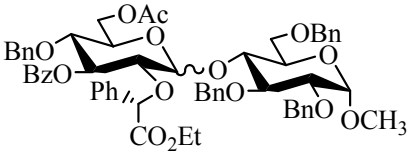
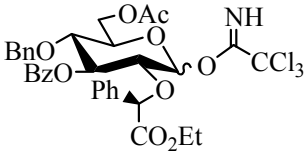
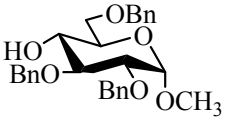
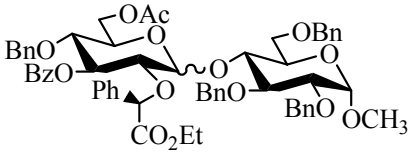


Benzoylation of **2*R*** followed by acetolysis of the 1,6-anhydro-bridge of the resulting **6*R*** delivered the acetate **7*R***, which was converted into the trichloroacetimidate **9*R*** by a standard two-step procedure.[3] The allyloxycarbonyl (Alloc) protected derivative **13*R*** could be prepared by a similar approach by first reacting **2*R*** with allyloxycarbonyl chloride in the presence of *N*,*N*,*N*,*N*-tetramethylethylenediamine (TMEDA) to furnish **10*R*** which was converted into the anomeric trichloroacetimidate by standard chemical manipulation. The glucosyl donors **9S** and **13S** were prepared in a similar fashion using **2S** as the starting material.

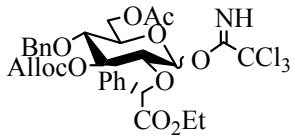
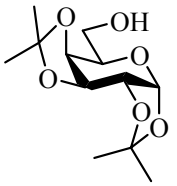
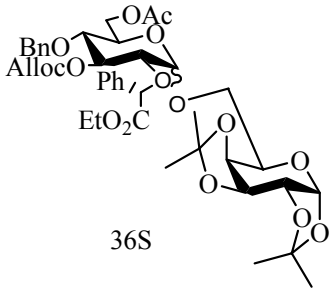
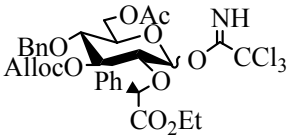
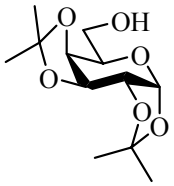
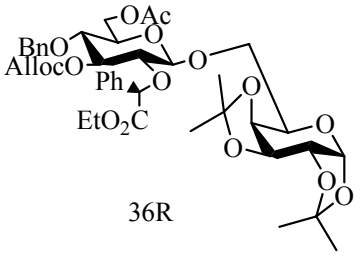
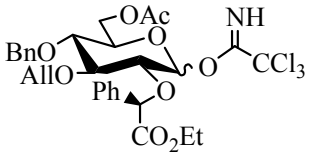
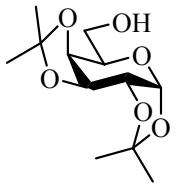
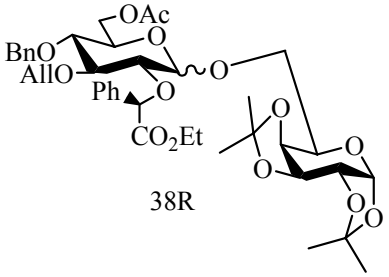
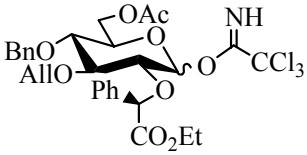
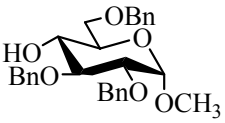
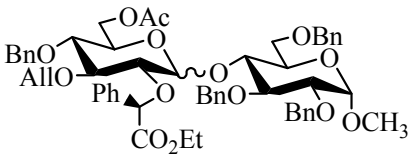


Having a number of differently protected glycosyl donors at hand, attention was focused on glycosylations with the glycosyl acceptors **23** and **24**. Thus, coupling of **9R** with **23** using a catalytic amount of trimethylsilyl triflate (TMSOTf) in dichloromethane (DCM) at –78 °C delivered the disaccharide **33R** mainly as the β-glycoside in high yield. At this low temperature, the reaction was completed within 15 min, indicating that the glycosyl donor **9R** is highly reactive. Dilution of the reaction mixture led to a small increase in anomeric selectivity, whereas higher reaction temperatures led to reductions of α-anomeric selectivity. As anticipated, the coupling of **9S** with **23** under similar reaction conditions gave **33*S*** as mainly the α-anomer. The fact that an inversion of configuration of the asymmetric center of the auxiliary led to a reversal of the stereochemical outcome of the glycosylation supports the proposed mode of participation. Various glycosylations were conducted to test the generality of the

approach. Coupling of **9R** with **24** gave mainly **34R** as the β-glycoside whereas **9S** with **24** delivered **34S** as the α-glycoside

Donor	Acceptor	Product	α/β Ratio (yield, %)
 9S	 24	 34S	only α (93%)
 9R	 24	 34R	only β (84%)
 9S	 23	 33S	7:1 (88%)
 9R	 23	 33R	1:3 (94%)

. Coupling of **13R** with **24** gave mainly the β-glycoside whereas **13S** with **24** resulted in the α-glycoside. Coupling of **17R** with **24** gave α/β mixture in (1/5) ratio and 90% yield. When **17R** was coupled with **23** an α/β mixture was obtained in (1/4) ratio.

Donor	Acceptor	Product	α/β Ratio (yield, %)
 13S	 24	 36S	10:7 (79%)
 13R	 24	 36R	only β (90%)
 17R	 24	 38R	1:5 (75%)
 17R	 23	 37R	1:4 (80%)

Conclusions:

For the first time it has been demonstrated that the anomeric selectivity of a glycosylation can be achieved by using a chiral auxiliary. This new method for anomeric control is especially suited for the introduction of α-glucosides. It is to be expected that a systematic optimization of the structure of the auxiliary will result in a stereospecific glycosylation protocol. In particular, an improved auxiliary may be obtained by increasing its nucleophilicity or reducing the flexibility of rotatable bonds. Only such a method will help in realizing the full potential of polymer supported and one-pot multi-step oligosaccharide syntheses for routine use for a large number of oligosaccharide targets.

Experimental Section

General Methods and Materials

All reactions were carried out under a positive pressure of argon unless otherwise noted. All chemicals were purchased from commercial suppliers and used without further purification unless otherwise noted. Dichloromethane was distilled from calcium hydride under nitrogen. Toluene was distilled from molten sodium under nitrogen. Dimethylformamide (DMF) was distilled from barium oxide under nitrogen. Column chromatography was performed on silica gel 60 (EM Science, 70–230 mesh). Reactions were monitored by TLC on kieselgel 60 F254 (EM Science), and the compounds were detected by examination under UV light and visualized by dipping the plates in a cerium sulfate/ammonium molybdate solution followed by heating. Organic solutions were concentrated by rotary evaporation below 40 °C under reduced pressure. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Inova 300 spectrometer equipped with Sun workstations. Chemical shifts are reported in parts per million (ppm).Data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, *t* = triplet, dd = double of doublet, m = multiplet and/or multiple resonances), integration, coupling constant in Hertz (Hz). High-resolution mass spectrometry was run in a JMS SX/SX102A tandem mass spectrometer, equipped with FAB source. The matrix used was DHB and the internal standards ultramark 1621 and PEG.

1,6-Anhydro-3-*O*-benzoyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-β-D-glucopyranose (6*R*). Tetramethyl ethylenediamine (117 μL, 0.78 mmol) was added to a solution of **2*R*** (250 mg, 0.60 mmol) and benzoyl chloride (139 μL, 1.20 mmol) in dichloromethane (10 mL) at 0 °C. After stirring the reaction mixture for 1 h at 0 °C, it was allowed to warm to room temperature and stirring was continued for 2 h. The reaction mixture was quenched with an aqueous saturated solution of $NaHCO_3$ (10 mL), and then diluted with dichloromethane (10 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 3/1) to afford **6*R*** (296 mg, 95%): colorless syrup, R_f = 0.65 (ethyl acetate/hexane, 1/1); $[\alpha]^{20}_D$ = -27° (*c* = 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, 2H, *J* = 8.0 Hz), 7.26–7.60 (m, 13H), 5.51 (s, 1H), 5.38 (s, 1H), 5.29 (d, 1H, *J* = 5.5 Hz), 4.94 (dd, 1H, *J* = 12.5 Hz, CH*H*Ph), 4.77 (dd, 1H, *J* = 12.5 Hz, C*H*HPh), 4.65 (d, 1H, *J* = 5.5 Hz), 4.15 (m, 2H, COOC*H*$_2$CH$_3$), 3.97 (d, 1H, *J* = 7.5 Hz), 3.76 (t, 1H, *J* = 7.0 Hz), 3.46 (s, 1H), 3.40 (s, 1H), 1.19 (t, 3H, *J* = 8.5 Hz, COOCH$_2$C*H*$_3$); HR MALDI-TOF MS m/z calcd for $C_{30}H_{30}O_8$ $[M+Na]^+$ 541.1838, found 541.1827.

Acetyl 6-*O*-acetyl-3-*O*-benzoyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-α-D-glucopyranose (7*R*). Trimethylsilyl trifluoromethanesulfonate (0.2 μL, 0.01 mmol) was added to a solution of **6*R*** (215 mg, 0.41 mmol) in acetic anhydride (3 mL) at 0 °C. After stirring the reaction mixture for 30 min at 0 °C, it was quenched with an aqueous saturated solution of $NaHCO_3$ (10 mL) and then diluted with dichloromethane (10 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (20% ethyl acetate in

hexane) to afford **7*R*** (244 mg, 96%): colorless syrup, R_f = 0.62 (ethyl acetate/hexane, 1/1); $[\alpha]^{20}_D$ = +97° (*c* = 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13–8.18 (m, 15H, Ar), 6.26 (d, 1H, *J* = 3.5 Hz, H-1), 5.87 (t, 1H, *J* = 10.0 Hz, H-3), 4.98 (s, 1H, >CHPh), 4.60 (d, 1H, *J* = 11.0 Hz, CH*H*Ph), 4.49 (d, 1H, *J* = 11.0 Hz, C*H*HPh), 4.27-4.28 (m, 1H, H-6_b), 4.03-4.05 (m, 1H, H-5), 3.94-3.97 (m, 2H, COOCH_2CH$_3$), 3.89 (dd, 1H, *J* = 3.5 Hz, 10.0 Hz, H-2), 3.84-3.88 (m, 1H, H-6_a), 3.77 (t, 1H, *J* = 10.0 Hz, H-4), 2.07 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 0.97 (t, 3H, *J* = 7.5 Hz, $COOCH_2CH_3$); HR MALDI-TOF MS m/z calcd for $C_{34}H_{36}O_{11}$ $[M+Na]^+$ 643.2155, found 643.2138.

6-*O*-Acetyl-3-*O*-benzoyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-α-D-glucopyranosyl trichloroacetimidate (9*R*). Hydrazinium acetate (33 mg, 0.36 mmol) was added to a solution of compound **7*R*** (205 mg, 0.33 mmol) in DMF (5 mL) at room temperature. After stirring for 18 h, the reaction mixture was quenched with an aqueous saturated solution of $NaHCO_3$ (10 mL), and diluted with ethyl acetate (10 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo.* The residue was purified by flash silica gel column chromatography (hexane/ethyl acetate; 3/1) to afford **8*R*** (168 mg, 88%). Trichloroacetonitrile (290 μL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 17 μL) were added to a solution of **8*R*** (168 mg, 0.29 mmol) in dichloromethane (5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and after which it was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 3/1) to afford **9*R***. (199 mg, 95%): yellowish syrup, R_f = 0.65 (ethyl acetate/hexane, 1/1); $[\alpha]^{20}_D$ = +104° (*c* = 0.8, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (s, 1H, =NH), 7.13–8.18 (m, 15H, Ar), 6.47 (d, 1H, *J* = 3.6 Hz, H-1), 5.97 (t, 1H, *J* = 9.6 Hz, H-3), 5.04 (s, 1H, >CHPh), 4.61 (d, 1H, *J* = 10.5 Hz,

CH*H*Ph), 4.51 (d, 1H, *J* = 10.5 Hz, C*H*HPh), 4.28-4.31 (m, 1H, H-5), 4.17-4.20 (m, 1H, H-6_a), 4.12 (q, 2H, *J* = 7.2 Hz, COOC*H*$_2$CH$_3$), 3.99 (dd, 1H, *J* = 3.6, 9.9 Hz, H-2), 3.92-3.94 (m, 1H, H-6_b), 3.83 (t, 1H, *J* = 9.9 Hz, H-4), 2.04 (s, 3H, COCH$_3$), 1.20 (t, 3H, *J* = 7.2 Hz, COOCH$_2$C*H*$_3$).

1,6-Anhydro-3-*O*-allyloxycarbonyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-*β*-D-glucopyranose (10*R*). Tetramethyl ethylenediamine (98 μL, 0.65 mmol) was added to a solution of **2*R*** (207 mg, 0.50 mmol) and allyloxycarbonyl chloride (106 μL, 1.0 mmol) in dichloromethane (10 mL) at 0 °C. Then the reaction mixture was warmed to room temperature and stirred for 2 hours. The reaction mixture was quenched with an aqueous saturated solution of NaHCO$_3$ (10 mL) and then diluted with dichloromethane (10 mL). The organic phase was dried (MgSO$_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 3/1) to afford **10*R*** (217 mg, 87%): colorless syrup, R_f = 0.58 (ethyl acetate/hexane, 1/1); $[\alpha]^{20}_D = +17^o$ (*c* = 0.8, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.26-7.48 (m, 10H, Ar), 5.89–5.97 (m, 1H, C*H*=CH$_2$), 5.41 (s, 1H, H-1), 5.36 (d, 1H, *J* = 17.0 Hz, CH*H*=CH), 5.29 (d, 1H, *J* = 10.5 Hz, C*H*H=CH), 5.21 (s, 1H, >CHPh), 4.96 (s, 1H), 4.84 (d, 1H, *J* = 12.5 Hz, CH*H*Ph), 4.70 (d, 1H, *J* = 12.0 Hz, C*H*HPh), 4.64 (d, 2H, *J* = 6.0 Hz), 4.59 (d, 1H, *J* = 5.5 Hz), 4.14–4.19 (m, 2H, COOC*H*$_2$CH$_3$), 3.85 (d, 1H, *J* = 12.5 Hz), 3.67 (dd, 1H, *J* = 10.0, 12.0 Hz), 3.37 (d, 2H, *J* = 8.5 Hz, H-2, H-6_b), 1.19 (t, 3H, *J* = 7.0 Hz, COOCH$_2$C*H*$_3$); HR MALDI-TOF MS m/z calcd for $C_{27}H_{30}O_9$ $[M+Na]^+$ 521.1787, found 521.1762.

Acetyl 6-*O*-acetyl-3-*O*-allyloxycarbonyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-α-D-glucopyranose (11*R*). Trimethylsilyl trifluoromethanesulfonate (0.2 μL, 0.01 mmol) was added to a solution of **10*R*** **(**175 mg, 0.35 mmol) in acetic anhydride (3 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. After which it was quenched with an aqueous saturated solution of $NaHCO_3$ (10 mL) and then diluted with dichloromethane (10 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo.* The residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 3/1) to afford **11*R*** (206 mg, 98%): colorless syrup, R_f = 0.59 (ethylacetate/hexane, 1/1); $[\alpha]^{20}_D$ = -67^o (*c* = 2.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26–7.38 (m, 10H, Ar), 6.21 (d, 1H, *J* = 3.0 Hz, H-1), 5.93-6.03 (m, 1H, C*H*=CH_2), 5.39 (t, 1H, *J* = 9.5 Hz, H-3), 5.29 (dd, 1H, *J* = 7.5, 18.0 Hz, CH=CH*H*), 5.25 (dd, 1H, *J* = 10.5, 18.0 Hz, CH=C*H*H), 5.00 (s, 1H, >CHPh), 4.72 (d, 1H, *J* = 11.0 Hz, C*H*HPh), 4.68–4.73 (m, 2H, OC*H*HCH=CH_2, OCH*H*CH=CH_2), 4.55 (d, 1H, *J* = 11.0 Hz, CH*H*Ph), 4.21–4.28 (m, 2H, H-6_a, H-6_b), 4.15 (q, 2H, *J* = 7.0 Hz, COOC$H_2$$CH_3$), 3.95–3.97 (m, 1H, H-5), 3.73 (dd, 1H, *J* = 3.0, 9.5 Hz, H-2), 3.66 (t, 1H, *J* = 9.5 Hz, H-4), 2.02 (s, 6H, $COCH_3$), 1.19 (t, 3H, *J* = 7.0 Hz, $COOCH_2CH_3$); HR MALDI-TOF MS m/z calcd for $C_{31}H_{36}O_{12}$ $[M+Na]^+$ 623.2104, found 623.212

6-*O*-Acetyl-3-*O*-allyloxycarbonyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-α-D-glucopyranosyl trichloroacetimidate (13*R*). Hydrazinium acetate (28 mg, 0.30 mmol) was added to a solution of compound **11*R*** (165 mg, 0.27 mmol) in DMF (5 mL) at room temperature. After stirring for 18 h, the reaction mixture was then quenched with an aqueous saturated solution of $NaHCO_3$ (10 mL), and diluted with ethyl acetate (10 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo.* The residue was

purified by flash silica gel column chromatography (hexane/ethyl acetate, 3/1) to afford **12*R*** (130 mg, 86%). Trichloroacetonitrile (230 μL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 14 μL) were added to a solution of **12*R*** (130 mg, 0.23 mmol) in dichloromethane (5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was concentrated *in vacuo* and the the residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 3/1) to afford **13*R***. (155 mg, 95%): R_f = 0.74 (ethyl acetate/hexane, 1/1); $[\alpha]^{20}_D$ = +53° (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (s, 1H, =NH), 7.26-7.47 (m, 10H, Ar), 6.43 (d, 1H, *J* = 3.5 Hz, H-1), 5.92-5.99 (m, 1H, C*H*=CH_2), 5.50 (t, 1H, *J* = 10.0 Hz, H-3), 5.38–5.41 (m, 1H, CH=C*H*H), 5.24-5.26 (m, 1H, CH=CH*H*), 5.06 (s, 1H, >CHPh), 4.74 (d, 1H, *J* = 10.5 Hz, C*H*HPh), 4.70-4.75 (m, 2H, OC*H*HCH=CH_2, OCH*H*CH=CH_2), 4.57 (d, 1H, *J* = 10.5 Hz, C*H*HPh), 4.24–4.27 (m, 2H, H-6_a, H-6_b), 4.14–4.17 (m, 2H, COOC$H_2$$CH_3$), 4.10 (d, 1H, *J* = 13.5 Hz, H-5), 3.82 (dd, 1H, *J* = 3.0, 9.5 Hz, H-2), 3.71 (t, 1H, *J* = 9.5 Hz, H-4), 2.02 (s, 3H, $COCH_3$), 1.20 (t, 3H, *J* = 7.0 Hz, $COOCH_2CH_3$).

General Procedure for the Glycosylations

A mixture of glycosyl donor **9R** or **9*S*** (20 mg, 0.03 mmol), glycosyl acceptor (0.036 mmol) and activated molecular sieves (4Å) in dichloromethane (10 mL) was stirred at room temperature for 1 h under an atmosphere of argon. After cooling the mixture to -78 °C, trimethylsilyl trifluoromethanesulfonate (2.2 μL, 0.012 mmol) was added, and the reaction mixture was stirred at -78 °C for 1 h and then allowed to warm to 0 °C over a period of 1 h. The reaction mixture was quenched with an aqueous saturated solution of $NaHCO_3$ (10 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was

concentrated *in vacuo*. The residue was purified by silica gel column chromatography (dichloromethane/hexane/ethyl acetate = 2/2/1).

6-*O*-Acetyl-3-*O*-benzoyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-*β*-D-glucopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose (34*Rβ*). $[\alpha]^{20}_D$ = -32° (c = 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13–8.22 (m, 15H, Ar), 5.63 (s, 1H, >CHPh), 5.63 (t, 1H, *J* = 9.5 Hz, H-3'), 5.53 (d, 1H, *J* = 5.0 Hz, H-1), 4.60 (d, 1H, *J* = 10.5 Hz, C*H*HPh), 4.58 (d, 1H, *J* = 7.5 Hz, H-1'), 4.55-4.57 (m, 1H, H-5'), 4.48 (d, 1H, *J* = 10.5 Hz, CH*H*Ph), 4.34 (dd, 1H, *J* = 2.5, 12.5 Hz, H-3), 4.31 (dd, 1H, *J* = 2.5, 5.0 Hz, H-2), 4.26 (dd, 1H, *J* = 4.0, 12.5 Hz, H-4), 4.14-4.15 (m, 1H, H-$6_{a'}$), 4.05 (dd, 1H, *J* = 4.0, 11.0 Hz, H-6_a), 4.01–4.03 (m, 1H, H-6_b), 3.94 (q, 2H, *J* = 7.0 Hz, COOC*H*$_2$CH$_3$), 3.79 (dd, 1H, *J* = 2.0, 9.5 Hz, H-2'), 3.73 (t, 1H, *J* = 9.5 Hz, H-4'), 3.61-3.65 (m, 2H, H-5, H-$6_{b'}$), 2.07 (s, 3H, $COCH_3$), 1.52 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 0.97 (t, 3H, *J* = 7.0 Hz, $COOCH_2CH_3$); HR MALDI-TOF MS m/z calcd for $C_{44}H_{52}O_{15}$ $[M+Na]^+$ 843.3204, found 843.3197.

6-*O*-Acetyl-3-*O*-allyloxycarbonyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-*β*-D-glucopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose (36*Rβ*). $[\alpha]^{20}_D$ = -25° (c = 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26–7.54 (m, 10H, Ar), 5.95-6.01 (m, 1H, C*H*=CH_2), 5.63 (s, 1H, >CHPh), 5.52 (d, 1H, *J* = 5.0 Hz, H-1), 5.41 (dd, 1H, *J* = 1.0, 17.0 Hz, CH=CH*H*), 5.24 (d, 1H, *J* = 10.0 Hz, CH=C*H*H), 5.14 (t, 1H, *J* = 9.5 Hz, H-3'), 4.73–4.75 (m, 2H, OC*H*HCH=CH_2, OCH*H*CH=CH_2), 4.74 (d, 1H, *J* = 11.0 Hz, C*H*HPh), 4.54 (d, 1H, *J* = 11.0 Hz, CH*H*Ph), 4.50 (d, 1H, *J* = 7.5 Hz, H-4), 4.28–4.31 (m, 2H, H-2, H-6_a), 4.21–4.25 (m, 1H, H-6_b), 4.13–4.17 (m, 3H, H-5, COOC*H*$_2$CH$_3$), 4.01 (d, 1H, *J* = 7.0 Hz, H-1'), 3.67 (t, 1H, *J* = 9.5 Hz, H-4'), 3.61 (dd,

1H, *J* = 7.0, 9.5 Hz, H-2'), 3.53–3.56 (m, 2H, H-3, H-5'), 2.03 (s, 3H, $COCH_3$), 1.51 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.17 (t, 3H, *J* = 7.5 Hz, $COOCH_2CH_3$); HR MALDI-TOF MS m/z calcd for $C_{41}H_{52}O_{16}$ $[M+Na]^+$ 823.3153, found 823.3146.

6-*O*-Acetyl-3-*O*-allyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-α-D-glucopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose (38*R*α). Selected ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.42 (s, 1H, H-1'), 1.93 (s, 3H, $COCH_3$), 1.20 (t, 3H, *J* = 7.2 Hz, $COOCH_2CH_3$).

6-*O*-Acetyl-3-*O*-allyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-β-D-glucopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose (38*R*β). $[\alpha]^{20}_D$ = -24° (c = 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26–7.58 (m, 10 H, Ar), 6.13 (m, 1H, C*H*=CH), 5.71 (s, 1H, >CHPh), 5.54 (d, 1H, *J* = 4.5 Hz, H-1), 5.36 (d, 1H, *J* = 17.0 Hz, CH*H*=CH), 5.20 (d, 1H, *J* = 10.5 Hz, C*H*H=CH), 4.94 (d, 1H, *J* = 10.5 Hz, H-1'), 4.81 (dd, 1H, *J* = 5.5 Hz, OCH*H*CH=CH_2), 4.57 (t, 1H, *J* = 9.5 Hz, H-2'), 4.55 (d, 1H, *J* = 3.5 Hz, H-3), 4.39 (d, 1H, *J* = 7.5 Hz, OC*H*HCH=CH_2), 4.31 (d, 1H, *J* = 11.0 Hz, C*H*HPh), 4.26 (d, 1H, *J* = 11.0 Hz, CH*H*Ph), 4.24 (d, 1H, *J* = 4.5 Hz, H-2), 4.20 (q, 2H, *J* = 7.0 Hz, $COOCH_2CH_3$), 4.12 (t, 1H, *J* = 9.5 Hz, H-3'), 4.01–4.02 (m, 2H, H-6_b, H-5'), 3.57–3.62 (m, 2H, H-6_a, H-$6_{a'}$), 3.49–3.53 (m, 2H, H-5, H-6_b'), 3.43–3.45 (m , 2H, H-4, H-4'), 2.02 (s, 3H, $COCH_3$), 1.53 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.18 (t, 3H, *J* = 7.0 Hz, $COOCH_2CH_3$); HR MALDI-TOF MS m/z calcd for $C_{40}H_{52}O_{14}$ $[M+Na]^+$ 779.3255, found 779.3271.

6-*O*-Acetyl-3-*O*-benzoyl-4-*O*-benzyl-2-*O*-(*S*)-ethoxycarbonylbenzyl-*α*-D-glucopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose

(34*Sα*). $[\alpha]^{20}_{D}$ = +44° (c = 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12–8.02 (m, 15H, Ar), 5.88 (t, 1H, *J* = 9.5 Hz, H-3'), 5.52 (d, 1H, *J* = 5.0 Hz, H-1), 5.17 (d, 1H, *J* = 3.5 Hz, H-1'), 5.03 (s, 1H, >CHPh), 4.61 (dd, 1H, *J* = 2.5, 7.5 Hz, H-3), 4.53 (d, 1H, *J* = 11.0 Hz, CH*H*Ph), 4.40 (d, 1H, *J* = 11.0 Hz, C*H*HPh), 4.38 (dd, 1H, *J* = 2.5, 8.5 Hz, H-4), 4.29–4.32 (m, 3H, H-2, H-6_a, H-6_b), 4.13–4.15 (m, 1H, H-5'), 4.11–4.13 (m, 1H, H-5), 4.08 (q, 2H, COOC$H_2$$CH_3$), 3.81–3.83 (m, 2H, H-$6_{a'}$, H-$6_{b'}$), 3.64 (t, 1H, *J* = 9.5 Hz, H-4'), 3.60 (dd, 1H, *J* = 3.5, 9.5 Hz, H-2'), 2.06 (s, 3H, $COCH_3$), 1.56 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.12 (t, 3H, *J* = 7.5 Hz, $COOCH_2CH_3$); HR MALDI-TOF MS m/z calcd for $C_{44}H_{52}O_{15}$ $[M+Na]^+$ 843.3204, found 843.3192.

6-*O*-Acetyl-3-*O*-allyloxycarbonyl-4-*O*-benzyl-2-*O*-(*S*)-ethoxycarbonylbenzyl-*β*-D-glucopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose

(36*Sβ*). $[\alpha]^{20}_{D}$ = -60° (c = 0.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22-7.33 (m, 10H, Ar), 5.91-5.96 (m, 1H, CH_2=C*H*), 5.61 (s, 1H, >CHPh), 5.55 (d, 1H, *J* = 5.0 Hz, H-1), 5.37 (d, 1H, *J* = 17.5 Hz, CH*H*=CH), 5.26 (d, 1H, *J* = 11.0 Hz, C*H*H=CH), 5.14 (t, 1H, *J* = 10.0 Hz, H-3'), 4.59–4.64 (m, 5H, H-3, H-1', OCH*H*CH=CH_2, OC*H*HCH=CH_2, CH*H*Ph), 4.20 (d, 1H, *J* = 11.0 Hz, C*H*HPh), 4.33 (dd, 1H, *J* = 2.0, 5.0 Hz, H-2), 4.16-4.19 (m, 2H, H-6_b', H-6_a), 4.05–4.07 (m, 2H, H-6'$_a$, H-6_b), 3.46 (t, 1H, *J* = 9.5 Hz, H-4'), 3.28 (t, 1H, *J* = 9.5 Hz, H-2'), 2.00 (s, 3H, $COCH_3$), 1.60 (s, 3H, CH_3), 1.55 (s, 6H, CH_3), 1.45 (s, 3H, CH_3), 1.17 (t, 3H, *J* = 7.5 HZ, $COOCH_2CH_3$); HR MALDI-TOF MS m/z calcd for $C_{41}H_{52}O_{16}$ $[M+Na]^+$ 823.3153, found: 823.3136.

6-*O*-Acetyl-3-*O*-allyloxycarbonyl-4-*O*-benzyl-2-*O*-(*S*)-ethoxycarbonylbenzyl-*α*-D-glucopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose (36*Sα*). ^{1}H

NMR (500 MHz, $CDCl_3$) δ 7.25–7.40 (m, 10H, Ar), 5.81–5.88 (m, 1H, C*H*=CH_2), 5.51 (d, 1H, *J* = 5.0 Hz, H-1), 5.40 (t, 1H, *J* = 10.0 Hz, H-3'), 5.31 (d, 1H, *J* = 17.0 Hz, CH=CH*H*), 5.21 (d, 1H, *J* = 11.0 Hz, CH=C*H*H), 5.16 (d, 1H, *J* = 3.0 Hz, H-1'), 5.12 (s, 1H, >CHPh), 4.61–4.63 (m, 2H, H-3, H-5), 4.49–4.52 (m, 2H, OCH*H*CH=CH_2, OC*H*HCH=CH_2), 4.38 (d, 1H, *J* = 8.5 Hz, H-4), 4.30 (d, 1H, *J* = 5.0 Hz), 4.24–4.31 (m, 3H, H-2, H-6_a, H-6_b), 4.14 (q, 2H, COOC$H_2$$CH_3$), 4.03–4.07 (m, 1H, H-5'), 3.77–3.88 (m, 2H, H-$6_{a'}$, H-$6_{b'}$), 3.54 (t, 1H, *J* = 10.0 Hz, H-4'), 3.51 (dd, 1H, *J* = 3.0, 10.0 Hz, H-2'), 2.03 (s, 3H, $COCH_3$), 1.53 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.19 (t, 3H, *J* = 7.0 Hz, $COOCH_2CH_3$); HR MALDI-TOF MS m/z calcd for $C_{41}H_{52}O_{16}$ $[M+Na]^+$ 823.3153, found 823.3175.

Methyl (6-*O*-acetyl-3-*O*-allyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-α–D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (37*R*α). Selected ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.57 (d, 1H, *J* = 3.6 Hz, H-1'), 3.34 (s, 3H, CH_3), 1.78 (s, 3H, $COCH_3$), 1.16 (t, 3H, *J* = 7.2 Hz, $COOCH_2CH_3$).

Methyl (6-*O*-acetyl-3-*O*-allyl-4-*O*-benzyl-2-*O*-(*R*)-ethoxycarbonylbenzyl-β–D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (37*R*β). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.46 (d, 1H, *J* = 7.5 Hz, H-1'), 3.43 (s, 3H, CH_3), 1.98 (s, 3H, $COCH_3$), 1.19 (t, 3H, *J* = 7.2 Hz, $COOCH_2CH_3$).

Methyl (6-*O*-acetyl-3-*O*-benzoyl-4-*O*-benzyl-*2*-*O*-(*R*)-ethoxycarbonylbenzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (33*R*α). Selected ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.84 (d, 1H, *J* = 3.6 Hz, H-1'), 3.39 (s, 3H, CH_3), 2.03 (s, 3H, $COCH_3$), 1.22 (t, 3H, *J* = 7.2 Hz, $COOCH_2CH_3$).

Methyl (6-*O*-acetyl-3-*O*-benzoyl-4-*O*-benzyl-*2*-*O*-(*R*)-ethoxycarbonylbenzyl-*β*-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*α*-D-glucopyranoside (33*Rβ*). Selected ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.62 (d, 1H, *J* = 7.5 Hz, H-1'), 3.37 (s, 3H, CH_3), 1.96 (s, 3H, $COCH_3$), 1.18 (t, 3H, *J* = 7.2 Hz, $COOCH_2CH_3$).

Methyl (6-*O*-acetyl-3-*O*-benzoyl-4-*O*-benzyl-*2*-*O*-(*S*)-ethoxycarbonylbenzyl-*α*-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*α*-D-glucopyranoside (33*Sα*). $[\alpha]^{20}_D$ = +38° (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80–7.52 (m, 30H, Ar), 5.83 (d, 1H, *J* = 3.5 Hz, H-1), 5.75 (t, 1H, *J* = 9.5 Hz, H-3'), 5.04 (d, 1H, *J* =12.0 Hz, C*H*HPh), 4.92 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.90 (d, 1H, *J* = 12.0 Hz, C*H*HPh), 4.88 (s, 1H, >CHPh), 4.69 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.61 (d, 1H, *J* = 12.0 Hz, C*H*HPh), 4.56 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.55 (d, 1H, *J* = 3.5 Hz, H-1'), 4.43 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.32 (d, 1H, *J* = 12.0 Hz, C*H*HPh), 4.04–4.06 (m, 2H, H-6_a, H-6_b), 3.83–3.97 (m, 4H), 3.71 (t, 1H, *J* = 9.5 Hz, H-4), 3.60-3.63 (m, 3H), 3.51 (t, 1H, *J* = 9.5 Hz, H-4'), 3.46 (dd, 1H, *J* = 3.5, 9.5 Hz, H-2'), 3.43 (dd, 1H, *J* = 3.5, 9.5 Hz, H-2), 3.32 (s, 3H, OCH_3), 1.91 (s, 3H, $COCH_3$), 0.98 (t, 3H, *J* = 7.0 Hz, $COOCH_2CH_3$); HR MALDI-TOF MS m/z calcd for $C_{60}H_{64}O_{15}$ $[M+Na]^+$ 1047.4142, found 1047.4138.

Methyl (6-*O*-acetyl-3-*O*-benzoyl-4-*O*-benzyl-*2*-*O*-(*S*)-ethoxycarbonylbenzyl-*β*-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-*α*-D-glucopyranoside (33*Sβ*). Selected ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.86–7.40 (m, 30H, Ar), 5.91 (d, 1H, *J* = 7.5 Hz, H-1'), 3.38 (s, 3H, CH_3), 1.66 (s, 3H, $COCH_3$), 1.05 (t, 3H, *J* = 7.5 Hz, $COOCH_2CH_3$).

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