DEVELOPING A SELECTIVE INHIBITOR OF HUMAN GOLGI ALPHA-MANNOSIDASE II AND CARBOHYDRATE BASED VACCINES TARGETING BIOTERRORISM WEAPONS

by

WEI ZHONG

(Under the Direction of Geert-Jan Boons)

ABSTRACT

Inhibition of the mannose trimming enzyme human Golgi α-mannosidase II (HGMII) that acts late in the *N*-glycan processing pathway, provides one route to blocking the oncogene-induced changes in cell surface oligosaccharide structures. HGMII selectively cleaves α(1→3) and α(1→6) mannosyl residues present in its natural substrate $GlcNAcMan_5GlcNAc_2$. It has been proposed that HGMII has an extended binding site recognizing a large part of the oligosaccharide. To probe the substrate requirements of HGMII, we have synthesized a range of part-structures of $GlcNAcMan_5GlcNAc_2$ and determined kinetic parameters for hydrolysis by HGMII.

Mannostatin A is a potent inhibitor of HGMII. The thiomethyl moiety is a feature that is not observed in any other glycosidase inhibitors. It has been proposed that the sulfur atom and ε-CH_3 group of methionine residues are involved in several different interactions important for protein stability. To probe the interactions of the thiomethyl function with dGMII, Mannostatin B and analogs, which contain hydroxyl, methoxy or deoxy, respectively instead of the thiomethyl moiety, were prepared. The ability of the compounds to inhibit dGMII has been examined.

The glycoprotein BclA, an important constituent of the exosporium of *Bacillus anthracis* spores, is substituted with an oligosaccharide composed of a β-L-rhamnoside substituted with anthrose, a potential species-specific marker for *B. anthracis*. To study the antigenicity of anthrose, syntheses of an anthrose-containing trisaccharide and a series of structurally related analogues were developed. Serum antibodies of rabbits immunized with live or irradiated spores of *B. anthracis* Sterne $34F_2$ were able to recognize the synthetic trisaccharide–mcKLH conjugate. Inhibition using the trisaccharide analogues demonstrated that the isovaleric acid moiety of anthrose is an important structural motif for antibody recognition.

Francisella tularensis, the etiologic agent of tularemia in humans and animals, has been classified as a top-priority bio-terrorism agent. Recently, the structure of the lipopolysaccharide of *F. tularensis* was determined. We developed a highly convergent synthesis of a number of truncated structures to determine the smallest part structure of the core oligosaccharide, which can elicit antibodies that recognize LPS from *F. tularensis*. Such a structure will be attractive to be further developed as a vaccine candidate for tularemia.

INDEX WORDS: Oligosaccharides, Thioglycoside, Glycosidation, Golgi α-mannosidase II, *N*-glycan, Mannostatin A, Inhibitors, Structure-Activity-Relationships, *Bacillus anthracis*, Anthrax, Glycoconjuates, Vaccines, *Francisella tularensis*, Lipopolysaccharide

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DEDICATION

This thesis is dedicated to my parents, Zhibin Zhong and Chaoming Li, my wife, Li Jing, and my daughter, Athena Zhong for their unconditional support and love.

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ABBREVIATIONS

Ac ..... Acetyl

Ac_2O ..... Acetic anhydride

AgOTf ..... Silver triflate

AIBN ..... 2,2′-Azobis(2-methylpropionitrile)

All ..... Allyl

Am ..... Acetylmandelate

BDA Butane-2,3-diacetal

Bn ..... Benzyl

BSA ..... Bovine serum albumin

BSM ..... Benzenesulfinyl morpholine

BSP ..... 1-Benzenesulfinyl piperidine

BTF ..... (Trifluoromethyl)benzene

Bz ..... Benzoyl

CDA Cyclohexane-1,2-diacetal

ClAc ..... Chloroacetyl

COSY ..... Correlation Spectroscopy

DAST ..... (Diethylamino)sulfur trifluoride

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

DCC ..... *N,N*′-Dicyclohexylcarbodiimide

DCE 1,2-Dichloroethane

DCM ..... Dichloromethane

DDQ 2,3-Dichloro-5,6-dicyano-p-benzoquinone

DIPEA ..... Diisopropylethylamine

DMAP ..... 4-(Dimethylamino)pyridine

DMF ..... *N,N*-Dimethylformamide

DMSO ..... Dimethyl sulfoxide

DMTST ..... Dimethyl(methylthio) sulfonium triflate

DTBMP 2,6-Di-*tert*-butyl-4-methylpyridine

EDTA ..... Ethylene diamine tetraacetate

ELISA ..... Enzyme-Linked ImmunoSorbent Assay

Et ..... Ethyl

Fmoc 9-Fluorenylmethoxycarbonyl

HATU ..... *O*-(7-azabenzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate

HMBC ..... Heteronuclear Multiple Bond Correlation

HMDS ..... Hexamethyldisilane

HOAt 1-Hydroxy-7-Azabenzotriazole

HSQC ..... Heteronuclear Single Quantum Correlation

IAD ..... Intramolecular aglycon delivery

IDCP ..... Iodonium dicollidine perchlorate

KDO ..... 3-Deoxy-D-manno-octulosonic acid

KLH ..... Keyhole Limpet Hemocyanin

Lev ..... Levulinoyl

LVS ..... Live attenuated vaccine

mCPBA ..... 3-Chloroperbenzoic acid

Me ..... Methyl

MeCN ..... Acetonitrile

MeOTf ..... Methyl triflate

MMPP ..... Magnesium monoperoxyphthalate

MPBT ..... *S*-(4-methoxyphenyl) benzenethiosulfinate

NBS ..... *N*-bromosuccinimide

NIS ..... *N*-iodosuccinimide

Ph ..... Phenyl

PhthN ..... Phthalimido

PhthNSEt ..... *N*-(ethylthio)phthalimide

Piv ..... Pivaloyl

PMB ..... *p*-Methoxybenzyl

pMP ..... *p*-Methoxyphenyl

PTC ..... Phase transfer catalyzed

Py ..... Pyridine

SBAP ..... Succinimidyl 3-(bromoacetamido) propionate

SBox ..... *S*-benzoxazolyl

SE ..... Trimethylsilyl ethyl

STaz ..... *S*-thiazolinyl

TBAI ..... Tetrabutylammonium iodide

TBDMS ..... *Tert*-Butyldimethylsilyl

TBDPS ..... *Tert*-Butyldiphenylsilyl

TBPA .....Tris-(4-bromophenyl) ammoniumylhexachloroantimonate

TEA.....Triethyl amine

Tf_2O..... Triflic anhydride

TFA .....Trifluoroacetic acid

TfOH .....Trifluoromethanesulfonic acid

THF .....Tetrohydrofuran

TMSOTf.....Trimethylsilyl triflate

TOCSY .....Total Correlated Spectroscopy

Tol ..... *p*-Tolyl

Tr.....Triphenylmethyl

Troc ..... Trichloroethyloxycarbonyl

Ts..... Tosyl

TTBP.....2,4,6-tri-*tert*-butylpyrimidine

Z ..... Benzyloxycarbonyl

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Preparation and *O*-glycosidation of Thioglycosides

Alkyl and aryl thioglycosides are versatile building blocks for oligosaccharide synthesis.[1] Due to their excellent chemical stability, anomeric thio groups offer efficient protection of the anomeric center. However, in the presence of soft electrophiles, thioglycosides can be activated and used in direct glycosylations. Other attractive features of thioglycosides include their ability to be transformed into a range of other glycosyl donors and act as acceptors in glycosylation reactions, which make thioglycosides particularly suitable for use in chemoselective, orthogonal and iterative glycosylations.[2] This chapter reviews these properties of thioglycosides in detail.

1. Preparation of thioglycosides

Many methods exist for the efficient preparation of thioalkyl- and aryl glycosides (**Table 1.1**). Among these approaches, (Lewis) acid-mediated thiolysis of per-acetylated sugars is the most commonly employed route. Lemieux and co-workers[3,4] were the first to demonstrate the efficiency of this reaction by preparing several 1,2-*trans* ethyl 1-thioglycopyranosides using ethanethiol as a solvent and zinc chloride as a non-protic acid catalyst. A number of other catalysts have been reported, such as trimethylsilyl triflate (TMSOTf),[5] boron trifluoride diethyl etherate,[5-10] tin(IV) chloride,[11] titanium tetrachloride,[12-14] iron(III) chloride,[15] MoO_2Cl_2[16] and *p*-toluenesulfonic acid.[6] The use of phosphorus oxychloride for the thioglycosidation of

β-per-*O*-acetates has also been described,[17] however, this procedure gave poor selectivities and yields. Zirconium (IV) chloride[18,19] is an efficient catalyst in thioglycosylations leading mainly to the formation of per-acetylated 1,2-*trans* 1-thio-glycosides starting from the corresponding 1,2-*trans* acetylated saccharides. However, the preparation of per-acetylated 1,2-*trans* 1-thiomannosides proceeded in a disappointing yield.

Treatment of p-methoxyphenyl (pMP) glycosides prepared from the corresponding 1-*O*-acetyl sugars using boron trifluoride etherate as promoter in combination with thiophenol gave the corresponding thioglycosides in high yield and high 1,2-*trans* selectivity.[20] Sequential per-*O*-acetylation and thioglycosidation of unprotected reducing sugars using a stoichiometric quantity of acetic anhydride and alkyl- or aryl-thiols has been reported. These reactions, which are catalyzed by BF_3 etherate[21,22] or $HClO_4$,[23] constitute an efficient one-pot method for the synthesis of acetylated 1-thioglycosides.

1,2-*Trans* alkyl and aryl 1-thioglycosides have also been prepared by reaction of acylated glycosyl halides with thiols,[24-33] disulfides,[34] or alternatively, by *S*-alkylation of tetra-*O*-acetyl-1-thiosugars.[35] A convenient and simple approach for the stereoselective synthesis of 1,2-*trans* 1-thioglycosides is based on the utilization of glycosyl isothiourea derivatives as precursors.[36] Conversion of 2,3,4,6-tetra-*O*-acetyl-1-thio-*β*-D-hexapyranoses into their pseudothiourea derivatives[37] followed by treatment with alkyl iodides (bromides) under basic conditions provides an efficient method for the synthesis of alkyl 1-thio-β-D-glucosides.[38] Recently, this procedure was successfully used for the synthesis of thio-linked oligosaccharides.[39] A simple and efficient procedure for the synthesis of thioglycosides has been

achieved by reaction of glycosylisothiouronium salts with alkyl or heteroaryl halides under microwave irradiation, which allows short reaction times. The yields of the products were comparable to conventional methods.[40] Mild and stereoselective arylthio glycoside syntheses have also been accomplished by displacement of glycosyl halides under phase transfer catalyzed conditions.[41, 42] Hanessian and coworkers reported a direct conversion of alkyl *O*-glycosides to their corresponding thioglycosides.[43] This reaction was applied recently by Liu and co-workers for the synthesis of various thioglycosyl building blocks.[44]

Table 1.1. Methods for the preparation of thioglycosides

starting material	**reagents**	**reference**
peracetylated hexapyranoside	thiol, (Lewis) acid	3-20
acylated glycosyl halide	thiolate anion	24-34
acylated glycosyl halide	i) a) thiourea b) H_2O, K_2CO_3, ii) alkylation	36-40
acylated glycosyl halide	i) thioacetamide, ii) RBr, Phase transfer catalyst	41, 42
unprotected sugar	Ac_2O, acid, Arene thiols	21-23
dithioacetal	i) partial hydrolysis, ii) alkylation	37, 45
acylated 1-thioaldose	Alkyl halides	35
acylated 1-thioaldose	i) diazonium salt, ii) Δ	46
acylated glycosyl xanthates	sodium iodide	48
acylated glycosyl thiocyanates	Grignard reagent	49
acylated 1-thioaldoses	alkene, AIBN	47
1-*O*-alkyl glycosides	$PhSSiMe_3$, ZnI_2, Bu_4NI	43, 44

Partial hydrolysis of dithioacetals has been found useful for the preparation of anomers not obtained by the methods discussed above and for the preparation of furanosidic thioglycosides.[37,45]

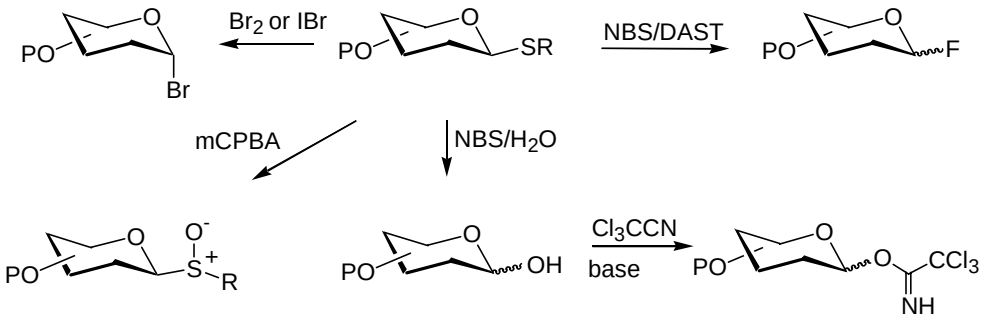
Aryl thioglycosides can be obtained by reaction of 1-thioglycopyranosides with diazonium salts, followed by thermal decomposition of the intermediate diazo-product.[46] Acylated 1-thio-aldoses react with alkenes in the presence of AIBN to give acylated alkyl 1-thio-glycopyranosides.[47] Thermal decomposition of glycosyl xanthates, which can be prepared by treating acylated glycopyranosyl halides with potassium alkyl- or benzyl xanthate, gives the corresponding 1-thio-glycosides.[48] Acylated glycopyranosyl thiocyanates can be prepared by reaction of acylated glycopyranosyl halides with potassium thiocyanate.[49] Treatment of the resulting product with grignard reagents led to the formation of alkyl and acyl thioglycosides.

2. Indirect Use of Thioglycosides in Glycosidations

Thioglycosides can be transformed into a range of other glycosyl donors (**Scheme 1.1**). For example, treatment of a thioglycoside with bromine gives a glycosyl bromide, which after work-up can be used in a Hg(II), Ag(I),[50] or phosphine oxide[51] promoted glycosylations. Iodine monobromide, an efficient reagent for the conversion of both activated and deactivated thioglycosides into glycosyl bromides, also permits the glycosylation via a bromide intermediate.[52]

A glycosyl bromide can also be prepared *in-situ* followed by glycosylation by reaction with $(Bu_4N)_2CuBr_4$ and AgOTf[8,53] or Et_4NBr and *N,N,N',N'*-tetramethylurea.[54] In an alternative approach, AgOTf/Br_2 was used as the activation reagent. A thioglycoside can also be converted into a glycosyl fluoride by treatment with *N*-bromosuccinimide/(diethylamino)sulfur trifluoride (NBS/DAST),[55-57] or hydrolyzed to give the corresponding aldose using a number of reagents such as *N*-bromosuccinimide (NBS) or NIS in wet acetone,[58-60] $AgNO_3$ in wet acetone,[61,62]

NBS/$NaHCO_3$ (aq) or $CaCO_3$ (aq) in THF,[63] NBS/HCl,[64] $nBu_4NIO_4/TrB(C_6H_5)_4$, nBu_4NIO_4/ trifluoromethanesulfonic acid (TfOH), $nBu_4NIO_4/HClO_4$,[65] $(NH_4)_6Mo_7O_{24}\cdot 4H_2O–H_2O_2$ with $HClO_4/NH_4Br$,[66] $V_2O_5–H_2O_2/NH_4Br$,[67] chloramine-T,[68] NIS/TfOH[69] and NIS/TFA.[70] The resulting hemiacetal are suitable substrates for the preparation of anomeric trichloroacetimidates.[71-73] Finally, another approach involves oxidation of a thioglycoside to the corresponding sulfoxide using mCPBA,[74-78] hydrogen peroxide–acetic anhydride–SiO_2,[76] oxone,[79,80] selectfluor,[81] magnesium monoperoxyphthalate (MMPP),[82] or *tert*-butyl hydroperoxide.[83] The resulting compound can then be activated with triflic anhydride at low temperature to give glycosides.[74,76,84-88]



Scheme 1.1. Leaving group interconversions of thioglycosides

3. Direct Use of Thioglycosides in Glycosidations

Ferrier and co-workers reported, for the first time, the use of thioglycosides in direct glycosylations.[89] A number of phenyl 1-thioglucopyranosides were solvolyzed in methanol in the presence of $Hg(OAc)_2$ to give the corresponding methyl glycosides. These reactions proceeded with inversion of anomeric configuration and gave only acceptable yields when reactive sugar alcohols were employed. For example, reaction of phenyl 1-thioglucopyranosides with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose gave an α-linked disaccharide in a yield

of 54%. Several other heavy metal salt promoters (**Table 1.2**) have been proposed for the activation of thioglycosides: a notable example being $Pd(ClO_4)_2$ which was used by Woodward[90] for the synthesis of Erythromycin A, and by Wuts[91] for the preparation of Avermectin.

Despite these important achievements, heavy metal salt-mediated activation of thioglycosides did not give consistently high yields and consequently did not find wide application in glycosidic bond chemistry. Lönn demonstrated[12] that methyl triflate is an efficient thiophilic promoter and glycosylations mediated by this reagent usually gave good yields of glycosides. For example, thioglycosides activated with MeOTf were applied for the preparation of a saccharide component of a glycoprotein isolated from fucosidosis patients and for the preparation of phytoelicitor oligosaccharides involved in the recognition and defense of soybean plants against infections by *Phytophthora megasperma*.

Methyl triflate is highly toxic and can methylate hydroxyls when glycosyl acceptors of low reactivity are used. Intensive research has focused on finding alternative reagents with more favorable properties, and today the most commonly used reagents include dimethyl(methylthio) sulfonium triflate (DMTST),[92] *N*-iodosuccinimide-triflic acid (NIS-TfOH) or NIS/TMSOTf,[93,94] iodonium dicollidine perchlorate (IDCP),[95,96] and phenylselenyl triflate (PhSeOTf).[97,98] Activation of thioglycosides involves the reaction of an electrophilic species with the sulfur lone-pair, resulting in the formation of a sulfonium intermediate. The latter intermediate is an excellent leaving-group and can be displaced by a sugar hydroxyl.

Table 1.2. Glycosidation of thioglycosides

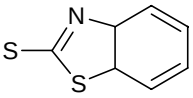
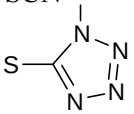
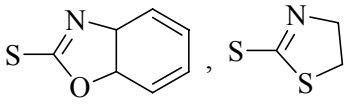
activator	SR	reference
$HgSO_4$	SPh	89
$HgCl_2$	SEt, SPh	89, 251
PdHgOTf	SPh	252
$Hg(OBz)_2$	SPh	253
$Hg(NO_3)_2$	SPh	254
$Cu(OTf)_2$		255
$Pd(ClO_4)_2$	SPy	90, 91
$CuBr_2/Bu_4NBr/AgOTf$	SMe, SEt	8, 53
PhSeOTf	SMe	97, 98
N-(phenylseleno)phthalimide/TMSOTf	SMe, SPh	256
$AgOTf/Br_2$	SEt	8
NBS	SPh	257
NIS/TfOH	SMe, SEt, SPh	93, 94
IDCP	SEt	95, 96
IDCT	SEt	258
I_2	SMe	259
$PhIO/Tf_2O$	SMe	260
$NOBF_4$	SMe	261, 262
MeI	SPy	263
MeOTf	SEt	12
PhSOTf	SMe, SEt, SPh	264
DMTST	SMe, SEt, SPh	92
$TrClO_4$	SCN	265-267
AgOTf		268
TBPA	SEt, SPh	102, 103
e	SPh	104-106

Table 1.2. Glycosidation of thioglycosides (cont.)

activator	SR	reference
$TrB(C_6F_5)_4/I_2$/DDQ	SEt	107
$TrB(C_6F_5)_4/NaIO_4$	SMe, SEt, SPh	108, 110
$TrB(C_6F_5)_4$/PhthNSEt	SEt	109
NBS/TfOH	SPh	269
NBS/Me_3SiOTf	SEt, SPh	270
1-Fluoropyridinium triflates	SEt	271
NIS/$HClO_4$-Silica	STol	272
NBS/strong acid salts	SMe, SPh	273
IX/AgOTf (X=Cl, Br)	SMe, SEt, SPh	274, 275, 111
I_2/hexamethyldisilane (HMDS) or IX (X=Cl, Br)	SMe	112, 113
NIS or NBS/ $TrB(C_6F_5)_4$	SEt	276
Ph_2SO/Tf_2O	SPh	87, 99
N-(Phenylthio)-ε-caprolactam	STol	277
Benzenesulfinyl morpholine/ triflic anhydride (BSM/Tf_2O)	STol	101
N-phenylselenophthalimide-$Mg(ClO_4)_2$/ PhIO-$Mg(ClO_4)_2$	SMe, SPh	278
S-(4-methoxyphenyl) benzenethiosulfinate/ triflic anhydride (MPBT/Tf_2O)	SPh	100
1-benzenesulfinyl piperidine/ 2,4,6-tri-tertbutylpyrimidine/ triflic anhydride (BSP/TTBP/Tf_2O)	SEt, SPh	85
AgOTf		114-118

Recently, a number of thiophilic activators that can activate thioglycosides of low reactivity at low temperature has been described. For example, thiophilic promoter systems such as diphenylsulfoxide,[87,99] *S*-(4-methoxyphenyl) benzenethiosulfinate (MPBT),[100] benzenesulfinyl morpholine (BSM)[101] or 1-benzenesulfinyl piperidine/2,4,6-tri-*tert*-butylpyrimidine

(BSP/TTBP)[85] in combination with triflic anhydride (Tf_2O), provide high yields of products for difficult glycosylations.

Thioglycosides can also be activated by a one-electron transfer reaction from sulfur to the activating reagent tris-(4-bromophenyl)ammoniumyl hexachloroantimonate ($TBPA^+$).[102,103] The use of this promoter was inspired by an earlier report where activation was achieved under electrochemical conditions to give an intermediate S-glycosyl radical cation intermediate,[104] and the reactivity and mechanism have also been explored.[105,106]

A combined use of trityl tetrakis(pentafluorophenyl) borate [$TrB(C_6F_5)_4$], iodine (I_2) and 2, 3-dichloro-5, 6-dicyano-*p*-benzoquinone (DDQ) effectively activates thioglycosides of low reactivity while a combined use of trityl tetrakis(pentafluorophenyl) borate and *N*-(ethylthio)phthalimide (PhthNSEt) activates highly reactive thioglycosides. Use of trityl tetrakis(pentafluorophenyl) borate and $NaIO_4$ as co-oxidant, can activate thioglycosides as well. Selective use of trityl salts to activate thioglycosides has been applied in a one pot glycosylation.[107-110] ICl/AgOTf works well for glycosylations with thioglycosyl donors having a participating group at C-2 of the glycosyl donor, whereas IBr/AgOTf is superior for glycosyl donors having a non-participating group at this position. The interhalogens in combination with silver triflate have been applied in the synthesis of bislactam analogues of Ganglioside GD3. IX promoter systems offer convenient handling of reagents and do not produce byproducts such as *N*-succinimide, which is released in the popular NIS/TMSOTf promoted glycosylations.[111-113]

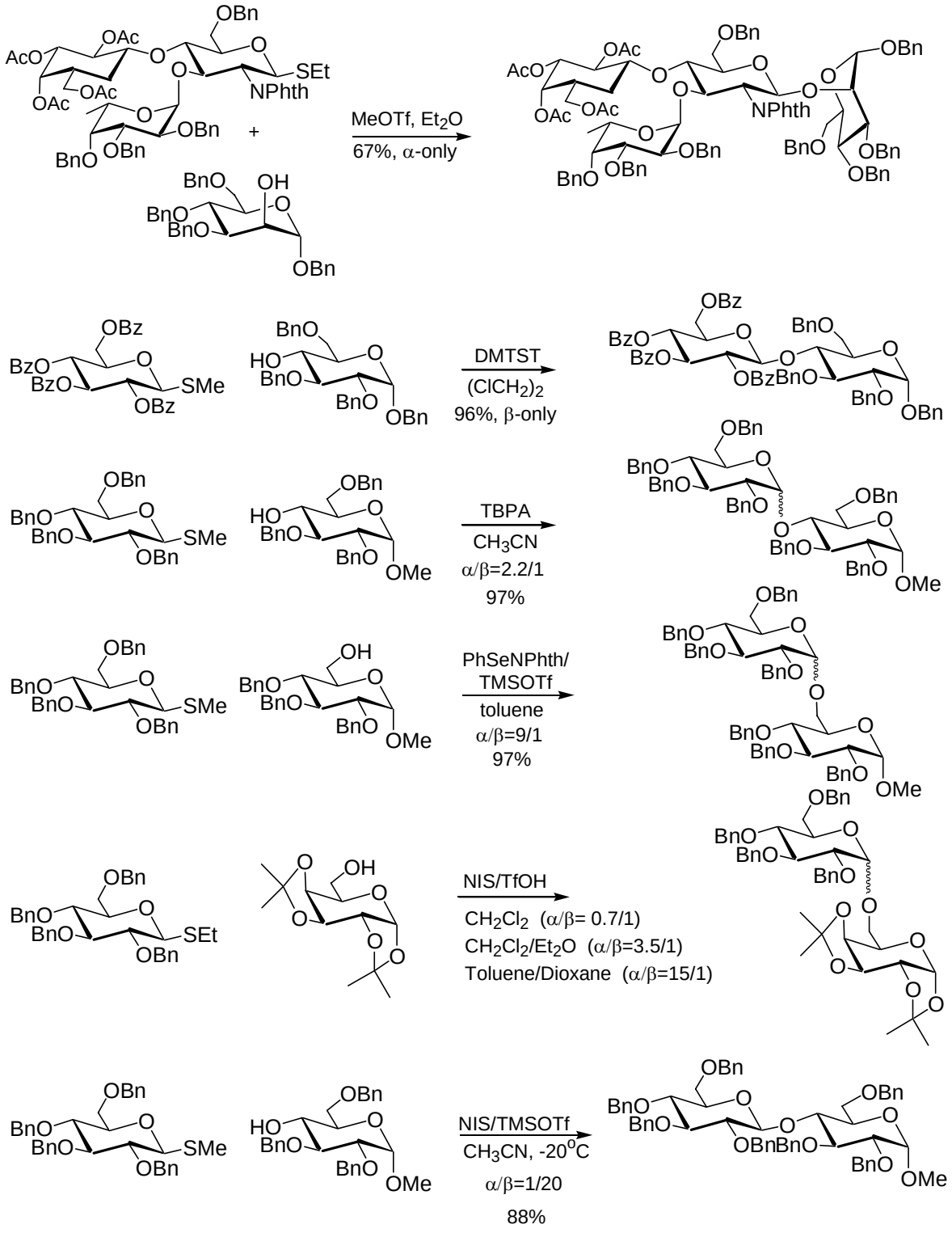
S-Benzoxazolyl (SBox) and, especially, S-thiazolinyl (STaz) moieties are sufficiently stable for use in anomeric protection. These derivatives can, however, be activated under mild conditions

using silver triflate.[114-118]

4. Anomeric Control in Glycosidations of Thioglycosides

The protecting group at C-2 of a glycosyl donor is an important determinant of the stereochemical outcome of a glycosylation.[119-121] In general, participating groups at C-2 such as *O*-acetyl, *O*-benzoyl and *N*-phthaloyl lead to the formation of 1,2-*trans* glycosides, whereas non-participating groups such as benzyl ethers, give mixtures of anomers (**Scheme 1.2**). The anomeric outcome of glycosylations with glycosyl donors having a non-participating group at C-2 is markedly influenced by the nature of the solvent.[122] In general, solvents of low polarity are thought to increase α-selectivity by suppression of the formation of oxa-carbenium ions. Solvents of moderate polarity, such as mixtures of toluene and nitromethane are highly beneficial when the glycosyl donors have participating C-2 substituents. It is likely that these solvents stabilize the positively charged intermediates.

Mechanistic studies[123] have shown that thioglycosides can undergo *in-situ* anomerization in the presence of iodonium ion catalysts. It has been demonstrated that this anomerization proceeds by intermolecular exchange of alkyl thio groups. Increase in steric bulk of the leaving group resulted in incomplete or no anomerization. It has been proposed that this anomerization process is important for the stereochemical outcome of glycosylations.[123]



Scheme 1.2. Stereoselective glycosidations of thioglycosides

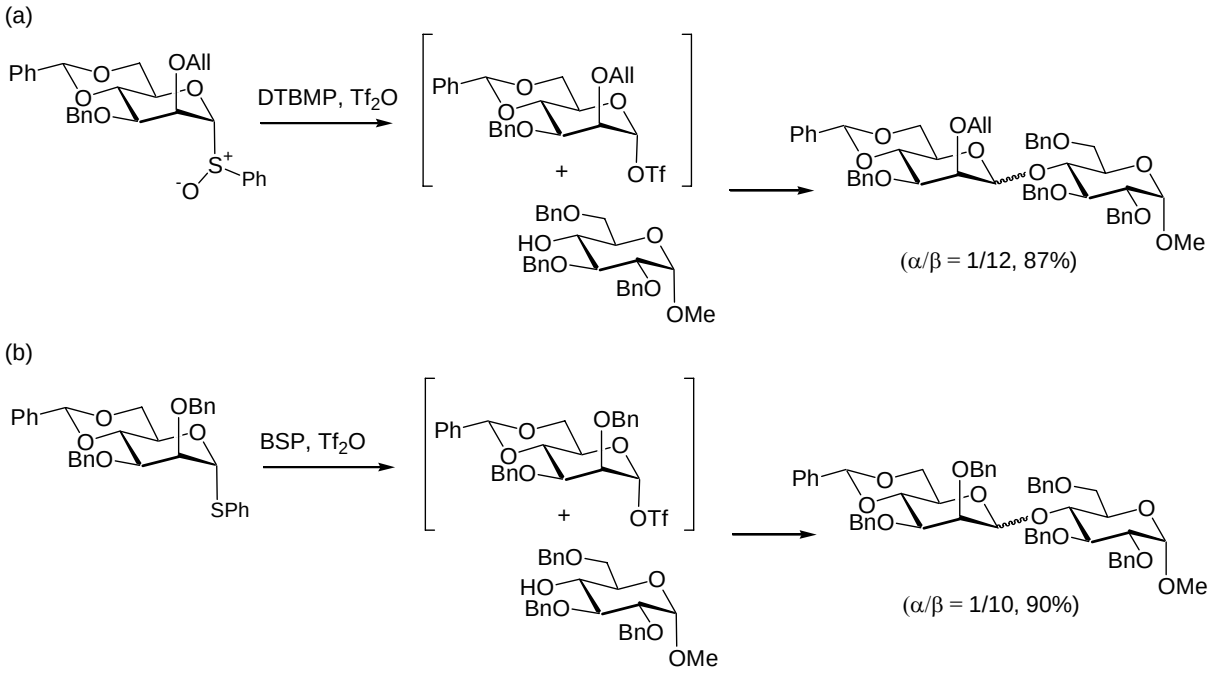
Some solvents form complexes with the oxa-carbenium ion intermediates thereby affecting the stereoselectivity of glycosylations. For example, diethyl ether is known to increase α-anomeric

selectivity, presumably by formation of a diethyl oxonium-ion intermediate. The β-configuration of this intermediate is probably favored due to steric reasons. Nucleophilic displacement with inversion of configuration will then give an α-glycoside. Boons and co-workers showed that iodonium-ion mediated glycosidations of thioglycosides in toluene/1,4-dioxane give much higher α-selectivities than when conventional glycosylation solvents are employed.[124] Furthermore, it was shown that the iodonium-ion source, glycosyl donor/acceptor ratio of and presence of molecular sieves also have major impacts on the stereochemical outcome of a glycosylation.

Acetonitrile is another participating solvent, which in many cases leads to the formation of an equatorially-linked glycoside.[125-131] It has been proposed that these reactions proceed *via* an α-nitrilium ion intermediate. It is not well understood why the nitrilium ion adopts an axial-orientation; however, spectroscopic studies support the proposed anomeric configuration.[130,131] It is known that nucleophilic substitution of the α-nitrilium ion by an alcohol leads to β-glycosidic bonds and the best β-selectivities are obtained when reactive alcohols at low reaction temperatures are employed. Unfortunately, mannosides give poor anomeric selectivities under these conditions.

β-mannosides are difficult to introduce because the axial C-2 substituent of a mannosyl donor sterically and electronically disfavors nucleophilic attack from the β-face. β-Mannosides have been obtained by the direct substitution of α-glycosyl triflates, which are conveniently prepared by treatment of an anomeric sulfoxide with triflic anhydride (Tf_2O) or thioglycosides with NIS (**Scheme 1.3a**).[128,132-134] An α-triflate is formed because this anomer is stabilized by a strong endo-anomeric effect. Upon addition of an alcohol, the triflate is displaced in an S_N2 fashion

resulting in the formation of a β-mannoside. A mixture of anomers is obtained when triflic anhydride is added to a mixture of a sulfoxide and alcohol. In this case, it is very likely that the glycosylation proceeds through an oxa-carbenium ion since triflate formation is less likely due to the greater nucleophilicity of an alcohol.



Scheme 1.3. Glycosidation of intermediate α–triflates

Another prerequisite of β-mannoside formation is the protection of the mannosyl donor as a 4,6-*O*-benzylidene acetal. Although this observation is difficult to rationalize it is has been suggested that oxa-carbenium ion formation is disfavored because of torsional strain engendered on going to the half-chair conformation of this intermediate. Crich and co-workers employed α-deuterium kinetic isotope effects to unravel the mechanism of 4,6-O-benzylidene-directed β-mannosylation. It was found that a torsionally disarming benzylidene acetal opposed rehybridization at the anomeric carbon, thereby shifting the complete set of equilibria toward the covalent triflate and away from the solvent-separated ion pair (SSIP), resulting in minimization

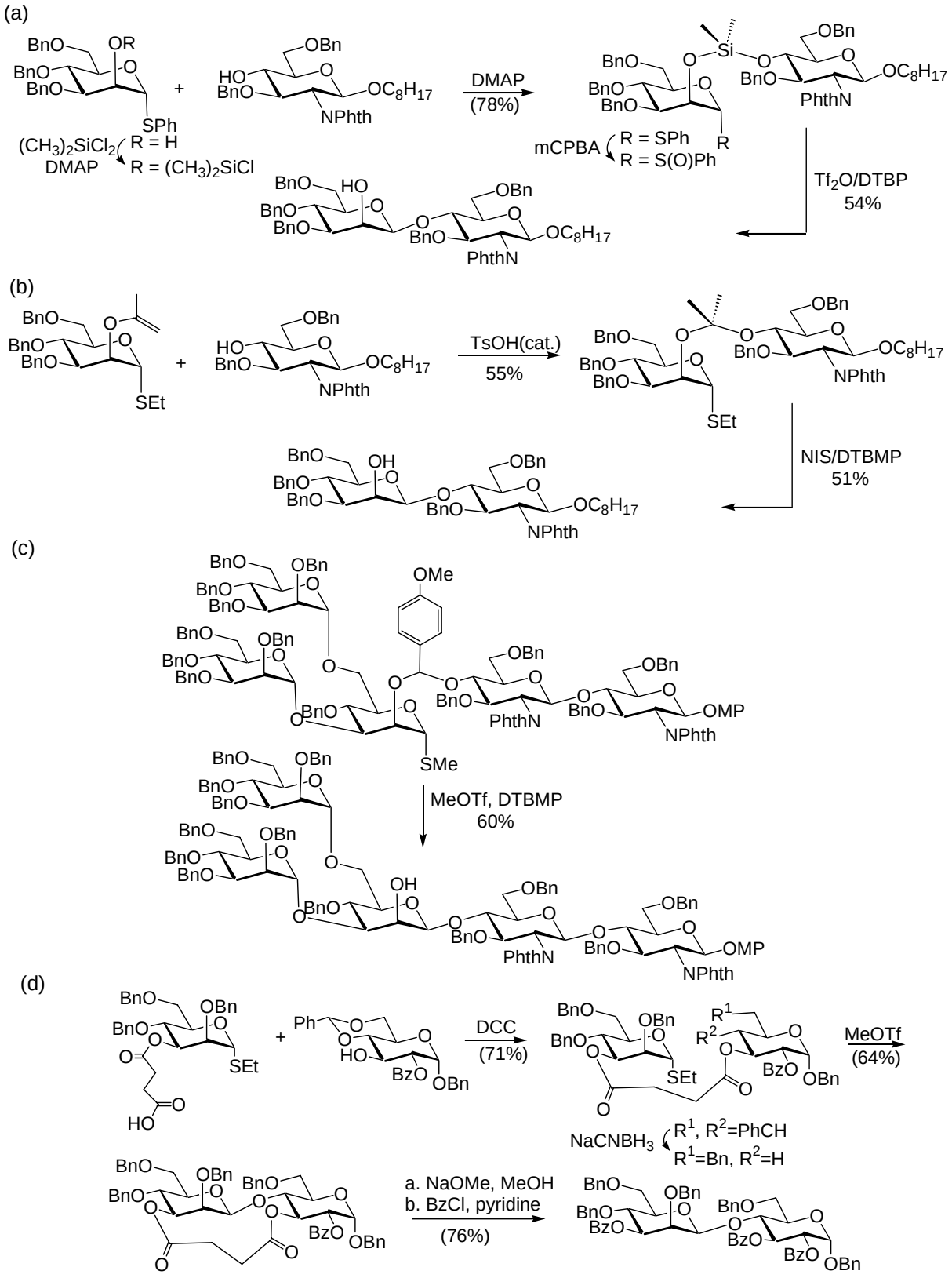
of α-glycoside formation.[135]

Recently, powerful and metal-free thiophilic reagents have been shown to readily activate thioglycosides via glycosyl triflates leading to β-mannosides. For example, a combination of BSP and Tf_2O in the presence of TTBP[85] or MPBT and Tf_2O in the presence of DTBMP[100] at low temperature has been used to prepare β-mannosides in good yield and high β-anomeric selectivity. It was also found that 2-*O*-propargyl ethers were advantageous in the 4,6-*O*-benzylidene acetal directed β-mannosylations (**Scheme 1.3b**).[136,137] This approach has been applied to the synthesis of β-mannans from *Rhodotorula glutinis*, *Rhodotorula mucilaginosa*, and *Leptospira biflexa*.[138] Van Boom and coworkers developed the very potent thiophilic glycosylation promoter system, diphenylsulfoxide in combination with triflic anhydride, to activate thioglycosides for β-mannosylation.[87,99,139] Furthermore, Demchenko and coworkers found that the stereoselectivity of β-mannosylation can be improved when a participating moiety at C-4 (*O*-anisoyl, *O*-thiocarbamoyl) is employed. This improvement was achieved in glycosidations of *S*-ethyl and, especially, *S*-benzoxazolyl (SBox) mannosides.[140]

Stork[141,142] and Hindsgaul[143-145] reported independently the preparation of β-mannosides in a highly stereoselective manner by intramolecular aglycon delivery. In this approach, a sugar alcohol (ROH) is first linked *via* an acetal or silicon tether to the C-2 position of a mannosyl donor and subsequent activation of the anomeric center of this adduct forces aglycon delivery from the β-face of the glycosyl donor. The remnant of the tether hydrolyses during the work-up procedure (**Scheme 1.4a)**. A silicon tether was easily introduced by conversion of a glycosyl acceptor into a corresponding chlorodimethyl silyl ether and subsequent reaction with the C-2

hydroxyl of a donor to give the silicon tethered compound.[141,142] Oxidation of the phenylthio-group yielded a phenylsulfoxide, which upon activation with Tf_2O resulted in the selective formation of a β-mannoside in a 61% overall yield. Alternatively, direct activation of thioglycosides also resulted in the formation of β-mannosides. Acetal tethers could easily be prepared by treatment of equimolar amounts of a 2-propenyl ether derivative of a saccharide with a sugar hydroxyl in the presence of a catalytic amount of acid (**Scheme 1.4b**).[143-145] Activation of the anomeric thio moiety of the tethered compound with *N*-iodosuccinimide (NIS) in dichloromethane resulted in the formation of β-linked disaccharides. In this reaction, no α-linked disaccharide could be detected. It is of interest to note that when this reaction was performed in the presence of methanol, no methyl glycosides were obtained. This experiment indicates that the glycosylation proceeds *via* a concerted reaction and not by addition to an anomeric oxa-carbenium ion.

Fairbanks modified the intramolecular aglycon delivery to achieve stereospecific 1,2-*cis* glycosylation via 2-O-vinyl thioglycosides, which were synthesized from the corresponding alcohols by Ir-catalyzed transvinylation with vinyl acetate, followed by iodine-mediated tethering of a range of primary and secondary carbohydrate acceptors, and finally intramolecular aglycon delivery (IAD).[146-150] The use of such an intramolecular glycosylation strategy furnished the desired α-gluco and β-manno disaccharides in a stereoselective manner.[146-149] The methodology has been applied for the synthesis of a tetrasaccharide derived from *N*-linked glycans.[150]

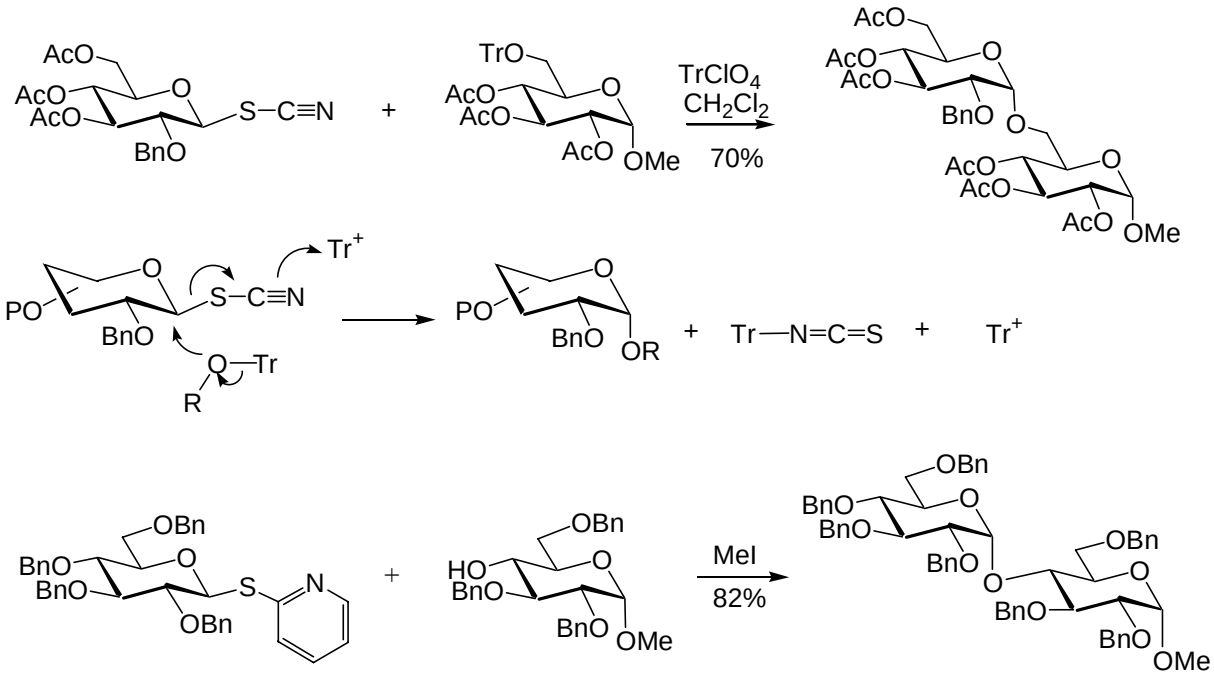


Scheme 1.4. Synthesis of β-mannosides by intramolecular aglycon delivery

An intramolecular acetal has also been introduced by treatment of a mixture of a 1-thio-mannoside, having a methoxybenzyl protecting group at C-2 and an alcohol with DDQ[71] (**Scheme 1.4c**). Activation of the thioglycoside with methyl triflate gave a β-mannoside as the only anomer. This approach was employed for the synthesis of the core pentasaccharide of N-linked glycoproteins.

Ziegler and Lemanski prearranged a glycoside by employing a succinyl tether between C-6 of a mannosyl donor and C-3 of glucosyl acceptor.[151,152] They found that the nature of the glycosyl acceptor and the length of the tether affected the anomeric selectivity of the intramolecular mannosylation (**Scheme 1.4d**).[153]

Kochetkov and coworkers have reported[154-156] an efficient approach for the synthesis of 1,2-*cis*-pyranosides employing 1,2-*trans*-glycosyl thiocyanates as glycosyl donors and tritylated sugar derivatives as glycosyl acceptors (**Scheme 1.5**). This coupling is initiated by reaction of the nitrogen of the thiocyanate with trityl cation from $TrClO_4$. This results in leaving group departure accompanied by simultaneous nucleophilic attack by a trityl protected sugar alcohol to give an α-glycoside. It appears that this reaction proceeds by clean S_N2 inversion of configuration at the anomeric center. Mereyala and co-workers[157-159] used 2-pyridyl 1-thioglycosides having a non-participating C-2-substituent as glycosyl donors and methyl iodide as an activator to achieve stereoselective *α*-glycosylations in the D-gluco- and D-galacto-series. The reaction is proposed to proceed *via* the electrophilic activation of the glycosyl donor by methyl iodide followed by the formation of sulfenium salt. An alcohol displaces the latter intermediate via a S_N2 mechanism.



Scheme 1.5. Glycosidations of thioglycosides by inversion of configuration

2-Thio-sialyl glycosides (**Scheme 1.6a**) are commonly used for the preparation of α-sialyl glycosides.[97,98,160-163] The best yields and anomeric selectivities have been obtained when partially protected galactosyl acceptors are employed. Furthermore, it has been found that the reactivity of sialyl thioglycosyl donors can be significantly increased by acetylation of the acetamido group[164] (**Scheme 1.6b**). This modification enables the efficient synthesis of α-(2→8)-dimers of Neu5Ac.[165]

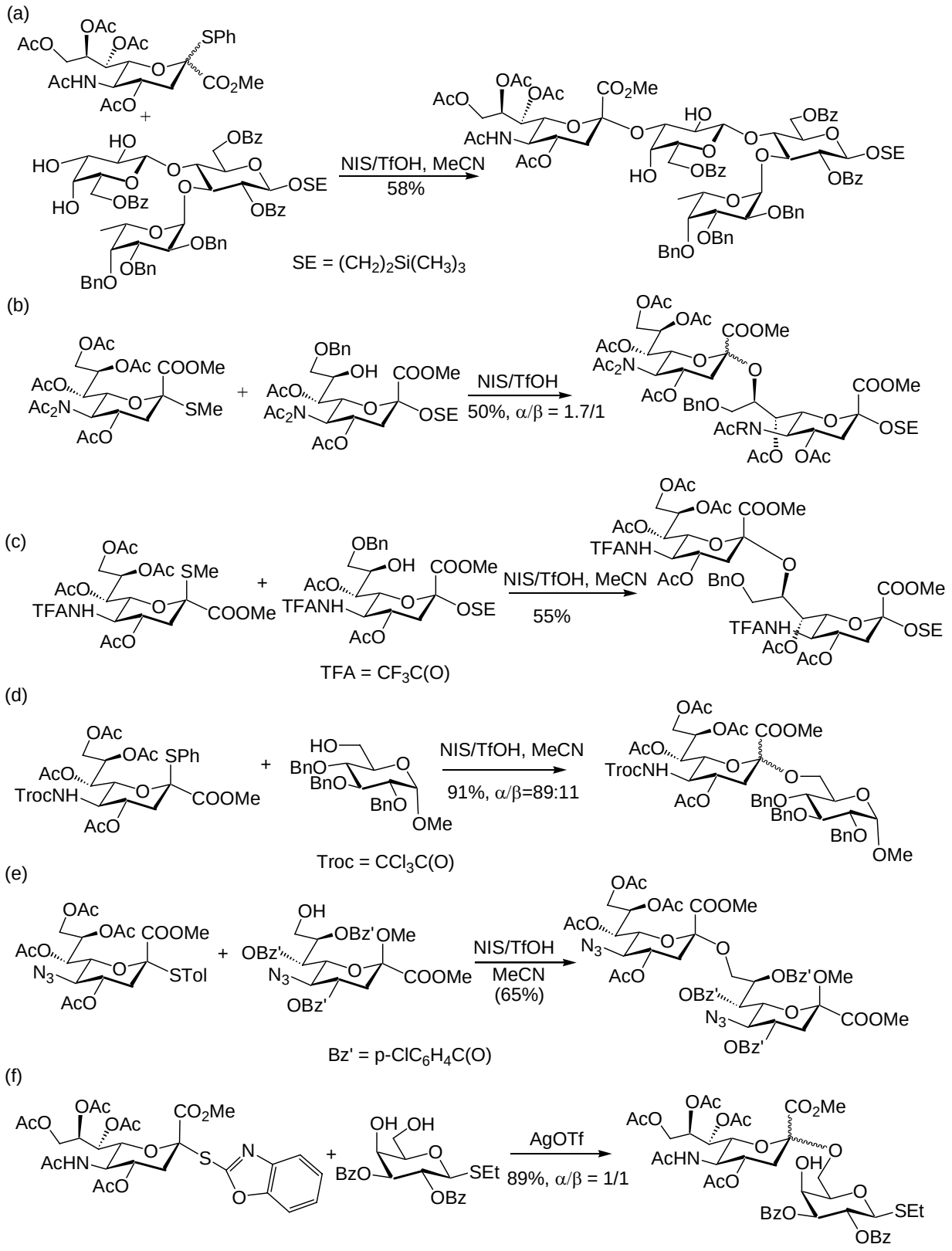
Boons and coworkers[165,166] modified the C-5 amino group of 2-methyl and 2-thiophenyl sialosides into *N*-TFA derivatives, which provided a glycosyl donor that gives good yields and high α-anomeric selectivities in direct sialylations with a wide range of glycosyl acceptors of differing reactivities (**Scheme 1.6c**). Sialyl acceptor protected as an *N*-TFA derivative gave the best yields and it was postulated that lower nucleophilicity of the TFA-protected amino

functionalities and enhanced reactivity affects the efficiency of the glycosylations.

Takahashi and coworkers described an effective sialylation method utilizing the *N*-Fmoc, *N*-Troc, and *N*-trichloroacetyl-β-thiophenyl sialosides (**Scheme 1.6d**).[167] It was found that the *N*-Troc derivative of N-acetylneuraminic acid performed better than the corresponding *N*-Fmoc derivative. An *N*-Troc β-thiosialoside was applied for the synthesis of glycosyl amino acids by one-pot glycosylation.[167] Importantly, it was found that the *N*-Troc protecting group could be converted into an acetamido moiety without causing racemization of the peptide.

Another effective α-selective sialylation involves the use of a 5-*N*, 4-*O*-carbonyl-protected sialyl donor, which could efficiently be used for the preparation of an α(2, 8)-tetrasialoside. It was found that the 5-*N*, 4-*O*-carbonyl protecting group improves the reactivity of the C-8 hydroxyl group of the sialyl acceptor.[168]

Wong and coworkers showed that 5-azido sialyl donors protected with *O*-acetyl ester are useful for α-selective glycosylations of primary hydroxyls (**Scheme 1.6e**).[169] It was proposed that the linear and electron withdrawing nature of the C-5 azido moiety stabilizes the reactive axial acetonitrile adduct to allow the incoming nucleophile to approach the α-face in an S_N2-like fashion. In addition, a chemoselective glycosylation method has been developed for the synthesis of NeuAcα- (2→9) NeuAc as thioglycoside donor for use in subsequent glycosylations.[169]



Scheme 1.6. Glycosidations of thioglycosides of Neu5Ac

Recently, De Meo and coworker described two novel sialyl donors bearing a thioimidoyl moiety

as leaving group (**Scheme 1.6f**).[170] The SBox and STaz sialosides proved to be excellent glycosyl donors when activated with MeOTf or AgOTf. In general, good yields and stereoselectivities were observed with a number of glycosyl acceptors ranging from highly reactive primary to less reactive secondary hydroxyls. The most attractive feature of thiomidoyl moieties is that they can be selectively activated in the presence of thioglycosides using AgOTf as promoter.

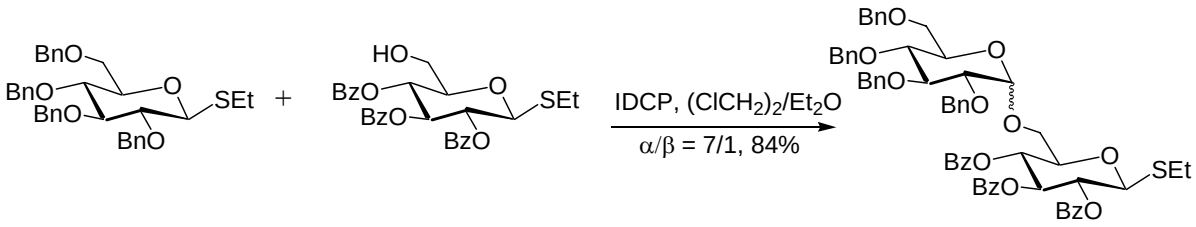
In brief, the use of acetonitrile as solvent and selection of an appropriate C-5 amino protecting group and reactive promoter system are critical for achieving high α–selectivities and yields in the synthesis of sialosides.

5. Glycosylation Strategies using Thioglycosides

Chemoselective glycosylations.

Van Boom and co-workers showed that the reactivity of thioglycosides can be controlled by selection of appropriate protecting groups. It was found that a C-2 ether protecting group activates and a C-2 ester deactivates the anomeric center.[96] This difference in reactivity was exploited for attractive chemoslective glycosylations. For example, iodonium-ion mediated coupling of a fully benzylated thioglycoside with a partially benzoylated thioglycosyl acceptor gave a disaccharide mainly as the α-anomer in a yield of 84% (**Scheme 1.7**). It has been established that the resulting disarmed thioglycosyl disaccharide can be readily activated using the strong thiophilic promoter NIS/TfOH. Subsequent coupling with a glycosyl acceptor gives a trisaccharide.[93,171-175] The chemoselective glycosylation approach was rationalized as follows: electron density on the anomeric sulfur atom in a 2-*O*-acyl ethylthio glycoside is decreased due

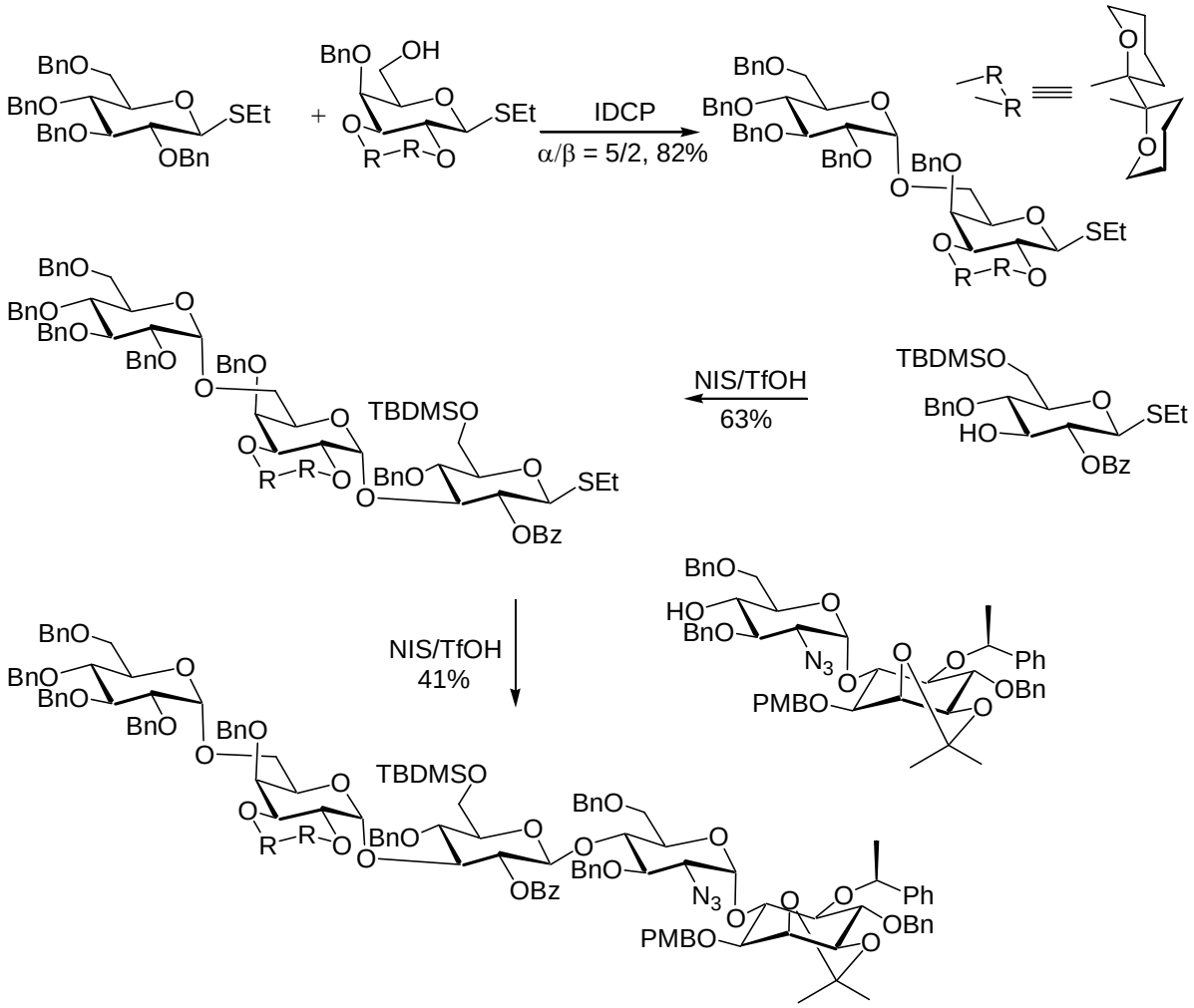
to inductive effects by the electron withdrawing ester functionality at C-2. As a result, nucleophilic complexation of the anomeric thio group with iodonium ions decreases and the thioglycoside can be regarded as disarmed with respect to an armed 2-*O*-alkyl thioglycoside. It is important to note that Fraser-Reid and co-workers introduced the armed–disarmed glycosylation protocol using n-pentenyl glycosides as glycosyl donors and acceptors.[94,176,177]



Scheme 1.7. Armed-disarmed glycosylations of thioglycosides

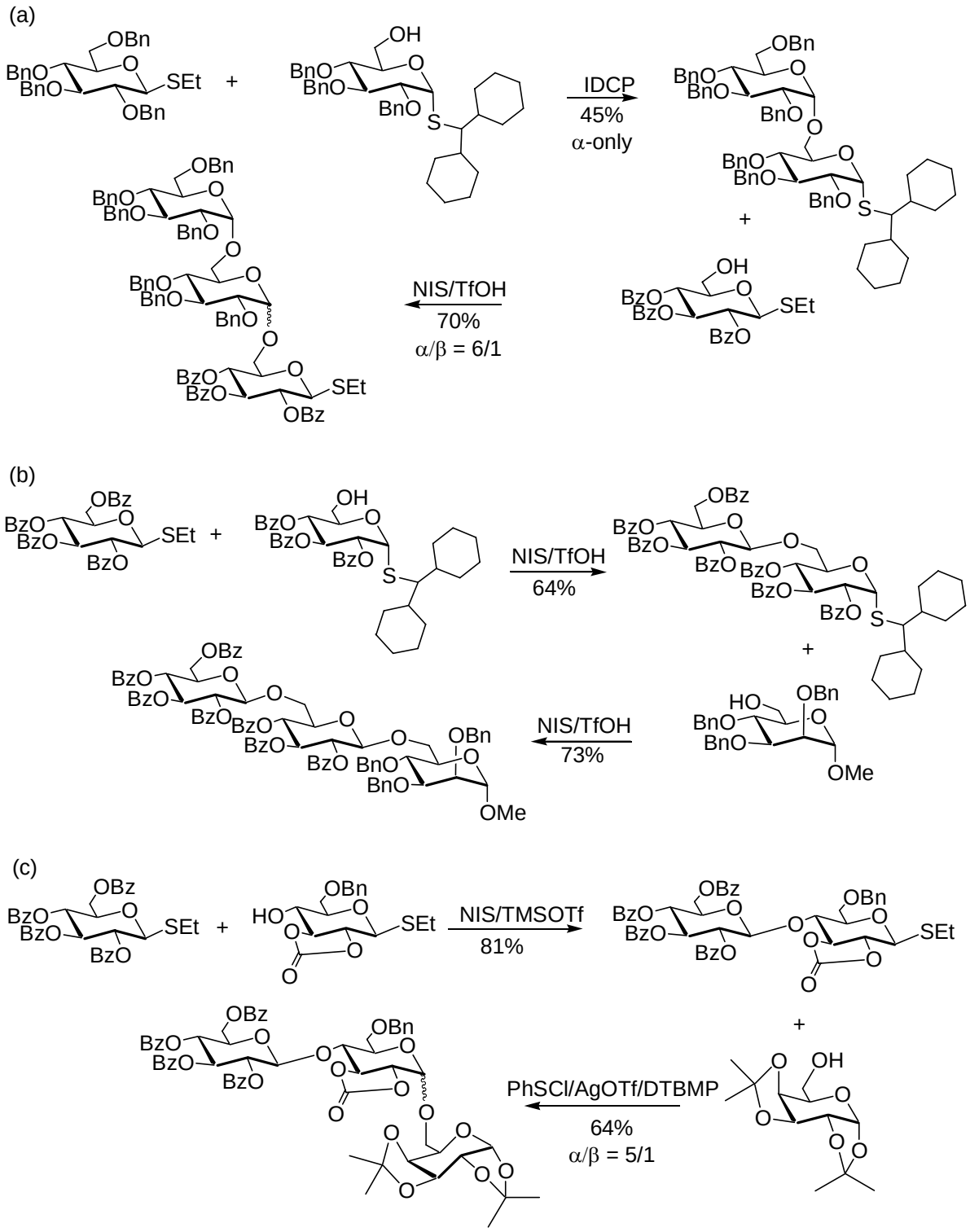
Ley and co-workers proposed [178-186] that the armed-disarmed glycosylation strategy could gain versatility by further tuning of glycosyl donor leaving group ability. In this respect, a dispiroketal or a butane-2,3-diacetal (BDA) protecting group has a marked effect on the reactivity of the anomeric center. It was found that thioglycosides protected with these functionalities have reactivities between an armed C-2 alkylated thioglycoside and a disarmed C-2 acylated thioglycoside (**Scheme 1.8**). For example, the three levels of anomeric reactivity were exploited for the preparation of a protected pseudo-pentasaccharide unit common to the variant surface glycoprotein of *Trypanosoma brucei*.[178] Thus, iodonium dicollidine perchlorate (IDCP) mediated chemoselective glycosylation of benzylated thioglycosyl donor with dispiroketal protected acceptor gave a disaccharide in excellent yield (82%, α/β = 5/2). Further chemoselective glycosylation of the torsionally-deactivated glycosyl donor with an electronically deactivated acceptor in the presence of the more powerful activator NIS/TfOH gave a 63% yield

of trisaccharide as one isomer. The pseudo-pentasaccharide was obtained by NIS/TfOH mediated condensation of the trisaccharide donor with a pseudo-disaccharide acceptor.



Scheme 1.8. Chemoselective glycosidations of thioglycosides

In the armed-disarmed glycosylation approach, the leaving group ability is controlled by protecting groups (ether/dispiroketal/ester). It may, however, be advantageous to control anomeric reactivity by means of modifying the leaving group. Boons and co-workers[187,188] showed that the bulkiness of the anomeric thio group has a marked effect on glycosyl donor reactivity and provides an opportunity to produce a new range of differentially reactive coupling substrates.



Scheme 1.9. chemoselective glycosidations with sterically deactivated thioglycosides

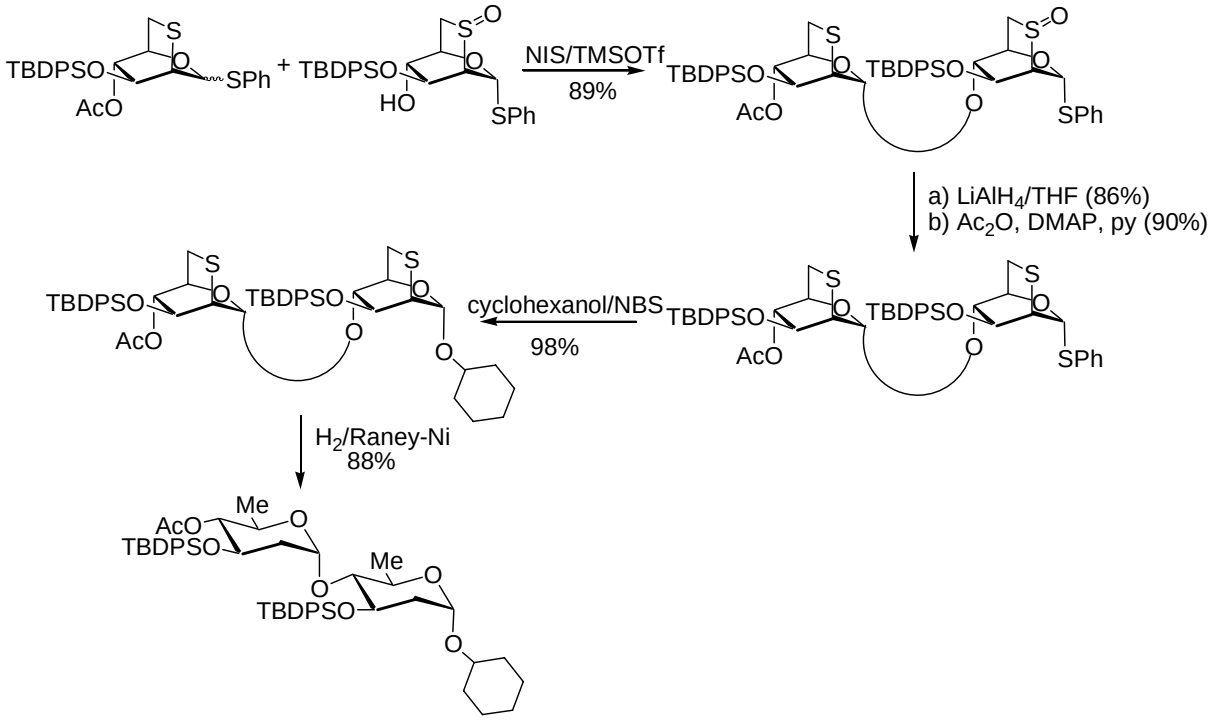
For example, IDCP mediated chemoselective glycosylation of a fully benzylated ethyl

thioglycosyl donor with a partially benzylated dicyclohexylmethyl thioglycosyl acceptor gave a disaccharide in a yield of 45% as one anomer (**Scheme 1.9**). Further chemoselective coupling of the resulting sterically deactivated donor with an electronically deactivated glycosyl acceptor in the presence of the more powerful promoter system NIS/TfOH gave a trisaccharide in yield of 70%. In both glycosylations, no self-condensed or polymeric products were detected (**Scheme 1.9a**). These experiments show that the reactivity of a C-2 benzylated dicyclohexylmethyl thioglycoside is between ethyl thioglycosides having a fully armed ether and disarmed ester protecting group on C-2. This new approach to tuning thioglycoside reactivity was employed for the preparation of a phytoalexin-elicitor active oligosaccharide and its photoreactive derivatives (**Scheme 1.9b**).[189]

The *trans*-2,3-cyclic carbonate function was introduced as a non-participating thioglycoside, which deactivates the anomeric center of thioglycosides by both electronic and conformational effects. These thioglycosides are significantly less reactive than corresponding thioglycosides having ester-protecting groups at C-2.[190] Thioglycosides protected as a *trans*-2,3-cyclic carbonate remain intact upon treatment with thiophilic promoters such as NIS/TMSOTf, NIS/AgOTf and MeOTf. However, the activator PhSOTf, generated *in-situ* by the reaction of PhSCl with AgOTf, can activate these thioglycosides. It was concluded that thioglycosides protected as trans-2,3-cyclic carbonates have significantly lower anomeric reactivities compared to fully acylated and *N*-acyl protected thioglycosides. As a result, these derivatives can be used as acceptors in chemoselective glycosylations with a wide range of C-2 alkylated or acylated thioglycosyl donors (**Scheme 1.9c**). An interesting feature of these disarmed donors is that they

permit the introduction of a 1,2-*cis* glycosides whereas this is not possible with classical 2-acyl disarming derivatives.

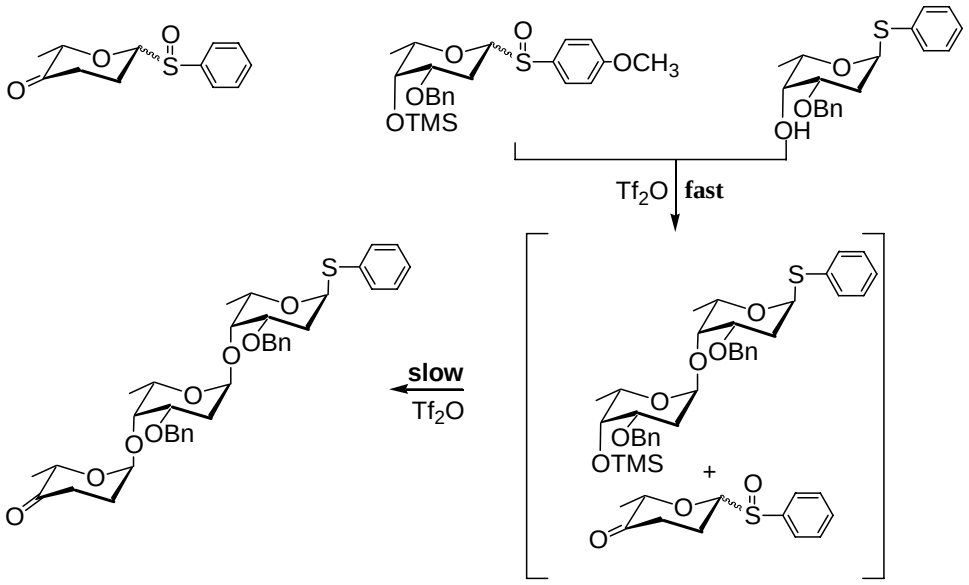
Toshima and co-workers developed a strategy for the chemoselective activation of thioglycosides for the preparation of 2,6-dideoxy glycosides.[191-195] Thus, the activated 2,6-anhydro-2-thioglycoside was coupled with the deactivated 2,6-anhydro-2-sulfinyl substrate to afford a disaccharide (**Scheme 1.10**). The resulting compound was converted into its active 2-thio-analogue by reduction of the sulfinyl moiety and condensation with cyclohexanol. Reductive removal of the thio-bridge afforded a 2,2'6,6'-tetra-deoxy-disaccharide, which corresponds to the saccharide moiety of the biologically important Avermectin antibiotic.



Scheme 1.10. Thioglycosides for the preparation of 2,6-di-deoxy-glycosides

Several methods for chemoselective glycosylations by one-pot procedures have been reported. For example, Kahne and co-workers[74] described a glycosylation method, which is based on

selective activation of anomeric sulfoxides with triflic anhydride (Tf_2O) or triflic acid (TfOH). Mechanistic studies have revealed that the rate-limiting step in this reaction is triflation of the phenyl sulfoxide. Therefore, the reactivity of the glycosyl donor can be influenced by the nature of the substituent of the *para*-position of the phenyl ring and the following reactivity order was established OMe > H > NO_2. Interestingly, the reactivity difference between a *p*-methoxyphenyl sulfenyl glycoside and an unsubstituted phenylsulfenyl glycoside is sufficient to permit selective activation. In addition, silyl ethers are appropriate glycosyl acceptors when catalytic triflic acid is used as the activating reagent but these compounds react more slowly than corresponding alcohols. These observations allowed for a one-pot synthesis of a trisaccharide from a mixture of three monosaccharides (**Scheme 1.11**).[84] Thus, treatment of the mixture with triflic acid resulted in the formation of the expected trisaccharide in a 25% yield. No other trisaccharides were isolated and the only other coupling product was a disaccharide.



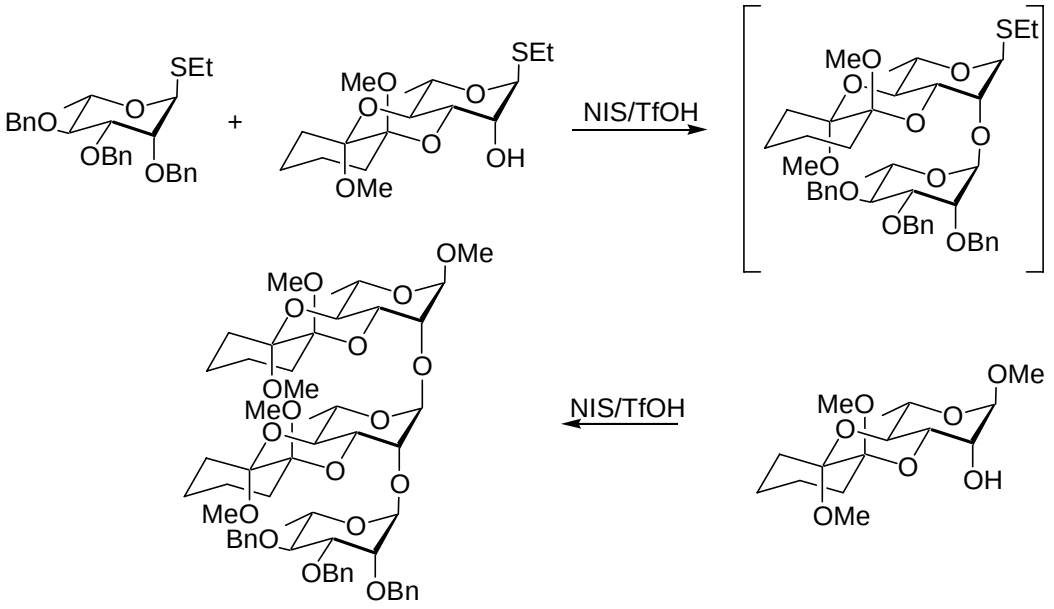
Scheme 1.11. Chemoselective glycosylations of anomeric sulfoxides by a one-pot procedure

The products of the reaction indicate that the glycosylation takes place in a sequential manner. First, the most reactive *p*-methoxyphenylsulfenyl glycoside was activated and reacted with the sugar alcohol and not with the silyl ether. In the second stage of the reaction, the less reactive silyl ether of the disaccharide reacted with the less reactive sulfoxide to give the trisaccharide. The phenylthio group of the trisaccharide could be oxidized to a sulfoxide, which could be used in a subsequent glycosylation to give a part structure of the natural product Ciclumycin. Despite the relatively low yield of the coupling reactions, this methodology provides an efficient route to this compound.

Several variations of the one-pot multi-step glycosylation concept have been reported. For example, Ley and co-workers[179,196] prepared a trisaccharide derived from the common polysaccharide antigen of group B *Streptococcus* by a facile one-pot two-step synthesis (**Scheme 1.12)**. In this strategy, a benzylated activated thioglycosyl donor was chemoselectively coupled with the less reactive cyclohexane-1,2-diacetal (CDA) protected thioglycosyl acceptor to give a disaccharide. Next, a second acceptor and additional activator were added to the reaction mixture, which resulted in the clean formation of a trisaccharide. The lower reactivity of the CDA protected thioglycoside reflects the torsional strain inflicted upon the developing cyclic oxa-carbenium ion, the planarity of which is opposed by the cyclic protecting group.

The one-pot two-step glycosylation strategy allows the construction of several glycosidic bonds without time-consuming work-up and purification steps. It should, however, be realized that this type of reaction will only give satisfactory results when all the glycosylations are high yielding and highly stereoselective. For example, by exploiting neighboring group participation, it is

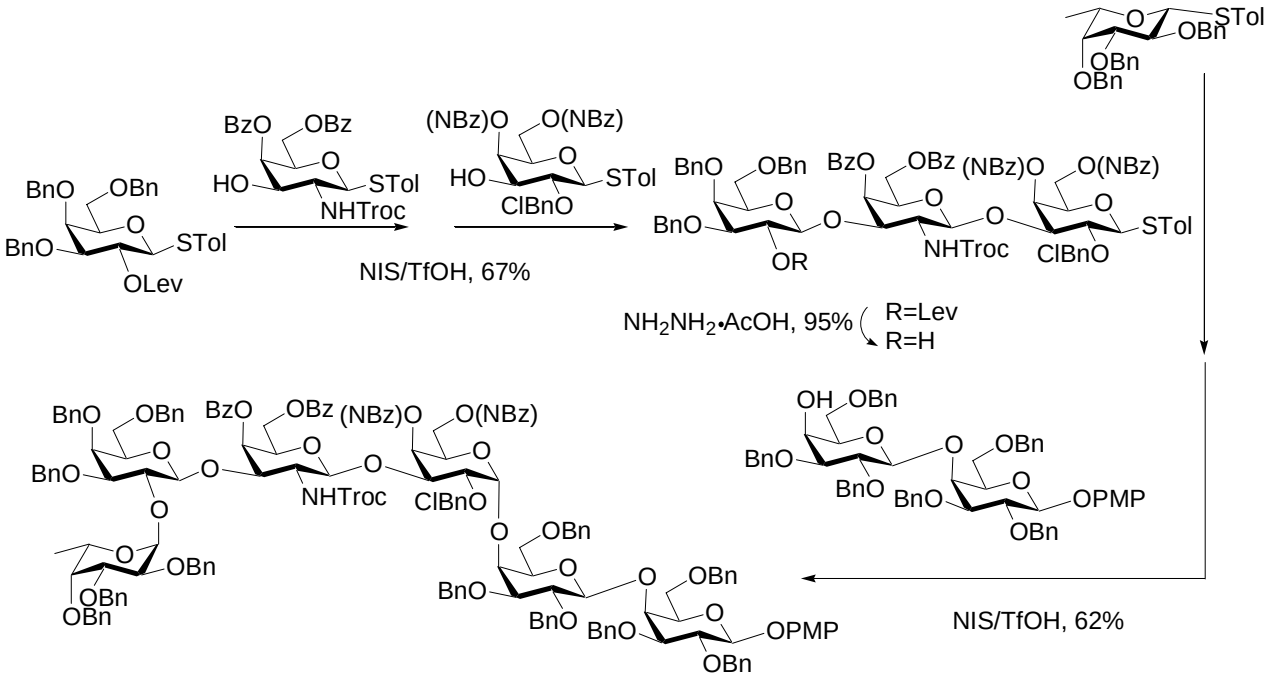
relatively easy to selectively install 1,2-*trans* glycosides. Also, in general mannosides give very high α-selectivities. Other types of glycosidic linkages may, however, pose problems.



Scheme 1.12. One-pot multi-step glycosidations of thioglycosides

Wong and co-workers have pursued an approach using HPLC for the rapid and precise measurement of relative reactivities of thioglycosyl donors. It was found that the nature of the saccharide, the position and type of protecting groups contribute to anomeric reactivity. This information was employed to create a database of thioglycosyl reactivities, which can be used to select glycosyl donors and acceptors for easy and rapid one-pot assemblies of various linear and branched oligosaccharide structures.[197] The data-base has been successfully employed for one-pot multi-step preparations of oligosaccharide libraries,[198,199] and complex oligosaccharides such as Globo-H,[200] fucosyl GM1,[201] sialyl Lewis X,[202] oligolactosmine,[203] α-Gal pentasaccharide,[204] oligomannan,[205] and Lewis Y.[206] The "OptiMer" computer program was developed to guide the selection of appropriate thioglycosyl building blocks that have

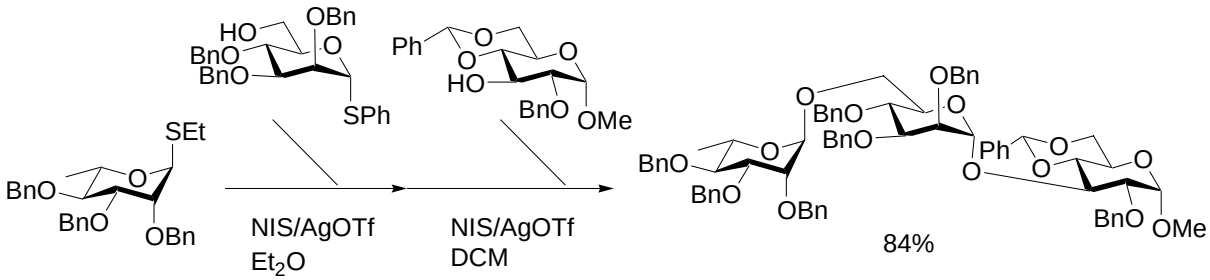
sufficiently different reactivities for one pot multi-step glycosylations. For example, the program aided in the selection of appropriate building blocks for the convenient synthesis of the tumor associated hexasaccharide Globo-H. The reactivity of the building blocks was tuned by using electron-donating groups such as benzyl ether and 2,2,2-trichloroethylcarbamate and electron-withdrawing protecting groups such as benzoyl, p-nitrobenzoyl (NBz), and o-chlorobenzyl ethers (ClBn) (**Scheme 1.13**).



Scheme 1.13. One-pot synthesis of the Globo-H hexasaccharide

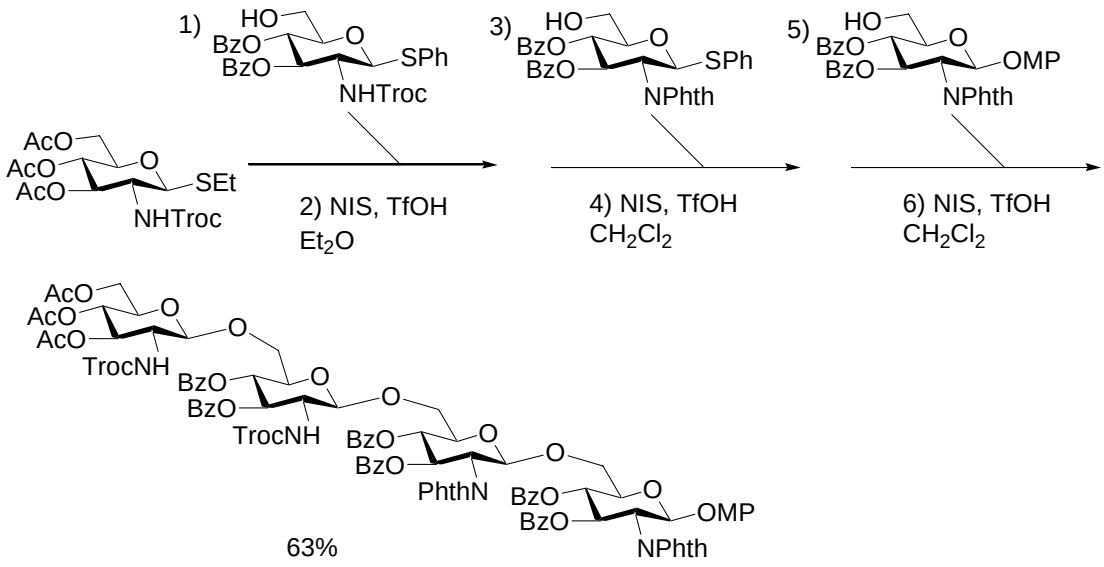
A one-pot two-step glycosylation to give a trisaccharide was accomplished by simply changing the solvent system.[207] In this approach, the solvent controls anomeric selectivity and also rate of glycosylation. Thus, when a reactive ethyl thiorhamnoside and a less reactive thiophenyl mannoside were dissolved in diethyl ether, only the rhamnosyl donor was activated by promoter system NIS/AgOTf to give the corresponding thiophenyl disaccharides in an almost quantitative yield. After adding a glucosyl acceptor and additional promoter dissolved in CH_2Cl_2, the

intermediate thiophenyl disaccharide donor was activated by NIS/TMSOTf leading to the formation of a trisaccharide in high yield and stereoselectivity. Thus, by tuning the reactivity of acceptors and donors and performing the first glycosylation in diethyl ether (low glycosylation rate) and the second in CH_2Cl_2/Et_2O (higher glycosylation rate), a trisaccharide could be prepared by a one-pot two-step procedure (**Scheme 1.14**).



Scheme 1.14. solvent reactivity effects in one-pot oligosaccharide synthesis

Baasov and coworkers achieved an efficient synthesis of an oligosaccharide using a one-pot procedure whereby the reactivity of glycosyl donors and acceptors were tuned by a combination of the nature of the C-2 amino protecting group (Troc, Phth) and anomeric leaving group (ethylthio and phenylthio).[208] In addition, by exploiting solvent reactivity effects, an ethyl 1-thioglycoside could be activated in the presence of a phenyl 1-thioglucosyl acceptor. Thus, the synthesis exploited the observation that NTroc protected thioglycosides are significantly more reactive than their NPhth protected counterparts. Furthermore, successful synthesis of the target tetrasaccharide exploited the higher reactivity of thioethyl glucosides compared to similar thiophenyl glycosides. As a result, the desired tetraglucosamine could be prepared in an overall yield of 63% by a one-pot three-step glycosylation (**Scheme 1.15**).

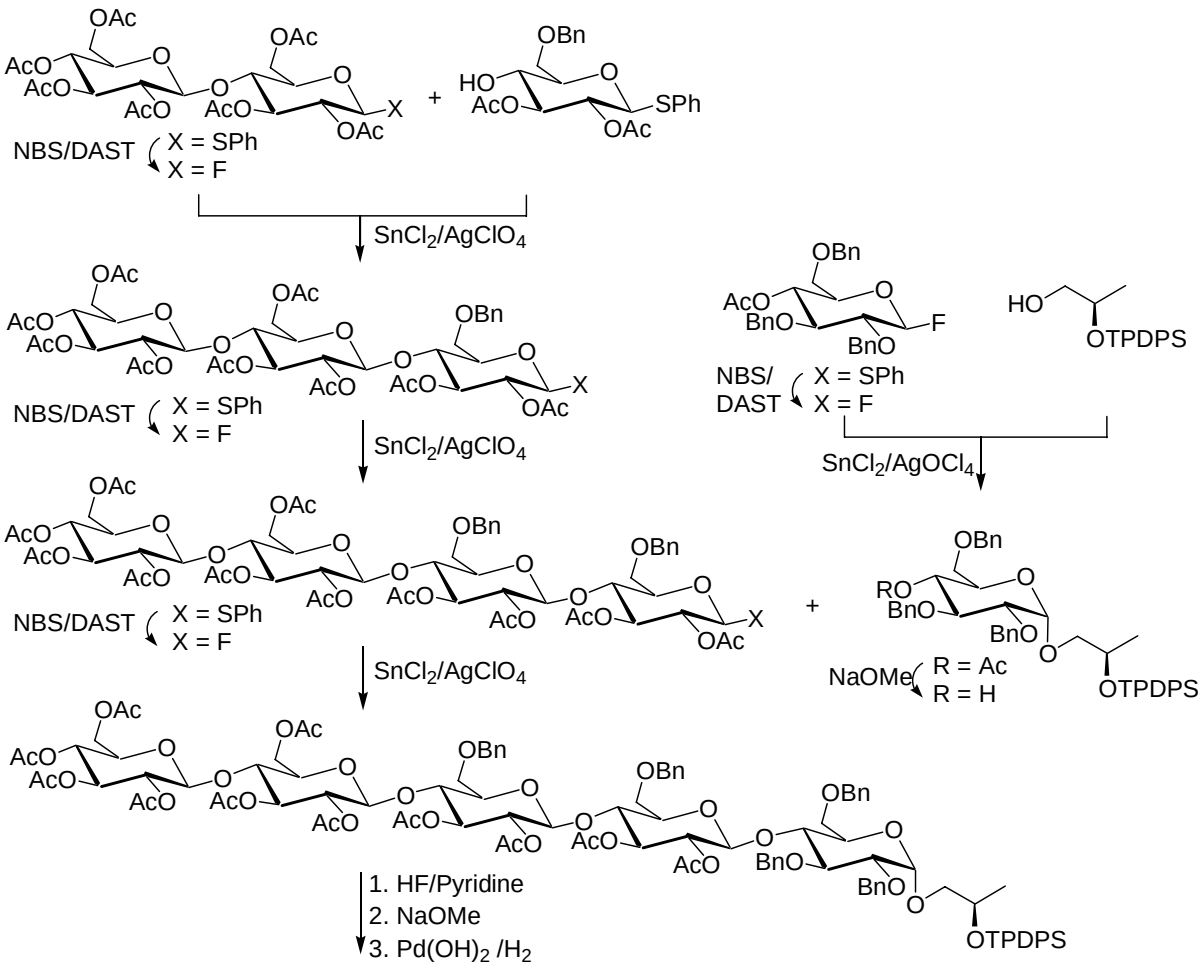


Scheme 1.15. One-pot synthesis of glucosamine oligosaccharide

Orthogonal and Semi-orthogonal Glycosylations

Orthogonal glycosylations use glycosyl donors and acceptors that have different anomeric groups (*e.g.* X = F and Y = SR), which can be activated without affecting the other one. These synthetic approaches are attractive since no or very few protecting group manipulations are involved during the assembly of a complex oligosaccharide.

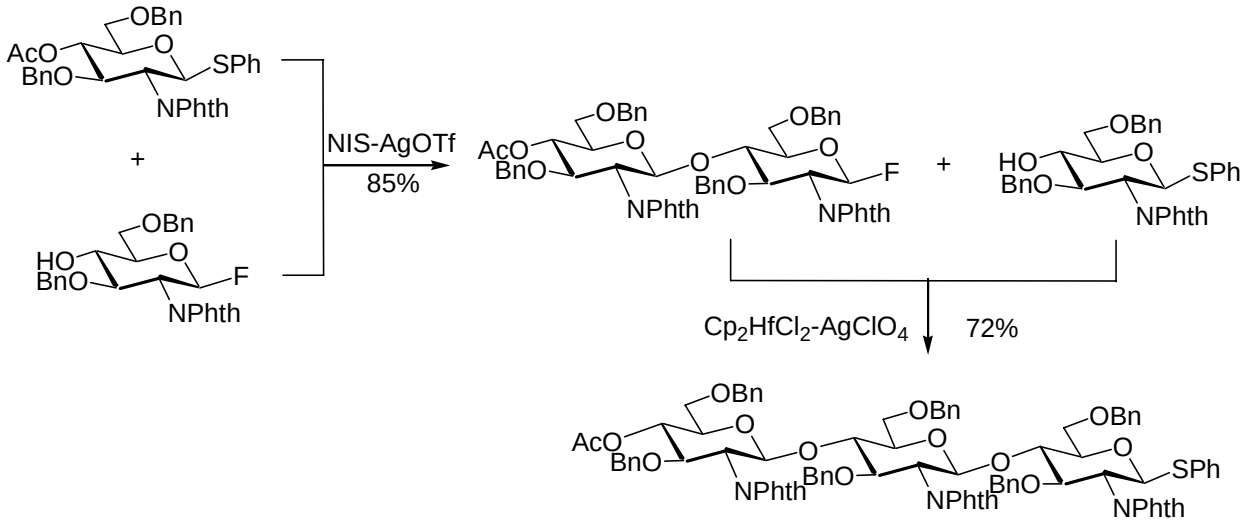
Nicolaou and coworkers[209-214] have described a two-stage glycosylation strategy whereby a thioglycoside is converted into a glycosyl fluoride donor, which is then employed as a glycosyl donor for coupling with a thioglycosyl acceptor. The procedure can be repeated by conversion of the anomeric thio group of the oligosaccharide into an anomeric fluoride which can be used in a further coupling reaction. This glycosylation strategy was exploited for the preparation of *Rhynchosporides* and key reactions are depicted in **Scheme 1.16**.



β-D-Glcp-(1-4)-β-D-Glcp-(1-4)-β-D-Glcp-(1-4)-β-D-Glcp-(1-4)-α-D-Glcp-(1-1)-1,2-propane diol

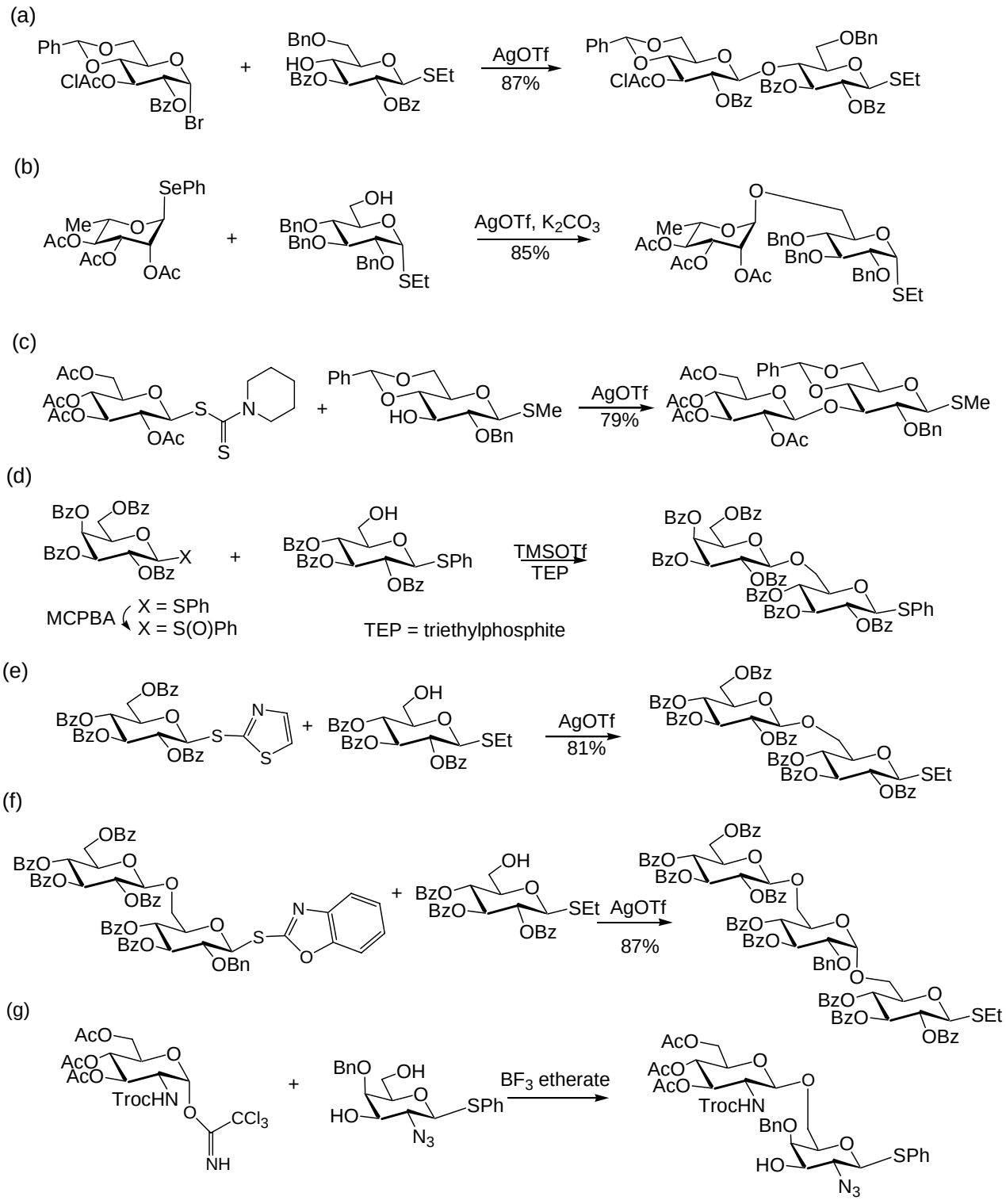
Scheme 1.16. Two-stage activation of thioglycosides

In a further improved orthogonal glycosylation strategy,[215] thioglycosides and glycosyl fluorides act as glycosyl donors and acceptors and are coupled with each other in a chemoselective manner.[215] An example of this strategy is depicted in **Scheme 1.17**, in which the synthesis of β(1→4)-2-acetamido-2-deoxy-D-glucose linked oligosaccharides is described. Thus, a thioglycosyl donor was coupled with a glycosyl fluoride acceptor using a thiophilic promoter. Next, the resulting glycosyl fluoride acted as glycosyl donor and coupled with a thioglycosyl acceptor. Reiteration of the process leads to the rapid build-up of long-chain oligosaccharides.



Scheme 1.17. Orthogonal glycosylations of thioglycosides and glycosyl fluorides

Several other examples have been reported in which thioglycosides were used as glycosyl acceptors. Thus, thioglycosides containing free hydroxyls can be coupled chemoselectively with glycosyl bromides and chlorides in the presence of silver triflate or tin(II) chloride-silver perchlorate as the promoter system.[12,53,56,162,218-221] Such a synthesis is shown in **Scheme 1.18a,** in which a glycosyl bromide is coupled with the thioglycosyl acceptor to afford a thioglycosyl disaccharide. The glycosyl bromide was obtained from the corresponding 1-thioglycoside by treatment with Br_2. It was shown[222-224] that phenyl selenoglycosides can be selectively activated in the presence of ethyl thioglycosides using silver triflate/potassium carbonate (or silver carbonate) as the promoter system (**Scheme 1.18b**). Garegg and co-workers reported the use of glycosyl 1-piperidinecarbodithioates in combination with thioglycosides (**Scheme 1.18c**).[225] Per-acetylated piperidinecarbodithioate donor could be selectively activated in the presence of the thioglycosyl acceptor by using silver triflate as the promoter to afford a thioglycosyl disaccharide.



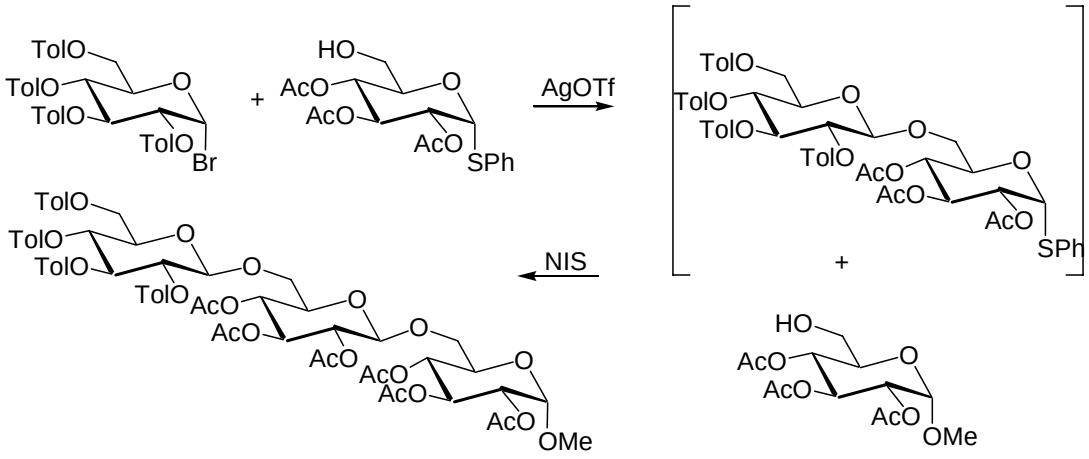
Scheme 1.18. Glycosylations with thioglycosyl acceptors

Kahne and co-workers reported a glycosylation approach in which glucosyl sulfoxides are

activated in the presence of thioglycosides (**Scheme 1.18d**).[279-281] Next, the resulting thioglycosyl disaccharides can be activated using a thiophilic promoter or converted into the corresponding sulfoxides. Demchenko and co-workers have studied a series of thioimidate based glycosyl donors, such as S-Benzoxazolyl (SBox) and S-thiazolinyl (STaz) glycosides, which can be activated by AgOTf or $Cu(OTf)_2$ in the presence of ethyl 1-thioglycosides (**Scheme 1.18e,f**).[115,116] In addition, a strategy was developed whereby anomeric reactivities were reduced by metal complexation with the anomeric group.[227] Furthermore, STaz glycosides can selectively be activated over conventional 1-thioglycosides and O-pentenyl glycosides, while bromides, trichloroacetimidates, and 1-thioglycosides can be activated over the STaz moiety.[228] The reaction of thioglycosyl acceptors with trichloroacetimidates has also been described (**Scheme 1.18g**).[216, 226]

Orthogonol and semi-orthogonal glycosylations have also been performed in one-pot multi-step fashion. For example, Takahashi and co-workers[216] reported a one-pot two-step glycosylation in which the difference in reactivity between glycosyl donors and acceptors was accomplished through the use of two types of anomeric leaving groups with different reactivities (**Scheme 1.19**). Thus, a glycosyl bromide was coupled with a thioglycosyl acceptor in the presence of silver triflate to give a disaccharide. While the anomeric thiophenyl groups are stable to silver triflate (AgOTf), addition of both the second activator (NIS) and the glycosyl acceptor promoted selective activation of the glycosyl donor, resulting in the formation of a trisaccharide (84% overall yield). In this example, the stereochemical outcome of the glycosylations was controlled by the neighboring group participation of the 2-*O*-toluoyl (Tol) and acetyl protecting

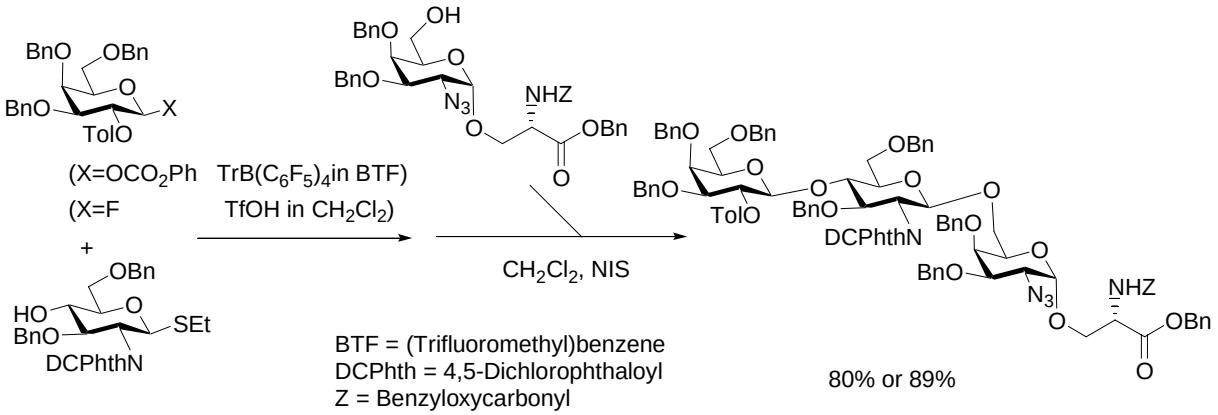
groups. A similar one-pot two-step glycosylation procedure was employed for the preparation of an elicitor-active hexaglycoside.



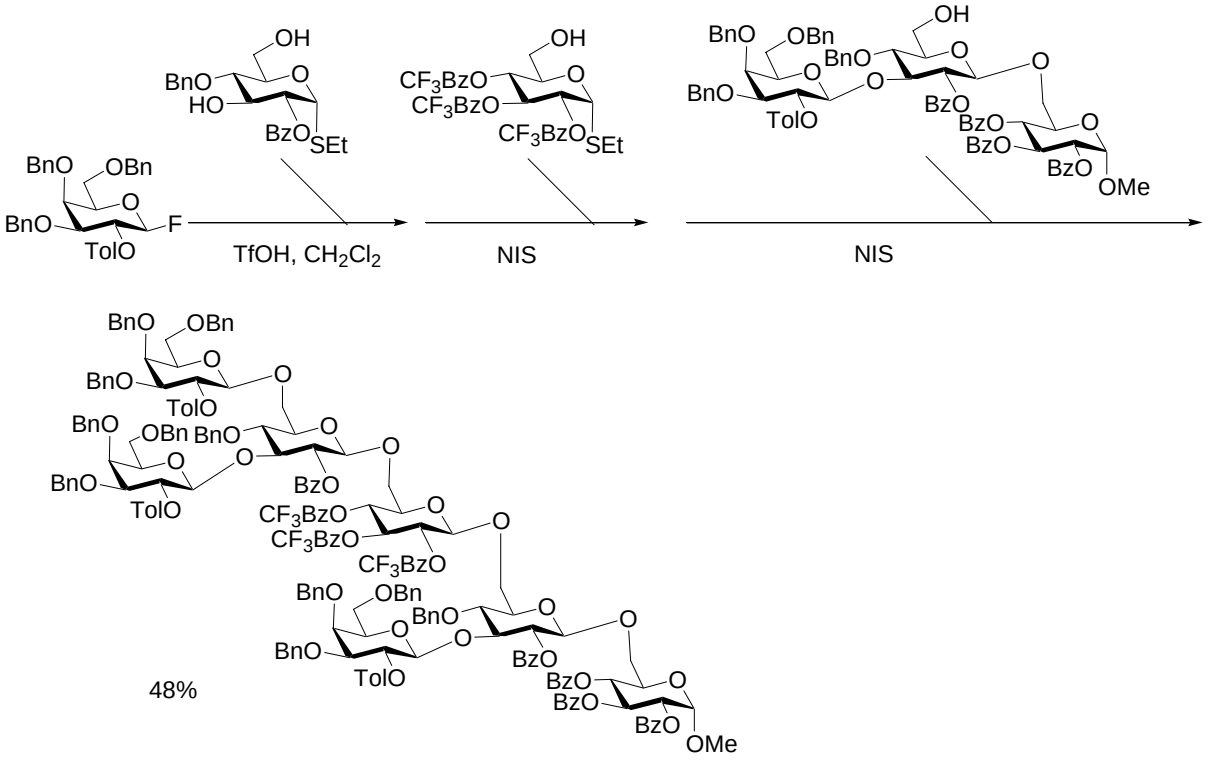
Scheme 1.19. One-pot multi-step glycosylations with thioglycosides and glycosyl bromides

Mukaiyama and co-workers have reported a one-pot assembly of a mucin related F1α antigen using anomeric fluorides and carbonates.[51] After careful evaluation of solvent systems, promoters and reaction temperatures, fully protected F1α antigen was synthesized by a one-pot sequential glycosylation using a galactosyl phenyl carbonate or fluoride, a thioglycoside, and a glycosyl amino acid (**Scheme 1.20**). In the first step, the phenylcarbonate or fluoride donor was coupled with the ethyl thioglucoside in the presence of $TrB(C_6F_5)_4$ or TfOH, respectively. After TLC analysis indicated complete consumption of the glycosyl donor, consecutive addition of the terminal glycosyl amino acid and NIS provided the target trisaccharide in high yield (80% and 89% respectively). In a similar manner, an anomeric fluoride donor and two different thioglycosides were employed for the preparation of a phytoalexin elicitor heptasaccharide (**Scheme 1.21**).[217] Thus, TfOH-catalyzed double glycosylation of the fluoride with the ethyl thioglycosyl acceptor gave a trisaccharide, which was coupled with the highly deactivated

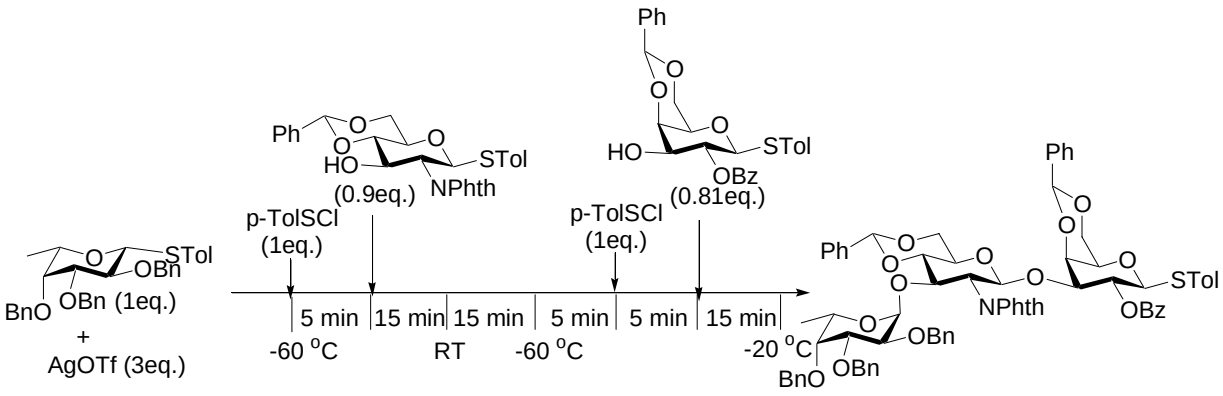
p-(trifluoromethyl)benzoyl (CF_3Bz) protected thioglycoside to afford a tetraglucoside intermediate as the major product. Next, consecutive addition of a trisaccharide acceptor and NIS led to the formation of a heptasaccharide in an overall yield of 48%. Thus, four glycosidic linkages were stereoselectively introduced in a one-pot manner.



Scheme 1.20. One pot synthesis of mucin related F1α antigen



Scheme 1.21. Mukaiyama one pot synthesis of phytoalexin elicitor active heptasaccharide

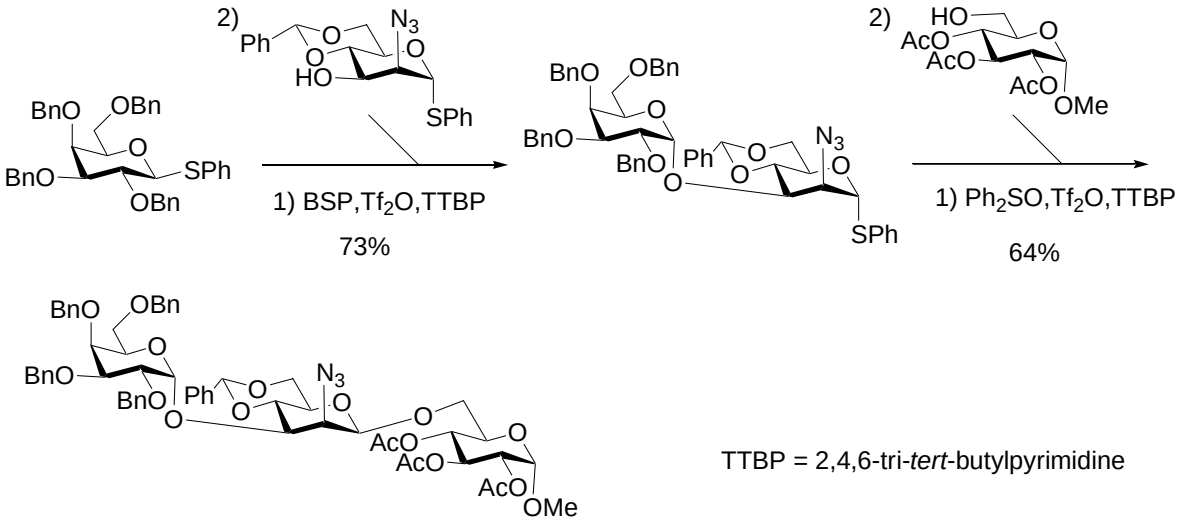


Scheme 1.22. Pre-activation of p-tolyl thioglycoside in one pot oligosaccharide synthesis

Huang and co-workers designed a general one-pot multi-step glycosylation approach independent of differential glycosyl donor and acceptor reactivities. The new and elegant method is based on pre-activation of a thioglycosyl donor to give a reactive intermediate in the absence of the acceptor. Subsequently, a thioglycosyl acceptor can be added to the activated donor leading to the formation of a coupling product.[229] The resulting thioglycoside can be activated and employed in a subsequent glycosylation with a thioglycosyl acceptor. For example, a trisaccharide was assembled which contains the biologically relevant Fuc-α1,3-GlcNAc and GlcNAc-β1,3-Gal moieties. Thus, pre-activation of the toluyl thiofucoside by *p*-TolSOTf at -60^{o}C was followed by the addition of a phthaloyl protected thioglycosyl acceptor. Over a period of 15 minutes, the reaction mixture was allowed to warm to room temperature during which time a disaccharide was formed. After cooling the reaction mixture to -60^{o}C, the disaccharide was pre-activated with *p*-TolSOTf followed by addition of the thiogalactosyl acceptor, producing a trisaccharide in 59% overall yield within a period of 1 hour. (**Scheme 1.22**). The trisaccharide carrying an anomeric *p*-thiotolyl moiety could be utilized as glycosyl donor for the synthesis of

Le^x containing oligosaccharides. Excellent anomeric stereoselectivities were obtained in each glycosylation.

Van Boom and coworkers described a similar pre-activation strategy for thioglycosides. It was found that diphenylsulfoxide in combination with triflic anhydride provides a very potent thiophilic reagent capable of activating deactivated thioglycosides.[87,99] A novel chemoselective condensation sequence was developed in which a benzylated reactive thioglycosyl donor was selectively activated by a mild thiophilic promoter and chemoselectively condensed with a relatively unreactive thioglycosyl donor (**Scheme 1.23**). Addition of the acceptor and the more reactive promoter system Ph_2SO/Tf_2O led to the formation of a trisaccharide. The side products formed from the 1-Benzenesulfinyl piperidine (BSP)/Tf_2O activation system were quenched by the addition of triethyl phosphite after each glycosylation to avoid activation of the acceptor and glycosylation product.[87]



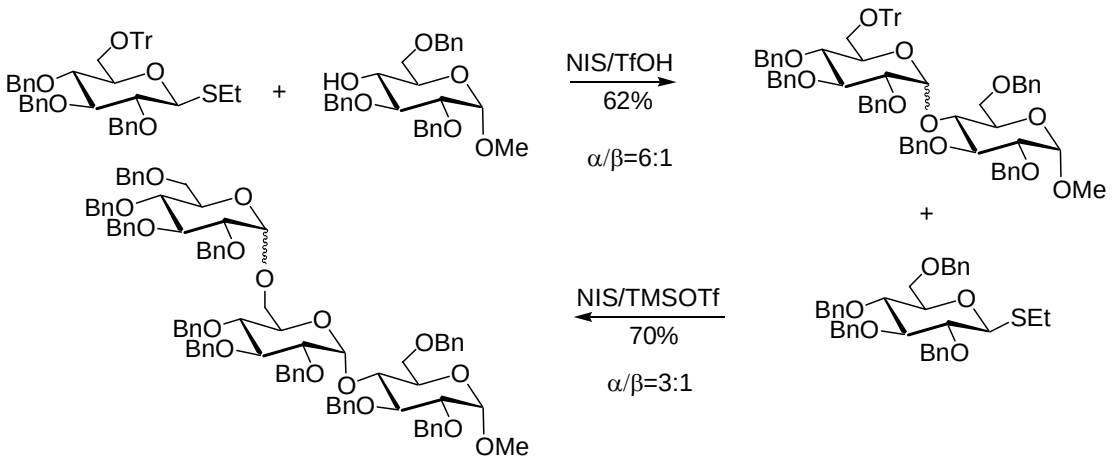
Scheme 1.23. Chemoselective glycosylation by preactivation strategy using Ph_2SO/Tf_2O promoter

Finally, a novel sequential glycosylation procedure has been reported using 1-hydroxyl and

thioglycosyl donors.[230] Yamago and co-workers reported a broad substrate scope utilizing the BSP/Tf_2O promoter system to pre-activate thioglycosyl donors.[231]

Two-directional glycosylation strategies

The overall efficiency of chemoselective and orthogonal glycosylations is compromised by the linear nature of the glycosylation sequence and the fact that the growing oligosaccharide chain acts in each reaction as glycosyl donor. These problems can be addressed by two-directional glycosylation strategies. In such an approach, a thiosaccharide building block can act as glycosyl donor as well as glycosyl acceptor. These properties enable oligosaccharide assembly in a very flexible and highly convergent manner. For example, coupling of a tritylated thioglycosyl donor with an acceptor having an 4-hydroxyl afforded a disaccharide in a yield of 62% (α/β = 6/1) (**Scheme 1.24**).[232]



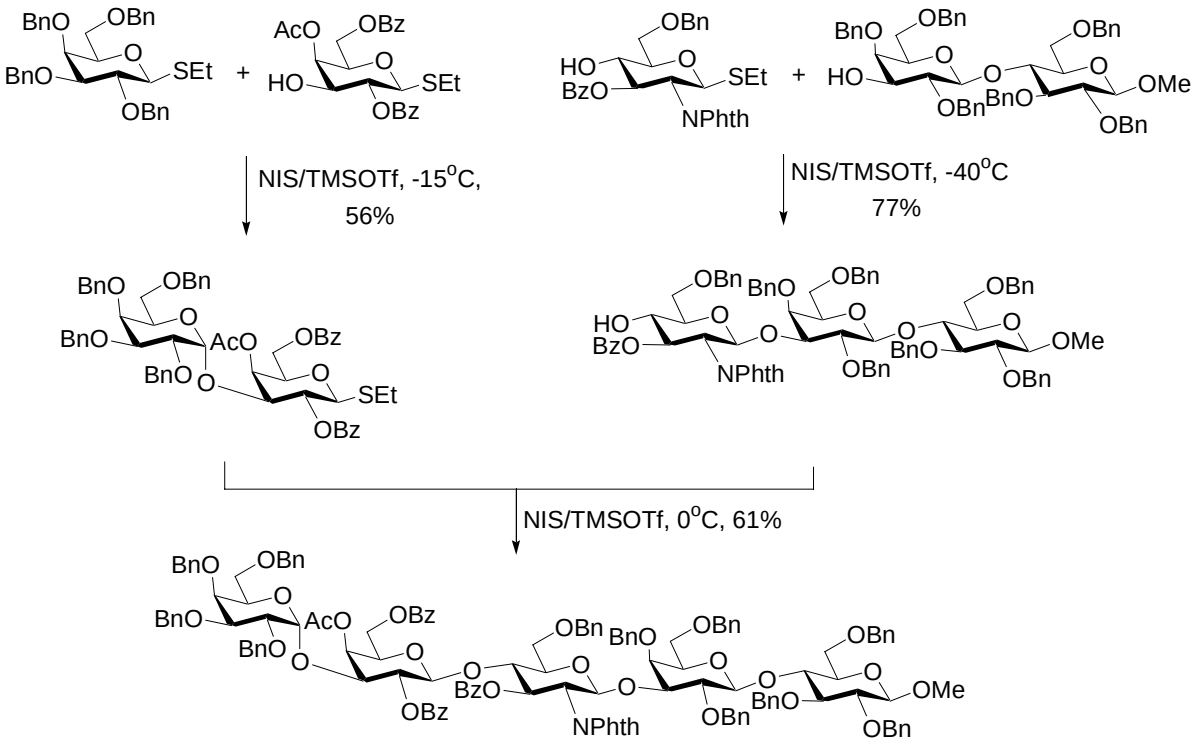
Scheme 1.24. Two-directional glycosylations with tritylated thioglycosides

In this case, the 6-*O*-trityl group improved the α-selectivity of the glycosylation due to steric effects. The trityl ether of the resulting product can act as an acceptor when glycosylated with a

fully benzylated thioglycosyl donor using NIS and a stochiometric amount of TMSOTf as the activator to give a trisaccharide in excellent yield as a mixture of anomers (α/β =3/1).

A two-direction glycosylation strategy can also be performed by regioselective glycosylation between glycosyl donors and acceptors both of which contain a free hydroxyl group to give di- and tri-saccharides.[233] The products of these glycosylations can immediately be employed as glycosyl acceptors in subsequent glycosylations without the need to perform protecting group manipulations. In combination with the previously reported chemoselective glycosylations, this methodology provides a powerful method to assemble oligosaccharides in a highly convergent manner, avoiding protecting group manipulations at the oligosaccharide stage. A prerequisite of regioselective glycosylation is that the acceptor's hydroxyl functionality must be substantially more reactive than the hydroxyl group of the glycosyl donor. Differences in reactivity may be achieved by primary *vs.* secondary or equatorially *vs.* axial disposition of hydroxyl groups. The new methodology was employed for the preparation of a pentasaccharide involved in the hyper-acute rejection response in xenotransplantation (**Scheme 1.25**).[234] Thus, the α-linked Gal(1→3)Gal dimer was obtained by an armed-disarmed chemoselective glycosylation using NIS/TMSOTf as promoter and toluene/1, 4-dioxane as reaction solvent. The right hand trisaccharide was obtained in a good yield of 77% by a NIS/TMSOTf mediated glycosylation between a lactoside acceptor and partially protected 2-deoxy-phthalimido-glucosyl acceptor. No self-condensation of the glycosyl donor was observed due to deactivation of the 4-OH by the neighboring benzoyl group. The pentasaccharide was obtained by coupling of the

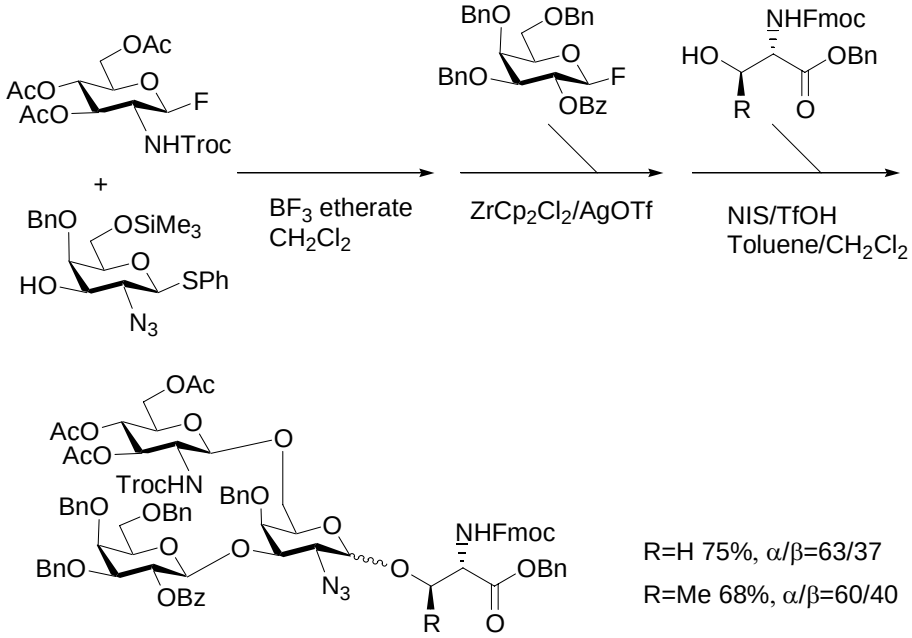
thio-disaccharide and trisaccharide acceptor in the presence of NIS/TMSOTf. Two-directional glycosylations using thioglycosides have also been performed on solid support.[235]



Scheme 1.25. The preparation of the Galili pentasaccharide using thioglycosyl donors and acceptors in a two-directional glycosylation strategy

Takahashi and co-workers described the one-pot synthesis of core-2 branched oligosaccharides.[236] It was found that boron trifluoride complexed with a trimethylsilyl ether would enhance the nucleophilicity of the silyl ether. As a result, glycosylations of the 6-O-TMS modified acceptor with a glycosyl fluoride provided selectively glycosylation at C-6 of the thioglycosyl acceptor without glycosylation of the C-3 hydroxyl. Therefore, a chemoselective glycosylation was performed between 6-O-silyl-4-benzyl-2-azido-thiogalactoside and a glycosyl fluoride in the presence of BF_3 etherate, followed by sequential coupling of the remaining secondary hydroxyl group with galactosyl fluoride in the presence of $ZrCp_2Cl_2$/AgOTf to

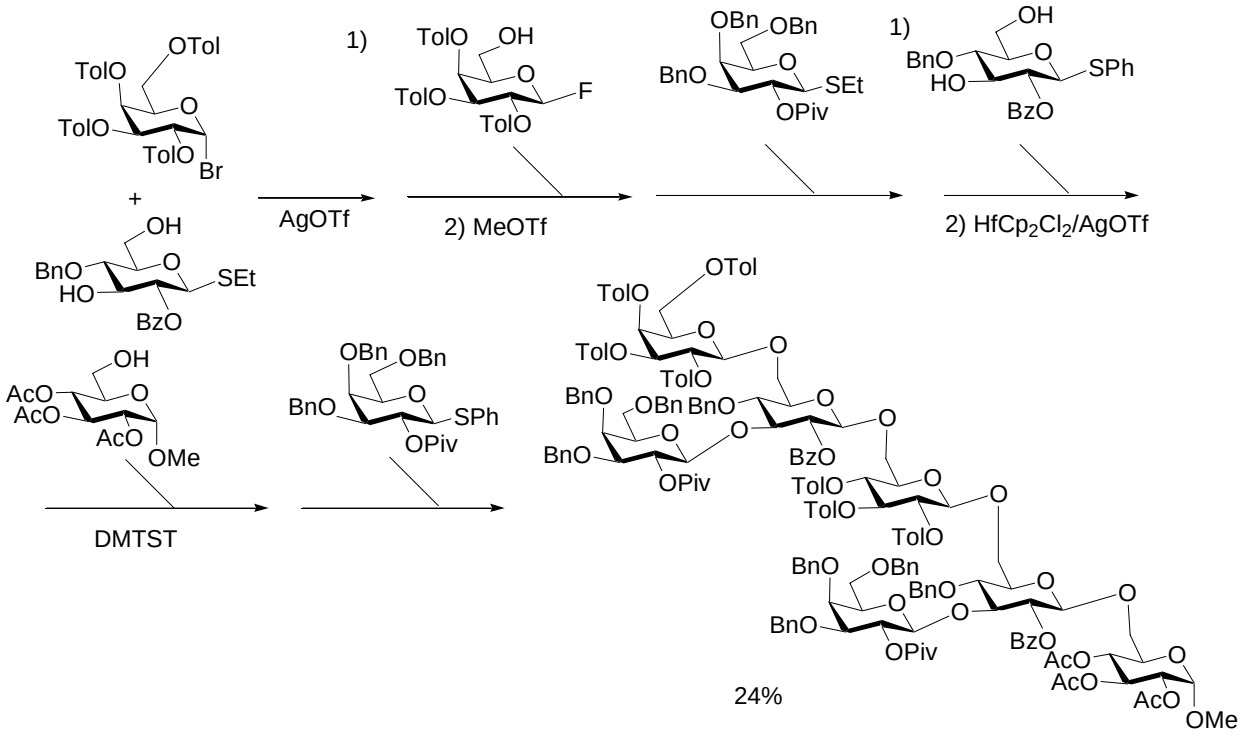
provide the desired trisaccharide. Subsequent NIS/TfOH promoted glycosidation of the thioglycoside with amino acids provided products in good yield (**Scheme 1.26**).



Scheme 1.26. One-pot synthesis of core 2 class amino acids

The same group also prepared a phytoalexin elicitor heptamer by a one-pot six-step glycosylation protocol providing the most impressive example of the potential of chemoselective glycosylation technology.[216, 237] The sequential addition of seven reaction components with six appropriate activators resulted in the one-pot six-step glycosylation. First, a toluoyl (Tol) protected galactosyl bromide, in combination with AgOTf, ensured the regioselective glycosylation of the primary alcohol of thioglucoside diol. Next, the resulting 1-thioglycoside acted as a glycosyl donor in a coupling with a galactosyl fluoride acceptor using large excess of MeOTf to avoid self-condensation. A third glycosylation of the C3-hydroxyl was achieved using thioglucoside. Fourth, $HfCp_2Cl_2$–AgOTf mediated coupling of the branched tetraglucoside to the third thioglycosidic building block was performed regioselectively. Next, the terminal glucose

acceptor was condensed with the resulting pentasaccharide thiophenyl donor using a large excess of Dimethyl(methylthio) sulfonium triflate (DMTST). Last, the heptasaccharide, the largest oligosaccharide amongst the reaction products, was formed via the second β-glucosidic linkage. The final compound was purified by size exclusion chromatography in a good yield of 24% (**Scheme 1.27**).



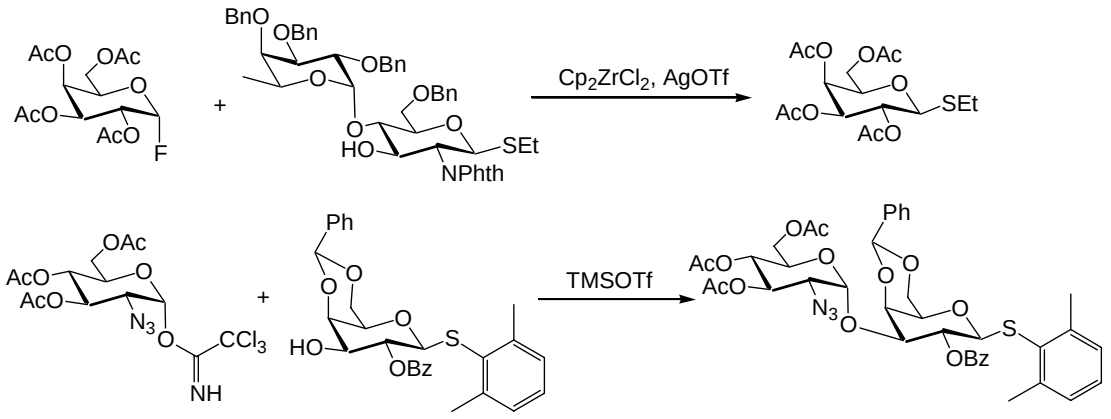
Scheme 1.27. Takahashi one pot synthesis of phytoalexin elicitor active heptasaccharide

Aglycon Transfer

While thioglycosides have been successfully employed as glycosyl acceptors, at times this type of glycosylation is plagued by aglycon transfer.[2,236,238-249] The aglycon transfer process is shown to affect both armed and disarmed thioglycosides, it causes anomerization of the carbon-sulfur bond of a thioglycoside, and destroys the product of a glycosylation reaction. This side reaction is especially important to consider when carrying out complex reactions such as solid-phase

glycosylations, one-pot or orthogonal multi-component glycosylations, and construction of carbohydrate libraries. For example, an intermolecular aglycon transfer reaction was observed in a Cp_2ZrCl_2/AgOTf mediated coupling of a glycosyl fluoride donor and a 1-thiodisaccharide acceptor (**Scheme 1.28**).[244] It was rationalized as follows: the acyloxonium ion generated after activation of the glycosyl fluoride attacked the sulfur instead of the sterically hindered alcohol, leading to the formation of β-thioglycoside. Aglycon transfer could be avoided by employing a less reactive 1-thioglycosyl acceptor.

To prevent aglycon transfer, Gildersleeve and coworker examined a number of modified aglycons.[250] It was found that the 2, 6-dimethylphenyl 1-thio moiety was effectively blocking the transfer in a variety of model studies and glycosylation reactions. The DMP group can be installed in one step from a commercially available 2, 6-dimethylthiophenol and is useable as a glycosyl donor.



Scheme 1.28. Aglycon transfer of thioglycosides

General procedure for synthesis of thioglycosides from peracetylated hexapyranosides promoted by BF_3-etherate[5-10]

To a solution of peracetylated hexapyranoside (1.0 equiv.), ethanethiol (1.2 equiv.), and freshly activated 4 Å powdered molecular sieves in dichloromethane (4.0 mL/mmol) was added BF_3-Et_2O (2.0 equiv.) dropwise at 0 °C under an argon atmosphere. The reaction mixture was stirred for 2 h at room temperature until TLC analysis indicated that the reaction had gone to completion. The solution was filtered through celite, and washed with dichloromethane. The filtrate was washed with saturated aqueous $NaHCO_3$ and H_2O. The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated to dryness. Purification of the crude product by column chromatography over silica gel afforded the target compound.

General procedure for synthesis of thioglycosides by displacement of acylated glycosyl bromide with thiolate anion[34]

To a solution of diaryl or diaralkyldisulfide (1.0 equiv.) in CH_3CN (5.0 ml/mmol) was added zinc-dust (1.0 equiv.) followed by fused $ZnCl_2$ (0.2 equiv.). The reaction mixture was placed in a pre-heated oil bath at 70 °C for 45 min during which time the reaction mixture became turbid indicating the formation of zinc thiolate. A solution of acylated glycosyl bromide (2.0 equiv.) in CH_3CN (2.5 ml/mmol) was added to the turbid reaction mixture which was then stirred at 70 °C until TLC analysis indicated that the reaction had gone to completion. The reaction mixture was concentrated *in vacuo* and the residue dissolved in dichloromethane. The organic layer was washed with saturated aqueous $NaHCO_3$ and H_2O, dried ($MgSO_4$), filtered and the filtrate was concentrated to dryness. Purification of the crude product by column chromatography over silica gel afforded the target compound.

General procedure for synthesis of sialyl thioglycosides using TMSSMe and TMSOTf[165]

To a solution of methyl 2,4,7,8,9-penta-*O*-acetyl-5-(*N*-acetylacetamido)-3,5-dideoxy-D-glycero-α,β-D-*galacto*-non-2-ulopyranosonate (1.0 equiv.), and freshly activated 4 Å powdered molecular sieves in 1,2-dichloroethane (2.0 mL/mmol) was added TMSSMe (1.4 equiv.) and TMSOTf (0.75 equiv.). The reaction mixture was stirred for 4.5 h at 50 ^{o}C and a further 16 h at room temperature. The solution was filtered through celite, and washed with dichloromethane. The filtrate was washed with saturated aqueous $NaHCO_3$ and H_2O, dried ($MgSO_4$), filtered and the filtrate was concentrated to dryness. Purification of the crude product by column chromatography over silica gel afforded methyl [methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamido)-3,5-dideoxy-2-thiol-D-glycero-α,β-D-*galacto*-non-2-ulopyranosid]onate as α/β mixture (1:1).

General procedure for activation of thioglycosides with Ph_2SO/Tf_2O[87,99]

To a solution of thioglycoside (1.0 equiv.), Ph_2SO (2.8 equiv.), and TTBP (3.0 equiv.) in dichloromethane (4.0 mL/mmol) was added trifluoromethanesulfonic anhydride (1.4 equiv.) at -60 ^{o}C under an argon atmosphere. The reaction mixture was stirred for 5 min, after which a solution of the glycosyl acceptor (1.5 equiv.) in dichloromethane (2.0 mL/mmol) was added. The mixture was stirred at -60 ^{o}C for 1 h, after which it was slowly warmed to room temperature and quenched by the addition of saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried ($MgSO_4$), filtered and the filtrate was concentrated to dryness. Purification of the crude product by column chromatography over silica gel afforded the product.

General procedure for activation of thioglycosides with BSP/TTBP/Tf_2O[85]

To a solution of thioglycoside (1.0 equiv.), 1-benzenesulfinyl piperidine (1.0 equiv.), TTBP (2.0 equiv.), and freshly activated 3 Å powdered molecular sieves in dichloromethane (25.0 mL/mmol) was added trifluoromethanesulfonic anhydride (1.1 equiv.) at -60 °C under an argon atmosphere. The reaction mixture was stirred for 5 min, after which a solution of the glycosyl acceptor (1.5 equiv.) in dichloromethane (4.0 mL/mmol) was added. The reaction mixture was stirred at -60 °C for 2 min, after which it was slowly warmed to room temperature and quenched by the addition of saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried ($MgSO_4$), filtered and the filtrate was concentrated to dryness. Purification of the crude product by column chromatography over silica gel afforded the product.

General procedure for activation of sialyl thioglycosides with NIS/TfOH[165,166]

To a solution of sialyl thioglycoside (3.0 equiv.), glycosyl acceptor (1.0 equiv.), and freshly activated 3 Å powdered molecular sieves in MeCN (30.0 mL/mmol) was added NIS (6.0 equiv.) and TfOH (0.6 equiv.) at -35 °C under an argon atmosphere. The reaction mixture was stirred for 5 min until TLC analysis indicated that the reaction had gone to completion. The solution was filtered through celite, and washed with dichloromethane. The filtrate was washed with aqueous $Na_2S_2O_3$ (20%) and H_2O. The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated to dryness. Purification of the crude product by column chromatography over silica gel afforded the product.

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CHAPTER 2

PROBING THE SUBSTRATE SPECIFICITY OF HUMAN GOLGI α-MANNOSIDASE II USING SYNTHETIC OLIGOSACCHARIDES[1]

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Abstract

Inhibition of the mannose trimming enzyme human Golgi α-mannosidase II (HGMII) provides one route to blocking the oncogene-induced changes in cell surface oligosaccharide structures. HGMII selectively cleaves α(1→3) and α(1→6) mannosyl residues present in its natural substrate $GlcNAcMan_5GlcNAc_2$. It has been proposed that HGMII has an extended binding site recognizing a large part of the oligosaccharide. In order to probe the substrate requirements of HGMII, we have synthesized a range of part-structures of $GlcNAcMan_5GlcNAc_2$ and determined kinetic parameters for hydrolysis by HGMII. X-ray crystallography study is still ongoing.

Introduction

Aberrant glycosylation of glycoproteins and glycolipids is a molecular change typical of malignant transformations. For example, human cancers of breast, colon and melanomas often over-express the glycosyl transferase N-acetylglucosaminyl transferase V (GlcNAc-TV). This enzyme introduces a specific branching N-acetylglucosamine moiety at *N*-linked oligosaccharides of glycoproteins thereby forming the biosynthetic precursor for polylactosamine chains. It has been proposed that these polysaccharides on integrins and cadherins or adhesion receptors facilitate focal adhesion turnover, cell migration and tumor metastasis.[1,2]

There is evidence that inhibition of GlcNAc-TV may be a useful for the treatment of malignancies.[3] For example, mammary tumor growth and metastases induced by polyomavirus middle T-oncogene was considerably reduced in GlcNAc-TV deficient mice. In addition, somatic tumor cell mutants with a deficiency in UDP-Gal transport activity show a large reduction of

malignancy indicating that blocking of lactosamine extension in *N*- and *O*-glycans may be effective for cancer treatment.

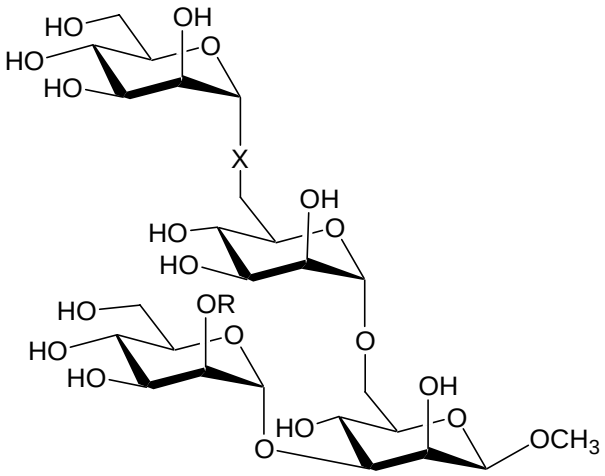
In general, it has been difficult to design and synthesize potent and cell permeable inhibitors of glycosyl transferases. Therefore, efforts to blocking the biosynthesis of polylactosamine chains of *N*-linked glycoproteins have not focused on GlcNAc-TV but instead on inhibitors of glycosidases that act earlier in the biosynthesis of N-glycan to prevent the formation of the biosynthetic pathway primer of GlcNAc-TV. Most efforts in the field have focused on Golgi α-mannosidase II (HGMII), which trims two mannosyl residues from $GlcNAcMan_5GlcNAc_2$ to form the core $GlcNAcMan_3GlcNAc_2$ moiety.[4]

GMII is a retaining glycosylhydrolase, which employs a two-stage mechanism involving two carboxylic acids positioned within the active site to act in concert: one as a catalytic nucleophile and the other as a general acid/base catalyst.[5-9] Protonation of the exocyclic glycosyl oxygen of a substrate molecule leads to bond-breaking and simultaneous attack of the catalytic nucleophile to form a glycosyl enzyme intermediate. Subsequent hydrolysis of the covalent intermediate by a nucleophilic water molecule gives an α-mannose product with overall retention of configuration. Studies with 5-fluoro pseudo-substrates and deuterium labeled substrates have shown that the transition states on either side of the covalent intermediate have marked oxacarbenium ion character.[5-9] Furthermore, the enzyme is highly specific for the presence of a single GlcNAc moiety attached in a β (1, 2) linkage to the α (1, 3)-Man arm of the $GlcNAcMan_5GlcNAc_2$ substrate.

The alkaloids swainsonine and mannostatin are potent inhibitors of Golgi α-mannosidase II.

These compounds inhibit, however, lysosomal α-mannosidases with potencies equal to that of the Golgi α-mannosidase II and therefore induce symptoms similar to that of lysosomal storage disease.[2,10] Attempts to develop more selective inhibitors of GMII by chemically modifying swainsonine or mannostatin have been met with failure. It is to be expected an understanding the molecular basis of the substrates specificities of these enzymes may provide unique opportunities for the design of more selective inhibitors.

In order to probe the substrate requirements of HGMII, we have synthesized compounds **1**, **2**, **3** and **4** (**Figure 2.1**) which will be employed for co-crystallization studies *Drosophilia* Golgi α-mannosidase II (dGMII).



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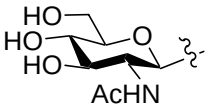
3: R=  , X=O

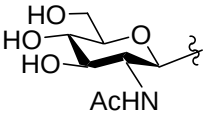
4: R=  , X=S

Figure 2.1. Synthetic targets derived from $GlcNAcMan_5GlcNAc_2$

Results and Discussion

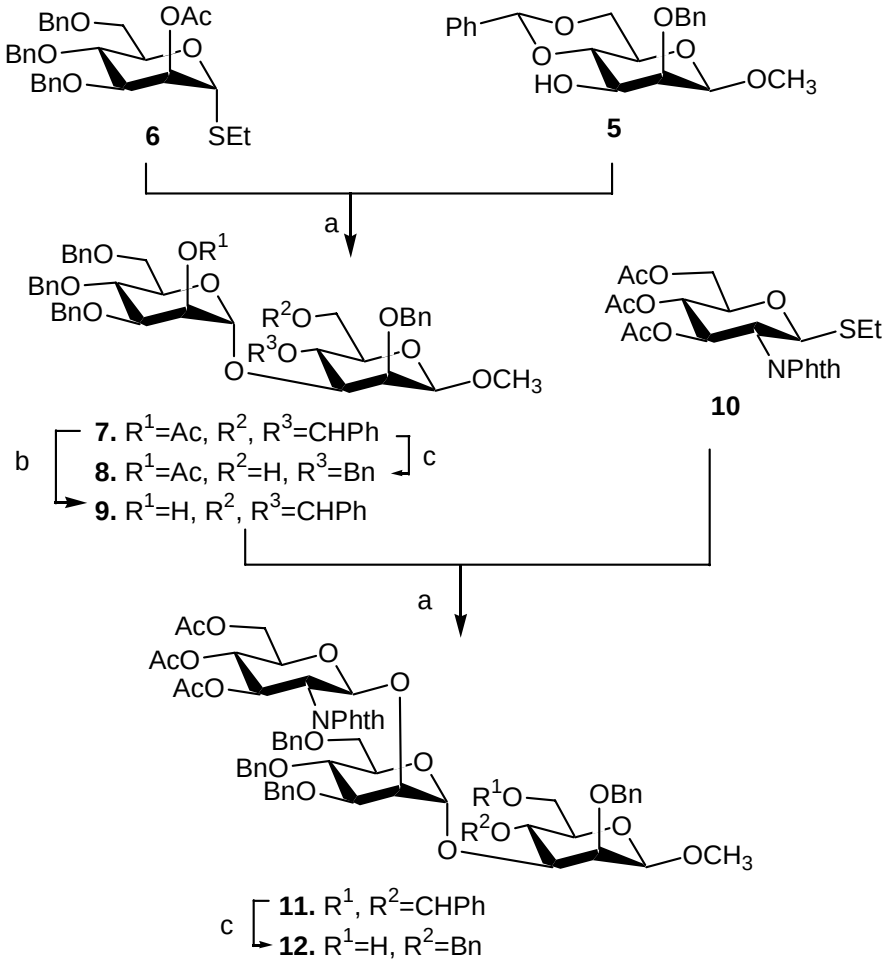
Synthesis

Tetrasaccharide **1** without β (1, 2)-linked GlcNAc moiety is derived form the natural substrate of GMII and contains the α (1, 6)-linked Man that is cleaved by the enzyme. Compound **2** is derived from **1**, however, the α (1, 6)-linked Man moiety is modified as a 1-thio-α-mannoside. Pentasaccharide **3** is derived form the natural substrate of GMII and contains the α (1, 6)-linked Man that is cleaved by the enzyme and the β (1, 2)-linked GlcNAc moiety which is required for recognition. Compound **4** is derived from **3**, however, the α (1, 6)-linked Man moiety is modified as a 1-thio-α-mannoside. Compounds **2** and **4** are resistant towards hydrolysis by mannosidases, which make them attractive derivatives for co-crystallographic studies with wild type dGMII.

Compounds **1** and **2** were prepared by a convergent 2+2 glycosylation strategy using glycosyl acceptor **8** and glycosyl donors **18** and **21**, respectively (**Scheme 2.1**). The disaccharide acceptor **8** could easily be prepared from known monosaccharide **5** and **6**. Compounds **3** and **4** were prepared by a convergent 3+2 glycosylation strategy using glycosyl acceptor **12** and glycosyl donors **18** and **21**, respectively (**Scheme 2.1**). The trisaccharide acceptor **12** could easily be prepared from known monosaccharides **5**, **6** and **10**. The thio-linked mannosyl donor **21** was obtained by a nucleophilic displacement of triflate **15** with a thioaldose prepared *in-situ* deacetylation of **19** followed by a two-step conversion of the trimethylethyl glycoside of the resulting compound into a trichloroacetimidate (**Scheme 2.2**).

Thus, NIS/TfOH[11] mediated coupling of thioglycoside **6**[12] with acceptor **5**[13] (**Scheme 2.1**) gave disaccharide **7** in an excellent yield of 80%. Neighboring group participation by the C-2

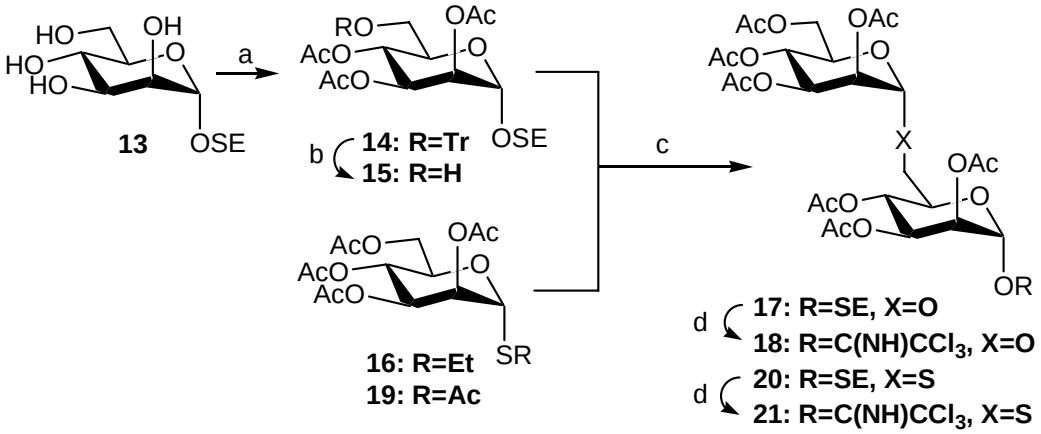
acetyl ester of **6** ensured exclusive formation of the α-anomer. Next, the acetyl ester of **7** was removed by treatment with NaOMe in methanol to glycosyl acceptor **9,** which was employed in a NIS/TfOH mediated coupling with thioglycoside **10**[14] to give trisaccharide **11** in a yield of 76%. The bulky C-2 phthalidene of **10** ensured that only the β anomer was formed. The benzylidene rings of **7** and **11** were selectively opened by treatment with borane in THF and Bu_2BOTf in DCM[15] resulted in the formation of glycosyl acceptor **8** and **12** having a C-4 benzyl ether and a C-6 hydroxyl.



Scheme 2.1. Reagents and conditions: a) NIS, TfOH, DCM, 0℃, **7**, 80%, **11**, 76%; b) NaOMe, MeOH, rt, 89%; c) 1 M BH_3 in THF, 1 M Bu_2BOTf in DCM, 0℃, **8**, 70%, **12**, 67%.

Next, attention was focused on the preparation of glycosyl donors **18** and **21**. Thus, glycosyl acceptor **15** was obtained by regioselective tritylation of the C-6 hydroxyl of mannoside **13**[16] using trityl chloride and pyridine followed acetylation and trityl ether removal employing $FeCl_3 \cdot 6H_2O$ in DCM. Next, glycosylation of **15** with **16** using NIS/TfOH as the promoter gave disaccharide **17** in an excellent yield of 76% as exclusively the α-anomer. The 1-thio-α-mannoside **21** was prepared by treatment of **15** with triflic anhydride in the presence of 2, 6-lutidine in DCM followed by displaced of the triflate of the resulting **15**, with 2, 3, 4, 6-tetra-O-acetyl-1-thio-α-D-mannose, which was prepared by *in-situ* S-deacetylation of **19**[17] using diethylamine in DMF.

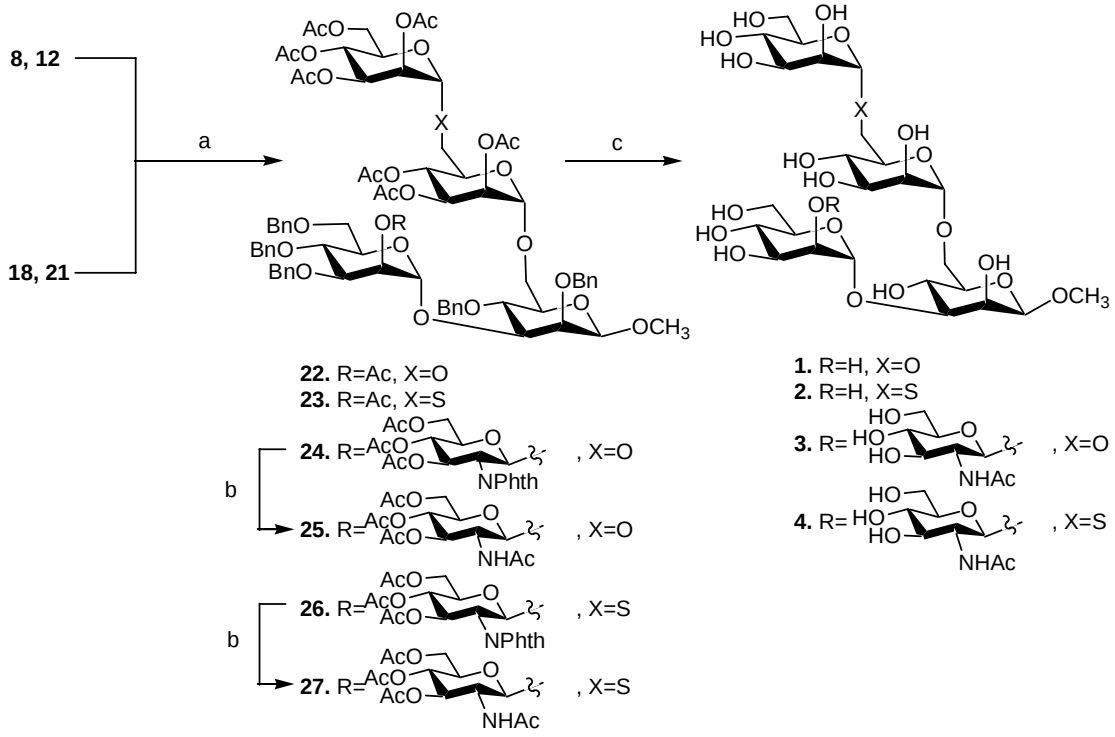
The requisite trichloroacetimidate **18** and **21** were obtained by hydrolysis of the trimethylsilyl ethyl glycoside of **17** and **20**, respectively using trifluoracetic acid in DCM followed by treatment of the resulting lactols with trichloroacetonitrile in the presence of DBU.[18,19]



Scheme 2.2. Reagents and conditions: a) 1. TrCl, Pyridine, 80℃; 2. Ac_2O, Pyridine, rt, 96% (2 steps); b) $FeCl_3 \cdot 6H_2O$, DCM, rt, 82%; c) i. **15**, **16**, NIS, TfOH, DCM, 0℃, 76%; ii. 1. **15**, Tf_2O, 2, 6-lutidine, DCM, -40℃; 2. **19**, Diethylamine, DMF, 0℃, 73% (2steps); d) 1. TFA, DCM, rt; 2. trichloroacetonitrile, DBU, DCM, rt, **18**, 83%, **21**, 86% (2 steps).

A TMSOTf promoted glycosylation of glycosyl acceptor **8** with glycosyl donors **18** or **21** in

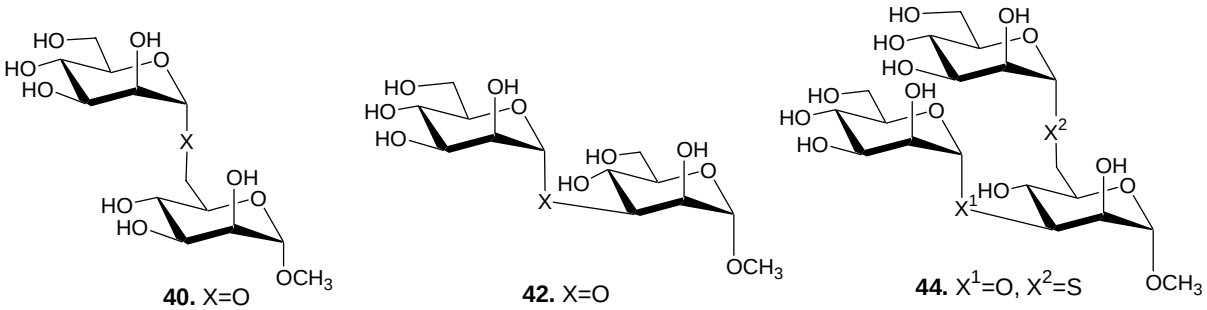
DCM at room temperature gave tetrasaccharides **22** and **23**, and glycosylation of glycosyl acceptor **12** with glycosyl donors **18** or **21** in DCM at room temperature gave pentasaccharides **24** and **26,** respectively in excellent yields. Finally, cleavage of phthalimido group of **24** and **26** was accomplished by treatment with hydrazine in ethanol followed by *N*-acetylation with acetic anhydride in pyridine to afford compounds **25** and **27**, respectively. Finally, deprotection of **22**, **23**, **25** and **27** was accomplished by a two-step procedure entailed treatment with NaOMe in methanol to hydrolyze the acetyl and Birch reduction to remove the benzyl ethers.



Scheme 2.3. Reagents and conditions: a) TMSOTf, DCM, rt, **22**, 83%, **23**, 81%, **24**, 80%, **26**, 83%; b) 1. $H_2NNH_2 \cdot H_2O$, EtOH, 90℃; 2. Ac_2O, Pyridine, rt, **25**, 96%, **27**, 89% (2 steps); c) 1. NaOMe, MeOH, rt; 2. Na (s), NH_3 (l), THF, -78℃, **1**, 87%, **2**, 90%, **3**, 87%, **4**, 86% (2 steps).

Compound **40** contains the α (1, 6)-linked Man that is cleaved by the enzyme, while compound **42** contains the α (1, 3)-linked Man that is cleaved by the enzyme. Compound **41** is derived from **40**, however, the α (1, 6)-linked Man moiety is modified as a 1-thio-α-mannoside. Compound **43**

is derived from **42**, however, the α (1, 3)-linked Man moiety is modified as a 1-thio-α-mannoside. Compound **44** contains the α (1, 3)-linked Man that is cleaved by the enzyme, while the α (1, 6)-linked Man moiety is modified as a 1-thio-α-mannoside that is resistant towards hydrolysis by mannosidases. Compound **45** contains the α (1, 6)-linked Man that is cleaved by the enzyme, while the α (1, 3)-linked Man moiety is modified as a 1-thio-α-mannoside that is resistant towards hydrolysis by mannosidases. These compounds are attractive derivatives for co-crystallographic studies with wild type dGMII to explore the catalytic site of the enzyme (**Figure 2.2**).



40. X=O

41. X=S

42. X=O

43. X=S

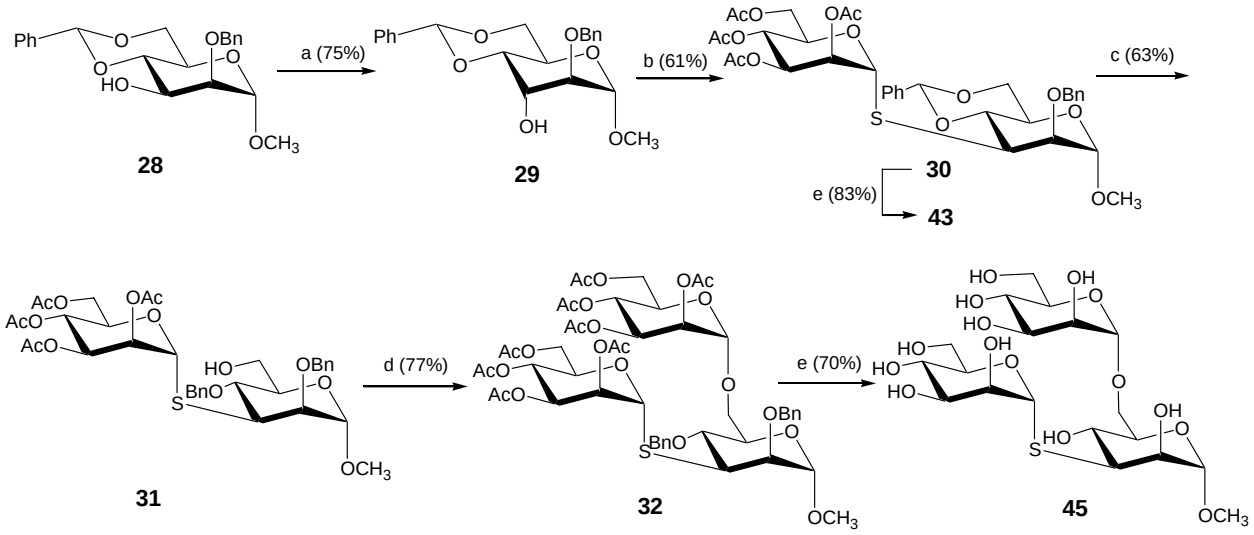
44. X^1=O, X^2=S

45. X^1=S, X^2=O

Figure 2.2. Substrates and inhibitors derived from $GlcNAcMan_5GlcNAc_2$

The 1-thio-α-mannoside **30** was prepared by treatment of **29** (**Scheme 2.4**), which was synthesized from known compound **28**[20] by conversion of C-3 hydroxyl from equatorial to axial, with triflic anhydride in the presence of pyridine in DCM followed by displaced of the triflate of the resulting **29**, with 2, 3, 4, 6-tetra-O-acetyl-1-thio-α-D-mannose, which was prepared by *in-situ* S-deacetylation of **19** using diethylamine in DMF. Then, deprotection of **30** was accomplished by a two-step procedure entailed treatment with NaOMe in methanol to hydrolyze the acetyl and Birch reduction to remove the benzyl ethers to afford compound **43**. The

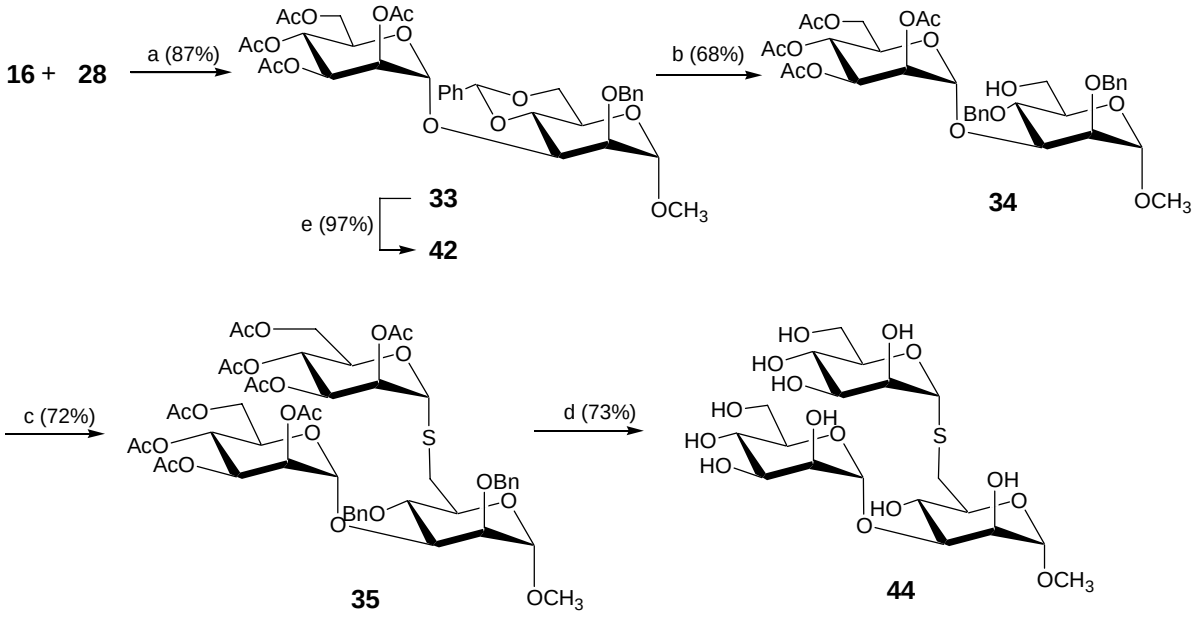
benzylidene ring of **30** was selectively opened by treatment with borane in THF and Bu_2BOTf in DCM resulted in the formation of glycosyl acceptor **31** having a C-4 benzyl ether and a C-6 hydroxyl. Next, NIS/TfOH mediated coupling of thioglycoside **16** with acceptor **31** gave trisaccharide **32** in an excellent yield of 77%. Neighboring group participation by the C-2 acetyl ester of **16** ensured exclusive formation of the α-anomer. Finally, deprotection of **32** was accomplished by a two-step procedure entailed treatment with NaOMe in methanol to hydrolyze the acetyl and Birch reduction to remove the benzyl ethers to afford compound **45**.



Scheme 2.4. Reagents and conditions: a) 1. DMSO, 1:2 Ac_2O/DMSO, 2. $NaBH_4$, 1:1 DCM/MeOH; b) 1. Tf_2O, 1:2 pyridine/DCM, 2. **19**, DMF, diethylamine, 0 ^oC; c) BH_3 in THF, Bu_2BOTf; d) **16**, NIS, TfOH, DCM, 0 ^oC; e) 1. NaOMe, MeOH, 2. Na/NH_3(l), -78 ^oC

NIS/TfOH mediated coupling of thioglycoside **16** with acceptor **28** (**Scheme 2.5**) gave disaccharide **33** in an excellent yield of 87%. Neighboring group participation by the C-2 acetyl ester of **16** ensured exclusive formation of the α-anomer. Then, deprotection of **33** was accomplished by a two-step procedure entailed treatment with NaOMe in methanol to hydrolyze the acetyl and palladium(0) catalyzed hydrogenation to remove the benzyl ethers to afford compound **42**. The benzylidene ring of **33** was selectively opened by treatment with borane in

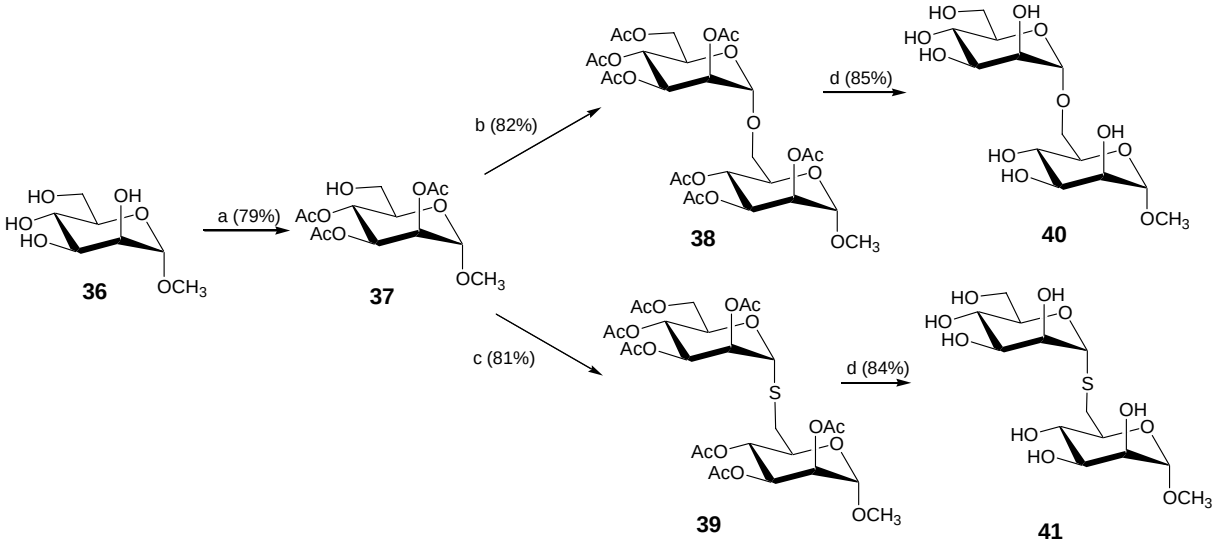
THF and Bu_2BOTf in DCM resulted in the formation of glycosyl acceptor **34** having a C-4 benzyl ether and a C-6 hydroxyl. Then, the 1-thio-α-mannoside **35** was prepared by treatment of **34** with triflic anhydride in the presence of 2, 6-lutidine in DCM followed by displaced of the triflate of the resulting **34**, with 2, 3, 4, 6-tetra-O-acetyl-1-thio-α-D-mannose, which was prepared by *in-situ* S-deacetylation of **19** using diethylamine in DMF. Finally, deprotection of **35** was accomplished by a two-step procedure entailed treatment with NaOMe in methanol to hydrolyze the acetyl and Birch reduction to remove the benzyl ethers to afford compound **44**.



Scheme 2.5. Reagents and conditions: a) NIS, TfOH, DCM, 0 ^{o}C; b) BH_3 in THF, Bu_2BOTf; c) 1. Tf_2O, 2,6-lutidine, 2. **19**, diethylamine, DMF, 0 ^{o}C; d) 1. NaOMe/MeOH, 2. Na/NH_3(l), -78 ^{o}C; e) 1. NaOMe/MeOH, 2. Pd/C, ethanol

Compounds **38** and **39** were synthesized from compound **37** (**Scheme 2.6**), which was obtained by regioselective tritylation of the C-6 hydroxyl of mannoside **36** using trityl chloride and pyridine followed acetylation and trityl ether removal employing $FeCl_3 \cdot 6H_2O$ in DCM. NIS/TfOH mediated coupling of thioglycoside **16** with acceptor **37** gave disaccharide **38** in an

excellent yield of 82%. Neighboring group participation by the C-2 acetyl ester of **16** ensured exclusive formation of the α-anomer. Deprotection of **38** was accomplished by treatment with NaOMe in methanol to hydrolyze the acetyl to afford compound **40**. The 1-thio-α-mannoside **39** was prepared by treatment of **37** with triflic anhydride in the presence of 2, 6-lutidine in DCM followed by displaced of the triflate of the resulting **37**, with 2, 3, 4, 6-tetra-O-acetyl-1-thio-α-D-mannose, which was prepared by *in-situ* S-deacetylation of **19** using diethylamine in DMF. Finally, deprotection of **39** was accomplished by treatment with NaOMe in methanol to hydrolyze the acetyl to afford compound **41**.



Scheme 2.6. Reagents and conditions: a) 1. TrCl, pyridine, 2. Ac_2O, pyridine, 3. $FeCl_3$, DCM; b) **16**, NIS, TfOH, DCM; c) 1. Tf_2O, 2,6-lutidine, DCM, 2. **19**, diethylamine, DMF; d) NaOMe/MeOH

Biological evaluation of substrates

The Kms of substrates are more than 10mM indicating that these compounds are poor substrates and inhibitors.

Co-crystallographic studies with wild type dGMII

X-ray crystallography studies are still ongoing.

Conclusion

Glycosidases play important roles in the biosynthesis of glycoproteins. Understanding the catalytic and extended binding sites could help in the design and synthesis of more potent and selective inhibitors. Successfully synthesized substrates **1**, **3**, **40** and **42** and inhibitors **2**, **4**, **41**, **43**, **44** and **45** are very valuable compounds in the investigation of catalytic and extended binding sites of golgi α-mannosidase II. X-ray crystallography studies, which are still ongoing, could aid in a better understanding of the catalytic and extended binding sites of Golgi α-mannosidase II.

Experimental Section

General:

^{1}H-NMR spectra were recorded in $CDCl_3$ or D_2O on a Varian Merc-300 or Varian Inova-500 spectrometers equipped with Sun workstations at 300K. TMS (δ_H=0.00) or D_2O (δ_H=4.67) was used as the internal reference. ^{13}C-NMR spectra were recorded in $CDCl_3$ or D_2O at 75MHz on Varian Merc-300 spectrometer, using the central resonance of $CDCl_3$ (δ_C=77.0) as the internal reference. COSY, HSQC, HMBC and TOCSY experiments were used to assist assignment of the products. The different monosaccharide units are referred to as a, b, c, d, and e respectively, with a denoting the reducing end monosaccharide. Mass spectra were obtained on Applied Biosystems Voyager DE-Pro MALDI-TOF (no calibration) and Bruker Daltonics 9.4T (FTICR, external calibration with BSA). Optical rotatory power was obtained on Jasco P-1020 polarimeter at 300 K.

Chemicals were purchased from Aldrich or Fluka and used without further purification. DCM was distilled from calcium hydride; THF from sodium; MeOH from magnesium and iodine.

Aqueous solutions are saturated unless otherwise specified. Molecular sieves were activated at 350℃ for 3 h *in vacuo*. All the reactions were performed under anhydrous conditions under argon and monitored by TLC on Kieselgel 60 F254 (Merck). Detection was by examination under UV light (254 nm) and by charring with 10 % sulfuric acid in methanol. Silica gel (Merck, 70-230 mesh) was used for chromatographies. Iatrobeads 6RS-8060 was purchased from Bioscan. Bio-Gel P-2 Gel was purchased from Bio-Rad Laboratories.

General procedure for glycosidations with thioglycosides 6, 10 or 16.

A mixture of glycosyl acceptor (1 eq.), glycosyl donor (1.1 eq.) and 4Å powdered molecular sieves in DCM (0.06 mol/L) was stirred at 0℃ for 1h. Subsequently, NIS (1.1 eq.) and TfOH (0.2 eq.) were added. The mixture was stirred at 0℃ for 1 h, and then neutralized with triethylamine. The solution was filtered through celite, washed with MeOH/DCM (5:95, v/v), and the combined filtrates were concentrated to dryness. The residue was dissolved in DCM, and the solution was washed with $Na_2S_2O_3$ (1 M) and water. The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the crude product by column chromatography on silica gel afforded the products **7**, **11**, **17**, **32**, **33** or **38**.

General procedure for synthesis of glycosyl trichloroimidate 18 or 21.

To a solution of **17** or **20** (1 eq.) in DCM (0.12 mol/L) was added trifluoacetic acid (200 eq.) under argon. The reaction mixture was stirred at room temperature for 5 h, and then concentrated to dryness. The residue was co-evaporated with EtOAc/toluene (1:1) and toluene (twice). To the resulting residue in anhydrous DCM (0.06 mol/L) under argon was added trichloroacetonitrile (10 eq.) and DBU (0.2 eq.). The reaction mixture was stirred at room temperature for 2 h, and

then concentrated to dryness. Purification of the crude product by fast column chromatography on silica gel afforded the trichloroimidates **18** or **21** as colorless oils.

General procedure for glycosidations with glycosyl trichloroimidates 18 or 21.

A mixture of glycosyl acceptor (1 eq.), glycosyl donor (1.1 eq.) and 4Å powdered molecular sieves in DCM (0.06 mol/L) was stirred at 0℃ for 1 h, and then cooled to -20℃. Subsequently, TMSOTf (0.2 eq.) was added and the reaction mixture was allowed to warm to room temperature and stirred for 3 h. After neutralize with triethylamine, the mixture was filtered through celite, and the filtrate was washed with MeOH/DCM (5:95, v/v). The combined filtrates were concentrated to dryness, and the residue was purified by column chromatography on silica gel afforded the product **22**, **23**, **24** or **26** as colorless oils.

General procedure for reduction of benzylidene acetals 7, 11, 30 or 33.

To a dry flask containing **7**, **11**, **30** or **33** (1 eq.) was added a solution of 1 M BH_3 in THF(10 eq.) at 0℃, and then the solution was stirred for 5 min. A solution of 1 M Bu_2BOTf in DCM (1 eq.) was then added to the clear solution dropwise. After 30 min at 0℃, TLC showed the disappearance of starting material. TEA was then added followed by careful addition of methanol until evolution of H_2 had ceased. The mixture was co-evaporated with methanol three times. Purification of the crude product by column chromatography over silica gel afforded **8**, **12**, **31** or **34** as white solids.

General procedure for glycosidation with thioacetyl mannoside 19.

To a solution of **15**, **34** or **37** (1 eq.) and 2, 6-lutidine (1.2 eq.) in DCM (0.2 mol/L) was added trifluoromethanesulfonic anhydride (1.5 eq.). The reaction mixture was stirred at -40℃ for 1 h,

and then quenched by the addition of saturated aqueous $NaHCO_3$. The resulting mixture was extracted with ether for 3 times. The combined organic phases were dried ($MgSO_4$), filtered, and the filtrate was concentrated to dryness below 30℃. A solution of the residue and **19** (0.9 eq.) in DMF (0.1 mol/L) was cooled (0℃) and placed under argon. Liquid diethyl amine (2.3 mol/L) was added slowly. The reaction mixture was stirred at 0℃ for 24 h and at room temperature for 5 h, and then concentrated to dryness. The residue was dissolved in EtOAc, and the solution was washed with H_2O and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. Purification of the crude product by column chromatography on silica gel afforded the desired product **20**, **35** or **39** as colorless oils.

General procedure for phthalimido removal followed by acetylation 24 or 26.

To a solution of **24** or **26** (1 eq.) in EtOH (0.02 mol/L) was added $H_2NNH_2 \cdot H_2O$ (80 eq.). The reaction mixture was stirred at 90℃ for 24 h, and then concentrated to dryness. The residue was dissolved in Ac_2O (40 eq.) and pyridine (40 eq.). The reaction mixture was stirred at room temperature for 12 h, and then concentrated to dryness. Purification of the crude product by column chromatography on silica gel afforded **25** or **27** as colorless oils.

General precedure for global deprotection of 22, 23, 25, 27, 30, 32 or 35.

To a solution of **22**, **23**, **25**, **27, 30**, **32** or **35** (1 eq.) in dry MeOH (0.06 mol/L) was added NaOMe (pH=8-10). The reaction mixture was stirred at room temperature overnight, and then neutralized by the addition of Dowex 650 H^+. The solution was filtered through celite, washed with MeOH/DCM (1:1). The combined filtrates were concentrated to dryness. Purification of the crude product by Iatrobeads afforded the desired deacetylated product. A solution of the partially

deprotected compound (1 eq.) in THF (0.02 mol/L) was added to NH_3 (l) at -78℃. Small pieces of sodium were added until the reaction mixture remained blue. The reaction mixture was stirred at -78℃ for 20 min, and then added ammonium chloride until the blue color disappeared. The NH_3 (l) was allowed to evaporate slowly and the remaining solution was concentrated to dryness. The residue was dissolved with milli-Q water. The aqueous solution was loaded on a P2 column to afford **1**, **2**, **3**, **4, 43, 44** or **45** as white solids.

Methyl *O*-(2-*O*-acetyl-3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4, 6-di-*O*-benzylidene-β-D-mannopyranoside (7):

The compound **7** was synthesized according to the general procedure for the glycosidation with thioglycoside **6**. Glycosyl donor **6** (1.70 g, 3.17 mmol), glycosyl acceptor **5** (1.07 g, 2.87 mmol) and 4Å powdered molecular sieves (2.80 g) in DCM (36 mL) in the presence of NIS (0.79 g, 3.49 mmol) and TfOH (58 μL, 0.64 mmol) gave **7** as colorless oil (1.95 g, 80%). R_f =0.42 (Hexnae/EtOAc, 2:1); $[\alpha]_D^{27}$= +24.1 ($CHCl_3$, c=3.4 mg/mL); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 2.02 (s, 3H, OC(O)CH_3), 3.26 (m, 1H, H-5_a), 3.44 (s, 3H, OCH_3), 3.60-3.74 (m, 4H, H-4_b, H-5_b, H-6_b, H-$6_{b'}$), 3.80-3.88 (m, 4H, H-2_a, H-3_a, H-6_a, H-3_b), 4.12(t, 1H, $J_{3,4}$=9.6, $J_{4,5}$=9.6 Hz, H-4_a), 4.25 (dd, 1H, $J_{5,6}$=4.8, $J_{6,6'}$=10.5 Hz, H-$6_{a'}$), 4.27 (s, 1H, H-1_a), 4.33-4.41 (m, 3H, PhCH*H*), 4.49 (d, 1H, *J*=12.0 Hz, PhCH*H*), 4.59 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.71 (d, 1H, *J*=12.3 Hz, PhCH*H*), 4.80 (d, 1H, *J*=12.3 Hz, PhCH*H*), 4.82 (d, 1H, *J*=10.5 Hz, PhCH*H*), 5.23 (d, 1H, $J_{1,2}$=1.8 Hz, H-1_b), 5.54 (s, 2H, H-1_b, PhC*H*), 7.08-7.37 (m, 25H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= 21.0 (OC(O)*C*H_3), 57.4 (O*C*H_3), 67.3 (C-5_a), 68.1 (C-2_b), 68.5 (C-6_a), 69.0 (C-6_b), [71.6, 73.3, 74.9, 75.1 (Ph*C*H_2)], 72.0 (C-5_b), 74.2 (C-4_b), 75.0 (C-3_a), 77.9 (C-2_a), 78.0 (C-3_b), 78.7 (C-4_a), 99.8

(C-1_b), 101.1 (Ph*C*H), 103.2 (C-1_a), [125.9, 127.5, 127.6, 127.7, 128.0, 128.1, 128.2, 128.3, 128.4, 128.7, 137.2, 137.8, 138.2, 138.3, 138.5 (C_{arom})], 169.9 (O*C*(O)CH_3); MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 869.3, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 869.3503, $C_{50}H_{54}O_{12}$ calcd for $[M+Na]^+$ 869.3513.

Methyl *O*-(2-*O*-acetyl-3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2, 4-di-*O*-benzyl-β-D-mannopyranoside (8):

The compound **8** was synthesized according to the general procedure for reduction of benzylidene acetal **7**. Treatment of **7** (0.13 g, 0.15 mmol) with a solution of 1 M BH_3 in THF (1.5 mL) and a solution of 1 M Bu_2BOTf in DCM (0.15 mL) gave **8** as colorless oil (0.091 g, 70%). R_f=0.3 (Hexnae/EtOAc, 1:1). $[\alpha]_D^{27}$= -23.3 ($CHCl_3$, c=5.8 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 2.00 (s, 3H, OC(O)C*H*$_3$), 3.23 (m, 1H, H-5_a), 3.40 (s, 3H, OCH_3), 3.56 (m, 2H, H-6_b, H-$6_{b'}$), 3.67-3.71 (m, 4H, H-2_a, H-6_a, H-4_b, H-$5_{b'}$), 3.79-3.86 (m, 3H, H-3_a, H-$6_{a'}$, H-3_b), 3.89 (t, 1H, $J_{3,4}$=9.6, $J_{4,5}$=9.6 Hz, H-4_a), 4.23 (s, 1H, H-1_a), 4.35-4.40 (m, 3H, PhCH*H*), 4.45 (d, 1H, *J*=12.3 Hz, PhCH*H*), 4.55 (d, 1H, *J*=11.1 Hz, PhCH*H*), 4.56 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.67 (d, 1H, *J*=12.6 Hz, PhCH*H*), 4.70 (d, 1H, *J*=11.1 Hz, PhCH*H*), 4.76 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.85 (d, 1H, *J*=12.0 Hz, PhCH*H*), 5.13 (s, 1H, H-1_b), 5.41 (s, 1H, H-2_b), 7.08-7.30 (m, 25H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= 21.0 (OC(O)*C*H_3), 57.3 (O*C*H_3), 62.1 (C-6_a), 68.7 (C-2_b), 69.1 (C-6_b), [71.9, 73.4, 74.3, 74.8, 75.2 (Ph*C*H_2)], 72.1 (C-5_b), 74.2 (C-4_b), 75.1 (C-4_a), 75.8 (C-5_a), 77.2 (C-3_a), 77.9 (C-3_b), 79.9 (C-2_a), 99.7 (C-1_b), 102.7 (C-1_a), [127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.9, 137.7, 137.8, 138.1, 138.5 (C_{arom})], 170.0 (O*C*(O)CH_3); MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 871.3, MALDI-FTICR/MS: *m/z*: found

$[M+Na]^+$ 871.3662, $C_{50}H_{56}O_{12}$ calcd for $[M+Na]^+$ 871.3669

Methyl *O*-(3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4, 6-di-*O*-benzylidene-β-D-mannopyranoside (9):

To a solution of **7** (0.58 g, 0.67 mmol) in dry DCM/MeOH (12 mL, 1:1) was added NaOMe (pH=8-10). The mixture was stirred at room temperature for 4 h, and concentrated to dryness *in vacuo*. Purification of the crude product by column chromatography over silica gel (Hexnae/EtOAc, 3:1) afforded **9** as white solid (0.49 g, 89%). R_f =0.31 (Hexnae/EtOAc, 2:1). $[\alpha]_D^{27}$= -34.2 ($CHCl_3$, c=3.1 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 3.27 (m, 1H, H-5_a), 3.42 (s, 3H, OCH_3), 3.59-3.76 (m, 4H, H-3_b, H-4_b, H-6_b, H-$6_{b'}$), 3.80-3.87 (m, 2H, H-2_a, H-6_a), 3.93 (dd, 1H, $J_{2,3}$=2.7, $J_{3,4}$=9.9 Hz, H-3_a), 4.03-4.09 (m, 2H, H-4_a, H-2_b), 4.22 (d, 1H, $J_{5,6}$=4.5 Hz, H-$6_{a'}$), 4.26 (s, 1H, H-1_a), 4.44 (d, 2H, *J*=11.4 Hz, PhCH*H*), 4.47 (d, 1H, *J*=12.0 Hz, PhCH*H*), 4.53 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.56 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.72 (d, 1H, *J*=12.0 Hz, PhCH*H*), 4.74 (d, 1H, *J*=11.1 Hz, PhCH*H*), 4.83 (d, 1H, *J*=12.3 Hz, PhCH*H*), 5.25 (d, 1H, $J_{1,2}$=1.5 Hz, H-1_b), 5.50 (s, 1H, PhC*H*), 7.06-7.39 (m, 25H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= 57.4 (O*C*H_3), 67.3 (C-5_a), 68.0 (C-2_b), 68.6 (C-6_a), 69.2 (C-6_b), [71.8, 73.4, 74.9, 75.1 (Ph*C*H_2)], 71.9 (C-5_b), 74.3 (C-4_b), 75.2 (C-3_a), 77.8 (C-2_a), 78.7 (C-4_a), 79.9 (C-3_b), 100.3 (C-1_b), 101.5 (Ph*C*H), 103.3 (C-1_a), [125.9, 127.5, 127.6, 127.7, 127.8, 127.9, 128.1, 128.2, 128.3, 128.5, 128.9, 137.3, 137.7, 138.3, 138.4, 138.5 (C_{arom})]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 827.3, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 827.3392, $C_{48}H_{52}O_{11}$ calcd for $[M+Na]^+$ 827.3407.

Methyl *O*-(3, 4, 6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4, 6-di-*O*-benzylidene-β-

D-mannopyranoside (11):

The compound **11** was synthesized according to the general procedure for the glycosidation with thioglycoside **10**. Glycosyl donor **10** (0.32 g, 0.67 mmol), glycosyl acceptor **9** (0.49 g, 0.61 mmol) and 4Å powdered molecular sieves (0.81 g) in DCM (20 mL) in the presence of NIS (0.17 g, 0.74 mmol) and TfOH (12 μL, 0.13 mmol) gave **11** as colorless oil (0.56 g, 76%). R_f =0.57 (Hexnae/EtOAc, 1:1). $[\alpha]_D^{27}$= -33.7 ($CHCl_3$, c=3.1 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= [1.79, 1.93, 2.00 (3×s, 9H, OC(O)C*H*$_3$)], 2.19 (m, 1H, H-5$_c$), 2.61 (dd, 1H, $J_{5,6}$=6.6, $J_{6,6'}$=10.8 Hz, H-6$_b$), 3.20-3.44 (m, 4H, H-5$_a$, H-4$_b$, H-5$_b$, H-6$_{b'}$), 3.42 (s, 3H, OC*H*$_3$), 3.67-4.00 (m, 8H, H-2$_a$, H-3$_a$, H-4$_a$, H-6$_a$, H-2$_b$, H-3$_b$, H-6$_c$, H-6$_{c'}$), 4.24 (s, 1H, H-1$_a$), 4.21-4.27 (m, 4H, H-6$_{a'}$, H-2$_c$, PhCH*H*), 4.31 (d, 1H, *J*=11.7 Hz, PhCH*H*), 4.35 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.56 (d, 1H, *J*=12.0 Hz, PhCH*H*), 4.65 (d, 1H, *J*=13.5 Hz, PhCH*H*), 4.71 (d, 1H, *J*=12.6 Hz, PhCH*H*), 4.74 (d, 1H, *J*=10.8 Hz, PhCH*H*), 4.92-4.99 (m, 3H, H-1$_b$, H-1$_c$, H-4$_c$), 5.48 (s, 1H, PhC*H*), 5.52 (t, 1H, $J_{2,3}$=9.9, $J_{3,4}$=9.9 Hz, H-3$_c$), 6.96-7.67 (m, 29H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.5, 20.6, 20.7 (OC(O)*C*H$_3$)], 54.2 (C-2$_c$), 57.5 (O*C*H$_3$), 61.3 (C-6$_c$), 67.1 (C-5$_a$), 68.7 (C-4$_c$), 68.8 (C-6$_a$), 69.8 (C-6$_b$), 70.3 (C-3$_c$), 70.7 (C-5$_c$), [71.1, 74.8×3 (Ph*C*H$_2$)], 71.8 (C-2$_b$), 71.9 (C-5$_b$), 72.5 (C-3$_a$), 74.0 (C-4$_b$), 77.2 (C-2$_a$), 77.8 (C-3$_b$), 78.9 (C-4$_a$), 95.5 (C-1$_c$), 97.7 (C-1$_b$), 102.6 (Ph*C*H), 103.2 (C-1$_a$), [123.5, 123.6, 127.0, 127.2, 127.3, 127.5, 127.6, 127.8, 128.0, 128.1, 128.2, 128.3, 128.7, 130.1, 133.9, 137.6, 138.0, 138.3, 138.4, 138.7 (C_{arom})], [167.2, 167.8 (*C*=O, Phth)], [169.2, 170.1, 170.7 (O*C*(O)CH$_3$)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 1244.4, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1244.4464, $C_{68}H_{71}NO_{20}$ calcd for $[M+Na]^+$ 1244.4467.

Methyl *O*-(3, 4, 6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2, 4-di-*O*-benzyl-β-D-mannopyranoside (12):

The compound **12** was synthesized according to the general procedure for reduction of benzylidene acetal **11**. Treatment of **11** (0.21 g, 0.17 mmol) with a solution of 1 M BH_3 in THF (1.7 mL) and a solution of 1 M Bu_2BOTf in DCM (0.17 mL) gave **12** as white solid (0.14 g, 67%). R_f=0.3 (Hexnae/EtOAc, 1:1). $[\alpha]_D^{27}$= -135.0 ($CHCl_3$, c=1.4 mg/mL). 1H NMR ($CDCl_3$, 300 MHz): δ= [1.78, 1.93, 1.97 (3×s, 9H, OC(O)CH_3)], 2.55 (m, 1H, H-5_c), 2.68 (dd, 1H, $J_{5,6}$=6.9, $J_{6,6'}$=10.8 Hz, H-6_b), 3.21-3.36 (m, 3H, H-5_a, H-4_b, H-$6_{b'}$), 3.35 (s, 3H, OCH_3), 3.49-3.97 (m, 10H, H-2_a, H-3_a, H-4_a, H-6_a, H-$6_{a'}$, H-2_b, H-3_b, H-5_b, H-6_c, H-$6_{c'}$), 4.28 (s, 1H, H-1_a), 4.26-4.33 (m, 2H, H-2_c, PhCH*H*), 4.42 (d, 1H, *J*=11.4 Hz, PhCH*H*), 4.52-4.78 (m, 8H, PhCH*H*), 4.92 (s, 1H, H-1_b), 4.98 (t, 1H, $J_{3,4}$=9.3, $J_{4,5}$=9.9 Hz, H-4_c), 5.08 (d, 1H, $J_{1,2}$=8.4 Hz, H-1_c), 5.55 (t, 1H, $J_{2,3}$=10.8, $J_{3,4}$=9.3 Hz, H-3_c), 6.98-7.54 (m, 29H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.5, 20.6, 20.7 (OC(O)*C*H_3)], 54.3 (C-2_c), 57.2 (O*C*H_3), 61.6 (C-6_c), 61.8 (C-6_a), 68.6 (C-4_c), 69.9 (C-6_b), 70.4 (C-3_c), [71.2, 73.9, 74.2, 74.6, 74.7 (Ph*C*H_2)], 72.2 (C-5_c), 72.7 (C-2_b, C-5_b), 73.3 (C-3_a), 74.1 (C-4_b), 74.6 (C-2_a), 75.7 (C-5_a), 77.7 (C-3_b), 80.6 (C-4_a), 96.0 (C-1_c), 98.8 (C-1_b), 102.6 (C-1_a), [123.5, 123.6, 126.3, 127.1, 127.3, 127.6, 127.7, 128.1, 128.2, 128.7, 131.5, 133.9, 138.0, 138.3, 138.4, 138.5, 138.6 (C_{arom})], [167.2, 167.8 (*C*=O, Phth)], [169.3, 170.1, 170.6 (O*C*(O)CH_3)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 1246.4, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1246.4624, $C_{68}H_{73}NO_{20}$ calcd for $[M+Na]^+$ 1246.4624.

2-(Trimethylsilyl)ethyl 2, 3, 4-tri-*O*-acetyl-6-*O*-triphenylmethyl-α-D-mannopyranoside (14):

To a solution of **13** (0.62 g, 2.21 mmol) in pyridine (5 mL) was added TrCl (0.93 g, 3.34 mmol).

The reaction mixture was stirred at 80℃ for 2 h, and then concentrated to dryness. To the solution of this residue in pyridine (7 mL) was added acetic anhydride (1.25 mL). The reaction mixture was stirred at room temperature for 6 h, and then concentrated to dryness. The residue was co-evaporated with methanol (3×20 mL) and toluene (3×20 mL). The residue was dissolved in DCM (30 mL), and then washed with 1N HCl (1×50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. Purification of the crude product by column chromatography over silica gel (Hexnae/EtOAc, 3:1) afforded **14** as yellow oil (1.37 g, 96%). R_f =0.20 (Hexnae/EtOAc, 3:1). $[\alpha]_D^{27}$= +30.1 ($CHCl_3$, c=10.5 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 0.04-0.07 (3×s, 9H, SiC*H*$_3$), 0.98-1.06 (m, 2H, SiC*H*$_2$), 1.74, 1.97, 2.17 (3×s, 9H, OC(O)C*H*$_3$), 3.15-3.23 (m, 2H, H-6, H-6'), 3.63 (m, 2H, OCH_2), 3.88-3.97 (m, 2H, H-5, $OCH_{2'}$), 4.89 (s, 1H, H-1), 5.18-5.25 (m, 2H, H-2, H-4), 5.32 (dd, 1H, *J*=3.3, 10.2 Hz, H-3), 7.20-7.47 (m, 15H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= -1.3, 1.0 (Si*C*H$_3$), 17.8 (*C*H$_2$Si), [20.6, 20.7, 20.9 (OC(O)*C*H$_3$)], 62.8 (C-6), 65.4 (O*C*H$_2$), 66.8 (C-4), 69.5 (C-3), 70.1 (C-2), 70.2 (C-5), 86.6 (Ph$_3$*C*), 96.6 (C-1), [126.9, 127.8, 128.7, 143.8 (C_{arom})], [169.4, 170.0, 170.2 (O*C*(O)CH$_3$)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 671.2, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 671.2636, $C_{36}H_{44}O_9Si$ calcd for $[M+Na]^+$ 671.2652.

2-(Trimethylsilyl)ethyl 2, 3, 4-tri-*O*-acetyl-α-D-mannopyranoside (15):

To a solution of **14** (0.70 g, 1.08 mmol) in DCM (10 mL) was added $FeCl_3 \cdot 6H_2O$ (1.02 g, 3.77 mmol). The reaction mixture was stirred at room temperature for 1 h, and then diluted with DCM (20 mL), washed with water (30 mL).The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness. Purification of the crude product by column chromatography over silica

gel (Hexnae/EtOAc, 2:1) afforded **15** as white solid (0.36 g, 82%). R_f=0.35 (Hexnae/EtOAc, 1:1). $[\alpha]_D^{27}$= +27.3 ($CHCl_3$, c=1.2 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= -0.01-0.05 (3×s, 9H, SiCH_3), 0.92-0.98 (m, 2H, SiCH_2), [1.98, 2.06, 2.13 (3×s, 9H, OC(O)CH_3)], 3.47-3.82 (m, 5H, H-5, H-6, H-6', OCH_2), 4.82 (s, 1H, H-1), 5.18-5.25 (m, 2H, H-2, H-4), 5.38 (dd, 1H, $J_{2,3}$=3.3, $J_{3,4}$=10.2 Hz, H-3); ^{13}C NMR (75MHz, $CDCl_3$): δ= [-1.5×3 (SiCH_3)], 17.8 (CH_2Si), [20.6, 20.7, 20.8 (OC(O)CH_3)], 61.3 (C-6), 65.7 (OCH_2), 66.5 (C-4), 68.9 (C-3), 69.8 (C-2), 70.5 (C-5), 97.0 (C-1), [169.8, 170.1, 170.8 (OC(O)CH_3)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 429.2, $C_{17}H_{30}O_9Si$ calcd for $[M+Na]^+$ 429.1557.

2-(Trimethylsilyl)ethyl *O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2, 3, 4-tri-*O*-acetyl-α-D-mannopyranoside (17):

The compound **17** was synthesized according to the general procedure for the glycosidation with thioglycoside **16**. Glycosyl donor **16** (0.24 g, 0.61 mmol), glycosyl acceptor **15** (0.21 g, 0.52 mmol) and 4Å powdered molecular sieves (0.45 g) in DCM (8 mL) in the presence of NIS (0.15 g, 0.67 mmol) and TfOH (12 μL, 0.12 mmol) gave **17** as colorless oil (0.29 g, 76%). R_f=0.31 (Hexnae/EtOAc, 1:1). $[\alpha]_D^{27}$= +48.3 ($CHCl_3$, c=1.6 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= [0.02×2, 0.05 (2×s, 9H, SiCH_3)], 0.92-0.99 (m, 2H, SiCH_2), [1.95, 1.97, 2.02, 2.04, 2.09, 2.10, 2.13, 2.14 (7×s, 21H, OC(O)CH_3)], 3.48-3.58 (m, 2H, H-6_a, OCH_2), 3.73-3.85 (m, 2H, H-$6_{a'}$, $OCH_{2'}$), 3.94 (m, 1H, H-5_a), 4.04-4.11 (m, 2H, H-5_b, H-6_b), 4.24 (dd, 1H, $J_{5,6}$=5.1, $J_{6,6'}$=12.0 Hz, H-$6_{b'}$), 4.78 (s, 1H, H-1_a), 4.84 (s, 1H, H-1_b), 5.18-5.36 (m, 6H, H-2_a, H-3_a, H-4_a, H-2_b, H-3_b, H-4_b); ^{13}C NMR (75MHz, $CDCl_3$): δ= [-1.5×2, 1.0 (SiCH_3)], 17.8 (CH_2Si), [20.6, 20.7, 20.9 (OC(O)CH_3)], 62.4 (C-6_b), 65.8 (OCH_2), 66.0 (C-4_b), 66.7 (C-4_a), 66.8 (C-3_b), 68.6 (C-6_a), 69.0

(C-5_b), 69.2 (C-3_a, C-5_a), 69.4 (C-2_b), 69.8 (C-2_a), 96.8 (C-1_a), 97.7 (C-1_b), [169.5, 169.9, 170.3, 170.6 (O*C*(O)CH_3)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 759.3, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 759.2487, $C_{31}H_{48}O_{18}Si$ calcd for $[M+Na]^+$ 759.2508.

Trichloroacetimidate *O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2, 3, 4-tri-*O*-acetyl-α-D-mannopyranoside (18):

The compound **18** was synthesized according to the general procedure for synthesis of glycosyl trichloroacetimidate **18**. Treatment of **17** (0.25 g, 0.34 mmol) in DCM (3 mL) with trifluoacetic acid (5.9 mL, 79.43 mmol) followed by treatment of the residue in DCM (8 mL) with trichloroacetonitrile (0.34 mL, 3.4 mmol) and DBU (10 μL, 0.068 mmol) gave **18** as colorless oil (0.22 g, 83%). R_f=0.45 (Hexnae/EtOAc, 1:1). $[\alpha]_D^{27}$= +30.9 ($CHCl_3$, c=7.5 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= [1.91, 1.95, 1.99, 2.02, 2.04, 2.08, 2.14 (7×s, 21H, OC(O)C*H*$_3$)], 3.58 (dd, 1H, $J_{5,6}$=2.4, $J_{6,6'}$=10.8 Hz, H-6_a), 3.73 (dd, 1H, $J_{5,6}$=5.7, $J_{6,6'}$=10.8 Hz, H-$6_{a'}$), 4.00-4.15 (m, 3H, H-5a, H-5b, H-6b), 4.24 (dd, 1H, $J_{5,6}$=4.8, $J_{6,6'}$=12.6 Hz, H-$6_{b'}$), 4.77 (d, 1H, $J_{1,2}$=1.5 Hz, H-1_b), 5.19-5.41 (m, 6H, H-2_a, H-3_a, H-4_a, H-2_b, H-3_b, H-4_b), 6.16 (d, 1H, $J_{1,2}$=1.5 Hz, H-1_a), 8.76 (s, 1H, OC(N*H*)CCl_3); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.6, 20.7, 20.8, 20.9 (OC(O)*C*H$_3$)], 62.3 (C-6_b), 65.7 (C-4_a), 66.0 (C-4_b), 66.2 (C-6_a), 67.9 (C-2_a), 68.5 (C-5_b), 68.8 (C-3_a), 68.9 (C-2_b), 69.4 (C-3_b), 71.9 (C-5_a), 90.5 (OC(NH)*C*Cl_3), 94.3 (C-1_a), 97.5 (C-1_b), 159.7 (O*C*(NH)CCl_3), [169.6, 169.7, 169.8, 169.9, 170.0, 170.6 (O*C*(O)CH_3)].

2-(Trimethylsilyl)ethyl *S*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2, 3, 4-tri-*O*-acetyl-α-D-mannopyranoside (20):

The compound **20** was synthesized according to the general procedure for glycosidation with

thioacetyl mannoside **19**. Treatment of **15** (0.35 g, 0.81 mmol) and 2, 6-lutidine (0.13 ml, 1.08 mmol) in DCM (4 mL) with trifluoromethanesulfonic anhydride (0.22 ml, 1.29 mmol) followed by treatment the triflate residue and **19** (0.25 g, 0.62 mmol) in DMF (7 mL) at 0℃ under argon with liquid diethyl amine (0.3 mL) gave **20** as colorless oil (0.34 g, 73%). R_f =0.29 (Hexnae/EtOAc, 1:1). $[\alpha]_D^{27}$= +65.5 ($CHCl_3$, c=6.7 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 0.04-0.07 (2×s, 9H, SiC*H*$_3$), 0.93-1.00 (m, 2H, SiC*H*$_2$), [1.99×2, 2.05×2, 2.12, 2.15, 2.16 (5×s, 21H, OC(O)C*H*$_3$)], 2.72 (dd, 1H, $J_{5,6}$=7.2, $J_{6,6'}$=13.8 Hz, H-6$_a$), 2.87 (dd, 1H, $J_{5,6}$=3.0, $J_{6,6'}$=13.8 Hz, H-6$_{a'}$), 3.55 (m, 1H, OCH_2), 3.85 (m, 1H, $OCH_{2'}$), 3.97 (m, 1H, H-5$_a$), 4.10 (dd, 1H, $J_{5,6}$=1.8, $J_{6,6'}$=12.0 Hz, H-6$_b$), 4.31 (dd, 1H, $J_{5,6}$=4.8, $J_{6,6'}$=12.0 Hz, H-6$_{b'}$), 4.39 (m, 1H, H-5$_b$), 4.78 (s, 1H, H-1$_a$), 5.18-5.36 (m, 7H, H-2$_a$, H-3$_a$, H-4$_a$, H-1$_b$, H-2$_b$, H-3$_b$, H-4$_b$); ^{13}C NMR (75MHz, $CDCl_3$): δ= [-1.4×2, 1.0 (Si*C*H$_3$)], 17.8 (*C*H$_2$Si), [20.6, 20.7, 20.8, 20.9 (OC(O)*C*H$_3$)], 32.0 (C-6$_a$), 62.3 (C-6$_b$), 65.9 (O*C*H$_2$), 66.2 (C-4$_a$), [68.8, 69.0, 69.1 (C-3$_a$, C-3$_b$, C-4$_b$)], 69.3 (C-5$_b$), 69.5 (C-5$_a$), 69.8 (C-2$_a$), 70.8 (C-2$_b$), 82.6 (C-1$_b$), 96.8 (C-1$_a$), [169.6, 169.7, 169.8, 169.9, 170.0, 170.2, 170.6 (O*C*(O)CH$_3$)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 775.2, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 775.2271, $C_{31}H_{48}O_{17}SSi$ calcd for $[M+Na]^+$ 775.2279.

Trichloroacetimidate *S*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2, 3, 4-tri-*O*-acetyl-α-D-mannopyranoside (21):

The compound **21** was synthesized according to the general procedure for synthesis of glycosyl trichloroacetimidate **21**. Treatment of **20** (0.21 g, 0.28 mmol) in DCM (2.5 mL) with trifluoacetic acid (4.9 mL, 65.97 mmol) followed by treatment of the residue in DCM (6 mL) with trichloroacetonitrile (0.28 mL, 2.8 mmol) and DBU (8.2 μL, 0.056 mmol) gave **21** as colorless

oil (0.19 g, 86%). R_f=0.70 (Hexnae/EtOAc, 3:2). $[\alpha]_D^{27}$= +65.2 ($CHCl_3$, c=9.5 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= [1.91, 1.94, 1.98, 2.00, 2.04, 2.09, 2.13 (7×s, 21H, OC(O)C*H*$_3$)], 2.64 (dd, 1H, $J_{5,6}$=6.0, $J_{6,6'}$=14.4 Hz, H-6$_a$), 2.88 (dd, 1H, $J_{5,6}$=2.7, $J_{6,6'}$=14.4 Hz, H-6$_{a'}$), 4.04 (dd, 1H, $J_{5,6}$=4.5, $J_{6,6'}$=11.7 Hz, H-6$_b$), 4.11 (m, 1H, H-5$_a$), 4.22 (dd, 1H, $J_{5,6}$=4.8, $J_{6,6'}$=11.7 Hz, H-6$_{b'}$), 4.30 (m, 1H, H-5$_b$), 5.16-5.38 (m, 7H, H-2$_a$, H-3$_a$, H-4$_a$, H-1$_b$, H-2$_b$, H-3$_b$, H-4$_b$), 6.17 (d, 1H, $J_{1,2}$=1,5 Hz, H-1$_a$), 8.72 (s, 1H, OC(N*H*)CCl_3); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.6, 20.7, 20.9 (OC(O)*C*H$_3$)], 31.5 (C-6$_a$), 62.3 (C-6$_b$), 66.2 (C-4$_a$), [67.7, 67.8, 68.6 (C-3$_a$, C-3$_b$, C-4$_b$)], 69.1 (C-5$_b$), 69.3 (C-2$_b$), 70.8 (C-2$_a$), 72.2 (C-5$_a$), 82.5 (C-1$_b$), 90.5 (OC(NH)*C*Cl_3), 94.2 (C-1$_a$), 159.6 (O*C*(NH)CCl_3), [169.7, 169.8, 169.9, 170.6 (O*C*(O)CH_3)].

Methyl *O*-(2-*O*-acetyl-3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*O*-(2, 3, 4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2, 4-di-*O*-benzyl-β-D-mannopyranoside (22):

The compound **22** was synthesized according to the general procedure for the glycosidation with glycosyl trichloroacetimidate **18**. Glycosyl donor **18** (33.0 mg, 42.3 μmol), glycosyl acceptor **8** (30.0 mg, 35.3 μmol) and 4Å powdered molecular sieves (63 mg) in DCM (2 mL) in the presence of TMSOTf (1.5 μL, 8.3 μmol) gave **22** as colorless oil (43.0 mg, 83%). R_f =0.45 (Hexnae/EtOAc, 1:2). $[\alpha]_D^{27}$= +20.4 ($CHCl_3$, c=2.0 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= [1.91, 1.94, 1.95, 1.98, 1.99, 2.02, 2.03, 2.08 (8×s, 24H, OC(O)C*H*$_3$)], 3.36-3.53 (m, 7H, H-5$_a$, H-6$_b$, H-6$_{b'}$, H-6$_{c'}$, OCH_3), 3.68-3.83 (m, 9H, H-2$_a$, H-3$_a$, H-4$_a$, H-6$_a$, H-6$_{a'}$, H-3$_b$, H-4$_b$, H-5$_b$, H-6$_c$), 3.94-4.03 (m, 3H, H-5$_c$, H-5$_d$, H-6$_d$), 4.21-4.23 (m, 2H, H-1$_a$, H-6$_{d'}$), 4.36-4.89 (m, 12H, PhCH*H*, H-1$_c$, H-1$_d$), 5.09 (s, 1H, H-1$_b$), 5.21-5.29 (m, 6H, H-2$_c$, H-3$_c$, H-4$_c$, H-2$_d$, H-3$_d$, H-4$_d$),

5.40 (s, 1H, H-2_b), 7.06-7.26 (m, 25H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.6, 20.7, 20.8, 20.9 (OC(O)*C*H_3)], 57.1 (O*C*H_3), 62.4 (C-6_d), [66.0, 66.3, 66.4, 66.5, 68.6, 68.7, 68.8, 69.0, 69.1, 69.2, 69.3, 69.4 (C-6_a, C-2_b, C-6_b, C-2_c, C-3_c, C-4_c, C-5_c, C-6_c, C-2_d, C-3_d, C-4_d, C-5_d)], [71.9, 72.1, 73.3, 74.0, 74.2, 74.8, 74.9, 75.0, 75.4 (C-4_a, C-5_a, C-4_b, C-5_b, Ph*C*H_2×5)], 77.2 (C-3_a), 77.9 (C-3_b), 80.1 (C-2_a), [97.2, 97.9 (C-1_c, C-1_d)], 99.7 (C-1_b), 102.5 (C-1_a), [127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 128.2, 128.3, 128.4, 137.7, 137.8, 138.1, 138.6 (C_{arom})], [169.5, 169.6, 169.7, 169.8, 169.9, 170.0, 170.1, 170.6 (O*C*(O)CH_3)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 1489.5, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1489.5455, $C_{76}H_{90}O_{29}$ calcd for $[M+Na]^+$ 1489.5466.

Methyl *O*-(2-*O*-acetyl-3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*S*-(2, 3, 4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2, 4-di-*O*-benzyl-β-D-mannopyranoside (23):

The compound **23** was synthesized according to the general procedure for the glycosidation with glycosyl trichloroacetimidate **21**. Glycosyl donor **21** (91.1 mg, 114.3 μmol), glycosyl acceptor **8** (88.0 mg, 103.7 μmol) and 4Å powdered molecular sieves (0.18 g) in DCM (5 mL) in the presence of TMSOTf (4.2 μL, 23.2 μmol) gave **23** as colorless oil (124.6 mg, 81%). R_f=0.18 (Hexnae/EtOAc, 3:2). $[\alpha]_D^{27}$= +11.1 ($CHCl_3$, c=1.4 mg/mL). ^{1}H NMR (500 MHz, $CDCl_3$): δ= [1.90, 1.91, 1.94, 1.97, 1.98, 2.03, 2.05, 2.06 (8×s, 24H, OC(O)C*H*$_3$)], 2.59 (dd, 1H, $J_{5,6}$=6.0, $J_{6,6'}$=14.0 Hz, H-6_c), 2.80 (dd, 1H, $J_{5,6}$=3.0, $J_{6,6'}$=14.0 Hz, H-$6_{c'}$), 3.34 (m, 1H, H-5_a), 3.41 (s, 3H, OC*H*$_3$), 3.52 (m, 2H, H-6_b, H-$6_{b'}$), 3.65-3.85 (m, 8H, H-2_a, H-3_a, H-4_a, H-6_a, H-$6_{a'}$, H-3_b, H-4_b, H-5_b), 3.97 (m, 1H, H-5_c), 4.02 (dd, 1H, $J_{5,6}$=2.0, $J_{6,6'}$=12.0 Hz, H-6_d), 4.20 (s, 1H, H-1_a), 4.26 (dd, 1H, $J_{5,6}$=5.0, $J_{6,6'}$=12.0 Hz, H-$6_{d'}$), 4.30 (m, 1H, H-5_d), 4.37 (d, 1H, *J*=12.0 Hz, PhCH*H*),

4.38 (d, 1H, *J*=10.5 Hz, PhCH*H*), 4.41 (d, 1H, *J*=11.5 Hz, PhCH*H*), 4.46 (d, 1H, *J*=12.0 Hz, PhCH*H*), 4.53 (d, 1H, *J*=11.5 Hz, PhCH*H*), 4.58 (d, 1H, *J*=10.5 Hz, PhCH*H*), 4.65 (d, 1H, *J*=12.5 Hz, PhCH*H*), 4.76 (s, 1H, H-1_c), 4.77 (d, 1H, *J*=11.0 Hz, PhCH*H*), 4.81 (d, 1H, *J*=12.5 Hz, PhCH*H*), 4.85 (d, 1H, *J*=11.0 Hz, PhCH*H*), 5.09 (s, 1H, H-1_b), 5.19-5.31 (m, 7H, H-2_c, H-3_c, H-4_c, H-1_d, H-2_d, H-3_d, H-4_d), 5.39 (dd, 1H, $J_{1,2}$=1.0, $J_{2,3}$=3.5 Hz, H-2_b), 7.05-7.28 (m, 25H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.5, 20.6, 20.7, 20.8, 20.9 (OC(O)*C*H_3)], 31.7 (C-6_c), 57.1 (O*C*H_3), 62.2 (C-6_d), 66.2 (C-6_a), 66.4 (C-4_c), [68.4, 68.7, 68.9, 69.0×2, 69.2, 69.3, 69.4 (C-2_b, C-6_b, C-2_c, C-3_c, C-5_c, C-3_d, C-4_d, C-5_d)], 70.8 (C-2_d), [71.9, 72.0, 73.3, 73.9, 74.2, 74.7, 74.9, 75.0, 75.4 (C-4_a, C-3_b, C-4_b, C-5_b, Ph*C*H_2×5)], 77.2 (C-3_a), 77.9 (C-3_b), 80.1 (C-2_a), 82.6 (C-1_d), 97.1 (C-1_c), 99.7 (C-1_b), 102.5 (C-1_a), [127.2, 127.4, 127.5, 127.6, 127.7, 127.8, 128.1, 128.2, 128.3, 128.4, 137.7, 137.8, 138.1, 138.5, 138.6 (C_{arom})], [169.6, 169.7, 169.8, 169.9, 170.0, 170.6 (O*C*(O)CH_3)]. MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 1505.5, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1505.5233, $C_{76}H_{90}O_{28}S$ calcd for $[M+Na]^+$ 1505.5237.

Methyl *O*-(3, 4, 6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*O*-(2, 3, 4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2, 4-di -*O*-benzyl-β-D-mannopyranoside (24):

The compound **24** was synthesized according to the general procedure for the glycosidation with glycosyl trichloroacetimidate **18**. Glycosyl donor **18** (68.0 mg, 87.1 μmol), glycosyl acceptor **12** (96.8 mg, 79.1 μmol) and 4Å powdered molecular sieves (0.17 g) in DCM (4 mL) in the presence of TMSOTf (3.2 μL, 17.7 μmol) gave **24** as colorless oil (116.7 mg, 80%). R_f=0.28

(Hexnae/EtOAc, 2:3). $[\alpha]_D^{27}$= +24.9 ($CHCl_3$, c=7.8 mg/mL). ^{1}H NMR (500 MHz, $CDCl_3$): δ= [1.90, 1.93×2, 1.94, 1.96×3, 2.02, 2.07, 2.08 (7×s, 30H, OC(O)CH_3)], 2.45 (m, 1H, H-5_c), 2.70 (dd, 1H, $J_{5,6}$=7.5, $J_{6,6'}$=10.5 Hz, H-6_b), 3.27 (d, 1H, $J_{6,6'}$=10.5 Hz, H-$6_{b'}$), 3.36 (s, 3H, OCH_3), 3.31-3.40 (m, 2H, H-4b, H-5a), 3.44-3.50 (m, 2H, H-5b, H-6d), 3.56-3.75 (m, 6H, H-2_a, H-4_a, H-6_a, H-$6_{a'}$, H-3_b, H-$6_{d'}$), 3.78-3.89 (m, 4H, H-3_a, H-2_b, H-6_c, H-5_d), 3.91-3.95 (m, 2H, H-$6_{c'}$, H-5_e), 4.00 (d, 1H, $J_{6,6'}$=12.0 Hz, H-6_e), 4.12 (s, 1H, H-1a), 4.21 (dd, 1H, $J_{5,6}$=5.0, $J_{6,6'}$=12.0 Hz, H-$6_{e'}$), 4.26-4.31 (m, 2H, H-2_c, PhCH*H*), 4.30 (d, 1H, *J*=11.0 Hz, PhCH*H*), 4.53-4.57 (m, 5H, PhCH*H*), 4.68 (d, 1H, *J*=13.0 Hz, PhCH*H*), 4.76 (s, 1H, H-1_d), 4.77-4.79 (m, 2H, PhCH*H*), 4.83 (s, 1H, H-1_e), 4.89 (s, 1H, H-1_b), 4.97 (t, 1H, J3, 4=9.5, J4, 5=10.0 Hz, H-4c), 5.02 (d, 1H, J1, 2=8.5 Hz, H-1c), 5.19-5.31 (m, 6H, H-2_d, H-3_d, H-4_d, H-2_e, H-3_e, H-4_e), 5.53 (t, 1H, $J_{2,3}$=10.5, $J_{3,4}$=9.5 Hz, H-3_c), 6.98-7.54 (m, 29H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.5, 20.6, 20.8 (OC(O)*C*H_3)], 54.2 (C-2_c), 57.1 (O*C*H_3), 61.6 (C-6_c), 62.4 (C-6_e), [66.0, 66.2 (C-4_d, C-4_e), [66.2, 66.4 (C-6_a, C-6_d)], 68.5 (C-4_c), [68.6, 68.9 (C-5_d, C-5_e)], [69.0, 69.2, 69.3, 69.4 (C-2_d, C-3_d, C-2_e, C-3_e)], 69.8 (C-6_b), 70.4 (C-3_c), 71.2 (C-5_c), 72.2 (C-5_b), 72.7 (C-2_b), 73.4 (C-3_a), 74.1 (C-4_b), [71.3, 73.9, 74.0×2, 74.7 (Ph*C*H_2×5)], 75.0 (C-5_a), 75.1 (C-2_a), 77.7 (C-3_b), 80.8 (C-4_a), 96.0 (C-1_c), 97.2 (C-1_e), 97.8 (C-1_d), 98.9 (C-1_b), 102.6 (C-1_a), [126.3, 127.1, 127.2, 127.3, 127.7, 128.1, 128.2, 128.3, 128.7, 138.0, 138.3, 138.4, 138.5, 138.7 (C_{arom})], [169.3, 169.5, 169.7, 169.9, 170.0, 170.1, 170.6, 170.7 (O*C*(O)CH_3)]. MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 1864.7, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1864.6423, $C_{94}H_{107}NO_{37}$ calcd for $[M+Na]^+$ 1864.6420.

Methyl *O*-(3, 4, 6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→2)-*O*-(3, 4,

6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*O*-(2, 3, 4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2, 4-di-*O*-benzyl-β-D-mannopyranoside (25):

The compound **25** was synthesized according to the general procedure for the phthalimido removal followed by acetylation **24**. Treatment of **24** (88.2 mg, 47.9 μmol) in EtOH (2 mL) with $H_2NNH_2 \cdot H_2O$ (0.2 mL, 4.12 mmol) followed by Ac_2O (0.5 mL) and pyridine (0.5 mL) gave **25** as colorless oil (68.1 mg, 81%). R_f =0.22 (Hexnae/EtOAc, 1:5). $[\alpha]_D^{27}$= +62.2 ($CHCl_3$, c=1.0 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.73 (s, 3H, NHC(O)C*H*$_3$), [1.92, 1.95×2, 1.98×2, 1.96×2, 2.03, 2.07×2 (6×s, 30H, OC(O)C*H*$_3$)], 2.70 (m, 1H, H-6$_b$), 3.41 (s, 3H, OC*H*$_3$), 3.31-3.67 (m, 7H), 3.77-4.11 (m, 10H), 4.18-4.22 (m, 2H), 4.31-4.46 (m, 5H), 4.59-4.85 (m, 11H), 4.93-4.98 (m, 3H), 5.18-5.32 (m, 6H), 7.05-7.37 (m, 25H); MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 1776.6, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1776.6477, $C_{88}H_{107}NO_{36}$ calcd for $[M+Na]^+$ 1776.6471.

Methyl *O*-(3, 4, 6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-*O*-(3, 4, 6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*S*-(2, 3, 4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2, 4-di-*O*-benzyl-β-D-mannopyranoside (26):

The compound **26** was synthesized according to the general procedure for the glycosidation with glycosyl trichloroacetimidate **21**. Glycosyl donor **21** (86.0 mg, 107.9 μmol), glycosyl acceptor **12** (120.1 mg, 98.1 μmol) and 4Å powdered molecular sieves (0.21 g) in DCM (4 mL) in the presence of TMSOTf (3.9 μL, 21.5 μmol) gave product **26** as colorless oil (151.3 mg, 83%). R_f

=0.24 (Hexnae/EtOAc, 2:3). $[\alpha]_D^{27}$= +36.0 ($CHCl_3$, c=6.0 mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= [1.92×2, 1.94, 1.96, 1.97×2, 1.98, 2.03, 2.05, 2.07 (8×s, 30H, OC(O)C*H*$_3$)], 2.44 (m, 1H, H-5$_c$), 2.57 (dd, 1H, J5, 6=5.7, J6, 6'=13.8 Hz, H-6d), 2.68 (dd, 1H, $J_{5,6}$=7.2, $J_{6,6'}$=10.8 Hz, H-6$_b$), 2.77 (d, 1H, J6, 6'=13.8 Hz, H-6d'), 3.27 (d, 1H, $J_{6,6'}$=10.8 Hz, H-6$_{b'}$), 3.36 (s, 3H, OC*H*$_3$), 3.29-3.38 (m, 2H, H-4b, H-5a), 3.46-4.02 (m, 12H, H-2$_a$, H-3a, H-4$_a$, H-6$_a$, H-6$_{a'}$, H-2b, H-3$_b$, H-5b, H-6c, H-6c', H-5d, H-6$_e$), 4.11 (s, 1H, H-1a), 4.23-4.31 (m, 4H, H-2$_c$, H-5e, H-6$_{e'}$, PhCH*H*), 4.44 (d, 1H, *J*=11.1 Hz, PhCH*H*), 4.53-4.58 (m, 5H, PhCH*H*), 4.68 (d, 1H, *J*=13.2 Hz, PhCH*H*), 4.75 (s, 1H, H-1$_d$), 4.76-4.82 (m, 2H, PhCH*H*), 4.89 (s, 1H, H-1$_b$), 4.96 (t, 1H, J3, 4=9.3, J4, 5=10.2 Hz, H-4c), 5.01 (d, 1H, J1, 2=8.1 Hz, H-1c), 5.17-5.29 (m, 7H, H-2$_d$, H-3$_d$, H-4$_d$, H-1e, H-2$_e$, H-3$_e$, H-4$_e$), 5.52 (t, 1H, $J_{2,3}$=10.5, $J_{3,4}$=9.3 Hz, H-3$_c$), 6.84-7.54 (m, 29H, H$_{arom}$); ^{13}C NMR (125MHz, $CDCl_3$): δ= [20.5, 20.6, 20.7, 20.8, 20.9, 21.0 (OC(O)*C*H$_3$)], 32.2 (C-6$_d$), 54.2 (C-2$_c$), 57.1 (O*C*H$_3$), 62.3 (C-6$_c$), 62.4 (C-6$_e$), [66.2×2, 66.3 (C-6$_a$, C-4$_d$, C-4$_e$)], 68.4 (C-4$_c$), [68.6, 68.9 (C-5$_d$, C-5$_e$)], [69.0, 69.2, 69.4, 69.5 (C-2$_d$, C-3$_d$, C-2$_e$, C-3$_e$)], 70.4 (C-6$_b$), 70.8 (C-3$_c$), 71.2 (C-5$_c$), 72.3 (C-5$_b$), 72.7 (C-2$_b$), 73.4 (C-3$_a$), 74.1 (C-4$_b$), [71.4, 73.9, 74.0×2, 74.7 (Ph*C*H$_2$×5)], 74.9 (C-5$_a$), 75.2 (C-2$_a$), 77.7 (C-3$_b$), 80.8 (C-4$_a$), 82.5 (C-1$_e$), 96.0 (C-1$_c$), 97.2 (C-1$_d$), 98.9 (C-1$_b$), 102.6 (C-1$_a$), [126.3, 127.1, 127.2, 127.3, 127.7, 128.1, 128.2, 128.3, 128.7, 138.0, 138.3, 138.4, 138.5, 138.7 (C$_{arom}$)], [169.3, 169.7, 169.8, 169.9, 170.0, 170.1, 170.6, 170.7, 170.8, 171.1 (O*C*(O)CH$_3$)]. MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 1880.7, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1880.6178, $C_{94}H_{107}NO_{36}S$ calcd for $[M+Na]^+$ 1880.6191.

Methyl *O*-(3, 4, 6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→2)-*O*-(3, 4,

6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-*S*-(2, 3, 4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2, 4-di-*O*-benzyl-β-D-mannopyranoside (27):

The compound **27** was synthesized according to the general procedure for the phthalimido removal followed by acetylation **26**. Treatment of **26** (96.3 mg, 51.8 μmol) in EtOH (2 mL) with $H_2NNH_2 \cdot H_2O$ (0.2 mL, 4.12 mmol) followed by Ac_2O (0.5 mL) and pyridine (0.5 mL) gave **27** as colorless oil (81.6 mg, 89%). R_f =0.18 (Hexnae/EtOAc, 1:3). $[\alpha]_D^{27}$= +94.9 ($CHCl_3$, c= 2.8mg/mL). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.73 (s, 3H, NHC(O)CH_3), [1.92×4, 1.95, 1.96, 1.98, 2.03, 2.05, 2.06 (7×s, 30H, OC(O)CH_3)], 2.58 (dd, 1H, $J_{5,6}$=6.3, $J_{6,6'}$=13.8 Hz, H-6_d), 2.66-2.76 (m, 2H, H-6_b, H-$6_{d'}$), 3.42 (s, 3H, OCH_3), 3.38-3.82 (m, 10H), 3.88-4.02 (m, 5H), 4.12 (d, 1H, J=7.8 Hz), 4.22-4.46 (m, 7H), 4.59-4.71 (m, 5H), 4.81-4.86 (m, 4H), 4.95-5.02 (m, 3H), 5.18-5.31 (m, 7H), 7.04-7.40 (m, 25H); MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 1792.6, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1792.6227, $C_{88}H_{107}NO_{35}S$ calcd for $[M+Na]^+$ 1792.6242.

Methyl *O*- (α-D-mannopyranosyl)-(1→3)-*O*-[(α-D-mannopyranosyl)-(1→6)-*O*-(α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (1):

The compound **1** was synthesized according to the general procedure for the global deprotection of **22**. Treatment of **22** (32.2 mg, 21.9 μmol) in MeOH/DCM (0.5 mL: 0.5 mL, v/v) with NaOMe (pH=8-10) gave deacetylated product (24.8 mg, 21.9 μmol, quantitive). Treatment of the partially deprotected compound (24.8 mg, 21.9 μmol) in THF (1 mL) with Na (s) in NH_3 (l) gave **1** as white solid (13.0 mg, 87%). ^{1}H NMR (500 MHz, D_2O): δ= 3.42 (s, 3H, OCH_3), 3.44-3.47 (m,

1H), 3.54-3.90 (m, 21H), 3.97 (s, 1H, H2-$_b$), 4.04 (d, 1H, J=3.0 Hz, H-2$_a$), 4.49 (s, 1H, H-1$_a$), [4.80, 4.81 (2×s, 2H, H-1$_c$, H-1$_d$)], 5.00 (s, 1H, H-1$_b$); ^{13}C NMR (75MHz, D_2O): δ= 57.0 (O*C*H$_3$), 61.2×2, 65.5, 65.9, 66.0, 66.8, 67.0×2, 70.1, 70.2×2, 70.6, 70.8, 70.9, 71.0, 71.3, 72.9, 73.5, 74.2, 80.9, [99.5, 99.6 (C-1$_c$, C-1$_d$)], 101.2 (C-1$_a$), 102.6 (C-1$_b$); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=160.3 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=172.3 Hz, ($J_{H\text{-}1c\text{-}C\text{-}1c}$, $J_{H\text{-}1d\text{-}C\text{-}1d}$ =171.5, 172.0 Hz). MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 703.3, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 703.2258, $C_{25}H_{44}NO_{21}$ calcd for $[M+Na]^+$ 703.2273.

Methyl *O*- (α-D-mannopyranosyl)-(1→3)-*O*-[(α-D-mannopyranosyl)-(1→6)-*S*-(α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (2):

The compound **2** was synthesized according to the general procedure for the global deprotection of **23**. Treatment of **23** (55.1 mg, 37.1 μmol) in MeOH/DCM (0.5 mL: 0.5 mL, v/v) with NaOMe (pH=8-10) gave deacetylated product (42.6 mg, 37.1 μmol, quantitive). Treatment of the partially deprotected compound (42.6 mg, 37.1 μmol) in THF (1 mL) with Na (s) in NH_3 (l) gave **2** as white solid (23.2 mg, 90%). ^{1}H NMR (500 MHz, D_2O): δ= 2.72 (dd, 1H, $J_{5,6}$=8.5, $J_{6,6'}$=13.5 Hz, H-6$_c$), 3.14 (d, 1H, $J_{6,6'}$=13.5 Hz, H-6$_{c'}$), 3.47 (s, 3H, OC*H*$_3$), 3.49-3.52 (dd, 1H, J=3.5, 10.0 Hz), 3.55-3.65 (m, 3H), 3.67-3.79 (m, 9H), 3.82-3.85 (m, 3H), 3.95 (s, 1H, H-2$_c$), 3.97-4.00 (m, 2H), 4.01 (s, 2H, H2-$_b$, H-2$_d$), 4.09 (d, 1H, J=2.5 Hz, H-2$_a$), 4.53 (s, 1H, H-1$_a$), 4.82 (s, 1H, H-1$_c$), 5.05 (s, 1H, H-1$_b$), 5.29 (s, 1H, H-1$_d$); ^{13}C NMR (75MHz, D_2O): δ= 31.5 (C-6$_c$), 57.0 (O*C*H$_3$), 61.0, 61.1, 65.5, 65.9, 66.9, 67.3, 69.7, 70.0, 70.1, 70.2, 70.5, 70.7, 71.1, 71.2, 71.8, 73.3, 73.5, 74.2, 80.9, 84.3 (C-1$_d$), 99.5 (C-1$_c$), 101.2 (C-1$_a$), 102.6 (C-1$_b$); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=160.3 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=172.5 Hz, $J_{H\text{-}1c\text{-}C\text{-}1c}$=172.0 Hz, $J_{H\text{-}1d\text{-}C\text{-}1d}$=168.3 Hz.

MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 719.2, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 719.2027, $C_{25}H_{44}NO_{20}S$ calcd for $[M+Na]^+$ 719.2044.

Methyl *O*-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→3)-*O*-[(α-D-mannopyranosyl)-(1→6)-*O*-(α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (3):

The compound **3** was synthesized according to the general procedure for the global deprotection of **25**. Treatment of **25** (67.1 mg, 38.2 μmol) in MeOH/DCM (0.5 mL: 0.5 mL, v/v) with NaOMe (pH=8-10) gave deacetylated product (51.0 mg, 38.2 μmol, quantitive). Treatment of the partially deprotected compound (51.0 mg, 38.2 μmol) in THF (1 mL) with Na (s) in NH_3 (l) gave **3** as white solid (29.4 mg, 33.3 μmol, 87%). ^{1}H NMR (600 MHz, D_2O): δ= 1.94 (s, 3H, NHC(O)C*H*$_3$), 3.34-3.40 (m, 2H), 3.42 (s, 3H, OC*H*$_3$), 3.43-3.50 (m, 2H), 3.50-3.75 (m, 14H), 3.78-3.87 (m, 6H), 3.90 (m, 1H), 4.04 (d, 1H, *J*=3.0 Hz, H-2$_a$), 4.09 (s, 1H, H2-$_b$), 4.44 (d, 1H, $J_{1,2}$=8.4 Hz, H-1$_c$), 4.48 (s, 1H, H-1$_a$), [4.79, 4.80 (2×s, 2H, H-1$_d$, H-1$_e$)], 5.01 (s, 1H, H-1$_b$); ^{13}C NMR (125MHz, D_2O): δ= 22.5 (NHC(O)*C*H$_3$), 55.5 (C-2$_c$), 57.0 (O*C*H$_3$), 60.8, 61.1, 61.8, 65.5, 65.8, 66.0, 66.7, 66.9, 67.4, 69.6, 70.0, 70.1, 70.2×2, 70.7, 70.9, 71.0, 72.9, 73.5, 73.6, 74.1, 76.0, 76.7 (C-2$_b$), 80.8, [99.5, 99.6×2 (C-1$_b$, C-1$_d$, C-1$_e$)], 99.8 (C-1$_c$), 101.2 (C-1$_a$), 175.0 (NH*C*(O)CH$_3$); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=160.3 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=172.0 Hz, ($J_{H\text{-}1d\text{-}C\text{-}1d}$, $J_{H\text{-}1e\text{-}C\text{-}1e}$ =171.5, 173.1 Hz).

MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 906.3, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 906.3064, $C_{25}H_{44}NO_{20}S$ calcd for $[M+Na]^+$ 906.3067.

Methyl *O*-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-

(1→3)-*O*-[(α-D-mannopyranosyl)-(1→6)-*S*-(α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside (4):

The compound **4** was synthesized according to the general procedure for the global deprotection of **27**. Treatment of **27** (78.8 mg, 44.5 μmol) in MeOH/DCM (0.5 mL: 0.5 mL, v/v) with NaOMe (pH=8-10) gave deacetylated product (60.1 mg, 44.5 μmol, quantitive). Treatment of the partially deprotected compound (60.1 mg, 44.5 μmol) in THF (1 mL) with Na (s) in NH_3 (l) gave **4** as white solid (34.4 mg, 86%). ^{1}H NMR (800 MHz, D_2O): δ= 1.92 (s, 3H, NHC(O)CH_3), 2.64 (dd, 1H, $J_{5,6}$=8.8, $J_{6,6'}$=14.4 Hz, H-6_d), 3.06 (d, 1H, $J_{6,6'}$=14.4 Hz, H-$6_{d'}$), 3.32-3.34 (m, 2H), 3.39 (m, 1H), 3.40 (s, 3H, OCH_3), 3.43-3.45 (m, 2H, H-4_a, H-3_c), 3.49-3.52 (m, 2H), 3.55-3.58 (m, 2H, H-2_c, H-4_d), 3.61-3.64 (m, 4H), 3.66-3.72 (m, 6H), 3.77-3.80 (m, 3H), 3.88 (s, 1H, H-2_d), 3.89-3.93 (m, 2H), 3.94 (s, 1H, H-2_e), 4.02 (d, 1H, *J*=3.2 Hz, H-2_a), 4.07 (s, 1H, H-2_b), 4.42 (d, 1H, $J_{1,2}$=8.8 Hz, H-1_c), 4.47 (s, 1H, H-1_a), 4.76 (s, 1H, H-1_d), 5.00 (s, 1H, H-1_b), 5.22 (s, 1H, H-1_e); ^{13}C NMR (75MHz, D_2O): δ= 22.5 (NHC(O)CH_3), 31.5 (C-6_d), 55.5 (C-2_c), 57.0 (OCH_3), 60.8, 61.0, 61.8, 65.5, 66.0, 67.3 (C-4_d), 67.4, 69.6, 69.7, 70.0 (C-4_c), 70.1 (C-2_d), 70.2 (C-2_a), 70.7, 71.0, 71.2 (C-2_e), 73.3, 73.5, 73.6, 74.1, 76.0, 76.6 (C-2_b), 80.7, 84.3 (C-1_e), [99.5, 99.6 (C-1_b, C-1_d)], 99.8 (C-1_c), 101.2 (C-1_a), 175.0 (NHC(O)CH_3); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=159.8 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=171.0 Hz, ($J_{H\text{-}1d\text{-}C\text{-}1d}$, $J_{H\text{-}1e\text{-}C\text{-}1e}$ =172.0, 168.3 Hz). MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 922.3, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 922.2837, $C_{25}H_{44}NO_{20}S$ calcd for $[M+Na]^+$ 922.2838.

Methyl 2-*O*-benzyl-4, 6-di-*O*-benzylidene-α-D-altropyranoside (29):

To a solution of **28** (0.93 g, 2.5 mmol) in DMSO (5 mL) was added 1:2 Ac_2O/DMSO (10 mL)

and the mixture was stirred for 16 h at room temperature, and then concentrated to dryness. To a solution of the residue in 1:1 DCM/MeOH (60 mL) was added $NaBH_4$ (4.60 g, 0.12 mol). The reaction mixture was stirred at 5-10 ℃ for 5 h, and then concentrated to dryness. The residue was dissolved in DCM (60 mL), and the solution was washed with 5% citric acid solution (60 mL), saturated $NaHCO_3$ (60 mL) and water (60 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated to dryness. Purification of the crude product by column chromatography over silica gel afforded **29** as colorless oil (0.70 g, 75%). R_f =0.30 (Hexnae/EtOAc, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 2.81 (d, 1H, *J*=7.2 Hz, OH), 3.35 (s, 3H, OC*H*$_3$), 3.66 (d, 1H, $J_{2,3}$=3.0 Hz, H-2), 3.76 (t, 1H, $J_{5,6}$=10.2, $J_{6,6'}$=10.2 Hz, H-6), 3.92 (dd, 1H, $J_{3,4}$=3.0, $J_{4,5}$=9.6 Hz, H-4), 4.07-4.15 (m, 2H, H-3, H-5), 4.27 (dd, 1H, $J_{5,6}$=4.8, $J_{6,6'}$=10.2 Hz, H-6'), 4.58 (dd, 2H, *J*=12.0 Hz, PhCH*H*), 4.66 (s, 1H, H-1), 5.57 (s, 1H, PhC*H*), 7.18-7.44 (m, 10H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= 5 5.8 (O*C*H$_3$), [58.5, 67.3 (C-3, C-5)], 69.4 (C-6), 72.8 (Ph*C*H$_2$), 76.8 (C-4), 76.9 (C-2), 100.3 (C-1), 102.5 (Ph*C*H), [126.2, 127.8, 128.1, 128.3, 128.6, 129.1, 137.2, 137.3 (C_{arom})]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 395.1, $C_{21}H_{24}O_6$ calcd for $[M+Na]^+$ 395.1471.

Methyl *S*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4, 6-di-*O*-benzylidene-α-D-mannopyranoside (30):

To a solution of **29** (0.14 g, 0.38 mmol) and freshly distilled pyridine (2.9 mL) in dry DCM (5.8 mL) was added trifluoromethanesulfonic anhydride (0.1 ml, 0.57 mmol) at -20℃. The reaction mixture was stirred for 2 h at room temperature, and then quenched by the addition of H_2O (0.2 mL). The resulting mixture was extracted with EtOAc (3×80 mL) and washed with 1 N HCl (70

mL), saturated $NaHCO_3$ (70 mL) and H_2O (70 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated to dryness below 30℃. To a solution of this residue and **19** (0.13 g, 0.32 mmol) in DMF (3 mL) at 0℃ under argon was added liquid diethyl amine (0.15 mL) slowly. The reaction mixture was stirred at 0℃ for 24 h and at room temperature for 5 h, and then concentrated to dryness. The residue was dissolved in EtOAc (30 mL), and the solution was washed with H_2O (30 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the crude product by column chromatography over silica gel (toluene/acetone, 10:1) afforded the **30** as colorless oil (0.14 g, 61%). R_f =0.33 (toluene/acetone, 9:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= [1.90, 1.95, 1.98, 2.01 (4×s, 12H, OC(O)C*H*$_3$)], 3.28 (s, 3H, OC*H*$_3$), 3.42 (dd, 1H, $J_{2,3}$=3.0, $J_{3,4}$=10.8 Hz, H-3$_a$), 3.64 (d, 1H, $J_{2,3}$=3.0 Hz, H-2$_a$), 3.67-3.77 (m, 2H, H-5$_a$, H-6$_a$), 3.97-4.05 (m, 2H, H-4$_a$, H-6$_b$), 4.12-4.25 (m, 3H, H-6$_{a'}$, H-5$_b$, H-6$_{b'}$), 4.60 (s, 1H, H-1$_a$), 4.55-4.64 (dd, 2H, *J*=12.0 Hz, PhCH*H*), 5.13-5.15 (m, 2H, H-3$_b$, H-4$_b$), 5.37 (s, 1H, H-2$_b$), 5.53 (s, 1H, PhC*H*), 5.59 (s, 1H, H-1$_b$), 7.19-7.34 (m, 10H, H$_{arom}$); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.9 (OC(O)*C*H$_3$)], 45.2 (C-3$_a$), 55.0 (O*C*H$_3$), 62.7 (C-6$_b$), 65.4 (C-5$_a$), 66.9 (C-4$_b$), [68.9 (C-6$_a$, C-5$_b$)], 69.5 (C-3$_b$), 70.8 (C-2$_b$), 73.8 (Ph*C*H$_2$), 79.9 (C-2$_a$), 80.3 (C-4$_a$), 83.1 (C-1$_b$), 98.3 (C-1$_a$), 101.7 (Ph*C*H), [126.1, 128.1, 128.3 128.7, 129.1, 137.5 (C$_{arom}$)], [170.0, 170.1 (O*C*(O)CH$_3$)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 741.2, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 741.2190, $C_{35}H_{42}O_{14}S$ calcd for $[M+Na]^+$ 741.2193.

Methyl *S*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2, 4-di-*O*-benzyl-α-D-mannopyranoside (31):

The compound **31** was synthesized according to the general procedure for reduction of

benzylidene acetal **30**. Treatment of **30** (48 mg, 0.067 mmol) with a solution of 1 M BH_3 in THF (0.66 mL) and a solution of 1 M Bu_2BOTf in DCM (0.066 mL) gave **31** as white solid (31 mg, 63%). R_f=0.25 (toluene/acetone, 4:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= [1.90, 1.94, 1.98, 2.00 (4×s, 12H, OC(O)CH_3)], 3.24 (s, 3H, OCH_3), 3.26 (dd, 1H, $J_{2,3}$=3.5, $J_{3,4}$=10.5 Hz, H-3_a), 3.51-3.56 (m, 2H, H-2_a, H-5_a), 3.70 (dd, 1H, $J_{5,6}$=4.0, $J_{6,6'}$=12.0 Hz, H-6_a), 3.76 (d, 1H, $J_{6,6'}$=12.0 Hz, H-$6_{a'}$), 3.83 (t, 1H, $J_{3,4}$=10.5, $J_{4,5}$=9.5 Hz, H-4_a), 3.94 (d, 1H, $J_{6,6'}$=10.5 Hz, H-6_b), 4.10-4.16 (m, 2H, H-5_b, H-$6_{b'}$), 4.77 (d, 1H, *J*=11.0 Hz, PhCH*H*), 4.52-4.64 (m, 3H, PhCH*H*), 5.10 (m, 2H, H-3_b, H-4_b), 5.31 (s, 1H, H-2_b), 5.40 (s, 1H, H-1_b), 7.10-7.35 (m, 10H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.5, 20.6, 20.7, 20.8 (OC(O)*C*H_3)], 47.9 (C-3_a), 54.6 (O*C*H_3), 62.2 (C-6_a), 62.5 (C-6_b), 66.6 (C-4_b), 68.6 (C-5_b), 69.4 (C-3_b), 70.3 (C-2_b), 72.9 (C-5_a), [73.1, 75.6 (2×Ph*C*H_2)], 76.2 (C-4_a), 79.5 (C-2_a), 83.4 (C-1_b), 97.2 (C-1_a), [127.8, 127.9, 128.0, 128.2, 128.4, 137.3, 137.7 (C_{arom})], [169.5, 169.7, 169.8, 170.7 (O*C*(O)CH_3)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 743.2, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 743.2347, $C_{35}H_{44}O_{14}S$ calcd for $[M+Na]^+$ 743.2349.

Methyl *S*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-[*O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2, 4-di-*O*-benzyl-α-D-mannopyranoside (32):

The compound **32** was synthesized according to the general procedure for the glycosidation with thioglycoside **16**. Glycosyl donor **16** (18 mg, 0.046 mmol), glycosyl acceptor **31** (27 mg, 0.038 mmol) and 4Å powdered molecular sieves (0.05 g) in DCM (1 mL) in the presence of NIS (11 mg, 0.051 mmol) and TfOH (1 μL, 0.01 mmol) gave **32** (31 mg, 77%) as colorless oil. R_f=0.5 (toluene/acetone, 3:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ= [1.90, 1.93, 1.96, 1.97, 1.99, 2.00, 2.07

(8×s, 24H, OC(O)CH_3)], 3.27 (m, 4H, H-3_a, OCH_3), 3.49 (d, 1H, $J_{2,3}$=2.0 Hz, H-2_a), 3.69 (m, 4H, H-4_a, H-5_a, H-6_a, H-$6_{a'}$), 3.92 (d, 1H, $J_{6,6'}$=13.5 Hz, H-6_b), 4.00-4.15 (m, 5H, H-5_b, H-$6_{b'}$, H-5_c, H-6_c, H-$6_{c'}$), 4.50 (d, 1H, *J*=11.5 Hz, PhCH*H*), 4.55 (d, 1H, *J*=11.0 Hz, PhCH*H*), 4.57 (s, 1H, H-1_a), 4.65 (d, 1H, *J*=11.5 Hz, PhCH*H*), 4.80 (d, 1H, *J*=11.0 Hz, PhCH*H*), 4.86 (s, 1H, H-1_c), 5.09 (m, 2H, H-3_b, H-4_b), 5.19 (m, 2H, H-2_c, H-4_c), 5.28 (m, 2H, H-2_b, H-3_c), 5.36 (s, 1H, H-1_b), 7.10-7.35 (m, 10H, H_{arom}); ^{13}C NMR (125 MHz, $CDCl_3$): δ= 20.7 (OC(O)*C*H_3), 48.1 (C-3_a), 54.5 (O*C*H_3), [62.3, 62.4 (C-6_b, C-6_c)], [66.7, 66.8, 67.6, 68.6, 68.7, 68.8, 69.5, 70.1, 70.3 (C-5_a, C-2_b, C-3_b, C-4_b, C-5_b, C-2_c, C-3_c, C-4_c, C-5_c)], 72.9 (C-4_a), [73.5, 76.5 (2×Ph*C*H_2)], 77.8 (C-6_a), 79.3 (C-2_a), 83.3 (C-1_b), 96.8 (C-1_a), 97.5 (C-1_c), [127.8, 128.0, 128.2, 128.3, 128.4, 137.4, 137.5 (C_{arom})], [169.4, 169.6, 169.8, 169.9, 170.7, 170.8 (O*C*(O)CH_3)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 1073.3, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1073.3295, $C_{49}H_{62}O_{23}S$ calcd for $[M+Na]^+$ 1073.3300.

Methyl *O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4, 6-di-*O*-benzylidene-α-D-mannopyranoside (33):

The compound **33** was synthesized according to the general procedure for the glycosidation with thioglycoside **16**. Glycosyl donor **16** (0.51 g, 1.30 mmol), glycosyl acceptor **28** (0.40 g, 1.08 mmol) and 4Å powdered molecular sieves (0.9 g) in DCM (18 mL) in the presence of NIS (0.32 g, 1.43 mmol) and TfOH (26 μL, 0.26 mmol) gave **33** as colorless oil (0.66 g, 87%). R_f=0.23 (Hexnae/EtOAc, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= [1.93, 1.99, 2.00 (4×s, 12H, OC(O)CH_3)], 3.29 (s, 3H, OCH_3), 3.71-3.83 (m, 4H, H-2_a, H-4_a, H-6_a, H-5_b), 3.98 (dd, 1H, $J_{5,6}$=2.1, $J_{6,6'}$=12.0 Hz, H-6_b), 4.08-4.21 (m, 4H, H-3_a, H-5_a, H-$6_{a'}$, H-$6_{b'}$), 4.69-4.72 (m, 3H, PhCH*H*, H-1_a),

5.12-5.18 (m, 2H, H-1_b, H-4_b), 5.31-5.38 (m, 2H, H-2_b, H-3_b), 5.53 (s, 1H, PhC*H*), 7.20-7.40 (m, 10H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.5, 20.6, 20.7 (OC(O)*C*H_3)], 54.8 (O*C*H_3), 62.4 (C-6_b), 63.9 (C-5_b), 66.2 (C-4_b), [68.6, 68.8, 68.9, 69.2 (C-4_a, C-6_a, C-2_b, C-3_b)], 73.4 (C-5_a), 73.5 (Ph*C*H_2), 77.1 (C-2_a), 78.8 (C-3_a), 98.5 (C-1_b), 99.9 (C-1_a), 101.2 (Ph*C*H), [125.9, 127.9, 128.0, 128.1, 128.6, 128.7, 137.2, 137.6 (C_{arom})], [169.6, 169.7, 169.8, 170.7 (O*C*(O)CH_3)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 725.2, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 725.2418, $C_{35}H_{42}O_{15}$ calcd for $[M+Na]^+$ 725.2421.

Methyl *O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2, 4-di-*O*-benzyl-α-D-mannopyranoside (34):

The compound **34** was synthesized according to the general procedure for reduction of benzylidene acetal **33**. Treatment of **33** (0.48 g, 0.68 mmol) with a solution of 1 M BH_3 in THF (8.6 mL) and a solution of 1 M Bu_2BOTf in DCM (0.86 mL) gave **34** as white solid (0.33 g, 68%). R_f=0.35 (Hexnae/EtOAc, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= [1.87, 1.93, 1.98, 1.99 (4×s, 12H, OC(O)C*H*$_3$)], 3.26 (s, 3H, OC*H*$_3$), 3.54 (m, 1H, H-5_a), 3.67-4.09 (m, 8H, H-2_a, H-3_a, H-4_a, H-6_a, H-$6_{a'}$, H-5_b, H-6_b, H-$6_{b'}$), 4.69 (s, 1H, H-1_a), 4.56-4.74 (m, 4H, PhCH*H*), 5.09-5.16 (m, 2H, H-1_b, H-4_b), 5.31-5.35 (m, 2H, H-2_b, H-3_b), 7.20-7.35 (m, 10H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.6, 20.7, 20.8 (OC(O)*C*H_3)], 54.9 (O*C*H_3), [62.0, 62.5, 66.2, 68.9, 69.0, 69.5, 72.3, 72.4 (C-2_a, C-4_a, C-5_a, C-6_a, C-2_b, C-3_b, C-4_b, C-5_b, C-6_b), [75.0, 75.2 (2×Ph*C*H_2)], 78.1 (C-3_a), 98.4 (C-1_a), 99.3 (C-1_b), [127.8, 127.8, 127.9, 128.4, 128.6, 137.8 (C_{arom})], [169.7, 169.8, 170.6 (O*C*(O)CH_3)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 727.3, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 727.2575, $C_{35}H_{44}O_{15}$ calcd for $[M+Na]^+$ 727.2578.

Methyl *O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-[*S*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)]-2, 4-di-*O*-benzyl-α-D-mannopyranoside (35):

The compound **35** was synthesized according to the general procedure for glycosidation with thioacetyl mannoside **19**. Treatment of **34** (0.188g, 0.27 mmol) and 2, 6-lutidine (0.04 ml, 0.34 mmol) in DCM (2 mL) with trifluoromethanesulfonic anhydride (0.068 ml, 0.37 mmol) followed by treatment the triflate residue and **19** (0.098 g, 0.24 mmol) in DMF (2.7 mL) at 0℃ under argon with liquid diethyl amine (0.12 mL) gave the desired **35** as colorless oil (0.18 g, 72%). R_f =0.39 (Hexnae/EtOAc, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ= [1.92, 1.97, 1.99, 2.02, 2.08 (8×s, 24H, OC(O)CH_3)], 2.69-2.73 (dd, 1H, $J_{5,6}$=8.0, $J_{6,6'}$=13.5 Hz, H-6_a), 2.96 (d, 1H, $J_{6,6'}$=13.5 Hz, H-$6_{a'}$), 3.28 (s, 3H, OCH_3), 3.67 (m, 2H, H-2_a, H-5_a), 3.80-3.87 (m, 3H, H-4_a, H-5_b, H-6_c), 3.96-4.06 (m, 3H, H-3_a, H-6_b, H-$6_{b'}$), 4.22 (dd, 1H, $J_{5,6}$=5.0, $J_{6,6'}$=12.0 Hz, H-$6_{c'}$), 4.30 (m, 1H, H-5_c), 4.56 (d, 1H, *J*=12.0 Hz, PhCH*H*), 4.69-4.76 (m, 3H, PhCH*H*), 5.03 (s, 1H, H-1_b), 5.12 (t, 1H, $J_{3,4}$=10.5, $J_{4,5}$=9.5 Hz, H-4_b), 5.21-5.33 (m, 6H, H-2_b, H-3_b, H-1_c, H-2_c, H-3_c, H-4_c), 7.19-7.34 (m, 10H, H_{arom}); ^{13}C NMR (75MHz, $CDCl_3$): δ= [20.5, 20.6, 20.7, 20.9 (OC(O)*C*H_3)], 32.4 (C-6_a), 54.9 (O*C*H_3), [62.3, 62.5 (C-6_b, C-6_c)], [66.2, 66.3, 68.9, 69.0, 69.1, 69.4, 69.5, 71.0, 71.1 (C-2_a, C-2_b, C-3_b, C-4_b, C-5_b, C-2_c, C-3_c, C-4_c, C-5_c)], [72.1, 75.3 (2×Ph*C*H_2)], 77.2 (C-5_a), [77.6, 78.4 (C-3_a, C-4_a)], 82.6 (C-1_c), 97.9 (C-1_a), 99.4 (C-1_b), [127.7, 127.8, 128.4, 128.6, 128.9, 137.6, 137.8 (C_{arom})], [169.6, 169.7, 169.8, 170.6 (O*C*(O)CH_3)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 1073.3, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 1073.3297, $C_{49}H_{62}O_{23}S$ calcd for $[M+Na]^+$ 1073.3300.

Methyl 2, 3, 4-tri-*O*-acetyl-α-D-mannopyranoside (37):

To a solution of methyl α-D-mannopyranoside **36** (1.0 g, 5.15 mmol) in pyridine (10 mL) was added TrCl (2.4 g, 8.62 mmol). The reaction mixture was stirred at 80℃ for 2 h, and then concentrated to dryness. To the solution of this residue in pyridine (8 mL) was added acetic anhydride (3.88 mL). The reaction mixture was stirred at room temperature overnight, and then concentrated to dryness. The residue was co-evaporated with methanol (3×20 mL) and toluene (3×20 mL). The residue was dissolved in DCM (30 mL), and then washed with saturated $NaHCO_3$ (1×50 mL), 1N HCl (1×50 mL) and brine (1×50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the crude product by column chromatography over silica gel (Hexnae/EtOAc, 3:1) afforded Methyl *O*-2, 3, 4-tri-*O*-acetyl-6-*O*-triphenylmethyl-α-D-mannopyranoside as yellow oil (2.75 g, 95%), To a solution of *O*-2, 3, 4-tri-*O*-acetyl-6-*O*-triphenylmethyl-α-D-mannopyranoside (1.98 g, 3.52 mmol) in DCM (30 mL) was added $FeCl_3 \cdot 6H_2O$ (3.0 g, 11.09 mmol). The reaction mixture was stirred at room temperature for 2 h, and then diluted with DCM (50 mL), washed with water (50 mL). The organic layer was dried ($MgSO_4$) filtered, and the filtrate was concentrated to dryness. Purification of the crude product by column chromatography over silica gel (Hexnae/EtOAc, 2:1) afforded **37** as white solid (0.94 g, 83%). R_f=0.24 (Hexnae/EtOAc, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.93, 2.01, 2.08 (3×s, 9H, OC(O)CH_3), 3.34 (s, 3H, OCH_3), 3.55-3.71 (m, 3H, H-5, H-6, H-6'), 4.66 (s, 1H, H-1), 5.14-5.20 (m, 2H, H-2, H-4), 5.32 (dd, 1H, $J_{2,3}$=3.3, $J_{3,4}$=10.2Hz, H-3); MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 343.1, $C_{13}H_{20}O_9$ calcd for $[M+Na]^+$ 343.1005.

Methyl *O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2, 3, 4-tri-*O*-acetyl-α-D-mannopyranoside (38):

The compound **38** was synthesized according to the general procedure for the glycosidation with thioglycoside **16**. Glycosyl donor **16** (0.16 g, 0.41 mmol), glycosyl acceptor **37** (0.11 g, 0.34 mmol) and 4Å powdered molecular sieves (0.3 g) in DCM (5 mL) in the presence of NIS (0.1 g, 0.44 mmol) and TfOH (10 μL, 0.1 mmol) gave **38** as colorless oil (0.18 g, 82%). R_f =0.50 (Hexnae/EtOAc, 1:2). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.91-2.09 (7×s, 21H, OC(O)C*H*$_3$), 3.36 (s, 3H, OC*H*$_3$), 3.51 (dd, 1H, $J_{5,6}$=2.4, $J_{6,6'}$=10.8 Hz, H-6$_a$), 3.72 (dd, 1H, $J_{5,6'}$=6.0, $J_{6,6'}$=10.8 Hz, H-6$_{a'}$), 3.88 (m, 1H, H-5$_a$), 4.00-4.09 (m, 2H, H-5$_b$, H-6$_b$), 4.20 (dd, 1H, $J_{5,6}$=5.4, $J_{6,6'}$=12.0 Hz, H-6$_{b'}$), 4.62 (s, 1H, H-1$_a$), 4.80 (s, 1H, H-1$_b$), 5.14-5.30 (m, 6H, H-2$_a$, H-3$_a$, H-4$_a$, H-2$_b$, H-3$_b$, H-4$_b$); MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 673.2, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 673.1952, $C_{27}H_{38}O_{18}$ calcd for $[M+Na]^+$ 673.1956.

Methyl *S*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-2, 3, 4-tri-*O*-acetyl-α-D-mannopyranoside (39):

The compound **39** was synthesized according to the general procedure for glycosidation with thioacetyl mannoside **19**. Treatment of **37** (0.125 g, 0.39 mmol) and 2, 6-lutidine (0.059 ml, 0.49 mmol) in DCM (2 mL) with trifluoromethanesulfonic anhydride (0.10 ml, 0.59 mmol) followed by treatment the triflate residue and **19** (0.144 g, 0.35 mmol) in DMF (4 mL) at 0℃ under argon with liquid diethyl amine (0.17 mL) gave **39** as colorless oil (0.192 g, 81%). R_f =0.41 (Hexnae/EtOAc, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= [1.92, 1.98, 2.00, 2.04, 2.05, 2.08, 2.10 (7×s, 21H, OC(O)C*H*$_3$)], 2.65 (dd, 1H, $J_{5,6}$=7.5, $J_{6,6'}$=13.8 Hz, H-6$_a$), 2.80 (dd, 1H, $J_{5,6}$=2.7, $J_{6,6'}$=13.8 Hz, H-6$_{a'}$), 3.35 (s, 3H, OC*H*$_3$), 3.87 (m, 1H, H-5$_a$), 4.00-4.09 (m, 2H, H-5$_b$, H-6$_b$), 4.21-4.31 (m, 2H, H-4$_b$, H-6$_{b'}$), 4.60 (s, 1H, H-1$_a$), 5.15-5.28 (m, 6H, H-2$_a$, H-3$_a$, H-4$_a$, H-1$_b$,

H-2_b, H-3_b); ^{13}C NMR (75MHz, $CDCl_3$): δ= [19.5, 19.6, 19.7, 19.8, 19.9 (OC(O)*C*H_3)], 30.9 (C-6_a), 54.4 (O*C*H_3), 60.9 (C-6_b), [61.2, 65.2, 67.7, 67.8, 68.1, 68.3, 68.4, 69.9 (C-2_a, C-3_a, C-4_a, C-5_a, C-2_b, C-3_b, C-4_b, C-5_b)], 81.5 (C-1_b), 97.4 (C-1_a), [168.6, 168.7, 168.8, 168.9, 169.1, 169.6 (O*C*(O)CH_3)]; MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 689.2, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 689.1722, $C_{27}H_{38}O_{17}S$ calcd for $[M+Na]^+$ 689.1727.

Methyl *O*-(α-D-mannopyranosyl)-(1→6)-α-D-mannopyranoside (40):

To a solution of **38** (0.173 g, 0.27 mmol) in dry MeOH (5 mL) was added NaOMe (pH=8-10). The reaction mixture was stirred at room temperature overnight, and then concentrated to dryness. The residue was dissolved with milli-Q water. The aqueous solution was loaded on a P2 column to afford **40** (81 mg, 85%) as white solid. ^{1}H NMR (300 MHz, D_2O): δ= 3.28 (s, 3H, OC*H*$_3$), 3.53-3.86 (m, 12H, H-2_a, H-3_a, H-4_a, H-5_a, H-6_a, H-$6_{a'}$, H-2_b, H-3_b, H-4_b, H-5_b, H-6_b, H-$6_{b'}$), 4.63 (s, 1H, H-1_a), 4.79 (s, 1H, H-1_b); ^{13}C NMR (75MHz, D_2O): δ= 54.9 (O*C*H_3), 61.1 (C-6_a), 65.7 (C-6_b), [66.7, 66.9 (C-4_a, C-4_b)], 70.0 (C-2_b), [70.1, 70.7 (C-2_a, C-3_b)], [70.8, 70.9 (C-3_a, C-5_b)], 72.9 (C-5_a), 99.6 (C-1_b), 101.2 (C-1_a); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=171 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=171 Hz. MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 379.2, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 379.1214, $C_{13}H_{24}O_{11}$ calcd for $[M+Na]^+$ 379.1216.

Methyl *S*-(α-D-mannopyranosyl)-(1→6)-α-D-mannopyranoside (41):

To a solution of **39** (0.059 g, 0.089 mmol) in dry MeOH (2 mL) was added NaOMe (pH=8-10). The reaction mixture was stirred at room temperature overnight, and then concentrated to dryness. The residue was dissolved with milli-Q water. The aqueous solution was loaded on a P2 column to afford **41** (28 mg, 84%) as white solid. ^{1}H NMR (500 MHz, D_2O): δ= 2.82 (dd, 1H, J_5,

$_{6}$=8.0, $J_{6,6'}$=14.0 Hz, H-6$_a$), 3.20 (dd, 1H, $J_{5,6}$=2.0, $J_{6,6'}$=14.0 Hz, H-6$_{a'}$), 3.43 (s, 3H, OCH_3), 3.64 (t, 1H, $J_{3,4}$=9.0, $J_{4,5}$=10.0 Hz, H-4$_b$), 3.69 (t, 1H, $J_{3,4}$=9.5, $J_{4,5}$=9.5 Hz, H-4$_a$), 3.73-3.83 (m, 4H, H-3$_a$, H-5$_a$, H-3$_b$, H-6$_b$), 3.91 (dd, 1H, $J_{5,6}$=2.5, $J_{6,6'}$=12.5 Hz, H-6$_{b'}$), 3.94 (dd, 1H, $J_{1,2}$=1.5, $J_{2,3}$=3.5 Hz, H-2$_a$), 4.02 (ddd, 1H, $J_{4,5}$=10.0, $J_{5,6}$=2.5, $J_{5,6'}$=6.5 Hz, H-5$_b$), 4.08 (dd, 1H, $J_{1,2}$=1.5, $J_{2,3}$=3.0 Hz, H-2$_b$), 4.74 (d, 1H, $J_{1,2}$=1.5 Hz, H-1$_a$), 5.36 (s, 1H, H-1$_b$); ^{13}C NMR (75MHz, D_2O): δ= 31.7 (C-6$_a$), 54.9 (O*C*H$_3$), 60.9 (C-6$_b$), 67.2 (C-4$_a$), 69.5 (C-4$_b$), 69.9 (C-2$_a$), [70.5, 71.1 (C-3$_a$, C-5$_a$)], 71.2 (C-3$_b$,), 71.7 (C-2$_b$), 73.3 (C-5$_b$), 84.5 (C-1$_b$), 101.1 (C-1$_a$); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=172 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=169 Hz. MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 395.1, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 395.0985, $C_{13}H_{24}O_{10}S$ calcd for $[M+Na]^+$ 395.0988.

Methyl *O*-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (42):

To a solution of **33** (0.166 g, 0.24 mmol) in dry MeOH (4 mL) was added NaOMe (pH=8-10). The reaction mixture was stirred at room temperature overnight, and then neutralized by the addition of Dowex 650 H^+. The solution was filtered through celite, washed with MeOH/DCM (1:1, v/v). The combined filtrates were concentrated to dryness. Purification of the crude product by Iatrobeads afforded the desired deacetylated product (0.122 g, 0.23 mmol, 97%). To a solution of the partially deprotected compound (0.116 g, 0.22 mmol) in ethanol (4 mL) was added Pd/C (cat.) under an atmosphere of hydrogen. The reaction mixture was stirred at room temperature overnight, and then filtered through Celite. The filtrate was concentrated to dryness. The residue was dissolved with milli-Q water. The aqueous solution was loaded on a P2 column to afford **42** (78 mg, quant.) as white solid. ^{1}H NMR (300 MHz, D_2O): δ= 3.32 (s, 3H, OCH_3), 3.54-3.83 (m, 10H, H-3$_a$, H-4$_a$, H-5$_a$, H-6$_a$, H-6$_{a'}$, H-3$_b$, H-4$_b$, H-5$_b$, H-6$_b$, H-6$_{b'}$), 3.98 (m, 2H,

H-2_a, H-2_b), 4.65 (s, 1H, H-1_a), 5.01 (s, 1H, H-1_b); ^{13}C NMR (75MHz, D_2O): δ= 54.8 (O*C*H_3), [60.9, 61.0 (C-6_a, C-6_b)], [66.2, 66.8 (C-4_a, C-4_b)], [69.6, 70.1, 70.4 (C-2_a, C-2_b, C-3_b)], [72.8, 73.4 (C-5_a, C-5_b)], 78.3 (C-3_a), 100.9 (C-1_a), 102.5 (C-1_b); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=172 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=173 Hz. MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 379.1, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 379.1213, $C_{13}H_{24}O_{11}$ calcd for $[M+Na]^+$ 379.1216.

Methyl *S*-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (43):

The compound **43** was synthesized according to the general procedure for the global deprotection of **30**. Treatment of **30** (0.021 g, 0.029 ㎜ol) in MeOH/DCM (0.5 mL: 0.5 mL, v/v) with NaOMe (pH=8-10) gave deacetylated product (14mg, 0.025 mmol, 87%). Treatment of the partially deprotected compound (12 mg, 0.022 mmol) in THF (1 mL) with Na (s) in NH_3 (l) at -78℃ gave **43** as white solid (7.7 mg, 95%). ^{1}H NMR (500 MHz, D_2O): δ= 3.13 (t, 1H, $J_{2,3}$=3.0, $J_{3,4}$=7.5 Hz, H-3_a), 3.31 (s, 3H, O*C*H_3), 3.53-3.58 (m, 3H, H-6_a, H-3_b, H-6_b), 3.78 (m, 2H, H-5_a, H-$6_{b'}$), 3.85 (d, 1H, $J_{2,3}$=3.0 Hz, H-2_a), 3.90 (m, 1H, H-5_b), 4.02 (d, 1H, $J_{2,3}$=2.0 Hz, H-2_b), 4.58 (s, 1H, H-1_a), 5.41 (s, 1H, H-1_b); ^{13}C NMR (75MHz, D_2O): δ= 52.6 (C-3_a), 57.3 (O*C*H_3), 63.6 (C-5_a), 63.8 (C-6_b), 73.6 (C-3_b), 73.8 (C-2_a), 74.3 (C-2_b), [69.4, 69.8, 76.1 (C-4_a, C-6_a, C-4_b)], 76.3 (C-5_b), 89.0 (C-1_b), 102.6 (C-1_a); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=172 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=170 Hz. MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 395.1, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 395.0987, $C_{13}H_{24}O_{10}S$ calcd for $[M+Na]^+$ 395.0988.

Methyl *O*-(α-D-mannopyranosyl)-(1→3)-[*S*-(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (44):

The compound **44** was synthesized according to the general procedure for the global

deprotection of **35**. Treatment of **35** (0.057 g, 0.054 mmol) in MeOH/DCM (1 mL: 1 mL, v/v) with NaOMe (pH=8-10) gave deacetylated product (32 mg, 0.045 mmol, 83%). Treatment of the partially deprotected compound (21 mg, 0.029 mmol) in THF (1 mL) with Na (s) in NH_3 (l) at -78℃ gave **44** as white solid (14 mg, 88%). ^{1}H NMR (300 MHz, D_2O): δ= 2.85 (dd, 1H, $J_{5,6}$=6.0, $J_{6,6'}$=14.1 Hz, H-6_a), 3.21 (d, 1H, $J_{6,6'}$=14.1 Hz, H-$6_{a'}$), 3.41 (s, 3H, OCH_3), 3.62-3.86 (m, 12H, H-3_a, H-4_a, H-5_a, H-3_b, H-4_b, H-5_b, H-6_b, H-$6_{b'}$, H-3_c, H-4_c, H-6_c, H-$6_{c'}$), 3.98-4.07 (m, 4H, H-2_a, H-2_b, H-2_c, H-5_c), 4.70 (s, 1H, H-1_a), 5.09 (s, 1H, H-1_b), 5.35 (s, 1H, H-1_c); ^{13}C NMR (75MHz, D_2O): δ= 31.8 (C-6_a), 55.0 (O*C*H_3), [61.0, 61.1, 66.9, 67.2, 68.8, 69.7, 70.2, 70.5, 71.1, 71.2, 71.8, 73.4, 73.5, 78.1 (C-2_a, C-3_a, C-4_a, C-5_a, C-2_b, C-3_b, C-4_b, C-5_b, C-6_b, C-2_c, C-3_c, C-4_c, C-5_c, C-6_c)], 84.7 (C-1_c), 101.0 (C-1_a), 102.5 (C-1_b); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=172 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=172 Hz, $J_{H\text{-}1c\text{-}C\text{-}1c}$=168 Hz. MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 557.2, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 557.1512, $C_{19}H_{34}O_{15}S$ calcd for $[M+Na]^+$ 557.1516.

Methyl *S*-(α-D-mannopyranosyl)-(1→3)-[*O*-(α-D-mannopyranosyl)-(1→6)]-α-D-mannopyranoside (45):

The compound **45** was synthesized according to the general procedure for the global deprotection of **32**. Treatment of **32** (0.018 g, 0.017 mmol) in MeOH/DCM (0.5 mL: 0.5 mL, v/v) with NaOMe (pH=8-10) gave deacetylated product (9 mg, 0.013 mmol, 77%). Treatment of the partially deprotected compound (9 mg, 0.013 mmol) in THF (1 mL) with Na (s) in NH_3 (l) at -78℃ gave **45** as white solid (6 mg, 91%). ^{1}H NMR (500 MHz, D_2O): δ= 3.23 (dd, 1H, $J_{2,3}$=2.5, $J_{3,4}$=10.0 Hz, H-3_a), 3.41 (s, 3H, OCH_3), 3.62-3.89 (m, 12H, H-4_a, H-5_a, H-6_a, H-$6_{a'}$, H-3_b, H-4_b,

H-6_b, H-$6_{b'}$, H-3_c, H-4_c, H-5_c, H-6_c), 3.98-4.01 (m, 4H, H-2_a, H-5_b, H-2_c, H-$6_{c'}$), 4.12 (dd, 1H, $J_{1,2}$=1.5, $J_{2,3}$=3.5 Hz, H-2_b), 4.68 (s, 1H, H-1_a), 4.89 (s, 1H, H-1_c), 5.50 (s, 1H, H-1_b); ^{13}C NMR (75MHz, D_2O): δ= 50.3 (C-3_a), 54.8 (O*C*H_3), [61.0, 61.1, 65.8, 66.5, 66.9, 67.3, 70.1, 70.7, 71.1, 71.3, 71.7, 71.8, 72.8, 73.5 (C-2_a, C-4_a, C-5_a, C-6_a, C-2_b, C-3_b, C-4_b, C-5_b, C-6_b, C-2_c, C-3_c, C-4_c, C-5_c, C-6_c)], 86.4 (C-1_b), 99.6 (C-1_c), 100.4 (C-1_a); 1D Coupling HSQC (500 MHz, D_2O): $J_{H\text{-}1a\text{-}C\text{-}1a}$=172 Hz, $J_{H\text{-}1b\text{-}C\text{-}1b}$=170 Hz, $J_{H\text{-}1c\text{-}C\text{-}1c}$=173 Hz. MALDI-TOF/MS: *m/z*: found $[M+Na]^+$ 557.2, MALDI-FTICR/MS: *m/z*: found $[M+Na]^+$ 557.1513, $C_{19}H_{34}O_{15}S$ calcd for $[M+Na]^+$ 557.1516.

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CHAPTER 3

SYNTHESIS OF MANNOSTATIN ANALOGS[1]

[1] Wei Zhong, Jinkeng Asong, Sameer Kawatkar, Jun Guo, Doug Kuntz and Geert-Jan Boons. To be submitted to *ChemBioChem*.

Abstract

Mannostatin A is a potent inhibitor of the mannose trimming enzyme Golgi α-mannosidase II (GMII), which atcs late in the *N*-glycan processing pathway. Inhibition of this enzyme provides a route to blocking the oncogene-induced changes in cell surface oligosaccharide structures. The thiomethyl moiety of Mannostatin A is a feature that is not observed in any other glycosidase inhibitors. It has been proposed that the sulfur atom and ε-CH_3 group of methionine residues are involved in several different interactions important for protein stability. To probe in detail the interactions of the thiomethyl function of Mannostatin A with *d*GMII, Mannostatin B and analogs, which contain hydroxyl, methoxy or deoxy, respectively instead of the thiomethyl of Mannostatin A, were prepared. The ability of the compounds to inhibit *d*GMII has been examined and the results rationalized by examining X-ray crystal structures of *d*GMII with the synthetic compounds.

Introduction

Protein-linked carbohydrates, including those attached to lipids and proteins through either a nitrogen atom (*N*-linked) or oxygen atom (*O*-linked), are among the most complex and diverse sets of post-translationally modified structures on intracellular and secreted proteins. In recent studies, *N*- and *O*- glycan structures have been shown to contribute to several aspects of biological recognition including cell adhesion, inflammatory reactions, hormone action, arthritis and viral infections.[1] Although some of the roles of *N*-glycans in cell adhesion have been identified, many details relating to changes in cell adhesion remain unclear.[1-4] The cell- and tissue-specific changes in cell surface oligosaccharides during development have indicated that

these structures may be involved in cell adhesion and migration events during embryogenesis.[1,5] Alterations in the branching and extension of *N*-glycans have also been found on the surfaces of cells that have undergone oncogenic transformation and these changes correlate with alterations in cell adhesion which contribute to the metastatic potential of malignant cells.[5] A model linking oncogene activation to the induction of oligosaccharide branching and extension at the cell surface has been developed over the last decade. Inhibition of the mannose trimming enzyme human Golgi α-mannosidase II (HGMII) that acts late in the *N*-glycan processing pathway, provides one route to blocking the oncogene-induced changes in cell surface oligosaccharide structures.[6] HGMII selectively cleaves α(1→3) and α(1→6) mannosyl residues present in the natural substrate $GlcNAcMan_5GlcNAc_2$.[7] Inhibition of HGMII can retard tumor progression *in vivo*.

HGMII is a retaining glycosyl hydrolase. The double displacement mechanism proposed by Koshland[8] for retaining glycosidases is a two-step process which involves two displacement reactions at the anomeric center. In the first glycosylation step, a catalytic nucleophile makes a backside attack at the anomeric carbon of the glycone through an oxacarbenium ion-like transition state, followed by the formation of a covalent glycosyl-enzyme (CGE) intermediate with inversion of stereochemistry at the anomeric center. A general acid catalysis provided by another carboxylic acid residue in the active site assists the departure of the aglycone. In the second deglycosylation step, nucleophilic attack of a water molecule results in the hydrolysis of the CGE intermediate. The incoming water is believed to have been deprotonated by the conjugate base of the catalytic acid residue. The overall reaction constitutes hydrolysis of a

glycosidic bond with net retention of the anomeric configuration. The transition states on either side of the covalent intermediate show oxocarbenium ion character as shown in studies using retaining mannosidases 5-fluoro-substituted pseudosubstrates and deuterium-labeled substrates.[9-13] Potent inhibitors of the class II α-mannosidases are considered to mimic oxacarbenium-like transition states. For example, the five-membered ring of swainsonine resembles a flattened six-membered ring that has been forced to attain an oxocarbenium-like structure, which is elucidated by the crystal structure of swainsonine complexed with Golgi α-mannosidase II from *Drosophila*.[14] However, swainsonine blocks oligosaccharide catabolism by inhibition of a related catabolic α-mannosidase found in lysosomes inducing a phenocopy of a lysosomal storage disease.[6,15] An alternative lead compound is required for development as a drug appropriate for antimetastatic therapy, which specifically inhibits HGMII without affecting the function of human lysosomal α-mannosidase.

Mannostatins A and B (**Figure 3.1**) are the first natural non-azasugar type α-mannosidase inhibitors to be isolated from the soil microorganism *Streptoverticillus*, and the most potent inhibitors of class II amannosidases reported[16] to date. Inhibitors such as mannostatins A and B possess an aminocyclopentitol structure and are reversible and competitive inhibitors, not showing the slow-binding phenomenon exhibited by other inhibitors such as swainsonine and its analogues. Mannostatin A effectively blocked the processing of influenza viral hemagglutin in cultured MDCK cells and caused the accumulation of hybrid type protein linked oligosaccharides, which is in agreement with blocking Golgi mannosidase II.

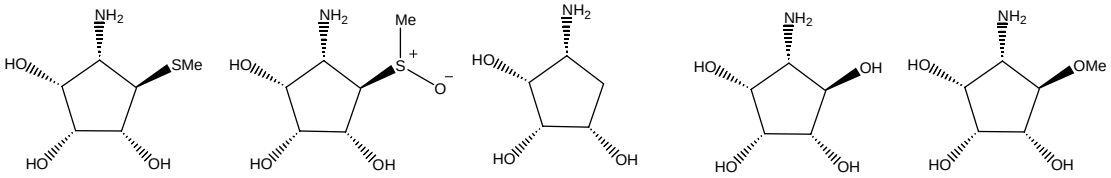
Recently, an X-ray crystal structure of *d*GMII in complex with Mannostatin A was reported.[24]

The five-membered ring of Mannostatin A adopted a $^{2}T_{1}$ twist envelope conformation, which was stacked against the aromatic ring of Trp95. The 3, 4 *cis*-diol complexed with a Zn^{2+} ion in the active site of dGMII resulting in T_6 coordination geometry. Furthermore, the amine of Mannostatin A formed hydrogen bonds with catalytic acid residue Asp204 and Asp341 and Tyr269. Data from SAR experiments has pointed to the importance of the amine and cis-diols in the inhibitory activity of mannostatin A and the crystal structure illustrated how these groups interact with the protein.[17-23]

The thiomethyl moiety of Mannostatin A, which is structurally similar to the side chain of a methionine residue, is a feature that is not observed in any other glycosidase inhibitors. It has been proposed that the sulfur atom and ε-CH_3 group of methionine residues are involved in several different interactions important for protein stability.[27-31] The methyl carbon of the thiomethyl moiety of Mannostatin A in the complex with GMII adopted two different conformations. In both conformations, the sulfur interacts with backbone oxygen of Arg876 through the π-orbital on the carbonyl oxygen and the antibonding σ* orbital of the S-C bond. In cf1 conformation, the polarizable methyl group is in a hydrophilic environment interacting with three water molecules and the π-system of the Arg228 side chain. On the other hand, the methyl group of the cf2 conformation is in a hydrophobic environment where it forms CH··π type interactions with the phenyl rings of Phe206 and Tyr727.

To probe in detail the interactions of the thiomethyl function of Mannostatin A with dGMII, Mannostatin B and analogs **3**, **4** and **5**, which contain hydroxyl, methoxy or deoxy, respectively instead of the thiomethyl of Mannostatin A, were prepared. The ability of the compounds to

inhibit dGMII has been examined and the results rationalized by examining X-ray crystal structures of dGMII with the synthetic compounds.



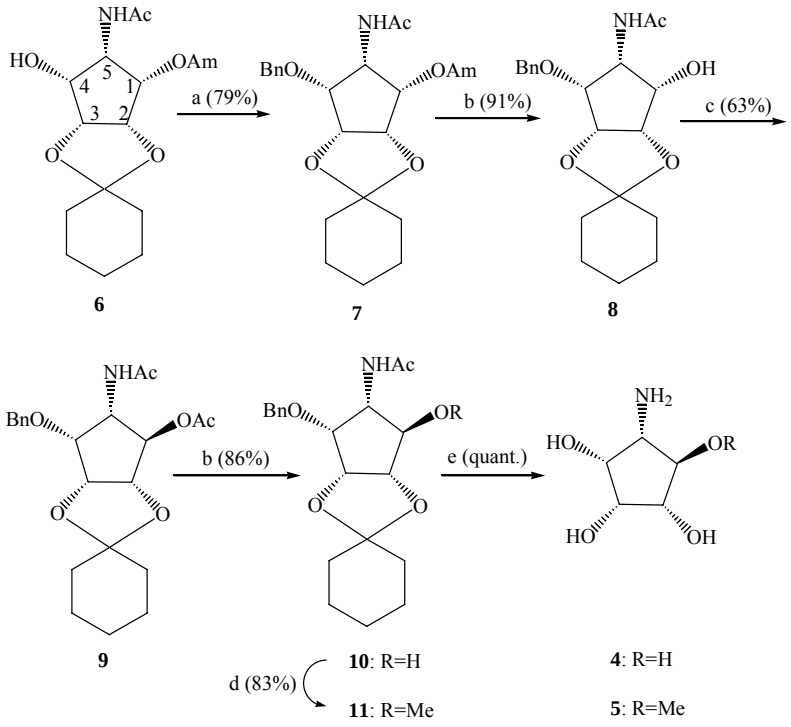
Mannostatin A **1** K_i=36nM | Mannostatin B **2** K_i=249nM | **3** K_i=265μM | **4** K_i=299nM | **5** K_i=76.4nM

Figure 3.1. Cyclopentitetrol inhibitors of Golgi α-mannosidase II.

Results and Discussion

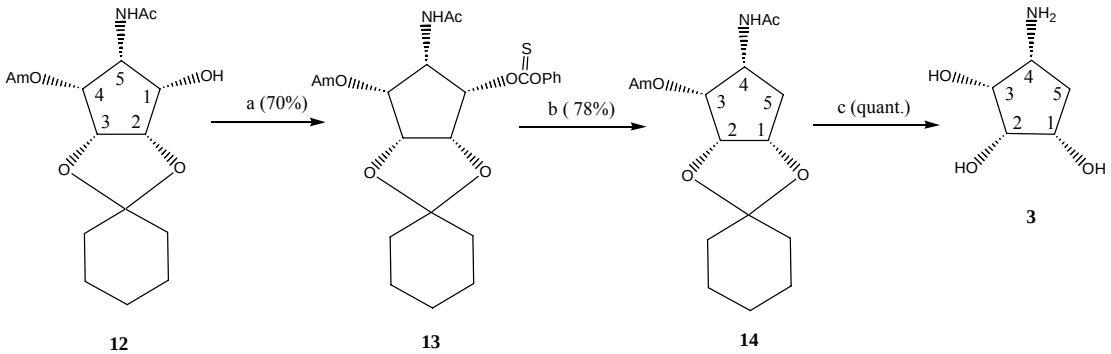
Synthesis

Optically pure compounds **6** and **12** were prepared by a modified literature procedures.[25, 26] The key steps of this approach were an aldol condensation of nitromethane with a dialdehyde derived from myo-inositol and a selective protection by an R-(-)-O-acetyl-mandelyl group.



Scheme 3.1. Reagents and conditions: a) BnBr, Ag_2O, THF, RT; b) NaOMe, MeOH, RT; c) 1) Tf_2O, Py, CH_2Cl_2, 0^oC; 2) Bu_4NOAc, toluene, RT; d) CH_3I, Ag_2O, MeCN, RT; e) 1. Pd/C, H_2 (g), $tBuOH/H_2O/AcOH$ 40:1:1, RT; 2. 1M HCl in H_2O/MeOH, reflux.

Thus, benzylation of **6** with benzyl bromide in the presence of freshly prepared silver (I) oxide in THF gave **7**, which was treated with NaOMe in methanol to remove the acetylmandelate (Am) ester to afford **8** (**scheme 3.1**). Inversion of configuration of C-2 hydroxyl of **8** was easily accomplished by triflation with triflic anhydride and pyridine in dichloromethane to give a triflate, which was immediately displaced tetrabutylammonium acetate in toluene using sonication to give acetate **9**. A small amount of elimination by-product was also isolated. The acetyl wster of **9** was cleaved with sodium methoxide in methanol to afford **10**, which was methylated with methyl iodide in the presence of freshly prepared silver (I) oxide to give **11.** Deprotection of **10** and **11** to give the target compounds **4** and **5** was accomplished by a two-step procedure involving catalytic hydrogenation over Pd/C to remove the benzyl ether followed by treatment with 1M HCl in a mixture of H_2O/MeOH to hydrolyze the cyclohexylidene acetal and convert the acetamido moiety into an amine.



Scheme 3.2. Reagents and conditions: a) DMAP, phenyl chlorothionoformate, acetonitrile, RT; b) AIBN, tributyltin hydride, toluene, reflux; c) 1M HCl in H_2O/MeOH, reflux.

Compound **3** could easily be prepared from **12** by treatment with DMAP and chlorothionoformate in acetonitrile to give phenylthiocarbonyl ester **13** (70%) which was

deoxygenated by heating under reflux in the presence of the free radical initiator AIBN and reducing reagent tributyltin hydride in toluene to afford **14** (**Scheme 3.2**). Deprotection of **14** was easily accomplished by heating under reflux in 1M HCl in H_2O/MeOH to give 5-deoxy-aminocyclopetititol **3** (quant.).

Enzymology

The rate of hydrolysis catalyzed by dGMII of different concentrations of 4-methylumbelliferyl α-D-mannopyranoside alone and in the presence of different concentrations of inhibitor was measured fluorometrically and Ki values were determined from Dixon plots. As can be seen in **Table 3.1**, Mannostatin A is a significantly more potent inhibitor than Mannostatin B. 1-Deoxyaminocyclopentitetrol **3**, which lacks the thiomethyl ether, is a very poor inhibitor indicating that the thiomethyl moiety of **1** makes important interactions with the binding site of the enzyme. Hydroxy and methoxy containing derivatives **4** and **5** gave Ki values that were eight and two-times larger than that of Mannostatin A.

Table 3.1. Inhibition constants (K_i) of compounds **1-5** for dGMII

Inhibitors	K_i (nM)
1	36
2	249
3	265×10^3
4	299
5	76

X-ray crystallography

In order to rationalize the inhibition data, X-ray crystal structures of compounds **2, 3, 4** and **5** complexed with dGMII are still ongoing.

Conclusion

Glycosidases play important roles in the biosynthesis of glycoproteins. Inhibitors of these enzymes may provide valuable lead compounds for drug discovery. Understanding the mode of inhibition by these compounds could help in the design and synthesis of more potent and selective inhibitors. However, the mode of inhibition sometimes is difficult to understand. As a result, only a few glycosidase inhibitors have been successfully developed as therapeutics.

Successfully synthesized mannostatin analogs **3**, **4** and **5** are very valuable compounds in the investigation of the role of the thiomethyl moiety of mannostatin A for SAR study. Computational studies and x-ray crystallography studies, which are still ongoing, could aid in a better understanding of the mode of inhibition by mannostatin A.

Experimental Section

^{1}H-NMR spectra were recorded in $CDCl_3$ or D_2O on Varian Merc-300 or Varian Inova-500 spectrometers equipped with Sun workstations at 300K. TMS (δ_H=0.00) or D_2O (δ_H=4.67) was used as the internal reference. ^{13}C-NMR spectra were recorded in $CDCl_3$ or D_2O at 75MHz on a Varian Merc-300 spectrometer, using the central resonance of $CDCl_3$ (δ_C=77.0) as the internal reference. COSY, HSQC and NOSEY experiments were used to assist assignment of the products. Mass spectra were obtained on an Applied Biosystems Voyager DE-Pro MALDI-TOF. Chemicals were purchased from Aldrich or Fluka and used without further purification. DCM

was distilled from calcium hydride; THF from sodium; and MeOH from magnesium and iodine. Aqueous solutions are saturated unless otherwise specified. All reactions were performed under anhydrous conditions under argon unless otherwise specified and monitored by TLC on Kieselgel 60 F254 (Merck). Detection was by examination under UV light (254 nm) and by charring with 10% sulfuric acid in methanol. Silica gel (Merck, 70-230 mesh) was used for chromatographies. Iatrobeads 6RS-8060 was purchased from Bioscan.

2, 3-*O*-Cyclohexylidene Derivative 7 of Respective (1*S*, 2*R*, 3*R*, 4*R*, 5*S*)-5-Acetamido-1-*O*-[(2*R*)-2-*O*-acetylmandelyl]-4-*O*-benzyl-1, 2, 3, 4-cyclopentanetetrol (7):

To a solution of **6** (0.30 g, 0.67 mmol) in dry THF (10 mL), was added benzyl bromide (0.16 mL, 1.34 mmol) and freshly prepared silver(I) oxide (1.55 g, 6.68 mmol). The flask was wrapped in aluminium foil to exclude light and the reaction mixture stirred at room temperature overnight, followed by filtration through celite. The filtrate was concentrated to dryness, and the residue was purified by column chromatography (toluene/acetone, 3:1) over silica gel to afford **7** (0.285 g, 79%) as colorless oil. R_f =0.46 (toluene/acetone, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.40-7.09 (m, 10H, Ph), 5.96 [s, 1H, PhC*H*(OAc)CO], 5.93 (d, 1H, $J_{5,NH}$=8.1 Hz, NH), 4.70 (dd, 1H, $J_{1,2}$=5.4, $J_{1,5}$=5.4 Hz, H-1), 4.64 (ddd, 1H, $J_{1,5}$=5.4, $J_{4,5}$=4.8 Hz, H-5), 4.59-4.49 (m, 4H, H-2, H-3, PhCH*H*), 3.60 (t, 1H, $J_{3,4}$=4.8, $J_{4,5}$=4.8 Hz, H-4), 2.11 (s, 3H, PhCH(C*H*$_3$CO)CO), 1.86 (s, 3H, C*H*$_3$CONH), 1.72-1.32 (m, 10H, C_6H_{10}) ppm. ^{13}C NMR (75MHz, $CDCl_3$): δ= 170.3, 169.3, 167.7 (*C*=O), 137.4-127.8 (C_{arom}), 113.4(C_6H_{10}), 79.1 (C-2), 77.2 (C-3), 74.2 (C-4), 74.1 (Ph*C*H(OAc)CO), 72.3 (Ph*C*H$_2$), 71.7 (C-1), 49.9 (C-5), [35.9, 33.2, 25.0, 24.0, 23.4 (C_6H_{10})], 23.1(*C*H$_3$CONH), 20.7 (PhCH(*C*H$_3$CO)CO) ppm. HRMS: *m/z*: found $[M+Na]^+$ 560.2257,

$C_{30}H_{35}NO_8$ calcd for $[M+Na]^+$ 560.2260.

2, 3-*O*-Cyclohexylidene Derivative 8 of Respective (1*S*, 2*R*, 3*R*, 4*R*, 5*S*)-5-Acetamido-4-*O*-benzyl-1, 2, 3, 4-cyclopentanetetrol (8):

To a solution of **7** (0.28 g, 0.52 mmol) in MeOH/DCM (5 mL: 5 mL, v/v), NaOMe (pH=8-10) was added. The reaction mixture was stirred at room temperature overnight, and then neutralized by the addition of Dowex 650 H^+. The solution was filtered through celite and washed with MeOH/DCM (1:1, v/v). The combined filtrates were concentrated to dryness. Purification of the residue by column chromatography (toluene/acetone, 3:1) over silica gel gave **8** (0.17 g, 91%) as a colorless oil. R_f=0.17 (toluene/acetone, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.27-7.19 (m, 5H, Ph), 6.29 (d, 1H, $J_{5,NH}$=8.1 Hz, NH), 4.62-4.52 (m, 3H, H-2, PhCH*H*), 4.45 (t, 1H, $J_{2,3}$=6.0, $J_{3,4}$=5.7 Hz, H-3), 4.37 (ddd, 1H, $J_{1,5}$=4.8, $J_{4,5}$=5.4 Hz, H-5), 3.89 (t, 1 H, $J_{3,4}$=5.7, $J_{4,5}$=5.4 Hz, H-4), 3.68 (t, 1H, $J_{1,2}$=4.5, $J_{1,5}$=4.8 Hz, H-1), 1.96 (s, 3H, C*H*$_3$CONH), 1.70-1.38 (m, 10H, C_6H_{10}) ppm. ^{13}C NMR (75MHz, $CDCl_3$): δ= 170.4 (*C*=O), 137.4-127.9 (C_{arom}), 113.4(C_6H_{10}), 79.4 (C-2), 77.2 (C-3), 75.2 (C-1), 72.6 (Ph*C*H$_2$), 70.7 (C-4), 52.6 (C-5), [35.8, 33.2, 25.0, 24.0, 23.4 (C_6H_{10})], 23.3 (*C*H$_3$CONH) ppm. HRMS: *m/z*: found $[M+Na]^+$ 384.1714, $C_{20}H_{27}NO_5$ calcd for $[M+Na]^+$ 384.1717.

2, 3-*O*-Cyclohexylidene Derivative 9 of Respective (1*R*, 2*R*, 3*R*, 4*R*, 5*S*)-5-Acetamido-1-O-acetyl-4-*O*-benzyl-1, 2, 3, 4-cyclopentanetetrol (9):

To a solution of **8** (0.17 g, 0.47 mmol) in pyridine (0.19 mL, 2.33 mmol) and dry DCM (5 mL) at 0℃, trifluoromethanesulfonic anhydride (0.12 mL, 0.71 mmol) was added slowly. The reaction mixture was stirred at 0℃ for 1 h, after which it was diluted with DCM and was washed with

H_2O and saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The residue was dissolved in toluene (20 mL), and tetrabutylammonium acetate (0.28 g, 0.92 mmol) was added. The reaction mixture was sonicated at room temperature for 16 hrs, and then concentrated to dryness. The residue was dissolved in DCM, and the solution washed with saturated $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated to dryness. Purification of the residue by column chromatography over silica gel (toluene/acetone, 2:1) gave **9** (0.12 g, 63%) as a colorless oil. R_f =0.42 (toluene/acetone, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.31-7.24 (m, 5H, Ph), 6.27 (d, 1H, $J_{5,NH}$=8.1 Hz, NH), 4.98 (d, 1H, $J_{1,5}$=2.4 Hz, H-1), 4.62-4.53 (m, 3H, H-3, PhCH*H*), 4.37 (ddd, 1H, $J_{1,5}$=2.4, $J_{4,5}$=5.7 Hz, H-5), 4.32 (d, 1H, $J_{2,3}$=6.0 Hz, H-2), 3.93 (t, 1H, $J_{3,4}$=5.4, $J_{4,5}$=5.7 Hz, H-4), 1.96 (s, 3H, CH_3CO), 1.89 (s, 3H, C*H*$_3$CONH), 1.72-1.30 (m, 10H, C_6H_{10}) ppm. ^{13}C NMR (75MHz, $CDCl_3$): δ= 170.4, 169.6 (*C*=O), 137.4-128.0 (C_{arom}), 113.3(C_6H_{10}), 81.9 (C-2), 79.3 (C-1), 78.8 (C-3), 76.6 (C-4), 72.5 (Ph*C*H$_2$), 54.3 (C-5), [35.9, 33.1, 25.0, 23.8, 23.4 (C_6H_{10})], 23.3 (*C*H$_3$CONH), 20.9 (*C*H$_3$CO) ppm. HRMS: *m/z*: found $[M+Na]^+$ 403.1992, $C_{22}H_{29}NO_6$ calcd for $[M+Na]^+$ 403.1995.

2, 3-*O*-Cyclohexylidene Derivative 10 of Respective (1*R*, 2*R*, 3*R*, 4*R*, 5*S*)-5-Acetamido-4-*O*-benzyl-1, 2, 3, 4-cyclopentanetetrol (10):

To a solution of **9** (0.12 g, 0.30 mmol) in MeOH/DCM (3 mL: 3 mL, v/v), was added NaOMe (pH=8-10). The reaction mixture was stirred at room temperature for 16 hrs, after which it was neutralized by the addition of Dowex 650 H^+. The solution was filtered through celite, and the residue was washed with MeOH/DCM (1:1, v/v). The filtrate was concentrated to dryness and

the residue was purified by column chromatography (toluene/acetone, 2:1) over silica gel to afford **9** (0.092 g, 86%) as a colorless oil. R_f=0.30 (toluene/acetone, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.28-7.09 (m, 5H, Ph), 6.39 (d, 1H, $J_{5,NH}$=6.3 Hz, NH), 4.64 (t, 1H, $J_{2,3}$=5.1, $J_{3,4}$=4.5 Hz, H-3), 4.56 (dd, 2H, *J*=12.0 Hz, PhCH*H*), 4.32 (d, 1H, $J_{2,3}$=5.1 Hz, H-2), 4.16 (ddd, 1H, $J_{1,5}$=1.5, $J_{4,5}$=4.5 Hz, H-5), 4.06 (t, 1H, $J_{3,4}$=4.5, $J_{4,5}$=4.5 Hz, H-4), 4.03 (d, 1H, $J_{1,5}$=1.5 Hz, H-1), 1.89 (s, 3H, C*H*$_3$CONH), 1.68-1.28 (m, 10H, C_6H_{10}) ppm. ^{13}C NMR (75MHz, $CDCl_3$): δ= 170.4 (*C*=O), 137.4-128.1 (C_{arom}), 112.5(C_6H_{10}), 84.1 (C-2), 79.1 (C-3), 78.1 (C-1), 76.7 (C-4), 72.3 (Ph*C*H$_2$), 57.6 (C-5), [35.9, 32.9, 25.1, 23.9, 23.4 (C_6H_{10})], 23.3 (*C*H$_3$CONH) ppm. HRMS: *m/z*: found $[M+Na]^+$ 384.1714, $C_{20}H_{27}NO_5$ calcd for $[M+Na]^+$ 384.1717.

2, 3-*O*-Cyclohexylidene Derivative 11 of Respective (1*R*, 2*R*, 3*R*, 4*R*, 5*S*)-5-Acetamido-4-*O*-benzyl-1-*O*-methyl-1, 2, 3, 4-cyclopentanetetrol (11):

To a solution of **10** (50 mg, 0.14 mmol) in dry acetonitrile (2 mL) was added methyl iodide (52 μL, 0.84 mmol) and freshly prepared silver(I) oxide (0.16 g, 0.69 mmol). The flask was wrapped in aluminium foil to exclude light. The reaction mixture was stirred at room temperature for 2 days, after which it was filtered through celite. The filtrate was concentrated to dryness and the residue was purified by column chromatography (toluene/acetone, 3:1) over silica gel to afford **11** (43 mg, 83%) as a colorless oil. R_f=0.45 (toluene/acetone, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.28-7.19 (m, 5H, Ph), 6.32 (d, 1H, $J_{5,NH}$=5.1 Hz, NH), 4.60-4.48 (m, 3H, H-3, PhC*H*$_2$), 4.30 (m, 2H, H-2, H-5), 3.93 (t, 1H, $J_{3,4}$=4.5, $J_{4,5}$=4.5 Hz, H-4), 3.54 (d, 1H, $J_{1,5}$=1.5 Hz, H-1), 3.34 (s, 3H, OCH_3), 1.90 (s, 3H, C*H*$_3$CONH), 1.69-1.28 (m, 10H, C_6H_{10}) ppm. ^{13}C NMR (75MHz, $CDCl_3$): δ= 169.5 (*C*=O), 137.3-128.0 (C_{arom}), 112.5 (C_6H_{10}), 86.9 (C-2), 82.0 (C-3), 79.1 (C-1),

76.6 (C-4), 72.1 (Ph*C*H_2), 57.6 (C-5), 53.5 (OCH_3), [36.0, 32.8, 25.0, 23.9, 23.4 (C_6H_{10})], 23.3 (*C*H_3CONH) ppm. HRMS: *m/z*: found $[M+Na]^+$ 375.2041, $C_{21}H_{29}NO_5$ calcd for $[M+Na]^+$ 375.2046.

1, 2-*O*-Cyclohexylidene Derivative 14 of Respective (1*S*, 2*S*, 3*R*, 4*R*)-4-Acetamido-3-*O*-[(2*R*)-2-*O*-acetylmandelyl]-5-deoxy-1, 2, 3-cyclohexanetriols (14):

DMAP (98 mg, 0.8 mmol) and phenyl chlorothionoformate (0.1 mL, 0.72 mmol) were subsequently added to a solution of **12** (60 mg, 0.13 mmol) in acetonitrile (0.6 mL). The reaction mixture was stirred for 3 hrs at room temperature, after which it was diluted by ethyl acetate (10 mL). The resulting solution was washed with water, dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the residue by column chromatography (toluene/acetone, 3:1) over silica gel afforded **13** (55 mg, 79%) as a colorless oil. R_f =0.7 (toluene/acetone, 1:1). A solution of precursor **13** (44 mg, 0.08 mmol) in dry toluene (4mL) was added to a solution of AIBN (4 mg, 0.023 mmol) and tributyltin hydride (60 μL, 0.23 mmol) in dry toluene (0.55 mL). The reaction mixture was refluxed for 2 hrs, after which it was cooled to room temperature, and concentrated *in vacuo*. Purification of the residue by column chromatography (toluene/acetone, 5:1) over silica gel afforded **14** (25 mg, 78%) as a colorless oil. R_f=0.36 (toluene/acetone, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.46-7.09 (m, 5H, Ph), 6.18 (d, 1H, $J_{5,NH}$=8.7 Hz, NH), 5.92 (s, 1H, PhC*H*(OAc)CO), 4.80 (t, 1H, $J_{2,3}$=5.4, $J_{3,4}$=4.8 Hz, H-3), 4.61-4.44 (m, 3H, H-1, H-2, H-4), 2.13 (s, 3H, PhCH(C*H*$_3$CO)CO), 1.93 (m, 1H, H-5), 1.79 (s, 3H, C*H*$_3$CONH), 1.45-1.19 (m, 10H, C_6H_{10}) ppm. ^{13}C NMR (75MHz, $CDCl_3$): δ= 170.5, 169.4, 167.9 (*C*=O), 133.3-128.1 (C_{arom}), 112.4 (C_6H_{10}), 77.7 (C-2), 77.1 (C-1), 74.6 (Ph*C*H(OAc)CO), 74.4 (C-3), 50.4 (C-4), 34.5 (C-5),

[35.8, 32.8, 24.9, 23.7, 23.3 (C_6H_{10})], 23.0 (*C*H_3CONH), 20.7 (PhCH(*C*H_3CO)CO) ppm. HRMS: *m/z*: found $[M+Na]^+$ 454.1858, $C_{23}H_{29}NO_7$ calcd for $[M+Na]^+$ 454.1862.

(1*R*, 2*R*, 3*R*, 4*R*, 5*S*)-5-Amino-1, 2, 3, 4-cyclopentanetetrol (4):

To a solution of **10** (40 mg, 0.11 mmol) in *tert*-butanol/H_2O/AcOH (40:1:1, 5 mL), was added Pd/C (cat.) under an atmosphere of hydrogen. The reaction mixture was stirred at room temperature for 16 hrs, and then filtered through celite. The filtrate was concentrated to dryness. The residue in 1M HCl in H_2O/MeOH (1:1, 8 mL) was refluxed for 2 hrs and then concentrated *in vacuo*. Purification of the residue over Iatro beads (acetonitrile/AcOH/H_2O, 5:1:1) afforded desired product **4** (15 mg, quant.) as a white solid. ^{1}H NMR (300 MHz, D_2O): δ= 4.24 (dd, 1H, $J_{3,4}$=4.5, $J_{4,5}$=6.6 Hz, H-4), 4.08 (t, 1H, $J_{1,5}$=6.6, $J_{1,2}$=6.9 Hz, H-1), 4.02 (t, 1H, $J_{2,3}$=4.8, $J_{3,4}$=4.5 Hz, H-3), 3.80 (dd, 1H, $J_{1,2}$=6.9, $J_{2,3}$=4.8 Hz, H-2), 3.35 (t, 1H, $J_{1,5}$=6.6, $J_{4,5}$=6.6 Hz, H-5) ppm. ^{13}C NMR (75MHz, D_2O): δ= 78.7 (C-3), 75.5 (C-2), 71.7 (C-4), 67.4 (C-1), 56.8 (C-5) ppm. HRMS: *m/z*: found $[M+H]^+$ 150.0776, $C_5H_{11}NO_4$ calcd for $[M+H]^+$ 150.0766.

(1*R*, 2*R*, 3*R*, 4*R*, 5*S*)-5-Amino-1-*O*-methyl-1, 2, 3, 4-cyclopentanetetrol (5):

To a solution of **11** (38 mg, 0.10 mmol) in *tert*-butanol/H_2O/AcOH (40:1:1, 5 mL), was added Pd/C (cat.) under an atmosphere of hydrogen. The reaction mixture was stirred at room temperature for 16 hrs, and then filtered through celite. The filtrate was concentrated to dryness. The residue in 1M HCl in H_2O/MeOH (1:1, 8 mL) was refluxed for 2 hrs and then concentrated *in vacuo*. Purification of the residue over Iatro beads (acetonitrile/AcOH/H_2O, 5:1:1) afforded **5** (17 mg, quant.) as a white solid. ^{1}H NMR (300 MHz, D_2O): δ= 4.22 (dd, 1H, $J_{3,4}$=4.2, $J_{4,5}$=5.7 Hz, H-4), 4.02 (t, 1H, $J_{2,3}$=4.2, $J_{3,4}$=4.2 Hz, H-3), 3.95 (t, 1H, $J_{1,2}$=4.8, $J_{2,3}$=4.2 Hz, H-2), 3.83 (t,

1H, $J_{1,5}$=5.7, $J_{1,2}$=4.8 Hz, H-1), 3.45 (m, 1H, H-5) ppm. ^{13}C NMR (75MHz, D_2O): δ= 88.8 (C-1), 74.4 (C-2), 72.3 (C-3), 68.4 (C-4), 58.1 (OCH_3), 55.3 (C-5) ppm. HRMS: *m/z*: found $[M+H]^+$ 164.0932, $C_6H_{13}NO_4$ calcd for $[M+H]^+$ 164.0923.

(1*S*, 2*S*, 3*R*, 4*R*)-4-Acetamido-5-deoxy-1, 2, 3-cyclohexanetriols (3):

A solution of **14** (25 mg, 0.06 mmol) in 1M HCl in H_2O/MeOH (1:1, 5 mL) was refluxed for 2 hrs and then concentrated *in vacuo*. Purification of the residue over Iatro beads (tert-butanol/AcOH/H_2O, 4:1:1) afforded **3** (11 mg, quant.) as a white solid. ^{1}H NMR (500 MHz, D_2O): δ= 4.11-4.06 (m, 2H, H-1, H-3), 3.91 (t, 1H, $J_{1,2}$=4.5, $J_{2,3}$=4.0 Hz, H-2), 3.58 (m, 1H, H-4), 2.46 (ddd, 1H, $J_{1,5}$=8.5, $J_{4,5}$=7.5, $J_{5,5'}$=15.0 Hz, H-5), 1.69 (ddd, 1H, $J_{1,5'}$=5.0, $J_{4,5'}$=6.0, $J_{5,5'}$=15.0 Hz, H-5') ppm. ^{13}C NMR (75MHz, D_2O): δ= 75.8 (C-2), [72.4, 72.3 (C-1, C-3)], 53.4 (C-4), 37.0 (C-5) ppm. HRMS: *m/z*: found $[M+H]^+$ 134.0826, $C_6H_{13}NO_4$ calcd for $[M+H]^+$ 134.0817.

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CHAPTER 4

SYNTHESIS AND ANTIGENIC ANALYSIS OF THE BC1A GLYCOPROTEIN OLIGOSACCHARIDE FROM THE *BACILLUS ANTHRACIS* EXOSPORIUM[1]

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Abstract

The glycoprotein BclA is an important constituent of the exosporium of *Bacillus anthracis* spores. This glycoprotein is substituted with an oligosaccharide composed of a β-l-rhamnoside substituted with the previously unknown terminal saccharide, 2-*O*-methyl-4-(3-hydroxy-3-methylbutanamido)-4,6-dideoxy-D-glucopyranose, also referred to as anthrose. Anthrose has not been found in spores of *B. cereus* and *B. thuringiensis*, making it a potential species-specific marker for *B. anthracis*. In order to study the antigenicity of anthrose, efficient syntheses of an anthrose-containing trisaccharide and a series of structurally related analogues were developed. The analogues lacked either the methyl ether at C-2 or contained modified C-4 amino functionalities of anthrose. The synthetic compounds were equipped with an aminopropyl spacer to facilitate conjugation to the carrier proteins mariculture Keyhole Limpet Hemocyanin (mcKLH) and bovine serum albumin (BSA). Serum antibodies of rabbits immunized with live or irradiated spores of *B. anthracis* Sterne $34F_2$ were able to recognize the synthetic trisaccharide–mcKLH conjugate. The specificity of the interaction was confirmed by competitive inhibition with the free and BSA-conjugated trisaccharides. Inhibition using the trisaccharide analogues demonstrated that the isovaleric acid moiety of anthrose is an important structural motif for antibody recognition. These data demonstrate that 1) anthrose is a specific antigenic determinant of the *B. anthracis* Sterne spore; 2) this antigen is presented to the immune system of rabbits receiving the anthrax live-spore vaccine; 3) synthetic analogues of the oligosaccharide retain the antigenic structure; and 4) the antigenic region is localized to specific terminal groups of the oligosaccharide. Collectively these data provide an important proof-of-concept step in the synthesis and development of spore specific reagents for detection and targeting of non-protein structures in *B. anthracis*.

Introduction

Bacillus anthracis is a gram-positive, spore-forming bacterium that causes anthrax in humans and other mammals.[1,2] Because of the high resilience of *Bacillus anthracis* spores to extremes of their environment they can persist for many years until encountering a signal to germinate.[3, 4] When spores of *B. anthracis* are inhaled or ingested they may germinate and establish populations of vegetative cells which release anthrax toxins, often resulting in the death of the host.[5] The relative ease by which *B. anthracis* may be weaponized and the difficulty in early recognition of inhalation anthrax due to the non-specific nature of its symptoms were demonstrated by the deaths of five people who inhaled spores from contaminated mail.[6–8] Consequently, considerable efforts are being directed towards the development of early disease diagnostics and there is a renewed interest in anthrax vaccines. Sterile, cell-free vaccines containing the protective antigen (PA) component of anthrax toxin have proven safe and effective.[9,10] The anthrax vaccine that provides the most comprehensive protection is, however, the *B. anthracis* Sterne $34F_2$ live-spore vaccine.[11,12] Although not licensed for human use in the US or EU, the live-spore vaccine has proven highly efficacious as a veterinary vaccine and similar live-spore preparations have been used extensively in humans and animals in eastern Europe and Asia.[13] Although these live-spore vaccines may elicit lower antitoxin antibodies than the licensed cell-free anthrax vaccines, their documented efficacy is attributed to additional adjuvant properties and as yet undefined protective epitopes contributed by the spores or outgrowing vegetative cells.[14] It is feasible, but as yet unexplored, that specific carbohydrate antigens may contribute to the enhanced efficacy of the live spore vaccines.

Spores of *B. anthracis* are enclosed by a prominentloose fitting layer called the exosporium, which consists of a paracrystalline basal layer composed of a number of different proteins and an

external hair-like nap.[15–19] The filaments of the nap are formed by the highly immunogenic glycoprotein BclA, which has a long, central collagen-like region containing multiple X-X-Gly repeats where X can be any amino acid.[20] Almost all of the repeating units contain a threonine (Thr) residue, which provides sites for potential glycosylation.[21,22] Recently, it was shown that the BclA glycoprotein contains an O-linked saccharide, the structure of which was determined by a combination of NMR spectroscopy and mass spectrometry.[23] The oligosaccharide is probably attached to the protein through a GalNAc moiety, which was lost during the hydrazine-mediated release from the BclA glycoprotein.[23] The structure of the tetrasaccharide is depicted in **Figure 4.1**.

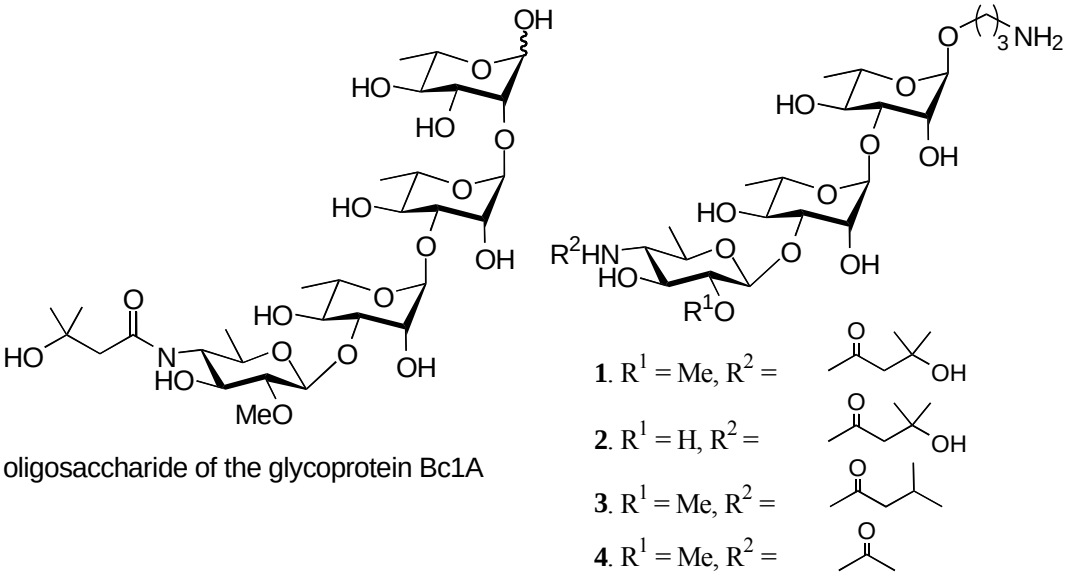


Figure 4.1. Oligosaccharide of the glycoprotein Bc1A and synthetic targets

The previously unknown non-reducing terminal saccharide, 2-*O*-methyl-4-(3-hydroxy-3-methylbutanmido)-4,6-dideoxy-d-glucopyranose, was named anthrose and has not been found in spores of *B. cereus* and *B. thuringiensis*, making it a potential species-specific marker for *B. anthracis*. It may also be a new target for therapeutic intervention

or vaccine development.[23]

Results and Discussion

Synthesis

To study the immunological properties of the oligosaccharide of BclA, we examined whether antisera from rabbits immunized with live or irradiated spores of *B. anthracis* Sterne $34F_2$ were able to recognize the synthetic anthrose-containing BclA oligosaccharide[24-27] and selected analogues. Although challenging, chemical synthesis offers an opportunity to obtain almost every oligosaccharide target in sufficient quantity and purity for these biological studies. Furthermore, chemical synthesis has the advantage that a target compound can be equipped with an artificial spacer for convenient conjugation to a carrier protein, and offers opportunities for obtaining analogues for structure-activity relationship studies.

Compounds **1**-**4** (**Figure 4.1**) were selected as targets for chemical synthesis. Compound **1** is derived from the oligosaccharide of BclA and contains an intact anthrose moiety. Compound **2** lacks the methyl ether at C-2 and derivatives **3** and **4** contain modified C-4 amino functionalities of anthrose. We anticipated that compound **1** conjugated to bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH) would be attractive material for determining whether live or irradiated spores of *B. anthracis* Sterne $34F_2$ can induce an anti-carbohydrate antibody response, and derivatives **2-4** valuable to examine which chemical moieties of anthrose are critical for binding with antibodies.

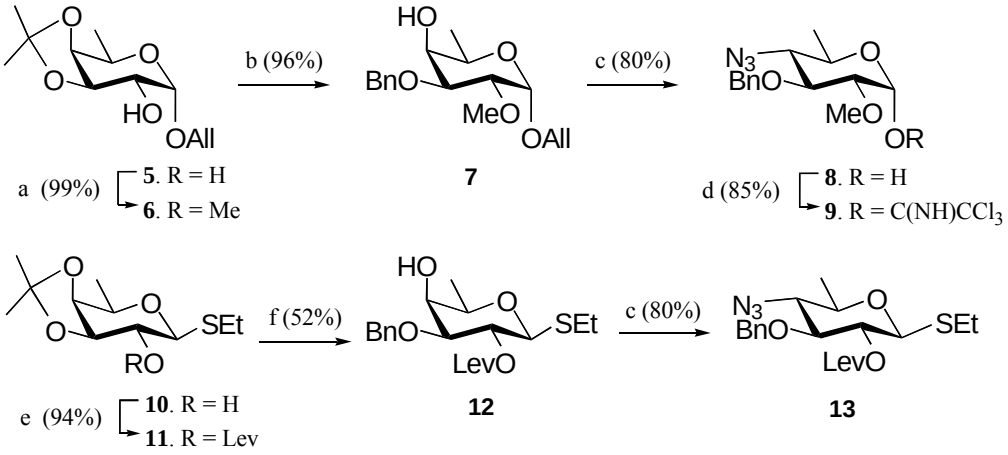
Compounds **1**-**4** were synthesized from monosaccharide precursors **14**, **15** and **9** or **13** (**Schemes 4.1 and 4.2**). Thus, glycosyl donor **14**[28] can be coupled with a benzyloxycarbonyl protected amino propyl spacer to give compound **16**, which immediately can be used in a subsequent glycosylation with rhamnoside **15** to give disaccharide **17**. After removal of the levulinoyl (Lev)

ester of **17**, the resulting glycosyl acceptor can be coupled with an appropriately protected anthrose donor. The benzoyl ester at C-2 of **15** will ensure that only α-glycosides will be obtained during glycosylation due to neighboring group participation.

The anthrose moieties of target compounds **1-4** are linked through a β-glycoside to the C-3 hydroxyl of the rhamnoside. Thus, an obvious strategy to introduce this moiety would be the use of a glycosyl donor which carries a selectively removable ester at C-2. At a late stage of the synthesis, this protecting group can be removed to reveal an alcohol, which can then be methylated. However, this strategy is complicated by the fact that the methylation has to be performed under neutral or mildly acidic conditions due to the presence of a number of base sensitive ester protecting groups. In general, such procedures provide relatively low yields of product, especially when applied to a complex compound. Alternatively, the methyl ether can be introduced at the monosaccharide stage by using strongly basic conditions; however, this approach may suffer from the formation of anomeric mixtures during the introduction of the anthrose glycoside. In order to examine both strategies, glycosyl donors **9** and **13** were prepared and coupled with glycosyl acceptor **18**. Compounds **9** and **13** contain an azido moiety at C-4, which at a late stage of the synthesis can be reduced to an amine and then acylated with different reagents to provide compounds **1-4**.

Glycosyl donor **9** was synthesized from selectively protected allyl 6-deoxygalactoside **5** (**Scheme 4.1**).[29] Thus, methylation of the C-2 hydroxyl of **5** could easily be accomplished by treatment of **5** with methyl iodide in the presence of sodium hydride to give compound **6** in a yield of 99 %. The 3,4-*O*-isopropylidene acetal of **6** could easily be removed by using aqueous acetic acid to give a diol, which was selectively benzylated at C-3 to give compound **7**, by first stannylene acetal formation by reaction with dibutyltin oxide in refluxing methanol followed by

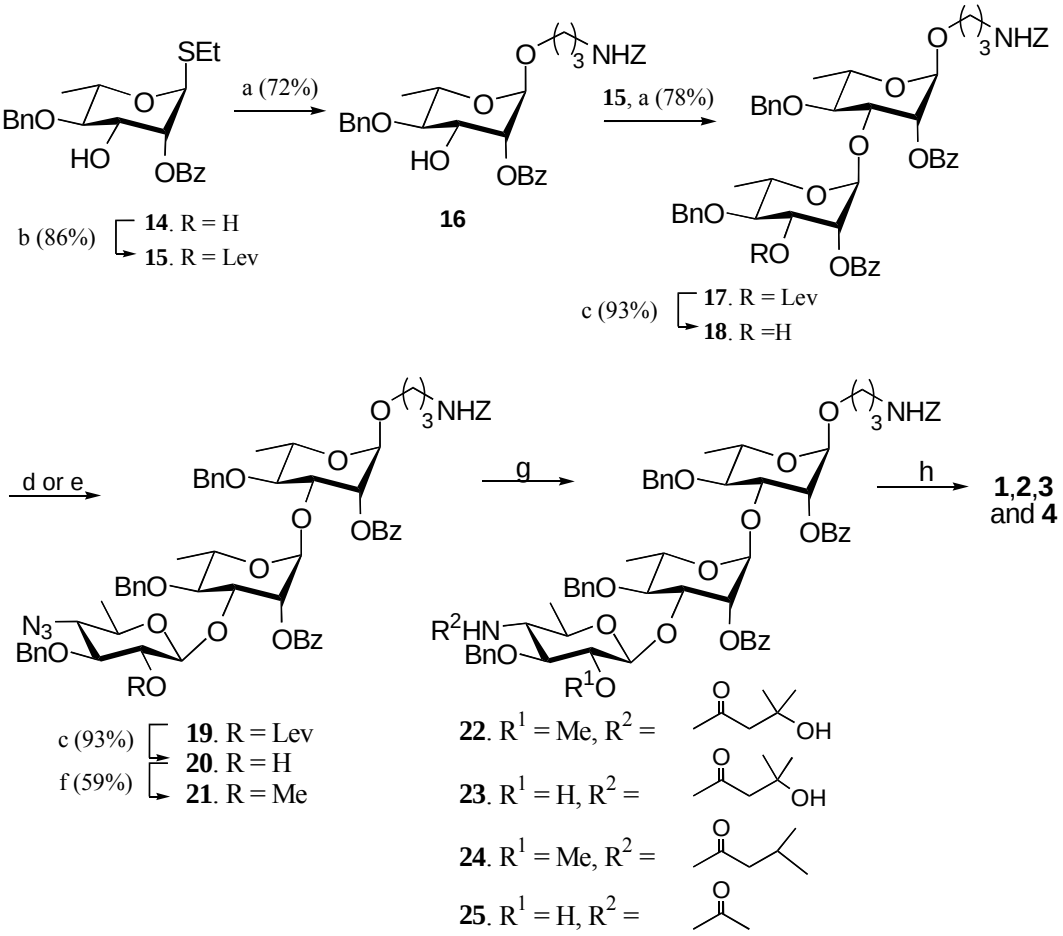
treatment with benzyl bromide and CsF in DMF.[30,31] Next, an azido group was introduced at C-4 with inversion of configuration to give compound **8** by conversion of the hydroxyl of **7** into a triflate by reaction with triflic anhydride and pyridine followed by displacement with sodium azide in DMF.[32] Fully protected **8** was converted into trichloroacetimidate **9** by removal of the anomeric allyl ether by treatment with $PdCl_2$ and NaOAc followed by reaction of the resulting lactol with trichloroacetonitrile in the presence of 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU).[33,34]



Scheme 4.1. Reagents and conditions: a) MeI, NaH, DMF, RT; b) 1) 60% HOAc in H_2O, 90 ℃, 2) Bu_2SnO, MeOH, reflux, 3) CsF, BnBr, DMF, RT; c) 1) Tf_2O, pyridine, CH_2Cl_2, 0 ℃, 2) NaN_3, DMF, 40 ℃; d) 1) $PdCl_2$, NaOAc, 90% HOAc/H_2O, RT, 2) trichloroacetonitrile, DBU, CH_2Cl_2, RT; e) levulinic acid, DCC, DMAP, CH_2Cl_2, RT; f) 1) 60% aq. HOAc, 90 ℃, 2) Bu_2SnO, toluene, reflux, 3) Bu_4NBr, BnBr, toluene, reflux.

Glycosyl donor **13** was synthesized from known thioglycoside **10**.[35] Thus, a levulinoyl (Lev) ester at C-2 of compound **10** was installed by treatment with levulinic acid, 1,3-dicyclohexylcarbodiimide (DCC), and 4-(dimethylamino)pyridine (DMAP) in CH_2Cl_2 to give compound **11** in excellent yield.[36] Next, the isopropylidene acetal of **11** was removed by treatment with aqueous acetic acid to give the corresponding diol. Attempts to selectively benzylate the C-3 hydroxyl of this compound by intermediate stannylene acetal formation, using conditions described for the preparation of **7**, gave **12** in a low yield due to cleavage of the Lev

ester. However, a moderate yield of **12** was obtained when the stannene acetal formation was performed by refluxing the diol and dibutyltin oxide in toluene followed by treatment with benzyl bromide and tetrabutylammonium bromide (Bu_4NBr). Finally, triflation of **12** followed by nucleophilic displacement with sodium azide gave the required thioglycosyl donor **13**.



Scheme 4.2. Reagents and conditions: a) $HO(CH_2)_3NHZ$, NIS, TfOH, CH_2Cl_2, 0 ℃; b) levulinic acid, DCC, DMAP, CH_2Cl_2, RT; c) $NH_2NH_2{\cdot}HOAc$, MeOH, CH_2Cl_2, RT; d) **13**, NIS, TfOH, CH_2Cl_2, 0 ℃, 76%; e) 9, BF3·Et2O, MeCN, -40 ℃, 86% α/β 1:4; f) MeI, Ag_2O, Me_2S, THF, RT; g) 1) 1,3-propanedithiol, TEA, pyridine, H_2O, 2) for **22**, **23**, **24**, HOAt, HATU, DIPEA, RT, 61–76%, for **25**, Ac_2O, pyridine, RT, **22**: 63%, **23**: 78%, **24**: 61%, **25**: 66%; h) 1) NaOMe, MeOH, RT, 2) Pd/C, H_2 (g), *t*BuOH/H_2O/AcOH 40:1:1, RT, **1**: 98%, **2**: 96%, **3**: 94 %, **4**: 92%.

Next, attention was focused on the preparation of rhamnosyl acceptor **18** and installment of the anthrose moiety. Thus, an *N*-iodosuccinimide/trifluoromethanesulfonic acid (NIS/TfOH) mediated glycosylation[37] of thioglycosyl donor **14** with benzyloxycarbonyl protected

aminopropanol gave spacer modified **16** as only the α-anomer. No self-condensation of **14** was observed due to a much higher glycosyl acceptor reactivity of *N*-benzyloxycarbonylaminopropanol. Compound **16** was immediately used in a second glycosylation with glycosyl donor **15**, using NIS/TfOH as the activator to give disaccharide **17** in a good yield. Next, the levulinoyl ester of **17** was selectively removed by treatment with hydrazine acetate,[36] to afford glycosyl acceptor **18** in a yield of 93 %. Coupling of trichloroacetimidate **9** with **18** in the presence of $BF_3 \cdot Et_2O$ in acetonitrile at -40 ℃ gave trisaccharide **21** in a good yield (86 %) as a 1:4 mixture of α/β-anomers. In this case, the modest β-selectivity was achieved by the formation of an intermediate α-nitrilium ion.[38,39] Anomerically pure **22** was obtained after reduction of the azido group of **21** to give an amine, which was acylated with 3-hydroxy-3-methyl-butyric acid using *O*-(7-azabenzotriazol-1-yl-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate/1-hydroxy-7-Azabenzotriazole/diisopropylethylamine (HATU/HOAt/DIPEA) as the activating reagent.

As expected, an NIS/TfOH mediated coupling of thioglycosyl donor **13** with acceptor **18** gave trisaccharide **19** as only the β-anomer due to the neighboring group participating Lev ester at C-2. The Lev group of **19** was selectively removed by treatment with hydrazine acetate[36] and the hydroxyl of the resulting trisaccharide **20** was methylated by treatment with methyl iodide and freshly prepared Ag_2O in the presence of dimethyl sulfide. Despite a prolonged reaction time, the product was obtained in a modest yield of 51 %. Thus, the advantage of using glycosyl donor **13** in trisaccharide formation was off-set by a low yielding methylation reaction.

Reduction of the C-4'' azido moiety of **21** followed by the coupling with 3-hydroxy-3-methyl-butyric acid gave compound **22**. Deprotection of **22** could easily be accomplished by a two-step procedure entailing removal of the benzoyl esters using sodium methoxide in methanol, followed

by cleavage of the benzyl ethers and benzyloxycarbamate by hydrogenation over Pd/C in a mixture of *tert*-butanol/water/acetic acid.

Analogue **2**, lacking a methyl ether at C-2 of anthrose, was prepared by reduction of the azido group of **20** followed by introduction of the 3-hydroxy-3-methyl-butyric acid moiety and deprotection using standard procedures. Compounds **3** and **4** were obtained by reduction of the azido moiety of **21** followed by acylation of the resulting amine using appropriate reagents to give compounds **24** and **25**, which were deprotected using standard procedures.

Preparation of carbohydrate-protein conjugates:

Trisaccharide **1** was linked to the carrier protein mariculture Keyhole Limpet Hemocyanin (mcKLH; Pierce Biotechnology, Rockford, IL) for immunological evaluation. To this end, the amino functionality of trisaccharide **1** was derivatized with an acetyl thioacetic acid moiety by reaction with *S*-acetylthioglycolic acid pentafluorophenyl ester to afford the corresponding thioacetate derivative, which after purification by size-exclusion chromatography, was directly de-*S*-acetylated using 7 % ammonia (g) in DMF just prior to conjugation. The de-*S*-acylation was performed under a strict argon atmosphere to prevent formation of the corresponding disulfide. KLH was activated with succinimidyl 3-(bromoacetamido) propionate (SBAP) in a sodium phosphate buffer (pH 7.2) containing 0.15 M sodium chloride and then purified by a centrifugal filter device with a nominal molecular-weight limit of 30 kDa. The bromoacetyl activated KLH (KLH-BrAc) was subsequently incubated with the thiolated trisaccharide in a 0.1 mM sodium phosphate buffer (pH 8.0) containing 5 mM ethylene diamine tetraacetate (EDTA). The afforded glycoconjugate (KLH-BrAc-**1**) carried 1042 copies of trisaccharide **1** per KLH molecule as determined by Lowry's protein concentration test and quantitative carbohydrate analysis by HPAEC-PAD. For the purpose of evaluating the binding specificity of antibodies

raised against the *B. anthracis* spores, the thiol derivative of trisaccharide **1** was conjugated to maleimide activated BSA (BSA-MI, Pierce Endogen, Inc.) in a phosphate buffer (pH 7.2) containing sodium azide and EDTA. After a reaction time of 2 h, the glycoprotein was purified using a centrifugal filter device with a nominal molecular weight cut-off of 10 kDa. The average number of trisaccharide copies per BSA molecule was determined to be 18:1. The same conjugation method and thiolated derivatives of trisaccharides **2**, **3**, and **4** were used to give the corresponding BSA-MI-**2**, BSA-MI-**3**, and BSA-MI-**4** glycoconjugates with a sacchride/protein ratio of 10:1, 9:1, and 4:1, respectively.

Antibody binding analyses:

To explore the immunogenicity of the saccharide moieties of BclA, rabbits were immunized four times at biweekly intervals with live or irradiated spores of *B. anthracis* Sterne $34F_2$. First, it was investigated whether the post-immune sera have the ability to recognize the synthetic anthrose-containing trisaccharide **1**. For this purpose, an ELISA was performed whereby microtiter plates were coated with the KLH-BrAc-**1** conjugate and serial dilutions of sera added. An anti-rabbit IgG antibody labeled with horseradish peroxidase was employed as a secondary antibody for colorimetric detection (OD, optical density). Binding was observed between the antisera and KLH-trisaccharide conjugate whereas no interaction was detected for native KLH, indicating that the saccharide epitopes of BclA are antigenic (**Figure 4.2a**). Rabbits immunized with irradiated Sterne$34F_2$ spores elicited lower but detectable titers of anti-saccharide antibodies. The fact that the irradiated spores elicited IgG antibodies indicates that the saccharide epitopes were not damaged during the irradiation process.

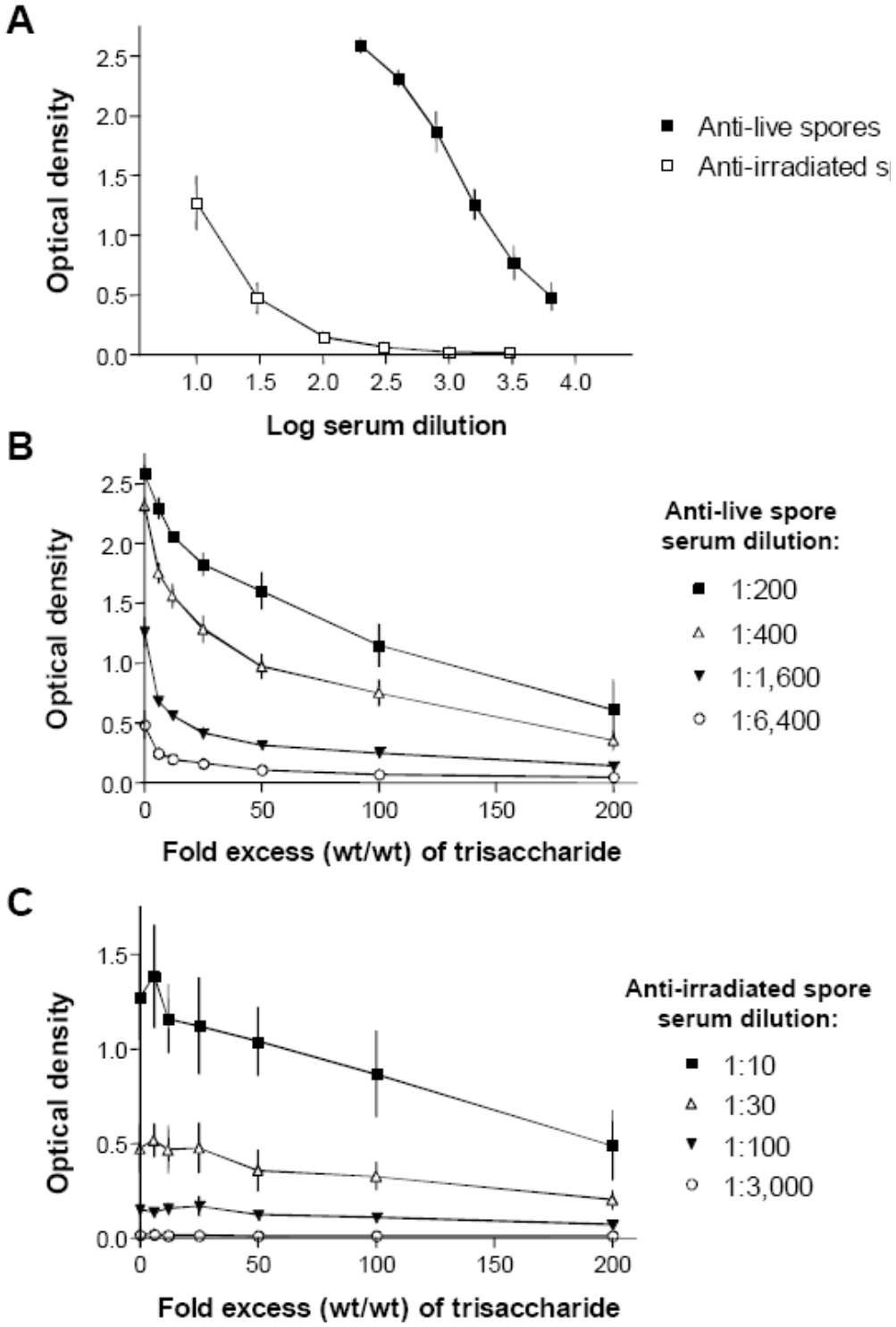


Figure 4.2. ELISA and competitive inhibition of anti-live and anti-irradiated spore anti-serum. Microtiter plates were coated with KLH-BrAc-**1** conjugate (0.5 mg per mL conjugate, corresponding to 0.03 mg per mL trisaccharide). Rabbit anti-live (1:200→1:6400 diluted) or anti-irradiated (1:10→1:3000 diluted) spore *B. antracis* Sterne 34F2 antiserum were applied to

coated microtiterplates (a). For the inhibition assay the serum was first mixed with free trisaccharide 1 (0–200-fold excess, wt/wt). Unspecific binding was tested with uncoated wells with 200-fold "excess" trisaccharide or 200-fold "excess" KLH (data not shown). The data are reported as the means ±SD of triplicate measurements.

Next, the specificity of the interaction of the antisera with the KLH-BrAc-**1** conjugate was further investigated using a competitive inhibition ELISA. Thus, microtiter plates were coated with the KLH-BrAc-**1** conjugate, and serial dilutions of antisera mixed with free trisaccharide **1** were added. As depicted in **Figure 4.2b**, a six-fold excess of trisaccharide **1** (as compared to a concentration of trisaccharide used for coating microtiter wells), resulted in a significant drop in OD at all serum dilutions tested. Also, increasing the excess of the competing trisaccharide **1** resulted in a further reduction in OD. It is evident that the inhibition is dose dependent, thus demonstrating that the interaction of the elicited antibodies with **1** is specific. The interaction of antisera from rabbits immunized with irradiated spores with **1** could also be inhibited in a dose response manner (**Figure 4.2c**).

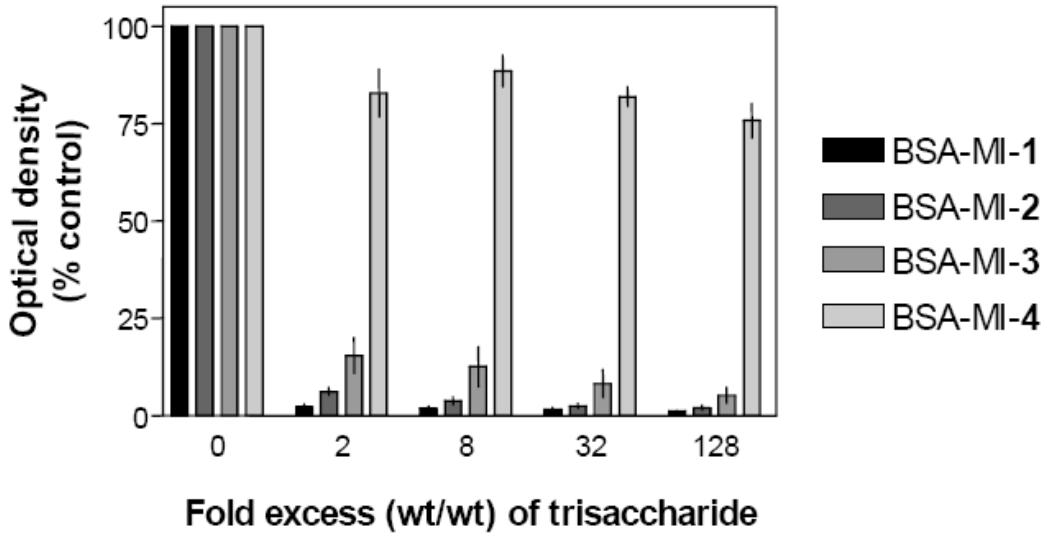


Figure 4.3. Competitive inhibition of anti-live spore antiserum binding to synthetic anthrose-containing trisaccharide by synthetic analogue conjugates. Microtiterplates were coated with KLH-BrAc-**1** conjugate (0.5 mg per mL conjugate corresponding to 0.03 mg per mL trisaccharide). Rabbit anti-live spore *B. anthracis* Sterne $34F_2$ antiserum (1:1600 dilute) was first

mixed with BSA–trisaccharide conjugates (0–128-fold excess, wt/wt based on carbohydrate concentration) and then applied to the coated microtiter plate. Unconjugated BSA mixed with anti-serum did not have any effect (data not shown). OD values were normalized for the OD values obtained without BSA-trisaccharide conjugate (0-fold "excess", 100%). Non-specific binding was tested with uncoated wells containing antiserum and buffer (data not shown). The data are reported as the means ±SD of triplicate measurements.

Having established that Sterne $34F_2$ spores are able to induce an anti-carbohydrate antibody response, we sought to further evaluate which structural motifs of the anthrose moiety are critical for antibody recognition. To this end, the ability of BSA-MI-**1** and BSA-MI-conjugates of the three structural analogues **2**, **3**, and **4** to inhibit the interaction of the antisera with KLH-BrAc-**1** was determined (**Figure 4.3**). For these experiments, BSA conjugates were employed in an effort to conserve synthetic material. Microtiter plates were again coated with the KLH-BrAc-**1** conjugate and treated with an antisera dilution of 1:1600. The importance of the 2''-*O*-methyl ether of anthrose was established using the BSA-MI-**2** conjugate. This conjugate carries trisaccharide analogue **2**, which lacks the 2''-*O*-methyl ether but has an intact *N*-(3-hydroxy-3-methyl-butyryl) moiety at C-4''. As shown in Figure 3, this conjugate is a potent inhibitor of antibody binding with as low as a 2-fold weight excess eliciting >95 % reduction in reporter signal, compared to the BSA-MI-conjugate carrying the native trisaccharide **1**, for which no significant difference in inhibition was observed in the concentration range investigated. These data indicate that the methyl ether is not critical for anti-spore antibody binding. To elucidate the importance of the 3-hydroxy-3-methyl-butyryl moiety of anthrose, conjugates BSA-MI-**3** and BSA-MI-**4** were prepared. Trisaccharide **3** carries a 3-methyl-butyryl moiety at the C-4$^{\prime\prime\prime}$, thus only lacking the hydroxyl group of the native C-4-moiety of the anthrose monosaccharide, whereas trisaccharide **4** is *N*-acetylated at the C-4'', thus lacking most of the 3-hydroxy-3-methyl-butyryl moiety. Interestingly, a two-fold excess of trisaccharide **3** reduced OD by 85 % compared to the control. In contrast, a similar concentration of analogue **4** resulted

in reduction in OD of only 17 %. Very high concentrations of BSA-MI-**4** were required to achieve considerable inhibition (a 500-fold excess of BSA-MI-**4** resulted in a 50 % drop in OD, data not shown). These results indicate that the 4''-(3-methylbutyryl)-moiety is an important structural motif of the authentic saccharide epitope on the surface of *B. anthracis* Sterne spores.

Conclusion

We have successfully synthesized an anthrose-containing trisaccharide and a series of structurally related analogs to study the antigenicity of anthrose. The analogues lacked either the methyl ether at C-2 or contained modified C-4 amino functionalities of anthrose. The synthetic compounds were equipped with an aminopropyl spacer to facilitate conjugation to the carrier proteins mariculture Keyhole Limpet Hemocyanin (mcKLH) and bovine serum albumin (BSA). Serum antibodies of rabbits immunized with live or irradiated spores of *B. anthracis* Sterne $34F_2$ were able to recognize the synthetic trisaccharide-mcKLH conjugate. The specificity of the interaction was confirmed by competitive inhibition with the free- and BSA-conjugated trisaccharides. Inhibition using the trisaccharide analogues demonstrated that the antigenic nature of the trisaccharide can be altered by modification of specific side groups in the terminal glycosyl structure and the isovaleric acid moiety of anthrose is an important structural motif for antibody recognition. These data demonstrate that 1) anthrose is a specific antigenic determinant of the *B. anthracis* Sterne spore and serum of rabbits immunized by live or irradiated spores of *B. anthracis* Sterne $34F_2$ recognize the trisaccharide **1**, which is derived from the glycoprotein BclA; 2) this antigen is presented to the immune system of rabbits receiving the anthrax live-spore vaccine; 3) synthetic analogues of the oligosaccharide retain the antigenic structure; and 4) the antigenic region is localized to specific terminal groups of the oligosaccharide. Collectively these data provide an important proof-of-concept step in the synthesis and development of

spore-specific reagents for detection and targeting of non-protein structures in *B. anthracis*.

The first significance of the observations is that, by using anti-live spore antisera and anti-irradiated spore antisera, the anthrose-containing trisaccharide of BclA is antigenic and exposed on the surface of *B. anthracis* Sterne $34F_2$ spores when presented in rabbits. The second is that we have located an important antigenic component of this reactivity in the terminal 3-methyl-butyryl structures of the saccharide and confirmed its specificity using synthetic saccharide analogues. These data provide an important proof-of-concept step in the development of spore-specific reagents for detection and targeting of non-protein structures in *B. anthracis*. These structures may in turn provide a foundation for directing immune responses to spore structures during the early stages of the *B. anthracis* infection process. Seeberger and co-workers reported that the anthrax oligosaccharide conjugated to KLH could elicit antibodies that recognize *B. anthracis* spores.[40] Our data are complementary to these findings in that *B. anthracis* spores elicit anti-carbohydrate antibodies, which may be harnessed for diagnosis. Ongoing studies will demonstrate whether these and additional saccharide structures are present and accessible on the spores from other *B. anthracis* isolates, including the highly virulent *B. anthracis* Ames and other B. *anthracis* cured of virulence plasmids pXO1 and pXO2.

Experimental Section

General:

^{1}H NMR spectra were recorded in $CDCl_3$ or D_2O on Varian Merc-300 or Varian Inova-500 spectrometers equipped with Sun workstations at 300 K. TMS (δ_H 0.00) or D_2O (δ_H 4.67) was used as the internal reference. ^{13}C NMR spectra were recorded in $CDCl_3$ or D_2O at 75 MHz on a Varian Merc-300 spectrometer, respectively, using the central resonance of $CDCl_3$ (δ_C 77.0) as the internal reference. COSY, HSQC, HMBC and TOCSY experiments were used to assist signal

assignment of the spectra. The different monosaccharide units are referred to as a, b, c, and d, respectively, with a denoting the reducing end monosaccharide. Mass spectra were obtained on Applied Biosystems Voyager DE-Pro MALDI-TOF (no calibration) and Bruker Daltonics 9.4T (FTICR, external calibration with BSA). Optical rotatory power was obtained on Jasco P-1020 polarimeter at 300 K.

Chemicals were purchased from Aldrich or Fluka and used without further purification. CH_2Cl_2, acetonitrile and toluene were distilled from calcium hydride; THF from sodium; and MeOH from magnesium and iodine. Mariculture keyhole limpet hemocyanin (mcKLH), maleimide activated bovine serum albumin (BSA-MI), and succinimidyl 3-(bromoacetamido)propionate (SBAP) were purchased from Pierce Endogen, Rockford, IL. Aqueous solutions are saturated unless otherwise specified. Molecular sieves were activated at 350 ℃ for 3 h in vacuo. All reactions were performed under anhydrous conditions under argon and monitored by TLC on Kieselgel 60 F254 (Merck). Detection was by examination under UV light (254 nm) and by charring with 10 % sulfuric acid in methanol. Silica gel (Merck, 70-230 mesh) was used for chromatographies. Iatrobeads 6RS-8060 was purchased from Bioscan.

General procedure for levulination:

A solution of DCC (6 equiv) and DMAP (0.015 equiv) in CH_2Cl_2 was added under argon to a solution of **10** or **14** (1 equiv) and levulinic acid (10 equiv) in CH_2Cl_2 (at a concentration of 0.06 mol saccharide per L). The reaction mixture was stirred at room temperature for 2 h, and then filtered through Celite. The filtrate was washed twice with water. The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the crude product by column chromatography on silica gel afforded the desired product **11** or **15**.

General procedure for isopropylidene removal:

A solution of **6** or **11** (1 equiv) in acetic acid/water (3:2, at a concentration of 0.5 mol saccharide per L) was heated under reflux at 90 ℃ for 15 min, and then concentrated to dryness. The residue was co-distilled with toluene twice. Purification of the crude product by column chromatography on silica gel afforded the desired diol product.

General procedure for introduction of the C-4 azide group:

Trifluoromethanesulfonic anhydride (1.5 equiv) was added slowly at 0 ℃ to a solution of **7** or **12** (1 equiv) in pyridine (10 equiv) and dry CH_2Cl_2 (at a concentration of 0.2 mol saccharide per L). The reaction mixture was stirred at 0 ℃ for 1 h, and then diluted with CH_2Cl_2. The solution was washed with H_2O and saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. To a solution of this residue in DMF (at a concentration of 0.08 mol saccharide per L) was added sodium azide (5 equiv). The reaction mixture was stirred at 40 ℃ overnight, and then concentrated to dryness. The residue was dissolved in ethyl acetate, and the solution was washed with saturated $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the crude product by column chromatography on silica gel afforded the desired product **8** or **13**.

General procedure for cleavage of the levulinoyl ester:

A solution of hydrazine acetate (1 equiv) in dry MeOH (0.4 mol L^{-1}) was added under argon to a solution of **17** or **19** (1 equiv) in dry CH_2Cl_2 (at a concentration of 0.04 mol saccharide per L). The reaction mixture was stirred at room temperature for 4 h, and then concentrated to dryness. The residue was dissolved in CH_2Cl_2, and then washed with water. The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the crude product by column chromatography on silica gel afforded the desired product **18** or **20**.

General procedure for azide reduction and introduction of C-4'' moiety:

TEA (15 equiv) was added to a solution of **20** or **21** (1 equiv) and 1, 3-propanedithiol (20 equiv) in pyridine (at a concentration of 0.014 mol saccharide per L) and H_2O (at a concentration of 0.1 mol saccharide per L). The reaction mixture was stirred at room temperature overnight, and then concentrated to dryness. The residue was co-evaporated with toluene twice and ethanol twice. Purification of the crude product by column chromatography on silica gel (CH_2Cl_2/MeOH/TEA 100:5:1) afforded the free amine compounds. α-Hydroxyisovaleric acid or isovaleric acid (2 equiv) was activated by HOAt (4 equiv) and HATU (4 equiv) in DMF (at a concentration of 0.01 mol saccharide per L) for 1 h, and then DIPEA (8 equiv) was added. The resulting yellow solution was added dropwise to the free amine compound (1 equiv) in DMF (at a concentration of 0.02 mol saccharide per L). The reaction mixture was stirred at room temperature for 4 h, and then concentrated to dryness. Purification of the crude product by column chromatography on silica gel afforded the desired product **22**, **23** or **24**. Alternatively, a solution of free amine (1 equiv) in Ac_2O (2 equiv), pyridine (2 equiv) and DMAP (0.1 equiv) was stirred at room temperature overnight, and then concentrated to dryness. The residue was co-evaporated with toluene twice. Purification of the crude product by column chromatography on silica gel afforded the desired product **25**.

General precedure for global deprotection:

NaOMe (pH 8-10) was added to a solution of **22**, **23**, **24** or **25** in dry MeOH (at a concentration of 0.06 mol saccharide per L). The reaction mixture was stirred at room temperature overnight, and then neutralized by the addition of Dowex 650 H^+. The suspension was filtered through Celite, and washed with MeOH/CH_2Cl_2 1:1. The combined filtrates were concentrated to dryness. Purification of the crude product by column chromatography on silica gel afforded the desired deacetylated product. To a solution of the partially deprotected compound in

tert-butanol/H_2O/AcOH (40:1:1, 0.01 mol L^{-1}) was added Pd/C (cat.) under an atmosphere of hydrogen. The reaction mixture was stirred at room temperature overnight, and then filtered through Celite. The filtrate was concentrated to dryness. Purification of the crude product by Iatro beads afforded the desired product **1-4**.

Allyl 3,4-*O*-isopropylidene-2-*O*-methyl-α-D-fucopyranoside (6):

NaH (3.25 g, 67.63 mmol, 50 % in mineral oil) was added to a solution of 5 (8.26 g, 33.81 mmol) in DMF (90 mL). The reaction mixture was stirred at 0℃ for 1 h, and then methyl iodide (4.21 mL, 67.62 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 6 h, and then poured into ice water. The solution was extracted with CH_2Cl_2 (100 mL) and washed with water (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the crude product by column chromatography on silica gel (hexane/EtOAc 4:1) afforded the desired product 6 as colorless oil (8.66 g, 99 %). R_f=0.74 (hexane/EtOAc 2:1); $[\alpha]_D^{27}$=+67.7 (c=3.6 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.26 (d, 3 H, $J_{5,6}$=6.3 Hz, H-6), [1.29, 1.47, 2 ×s (CH_3CCH_3)], 3.30 (dd, 1 H, $J_{1,2}$=3.6, $J_{2,3}$=8.1 Hz, H-2), 3.44 (s, 3 H, OCH_3), 3.94-4.10 (m, 3 H, H-4, H-5, OCH_2CHCH_2), 4.14 (dd, 1 H, J=5.4, 12.9 Hz, OCH'_2CHCH_2), 4.18 (dd, 1 H, $J_{2,3}$=8.1, $J_{3,4}$=5.7 Hz, H-3), 4.88 (d, 1 H, $J_{1,2}$=3.6 Hz, H-1), 5.16 (dd, 1 H, J=1.2, 10.2 Hz, OCH_2CHCH_2), 5.28 (dd, 1 H, J=1.5, 17.1 Hz, $OCH_2CHCH'_2$), 5.87 (m, 1 H, OCH_2CHCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 16.2 (C-6), [26.3, 28.3 (CH_3CCH_3)], 58.5 (OCH_3), 63.1 (C-5), 68.2 (OCH_2CHCH_2), 75.7 (C-4), 76.0 (C-3), 79.1 (C-2), 95.3 (C-1), 108.7 (CH_3CCH_3), 117.9 (OCH_2CHCH_2), 133.6 (OCH_2CHCH_2); MALDI-TOF/MS: m/z: calcd for $C_{13}H_{22}O_5Na$: 281.1365; found: 281.7 $[M+Na]^+$.

Allyl 3-*O*-benzyl-2-*O*-methyl-α-D-fucopyranoside (7):

Treatment of **6** (8.66 g, 33.53 mmol) in acetic acid/water (40.2 mL/26.8 mL) as described in the general procedures gave the diol as a white solid (7.39 g, 33.86 mmol, quantitative). R_f=0.30 (CH_2Cl_2/MeOH 19:1); $[\alpha]_D^{27}$=+4.9 (*c*=2.5 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.21 (d, 3 H, $J_{5,6}$=6.6 Hz, H-6), 2.56 (s, 1 H, OH), 3.40 (s, 3 H, OC*H*$_3$), 3.47 (dd, 1 H, $J_{1,2}$=3.0, $J_{2,3}$=9.6 Hz, H-2), 3.75 (s, 1 H, H-4), 3.89-3.97 (m, 2 H, H-3, H-5), 4.00 (dd, 1 H, *J*=6.3, 12.6 Hz, OC*H*$_2$CHCH$_2$), 4.14 (dd, 1 H, *J*=3.6, 12.9 Hz, OC*H'*$_2$CHCH$_2$), 4.99 (d, 1 H, $J_{1,2}$=3.0 Hz, H-1), 5.16 (d, 1 H, *J*=10.5 Hz, OCH$_2$CHC*H*$_2$), 5.28 (d, 1 H, *J*=17.1 Hz, OCH$_2$CHC*H'*$_2$), 5.87 (m, 1 H, OCH$_2$C*H*CH$_2$); ^{13}C NMR (75 MHz, $CDCl_3$): δ=16.1 (C-6), 57.7 (OCH_3), 65.6 (C-5), 68.2 (O*C*H$_2$CHCH$_2$), 69.4 (C-3), 71.5 (C-4), 77.9 (C-2), 94.5 (C-1), 117.9 (OCH$_2$CH*C*H$_2$), 133.8 (OCH$_2$*C*HCH$_2$); MALDI-TOF/MS: *m*/*z*: calcd for $C_{10}H_{18}O_5Na$: 241.1052; found: 241.7 [*M*+Na]$^+$.

Dibutyltin oxide (8.43 g, 33.86 mmol) was added to a solution of the diol (7.39 g, 33.8 mmol) in dry MeOH (300 mL). The reaction mixture was heated under reflux until the solution became clear. After cooling to room temperature, the reaction mixture was concentrated to dryness. Benzyl bromide (4.0 mL, 33.86 mmol) and CsF (5.15 g, 33.86 mmol) were added to a solution of the residue in DMF (130 mL). The reaction mixture was stirred at room temperature overnight, and then concentrated to dryness. The residue was dissolved in CH_2Cl_2 (100 mL), and the solution was washed with H_2O (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the crude product by column chromatography on silica gel (hexane/EtOAc 3:1) afforded the desired product **7** as colorless oil (10.03 g, 32.53 mmol, 96 %). R_f=0.34 (hexane/EtOAc 2:1); $[\alpha]_D^{27}$=+86.6 (*c*=2.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ= 1.21 (d, 3 H, $J_{5,6}$=6.5 Hz, H-6), 3.45 (s, 3 H, OC*H*$_3$), 3.55 (dd, 1 H, $J_{1,2}$=3.5, $J_{2,3}$=9.5 Hz, H-2), 3.59-3.78 (m, 2 H, H-3, H-5), 3.86 (dd, 1 H, $J_{2,3}$=7.0, $J_{3,4}$=7.0 Hz, H-4), 3.99 (dd, 1 H, *J*=7.0,

13.0 Hz, OCH_2CHCH$_2$), 4.12 (dd, 1 H, *J*=5.5, 13.0 Hz, OCH'_2CHCH$_2$), 4.61 (d, 1 H, *J*=12.0 Hz, PhCH_2), 4.72 (d, 1 H, *J*=12.0 Hz, PhCH'_2), 4.94 (d, 1 H, $J_{1,2}$=3.5 Hz, H-1), 5.15 (d, 1 H, *J*=10.5 Hz, OCH$_2$CHCH_2), 5.26 (d, 1 H, *J*=17.0 Hz, OCH$_2$CHCH''_2), 5.89 (m, 1 H, OCH$_2$CHCH$_2$), 7.19-7.29 (m, 5 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 16.1 (C-6), 58.9 (OCH$_3$), 65.3 (C-4), 68.2 (OCH$_2$CHCH$_2$), 70.2 (C-3), 72.7 (PhCH$_2$), 77.5 (C-2), 77.9 (C-5), 95.5 (C-1), 117.9 (OCH$_2$CHCH$_2$), [127.7, 127.8, 128.4, 133.9 (C_{arom})], 138.3 (OCH$_2$$CHCH_2$); MALDI-TOF/MS: *m*/*z*: calcd for $C_{17}H_{24}O_5Na$: 331.1521; found: 331.2 [*M*+Na]$^+$.

Allyl 4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-α-D-glucopyranoside (8):

Treatment of **7** (10.03 g, 32.53 mmol) in pyridine (28.6 mL, 0.33 mol) and CH_2Cl_2 (160 mL) with trifluoromethanesulfonic anhydride (8.2 mL, 48.66 mmol) followed by treatment of triflate residue in DMF (400 mL) with sodium azide (10.40 g, 0.16 mol) was performed according to the general procedure to give compound **8** as colorless oil (8.67 g, 80 %). R_f=0.41 (hexane/EtOAc 5:1); $[\alpha]_D^{27}$=+130.5 (*c*=2.4 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ= 1.30 (d, 3 H, $J_{5,6}$=6.5 Hz, H-6), 3.02 (t, 1 H, $J_{3,4}$=9.0, $J_{4,5}$=10.0 Hz, H-4), 3.27 (dd, 1 H, $J_{1,2}$=3.5, $J_{2,3}$=9.5 Hz, H-2), 3.44 (s, 3 H, OCH_3), 3.52 (m, 1 H, H-5), 3.72 (t, 1 H, $J_{2,3}$=9.5, $J_{3,4}$=9.0 Hz, H-3), 3.98 (dd, 1 H, *J*=7.0, 13.0 Hz, OCH_2CHCH$_2$), 4.12 (dd, 1 H, *J*=5.0, 13.0 Hz, OCH'_2CHCH$_2$), 4.72 (d, 1 H, *J*=10.5 Hz, PhCH_2), 4.84 (d, 1 H, *J*=10.5 Hz, PhCH'_2), 4.89 (d, 1 H, $J_{1,2}$=3.5 Hz, H-1), 5.18 (d, 1 H, *J*=10.5 Hz, OCH$_2$CHCH_2), 5.28 (d, 1 H, *J*=17.5 Hz, OCH$_2$CHCH'_2), 5.87 (m, 1 H, OCH$_2$CHCH$_2$), 7.19-7.35 (m, 5 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 18.4 (C-6), 58.7 (OCH$_3$), 66.1 (C-5), 67.9 (C-4), 68.2 (OCH$_2$CHCH$_2$), 75.5 (PhCH$_2$), 79.8 (C-3), 82.3 (C-2), 94.8 (C-1), 118.3 (OCH$_2$CHCH$_2$), [127.8, 128.2, 128.4, 133.6 (C_{arom})], 138.2 (OCH$_2$$CHCH_2$); MALDI-TOF MS: *m*/*z*: calcd for $C_{17}H_{23}N_3O_4Na$: 356.16; found: 356.7 [*M*+Na]$^+$.

Ethyl 3,4-*O*-isopropylidene-2-*O*-levulinoyl-1-thio-β-D-fucopyranoside (11):

Treatment of **10** (1.34 g, 5.40 mmol) and levulinic acid (5.53 mL, 54.00 mmol) in CH_2Cl_2 (90 mL) with DCC (6.69 g, 32.42 mmol) and DMAP (9.90 mg, 0.081 mmol) in CH_2Cl_2 (9 mL) according to the general procedure gave compound **11** as colorless oil (1.76 g, 94 %). R_f=0.71(hexane/EtOAc 1:1); $[\alpha]_D^{27}$=+1.3 (*c*=0.7 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ= 1.19 (t, 3 H, *J*=7.5 Hz, SCH_2CH_3), 1.28 (s, 3 H, CH_3), 1.35 (d, 3 H, $J_{5,6}$=7.0 Hz, H-6), 1.49 (s, 3 H, CH'$_3$), 2.12 (s, 3 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.53-2.78 (m, 6 H, $CH_3C(O)CH_2CH_2C(O)O$, SCH_2CH_3, $CH_3C(O)CH_2CH_2C(O)O$), 3.78-3.82 (m, 1 H, H-5), 3.98 (dd, $J_{3,4}$=5.5, $J_{4,5}$=2.5 Hz, H-4), 4.06 (dd, 1 H, $J_{2,3}$=7.5, $J_{3,4}$=5.5 Hz, H-3), 4.25 (d, 1 H, $J_{1,2}$=10.0 Hz, H-1), 4.92 (dd, 1 H, $J_{1,2}$=10.0, $J_{2,3}$=7.5 Hz, H-2); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 14.7 (SCH_2CH_3), 16.8 (C-6), [23.8, 26.4 (CH_3)], [27.8, 28.1 ($CH_3C(O)CH_2CH_2C(O)O$, SCH_2CH_3)], 29.8 ($CH_3C(O)CH_2CH_2C(O)O$), 38.0 ($CH_3C(O)CH_2CH_2C(O)O$), 71.8 (C-2), 72.7 (C-5), 76.4 (C-4), 77.2 (C-3), 82.2 (C-1), 171.7 ($CH_3C(O)CH_2CH_2C(O)O$), 206.3 ($CH_3C(O)CH_2CH_2C(O)O$); MALDI-TOF/MS: *m*/*z*: calcd for $C_{16}H_{26}O_6SNa$: 369.13; found: 369.5 $[M+Na]^+$.

Ethyl 3-*O*-benzyl-2-*O*-levulinoyl-1-thio-β-D-fucopyranoside (12):

Treatment of **11** (1.75 g, 5.05 mmol) in acetic acid/water (6.0 mL/4.0 mL) according to the general procedure for isopropylidene removal gave the diol as a white solid (1.55 g, quantitative). R_f=0.38(CH_2Cl_2/MeOH 19:1); $[\alpha]_D^{27}$=-3.5 (*c*=1.2 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.19 (t, 3 H, *J*=7.5 Hz, SCH_2CH_3), 1.28 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6), 2.13 (s, 3 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.51-2.86 (m, 6 H, $CH_3C(O)CH_2CH_2C(O)O$, SCH_2CH_3, $CH_3C(O)CH_2CH_2C(O)O$), 3.57-3.68 (m, 2 H, H-3, H-5), 3.75 (d, *J*=2.7 Hz, H-4), 4.32 (d, 1 H, $J_{1,2}$=9.9 Hz, H-1), 4.98 (t, 1 H, $J_{1,2}$=9.9, $J_{2,3}$=9.3 Hz, H-2); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.8 (SCH_2CH_3), 16.6 (C-6), 23.7 (SCH_2CH_3), 28.2 ($CH_3C(O)CH_2CH_2C(O)O$), 29.8 ($CH_3C(O)CH_2CH_2C(O)O$), 38.4 ($CH_3C(O)CH_2CH_2C(O)O$), [71.5, 71.8 (C-2, C-4)], 73.8 (C-3),

74.7 (C-5), 82.7 (C-1), 172.7 ($CH_3C(O)CH_2CH_2\mathit{C}(O)O$), 206.3 ($CH_3\mathit{C}(O)CH_2CH_2C(O)O$); MALDI-TOF/MS: *m*/*z*: calcd for $C_{13}H_{22}O_6SNa$: 329.10; found: 330.2 $[M+Na]^+$.

Dibutyl tin oxide (1.26 g, 5.06 mmol) was added to a solution of the diol (1.55 g, 5.06 mmol) in dry toluene (50 mL). The reaction mixture was heated under reflux with a Dean-Stark apparatus for 3 h, and then cooled to 60 ℃. Benzyl bromide (0.60 mL, 5.06 mmol) and tetrabutylammonium iodide (1.68 g, 5.06 mmol) were added and the resulting reaction mixture was heated under reflux for 3 h. After cooling to room temperature, the reaction mixture was concentrated to dryness. The residue was dissolved in EtOAc (50 mL), and the resulting solution was washed with H_2O (50 mL). The organic layer was dried ($MgSO_4$) filtered, and concentrated to dryness. Purification of the crude product by column chromatography on silica gel (hexane/EtOAc 2:1) afforded the desired product **12** as colorless oil (1.04 g, 52 %). R_f=0.43 (hexane/EtOAc 1:1); $[\alpha]_D^{27}$=-4.0 (*c*=0.8 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.16 (t, 3 H, *J*=7.5 Hz, $SCH_2C\mathit{H_3}$), 1.28 (d, 3 H, $J_{5,6}$=6.3 Hz, H-6), 2.12 (s, 3 H, $C\mathit{H_3}C(O)CH_2CH_2C(O)O$), 2.48-2.76 (m, 6 H, $CH_3C(O)CH_2C\mathit{H_2}C(O)O$, $SC\mathit{H_2}CH_3$, $CH_3C(O)C\mathit{H_2}CH_2C(O)O$), 3.43-3.54 (m, 2 H, H-3, H-5), 3.75 (d, *J*=3.0 Hz, H-4), 4.23 (d, 1 H, $J_{1,2}$=9.9 Hz, H-1), 4.57 (d, 1 H, *J*=12.0 Hz, $PhC\mathit{H_2}$), 4.61 (d, 1 H, *J*=11.1 Hz, $PhC\mathit{H'_2}$), 5.14 (t, 1 H, $J_{1,2}$=9.6, $J_{2,3}$=9.6 Hz, H-2), 7.19-7.31 (m, 5 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.7 ($SCH_2\mathit{C}H_3$), 16.6 (C-6), 23.4 ($S\mathit{C}H_2CH_3$), 28.1 ($CH_3C(O)CH_2\mathit{C}H_2C(O)O$), 29.9 ($\mathit{C}H_3C(O)CH_2CH_2C(O)O$), 37.9 ($CH_3C(O)\mathit{C}H_2CH_2C(O)O$), [69.1, 69.2 (C-2, C-4)], 71.7 ($Ph\mathit{C}H_2$), 74.5 (C-3), 79.7 (C-5), 82.9 (C-1), [127.9, 128.1, 128.5, 137.5 (C_{arom})], 171.7 ($CH_3\mathit{C}(O)CH_2CH_2C(O)O$), 206.3 ($CH_3C(O)CH_2CH_2\mathit{C}(O)O$); MALDI-TOF/MS: *m*/*z*: calcd for $C_{20}H_{28}O_6SNa$: 419.15; found: 419.5 $[M+Na]^+$.

Ethyl 4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-levulinoyl-1-thio-β-D-glucopyranoside (13):

Treatment of **12** (0.50 g, 1.26 mmol) in pyridine (1.0 mL, 12.61 mmol) and CH_2Cl_2 (6.5 mL) with trifluoromethanesulfonic anhydride (0.32 mL, 1.90 mmol) followed by treatment of triflate residue in DMF (16 mL) with sodium azide (0.41 g, 6.31 mmol) according to the general procedure for introduction of the C-4 azide group gave compound **13** as colorless oil (0.42 g, 79 %). R_f=0.32 (hexane/EtOAc 4:1); $[\alpha]_D^{27}$=+13.1 (*c*=0.5 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.67 (t, 3 H, *J*=7.5 Hz, SCH_2CH_3), 1.30 (d, 3 H, $J_{5,6}$=5.7 Hz, H-6), 2.10 (s, 3 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.44-2.49 (m, 2 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.57-2.67 (m, 4 H, SCH_2CH_3, $CH_3C(O)CH_2CH_2C(O)O$), 3.11-3.24 (m, 2 H, H-4, H-5), 3.48 (t, $J_{3,4}$=9.0, $J_{2,3}$=9.0 Hz, H-3), 4.27 (d, 1 H, $J_{1,2}$=9.9 Hz, H-1), 4.68 (d, 1 H, *J*=11.1 Hz, $PhCH_2$), 4.71 (d, 1 H, *J*=11.1 Hz, $PhCH'_2$), 4.94 (dd, 1 H, $J_{1,2}$=9.9, $J_{2,3}$=9.0 Hz, H-2), 7.22-7.29 (m, 5 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.8 (SCH_2CH_3), 18.7 (C-6), 23.9 (SCH_2CH_3), 28.0 ($CH_3C(O)CH_2CH_2C(O)O$), 29.8 ($CH_3C(O)CH_2CH_2C(O)O$), 37.8($CH_3C(O)CH_2CH_2C(O)O$), 67.7 (C-4), 72.2 (C-2), 74.9 ($PhCH_2$), 75.1 (C-5), 82.3 (C-3), 83.2 (C-1), [127.9, 128.0, 128.2, 128.4, 137.5 (C_{arom})], 171.5 ($CH_3C(O)CH_2CH_2C(O)O$), 206.1 ($CH_3C(O)CH_2CH_2C(O)O$); MALDI-TOF/MS: *m*/*z*: calcd for $C_{20}H_{27}N_3O_5SNa$: 444.15; found: 444.1 $[M+Na]^+$.

Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-levulinoyl-1-thio-α-L-rhamnopyranoside (15):

Treatment of **14** (4.93 g, 12.25 mmol) and levulinic acid (12.5 mL, 122.50 mmol) in CH_2Cl_2 (180 mL) with DCC (15.18 g, 73.57 mmol) and DMAP (22.45 mg, 0.18 mmol) in CH_2Cl_2 (18 mL) according to the general procedure for levulination gave compound **15** as colorless oil (5.29 g, 86 %). R_f=0.34 (hexane/EtOAc 3:1); $[\alpha]_D^{27}$=-18.9 (*c*=2.6 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.23 (t, 3 H, *J*=7.2 Hz, SCH_2CH_3), 1.33 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6), 2.02 (s, 3 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.35-2.39 (td, 2 H, *J*=6.9, 9.6 Hz, $CH_3C(O)CH_2CH_2C(O)O$), 2.46-2.72 (m, 4 H, SCH_2CH_3, $CH_3C(O)CH_2CH_2C(O)O$), 3.58 (t, 1 H, $J_{4,5}$=9.3, $J_{3,4}$=9.6 Hz, H-4),

4.18 (m, 1 H, H-5), 4.59 (d, 1 H, *J*=11.1 Hz, PhC*H*$_2$), 4.66 (d, 1 H, *J*=11.1 Hz, PhC*H'*$_2$), 5.22 (s, 1 H, H-1), 5.28 (dd, 1 H, $J_{2,3}$=3.3, $J_{3,4}$=9.6 Hz, H-3), 5.51 (dd, 1 H, $J_{1,2}$=1.5, $J_{2,3}$=3.3 Hz, H-2), 7.19-8.00 (m, 10 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.9 (SCH_2CH_3), 18.0 (C-6), 25.4 (SCH_2CH_3), 27.9 ($CH_3C(O)CH_2CH_2C(O)O$), 29.7 ($CH_3C(O)CH_2CH_2C(O)O$), 37.8($CH_3C(O)CH_2CH_2C(O)O$), 68.3 (C-5), 72.5 (C-2), 72.6 (C-3), 74.9 ($PhCH_2$), 78.9 (C-4), 81.9 (C-1), [127.8, 127.9, 128.4, 128.5, 129.7, 129.8, 133.4, 137.9 (C_{arom})], 165.5 (Ph*C*(O)O), 171.7 ($CH_3C(O)CH_2CH_2C(O)O$), 206.2 ($CH_3C(O)CH_2CH_2C(O)O$); MALDI-TOF/MS: *m*/*z*: found: 524.1 $[M+Na]^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{27}H_{32}O_7SNa$: 523.1766; found: 523.1761 $[M+Na]^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (16):

Glycosyl donor **14** (3.79 g, 9.42 mmol), 3-(*N*-benzyloxycarbonyl)aminopropanol (3.94 g, 18.83 mmol) and 4 Å powdered molecular sieves (7.73 g) in CH_2Cl_2 (150 mL) in the presence of NIS (2.33 g, 10.36 mmol) and TfOH (0.166 mL, 1.88 mmol) were reacted according to the general procedure for NIS glycosylation to give compound **16** as white solid (3.73 g, 72 %). R_f=0.26 (hexane/EtOAc 2:1); $[\alpha]_D^{27}$=+11.3 (*c*=1.8 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.32 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6), 1.73 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 2.11 (d, 1 H, *J*=4.5 Hz, O*H*), 3.24 (dd, 2 H, *J*=6.3, 12.6 Hz, $OCH_2CH_2CH_2NHZ$), 3.36-3.45 (m, 2 H, $OCH_2CH_2CH_2NHZ$, H-4), 3.65-3.75 (m, 2 H, $OCH'_2CH_2CH_2NHZ$, H-5), 4.12 (dd, 1 H, $J_{2,3}$=3.3, $J_{3,4}$=8.4 Hz, H-3), 4.69 (d, 1 H, *J*=11.1 Hz, PhC*H*$_2$), 4.76 (s, 1 H, H-1), 4.79 (d, 1 H, *J*=11.1 Hz, PhC*H'*$_2$), 4.85 (broad, 1 H, N*H*), 5.02 (s, 2 H, $PhCH_2OC(O)$), 5.25 (dd, 1 H, $J_{1,2}$=1.5, $J_{2,3}$=3.3 Hz, H-2), 7.18-7.99 (m, 15 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=18.2 (C-6), 29.6 ($OCH_2CH_2CH_2NHZ$), 38.6 ($OCH_2CH_2CH_2NHZ$), 65.6 ($OCH_2CH_2CH_2NHZ$), 66.6 (C-5), 67.6 ($PhCH_2OC(O)$), 70.5 (C-3),

73.2 (C-2), 75.2 (Ph*C*H_2), 81.6 (C-4), 97.5 (C-1), [127.9, 128.1, 128.4, 129.7, 129.9, 130.4, 133.3, 136.6, 138.1 (C_{arom})], 156.3 (PhCH_2O*C*(O)), 166.3(Ph*C*(O)O); MALDI-TOF/MS: *m*/*z*: found: 572.9 [*M*+Na]$^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{31}H_{35}NO_8Na$: 572.2260; found: 572.2259 [*M*+Na]$^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl *O*-(2-*O*-benzoyl-4-*O*-benzyl-3-*O*-levulinoyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (17):

Glycosyl donor **15** (3.04 g, 6.07 mmol), glycosyl acceptor **16** (3.03 g, 5.51 mmol) and 4 Å powdered molecular sieves (6.07 g) in CH_2Cl_2 (100 mL) in the presence of NIS (1.51 g, 6.71 mmol) and TfOH (0.11 mL, 1.22 mmol) was treated according to the general procedure for the linker glycosylation to give compound **17** as colorless oil (4.26 g, 78 %). R_f=0.34 (hexane/EtOAc 2:1); $[\alpha]_D^{27}$=+23.6 (c=1.8 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.11 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6b), 1.28 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6a), 1.72 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 1.99 (s, 3 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.32-2.39 (m, 2 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.50-2.67 (m, 2 H, $CH_3C(O)CH_2CH_2C(O)O$), 3.22 (dd, 2 H, *J*=6.0, 12.3 Hz, $OCH_2CH_2CH_2NHZ$), 3.39-3.49 (m, 2 H, $J_{3,4}$=9.6, $J_{4,5}$=9.6 Hz, $OCH_2CH_2CH_2NHZ$, H-4b), 3.56 (t, $J_{3,4}$=9.3, $J_{4,5}$=9.3 Hz, H-4a), 3.62-3.72 (m, 2 H, $OCH'_2CH_2CH_2NHZ$, H-5a), 3.84 (m, 1 H, H-5b), 4.17 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.0 Hz, H-3a), 4.45 (d, 1 H, *J*=11.4 Hz, $PhCH_2$), 4.50 (d, 1 H, *J*=11.4 Hz, $PhCH'_2$), 4.66 (d, 1 H, *J*=10.8 Hz, $PhCH''_2$), 4.80 (broad, 2 H, H-1a, N*H*), 4.95 (d, 1 H, *J*=10.8 Hz, $PhCH'''_2$), 4.99 (s, 2 H, $PhCH_2OC(O)$), 5.06 (s, 1 H, H-1b), 5.29 (d, 1 H, $J_{2,3}$=3.3 Hz, H-2a), 5.32 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.6 Hz, H-3b), 5.52 (dd, 1 H, $J_{1,2}$=1.8, $J_{2,3}$=3.0 Hz, H-2b), 7.05-8.00 (m, 25 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 17.8 (C-6b), 18.2 (C-6a), 28.0 ($CH_3C(O)CH_2CH_2C(O)O$), 29.7 ($OCH_2CH_2CH_2NHZ$, $CH_3C(O)CH_2CH_2C(O)O$)), 37.8

($CH_3C(O)CH_2CH_2C(O)O$), 38.5 ($OCH_2CH_2CH_2NHZ$), 65.6 ($OCH_2CH_2CH_2NHZ$), 66.5 ($PhCH_2OC(O)$), 67.9 (C-5a), 68.6 (C-5b), 70.7 (C-2b), 71.8 (C-3b), 72.7 (C-2a), [73.9, 75.8 ($PhCH_2$)], 78.2 (C-4b), 79.3 (C-3a), 79.8 (C-4a), 97.0 (C-1a), 99.7 (C-1b), [127.5, 127.7, 127.9, 128.2, 128.3, 128.4, 128.5, 129.5, 129.6, 129.7, 129.8, 133.4, 136.6, 137.9, 138.0 (C_{arom})], 156.3 ($PhCH_2OC(O)$), [165.3, 166.1 ($PhC(O)O$)], 171.7 ($CH_3C(O)CH_2CH_2C(O)O$), 206.2 ($CH_3C(O)CH_2CH_2C(O)O$); MALDI-TOF/MS: *m*/*z*: found: 1011.6 $[M+Na]^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{56}H_{61}NO_{15}Na$: 1010.3939; found: 1010.3932 $[M+Na]^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl *O*-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (18):

Treatment of **17** (4.26 g, 4.31 mmol) in CH_2Cl_2 (100 mL) with hydrazine acetate (397 mg, 4.31 mmol) in MeOH (10 mL) according to the general procedure for cleavage of the levulinoyl ester gave compound **18** as white solid (3.56 g, 93 %). R_f=0.42 (hexane/EtOAc 2:1); $[\alpha]_D^{27}$=+21.9 (*c*=2.2 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.17 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6b), 1.26 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6a), 1.72 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 3.22 (d, 2 H, *J*=6.0 Hz, $OCH_2CH_2CH_2NHZ$), 3.30-3.41 (m, 2 H, $J_{3,4}$=9.6, $J_{4,5}$=9.3 Hz, $OCH_2CH_2CH_2NHZ$, H-4b), 3.54 (t, 1 H, $J_{3,4}$=9.3, $J_{4,5}$=9.3 Hz, H-4a), 3.62-3.71 (m, 2 H, $OCH'_2CH_2CH_2NHZ$, H-5a), 3.78 (dd, 1 H, $J_{4,5}$=9.3 Hz, $J_{5,6}$=6.0 Hz, H-5b), 4.04 (dd, 1 H, $J_{2,3}$=2.1, $J_{3,4}$=9.6 Hz, H-3b), 4.18 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.0 Hz, H-3a), 4.57-4.63 (m, 3 H, $PhCH_2$), 4.77 (s, 1 H, H-1a), 4.86 (d, 1 H, *J*=10.8 Hz, $PhCH'_2$), 4.99 (s, 2 H, $PhCH_2OC(O)$), 5.11 (s, 1 H, H-1b), 5.28 (d, 1 H, $J_{2,3}$=3.0 Hz, H-2a), 5.33 (dd, 1 H, $J_{2,3}$=2.1 Hz, H-2b), 7.12-8.01 (m, 25 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=17.9 (C-6b), 18.1 (C-6a), 29.5 ($OCH_2CH_2CH_2NHZ$), 38.5 ($OCH_2CH_2CH_2NHZ$), 65.6 ($OCH_2CH_2CH_2NHZ$), 66.5 ($PhCH_2OC(O)$), 67.9 (C-5a), 68.3 (C-5b), 69.8 (C-3b), 72.8 (C-2b),

73.1 (C-2a), [74.0, 75.6 (Ph*C*H_2)], 77.6 (C-3a), 80.3 (C-4a), 81.1 (C-4b), 97.1 (C-1a), 99.5 (C-1b), [127.7, 127.8, 127.9, 128.2, 128.3, 128.4, 128.5, 129.6, 129.7, 129.8, 133.2, 133.3, 137.8, 138.1 (C_{arom})], 156.3 (PhCH_2O*C*(O)), [165.8, 165.9 (Ph*C*(O)O)]; MALDI-TOF/MS: *m*/*z*: found: 913.5 $[M+Na]^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{51}H_{55}NO_{13}Na$: 912.3571; found: 912.3559 $[M+Na]^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl *O*-(4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-levulinoyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (19):

Glycosyl donor **13** (80 mg, 0.19 mmol), glycosyl acceptor **18** (151 mg, 0.17 mmol) and 4 Å powdered molecular sieves (0.23 g) in CH_2Cl_2 (3 mL) in the presence of NIS (47 mg, 0.21 mmol) and TfOH (3 μL, 0.034 mmol) was treated according to the general procedure for the linker glycosylation to give compound **19** as colorless oil (161 mg, 76 %). R_f=0.30 (hexane/EtOAc 2:1); $[\alpha]_D^{27}$=+15.3 (*c*=0.8 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ= 0.89 (d, 3 H, $J_{5,6}$=6.5 Hz, H-6c), 1.06 (d, 3 H, $J_{5,6}$=6.5 Hz, H-6b), 1.27 (d, 3 H, $J_{5,6}$=6.5 Hz, H-6a), 1.73 (m, 2 H, OCH$_2$C*H$_2$*CH$_2$NHZ), 1.88 (s, 3 H, C*H$_3$*C(O)CH$_2$CH$_2$C(O)O), 1.99-2.10 (m, 2 H, CH$_3$C(O)CH$_2$C*H$_2$*C(O)O), 2.12-2.22 (m, 2 H, CH$_3$C(O)C*H$_2$*CH$_2$C(O)O), 2.76 (m, 1 H, H-5c), 2.91 (t, 1 H, $J_{3,4}$=9.5, $J_{4,5}$=10.0 Hz, H-4c), 3.17-3.23 (m, 3 H, OCH$_2$CH$_2$C*H$_2$*NHZ, H-3c), 3.41-3.44 (m, 2 H, OC*H$_2$*CH$_2$CH$_2$NHZ, H-4a), 3.56 (t, 1 H, $J_{3,4}$=9.5, $J_{4,5}$=9.5 Hz, H-4b), 3.66-3.74 (m, 3 H, OC*H'$_2$*CH$_2$CH$_2$NHZ, H-5a, H-5b), 3.97 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.5 Hz, H-3a), 4.20 (m, 2 H, H-1c, H-3b), 4.45 (d, 1 H, *J*=12.0 Hz, PhC*H$_2$*), 4.53 (d, 1 H, *J*=11.5 Hz, PhC*H'$_2$*), 4.61 (d, 1 H, *J*=12.0 Hz, PhC*H''$_2$*), 4.63 (d, 1 H, *J*=11.5 Hz, PhC*H'''$_2$*), 4.71 (d, 1 H, *J*=11.5 Hz, PhC*H''''$_2$*), 4.77 (s, 1 H, H-1a), 4.84 (m, 2 H, N*H*, PhC*H'''''$_2$*), 4.95 (t, 1 H, $J_{1,2}$=8.0, $J_{2,3}$=10.5

Hz, H-2c), 5.00 (s, 2 H, PhCH_2OC(O)), 5.14 (s, 1 H, H-1b), 5.30 (d, 1 H, $J_{2,3}$=3.0 Hz, H-2a), 5.32 (d, 1 H, $J_{2,3}$=3.0 Hz, H-2b), 7.13-8.05 (m, 30 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=17.7 (C-6c), 17.9 (C-6b), 18.1 (C-6a), 27.6 ($CH_3C(O)CH_2CH_2C(O)O$), 29.6 ($CH_3C(O)CH_2CH_2C(O)O$), 31.6 ($OCH_2CH_2CH_2NHZ$), 37.3 ($CH_3C(O)CH_2CH_2C(O)O$), 38.5 ($OCH_2CH_2CH_2NHZ$), 65.6 ($OCH_2CH_2CH_2NHZ$), 66.6 ($PhCH_2OC(O)$), 67.2 (C-4c), 67.8 (C-5a), 68.6 (C-5b), 70.6 (C-5c), 71.9 (C-2a), 72.7 (C-2b), [73.4, 74.3, 74.4 ($PhCH_2$)], 75.4 (C-2c), 77.2 (C-3b), 78.0 (C-3a), 79.6 (C-4a), 80.2 (C-4b), 80.9 (C-3c), 97.2 (C-1a), 98.8 (C-1b), 100.3 (C-1c), [127.0, 127.3, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 129.8, 129.9, 130.1, 133.0, 133.3, 133.4, 136.6, 137.5, 137.9, 138.6 (C_{arom})], 156.3 ($PhCH_2OC(O)$), [165.7, 165.8 ($PhC(O)O$)], 171.1 ($CH_3C(O)CH_2CH_2C(O)O$), 206.1 ($CH_3C(O)CH_2CH_2C(O)O$); MALDI-TOF/MS: *m*/*z*: found: 1271.7 $[M+Na]^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{69}H_{76}N_4O_{18}Na$: 1271.5052; found: 1271.4893 $[M+Na]^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl *O*-(4-azido-3-*O*-benzyl-4,6-dideoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl -α-L-rhamnopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (20):

Treatment of **19** (116 mg, 0.093 mmol) in CH_2Cl_2 (2.3 mL) with hydrazine acetate (8.6 mg, 0.093 mmol) in MeOH (0.23 mL) according to the general procedure for cleavage of the levulinoyl ester gave compound **20** as white solid (100 mg, 93 %). R_f=0.36 (hexane/EtOAc 2:1); $[\alpha]_D^{27}$=+11.0 (*c*=0.06 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ= 0.88 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6c), 1.13 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6b), 1.26 (d, 3 H, $J_{5,6}$=5.5 Hz, H-6a), 1.73 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 2.74 (m, 1 H, H-5c), 2.83 (t, 1 H, $J_{3,4}$=9.5, $J_{4,5}$=10.0 Hz, H-4c), 3.12 (t, 1 H, $J_{2,3}$=9.0, $J_{3,4}$=9.5 Hz, H-3c), 3.22 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 3.30 (t, 1 H, $J_{1,2}$=8.0, $J_{2,3}$=9.0 Hz, H-2c), 3.40 (m, 1 H, $OCH_2CH_2CH_2NHZ$), 3.49 (t, 1 H, $J_{3,4}$=9.0, $J_{4,5}$=10.0 Hz, H-4b), 3.54 (t, 1 H,

$J_{3,4}$=9.0, $J_{4,5}$=10.0 Hz, H-4a), 3.64-3.71 (m, 2 H, OCH''_2CH$_2$CH$_2$NHZ, H-5a), 3.78 (m, 1 H, H-5b), 4.05 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.5 Hz, H-3b), 4.08 (d, 1 H, $J_{1,2}$=8.0 Hz, H-1c), 4.21 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.0 Hz, H-3a), 4.56 (d, 1 H, J=10.5 Hz, PhCH_2), 4.60 (d, 1 H, J=10.5 Hz, PhCH'_2), 4.66 (d, 1 H, J=11.0 Hz, PhCH''_2), 4.71 (d, 1 H, J=12.0 Hz, PhCH'''_2), 4.75 (d, 1 H, J=12.0 Hz, PhCH''''_2), 4.76 (s, 1 H, H-1a), 4.83 (broad, 1 H, NH), 4.92 (d, 1 H, J=11.0 Hz, PhCH'''''_2), 5.00 (s, 2 H, PhCH_2OC(O)), 5.12 (s, 1 H, H-1b), 5.32 (d, 1 H, $J_{2,3}$=3.0 Hz, H-2a), 5.36 (d, 1 H, $J_{2,3}$=3.0 Hz, H-2b), 7.19-8.01 (m, 30 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=17.9 (C-6c), 18.1 (C-6a, C-6b), 29.6 (OCH$_2$CH$_2$CH$_2$NHZ), 38.5 (OCH$_2$CH$_2$CH$_2$NHZ), 65.6 (OCH$_2$CH$_2$CH$_2$NHZ), 66.6 (PhCH$_2$OC(O)), 66.9 (C-5c), 67.9 (C-5a), 68.7 (C-5b), 70.6 (C-4c), 72.5 (C-2a, C-2b), 74.6 (C-2c), [74.7, 75.0, 75.3 (PhCH$_2$)], 75.4 (C-3b), 77.6 (C-3a), 80.1 (C-4a, C-4b), 82.2 (C-3c), 97.1 (C-1a), 99.0 (C-1b), 103.0 (C-1c), [127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 129.8, 130.0, 133.3, 133.4, 136.6, 137.8, 138.0 (C_{arom})], 156.4 (PhCH$_2$OC(O)), [165.6, 165.8 (PhC(O)O)]; MALDI-TOF/MS: m/z: found: 1172.7 $[M+Na]^+$; MALDI-FTICR/MS: m/z: calcd for $C_{64}H_{70}N_4O_{16}Na$: 1173.4685; found: 1173.4588 $[M+Na]^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl *O*-(4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (21)

Method A: Sodium acetate (0.63 g, 7.68 mmol) and $PdCl_2$ (0.38 g, 2.14 mmol) was added to a solution of **8** (0.594 g, 1.78 mmol) in AcOH/H_2O 9:1 (60 mL). The reaction mixture mixture was stirred at room temperature overnight, and then filtered through Celite. The filtrate was concentrated to dryness and the residue was co-evaporated with toluene (2×60 mL). Purification of the crude product by column chromatography on silica gel (hexane/EtOAc 2:1) afforded the

hemiacetal compound. To a solution of this compound in CH_2Cl_2 (30 mL) was added trichloroacetonitrile (1.79 mL, 17.85 mmol) and DBU (0.11 mL, 0.74 mmol). The reaction mixture was stirred at room temperature for 5 h, and then concentrated to dryness. Purification of the crude product by column chromatography on silica gel (hexane/EtOAc 2:1 +0.5 %TEA) afforded imidate donor **9** as an α/β mixture 9:1 (0.622 g, 85 %). A mixture of acceptor **18** (1.22 g, 1.37 mmol), donor **9** (0.622 g, 1.51 mmol) and 4 Å powdered molecular sieves (1.85 g) in dry acetonitrile (23 mL) was stirred at 0 ℃ for 1 h, and then cooled to -40 ℃. A solution of $BF_3{\cdot}Et_2O$ (0.28 mL, 2.27 mmol) was added slowly. The mixture was stirred at -40 ℃ for 1 h, and then neutralized with triethylamine. The solution was filtered through Celite, washed with MeOH/CH_2Cl_2 5:95 (20 mL), and the combined filtrates were concentrated to dryness. Purification of the crude product by column chromatography (hexane/EtOAc 3:1) on silica gel afforded the desired product **21** as α/β 1:4 mixture (1.38 g, 86 %).

Method B: Methyl iodide (0.20 mL, 3.24 mmol) and silver(I) oxide (0.37 g, 1.60 mmol) were added to a solution of **20** (93 mg, 0.08 mmol) in THF (2 mL). Dimethyl sulfide (1 μL, 0.014 mmol) was added as catalyst. The flask was wrapped by aluminium foil to exclude light. The reaction mixture was stirred at room temperature overnight, and then filtered through Celite. The filtrate was concentrated to dryness. Purification of the crude product by column chromatography (hexane/EtOAc 3:1) on silica gel afforded the desired product **21** as colorless oil (48 mg, 51 %). R_f=0.56 (hexane/EtOAc 2:1); $[\alpha]_D^{27}$=+82.0 (*c*=0.2 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ= 0.83 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6c), 1.12 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6b), 1.24 (d, 3 H, $J_{5,6}$=5.5 Hz, H-6a), 1.73 (m, 2 H, $OCH_2C\mathit{H}_2CH_2NHZ$), 2.74 (m, 1 H, H-5c), 2.84 (t, 1 H, $J_{3,4}$=10.5, $J_{4,5}$=10.0 Hz, H-4c), 2.91 (t, 1 H, $J_{1,2}$=8.0, $J_{2,3}$=9.0 Hz, H-2c), 3.11 (t, 1 H, $J_{2,3}$=9.0, $J_{3,4}$=9.5 Hz, H-3c), 3.22 (m, 2 H, $OCH_2CH_2C\mathit{H}_2NHZ$), 3.36 (s, 3 H, OCH_3), 3.39 (m, 1 H,

OC*H*$_2$CH$_2$CH$_2$NHZ), 3.50 (t, 1 H, $J_{3,4}$=9.5, $J_{4,5}$=10.0 Hz, H-4b), 3.54 (t, 1 H, $J_{3,4}$=9.5, $J_{4,5}$=10.0 Hz, H-4a), 3.67 (m, 2 H, OC*H''*$_2$CH$_2$CH$_2$NHZ, H-5a), 3.76 (m, 1 H, H-5b), 4.10 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.5 Hz, H-3b), 4.20 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.5 Hz, H-3a), 4.31 (d, 1 H, $J_{1,2}$=8.0 Hz, H-1c), 4.52 (d, 1 H, *J*=11.0 Hz, PhC*H*$_2$), 4.59 (d, 1 H, *J*=10.5 Hz, PhC*H'*$_2$), 4.65 (d, 1 H, *J*=11.0 Hz, PhC*H''*$_2$), 4.72 (d, 1 H, *J*=11.5 Hz, PhC*H'''*$_2$), 4.75 (d, 1 H, *J*=12.0 Hz, PhC*H''''*$_2$), 4.76 (s, 1 H, H-1a), 4.80 (d, 1 H, *J*=10.5 Hz, PhC*H'''''*$_2$), 4.82 (broad, 1 H, N*H*), 5.00 (s, 2 H, PhC*H*$_2$OC(O)), 5.14 (s, 1 H, H-1b), 5.30 (d, 1 H, $J_{2,3}$=3.0 Hz, H-2a), 5.38 (d, 1 H, $J_{2,3}$=3.0 Hz, H-2b), 7.19-8.02 (m, 30 H, H$_{arom}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ=17.8 (C-6c), 18.0 (C-6b), 18.1 (C-6a), 29.6 (OCH$_2$*C*H$_2$CH$_2$NHZ), 38.5 (OCH$_2$CH$_2$*C*H$_2$NHZ), 60.4 (OCH_3), 65.6 (O*C*H$_2$CH$_2$CH$_2$NHZ), 66.6 (Ph*C*H$_2$OC(O)), 67.3 (C-5c), 67.9 (C-5a), 68.6 (C-5b), 70.2 (C-4c), 72.7 (C-2a), 73.2 (C-2b), [74.2, 75.2, 75.5 (Ph*C*H$_2$)], 75.9 (C-3b), 78.0 (C-3a), 80.0 (C-4a), 80.5 (C-4b), 82.5 (C-3c), 84.3 (C-2c), 97.1 (C-1a), 99.2 (C-1b), 102.9 (C-1c), [127.6, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 129.7, 129.8, 129.9, 130.1, 133.0, 133.3, 137.8, 137.9, 138.2 (C$_{arom}$)], 156.3 (PhCH$_2$O*C*(O)), [165.5, 165.8 (Ph*C*(O)O)]; MALDI-TOF/MS: *m*/*z*: found: 1187.8 [*M*+Na]$^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{65}H_{72}N_4O_{16}Na$: 1187.4841; found: 1187.4715 [*M*+Na]$^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl *O*-(4-(3-hydroxy-3-methylbutanamido)-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (22):

Treatment of **21** (0.71 g, 0.61 mmol), 1,3-propanedithiol (1.26 mL, 12.55 mmol) in pyridine (43 mL) and H_2O (6.1 mL) with TEA (1.28 mL, 9.15 mmol) according to the general procedure for azide reduction and introduction of C-4'' moitety gave free amine(0.69 g, 99 %). Treatment of

the free amine (0.47 g, 0.41 mmol) in DMF (20 mL) with β-hydroxyisovaleric acid (88 μL, 0.82 mmol) which was activated with HOAt (0.23 g, 1.64 mmol) and HATU (0.62 g, 1.64 mmol) in DMF (10 mL) for 1 h, and then added DIPEA (0.57 mL, 3.28 mmol) gave compound **22** as colorless oil (0.32 g, 63 %) and its α-isomer (76 mg, 15 %). R_f=0.26 (hexane/EtOAc 1:1); $[\alpha]_D^{27}$ =+9.4 (*c*=0.3 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ= 0.73 (d, 3 H, $J_{5,6}$=5.5 Hz, H-6c), 1.09 (s, 3 H, $(CH_3)_2C(OH)CH_2C(O)NH$), 1.12 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6b), 1.18 (s, 3 H, $(CH'_3)_2C(OH)CH_2C(O)NH$), 1.24 (d, 3 H, $J_{5,6}$=5.5 Hz, H-6a), 1.74 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 1.99 (d, 1 H, *J*=15.0 Hz, $(CH_3)_2C(OH)CH_2C(O)NH$), 2.09 (d, 1 H, *J*=15.0 Hz, $(CH_3)_2C(OH)CH'_2C(O)NH$), 2.91 (m, 1 H, H-5c), 2.98 (t, 1 H, $J_{1,2}$=8.0, $J_{2,3}$=8.5 Hz, H-2c), 3.15 (t, 1 H, $J_{2,3}$=8.5, $J_{3,4}$=9.0 Hz, H-3c), 3.22 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 3.38 (s, 3 H, OCH_3), 3.39 (m, 2 H, $OCH_2CH_2CH_2NHZ$, H-4c), 3.52 (t, 1 H, $J_{3,4}$=9.0, $J_{4,5}$=9.5 Hz, H-4b), 3.54 (t, 1 H, $J_{3,4}$=9.0, $J_{4,5}$=9.5 Hz, H-4a), 3.67 (m, 2 H, $OCH'_2CH_2CH_2NHZ$, H-5a), 3.76 (m, 1 H, H-5b), 4.12 (dd, 1 H, $J_{2,3}$=3.5, $J_{3,4}$=9.0 Hz, H-3b), 4.21 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.0 Hz, H-3a), 4.34 (d, 1 H, $J_{1,2}$=8.0 Hz, H-1c), 4.48 (d, 1 H, *J*=11.0 Hz, $PhCH_2$), 4.54 (d, 1 H, *J*=11.0 Hz, $PhCH'_2$), 4.60 (d, 1 H, *J*=10.5 Hz, $PhCH''_2$), 4.71 (d, 1 H, *J*=12.5 Hz, $PhCH'''_2$), 4.77 (s, 1 H, H-1a), 4.83 (d, 1 H, *J*=11.5 Hz, $PhCH''''_2$), 4.85 (broad, 1 H, NH), 4.95 (d, 1 H, *J*=11.0 Hz, $PhCH'''''_2$), 5.00 (s, 2 H, $PhCH_2OC(O)$), 5.15 (s, 1 H, H-1b), 5.30 (d, 1 H, $J_{2,3}$=3.0 Hz, H-2a), 5.39 (d, 1 H, $J_{2,3}$=3.5 Hz, H-2b), 7.14-8.00 (m, 30 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 17.7 (C-6c), 17.8 (C-6b), 18.1 (C-6a), 29.2 ($OCH_2CH_2CH_2NHZ$), [29.3, 29.7 ($(CH_3)_2C(OH)CH_2C(O)NH$)], 38.5 ($OCH_2CH_2CH_2NHZ$), 47.7 ($(CH_3)_2C(OH)CH_2C(O)NH$), 55.7 (C-4c), 60.3 (OCH_3), 65.6 ($OCH_2CH_2CH_2NHZ$), 66.6 ($PhCH_2OC(O)$), 67.9 (C-5a), 68.6 (C-5b), 69.4 ($(CH_3)_2C(OH)CH_2C(O)NH$), 70.6 (C-5c), 72.8 (C-2a), 73.1 (C-2b), [73.5, 74.1, 75.5 ($PhCH_2$)], 76.1 (C-3b), 78.1 (C-3a), 79.8 (C-3c), 80.0 (C-4a), 80.5 (C-4b), 84.4 (C-2c), 97.1 (C-1a), 99.2

(C-1b), 103.0 (C-1c), [127.5, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 129.7, 129.8, 129.9, 130.2, 133.0, 133.3, 137.9, 138.3, 138.5 (C_{arom})], 156.4 ($PhCH_2OC(O)$), [165.6, 165.9 ($PhC(O)O$)], 172.2 ($(CH_3)_2C(OH)CH_2C(O)NH$); MALDI-TOF/MS: *m*/*z*: found: 1261.4; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{70}H_{82}N_2O_{18}Na$: 1261.5460; found: 1261.5427 $[M+Na]^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl *O*-(4-(3-hydroxy-3-methylbutanamido)-3-*O*-benzyl-4,6-dideoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (23):

Treatment of **20** (21 mg, 0.018 mmol), 1,3-propanedithiol (0.04 mL, 0.40 mmol) in pyridine (1.28 mL) and H_2O (0.92 mL) with TEA (0.03 mL, 0.27 mmol) according to the general procedure for azide reduction and introduction of C-4'' moitety gave free amine (20 mg, 98 %). Treatment the free amine (20 mg, 0.018 mmol) in DMF (2 mL) with β-hydroxyisovaleric acid (4 μL, 0.037 mmol) which was activated with HOAt (10 mg, 0.074 mmol) and HATU (28 mg, 0.074 mmol) in DMF (1 mL) for 1 h, and then added DIPEA (26 μL, 0.15 mmol) gave compound **23** as colorless oil (17 mg, 78 %). R_f=0.61 (hexane/EtOAc 1:2); ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.79 (d, 3 H, $J_{5,6}$=6.5 Hz, H-6c), 1.11 (s, 3 H, $(CH_3)_2C(OH)CH_2C(O)NH$), 1.12 (d, 3 H, $J_{5,6}$=6.5 Hz, H-6b), 1.14 (s, 3 H, $(CH'_3)_2C(OH)CH_2C(O)NH$), 1.25 (d, 3 H, $J_{5,6}$=5.5 Hz, H-6a), 1.74 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 2.06 (d, 1 H, *J*=15.0 Hz, $(CH_3)_2C(OH)CH_2C(O)NH$), 2.14 (d, 1 H, *J*=15.0 Hz, $(CH_3)_2C(OH)CH'_2C(O)NH$), 2.98 (m, 1 H, H-5c), 3.21(m, 3 H, $OCH_2CH_2CH_2NHZ$, H-3c), 3.36-3.42 (m, 3 H, $OCH_2CH_2CH_2NHZ$, H-2c, H-4c), 3.52 (t, 1 H, $J_{3,4}$=9.0, $J_{4,5}$=9.5 Hz, H-4b), 3.54 (t, 1 H, $J_{3,4}$=9.0, $J_{4,5}$=10.0 Hz, H-4a), 3.68 (m, 2 H, $OCH'_2CH_2CH_2NHZ$, H-5a), 3.77 (m, 1 H, H-5b), 4.08 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.0 Hz, H-3b), 4.14 (d, 1 H, $J_{1,2}$=7.5 Hz, H-1c), 4.21 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.0 Hz, H-3a), 4.49 (d, 1 H, *J*=11.0

Hz, PhCH_2), 4.57 (d, 1 H, J=11.0 Hz, PhCH'_2), 4.60 (d, 1 H, J=10.5 Hz, PhCH''_2), 4.67 (d, 1 H, J=11.0 Hz, PhCH'''_2), 4.73 (d, 1 H, J=11.0 Hz, PhCH''''_2), 4.77 (s, 1 H, H-1a), 4.86 (broad, 1 H, NH), 4.94 (d, 1 H, J=10.5 Hz, PhCH'''''_2), 5.00 (s, 2 H, PhCH_2OC(O)), 5.13 (s, 1 H, H-1b), 5.32 (d, 1 H, $J_{2,3}$=3.0 Hz, H-2a), 5.38 (d, 1 H, $J_{2,3}$=3.5 Hz, H-2b), 5.43 (d, 1 H, J=9.0 Hz, $(CH_3)_2$C(OH)CH_2C(O)NH), 7.19-8.02 (m, 30 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=17.7 (C-6c), 17.9 (C-6b), 18.1 (C-6a), [29.3, 29.4 ($(CH_3)_2$C(OH)CH_2C(O)NH)], 29.7 (O$CH_2CH_2CH_2$NHZ), 38.5 (O$CH_2CH_2CH_2$NHZ), 47.8 ($(CH_3)_2$C(OH)CH_2C(O)NH), 55.3 (C-4c), 65.6 (O$CH_2CH_2CH_2$NHZ), 66.6 (PhCH_2OC(O)), 67.9 (C-5a), 68.7 (C-5b), 69.5 ($(CH_3)_2$C(OH)CH_2C(O)NH), 70.9 (C-5c), 72.7 (C-2a), 72.8 (C-2b), [72.5, 74.6, 75.4 (PhCH_2)], 74.9 (C-2c), 77.2 (C-3b), 78.0 (C-3a), 79.6 (C-3c), 80.0 (C-4a), 80.1 (C-4b), 97.1 (C-1a), 99.1 (C-1b), 103.1 (C-1c), [127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.6, 129.7, 129.8, 130.0, 133.1, 133.3, 137.9, 138.0, 138.4 (C_{arom})], 151.7 (PhCH_2OC(O)), [165.7, 165.9 (PhC(O)O)], 172.3 ($(CH_3)_2$C(OH)CH_2C(O)NH); MALDI-TOF/MS: m/z: calcd for $C_{69}H_{80}N_2O_{18}Na$: 1247.5304; found: 1249.7 $[M+Na]^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl *O*-(4-(3-methylbutanamido)-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-α-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (24):

The azide of compound **20** was reduced as described in the general procedures. Treatment of the free amine (0.12 g, 0.11 mmol) in DMF (5 mL) with DIPEA (0.15 mL, 0.86 mmol) and isovaleric acid (24 μL, 0.22 mmol) that was pre-activated with HOAt (57 mg, 0.42 mmol) and HATU (0.16 g, 0.42 mmol) in DMF (2.6 mL) for 1 h, gave compound **24** as colorless oil (78 mg, 0.064 mmol, 61 %) and its α-isomer (19 mg, 0.016 mmol, 15 %). R_f=0.39 (hexane/EtOAc 1:1);

$[\alpha]_D^{27}$=+18.3 (*c*=0.6 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 0.73 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6c), 0.78 (s, 3 H, $(CH_3)_2CHCH_2C(O)NH$), 0.82 (s, 3 H, $(CH'_3)_2CHCH_2C(O)NH$), 1.12 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6b), 1.24 (d, 3 H, $J_{5,6}$=5.5 Hz, H-6a), 1.71 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 1.80-1.98 (m, 3 H, $(CH_3)_2CHCH_2C(O)NH$, $(CH_3)_2CHCH_2C(O)NH$), 2.92 (m, 1 H, H-5c), 2.97 (t, 1 H, $J_{1,2}$=7.8, $J_{2,3}$=9.0 Hz, H-2c), 3.15-3.23 (m, 3 H, $OCH_2CH_2CH_2NHZ$, H-3c), 3.37 (s, 3 H, OCH_3), 3.39 (m, 2 H, $OCH_2CH_2CH_2NHZ$, H-4c), 3.51 (t, 1 H, $J_{3,4}$=9.0, $J_{4,5}$=9.0 Hz, H-4b), 3.56 (t, 1 H, $J_{3,4}$=9.0, $J_{4,5}$=9.0 Hz, H-4a), 3.66 (m, 2 H, $OCH'_2CH_2CH_2NHZ$, H-5a), 3.76 (m, 1 H, H-5b), 4.13 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.0 Hz, H-3b), 4.21 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.0 Hz, H-3a), 4.34 (d, 1 H, $J_{1,2}$=7.8 Hz, H-1c), 4.48 (d, 1 H, *J*=12.0 Hz, $PhCH_2$), 4.53 (d, 1 H, *J*=10.8 Hz, $PhCH'_2$), 4.60 (d, 1 H, *J*=10.8 Hz, $PhCH''_2$), 4.69 (d, 1 H, *J*=12.0 Hz, $PhCH'''_2$), 4.77 (s, 1 H, H-1a), 4.83 (d, 1 H, *J*=10.8 Hz, $PhCH''''_2$), 4.85 (broad, 1 H, NH), 4.96 (d, 1 H, *J*=10.8 Hz, $PhCH'''''_2$), 5.00 (s, 2 H, $PhCH_2OC(O)$), 5.15 (d, 1 H, $J_{1,2}$=1.2 Hz, H-1b), 5.30 (dd, 1 H, $J_{1,2}$=1.2, $J_{2,3}$=3.0 Hz, H-2a), 5.39 (dd, 1 H, $J_{1,2}$=1.8, $J_{2,3}$=3.0 Hz, H-2b), 7.18-8.06 (m, 30 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 17.6 (C-6c), 17.8 (C-6b), 18.1 (C-6a), [22.4, 22.5 ($(CH_3)_2CHCH_2C(O)NH$)], 25.9 ($(CH_3)_2CHCH_2C(O)NH$), 29.5 ($OCH_2CH_2CH_2NHZ$), 38.4 ($OCH_2CH_2CH_2NHZ$), 46.2 ($(CH_3)_2CHCH_2C(O)NH$), 55.7 (C-4c), 60.3 (OCH_3), 65.6 ($OCH_2CH_2CH_2NHZ$), 66.5 ($PhCH_2OC(O)$), 67.8 (C-5a), 68.5 (C-5b), 70.7 (C-5c), 72.7 (C-2a), 73.1 (C-2b), [73.3, 74.1, 75.5 ($PhCH_2$)], 76.0 (C-3b), 78.2 (C-3a), 79.7 (C-3c), 79.8 (C-4a), 80.4 (C-4b), 84.3 (C-2c), 97.0 (C-1a), 99.2 (C-1b), 103.0 (C-1c), [127.5, 127.6, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 129.7, 129.8, 129.9, 130.1, 133.0, 133.3, 136.6, 137.9, 138.3, 138.4 (C_{arom})], 156.3 ($PhCH_2OC(O)$), [165.6, 165.9 ($PhC(O)O$)], 172.2 ($(CH_3)_2CHCH_2C(O)NH$); MALDI-TOF/MS: *m*/*z*: found: 1245.4; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{70}H_{82}N_2O_{17}Na$: 1245.5511; found: 1245.5510 $[M+Na]^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl *O*-(4-acetamido-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (25):

The azide of compound **20** was reduced as described in the general procedures. Treatment the free amine (94 mg, 0.083 mmol) with acetic anhydride (0.016 mL, 0.17 mmol) in pyridine (0.014 mL, 0.17 mmol) and DMAP (1 mg, 0.008 mmol) gave compound **25** as colorless oil (64 mg, 66 %) and its α-isomer (17 mg, 17 %). R_f=0.25 (hexane/EtOAc 2:3); $[\alpha]_D^{27}$=+7.2 (*c*=0.4 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ= 0.72 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6c), 1.12 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6b), 1.23 (d, 3 H, $J_{5,6}$=6.5 Hz, H-6a), 1.70 (s, 3 H, C*H*$_3$C(O)NH), 1.73 (m, 2 H, OCH$_2$C*H*$_2$CH$_2$NHZ), 2.90 (m, 1 H, H-5c), 2.97 (t, 1 H, $J_{1,2}$=8.0, $J_{2,3}$=8.5 Hz, H-2c), 3.12 (t, 1 H, $J_{2,3}$=8.5, $J_{3,4}$=9.5 Hz, H-3c), 3.22 (m, 2 H, OCH$_2$CH$_2$C*H*$_2$NHZ), 3.33 (t, 1 H, $J_{3,4}$=9.5, $J_{4,5}$=10.0 Hz, H-4c), 3.38 (s, 3 H, OCH$_3$), 3.39 (m, 1 H, OC*H*$_2$CH$_2$CH$_2$NHZ), 3.51 (t, 1 H, $J_{3,4}$=9.0, $J_{4,5}$=10.0 Hz, H-4b), 3.54 (t, 1 H, $J_{3,4}$=9.5, $J_{4,5}$=8.5 Hz, H-4a), 3.67 (m, 2 H, OC*H'*$_2$CH$_2$CH$_2$NHZ, H-5a), 3.75 (m, 1 H, H-5b), 4.13 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.0 Hz, H-3b), 4.21 (dd, 1 H, $J_{2,3}$=2.5, $J_{3,4}$=9.5 Hz, H-3a), 4.34 (d, 1 H, $J_{1,2}$=8.0 Hz, H-1c), 4.48 (d, 1 H, *J*=11.5 Hz, PhC*H*$_2$), 4.53 (d, 1 H, *J*=11.0 Hz, PhC*H'*$_2$), 4.59 (d, 1 H, *J*=10.5 Hz, PhC*H''*$_2$), 4.70 (d, 1 H, *J*=12.0 Hz, PhC*H'''*$_2$), 4.77 (s, 1 H, H-1a), 4.81 (d, 1 H, *J*=11.0 Hz, PhC*H''''*$_2$), 4.83 (broad, 1 H, N*H*), 4.95 (d, 1 H, *J*=10.5 Hz, PhC*H'''''*$_2$), 5.00 (s, 2 H, PhC*H*$_2$OC(O)), 5.15 (s, 1 H, H-1b), 5.30 (d, 1 H, $J_{2,3}$=2.5 Hz, H-2a), 5.40 (d, 1 H, $J_{2,3}$=3.0 Hz, H-2b), 7.19-8.00 (m, 30 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 17.5 (C-6c), 17.8 (C-6b), 18.1 (C-6a), 23.5 (*C*H$_3$C(O)NH), 29.7 (OCH$_2$*C*H$_2$CH$_2$NHZ), 38.5 (OCH$_2$CH$_2$*C*H$_2$NHZ), 55.9 (C-4c), 60.3 (OCH$_3$), 65.6 (O*C*H$_2$CH$_2$CH$_2$NHZ), 66.6 (Ph*C*H$_2$OC(O)), 67.8 (C-5a), 68.5 (C-5b), 70.7 (C-5c), 72.8 (C-2a),

73.1 (C-2b), [73.5, 74.2, 75.5 (Ph*C*H_2)], 76.0 (C-3b), 78.3 (C-3a), 79.6 (C-3c), 79.8 (C-4a), 80.5 (C-4b), 84.5 (C-2c), 97.0 (C-1a), 99.3 (C-1b), 103.0 (C-1c), [127.6, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 129.7, 129.8, 129.9, 130.2, 133.0, 133.3, 136.6, 137.9, 138.3, 138.5 (C_{arom})], 156.3 (PhCH_2O*C*(O)), [165.6, 165.9 (Ph*C*(O)O)], 169.8 (CH_3*C*(O)NH); MALDI-TOF/MS: *m*/*z*: found: 1204.3; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{67}H_{76}N_2O_{17}Na$: 1203.5042; found: 1203.5040 [*M*+Na]$^+$.

3-Aminopropyl *O*-(4-(3-hydroxy-3-methylbutanamido)-4,6-dideoxy-2-*O*-methyl-β-D-glucopyranosyl)-(1→3)-*O*-(α-L-rhamnopyranosyl)-(1→3)-α-L-rhamnopyranoside (1):

Treatment of **22** (138.0 mg, 111.3 μmol) in MeOH/CH_2Cl_2 (2 mL/2 mL) with NaOMe (pH 8-10) according to the general procedure for global deprotection gave deacetylated product (110.1 mg, 96 %). Treatment of the partially deprotected compound (110.1 mg, 106.7 μmol) in *tert*-butanol/H_2O/AcOH (10 mL/0.25 mL/0.25 mL) with a catalytic amount of Pd/C under an atmosphere of hydrogen gave compound **1** as white solid (65.5 mg, 98 %). R_f=0.50 (CH_3CN/H_2O/AcOH 40:20:1); ^{1}H NMR (500 MHz, D_2O): δ=1.13 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6c), 1.21 (broad, 12 H, (C*H*$_3$)$_2$C(OH)CH_2C(O)NH, H-6a, H-6b), 1.92 (m, 2 H, OCH_2C*H*$_2$$CH_2NH_2$), 2.36 (s, 2 H, $(CH_3)_2$C(OH)C*H*$_2$C(O)NH), 3.00-3.15 (m, 4 H, OCH_2CH_2C*H*$_2$$NH_2$, H-2c, H-4c), 3.42-3.52 (m, 5 H, OC*H*$_2$$CH_2CH_2NH_2$, H-4a, H-4b, H-3c, H-5c), 3.53 (s, 3 H, OCH_3), 3.61 (m, 1 H, H-5a), 3.69-3.76 (m, 3 H, H-3a, H-5b, OC*H'*$_2$$CH_2CH_2NH_2$), 3.90 (d, 1 H, $J_{3,4}$=10.0 Hz, H-3b), 3.93 (s, 1 H, H-2a), 4.17 (s, 1 H, H-2b), 4.63 (d, 1 H, $J_{1,2}$=8.0 Hz, H-1c), 4.65 (s, 1 H, H-1a), 4.93 (s, 1 H, H-1b); ^{13}C NMR (75 MHz, D_2O): δ=[16.7, 16.8 (C-6a, C-6b)], 17.2 (C-6c), 26.8 (OCH_2*C*$H_2CH_2NH_2$), [28.2, 28.4 ((*C*H$_3$)$_2$C(OH)CH_2C(O)NH)], 37.6 (OCH_2CH_2*C*H_2NH_2), 49.0 ($(CH_3)_2$C(OH)*C*H_2C(O)NH), 56.7 (C-4c), 60.2 (OCH_3), 65.0 (O*C*$H_2CH_2CH_2NH_2$), 68.9

(C-5a), 69.4 (C-5b), 69.9 ($(CH_3)_2C$(OH)CH_2C(O)NH), 70.0 (C-2a), 70.3 (C-2b), [70.9, 71.2, 71.4, 72.9 (C-4a, C-4b, C-3c, C-5c)], 78.4 (C-3a), 79.7 (C-3b), 83.4 (C-2c), 99.8 (C-1a), 102.3 (C-1b), 103.8 (C-1c), 174.2 ($(CH_3)_2$C(OH)CH_2C(O)NH); MALDI-TOF/MS: *m*/*z*: found: 649.6; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{27}H_{50}N_2O_{14}Na$: 649.3160; found: 649.3156 $[M+Na]^+$.

3-Aminopropyl *O*-(4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-β -D-glucopyranosyl)-(1→3)-*O*-(α-L-rhamnopyranosyl)-(1→3)-α-L-rhamnopyranoside (2):

Treatment of **23** (17.0 mg, 13.9 μmol) in MeOH/CH_2Cl_2 (0.5 mL/0.5 mL) with NaOMe (pH 8-10) according to the general procedure for global deprotection gave the deacetylated product (14.0 mg, 99 %). Treatment of the partially deprotected compound (14.0 mg, 13.8 μmol) in *tert*-butanol/H_2O/AcOH (2 mL/0.05 mL/0.05 mL) with a catalytic amount of Pd/C under an atmosphere of hydrogen gave compound **2** as white solid (8.1 mg, 96 %). R_f=0.30 (CH_3CN/H_2O/AcOH 40:20:1); ^{1}H NMR (300 MHz, D_2O): δ= 1.11 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6c), 1.17 (broad, 12 H, (CH_3)$_2$C(OH)CH_2C(O)NH, H-6a, H-6b), 1.84 (m, 2 H, $OCH_2CH_2CH_2NH_2$), 2.33 (s, 2 H, $(CH_3)_2$C(OH)CH_2C(O)NH), 2.96 (m, 2 H, $OCH_2CH_2CH_2NH_2$), 3.11 (t, 1 H, $J_{3,4}$=7.2, $J_{4,5}$=7.2 Hz, H-4c), 3.27 (t, 1 H, $J_{1,2}$=7.8, $J_{2,3}$=8.4 Hz, H-2c), 3.36-3.60 (m, 6 H, $OCH_2CH_2CH_2NH_2$, H-4a, H-5a, H-4b, H-3c, H-5c), 3.64-3.75 (m, 3 H, H-3a, H-5b, $OCH'_2CH_2CH_2NH_2$), 3.86 (m, 2 H, H-2a, H-3b), 4.14 (s, 1 H, H-2b), 4.58 (d, 1 H, $J_{1,2}$=7.8 Hz, H-1c), 4.63 (s, 1 H, H-1a), 4.88 (s, 1 H, H-1b); ^{13}C NMR (75 MHz, D_2O): δ= [16.7, 17.2 (C-6a, C-6b, C-6c)], 27.1 ($OCH_2CH_2CH_2NH_2$), [28.2, 28.4 ($(CH_3)_2$C(OH)CH_2C(O)NH)], 37.6 ($OCH_2CH_2CH_2NH_2$), 49.0 ($(CH_3)_2$C(OH)CH_2C(O)NH), 56.7 (C-4c), 65.1 ($OCH_2CH_2CH_2NH_2$), 68.9 (C-5a), 69.1 (C-5b), 69.9 ($(CH_3)_2C$(OH)CH_2C(O)NH), 70.0 (C-2a), 70.3 (C-2b), [71.1, 71.3, 71.4, 73.5 (C-4a, C-4b, C-3c, C-5c)], 74.2 (C-2c), 78.4 (C-3a), 79.7 (C-3b), 99.8 (C-1a), 102.3

(C-1b), 103.6 (C-1c), 174.2 ($(CH_3)_2C(OH)CH_2C(O)NH$); MALDI-TOF/MS: *m*/*z*: found: 635.3; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{26}H_{48}N_2O_{14}Na$: 635.3003; found: 635.3000 $[M+Na]^+$.

3-Aminopropyl *O*-(4,6-dideoxy-4-(3-methylbutanamido)-2-*O*-methyl-β-D-glucopyranosyl)-(1→3)-*O*-(α-L-rhamnopyranosyl)-(1→3)-α-L-rhamnopyranoside (3):

Treatment of **24** (47.0 mg, 38.4 μmol) in MeOH/CH_2Cl_2 (0.5 mL/0.5 mL) with NaOMe (pH 8-10) according to the general procedure for global deprotection gave the deacetylated product (39.0 mg, 100 %). Treatment of the partially deprotected compound (39.0 mg, 38.4 μmol) in *tert*-butanol/H_2O/AcOH (4 mL/0.1 mL/0.1 mL) with a catalytic amount of Pd/C under an atmosphere of hydrogen gave compound **3** as white solid (22.1 mg, 94 %). R_f=0.40 (CH_3CN/H_2O/AcOH 60:20:1); ^{1}H NMR (500 MHz, D_2O): δ= 0.77 (m, 6 H, $(CH_3)_2CHCH_2C(O)NH$), 1.06 (d, 3 H, $J_{5,6}$=6.0 Hz, H-6c), 1.14 (m, 6 H, H-6a, H-6b), 1.84 (broad, 3 H, $OCH_2CH_2CH_2NH_2$, $(CH_3)_2CHCH_2C(O)NH$), 1.99 (m, 2 H, $(CH_3)_2CHCH_2C(O)NH$), 2.94-2.99 (m, 4 H, $OCH_2CH_2CH_2NH_2$, H-2c, H-4c), 3.33-3.46 (m, 5 H, $OCH_2CH_2CH_2NH_2$, H-4a, H-4b, H-3c, H-5c), 3.47 (s, 3 H, OCH_3), 3.55 (m, 1 H, H-5a), 3.63 (dd, 1 H, $J_{2,3}$=3.5, $J_{3,4}$=9.5 Hz, H-3a), 3.69 (m, 2 H, H-5b, $OCH'_2CH_2CH_2NHZ$), 3.83 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=10.0 Hz, H-3b), 3.87 (s, 1 H, H-2a), 4.12 (s, 1 H, H-2b), 4.57 (d, 1 H, $J_{1,2}$=8.5 Hz, H-1c), 4.61 (s, 1 H, H-1a), 4.86 (s, 1 H, H-1b); ^{13}C NMR (75 MHz, D_2O): δ= [16.7, 16.8 (C-6a, C-6b)], 17.2 (C-6c), [21.7, 21.8 ($(CH_3)_2CHCH_2C(O)NH$)], 22.3 (CH_3COOH), 26.2 ($(CH_3)_2CHCH_2C(O)NH$), 26.8 ($OCH_2CH_2CH_2NH_2$), 37.6 ($OCH_2CH_2CH_2NH_2$), 45.5 ($(CH_3)_2CHCH_2C(O)NH$), 56.7 (C-4c), 60.2 (OCH_3), 65.1 ($OCH_2CH_2CH_2NH_2$), 69.0 (C-5a), 69.4 (C-5b), 70.0 (C-2a), 70.1 (C-2b), [71.0, 71.3, 71.5, 73.0 (C-4a, C-4b, C-3c, C-5c)], 78.4 (C-3a), 79.8 (C-3b), 83.5 (C-2c), 99.9 (C-1a), 102.3 (C-1b), 103.8 (C-1c), 177.2 (CH_3COOH), 179.7 ($(CH_3)_2CHCH_2C(O)NH$);

MALDI-TOF/MS: *m*/*z*: found: 633.2; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{27}H_{50}N_2O_{13}Na$: 633.3211; found: 633.3207 $[M+Na]^+$.

3-Aminopropyl *O*-(4-acetamido-4,6-dideoxy-2-*O*-methyl-β-D-glucopyranosyl)-(1→3)-*O*-(α-L-rhamnopyranosyl)-(1→3)-α-L-rhamnopyranoside (4):

Treatment of **25** (27.2 mg, 23.0 μmol) in MeOH/CH_2Cl_2 (0.5 mL/0.5 mL) with NaOMe (pH 8-10) according to the general procedure for global deprotection gave the deacetylated product (22.9 mg, quantitative). Treatment of the partially deprotected compound (22.9 mg, 23.5 μmol) in *tert*-butanol/H_2O/AcOH (4 mL/0.1 mL/0.1 mL) with a catalytic amount of Pd/C under an atmosphere of hydrogen gave compound **4** as white solid (12.1 mg, 92 %). R_f=0.45 (CH_3CN/H_2O/AcOH 40:20:1); ^{1}H NMR (500 MHz, D_2O): δ= 1.04 (d, 3 H, $J_{5,6}$=5.5 Hz, H-6c), 1.13 (d, 3 H, $J_{5,6}$=6.5 Hz, H-6b), 1.16 (d, 3 H, $J_{5,6}$=6.5 Hz, H-6a), 1.87 (s, 3 H, C*H*$_3$C(O)NH), 1.89 (m, 2 H, $OCH_2CH_2CH_2NH_2$), 2.93-3.08 (m, 4 H, $OCH_2CH_2CH_2NH_2$, H-2c, H-4c), 3.34-3.46 (m, 5 H, $OCH_2CH_2CH_2NH_2$, H-4a, H-4b, H-3c, H-5c), 3.47 (s, 3 H, OCH_3), 3.55 (m, 1 H, H-5a), 3.64-3.71 (m, 3 H, H-3a, H-5b, $OCH'_2CH_2CH_2NHZ$), 3.83 (d, 1 H, $J_{3,4}$=10.0 Hz, H-3b), 3.87 (s, 1 H, H-2a), 4.12 (s, 1 H, H-2b), 4.59 (d, 1 H, $J_{1,2}$=8.0 Hz, H-1c), 4.61 (s, 1 H, H-1a), 4.87 (s, 1 H, H-1b); ^{13}C NMR (75 MHz, D_2O): δ= [16.8, 17.0 (C-6a, C-6b, C-6c)], 22.3 (CH_3COOH), 26.3 ($CH_3C(O)NH$), 26.8 ($OCH_2CH_2CH_2NH_2$), 37.6 ($OCH_2CH_2CH_2NH_2$), 56.9 (C-4c), 60.2 (OCH_3), 65.1 ($OCH_2CH_2CH_2NH_2$), 68.2 (C-5a), 69.0 (C-5b), 69.4 (C-2a), 70.0 (C-2b), [71.0, 71.3, 71.5, 73.0 (C-4a, C-4b, C-3c, C-5c)], 78.4 (C-3a), 79.7 (C-3b), 83.3 (C-2c), 99.9 (C-1a), 102.3 (C-1b), 103.8 (C-1c), 174.8 ($CH_3C(O)NH$), 178.4 (CH_3COOH); MALDI-TOF/MS: *m*/*z*: found: 591.2; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{24}H_{44}N_2O_{13}Na$: 591.2741; found: 591.2737 $[M+Na]^+$.

General procedure for *S*-acetylthioglycolylamido derivatization of the aminopropyl spacer:

The oligosaccharide **1** (10 mg, 0.016 mmol) was slurried in dry DMF (500 μL) and SAMA-OPfp (7.2 mg, 0.024 mmol) was added followed by dropwise addition of DIPEA (5.6 μL, 0.032 mmol). After stirring at room temperature for 2 h, the mixture was concentrated, co-evaporated twice with toluene and the residue purified by size-exclusion chromatography (Biogel P2 column, eluated with H_2O containing 1 % *n*-butanol) to give, after lyophilization, the corresponding thioacetate (10.6 mg, 0.0144 mmol, 90 %) as a white powder. In this manner, the thioacetamido derivatives of compounds **1-4** were prepared in yields of 85-95 %.

General procedure for *S*-deacetylation:

7 % NH_3 (g) in DMF solution (200 μL) was added to a solution of the thioacetate derivative corresponding to trisaccharide **1** (2.6 mg, 3.5 μmol) in ddH_2O (40 μL) and the mixture was stirred under argon atmosphere. The reaction was monitored by MALDI-TOF showing the product peak of $[M+Na]^+$. After 1 h the solvent was evaporated under high-vacuum. The thiol derivatized trisaccharide was further dried under high vacuum for 30 min and then used immediately in conjugation without further purification.

General procedure for the conjugation of thiol derivatized trisaccharides to BSA-MI:

The conjugations were performed as instructed by Pierce Endogen Inc. In short, the thiol derivative (2.5 equiv excess to available MI-groups on the protein), deprotected just prior to conjugation as described above, was dissolved in ddH_2O (100 μL) and added to a solution of the maleimide activated protein (2 mg) in conjugation buffer sodium phosphate pH 7.2 containing EDTA and sodium azide (200 μL). The mixture was incubated for 2 h at room temperature and then purified by Millipore Centriplus centrifugal filter devices with a 10 kDa molecular cut-off. All centrifugations were performed at 8 ℃ for 25 min, spinning at 13×g. The reaction mixture

was centrifuged and the filter washed with 10 mM Hepes buffer pH 6.5 (3×200 µL). The conjugate was retrieved and taken up in sodium phosphate buffer pH 7.4, 0.15 M sodium chloride (1 mL). This gave glycoconjugates with a carbohydrate/BSA ratio of 18:1 for trisaccharide **1**, 10:1 for 2''-OH-trisaccharide **2**, 9:1 for 4''-isovaleric acid trisaccharide **3** and 4:1 for trisaccharide 4''-HNAc-trisaccharide **4** as determined by Dubois' phenol-sulfuric acid total carbohydrate assay, quantitative monosaccharide analysis by HPAEC/PAD and Lowry protein concentration test.

Conjugation of thiol derivatized trisaccharide to KLH-BrAc:

A solution of KLH (15 mg) in 0.1 M sodium phosphate buffer pH 7.2 containing 0.15 M NaCl (1.5 mL) was added to a solution of SBAP (6 mg) in DMSO (180 µL). The mixture was incubated for 2 h at room temperature and then purified using Millipore Centriplus centrifugal filter devices with a molecular cut-off of 30 kDa. All centrifugations were performed at 8 ℃ for 25 min. spinning at 3000 rpm. The reaction mixture was centrifuged off and the filter washed with conjugation buffer (2×750 µL). The activated protein was retrieved by spinning at 3000 rpm for 15 min at 8 ℃ and taken up in 0.1 mM sodium phosphate buffer pH 8.0 containing 5 mM EDTA (2 mL). The activated protein was added to a vial containing de-S-acetylated trisaccharide (2.6 mg) and the mixture was incubated at room temperature for 18 h. Purification was achieved using centrifugal filters as described above for the BSA-MI-trisaccharide conjugates. This gave a glycoconjugate with 1042 trisaccharide residues/KLH molecule as determined by phenol/sulfuric acid total carbohydrate assay, quantitative monosaccharide analysis by HPAEC/PAD and Lowry protein concentration test.

Preparation of *Bacillus anthracis* Sterne 34 F_2 spores:

Bacillus anthracis Sterne $34F_2$ was obtained from the CDC culture collection. Spores of *B. anthracis* Sterne $34F_2$ were prepared from liquid cultures of PA medium[41] grown at 37 ℃, 200 rpm for six days. Spores were washed two times by centrifugation at 10 000×g in cold (4 ℃) sterile deionized water, purified in a 50 % Reno-60 (Bracco Diagnostics Inc., Princeton, NJ) gradient (10 000×g, 30 min, 4 ℃) and washed a further four times in cold sterile deionized water. After suspension in sterile deionized water, spores were quantified with surface spread viable cell counts on brain heart infusion (BHI) agar plates (BD BBL, Sparks, MD). Spore suspensions were stored in water at -80 ℃.

For the preparation of killed spores, 500-μL aliquots of spore suspensions in water, prepared as described above and containing approximately 3×10^8 CFU, were irradiated in 2.0 mL Sarstedt freezer tubes (Sarstedt, Newton, NC) in a gammacell irradiator with an absorbed dose of 2 million rads. Sterility after irradiation was monitored by spread-plating 10-μL aliquots of irradiated spore suspension on BHI agar plates. The plates were incubated for 72 h at 37 ℃ and monitored for colony growth. Absence of growth was taken as an indicator of sterility.

Preparation of antispore antiserum: All antisera were prepared in female New Zealand White rabbits (2.0-3.5 kg) purchased from Myrtle's Rabbitry (Thompson Station, TN). For antiserum production each of two rabbits were inoculated intramuscularly at two sites in the dorsal hind quarters with 0.5 mL of washed live-spore or irradiated spore inoculum (3×10^6 total spores). Rabbits were vaccinated at 0, 14, 28, and 42 days. Serum was collected prior to the first immunization (pre-immune serum) and at 7 and 14 d after each injection of antigen. Terminal bleeds were collected 14 d after the last immunization. All animal protocols were approved by

the CDC Animal Care and Use Committee (ACUC) and implemented under the direction of the CDC attending veterinarian.

Antibody-binding analyses:

Binding of rabbit anti-live spore antiserum to synthetic oligosaccharide conjugates was done by enzyme-linked immunosorbent assay (ELISA). Briefly, Immulon II-HB flat bottom 96-well microtiter plates (Thermo Labsystems, Franklin, MA) were coated with 100 μL per well of the KLH-BrAc-**1** conjugate at a concentration of 0.03 μg per mL of carbohydrate content, corresponding to 0.5 μg per mL by protein content, or by the protein mcKLH by itself (0.5 μg per mL protein) in coating buffer (0.01 M PBS, pH 7.4). Plates were washed three times in wash buffer (0.01 M PBS, pH 7.4, 0.1 % Tween-20) using an ELX405 microplate washer (BioTek Instruments Inc., Winooski, VT). Serial dilutions (100 μL per well) in blocking solution (0.01 M PBS, pH 7.4, 5 % skim milk, 0.5 % Tween-20) of either rabbit anti-spore antiserum from the day 49 bleed or pre-immune serum were then added and plates were incubated for 1 h 37 ℃. After incubation the plates were washed three times in wash buffer at which time a goat anti-rabbit IgG horseradish peroxidase conjugate (ICN Pharmaceuticals, Aurora, OH) was added (100 μL per well) and the incubation continued for 1 hour at 37 ℃. Plates were then washed three times in wash buffer and 100 μL per well of ABTS peroxidase substrate was added (KPL, Gaithersburg, MD). Color development was stopped after 15 minutes at 37 ℃ by addition of 100 μL per well of ABTS peroxidase stop solution (KPL, Gaithersburg, MD). Optical density (OD) values were read at a wavelength of 410 nm (490 nm reference filter) with a MRX Revelation microtiter plate reader (Thermo Labsystems, Franklin, MA).

To test for competitive inhibition, the rabbit anti-live spore antiserum or the rabbit anti-irradiated spore antiserum was added together with unconjugated trisaccharide in blocking solution at a 6-, 12-, 25-, 50-, 100-, or 200-fold weight excess compared to weight of carbohydrate used for coating. The negative control consisted of uncoated wells incubated with the respective antiserum plus trisaccharide **1** at a concentration corresponding to "200-fold excess" of trisaccharide.

To explore competitive inhibition by synthetic saccharide analogues conjugated to bovine serum albumin (BSA; Pierce Biotechnology, Rockford, IL), rabbit anti-live spore antiserum was diluted 1:1600 in blocking solution. For each well 100 µL of the serum were mixed with either 100 µL blocking solution or 100 µL of BSA-MI-conjugate in blocking solution with a concentration corresponding to a 2-, 4-, 8-, 16-, 32-, 64-, or 128-fold weight excess of carbohydrate compared to carbohydrate used for coating. The four conjugates tested were: BSA-MI-**1**, BSA-MI-**2**, BSA-MI-**3**, and BSA-MI-**4**. First the serum and then the BSA-saccharide conjugate solutions were added to an uncoated microtiter plate and mixed by pipetting up and down before the well contents were transferred to a coated plate. The microtiter plates were incubated and developed as described above.

The data are reported as the means ±SD of triplicate measurements.

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CHAPTER 5

SYNTHESIS OF THE CORE OLIGOSACCHARIDE OF *FRANCISELLA TULARENSIS*[1]

[1] Wei Zhong, Jin-Hwan Kim, Jin Park, Thomas Boltje and Geert-Jan Boons. To be submitted to *J. Am. Chem. Soc.*

Abstract

Francisella tularensis, the etiologic agent of tularemia (rabbit fever) in humans and animals, has been classified by the CDC as a top-priority (Class A) bio-terrorism agent. Tularemia transmits easily, has the capacity to inflict substantial morbidity and mortality on a large number of people and can induce widespread panic. Recently, the structure of the lipopolysaccharide of *F. tularensis* was determined. We developed a highly convergent synthesis of a number of truncated structures to determine the smallest part structure of the core oligosaccharide, which can elicit antibodies that recognize LPS from *F. Tularensis*. Such a structure will be attractive to be further developed as a vaccine candidate for tularemia. The target compound also presents an exciting opportunity to demonstrate how the new auxiliary-based methodology for the introduction of α-glycosidic linkages can be used in combination with the methodology for the formation of a β-glycosidic linkage.

Introduction

Francisella tularensis was isolated in 1911 in California, and is the etiologic agent of tularemia, a zoonotic disease which occurs throughout the northern hemisphere.[1] *F. tularensis* is a Gram-negative facultative intracellular pathogen that can survive and propagate within phagocytic cells. In nature, a disease cycle is maintained between wild animals such as rabbits, beavers, squirrels and water rats and biting vectors such as flies, ticks, mosquitoes and mites and the contaminated environment.[2] Classically, there are two types of *F. tularensis*: a highly virulent type A and moderately virulent type B. More recently, the species was re-classified into four distinct subspecies: *tularensis, holarctica, mediasiatica* and *novicida,* based on their marked

variations in virulence. Of these, only *F. tularensis* subspecies *tularensis* (Type A) and *holarctica* (Type B) cause disease in humans.

F. tularensis has been widely accepted as being a possible biological weapon and is classified by the CDC as a top-priority (Class A) bio-terrorism agent because of its extreme virulence, low infectious dose, ease of aerosol dissemination, and capacity to cause severe illness and death. Indeed, inhalation of as few as 10-50 cells may cause a deadly infection in humans. *F. tularensis* can survive for long periods of time under hash environmental conditions and may occur in different forms, of which the pneumonic form is associated with a high mortality of 30%. Aerosol dispersal is considered the most hazardous mode of transmission, as it would affect the most people. The World Health Organization estimated that 50 Kg of *F. tularensis* spread by aerosolization over an urban area would result in thousands of deaths. In history, *F. tularensis* infection has occurred in Europe and the former Soviet Union, arising from laboratory accidents by water-borne outbreak. The United States has experienced only epizootic cases.[3-6] The largest airborne dispersal of tularemia occurred in Sweden in the mid 1960s, resulting in the deaths of more than 600 farmers.[7]

In order to prevent *F. tularensis*, a live attenuated vaccine (LVS) against airborne disease was developed in the 1950s. Although it demonstrates the feasibility of vaccination against tularemia, the LVS strain is not licensed for use as a human vaccine because the nature of its attenuation may not be stable enough. Diagnoses are based on time consuming culture, serology or sophisticated molecular techniques. Therefore, improved vaccine candidates and rapid diagnostic tests are needed for this pathogen.

One strategy for the development of a tularemia vaccine is to immunize with the live vaccine or natural infection to identify the antigens responsible for induction of a protective response. In animal models, a few antigens recognized by immune sera or T cells have been assessed for protective efficacy. Lipopolysaccharide is the only protective antigen identified to date. Immunization with lipopolysaccharide induces protection against *F. tularensis* strains of low virulence.[8]

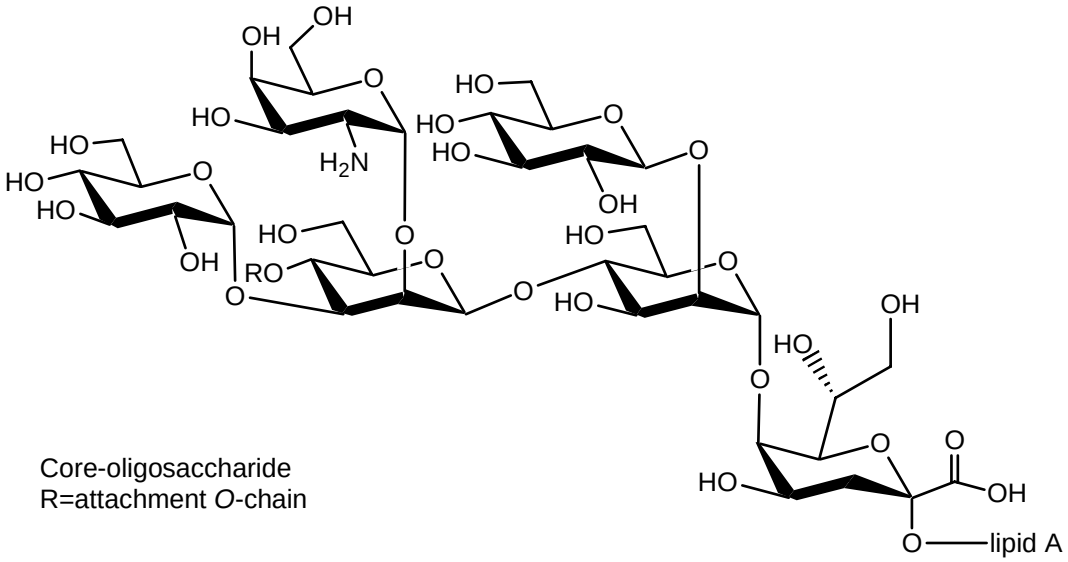


Figure 5.1. Core region of *Francisella tularensis*

Recently, the structure of the lipopolysaccharide of *F. tularensis* was determined (**Figure 5.1**) by NMR, mass spectrometry and chemical methods[9] and found to have a core region of unusual structure that is linked to the lipid A region by only one KDO moiety, instead of the usual two KDO residues. It does not contain heptosyl residues but contains a mannosyl moiety instead. The *O*-chain polysaccharide is composed of tetrasaccharide repeating units, which consist of two *N*-acetyl galactosamine uronamides and an *N*-acetyl quinovosamine and *N*-formyl-4-amino-quinovose moiety. *F. tularensis* also contains a capsular polysaccharide of

unknown structure. A conjugate of the O-chain of *F. tularensis* with BSA has shown promise as a vaccine candidate.[10]

Results and Discussion

Synthesis

In general, it is difficult to release core oligosaccharides from LPS for immunogical studies and vaccine development. Therefore, a chemically synthesized core oligosaccharide derived from *F. tularensis* LPS offers a unique approach to obtain an oligosaccharide target in sufficient quantity and purity to study the immunological properties of this unusual compound and develop a vaccine for tularemia. Furthermore, the target compound (**Figure 5.2**) can be equipped with an artificial spacer for convenient conjugation to a carrier protein offering an opportunity for a vaccine development. The target compound also presents an exciting opportunity to demonstrate how the new auxiliary-based methodology for the introduction of α-glycosidic linkages can be used in combination with the methodology for the formation of a β-glycosidic linkage. The mechanism can be demonstrated as the phenylsulfanyl moiety of the C-2 functionality performs neighboring group participation to give a quasi-stable anomeric sulfonium ion, which is only formed as a *trans*-decalin due to steric and electronic effects. Displacement of the sulfonium ion by a sugar hydroxyl leads exclusively to the formation of an α-glycoside.[13]

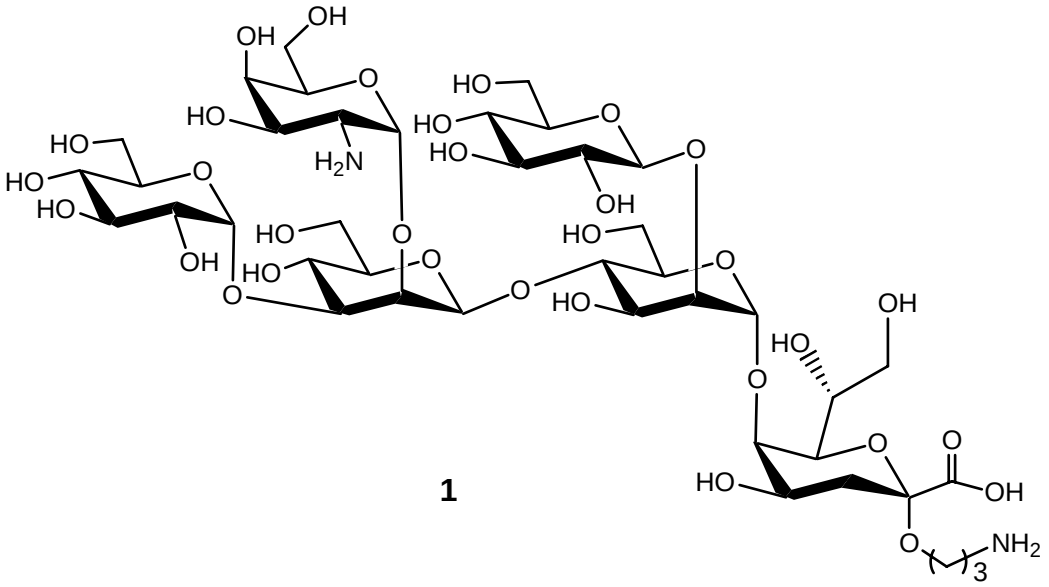


Figure 5.2. Synthetic target derived from core oligosaccharide *Francisella tularensis*

It was envisaged that the target compound **1** could be synthesized from monosaccharide precursors **3**, **4**, **11**, **12**, **16**, and **19** (**Figure 5.3**). Thus, glycosyl acceptor **3** can be coupled with glycosyl donor **4** having a C-2 (S)-(phenylthiomethyl)benzyl auxiliary to give disaccharide **5**. The chiral auxiliary at C-2 of **4** will ensure that only an α-glycoside will be obtained during glycosylation. Glycosyl acceptor **12** can be coupled with appropriately protected glycosyl donor **11** to give disaccharide **13**. The levulinoyl (Lev) ester at C-2 of **11** will ensure that only α-glycoside will be obtained during glycosylation due to neighboring group participation. After removal of the Fmoc protecting group of **13**, the resulting glycosyl acceptor can be coupled with disaccharide donor **5** resulting in the formation of tetrasaccharide **15**. Only β-glycoside will be obtained during glycosylation due to C-2 *p*-methoxybenzylidene (PMB) assisted intramolecular aglycon delivery. This reaction takes advantage of the facile formation of *S*-diastereomer of mixed acetal under oxidative condition. Subsequent activation of the mannose anomeric position

triggers intramolecular aglycon delivery to give β mannoside.[25,26] Glycosyl acceptor **15** can be coupled with glycosyl bromide donor **16** to give pentasaccharide **17**. Only α-glycoside will be obtained during glycosylation due to the hindered free hydroxyl group of **15** and the acetyl (Ac) withdrawing groups on the galactoside donor **16**. After removal of the levulinoyl (Lev) ester of **17**, the resulting glycosyl acceptor **18** can be coupled with a glucosyl donor **19**. Traditional participation by the benzoyl ester at C-2 of **19** will ensure that only β-glycoside will be obtained during glycosylation. Finally, removal of protecting groups of compound **20** will achieve the synthesis of target compound **1**.

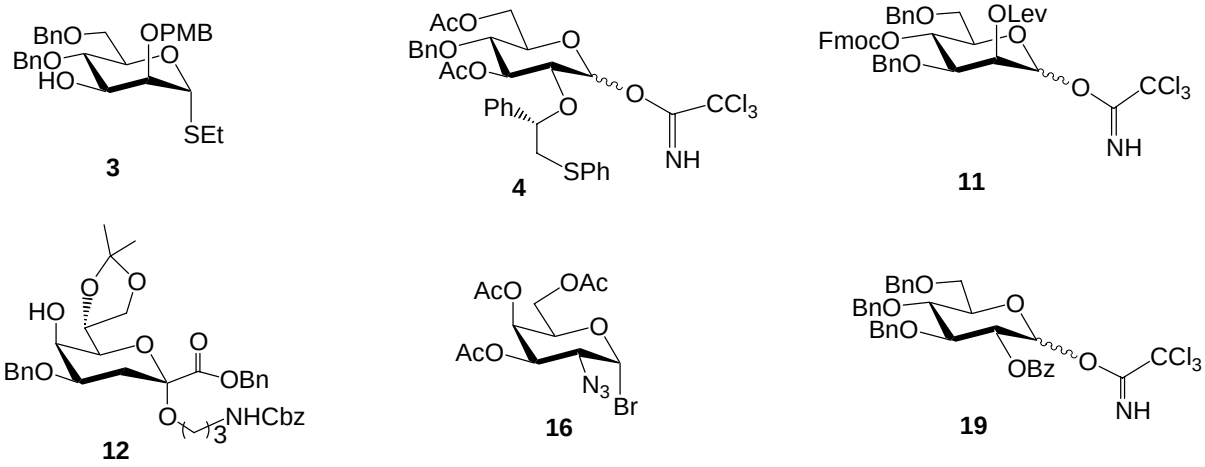
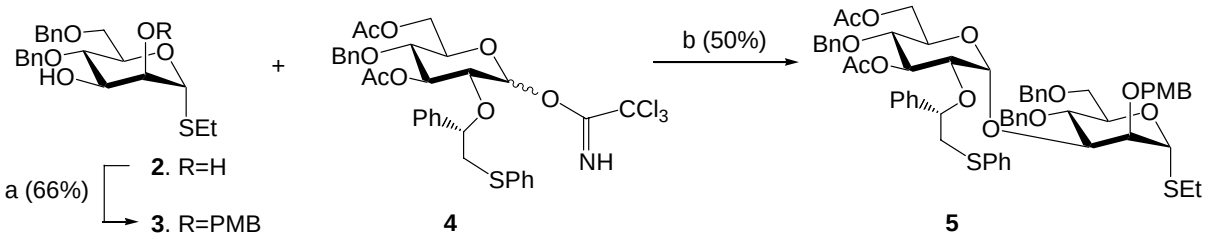


Figure 5.3. Building blocks for synthesis of synthetic target compound **1**

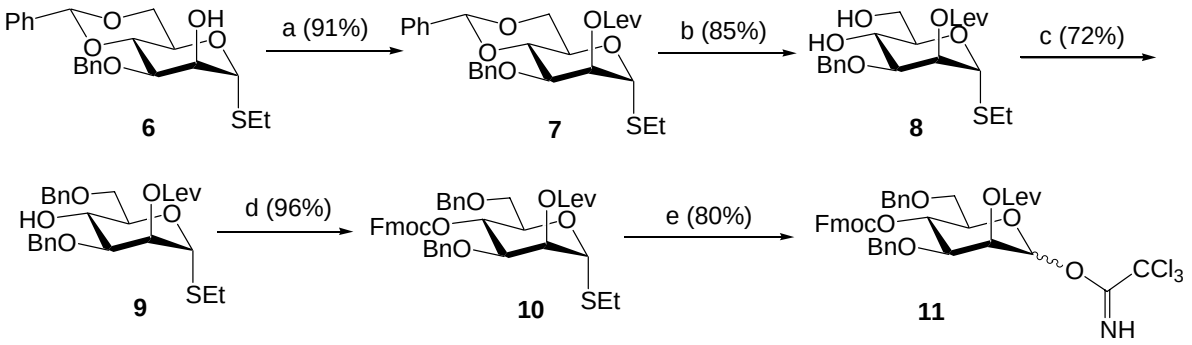
Thioglycoside **3**, having a C-3 free hydroxyl group, was synthesized from known thiomannoside **2**.[11] Regioselective protection of C-2 hydroxyl group was achieved by phase transfer reaction resulting in the formation of glycosyl acceptor **3** in a yield of 66 %.[12] Next, coupling of known trichloroacetimidate donor **4,** having a C-2 (S)-(phenylthiomethyl)benzyl auxiliary,[13] with glycosyl acceptor **3**, using a catalytic amount of TMSOTf in dichloromethane at -78 °C followed by gradual warming to 10 °C gave, after a reaction time of 3 hrs, disaccharides **5** as only the

α-glycoside (**Scheme 5.1**).



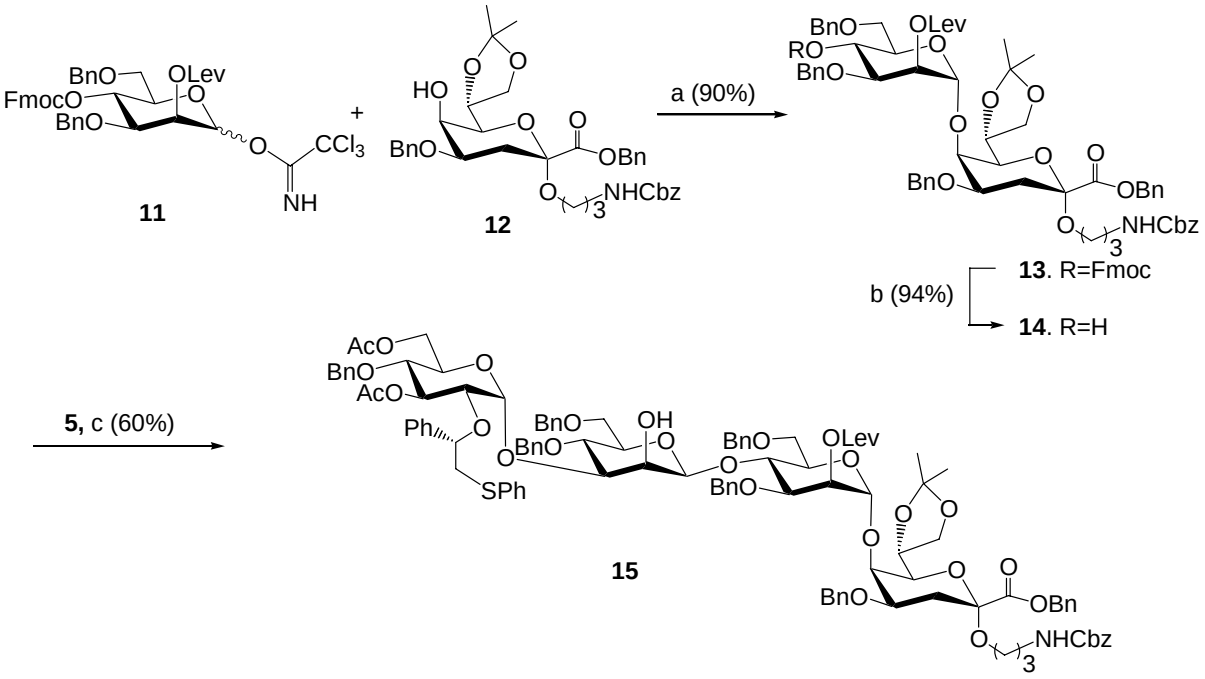
Scheme 5.1. Reagents and conditions: a) PMBCl, NaOH (aq.); b) TMSOTf, DTBMP, DCM, -78 ^{o}C to -20 ^{o}C

Next, attention was focused on the preparation of disaccharide acceptor **14** (**Scheme 5.3**). Glycosyl donor **11** was synthesized from known thioglycoside **6** (**Scheme 5.2)**.[14] Thus, a levulinoyl (Lev) ester at C-2 of compound **6** was installed by treatment with levulinic acid, 1,3-dicyclohexylcarbodiimide (DCC), and 4-(dimethylamino)pyridine (DMAP) in CH_2Cl_2 to give compound **7** in excellent yield.[15] Next, the benzylidene acetal of **7** could easily be removed by using aqueous acetic acid to give a diol **8**, which was selectively benzylated at C-6 to give compound **9**, by first stannylene acetal formation[16, 17] by reaction with dibutyltin oxide in refluxing toluene followed by treatment with benzyl bromide and tetrabutylammonium bromide (Bu_4NBr). Next, the C-4 hydroxyl group was protected by Fmoc by treatment with FmocCl in pyridine to give **10** in the yield of 96%. Finally, fully protected **10** was converted into trichloroacetimidate **11** by hydrolysis of the anomeric thioethyl by treatment with NBS in acetone/H_2O[18] followed by reaction of the resulting lactol with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[19,20]



Scheme 5.2. Reagents and conditions: a) levulinic acid,DCC, DMAP,DCM, rt; b) 90% AcOH/H_2O, reflux; c) 1: Bu_2SnO, toluene, reflux, 2: BnBr, TBAI, toluene; d) FmocCl, Pyridine, rt; e) 1: NBS, 90% Acetone/H_2O, 2: K_2CO_3, CCl_3CN, DCM, 0 ^oC

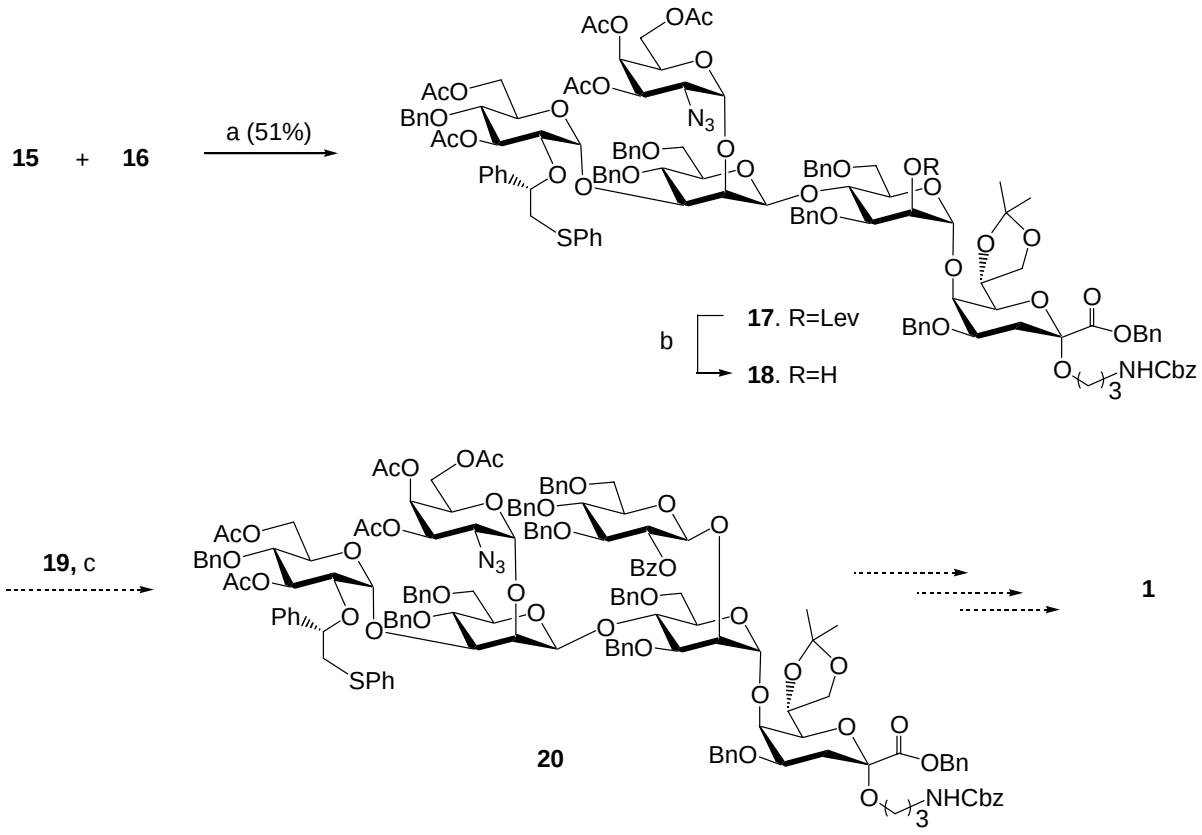
With glycosyl donor **10** and **11** in hand, attention was focused on the glycosylation of KDO acceptor **12**, which was synthesized by a modified procedure (Scheme 5.3).[21-23] An *N*-iodosuccinimide/trifluoromethanesulfonic acid (NIS/TfOH) mediated glycosylation[24] of thioglycosyl donor **10** with glycosyl acceptor **12** resulted in the formation of **13** as only the α-anomer in low yield. It appeared that the thioethyl donor was difficult to activate by the promoter system. Fortunately, coupling of trichloroacetimidate **11** with **12** in the presence of catalytic amount of TMSOTf in dichloromethane at 0 ℃ in the absence of molecular sieves gave disaccharide **13** in an excellent yield (90 %). It was also found that the use of molecular sieves resulted in a somewhat lower yield of 80%. Next, the Fmoc carbonate of **13** was selectively removed by treatment with triethylamine in DCM, to afford glycosyl acceptor **14** in a yield of 94 %. Then, 2-*O*-*p*-methoxybenzyl (PMB) protected mannosyl donor **5**, treatment with DDQ in the presence of **14**, was conversed into the mixed acetal via a quinonemethide-like species. Subsequent activation of the anomeric position by MeOTf affords β-*manno*-glycoside **15** as a single stereoisomer in a yield of 60% by intramolecular aglycon delivery.[25,26]



Scheme 5.3. Reagents and conditions: a) TMSOTf, DCM, 0 ^{o}C; b) TEA, DCM, rt; c) 1: DDQ, DCM, 0 ^{o}C; 2: MeOTf, DTBMP, DCE, 40 ^{o}C

Finally, an AgOTf mediated glycosylation[27] of known glycosyl bromide donor **16**[28] with glycosyl acceptor **15** resulted in the formation of **17** as only the α-anomer in a yield of 51%.

It is envisage that the chemical synthesis of the target compound can be completed as follows, the Lev group of **17** will be selectively removed by treatment with hydrazine acetate[15] and the hydroxyl of the resulting pentasaccharide **18** will be glycosylated with known glycosyl trichloroacetimidate donor **19**[29] in the presence of catalytic amount of TMSOTf in dichloromethane resulting in the formation of hexasaccharide **20**. Global deprotection of compound **20** will afford target compound **1** (**Scheme 5.4**).



Scheme 5.4. Reagents and conditions: a) AgOTf, DCM, -78 ^{o}C to 0 ^{o}C ; b) NH_2NH_2-AcOH, MeOH, DCM, rt; c) TMSOTf, DCM, 0 ^{o}C

Conclusion

We have successfully synthesized building blocks for the synthesis of hexasaccharide. One more glycosylation and removal of protecting groups are still ongoing. The synthetic compounds were equipped with an aminopropyl spacer to facilitate conjugation to the carrier proteins mariculture Keyhole Limpet Hemocyanin (mcKLH) and bovine serum albumin (BSA). Serum antibodies of rabbits immunized with a live attenuated vaccine (LVS) of F. tularensis will be used to see whether they can recognize the synthetic hexasaccharide-mcKLH conjugate.

Experimental Section

General:

^{1}H NMR spectra were recorded in $CDCl_3$ or D_2O on Varian Merc-300 or Varian Inova-500 spectrometers equipped with Sun workstations at 300 K. TMS (δ_H 0.00) or D_2O (δ_H 4.67) was used as the internal reference. ^{13}C NMR spectra were recorded in $CDCl_3$ or D_2O at 75 MHz on a Varian Merc-300 spectrometer, using the central resonance of $CDCl_3$ (δ_C 77.0) as the internal reference. COSY, HSQC, HMBC and TOCSY experiments were used to assist signal assignment of the spectra. The different monosaccharide units are referred to as a, b, c, d, e and f respectively, with a denoting the reducing end monosaccharide. Mass spectra were obtained on Applied Biosystems Voyager DE-Pro MALDI-TOF (no calibration) and Bruker Daltonics 7T (FTICR, external calibration with BSA). Optical rotatory power was obtained on Jasco P-1020 polarimeter at 300 K.

Chemicals were purchased from Aldrich or Fluka and used without further purification. CH_2Cl_2, acetonitrile and toluene were distilled from calcium hydride; THF from sodium; and MeOH from magnesium and iodine. Mariculture keyhole limpet hemocyanin (mcKLH), maleimide activated bovine serum albumin (BSA-MI), and succinimidyl 3-(bromoacetamido)propionate (SBAP) were purchased from Pierce Endogen, Rockford, IL. Aqueous solutions are saturated unless otherwise specified. Molecular sieves were activated at 350 ℃ for 3 h in vacuo. All reactions were performed under anhydrous conditions under argon and monitored by TLC on Kieselgel 60 F254 (Merck). Detection was by examination under UV light (254 nm) and by charring with 10 % sulfuric acid in methanol. Silica gel (Merck, 70-230 mesh) was used for chromatographies. Iatrobeads 6RS-8060 was purchased from Bioscan.

Ethyl 4, 6-di-*O*-benzyl-2-*O*-(*p*-methoxybenzyl)-1-thio-α-D-mannopyranoside (3):

To a solution of **2** (2.78 g, 6.87 mmol), Bu_4NBr (0.76 g, 2.36 mmol) and PMBCl (2.2 mL, 5.5 mmol) in CH_2Cl_2 (50 mL) under argon, was added aqueous NaOH (4.4 mL, 5% solution). The reaction mixture was heated under reflux for 18 hrs, after which it was cooled to room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and then washed with H_2O (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (hexane/EtOAc 4:1) afforded compound **3** as colorless oil (2.39 g, 66 %). R_f=0.33 (hexane/EtOAc 4:1); $[\alpha]_D^{27}$= +65.1 (*c*=0.52 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.19 (t, 3 H, *J*=7.5 Hz, SCH_2CH_3), 2.28 (d, *J*=9.3 Hz, 1 H, O*H*), 2.44-2.64 (m, 2 H, SCH_2CH_3), 3.61-3.68 (m, 2 H, H-4, H-6), 3.71-3.77 (m, 5 H, H-4, H-6', PMB), 3.85 (dt, 1 H, $J_{2,3}$=3.6, $J_{3,4}$=9.0, $J_{3,OH}$=9.3 Hz, H-3), 4.02-4.07 (m, 1 H, H-5), 4.36 (d, 1H, *J*=11.1 Hz, PhCH_2), 4.41 (d, 1H, *J*=12.3 Hz, PhCH_2), 4.42 (d, 1 H, *J*=11.1 Hz, PhCH_2), 4.58 (d, 1 H, *J*=12.3 Hz, PhCH_2), 4.63 (d, 1 H, *J*=11.1 Hz, PhCH_2), 4.76 (d, 1 H, *J*=11.1 Hz, PhCH_2), 5.40 (s, 1 H, H-1), 6.78-7.28 (m, 14 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 14.9 (SCH_2CH_3), 25.0 (SCH_2CH_3), 55.3 (CH_2PhOCH_3), 69.1 (C-6), 71.1 (C-5), [71.8, 73.3, 74.8 (3×PhCH_2)], 72.3 (C-3), 76.9 (C-4), 79.4 (C-2), 80.9 (C-1), [114.0, 127.5, 127.6, 127.7, 127.8, 128.2, 128.3, 129.5, 129.6, 138.3, 138.5, 159.5 (C_{arom})]; MALDI-TOF/MS: *m*/*z*: found: 547.2 [*M*+Na]$^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{30}H_{36}O_6SNa$: 547.2130; found: 547.2130 [*M*+Na]$^+$.

Ethyl *O*-{3, 6-di-*O*-acetyl-4-*O*-benzyl-2-*O*-[(1S)-phenyl-2-(phenylsulfanyl)ethyl]-α-D-glucopyranosyl}-(1→3)- 4, 6-di-*O*-benzyl-2-*O*-(*p*-methoxybenzyl)-1-thio-α-D-mannopyranoside (5):

A cooled (0 ℃) solution of Glycosyl donor **4** (0.65 g, 0.91 mmol) and 4 Å activated molecular sieves (0.5 g) in DCM (5 mL) was stirred for 30 min under an atmosphere of argon. After the mixture was cooled to -78 ℃, TMSOTf (165 μL, 0.91 mmol) was added dropwise. The reaction mixture was allowed to warm slowly to -20 ℃. After the donor was consumed, the reaction mixture was quenched by the addition of DTBMP (0.37 g, 1.8 mmol). A solution of glycosyl acceptor **3** (0.44 g, 0.84 mmol) and 4 Å activated molecular sieves (0.5 g) in DCM (5 mL) was added slowly. The reaction mixture was stirred at -20 ℃ for 3 hrs and then allowed to warm slowly to room temperature. The reaction mixture was quenched with aqueous saturated $NaHCO_3$ and separated. The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (CH_2Cl_2/acetone 300:1) afforded compound **5** as colorless oil (0.45 g, 50%). R_f=0.33 ((hexane/EtOAc 4:1); $[\alpha]_D^{27}$= +34.9 (*c*=0.3 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 1.23 (t, 3 H, *J*=7.5 Hz, SCH_2CH_3), 1.48 (s, 3 H, $CH_3C(O)$), 1.95 (s, 3 H, $CH_3C(O)$), 2.53-2.67 (m, 3 H, SCH_2CH_3, $PhCHOCH_2SPh$), 2.82 (dd, 1 H, *J*=7.2, 13.8 Hz, $PhCHOCH'_2SPh$), 3.26 (dd, 1 H, $J_{1,2}$=3.0, $J_{2,3}$=9.9 Hz, H-2b), 3.31 (t, 1 H, $J_{3,4}$=9.6, $J_{4,5}$=9.6 Hz, H-4b), 3.63 (s, 3 H, PMB), 3.70 (d, 1 H, $J_{6,6'}$=11.1 Hz, H-6a), 3.78 (dd, 1 H, $J_{5,6}$=4.8, $J_{6,6'}$=11.1 Hz, H-6a'), 3.95-4.08 (m, 4 H, H-2a, H-4a, H-6b, H-6b'), 4.12-4.22 (m, 4 H, H-3a, H-5a, H-5b, $PhCHOCH_2SPh$), 4.32-4.66 (m, 7 H, $PhCH_2$), 5.20 (d, 1 H, $J_{1,2}$=3.0 Hz, H-1b), 5.24 (d, 1 H, *J*=12.0 Hz, $PhCH_2$), 5.46 (s, 1 H, H-1a), 5.56 (t, 1 H, $J_{2,3}$=9.9, $J_{3,4}$=9.6 Hz, H-3b), 6.67-7.28 (m, 29 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=15.0 (SCH_2CH_3), [20.7 20.9 (2×$CH_3C(O)$)], 25.5 (SCH_2CH_3), 40.5 ($PhCHOCH_2SPh$), 55.1 (CH_2PhOCH_3), 62.9 (C-6b), 68.9 (C-5b), 69.3 (C-6a), [70.4, 73.2, 73.8, 74.6 (4×$PhCH_2$)], 72.2 (C-5a), 72.6 (C-3b), 74.2

(C-4a), 76.1 (C-4b), 78.6 (C-2b), 79.0 (C-2a), 80.6 (C-1a), 82.0 (C-3a), 82.5 (Ph*C*HOCH$_2$SPh), 98.7 (C-1b), [113.8, 125.7, 126.7, 126.8, 126.9, 127.3, 127.5, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.5, 128.6, 128.8, 129.0, 129.9, 136.1, 137.5, 138.4, 139.4, 140.7, 159.0 (C_{arom})], [169.6, 170.5 (2×CH$_3$*C*(O))]; MALDI-TOF/MS: *m*/*z*: found: 1095.4 [*M*+Na]$^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{61}H_{68}O_{13}S_2Na$: 1095.3999; found: 1095.4000 [*M*+Na]$^+$.

Ethyl 3-*O*-benzyl-4, 6-di-*O*-benzylidene-2-*O*-levulinoyl-1-thio-α-D-mannopyranoside (7):

To a solution of **6** (2.11 g, 5.24 mmol) and levulinic acid (5.36 mL, 52.4 mmol) in CH_2Cl_2 (90 mL) under argon, was added a solution of DCC (6.49 g, 31.45 mmol) and DMAP (9.6 mg, 0.08 mmol) in CH_2Cl_2 (9 mL). The reaction mixture was stirred at room temperature for 2 hrs, and then filtered through Celite. The filtrate was washed twice with water. The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (hexane/EtOAc 3:1) afforded compound **7** as colorless oil (2.39 g, 91 %). R_f=0.41 (hexane/EtOAc 2:1); $[\alpha]_D^{27}$= +58.7 (*c*=0.62 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.21 (t, 3 H, *J*=7.5 Hz, SCH$_2$C*H$_3$*), 2.11 (s, 3 H, C*H$_3$*C(O)CH$_2$CH$_2$C(O)O), 2.45-2.77 (m, 6 H, SC*H$_2$*CH$_3$, CH$_3$C(O)C*H$_2$*C*H$_2$*C(O)O), 3.79 (m, 1 H, H-6), 3.89 (dd, 1 H, $J_{2,3}$=3.3, $J_{3,4}$=9.6 Hz, H-3), 4.00 (t, 1 H, $J_{3,4}$=9.6 Hz, $J_{4,5}$=9.0, H-4), 4.12-4.20 (m, 2 H, H-5, H-6'), 4.58 (dd, 2 H, *J*=12.3 Hz, PhC*H$_2$*), 5.19 (d, 1 H, $J_{1,2}$=1.2 Hz, H-1), 5.37 (dd, 1 H, $J_{1,2}$=1.2, $J_{2,3}$=3.3 Hz, H-2), 5.56 (s, 1 H, PhC*H*), 7.18-7.45 (m, 10 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.9 (SCH$_2$*C*H$_3$), 25.5 (S*C*H$_2$CH$_3$), 28.1 (CH$_3$C(O)CH$_2$*C*H$_2$C(O)O), 29.8 (*C*H$_3$C(O)CH$_2$CH$_2$C(O)O), 38.0 (CH$_3$C(O)*C*H$_2$CH$_2$C(O)O), 64.5 (C-5), 68.6 (C-6), 71.7 (C-2), 72.2 (Ph*C*H$_2$), 74.1 (C-3), 78.7 (C-4), 83.4 (C-1), 101.6 (Ph*C*H), [126.1, 127.6, 127.7, 128.2, 128.3, 128.9, 137.4, 137.8

(C_{arom})], 171.9 ($CH_3C(O)CH_2CH_2\mathit{C}(O)O$), 206.3 ($CH_3\mathit{C}(O)CH_2CH_2C(O)O$); MALDI-TOF/MS: *m*/*z*: found: 523.1 [*M*+Na]$^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{27}H_{32}O_7SNa$: 523.1766; found: 523.1768 [*M*+Na]$^+$.

Ethyl 3-*O*-benzyl-2-*O*-levulinoyl-1-thio-α-D-mannopyranoside (8)

A solution of **7** (1.88 g, 3.76 mmol) in acetic acid/water (9:1, 15 mL) was heated under reflux at 90 ℃ for 1 hr, and then concentrated *in vacuo*. The residue was co-distilled with toluene twice. Purification of the residue by column chromatography over silica gel (CH_2Cl_2/MeOH 20:1) afforded compound **8** as colorless oil (1.32 g, 85 %). R_f=0.43 (CH_2Cl_2/MeOH 16:1); $[\alpha]_D^{27}$= +44.2 (*c*=0.2 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.22 (t, 3 H, *J*=7.5 Hz, $SCH_2C\mathit{H_3}$), 2.10 (s, 3 H, $C\mathit{H_3}C(O)CH_2CH_2C(O)O$), 2.35 (br, 2 H, O*H*), 2.47-2.70 (m, 6 H, $SC\mathit{H_2}CH_3$, $CH_3C(O)C\mathit{H_2}C\mathit{H_2}C(O)O$), 3.66 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.3 Hz, H-3), 3.77-3.79 (m, 2 H, H-6, H-6'), 3.84 (t, 1 H, $J_{3,4}$=9.3 Hz, $J_{4,5}$=9.6, H-4), 3.93-3.99 (m, 1 H, H-5), 4.59 (dd, 2 H, *J*=11.4 Hz, $PhC\mathit{H_2}$), 5.21 (d, 1 H, $J_{1,2}$=1.2 Hz, H-1), 5.34 (dd, 1 H, $J_{1,2}$=1.2, $J_{2,3}$=3.0 Hz, H-2), 7.19-7.31 (m, 5 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.8 ($SCH_2\mathit{C}H_3$), 25.6 ($S\mathit{C}H_2CH_3$), 28.1 ($CH_3C(O)CH_2\mathit{C}H_2C(O)O$), 29.8 ($\mathit{C}H_3C(O)CH_2CH_2C(O)O$), 38.0 ($CH_3C(O)\mathit{C}H_2CH_2C(O)O$), 62.5 (C-6), 67.2 (C-4), 69.9 (C-2), 71.5 ($Ph\mathit{C}H_2$), 72.2 (C-5), 77.8 (C-3), 82.5 (C-1), [128.1, 128.2, 128.6, 137.3 (C_{arom})], 171.9 ($CH_3C(O)CH_2CH_2\mathit{C}(O)O$), 206.3 ($CH_3\mathit{C}(O)CH_2CH_2C(O)O$); MALDI-TOF/MS: *m*/*z*: found: 435.2 [*M*+Na]$^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{20}H_{28}O_7SNa$: 435.1453; found: 435.1466 [*M*+Na]$^+$.

Ethyl 3, 6-di-*O*-benzyl-2-*O*-levulinoyl-1-thio-α-D-mannopyranoside (9)

Dibutyl tin oxide (3.38 g, 13.57 mmol) was added to a solution of the diol **8** (5.08 g, 10.15 mmol) in dry toluene (150 mL). The reaction mixture was heated under reflux with a Dean-Stark apparatus for 3 hrs, and then cooled to 60 ℃. Benzyl bromide (1.63 mL, 13.75 mmol) and tetrabutylammonium iodide (4.57 g, 13.75 mmol) were added and the resulting reaction mixture was heated under reflux for 3 hrs. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc (200 mL), and the resulting solution was washed with H_2O (200 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (hexane/EtOAc 2:1) afforded the desired product **9** as colorless oil (4.46 g, 72 %). R_f=0.5 (hexane/EtOAc 1:1); $[\alpha]_D^{27}$= +49.6 (c=0.56 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.21 (t, 3 H, J=7.5 Hz, SCH_2CH_3), 2.07 (s, 3 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.40 (d, 1 H, J=2.1 Hz, O*H*), 2.48-2.67 (m, 6 H, SCH_2CH_3, $CH_3C(O)CH_2CH_2C(O)O$), 3.65 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.3 Hz, H-3), 3.69-3.75 (m, 2 H, H-6, H-6'), 3.88 (td, 1 H, $J_{3,4}$=9.3 Hz, $J_{4,5}$=9.6, $J_{4,OH}$=2.1 Hz, H-4), 4.04-4.10 (m, 1 H, H-5), 4.38 (d, 1 H, J=11.4 Hz, $PhCH_2$), 4.50 (d, 1 H, J=12.0 Hz, $PhCH'_2$), 4.55 (d, 1 H, J=12.0 Hz, $PhCH''_2$), 4.59 (d, 1 H, J=11.4 Hz, $PhCH'''_2$), 5.23 (d, 1 H, $J_{1,2}$=1.5 Hz, H-1), 5.34 (dd, 1 H, $J_{1,2}$=1.5, $J_{2,3}$=3.0 Hz, H-2), 7.19-7.27 (m, 10 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.8 (SCH_2CH_3), 25.5 (SCH_2CH_3), 28.1 ($CH_3C(O)CH_2CH_2C(O)O$), 29.8 ($CH_3C(O)CH_2CH_2C(O)O$), 37.9 ($CH_3C(O)CH_2CH_2C(O)O$), 67.5 (C-4), 69.7 (C-6), 69.9 (C-2), [71.5, 73.5 (2×$PhCH_2$)], 71.5 (C-5), 77.8 (C-3), 82.4 (C-1), [127.5, 127.6, 128.0, 128.2, 128.3, 128.5, 137.4, 138.1 (C_{arom})], 171.9 ($CH_3C(O)CH_2CH_2C(O)O$), 206.2 ($CH_3C(O)CH_2CH_2C(O)O$);

MALDI-TOF/MS: *m*/*z*: found: 525.2 $[M+Na]^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{27}H_{34}O_7SNa$: 525.1923; found: 525.1924 $[M+Na]^+$.

Ethyl 3, 6-di-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-2-*O*-levulinoyl-1-thio-α-D-mannopyranoside (10)

To a solution of **9** (3.24 g, 6.45 mmol) and pyridine (2.09 mL, 25.8 mmol) in CH_2Cl_2 (50 mL), Fmoc chloride (3.34 g, 12.9 mmol) was added at 0 ℃. The reaction mixture was stirred at 0 ℃ for 4 hrs, and then diluted with CH_2CL_2 (50 mL). The solution was washed with 1 N HCl (50 mL) and saturated $NaHCO_3$ (50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (hexane/EtOAc 3:1) afforded the desired product **10** as colorless oil (4.49 g, 96%). R_f=0.29 (hexane/EtOAc 2:1); $[\alpha]_D^{27}$= +44.8 (*c*=1.23 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.21 (t, 3 H, *J*=7.5 Hz, SCH_2CH_3), 2.04 (s, 3 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.46-2.70 (m, 6 H, SCH_2CH_3, $CH_3C(O)CH_2CH_2C(O)O$), 3.53-3.62 (m, 2 H, H-6, H-6'), 3.84 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.6 Hz, H-3), 4.09 (t, 1 H, *J*=7.5 Hz, C*H*, Fmoc), 4.22-4.31 (m, 3 H, H-5, CH_2, Fmoc), 4.38 (d, 1 H, *J*=11.7 Hz, $PhCH_2$), 4.45 (d, 1 H, *J*=12.0 Hz, $PhCH'_2$), 4.49 (d, 1 H, *J*=12.0 Hz, $PhCH''_2$), 4.52 (d, 1 H, *J*=11.7 Hz, $PhCH'''_2$), 5.10 (t, 1 H, $J_{3,4}$=9.6 Hz, $J_{4,5}$=9.9, H-4), 5.23 (d, 1 H, $J_{1,2}$=1.5 Hz, H-1), 5.34 (dd, 1 H, $J_{1,2}$=1.5, $J_{2,3}$=3.0 Hz, H-2), 7.10-7.71 (m, 18 H, H_{arom}); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.8 (SCH_2CH_3), 25.5 (SCH_2CH_3), 28.2 ($CH_3C(O)CH_2CH_2C(O)O$), 29.7 ($CH_3C(O)CH_2CH_2C(O)O$), 37.9 ($CH_3C(O)CH_2CH_2C(O)O$), 46.7 (*C*H, Fmoc), 69.1 (C-6), 69.9 (C-5), 70.0 (CH_2, Fmoc), 70.2 (C-2), [71.5, 73.5 (2×$PhCH_2$)], 72.7 (C-4), 75.2 (C-3), 82.2 (C-1), [120.0, 125.1, 127.1, 127.5, 127.6, 127.7, 127.8, 127.9, 128.2, 128.3, 137.3, 137.9, 141.3, 143.2,

143.3 (C_{arom})], 154.5 (O*C*(O)O, Fmoc), 171.8 ($CH_3C(O)CH_2CH_2C(O)O$), 206.3 ($CH_3C(O)CH_2CH_2C(O)O$); MALDI-TOF/MS: *m*/*z*: found: 747.3 [*M*+Na]$^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{42}H_{44}O_9SNa$: 747.2604; found: 747.2601 [*M*+Na]$^+$.

Benzyl [N-benzyloxycarbonyl-3-amino-propyl 4-*O*-benzyl-3-deoxy-7, 8-*O*-isopropylidene-α-D-manno-octulopyranosid] onate (12)

This compound was synthesized according to a slightly modified procedure. [??] R_f=0.19 (hexane/EtOAc 2:1); $[\alpha]_D^{27}$= +5.7 (*c*=1.2 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.28 (s, 3 H, C*H*$_3$), 1.32 (s, 3 H, C*H'*$_3$), 1.63 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 1.91 (t, 1 H, $J_{3ax,3eq}$=12.9, $J_{3ax,4}$=11.7 Hz, H-3$_{ax}$), 2.12 (dd, 1 H, $J_{3ax,3eq}$=12.9, $J_{3eq,4}$=5.1 Hz, H-3$_{eq}$), 3.08-3.26 (m, 3 H, $OCH_2CH_2CH_2NHZ$, $OCH_2CH_2CH_2NHZ$), 3.41-3.47 (m, 2 H, $OCH'_2CH_2CH_2NHZ$, H-6), 3.81 (m, 1 H, H-4), 3.97 (dd, 1 H, $J_{8,8'}$=9.0, $J_{7,8}$=5.1 Hz, H-8), 4.01 (m, 1 H, H-5), 4.06 (dd, 1 H, $J_{8,8'}$=9.0, $J_{7,8'}$=6.0 Hz, H-8'), 4.36 (dd, 1 H, $J_{6,7}$=12.3, $J_{7,8}$=5.1, $J_{7,8'}$=6.0 Hz, H-7), 4.49 (dd, 2 H, *J*=11.7 Hz, PhC*H*$_2$), 5.02 (s, 2H, PhC*H*$_2$OC(O)NH), 5.13 (dd, 2 H, *J*=12.3 Hz, PhC*H*$_2$OC(O)), 5.22 (br, 1 H, N*H*), 7.19-7.26 (m, 15 H, H$_{arom}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ=[25.4, 26.6 (2×*C*H$_3$)], 29.2 ($OCH_2CH_2CH_2NHZ$), 32.1 (C-3), 38.2 ($OCH_2CH_2CH_2NHZ$), 61.2 ($OCH_2CH_2CH_2NHZ$), 64.2 (C-5), 66.5 (C-8), 66.5 (Ph*C*H$_2$OC(O)NH), 67.3 (Ph*C*H$_2$OC(O)), 70.4 (Ph*C*H$_2$), 72.4 (C-6), 73.0 (C-4), 74.1 (C-7), 99.0 (C-2), 109.2 (CH_3CCH_3), [127.7, 127.9, 128.0, 128.3, 128.4, 128.5, 128.6, 135.1, 136.7, 137.5 (C_{arom})], 156.4 ($PhCH_2OC(O)NH$), 167.9 ($PhCH_2OC(O)$); MALDI-TOF/MS: *m*/*z*: found: 672.3 [*M*+Na]$^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{36}H_{43}NO_{10}Na$: 672.2785; found: 672.2786 [*M*+Na]$^+$.

Benzyl {N-benzyloxycarbonyl-3-amino-propyl *O*-[3, 6-di-*O*-benzyl-4-*O*-

(9-fluorenylmethoxycarbonyl)-2-*O*-levulinoyl-α-D-mannopyranosyl]-(1→5)-4-*O*-benzyl -3-deoxy-7, 8-*O*-isopropylidene-α-D-manno-octulopyranosid} onate (13)

To a solution of **10** (3.57 g, 4.93 mmol) in Acetone/H_2O (9:1, 50 mL), NBS (1.78 g, 9.89 mmol) was added at 0 ℃. The reaction mixture was stirred at 0 ℃ for 30 min and then diluted with CH_2Cl_2 (100 mL). The solution was washed aqueous $Na_2S_2O_3$ (50 mL) and saturated $NaHCO_3$ (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (hexane/EtOAc 1:1) afforded the hydrolysis compound (2.68 g, 80%). To a solution of this compound in dry CH_2Cl_2 (20 mL) was added trichloroacetonitrile (3.95 mL, 39.37 mmol) and K_2CO_3 (0.82 g, 5.93 mmol). The reaction mixture was stirred at 0 ℃ for 2 hrs, and then concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (hexane/EtOAc 1:1 +0.5 %TEA) afforded imidate donor **11** as colorless oil (3.21 g, 99%). To a solution of glycosyl donor **11** (2.79 g, 3.38 mmol) and glycosyl acceptor **12** (1.83 g, 2.82 mmol) in dry CH_2Cl_2 (20 mL), TMSOTf (60 μL, 0.33 mmol) was added dropwise at 0 ℃. The reaction mixture was stirred at 0 ℃ for 30 min, and then neutralized with TEA. The reaction mixture was diluted with CH_2CL_2 (50 mL), and then washed with saturated $NaHCO_3$ (50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (hexane/EtOAc 2:1) afforded the desired product **13** as colorless oil (3.32 g, 90%). R_f=0.4 (hexane/EtOAc 1:1); $[\alpha]_D^{27}$= +28.5 (*c*=0.77 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.22 (s, 3 H, C*H*$_3$), 1.30 (s, 3 H, C*H'*$_3$), 1.61 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 1.98 (s, 3 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.02 (m, 1 H, H-3a$_{ax}$), 2.14 (dd, 1 H, $J_{3ax,3eq}$=12.9, $J_{3eq,4}$=4.8 Hz,

H-3a$_{eq}$), 2.52-2.62 (m, 4 H, $CH_3C(O)CH_2CH_2C(O)O$), 3.06 (m, 2H, H-6b, H-6b'), 3.14 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 3.20 (m, 1 H, $OCH_2CH_2CH_2NHZ$), 3.28 (m, 1 H, H-6a), 3.36 (m, 1 H, $OCH'_2CH_2CH_2NHZ$), 3.77 (m, 2 H, H-4a, H-8a), 3.87 (dd, 1 H, $J_{2,3}$=3.3, $J_{3,4}$=9.6 Hz, H-3b), 4.03-4.41 (m, 10 H, H-5a, H-7a, H-8a', H-5b, $PhCH_2$ (3 H), CH, CH_2, Fmoc), 4.45-4.51 (m, 3 H, $PhCH_2$), 5.01 (s, 2H, $PhCH_2OC(O)NH$), 5.05 (br, 1 H, NH), 5.10-5.26 (m, 4 H, H-1b, H-4b, $PhCH_2OC(O)$), 5.34 (d, 1 H, $J_{2,3}$=1.8 Hz, H-2b), 7.05-7.72 (m, 33 H, H$_{arom}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ=[24.7, 26.8 (2×CH_3)], 28.2 ($CH_3C(O)CH_2CH_2C(O)O$), 29.3 ($OCH_2CH_2CH_2NHZ$), 29.6 ($CH_3C(O)CH_2CH_2C(O)O$), 32.8 (C-3a), 37.9 ($CH_3C(O)CH_2CH_2C(O)O$), 38.3 ($OCH_2CH_2CH_2NHZ$), 46.8 (CH, Fmoc), 61.6 ($OCH_2CH_2CH_2NHZ$), 66.5 ($PhCH_2OC(O)NH$), 67.3 ($PhCH_2OC(O)$), 67.7 (C-8a), 67.9 (C-6b), 68.5 (C-2b), 69.4 (C-5b), 69.8 (CH_2, Fmoc), [70.4, 71.5, 73.4 (3×$PhCH_2$)], 71.9 (C-5a), 72.0 (C-4b), 72.4 (C-7a), 72.6 (C-4a), 74.6 (C-6a), 75.2 (C-3b), 98.2 (C-1b), 99.1 (C-2a), 109.7 (CH_3CCH_3), [120.0, 125.1, 127.1, 127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 128.0, 128.2, 128.3, 128.5, 128.6, 128.7, 135.0, 136.6, 137.7, 137.9, 138.0, 141.2, 143.4, 143.5 (C$_{arom}$)], 154.3 ($OC(O)O$, Fmoc), 156.3 ($PhCH_2OC(O)NH$), 167.8 ($PhCH_2OC(O)$), 171.7 ($CH_3C(O)CH_2CH_2C(O)O$), 206.2 ($CH_3C(O)CH_2CH_2C(O)O$); MALDI-TOF/MS: *m*/*z*: found: 1334.5 $[M+Na]^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{76}H_{81}NO_{19}Na$: 1334.5301; found: 1334.5301 $[M+Na]^+$.

Benzyl [N-benzyloxycarbonyl-3-amino-propyl *O*-(3, 6-di-*O*-benzyl-2-*O*-levulinoyl-α-D-mannopyranosyl)-(1→5)-4-*O*-benzyl-3-deoxy-7, 8-*O*-isopropylidene-α-D-manno -octulopyranosid] onate (14)

To a solution of **13** (1.0 g, 0.76 mmol) in CH2Cl2 (50 mL), TEA (12 mL) was added. The reaction mixture was stirred at room temperature overnight, and then concentrated *in vacuo*. Purification of the residue by column chromatography over silica gel (hexane/EtOAc 2:1) afforded the desired product **14** as colorless oil (0.78 g, 94%). R_f=0.36 (hexane/EtOAc 1:1); $[\alpha]_D^{27}$= +24.4 (*c*=0.43 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.21 (s, 3 H, C*H*$_3$), 1.30 (s, 3 H, C*H'*$_3$), 1.61 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 1.97 (m, 1 H, H-3a$_{ax}$), 2.02 (s, 3 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.14 (dd, 1 H, $J_{3ax,3eq}$=12.9, $J_{3eq,4}$=4.8 Hz, H-3a$_{eq}$), 2.37 (d, 1 H, *J*=2.1 Hz, O*H*), 2.51-2.62 (m, 4 H, $CH_3C(O)CH_2CH_2C(O)O$), 3.14 (m, 3 H, H-6b, $OCH_2CH_2CH_2NHZ$), 3.20 (m, 1 H, $OCH_2CH_2CH_2NHZ$), 3.28 (m, 2 H, H-6a, H-6b'), 3.39 (m, 1 H, $OCH'_2CH_2CH_2NHZ$), 3.68 (dd, 1 H, $J_{2,3}$=3.3, $J_{3,4}$=9.3 Hz, H-3b), 3.77 (m, 2 H, H-4a, H-8a), 3.91 (td, 1 H, $J_{3,4}$=9.3, $J_{4,5}$=9.9, $J_{4,OH}$=2.1 Hz, H-4b), 4.02 (m, 1 H, H-5b), 4.12-4.22 (m, 4 H, H-5a, H-7a, H-8a', $PhCH_2$), 4.28-4.55 (m, 5 H, $PhCH_2$), 5.01 (s, 2H, $PhCH_2OC(O)NH$), 5.06 (br, 1 H, N*H*), 5.08-5.22 (m, 3 H, H-1b, $PhCH_2OC(O)$), 5.34 (d, 1 H, $J_{2,3}$=1.8 Hz, H-2b), 7.14-7.30 (m, 25 H, H$_{arom}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ=[24.7, 26.8 (2×*C*H_3)], 28.1 ($CH_3C(O)CH_2CH_2C(O)O$), 29.3 ($OCH_2CH_2CH_2NHZ$), 29.7 ($CH_3C(O)CH_2CH_2C(O)O$), 32.9 (C-3a), 37.9 ($CH_3C(O)CH_2CH_2C(O)O$), 38.3 ($OCH_2CH_2CH_2NHZ$), 61.6 ($OCH_2CH_2CH_2NHZ$), 66.5 ($PhCH_2OC(O)NH$), 67.1 (C-4b), 67.3 ($PhCH_2OC(O)$), 67.7 (C-8a), 68.2 (C-2b), 69.1 (C-6b), [70.4, 71.6, 73.4 (3×Ph*C*H_2)], 71.0 (C-5b), 71.8 (C-5a), 72.4 (C-7a), 72.7 (C-4a), 74.6 (C-6a), 77.3 (C-3b), 98.6 (C-1b), 99.1 (C-2a), 109.6 (CH_3CCH_3), [127.3, 127.4, 127.5, 127.8, 128.0, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 135.1, 136.7, 137.8, 138.0, 138.2 (C$_{arom}$)], 156.3 ($PhCH_2OC(O)NH$), 167.9 ($PhCH_2OC(O)$), 171.8 ($CH_3C(O)CH_2CH_2C(O)O$), 206.1

($CH_3C(O)CH_2CH_2C(O)O$); MALDI-TOF/MS: *m*/*z*: found: 1112.5 $[M+Na]^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{61}H_{71}NO_{17}Na$: 1112.4620; found: 1112.4618 $[M+Na]^+$.

Benzyl {N-benzyloxycarbonyl-3-amino-propyl *O*-{3, 6-di-*O*-acetyl-4-*O*-benzyl-2-*O*-[(1S)-phenyl-2-(phenylsulfanyl)ethyl]-α-D-glucopyranosyl}-(1→3)-*O*-(4, 6-di-*O*-benzyl -α-D-mannopyranosyl)-(1→4)-*O*-(3, 6-di-*O*-benzyl-2-*O*-levulinoyl-α-D-mannopyranosyl)-(1→5)-4-*O*-benzyl-3-deoxy-7, 8-*O*-isopropylidene-α-D-manno-octulopyranosid} onate (15)

A mixture of DDQ (107 mg, 0.47 mmol), 4 Å molecular sieves (0.4 g) in CH_2Cl_2 (2 mL) was stirred at 0 ℃ for 30 min. The flask was wrapped by aluminum foil to exclude light. A solution of **5** (0.39 g, 0.36 mmol), **14** (0.36 g, 0.33 mmol) and 4 Å molecular sieves (0.8 g) in CH_2Cl_2 (2 mL) was added. The reaction mixture was stirred at room temperature for 4 h and then quenched with aqueous solution of ascorbic acid (0.7%)/citric acid (1.3)/NaOH (0.9%). The mixture was stirred for 5 min and then diluted with CH_2Cl_2. The mixture was filtered through Celite. The filtrate was washed with saturated $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was mixed with DTBMP (0.38 g, 1.85 mmol) and coevaporated with dry toluene three times. To a solution of the residue and 4 Å molecular sieves (1.5 g) in 1, 2-dichloroethane (40 mL), a solution of 1 M MeOTf in 1, 2-dichloroethane (0.16 mL) was added at 0 ℃. The reaction mixture was stirred at 45 ℃ for 24 h and then quenched with TEA. The mixture was diluted CH_2Cl_2 and then filtered through Celite. The filtrate was washed with saturated $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification of the residue by column chromatography on LH-20

(CH_2Cl_2/MeOH 1:1) afforded compound **15** as white solid (0.43 g, 60%). R_f=0.58 (hexane/EtOAc 1:1); $[\alpha]_D^{27}$= +51.3 (*c*=0.4 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ=1.21 (s, 3 H, C*H*$_3$), 1.30 (s, 3 H, C*H'*$_3$), 1.49 (s, 3 H, C*H*$_3$C(O)), 1.61 (m, 2 H, $OCH_2CH_2CH_2NHZ$), 1.85 (s, 3 H, C*H*$_3$C(O)), 1.95 (s, 3 H, C*H*$_3$C(O)$CH_2CH_2C(O)O$), 1.97 (m, 1 H, H-3a$_{ax}$), 2.10 (d, 1 H, *J*=1.0 Hz, O*H*), 2.14 (dd, 1 H, $J_{3ax,3eq}$=12.5, $J_{3eq,4}$=4.5 Hz, H-3a$_{eq}$), 2.32-2.50 (m, 4 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.69 (dd, 1 H, *J*=7.0, 13.5 Hz, PhCHOC*H*$_2$SPh), 2.83 (dd, 1 H, *J*=7.0, 13.5 Hz, PhCHOC*H'*$_2$SPh), 3.04 (d, 1 H, $J_{6,6'}$=11.0 Hz, H-6b), 3.14 (m, 3 H, H-6b', $OCH_2CH_2CH_2NHZ$), 3.21 (m, 1 H, $OCH_2CH_2CH_2NHZ$), 3.26-3.33 (m, 4 H, H-6a, H-3c, H-5c, H-2d), 3.37 (m, 2 H, H-4d, OC*H'*$_2$$CH_2CH_2NHZ$), 3.52 (dd, 1 H, $J_{5,6}$=5.0, $J_{6,6'}$=10.5 Hz, H-6c), 3.65 (d, 1 H, $J_{6,6'}$=10.5 Hz, H-6c'), 3.70-3.77 (m, 3 H, H-4a, H-8a, H-4c), 3.82 (dd, 1 H, $J_{2,3}$=3.0, $J_{3,4}$=9.0 Hz, H-3b), 3.86 (s, 1 H, H-2c), 4.05 (d, 1 H, *J*=10.0 Hz, H-4b), 4.10-4.23 (m, 8 H, H-5a, H-7a, H-8a', H-5b, H-6d, H-6d', PhC*H*OCH_2SPh, PhC*H*$_2$ (1 H)), 4.36-4.49 (m, 11 H, H-1c, H-5d, PhC*H*$_2$ (9 H)), 4.58 (d, 1 H, *J*=12.0 Hz, PhC*H*$_2$), 4.94 (d, 1 H, $J_{1,2}$=2.5 Hz, H-1d), 5.01 (s, 2H, PhC*H*$_2$OC(O)NH), 5.04 (br, 1 H, N*H*), 5.08 (s, 1 H, H-1b), 5.18 (dd, 2 H, *J*=12.0 Hz, PhC*H*$_2$OC(O)), 5.26 (d, 1 H, *J*=12.0 Hz, PhC*H*$_2$), 5.33 (s, 1 H, H-2b), 5.56 (t, 1 H, $J_{2,3}$=9.5, $J_{3,4}$=9.5 Hz, H-3d), 6.97-7.29 (m, 50 H, H$_{arom}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ=[20.7, 20.8 (2×*C*H_3C(O)), [24.7, 26.8 (2×*C*H_3)], 27.9 ($CH_3C(O)CH_2CH_2C(O)O$), 29.2 ($OCH_2CH_2CH_2NHZ$), 29.7 ($CH_3C(O)CH_2CH_2C(O)O$), 32.8 (C-3a), 37.8 ($CH_3C(O)CH_2CH_2C(O)O$), 38.3 ($OCH_2CH_2CH_2NHZ$), 41.3 (PhCHO*C*H_2SPh), 61.6 ($OCH_2CH_2CH_2NHZ$), 63.2 (C-6d), 66.5 (Ph*C*H_2OC(O)NH), 67.4 (Ph*C*H_2OC(O)), 67.7 (C-8a), 68.2 (C-6b), 68.9 (C-5d), 69.2 (C-2b), 69.4 (C-6c), 69.9 (C-2c), [70.4, 72.0, 73.1, 74.1, 74.6,

75.7 (6×Ph*C*H_2)], 70.7 (C-4b), 72.1 (C-5a), 72.3 (C-7a, C-5b), 72.7 (C-4a, C-4c), 73.5 (C-3d), 74.8 (C-6a), 76.0 (C-5c), 76.3 (C-4d), 77.1 (C-3b), 78.7 (C-2d), 82.2 (Ph*C*HOCH_2SPh), 85.5 (C-3c), 97.8 (C-1d), 98.4 (C-1b), 99.1 (C-2a), 99.3 (C-1c), 109.6 (CH_3*C*CH_3), [126.0, 126.7, 127.0, 127.2, 127.3, 127.4, 127.5, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.9, 129.1, 135.0, 136.1, 136.7, 137.5, 137.9, 138.2, 138.3, 138.5, 139.2, 140.6 (C_{arom})], 156.3 (PhCH_2O*C*(O)NH), 167.8 (PhCH_2O*C*(O)), [169.7, 170.5 (CH_3*C*(O))], 171.8 (CH_3C(O)CH_2CH_2*C*(O)O), 206.1 (CH_3*C*(O)CH_2CH_2C(O)O); MALDI-TOF/MS: *m*/*z*: found: 2002.8 [*M*+Na]$^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{112}H_{125}NO_{29}SNa$: 2002.7956; found: 2002.7954 [*M*+Na]$^+$.

Benzyl {N-benzyloxycarbonyl-3-amino-propyl *O*-{3, 6-di-*O*-acetyl-4-*O*-benzyl-2-*O*-[(1S) -phenyl-2-(phenylsulfanyl)ethyl]-α-D-glucopyranosyl}-(1→3)-*O*-[(3, 4, 6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1→2)-*O*-(4, 6-di-*O*-benzyl-α-D-mannopyranosyl) -(1→4)]-*O*-(3, 6-di-*O*-benzyl-2-*O*-levulinoyl-α-D-mannopyranosyl)-(1→5)-4-*O*-benzyl -3-deoxy-7, 8-*O*-isopropylidene-α-D-manno-octulopyranosid} onate (17)

A mixture of donor **16** (0.12 g, 0.3 mmol), acceptor **15** (60 mg, 0.03 mmol), DTBMP (123 mg, 0.6 mmol) and 4 Å molecular sieves (0.2 g) in CH_2Cl_2 (2 mL) was stirred at 0 ℃ for 30 min and then cooled to -78 °C. The flask was wrapped in aluminum foil to exclude light. AgOTf (115 mg, 0.45 mmol) was added and then allowed to warm to 0 ℃. The reaction mixture was stirred overnight and then quenched with saturated $NaHCO_3$. The mixture was filtered through Celite. The filtrate was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue by

column chromatography on LH-20 (CH_2Cl_2/MeOH 1:1) afforded compound **17** as white solid (35 mg, 51%). R_f=0.62 (hexane/EtOAc 1:1); $[\alpha]_D^{27}$= +60.5 (c=0.2 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ=1.27 (s, 3 H, CH_3), 1.37 (s, 3 H, CH'_3), 1.48 (s, 3 H, CH_3C(O)), 1.67 (m, 2 H, $OCH_2CH_2CH_2NHZ$), [1.92, 2.02, 2.03, 2.13 (4×s, 4×3 H, 4×CH_3C(O))], 2.06 (s, 3 H, CH_3C(O)$CH_2CH_2C(O)O$), 1.98 (m, 1 H, H-3a_{ax}), 2.22 (dd, 1 H, $J_{3ax,3eq}$=12.5, $J_{3eq,4}$=4.0 Hz, H-3a_{eq}), 2.37-2.57 (m, 4 H, $CH_3C(O)CH_2CH_2C(O)O$), 2.75 (dd, 1 H, J=7.0, 13.0 Hz, PhCHOCH_2SPh), 2.89 (dd, 1 H, J=7.0, 13.0 Hz, PhCHOCH'_2SPh), 3.12 (d, 1 H, $J_{6,6'}$=10.5 Hz, H-6b), 3.19 (m, 3 H, H-6b', $OCH_2CH_2CH_2NHZ$), 3.23-3.44 (m, 7 H, H-6a, H-3c, H-5c, H-2d, H-4d, $OCH_2CH_2CH_2NHZ$), 3.60-3.68 (m, 2 H, H-6c, H-2e), 3.70 (d, 1 H, $J_{6,6'}$=11.0 Hz, H-6c'), 3.78-3.86 (m, 3 H, H-4a, H-8a, H-4c), 3.89-3.95 (m, 2 H, H-2c, H-3b), 4.02-4.29 (m, 12 H, H-5a, H-7a, H-8a', H-4b, H-5b, H-6d, H-6d', H-5e, H-6e, H-6e', PhCHOCH_2SPh, PhCH_2 (1 H)), 4.43-4.57 (m, 11 H, H-1c, H-5d, PhCH_2 (9 H)), 4.64 (d, 1 H, J=12.5 Hz, PhCH_2), 4.98 (d, 1 H, $J_{1,2}$=3.0 Hz, H-1d), 5.10-2.27 (m, 6H, H-1b, PhCH_2OC(O)NH, NH, PhCH_2OC(O)), 5.31-5.35 (m, 3 H, H-1e, H-3e, PhCH_2 (1 H)), 5.39-5.44 (m, 2 H, H-2b, H-4e), 5.64 (t, 1 H, $J_{2,3}$=9.5, $J_{3,4}$=9.5 Hz, H-3d), 7.03-7.37 (m, 50 H, H_{arom}); ^{13}C NMR (125 MHz, $CDCl_3$): δ=[20.8, 3×20.9, 21.0 (5×CH$_3$C(O)), [24.7, 26.8 (2×CH$_3$)], 27.9 ($CH_3C(O)CH_2CH_2C(O)O$), 29.2 ($OCH_2CH_2CH_2NHZ$), 29.7 ($CH_3C(O)CH_2CH_2C(O)O$), 32.8 (C-3a), 37.8 ($CH_3C(O)CH_2CH_2C(O)O$), 38.3 ($OCH_2CH_2CH_2NHZ$), 41.3 (PhCHOCH_2SPh), 57.9 (C-2e), 61.4 (C-6e), 61.6 ($OCH_2CH_2CH_2NHZ$), 63.2 (C-6d), 66.5 (PhCH_2OC(O)NH), 67.2 (C-5e), 67.3 (C-4e), 67.4 (PhCH_2OC(O)), 67.7 (C-8a), 68.2 (C-6b), 68.5 (C-3e), 68.9 (C-5d), 69.2 (C-2b), 69.4 (C-6c), 69.9 (C-2c), [70.4, 72.0, 73.1, 74.1, 74.6, 75.2 (6×PhCH_2)], 70.7 (C-4b), 72.1

(C-5a), 72.3 (C-7a, C-5b), 72.7 (C-4a, C-4c), 73.5 (C-3d), 74.8 (C-6a), 76.0 (C-5c), 76.3 (C-4d), 77.1 (C-3b), 78.7 (C-2d), 82.2 (Ph*C*HOCH$_2$SPh), 85.5 (C-3c), 96.1 (C-1e), 97.8 (C-1d), 98.4 (C-1b), 99.3 (C-1c); MALDI-TOF/MS: *m*/*z*: found: 2315.9 [*M*+Na]$^+$; MALDI-FTICR/MS: *m*/*z*: calcd for $C_{124}H_{140}N_4O_{36}SNa$: 2315.8866; found: 2315.8864 [*M*+Na]$^+$.

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CHAPTER 6

CONCLUSIONS

Thioglycosides in oligosaccharide synthesis has been reviewed. The properties of thioglycosides including their ability to offer efficient protection of the anomeric center, to be used in direct glycosylations in the presence of soft electrophiles, and to be transformed into a range of other glycosyl donors and act as acceptors in glycosylation reactions, which make thioglycosides particularly suitable for use in chemoselective, orthogonal and iterative glycosylationsin was reviewed in detail.

Glycosidases play important roles in the biosynthesis of glycoproteins. Efforts to blocking the biosynthesis of polylactosamine chains of *N*-linked glycoproteins have not focused on GlcNAc-TV but instead on inhibitors of glycosidases that act earlier in the biosynthesis of N-glycan to prevent the formation of the biosynthetic pathway primer of GlcNAc-TV. Most efforts in the field have focused on Golgi α-mannosidase II (HGMII), which trims two mannosyl residues from $GlcNAcMan_5GlcNAc_2$ to form the core $GlcNAcMan_3GlcNAc_2$ moiety. Understanding the catalytic and extended binding sites could help in the design and synthesis of more potent and selective inhibitors. Successfully synthesized a range of part-structures of $GlcNAcMan_5GlcNAc_2$ substrates are very valuable compounds in the investigation of catalytic and extended binding sites of golgi α-mannosidase II. X-ray crystallography studies, which are still ongoing, could aid in a better understanding of the catalytic and extended binding sites of Golgi α-mannosidase II.

Mannostatin A is a potent inhibitor of the mannose trimming enzyme Golgi α-mannosidase II (GMII), which atcs late in the *N*-glycan processing pathway. Inhibition of this enzyme provides a

route to blocking the oncogene-induced changes in cell surface oligosaccharide structures. To probe in detail the interactions of the thiomethyl function of Mannostatin A with *d*GMII, Mannostatin B and analogs, which contain hydroxyl, methoxy or deoxy, respectively instead of the thiomethyl of Mannostatin A, were prepared. Computational studies and x-ray crystallography studies, which are still ongoing, could aid in a better understanding of the mode of inhibition by mannostatin A.

An anthrose-containing trisaccharide and a series of structurally related analogs have successfully been synthesized to study the antigenicity of anthrose. The analogues lacked either the methyl ether at C-2 or contained modified C-4 amino functionalities of anthrose. The synthetic compounds were equipped with an aminopropyl spacer to facilitate conjugation to the carrier proteins mariculture Keyhole Limpet Hemocyanin (mcKLH) and bovine serum albumin (BSA). Serum antibodies of rabbits immunized with live or irradiated spores of *B. anthracis* Sterne $34F_2$ were able to recognize the synthetic trisaccharide-mcKLH conjugate. The specificity of the interaction was confirmed by competitive inhibition with the free- and BSA-conjugated trisaccharides. Inhibition using the trisaccharide analogues demonstrated that the antigenic nature of the trisaccharide can be altered by modification of specific side groups in the terminal glycosyl structure and the isovaleric acid moiety of anthrose is an important structural motif for antibody recognition. These data demonstrate that 1) anthrose is a specific antigenic determinant of the *B. anthracis* Sterne spore and serum of rabbits immunized by live or irradiated spores of *B. anthracis* Sterne $34F_2$ recognize the trisaccharide 1, which is derived from the glycoprotein BclA; 2) this antigen is presented to the immune system of rabbits receiving the anthrax live-spore vaccine; 3) synthetic analogues of the oligosaccharide retain the antigenic structure; and 4) the antigenic region is localized to specific terminal groups of the oligosaccharide. Collectively these

data provide an important proof-of-concept step in the synthesis and development of spore-specific reagents for detection and targeting of non-protein structures in *B. anthracis*.

We developed a highly convergent synthesis of a number of truncated structures to determine the smallest part structure of the core oligosaccharide, which can elicit antibodies that recognize LPS from *F. Tularensis*. Such a structure will be attractive to be further developed as a vaccine candidate for tularemia. The target compound also presents an exciting opportunity to demonstrate how the new auxiliary-based methodology for the introduction of α-glycosidic linkages can be used in combination with the methodology for the formation of a β-glycosidic linkage. Building blocks for the synthesis of hexasaccharide have successfully been synthesized. One more glycosylation and removal of protecting groups are still ongoing. The synthetic compounds were equipped with an aminopropyl spacer to facilitate conjugation to the carrier proteins mariculture Keyhole Limpet Hemocyanin (mcKLH) and bovine serum albumin (BSA). Serum antibodies of rabbits immunized with a live attenuated vaccine (LVS) of F. tularensis will be used to see whether they can recognize the synthetic hexasaccharide-mcKLH conjugate.