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Oligomer-supported Solution Synthesis of Oligosaccharides Using Low Molecular Weight Poly(ethylene Glycol) Linkers

by

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To my loving wife Kui
For her patience and support

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ABSTRACT

A new approach has been studied for solution-phase oligosaccharide syntheses using low molecular weight poly(ethylene glycol) monomethyl ethers (MPEG, average M_n= 550 and 750) as oligomer supports. A complex branched mannononaose (Man_9) has been synthesized by this method in a very concise way to yield a large quantitiy of final product. Attempt has been made towards the synthesis of a heptasaccharide phytoalexin elicitor (HPE).

Model linear mannotetraose and branched mannopentaose were first tested using the novel HMB low molecular weight poly(ethylene glycol) linker. The use of this linker as the oligomer support retains the normal advantages of polymer-supported solution synthesis of oligosaccharides. Purification of the supported synthons by flash column chromatography on silica gel is greatly simplified. Another advantage of this approach is that the reaction can be monitored readily by the usual arsenal of spectroscopic techniques. Both compounds were obtained in over 10% overall yields.

In order to synthesize properly protected glycosyl donors for the construction of the mannononaose, the regioselective reductive ring opening of benzylidene acetals in carbohydrates with BH_3/Bu_2BOTf and regioselective acylation of hexopyranosides with pivaloyl chloride have been developed. BH_3/Bu_2OTf is an effective reagent to reductively cleave 4,6-*O*-benzylidene acetals of various hexopyranosides to the corresponding 4-*O*-benzyl ethers. 4,6-*O*-isopropylidene acetals can be similarly cleaved. Common protecting groups are stable to the reaction conditions. On the other hand, the regioselectivity in the acylation of hexopyranosides with pivaloyl chloride in pyridine was studied. Manno- and galacto-pyranosides were regioselectively acylated to give the 3-*O*-pivaloylated compounds in good yields. Both methodologies have been used to provide properly protected mannoside donors and acceptors for polymannan syntheses. The regioselective reductive ring opening of 4,6-*O*-benzylidene acetals of hexopyranosides has also been demonstrated

by concise syntheses of two building blocks of a heptasaccharide phytoalexin elicitor (HPE).

A concise synthesis of the mannan residue of a highly branched mannose type oligosaccharide present on the viral coat of HIV-1 from only two monosaccharide building blocks was achieved. The synthesis requires only five coupling steps instead of the seven or more steps required for the conventional block synthesis. In addition, even though the synthesis of HPE has not been completed to the last step, it showed that adjustment of the length of the poly(ethylene glycol) oligomer support could result in better overall yields. In both cases, ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC NMR spectroscopies have proven to be extremely useful in monitoring the progress of oligosaccharide construction. These syntheses demonstrate that oligomer-supported solution synthesis using low molecular weight poly(ethylene glycol) is a powerful tool for the rapid and efficient preparation of complex oligosaccharides.

RÉSUMÉ

Nous avons étudié une nouvelle approche pour la synthèse d'oligosaccharides en solution par l'utilisation des éthers monomethyliques de glycol polyéthylènique de faible masse moléculaire (MPEG, M_n = 550 et 750) en tant que supports oligomèriques. Nous avons synthétisé une mannononaose branché complexe (Man_9) par cette méthode, de manière concise, pour aboutir à une quantité importante de produit final. Par ailleurs, nous avons essayé de synthétiser un eliciteur de phytoalexin heptasaccaridique (HPE).

Les modèles de mannotetraose linéaire ainsi que le mannopentaose branché ont tout d'abord été testés avec le nouveau de glycol polyethylenique HMB de faible masse moléculaire comme lien. L'utilisation de ce lien comme oligomère de support permet de conserver les avantages habituels d'une synthèse d'oligosaccharides en solution supportée par des polymères. La purification des synthons supportés, par chromatographie sur gel de silice, en colonne, est grandement simplifiée. Par ailleurs, les techniques habituelles de spectroscopie permettent de suivre l'avancement de la réaction, ce qui constitue un autre avantage de cette méthode. Les deux composés ont été obtenus avec un rendement supérieur à 10%.

Pour synthétiser des donneurs glycosyliques suffisament protégés pour la construction du mannononaose, nous avons développé l'ouverture reductrice régio-sélective du cycle de l'acetal de benzylidène dans les carbohydrates par le BH_3/Bu_2BOTf et l'acylation des hexopyranosides par le chlorure de pivaloyl. Le BH_3/Bu_2BOTf est un réactif efficace pour le clivage par réduction des acetals de 4,6-*O*-benzylidène de plusieurs hexopyranosides pour aboutir au ethers de 4-*O*-benzyl correspondants. Les acetals de 4,6-*O*-isopropylidène peuvent être clivés de manière similaire. Les groupes protecteurs communs sont stables dans les conditions de la réaction. D'autre part, nous avons étudié la régio-sélectivité de l'acylation des hexopyranosides par le chlorure de pivaloyl dans la pyridine. L'acylation régio-sélective des manno- et galacto-pyranosides a donné les 3-*O*-

pivaloylatés avec un bon rendement. Ces deux méthodes ont été utilisées pour l'obtention de donneurs mannosides et d'accepteurs suffisamment protégés. L'ouverture réductrice régio-sélective des acetals de 4,6-*O*-benzylidène d'hexopyranosides a aussi été démontrée par des systèmes consis de deux blocs constructeurs d'un eliciteur de phytoalexin heptasaccaridique (HPE).

Nous avons réussi la synthèse d'un résidu mannan d'un polysaccharide de type mannose extrêmement branché présent sur le manteau du HIV-1 à partir de deux blocs monosaccharides seulement. La synthèse requiert seulement cinq étapes au lieu de sept par des méthodes habituelles. De plus, malgré le fait que nous n'ayons pas atteind la dernière étape de la synthèse du HPE, nous avons pu observer que l'ajustement de la longueur de l'oligomère support poly(ethylene glycol) peut engendrer de meilleurs rendements. Dans les deux cas, les spectroscopies RMN ^{1}H-^{1}H COSY et ^{1}H-^{13}C HMQC se sont montrées très utiles pour suivre l'évolution de la construction de l'oligosaccharide. Ces études montrent que les synthèses en solution supportées par oligomères, utilisant du glycol polyéthylènique de faible masse moléculaire, sont un outil puissant pour la préparation rapide et performante d'oligosaccharides complexes.

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List of Abbreviations

Å	angstrom
Ac	acetyl
All	allyl
anal.	(elemental) analysis
Ar	aromatic
Asn	asparagine
Bn	benzyl
Boc	*tert*-butoxycarbonyl
BOP	bis(2-oxo-3-oxazolidinyl)phosphinyl
br	broad (spectral)
BSA	bovine serum albumin
Bu	butyl
Bz	benzoyl
c	concentration (g/100mL)
Calcd	calculated
Cbz	benzyloxycarbonyl
CMP	(+)-cytidine-5'-monophosphate
COSY	homonulcear correlation spectroscopy
CPG	controlled pore glass
δ	chemical shift
d	doublet
D	dextrorotatory
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-dicyclohexylcarbodiimide
DEAD	diethyl azodicarboxylate

DIEA	N,N-diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	N,N-dimethylacetamide
DMTST	dimethyl(methylthio)sulfonium triflate
DTBP	2,6-di-*tert*-butylpyridine
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
equiv.	equivalent
Et	ethyl
FAB	fast atom bombardment
Fuc	fucose
Gal	galactose
GDP	guanosine 5'-diphosphate
Glc	glucose
GlcNAc	N-acetylglucosamine
Gly	glycine
h	hour
Hz	hertz
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMB	4-(hydroxymethyl)benzoic acid
HMQC	hetero multiple quantum correlation
HOBt	benzotriazol
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
J	coupling constant
i-Pr	isopropyl
Lev	levulinyl
m	multiplet (spectral)

M_n	number-average molecular weight (g/mol)
MALDI	matrix-assisted laser desorption ionization
Man	mannose
Me	methyl
min	minute
mp	melting point
MP	4-methoxyphenyl
MPEG	poly(ethylene glycol) monomethyl ether
MTBE	methyl *tert*-butyl ether
Ms	mesylate
MS	mass spectroscopy and molecular sieve
NBS	N-bromosuccinimide
NeuAc	N-acetylneuraminic acid
NIS	N-iodosuccinimide
NMR	nuclear magnetic resonance spectroscopy
PEG	poly(ethylene glycol)
Pent	pentenyl
Ph	phenyl
Phth	phthalyl
Piv	pivaloyl
ppm	parts per million
PS	polystyrene
psi	pounds per square inch
Py	pyridine
R_f	retardation factor
rt	room temperature
s	singlet (spectral)

SE	2-(trimethylsilyl)ethyl
t	triplet (spectral)
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TEA	triethylamine
TESOTf	triethylsilyl triflate
TFA	trifluoroacetic acid
TfOH	triflic acid
THF	tetrahydrofurane
TLC (or tlc)	thin layer chromatograpgy
TMSOTf	trimethylsilyl triflate
TOF	time of flight
Tri	triphenylmethyl
UDP	uridine 5'-diphosphate

CHAPTER 1

Solid-phase and Polymer-supported Solution Syntheses of Oligosaccharides - an Overview

1.0 Introduction.

Glycoconjugates are the most functionally and structurally diverse molecules in nature and it is now well established that protein- and lipid-bound saccharides play essential roles in many cellular processes.[1] The efficient preparation of oligosaccharides is of central importance for the application of these glycoconjugates in biological and pharmaceutical sciences. Compared to the synthesis of peptides and nucleic acids, the chemical synthesis of oligosaccharides poses a much greater challenge. A major problem in syntheses involving carbohydrates is the presence of several reactive hydroxyl groups in each sugar residue. To achieve an unambiguous synthesis, first, it is necessary to protect those hydroxyl groups that will not be involved in the reaction and to selectively remove the temporary protecting groups for subsequent glycosylation. Secondly, the anomeric stereoselectivity at each step has to be controlled, otherwise there will be too many stereoisomers. In order to minimize the number of steps for assembly of the monomeric glycosyl donors and acceptors to a complex oligosaccharide as well as to make the purification and identification of the intermediates easier, a highly convergent block synthetic strategy is always desired. Consequently most of the synthetic effort is directed towards the preparation of the monomeric glycosyl donors and acceptors. Thirdly, technical difficulty in the preparation of complex oligosaccharides is the chromatographic purification of the desired component after each glycosylation step. In classical liquid phase

syntheses, purification has proven to be a constant challenge. Thus, the syntheses of oligosaccharides are often very time- and labour-consuming.

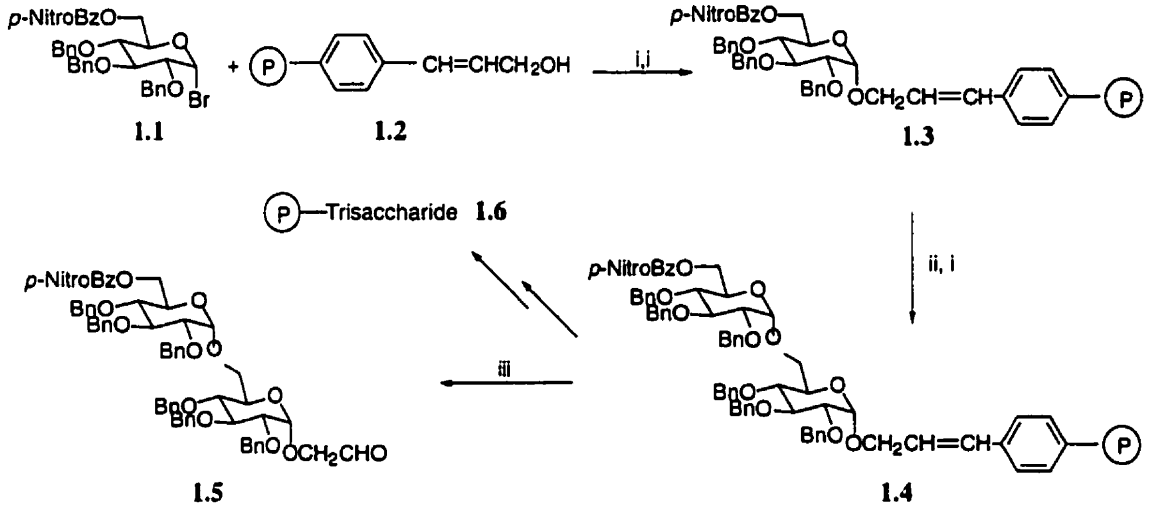
The use of crosslinked and functionalized polymer and inorganic supports have been extensively studied for the synthesis of polypeptides[2] and oligonucleotides.[3] The stepwise synthesis on solid support has many attractive features and should, in principle, be applicable to the preparation of oligosaccharides. The main advantages of the solid-phase synthesis are that (a) the growing molecule is covalently bonded to an insoluble support, purification is simplified at each step by filtering and washing; (b) the reaction rates can be increased by using a large excess of reagents which can be easily separated after the completion; and (c) automation of the synthesis is feasible.

1.1 Solid-phase Synthesis of Oligosaccharides.

1.1.1 Early Work.

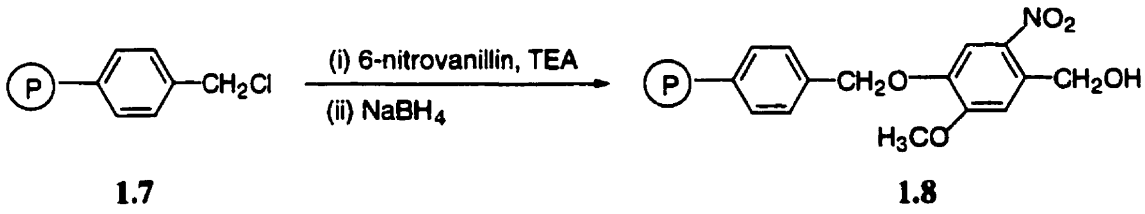
In the early seventies, Schuerch and co-workers[4] attempted solid-phase oligosaccharide synthesis using Merrifield's resin with cinnamyl alcohol functional groups. The polystyrene resin crosslinked with 1% divinylbenzene had a low degree of loading (<1 mmol/g) to avoid overcrowding of the reactive sites, and contained two different functionalities. The hydroxyl group was to attach the first sugar residue through formation of a glycosidic bond, while the double bond was used to allow for oxidative cleavage of the final product from its support under conditions which would not affect the oligosaccharide. The strategy for assembling the oligosaccharides included the use of a glycosyl donor, 2,3,4-tri-*O*-benzyl-6-*O*-(4-nitro-benzoyl)-α-D-glucopyranosyl bromide (**1.1**). In a typical reaction sequence shown in Scheme 1.1, coupling of the monosaccharide donor **1.1** to polymer **1.2** was achieved through a simple alcoholysis reaction. The glycosylation was performed at room temperature by a two-stage process which resulted in an ultimate

coupling yield of at least 90-95%. After quantitative removal of the temporary protecting group from (**1.3**), the coupling reaction was repeated. The second glycosylation reaction seemed to be easier than the first as yields of better than 90% could be obtained in a single alcoholysis reaction. The same reaction sequence, involving removal of the temporary protecting group followed by a single glycosylation, could be used to produce the polymer-bound trisaccharide (**1.6**) in excellent yield. Release of the di- or trisaccharides from their polymer supports was achieved in 51-91% yield by ozonolysis followed by reduction of the ozonide with dimethyl sulfide.



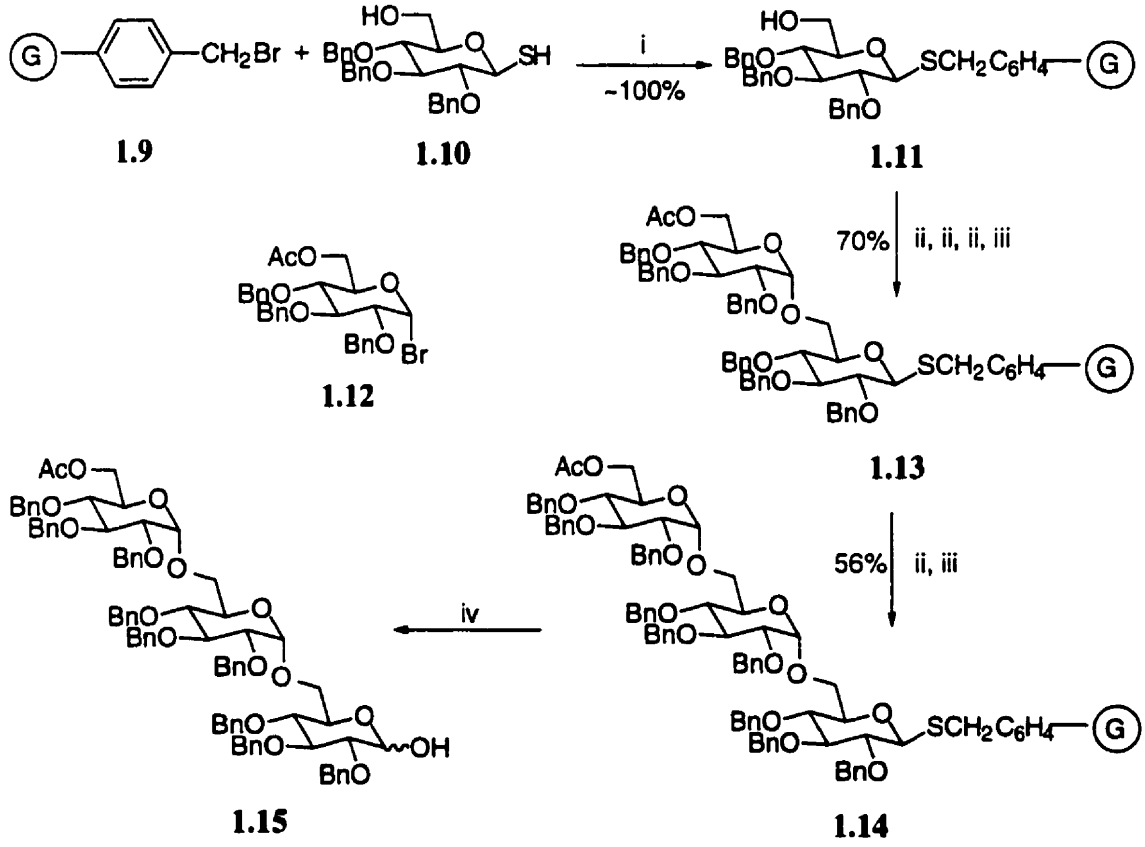
Reagents and conditions: (i) 4.0 equiv of **1.1**, 1.0 equiv of **1.2**, 2,6-lutidine, benzene, rt, 60 h; (ii) NaOEt, 2:1 benzene/EtOH, rt, 30 min; (iii) O_3, -78 °C, then CH_3SCH_3.

Scheme 1.1



Scheme 1.2

At the same time, Zehavi and co-workers[5] developed a new *O*-nitrobenzyl photocleavable linker for their solid-phase synthesis. The solid support (**1.8**) was prepared by substitition of the 2% crosslinked Merrifield's resin (**1.7**) with 6-nitrovanillin in the presence of triethylamine followed by reduction with sodium borohydride (Scheme 1.2). Their strategy for building the oligosaccharides was similar to the one used by Frechét and Schuerch.[4] Cleavage of the finished oligosaccharide from the support was accomplished by irradiation of a suspension of the polymer in dioxane at 320 nm. Unfortunately the cleavage reaction, which gave high yield in model studies, was much more sluggish and only partially released the saccharide from the support.



Reagents and conditions: (i) 1.0 equiv of **1.9**, 2.0 equiv of **1.10**, aqueous K_2CO_3; (ii) 3.0 equiv of **1.12**, 1.0 equiv of **1.11**, 2,6-lutidine, benzene, 55 °C, 72 h; (iii) NaOMe, MeOH, rt, 30 min; (iv) CH_3I/H_2O.

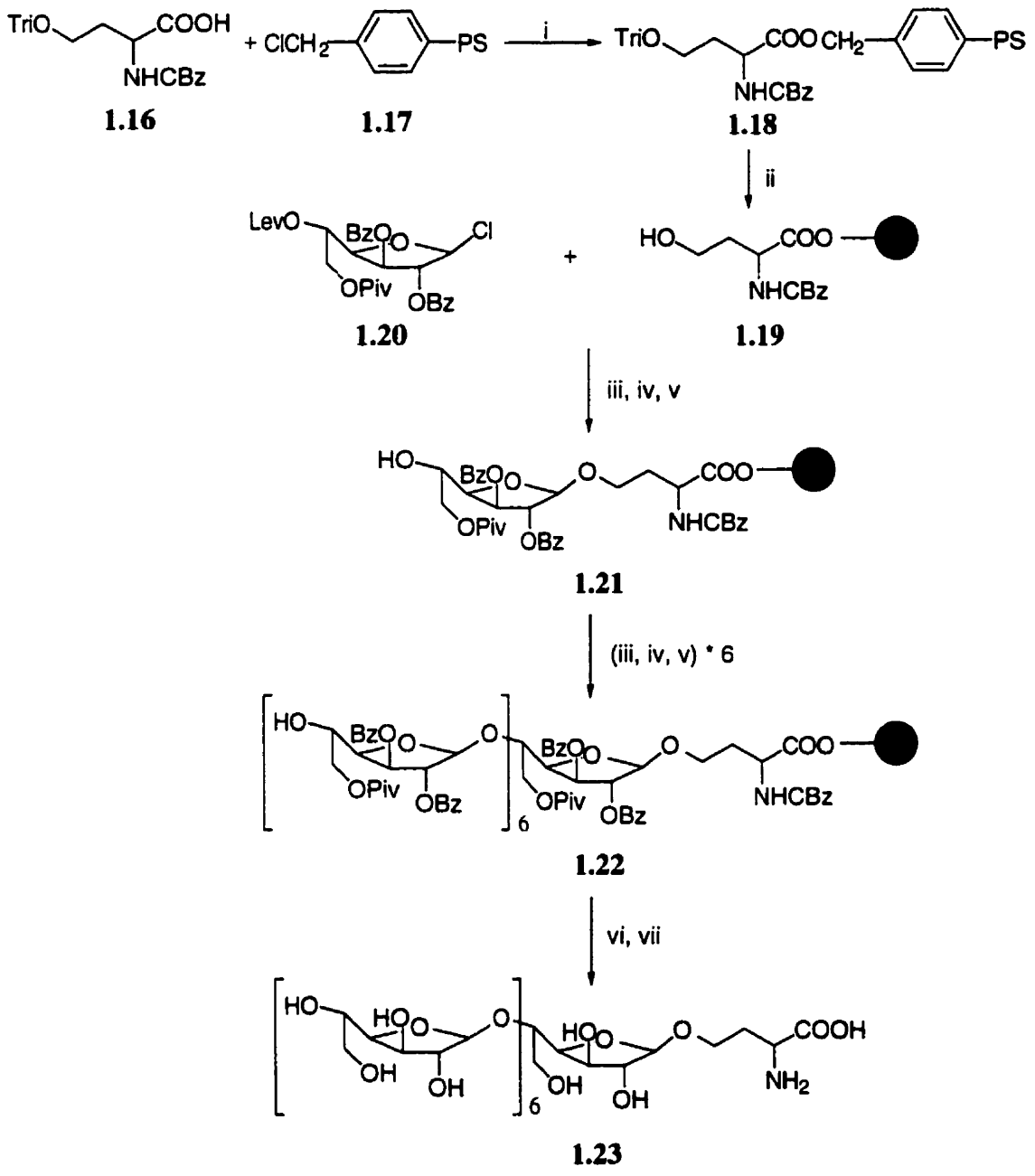
Scheme 1.3

Anderson and co-workers studied the use of the thioglycoside linkage as a way of anchoring the monosaccharide unit to a soluble linear polystyrene[6a], Merrifield's resin[6a] and glass support.[6b] In their glass support approach (Scheme 1.3), the porous glass beads were derivatized by reaction with p-bromomethylphenyltrichlorosilane. Coupling of the first sugar unit onto the support was accomplished by the reaction of the *p*-bromomethylphenyl glass (**1.9**) with 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranose (**1.10**) in the presence of aqueous potassium carbonate. The remainder of the sequence was similar to the glycosylation procedure of Fréchet and Schuerch[4] with 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**1.12**) as the donor. Cleavage of the trisaccharide from its glass support was carried out using a large excess of methyl iodide and water. The results of their study were very promising since the feasibility of the stepwise synthesis of oligosaccharides on glass was clearly demonstrated. Incomplete coupling and poor α/β selectivity in the glycosylation reaction, however, remained as major problems.

Other researchers also investigated different linkers on: a) Merrifield's resins,[7,8] b) soluble polystyrene resins[9] and c) derivatized glasses.[10] However, because of the lack of efficient glycoside coupling methods, little success had been achieved.

1.1.2 van Boom's Solid-phase Synthesis of a D-Galactofuranosyl Heptamer

In 1987, van Boom and co-workers reported the solid-phase synthesis of a naturally occuring β-(1-5)-linked D-galactofuranosyl heptamer containing the artificial linkage arm L-homoserine.[11] The synthetic approach is illustrated in Scheme 1.4. The partially protected L-homoserine **1.16** was attached to the Merrifield's resin (**1.17**) to afford the functionized polymer **1.18**. After removal of the trityl group of **1.18**, coupling of the glycosyl donor **1.20** to the alcohol **1.19** under Koenigs-Knorr conditions gave the homoserine glycoside **1.21**. It was reported that the glycosylation reaction did not go to completion and in order to minimize the formation of shorter fragments, the unreacted



Reagents and conditions: (i) Cs_2CO_3, DMF, 50 °C, 24 h; (ii) 10% TFA in CH_2Cl_2; (iii) 1.0 equiv of **1.19**, 3.0 equiv of $Hg(CN)_2/HgBr_2$, 2.0 equiv of **1.20**, 1:1 CH_3Cl/CH_3CN, 20 °C, 4 h; (iv) Ac_2O, Py, DMAP, rt, 10 min; (v) NH_2NH_2, AcOH, Py, 10 min; (vi) NH_4OH; (vii) H_2, Pd/C.

Scheme 1.4

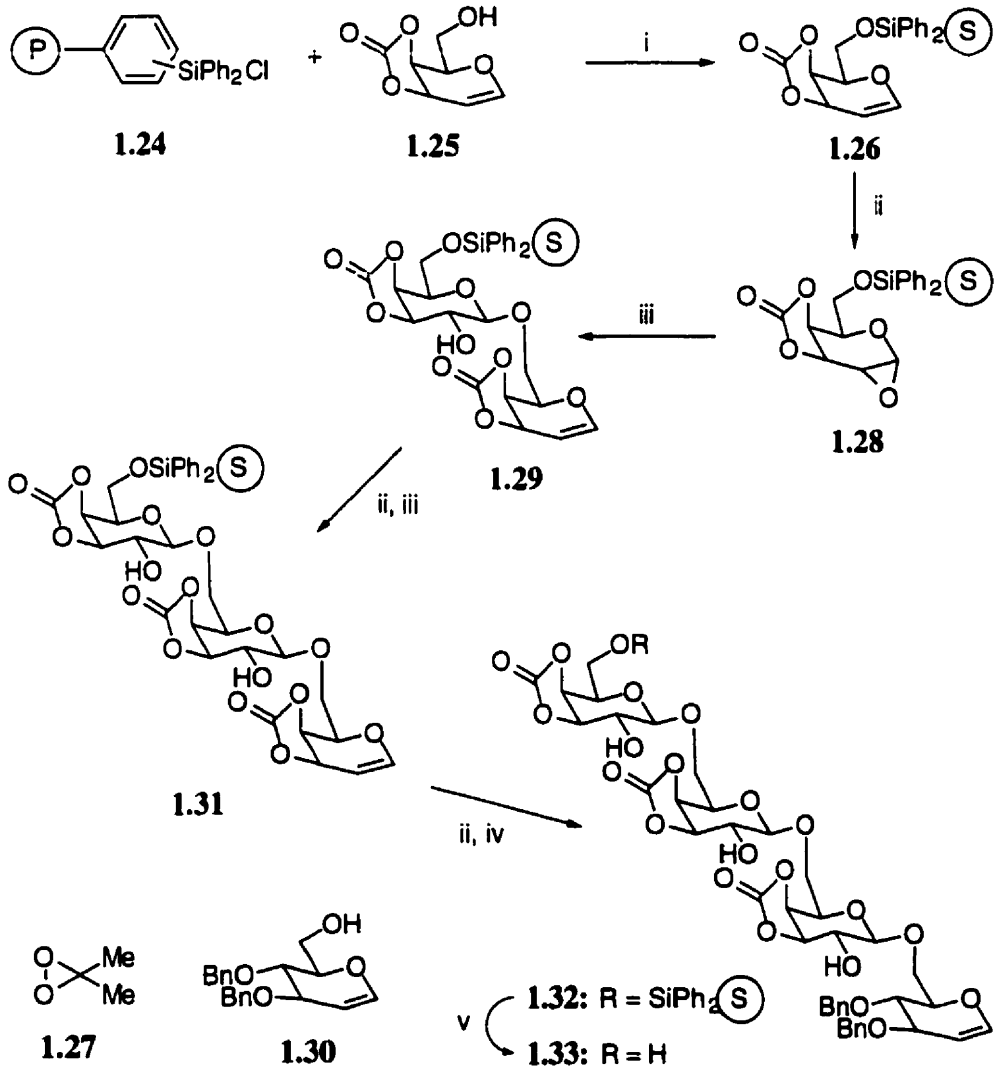
hydroxyl groups after each coupling reaction were capped by treatment with acetic anhydride in the presence of pyridine and DMAP. Deprotection of the levulinoyl groups was achieved by treatment with a hydrazine/pyridine/acetic acid mixture and the free hydroxyl groups were glycosylated with the donor **1.20**. After repeating this procedure six times, the polymer-bound heptasaccharide **1.22** was cleaved from the solid support by basic hydrolysis. At the end, removal of the benzyloxycarbonyl (Cbz) group by hydrogenolysis over Pd/C afforded the heptamer **1.23** in a 23% overall yield.

1.1.3 Danishefsky's Solid-Phase Synthesis of Oligosaccharides by the Glycal Assembly Methodology.

Danishefsky and co-workers reported a very interesting and succesful strategy of solid-phase synthesis of oligosaccharides in 1993.[12] Using the glycal assembly method, they synthesized the tetrasaccharide **1.33** on the derivatized copolymer of polystyrene cross-linked with 1% divinylbenzene in 32% overall yield (Scheme 1.5). The first glycal **1.25** was attached to the polymer support **1.24** by simple silylation. Exposure of the polymer-bound glycal **1.26** to 3,3-dimethyldioxirane (**1.27**) gave the 1,2-anhydrosugar **1.28**, which was coupled to the partially protected D-galactal **1.25** in the presence of zinc chloride to afford the polymer-bound disaccharide **1.29**. Repetition of the sequence, using acceptors **1.25** and **1.30** in sequence, followed by removal from the solid support with TBAF, provided the partially protected tetrasaccharide **1.33** in 32% overall yield, giving an average of ca.90% yield per coupling step.

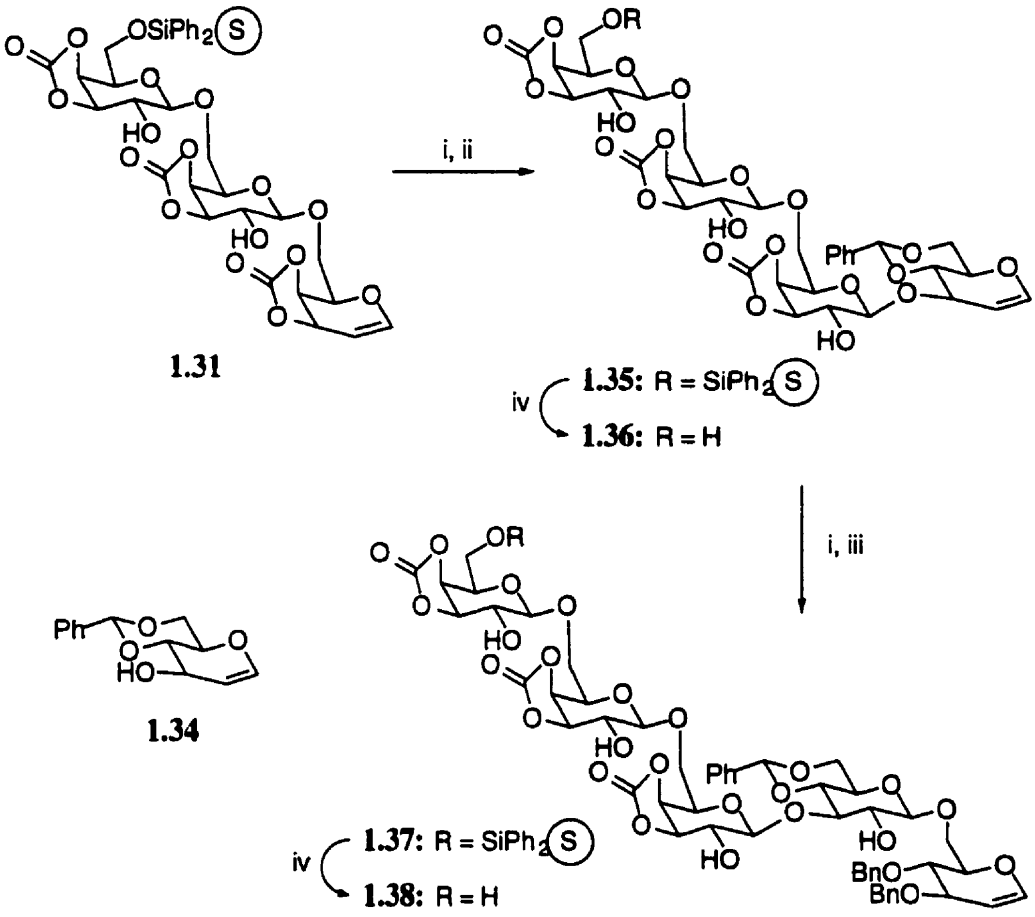
Less reactive secondary hydroxyl groups in acceptor glycals are also accommodated by this method (Scheme 1.6).[13] The polymer-bound trisaccharide glycal **1.31**, after epoxidization with 3,3-dimethyldioxirane (**1.27**), coupled with the D-glucal derivative **1.34** to yield **1.35**. Cleavage from the solid support with TBAF afforded the tetrasaccharide **1.36** in 66% overall yield from **1.26**. Subsequent epoxidation of the

tetrasaccharide glycal **1.35** with **1.27** gave the epoxide donor, which reacted with the acceptor **1.30** in the presence of $ZnCl_2$ to yield the polymer-pentasaccharide conjugate **1.37**. Release from the polymer support furnished the pentasaccharide **1.38** in 39% overall yield based on **1.26**.



Reagents and conditions: (i) $(i\text{-Pr})_2NEt$, CH_2Cl_2, DMAP; (ii) 10.0 equiv of **1.27** in CH_3COCH_3, CH_2Cl_2, 0 °C, 1 h; (iii) 10.0 to 20.0 equiv of **1.25**, 2.0 equiv of $ZnCl_2$, THF, 40 °C, 3 h; (iv) 10.0 to 20.0 equiv of **1.30**, 2.0 equiv of $ZnCl_2$, THF, 40 °C, 3 h; (v) TBAF, AcOH, THF.

Scheme 1.5



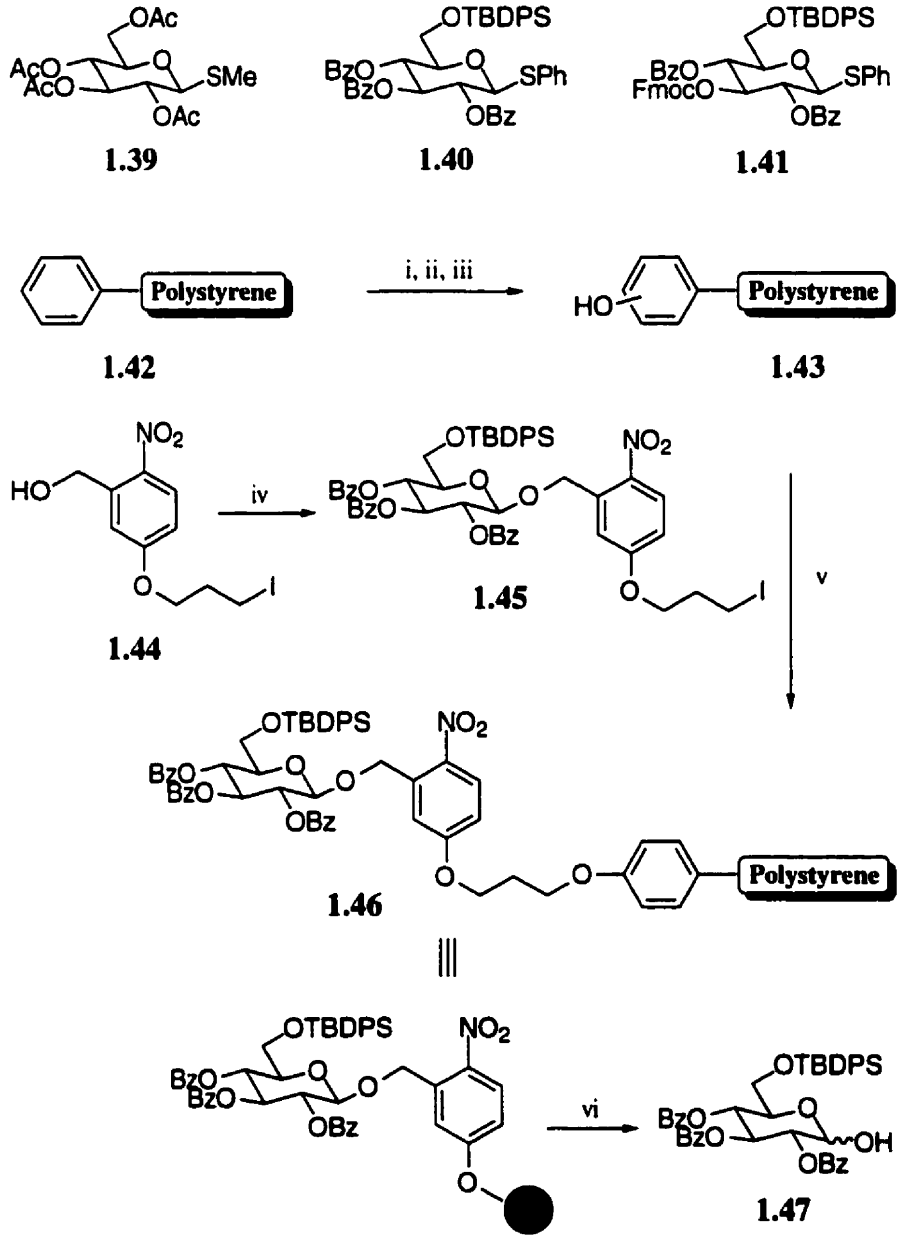
Reagents and conditions: (i) 10.0 equiv of **1.27** in CH_3COCH_3, CH_2Cl_3, 0 °C, 1 h; (ii) 10.0 to 20.0 equiv of **1.34**, 2.0 equiv of $ZnCl_2$, THF, 40 °C, 3 h; (iii) 10.0 to 20.0 equiv of **1.30**, 2.0 equiv of $ZnCl_2$, THF, 40 °C, 3 h; (v) TBAF, AcOH, THF.

Scheme 1.6

In order to characterize the reaction intermediates on the solid support, these researchers developed a technique to monitor the progress of solid-phase oligosaccharide synthesis by high-resolution Magic angle spinning NMR.[14] A model polymer-bound trisaccharide was synthesized and analyzed by ^1H, ^{13}C, and ^1H-^{13}C HMQC NMR spectroscopies. Only relative short acquisition time was required. It is believed that *the development of novel methodologies for the assembly of oligosaccharides on the solid support will undoubtedly benefit from this discerning "on-resin" analytical method.*[14]

Danishefsky *et al* [15] have also successfully extended their solid-phase oligosaccharide synthesis methodology to the solid-phase glycopeptide synthesis.

1.1.4 Nicolaou's Solid-phase Synthesis of A Heptasaccharide Phytoalexin Elicitor (HPE).

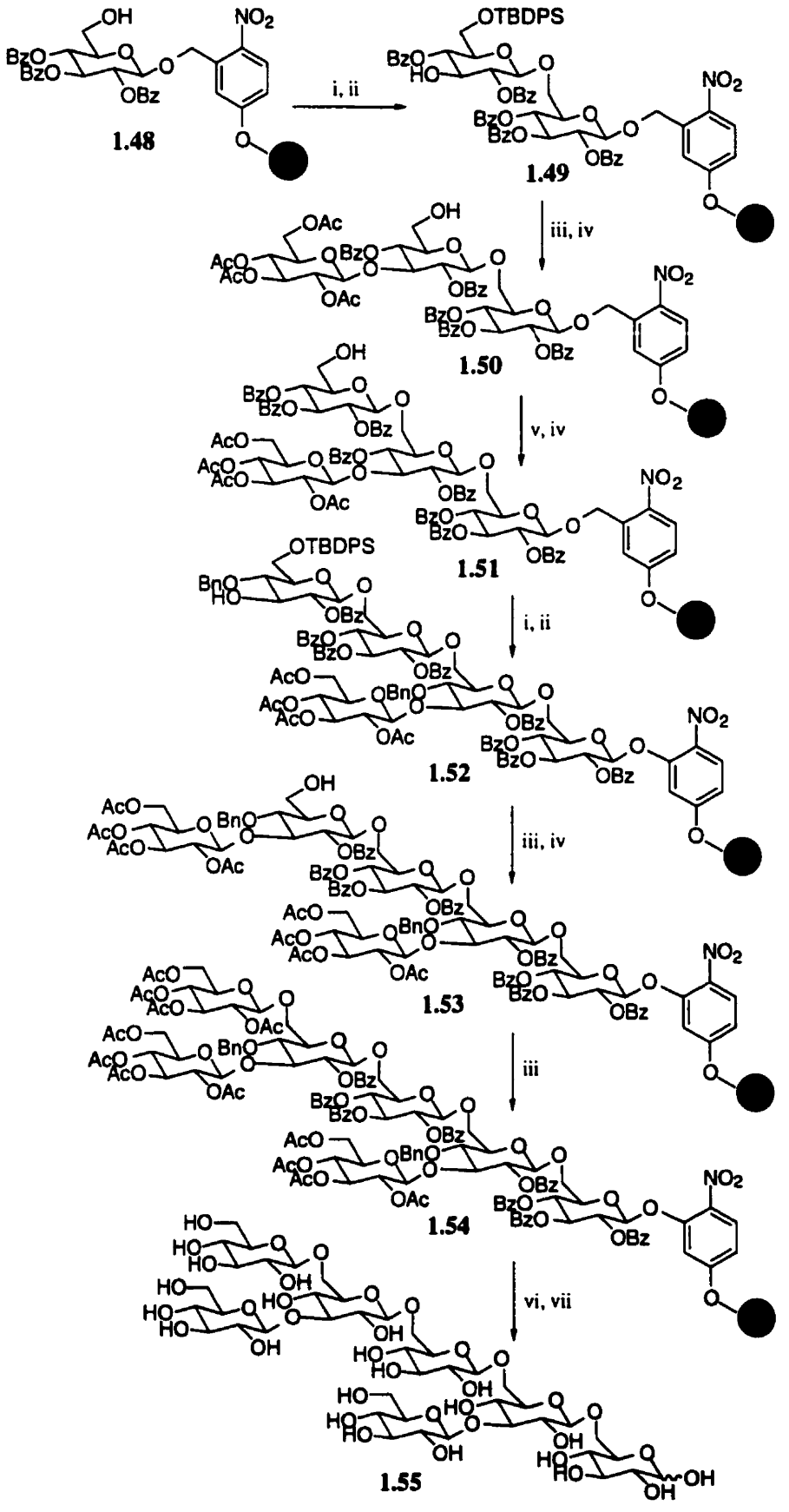


Reagents and conditions: (i) 1.3 equiv of *n*-BuLi, cyclohexane, 65 °C, 4 h; (ii) O_2, cyclohexane, 25 °C, 2 h; (iii) 2.0 equiv of PPh_3, THF, 25 °C, 12 h; (iv) 1.0 equiv of **1.40**, 1.5 equiv of **1.44**, 4.0 equiv of DMTST, 4Å MS, CH_2Cl_2, 25 °C, 4 h; (v) 2.0 equiv of **1.45**, 1.0 equiv of **1.43**, 2.0 equiv of Cs_2CO_3, DMF, 25 °C, 30 h.

Scheme 1.7

A recent disclosure by Nicolaou's group,[16] described a new method for the construction of complex oligosaccharides on a polystyrene resin. A photolabile *o*-nitrobenzyl ether tether was chosen as the linker for its ease of attachment and cleavage. Three monosaccharide building blocks **1.39**, **1.40**, and **1.41** shown in Scheme 1.7 were designed for the synthesis of the heptasaccharide phytoalexin elicitor (HPE). Low cross-linked polystyrene (**1.42**) was functionalized to phenolic polystyrene (**1.43**) by treatment with *n*-BuLi, oxygen and PPh_3. Glycosylation of **1.44** with the building block **1.40** in the presence of dimethylthiomethylsulfonium triflate (DMTST) gave the β-glycoside **1.45** as the only product in 95% yield. This D-glucoside derivative **1.45** was then attached to the cross-linked phenolic polystyrene **1.43** by a simple displacement reaction which proceeded in greater than 90% yield based on mass gain of the polymer. Efficient cleavage of the sugar unit from the polymer support was accomplished by irradiation of **1.46** in THF at 25 ^{o}C to furnish the partially protected D-glucose **1.47** in 95% yield.

Deprotection of the primary hydroxyl group in **1.46** with 30% hydrogen fluoride-pyridine provided **1.48**, which was coupled with the donor **1.41** (Scheme 1.8). Removal of the Fmoc protecting group at the 3-OH position with Et_3N in CH_2Cl_2 went smoothly and gave **1.49** in better than 95% yield. Coupling of **1.49** with the donor **1.39** followed by desilylation afforded **1.50**, which was converted to **1.51** by glycosylation with **1.40** and desilylation. Reiteration of these sequences eventually reached the polymer-bound branched heptasaccharide **1.54**. Photocleavage of the heptasaccharide from the resin, followed by treatment with NaOMe in MeOH and hydrogenolysis, furnished the target heptasaccharide **1.55** in ca. 20% overall yield.


1.48
i, ii
1.49
iii, iv
1.50
v, iv
1.51
i, ii
1.52
iii, iv
1.53
iii
1.54
vi, vii
1.55

Reagents and conditions: (i) 3.0 equiv of **1.41**, 12.0 equiv of DMTST, 4Å MS, CH_2Cl_2, 25 °C, 15 h; (ii) 20% Et_3N in CH_2Cl_2, 25 °C, 5 h; : (iii) 4.0 equiv of **1.39**, 16.0 equiv of DMTST, 4Å MS, CH_2Cl_2, 25 °C, 30 h; (iv) 30% $(HF)_x$•Py, THF, 25 °C, 15 h; (v) 3.0 equiv of **1.40**, 12.0 equiv of DMTST, 4Å MS, CH_2Cl_2, 25 °C, 15 h; (vi) *hv*, THF, 25 °C; (vii) NaOMe, 1:2 THF/MeOH, 15 h; (viii) H_2 (1 atm), Pd/C, MeOH, 25°C, 12 h.

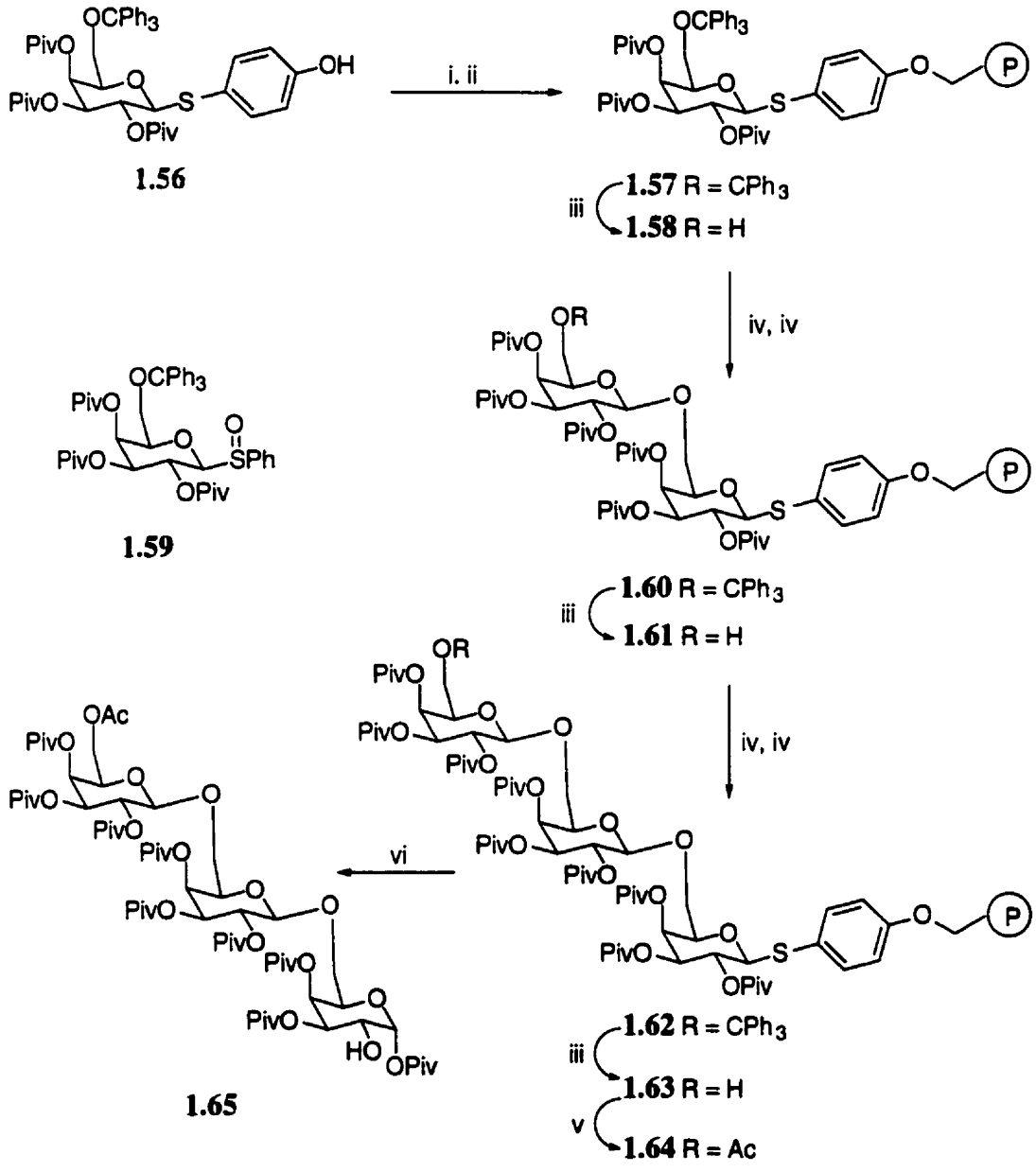
Scheme 1.8

1.1.5 Kahne's Approach Using Anomeric Sulfoxides As Glycosyl Donors.

Kahne and co-workers recently demonstrated the application of the sulfoxide glycosylation method to solid-phase oligosaccharide synthesis on the Merrifield's resin.[17] Their general strategy to solid-phase synthesis is illustrated in Scheme 1.9. The first sugar unit was anchored to the solid support using the cesium salt of a *p*-hydroxythiophenyl galactoside **1.56**. The thioether linkage is stable to the coupling condition and can be readily cleaved at the end with mercuric salts. Deprotection of the triphenylmethyl group of **1.57** afforded the polymer-bound acceptor **1.58**. Glycosylation of **1.58** with the D-galactosyl sulfoxide donor **1.59** was carried out at a remarkably low temperature, -60 °C. The reaction was monitored by hydrolyzing small aliquots of resin with mercuric trifluoroacetate and analyzing the hydrolysis product by TLC. In order to maximize the coupling yield, the glycosylation was repeated. Subsequent deprotection of the disaccharide **1.60** followed by another coupling with **1.59** provided **1.62**, which was detritylated, acetylated, and then cleaved from the resin to give **1.65** in 52% overall yield. They claimed that if one assumes a 70-75% yield for the detachment of the trisaccharide, then each of the other six steps must have proceeded to give better than a 94-95% yield on average.

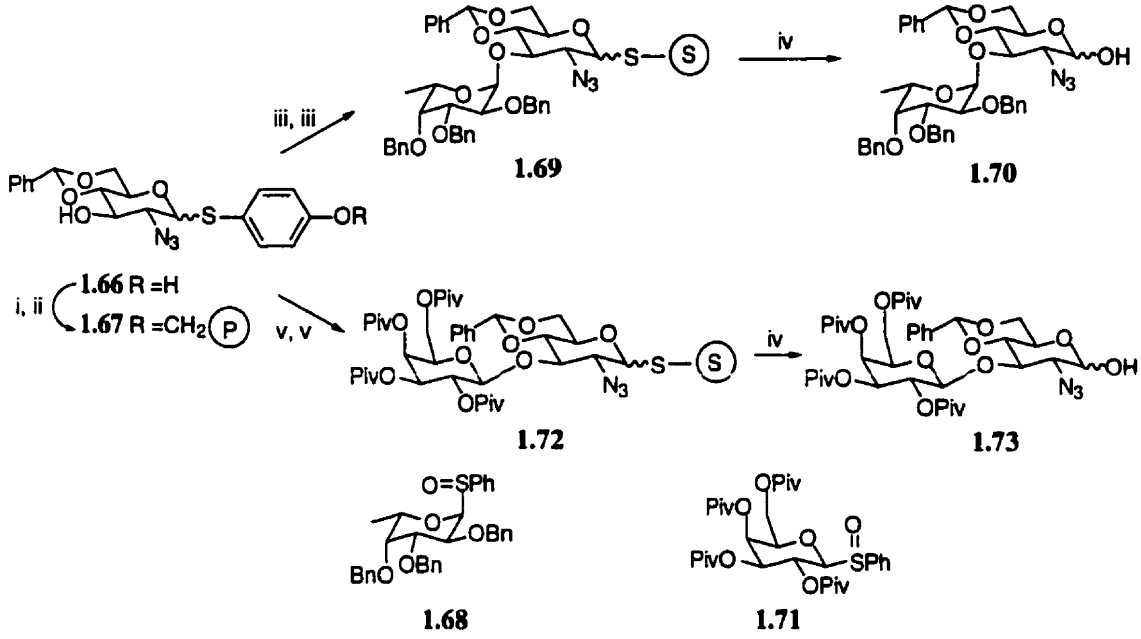
To demonstrate that the solid-phase methodology can also work on less reactive secondary hydroxyl groups, they synthesized disaccharide **1.70** and **1.73** (Scheme 1.10) from the polymer-bound monosaccharide **1.67** and two donors (**1.68**, **1.71**). The yields of both coupling reactions, after repeated glycosylation, were excellent. The work shows

that the sulfoxide glycosylation can be employed to make both α and β glycosidic bonds to secondary hydroxy groups in high yield and excellent selectivity on a solid support.



Reagents and conditions: (i) Me_3SiCl, Et_3N, THF, 0 to 25 °C, 50 min; (ii) ®-CH_2Cl, CsF, DMF, 60 °C, 24 h; : (iii) CF_3COOH, CH_2Cl_2; (iv) 4.0 equiv of **1.59**, 12.0 equiv of DBMP, 2.0 equiv of Tf_2O, CH_2Cl_2, -78 to -60 °C, 1 h; (v) Ac_2O, Py; (vi) $Hg(OCOCF_3)_2$, CH_2Cl_2, H_2O, rt, 5 h.

Scheme 1.9



Reagents and conditions: (i) Cs_2CO_3, MeOH, , rt, 3 h; (ii) ®-CH_2Cl, NMP, 55 °C, 24 h; (iii) 4.0 equiv of **1.68**, 12.0 equiv of DBMP, 2.0 equiv of Tf_2O, CH_2Cl_2, -78 to -60 °C, 1 h; (iv) $Hg(OCOCF_3)_2$, CH_2Cl_2, H_2O, rt, 5 h; (v) 4.0 equiv of **1.71**, 12.0 equiv of DBMP, 2.0 equiv of Tf_2O, CH_2Cl_2, -78 to -60 °C, 1 h.

Scheme 1.10

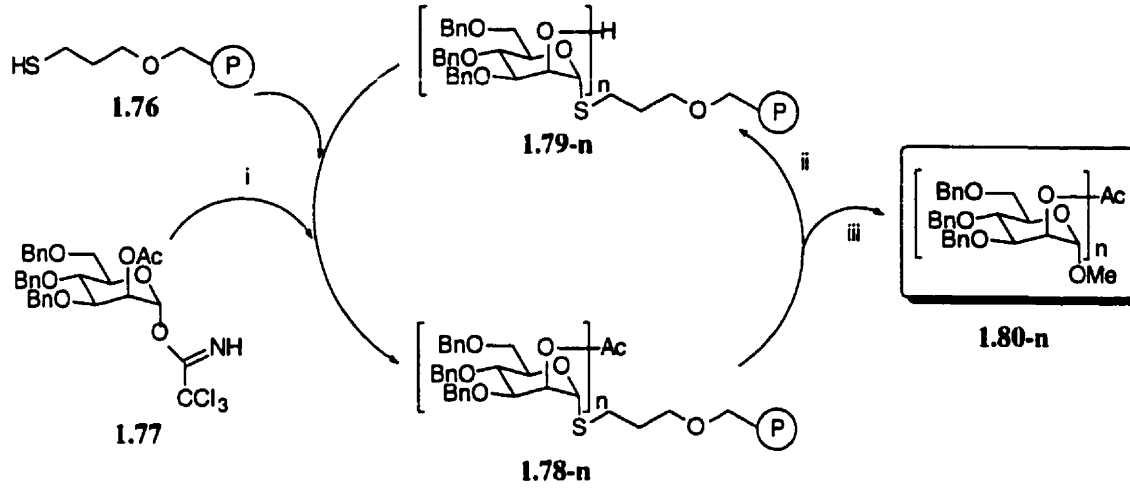
1.1.6 Schmidt's Solid Phase Glycosylation on an Alkyl Thiol Polymer.

Using an alkyl thiol linker coupled to chloromethylated 1% divinylbenzene-polystyrene copolymer, Schmidt *et al* introduced a novel strategy for solid phase glycosylation.[18] Resins were functionalized to allow relative high loadings of 0.1-0.6 mmol/g (Scheme 1.11). O-alkylation of the linker **1.74** with chloromethylated polystyrene/divinylbenzene copolymer **1.75** gave the thiol-functionalized resin using sodium hydride as base in combination with a crown ether (15-crown-5). Cleavage of monomethoxyltrityl group from the resin bound linker afforded the functionalized polymer **1.76**.

1.74 **1.75** **1.76**

Reagents and conditions: (i) **1.74**, NaH, CH_2Cl_2, 60 °C, 24 h; (ii) 5% TFA in CH_2Cl_2.

Scheme 1.11



Reagents and conditions: (i) 3.0 equiv of **1.77**, 0.3 equiv of TMSOTf, CH_2Cl_2, rt, 1 h; (ii) NaOMe, 10:1 CH_2Cl_2/MeOH, 2 h; (iii) 4.0 equiv of NBS, DTBP, 9:1 THF/MeOH, 90 min.

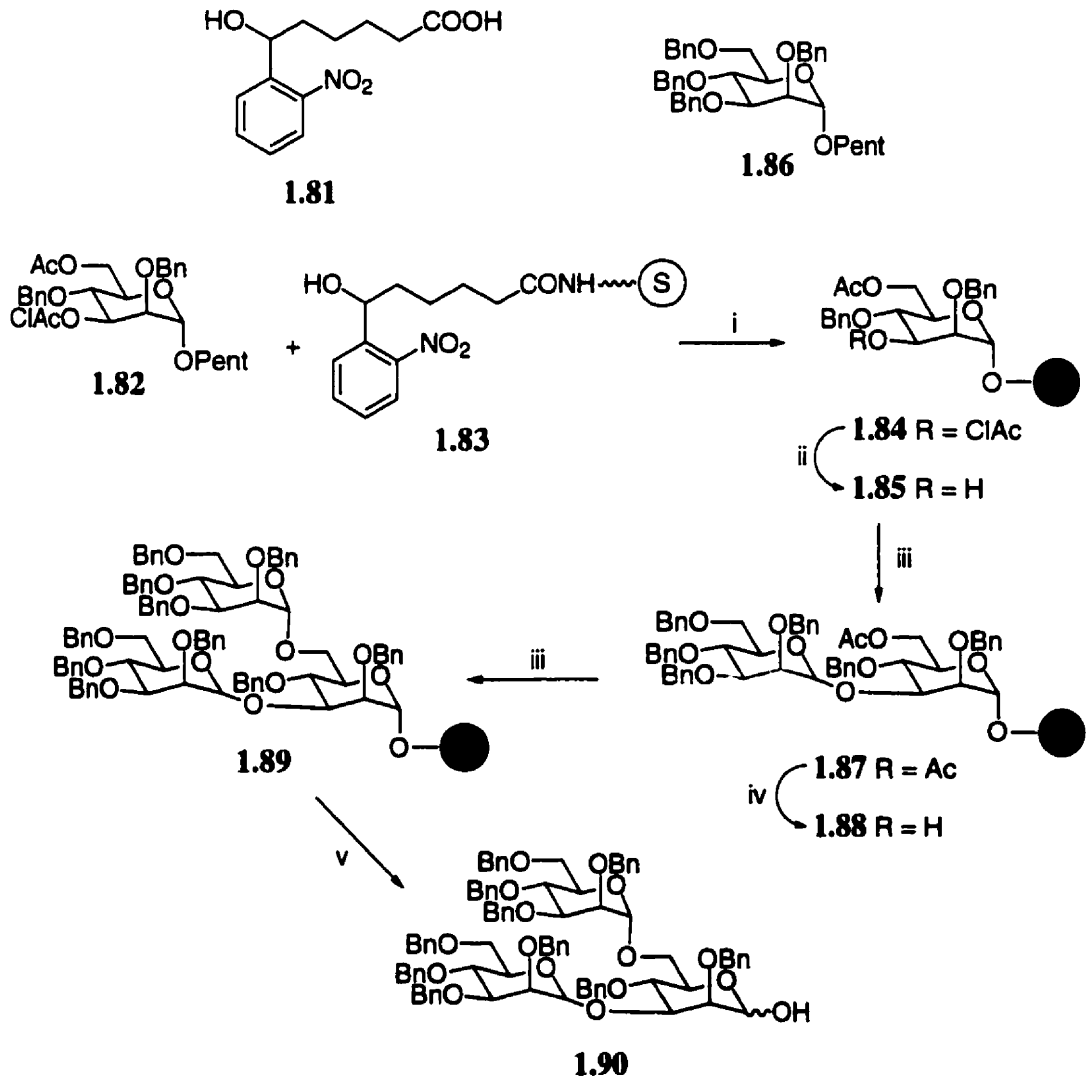
Scheme 1.12

The target oligomannosides were synthesized with the widely used trichloroacetimidate **1.77** as a donor and trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst. Glycosylation and deprotection of the functionalized resin were performed in a cyclic manner (Scheme 1.12). The deacetylation steps were performed in a 10:1 mixture of CH_2Cl_2 with 0.5 M sodium methoxide in MeOH to avoid shrinkage of the resin. Removal of the sodium methoxide was conducted effectively with THF containing traces of acetic acid and 15-crown-5. For the analytical cleavage, a resin sample was treated with silver triflate solution. After 15 minutes, the reaction products were analyzed directly from the supernatant either by TLC and HPLC or by MALDI-TOF mass spectrometry. For

preparative isolation of the products, cleavage was accomplished in THF/MeOH with NBS in the presence of 2,6-di-*tert*-butylpyridine (DTBP) yielding the methyl glycosides (**1.80-n**). The disaccharide, trisaccharide and tetrasaccharide were synthesized and isolated in an overall yield of 75%, 54% and 34%, respectively.

In an earlier communication,[19] they have also reported the synthesis of pentaglucosides in a similar manner.

1.1.7 Fraser-Reid's Approach Using A Photocleavavable Linker.



Reagents and conditions: (i) NIS, TESOTf, CH_2Cl_2, rt, 2 h; (ii) $CS(NH_2)_2$, methoxyethanol, 80 °C, 6 h; (iii) **1.86**, NIS, TESOTf, CH_2Cl_2, rt, 2 h; (iv) NaOMe/MeOH in THF; (v) *hv*, THF, 15 h.

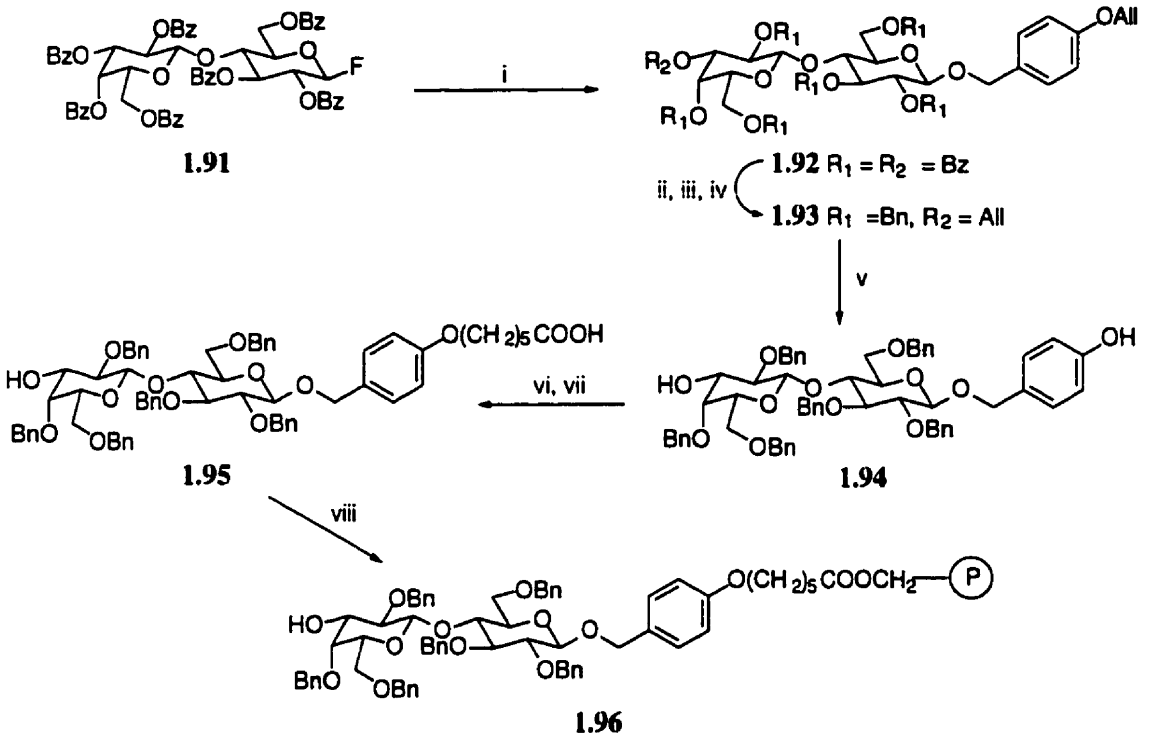
Scheme 1.13

In the recent publications[20, 21] from Fraser-Reid's group, they adopted a new *o*-nitrobenzyl photocleavable linker (**1.81**) for solid phase synthesis of oligosaccharide. Its usefulness was demonstrated by the synthesis of the branched trimannan **1.90** (Scheme 1.13). Pentenyl glycoside **1.82** was coupled to the photolabile resin **1.83** smoothly to afford the resin-bound monosaccharide **1.84** which underwent dechloroacetylation with thiourea to give **1.85**. Glycosylation of the 3-OH of **1.85** with the perbenzylated donor **1.86** afforded disaccharide **1.87**. Subsequent deacetylation of the 6-OH with NaOMe and coupling with the same mannoside donor (**1.86**) furnished the resin-bound trisaccharide **1.89**. Irradiation of the resin **1.89** in THF at 365 nm for 15 h gave the branched trimannan **1.90** in 42% overall yield, which translates into an average yield of ca.87% per coupling step.

1.1.8 Ogawa's Solid-phase Synthesis of Polylactosamine Oligosaccharide.

Employing the Merrifield's resin, the Japanese group chose the *p*-alkoxybenzyl type functionality as a linker.[22] Their strategy is based on the premise that assembled oligosaccharides can be cleaved from solid support either under acidic, basic, or oxidative conditions. The resin-bound lactose derivative was prepared as shown in Scheme 1.14. *p*-Allyloxybenzyl alcohol was glycosylated with the lactose fluoride **1.91** to afford the β-glycoside **1.92**, which was deacylated and subjected to dibutyltin oxide mediated regioselective allylation followed by benzylation to give **1.93**. Subsequent transformation into diol **1.94** was followed by Williamson ether formation and hydrolysis to yield the acid

1.95. Coupling with Merrifield's resin was performed in the presence of Cs_2CO_3 to afford the disaccharide-resin conjugate **1.96**.

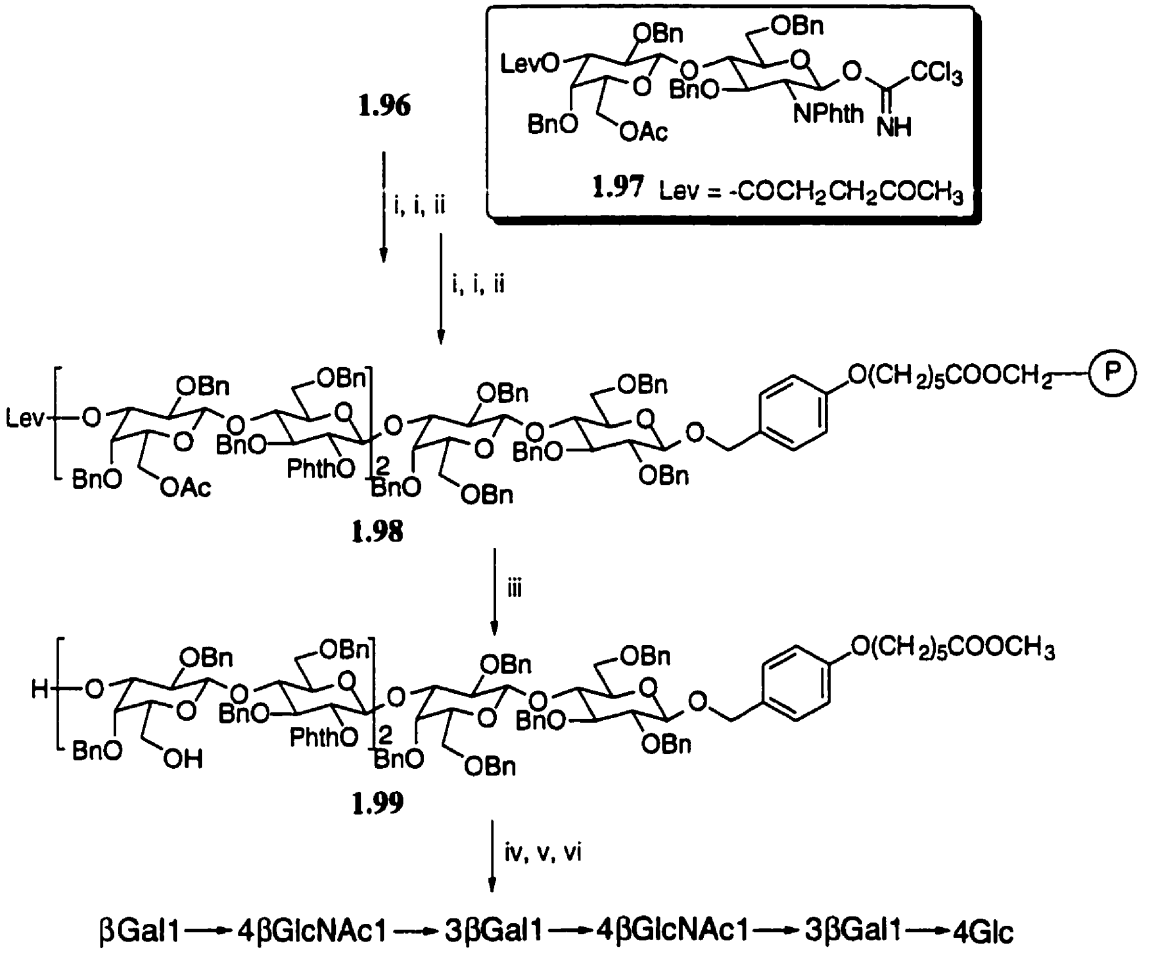


Reagents and conditions: (i) *p*-$AllOC_6H_4CH_2OH$, AgOTf, $SnCl_2$, 4Å MS, *s*-collidine/ $ClCH_2CH_2Cl$; (ii) NaOMe, MeOH; (iii) *n*-Bu_2SnO, toluene, reflux, then AllBr, *n*-Bu_4NBr; (iv) BnBr, NaH, DMF; (v) $Ir\{(COD)[PCH_3Ph_2]_2\}PF_6$, THF, then $HgCl_2$, HgO, CH_3COCH_3, H_2O; (vi) $Br(CH_2)_5COOEt$, Cs_2CO_3, DMF; (vii) NaOH, THF, EtOH, H_2O; (viii) Merrifield's resin, Cs_2CO_3, DMF.

Scheme 1.14

Elongation of the glycan chain was achieved using lactosamine trichloroacetimidate **1.97** as a glycosyl donor (Scheme 1.15). The resin-bound lactose derivative **1.96** was coupled with this donor twice and then subjected to delevulinoylation. Reiteration of the glycosylation-deprotection afford the resin-bound hexasaccharide **1.98**, which was cleaved from the polymer support under basic conditions to give the corresponding methyl

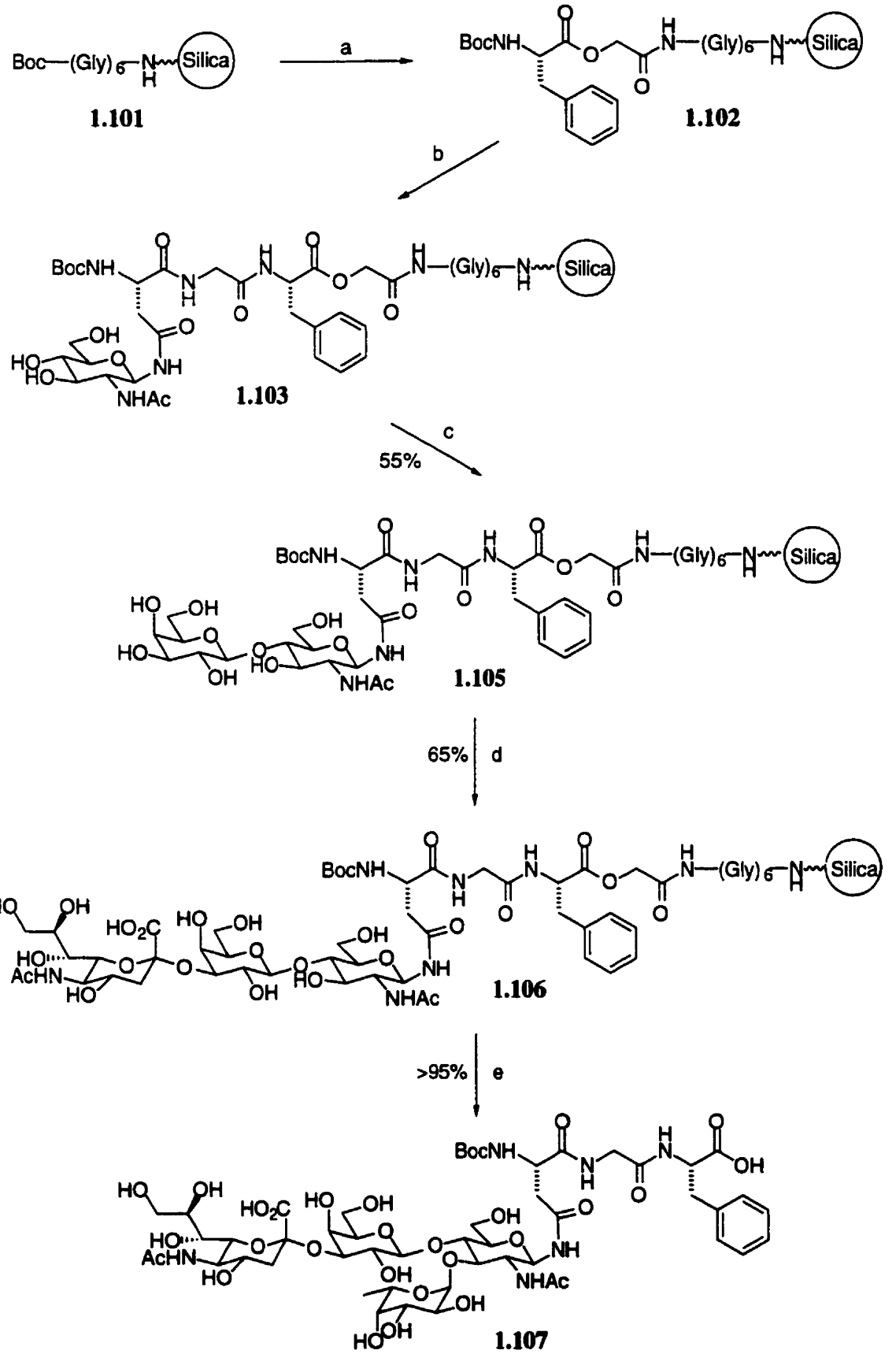
ester **1.99** in 56% overall yield. Complete deprotection of **1.99** into **1.100** was acccomplished in three steps in 56% yield.



Reagents and conditions: (i) 1.5 equiv of **1.97**, 0.2 equiv of TMSOTf, CH_2Cl_2, -78 °C, 2 h; (ii) NH_2NH_2•AcOH, EtOH; (iii) NaOMe, MeOH, 45 °C, 7 h; (iv) $H_2NCH_2CH_2NH_2$, MeOH; (v) Ac_2O, MeOH; (vi) H_2, $Pd(OH)_2$, MeOH, H_2O.

Scheme 1.15

1.1.9 Wong's Solid-phase Chemical-Enzymatic Synthesis of Glycopeptides and Oligosaccharides.


Boc—(Gly)6—N—Silica
a
BocNH
(Gly)6
Silica
1.101
1.102
b
OH
HO
HO
NH
NHAc
1.103
c
55%
1.105
65%
d
HO2C
AcHN
1.106
>95%
e
1.107

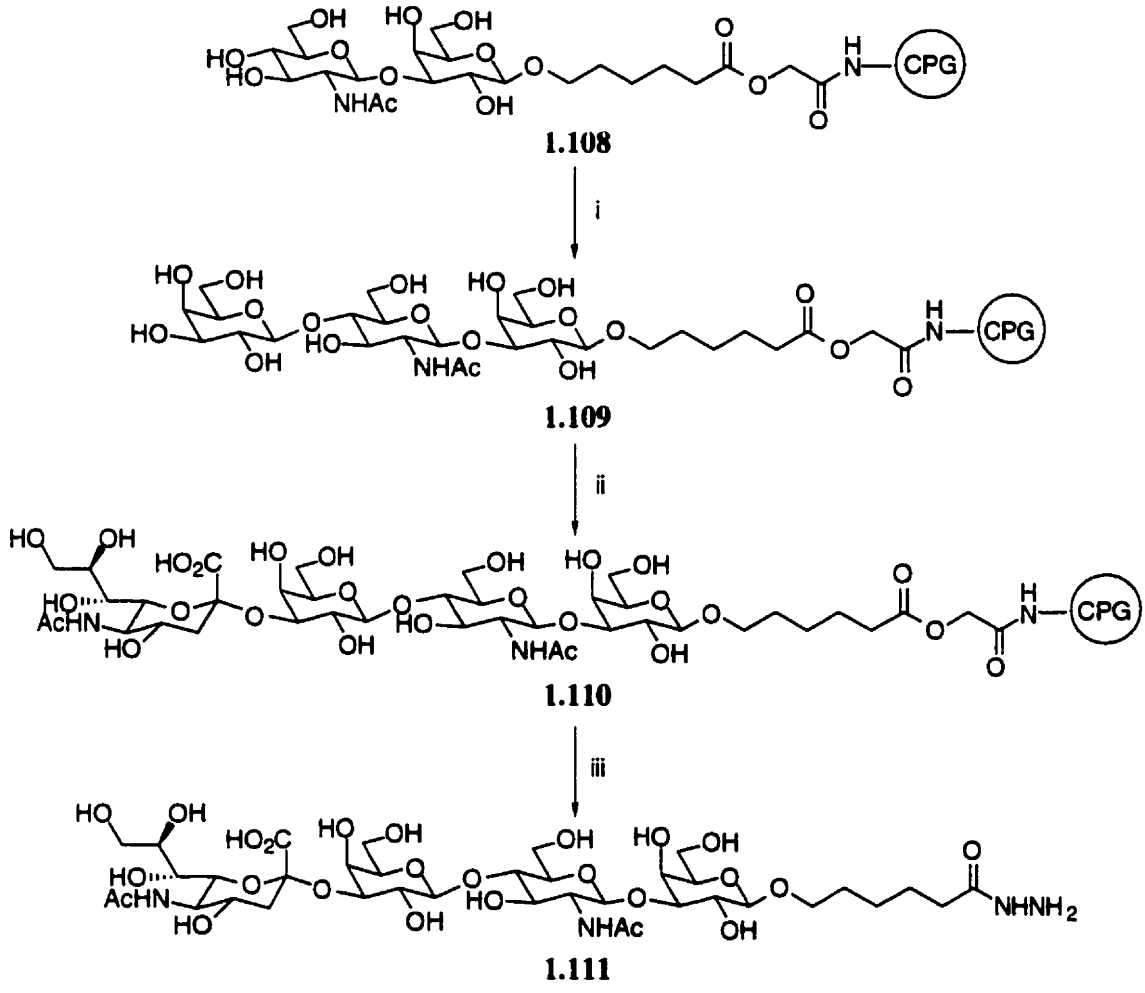
Reagents and conditions: (a) (i) 25% TFA in CH_2Cl_2; (ii) 7.0 equiv of O-(N-Boc-phenylalanyl)glycolic acid, BOP/HOBt, DIEA; (b) (i) 25% TFA in CH_2Cl_2; (ii) 7.0 equiv of Boc-Gly-OH, BOP/HOBt, DIEA; (iii) 25% TFA in CH_2Cl_2; (iv) 3.0 equiv of Boc-Asn(GlcNAcβ)-OH, BOP, DIEA; (c) β-1,4-galactosyltransferase, 1.5 equiv of UDP-Gal, 0.1 M HEPES (pH 7.0), 10 mM $MnCl_2$; (d) α-2,3-sialyltransferase, 1.5 equiv of CMP-NeuAc, 0.1 M HEPES (pH 7.0), 5 mM $MnCl_2$; (e) (i) α-chymotrypsin, H_2O (pH 7.0), (ii) ultrafiltration, (iii) α-1,3-fucosyltransferase, 2.5 equiv of GDP-Fuc, 0.1 M HEPES (pH 7.0).

Scheme 1.16

Although several methods are available for solid-phase glycopeptide synthesis, none of them involves the use of glycosyltransferase in solid-phase synthesis. Wong and co-workers developed a new strategy which takes advantage of a stepwise formation of peptide bonds chemically and glycosidic bonds enzymatically with glycosyltransferases on a silica-based solid support compatible with both organic and aqueous solvents.[23] The critical element is the attachment of a proper acceptor-spacer group with a cleavable bond to the solid support. Thus, enzymatic coupling can be effectively performed and the glycopeptides can be released.

Since aminopropyl silica is compatible with both aqueous and organic solvents, it was chosen among several solid supports they tested. A hexaglycine spacer was attached to the support and excess amino groups were then capped with acetyl groups. Introduction of an α-chymotrypsin-sensitive phenylalanyl ester bond enables the release of intermediates and final products from the support under mild conditions (Scheme 1.16). A short glycopeptide was synthesized chemically from **1.101** to give the glycosyl acceptor **1.103**, which after α-chymotrypsin-catalyzed hydrolysis yielded N-Boc-Asn(GlcNAcβ)-Gly-Phe-OH (**1.104**) as the only soluble product. The galactosylation of **1.103** was catalyzed by β-1,4-galactosyltransferase using UDP-galactose as a glycosyl donor, and the yield was about 55% based on the reverse-phase HPLC analysis. The subsequent sialylation of **1.105** was performed under similar conditions employing CMP-sialic acid and α-2,3-

sialyltransferase to afford the sialylated product in 65% yield. Upon released from the solid support by chymotrypsin, the glycopeptide was enzymatically fucosylated with GDP-fucose to give Sialyl Lewis X glycopeptide **1.107** in almost quantitative yield.



Reagents and conditions: (i) β-1,4-galactosyltransferase, UDP-Gal, 50 mM HEPES (pH 7.2), 5 mM $MnCl_2$; (ii) α-2,3-sialyltransferase, CMP-NeuAc, DTT, Triton-X, alkaline phosphatase, BSA, 50 mM sodium cacodylate buffer (pH 7.5); (iii) $H_2NNH_2 \cdot H_2O$, 24 h.

Scheme 1.17

In another example of the use of glycosyltransferase in oligosaccharide synthesis (Scheme 1.17),[24] Wong *et al* synthesized the tetrasaccharide **1.111** starting from the

disaccharide **1.108**, attached to controlled pore glass (CPG) via a spacer containing an ester bond. Two to three equivalents of UDP-Gal and CMP-NeuAc were used in the enzymatic glycosylation , and the conversion for each glycosylation step was found to be better than 98% based on the analysis of products cleaved by treatment of hydrazine.

Besides having the advantages of solid phase syntheses, a solid-phase enzymatic synthesis has the advantage that internal deletions in an oligosaccharide sequence are minimized. *A major disadvantage, however, is that the narrow substrate specificity of glycosyltransferases makes the synthesis of various modified oligosaccharides difficult.*[24]

1.1.10 Other Approaches to Solid-phase Oligasaccharide Synthesis.

De Napoli and co-workers[25] reported the synthesis of a disaccharide on Tentagel using a trichloroacetimidate donor. Rough *et al* [26]demonstrated that precursors of 6-deoxy di- and trisaccharide can be readily prepared in high yield by using a sulfonyl chloride resin as an insoluble support. Also, Paulsen, Meldal and co-workers[27] developed a solid-phase oligosaccharide synthesis on glycopeptides bound to a PEGA resin.

Although it has been reported[28] that the PEGA resin shrank after contact with the acqueous enzyme solution, Meldal, Hindsgaul and co-workers[29] have successfully carried out the enzymatic glycosylation of a glycopeptide on a PEGA resin in water.

1.2 Polymer-supported Solution Synthesis of Oligosaccharides.

The approaches described above represent recent advances in solid-phase oligosaccharide synthesis, which are based on significant improvements on solution-phase glycosylation methodologies in recent years. With respect to the classical oligosaccharide synthesis, newly developed solid-phase strategies do improve the coupling yields considerably and simplify the purification procedures in many cases. However, improved

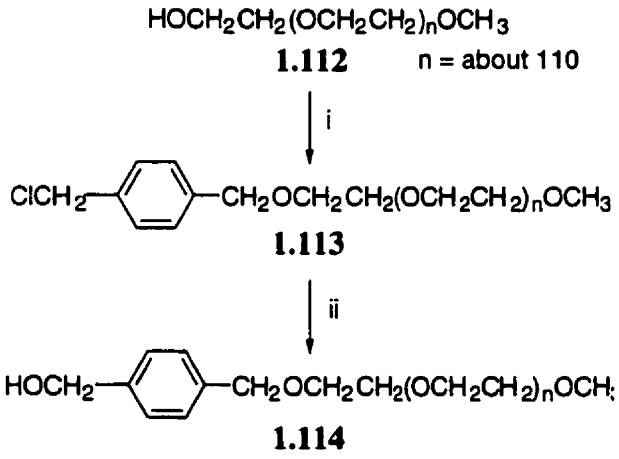
yields result from the use of a large excess of glycosyl donors or acceptors, which normally are quite difficult and expensive to prepare, and in most cases, are not possible to recycle. In addition, the solution-phase coupling methods have to be adapted to solid-phase methodologies. For example, sometimes coupling reactions failed on solid supports. Since overcrowding of the reactive sites can cause internal deletions in an oligosaccharide sequence, the loading of most solid supports is generally low and therefore, only a small amount of final products can be prepared. Furthermore, even the crosslinking degree of most solid supports (usually polystyrene) used is quite low, elongation of the large or branched oligosaccharide chains could still be steric hindered.

On the other hand, polymer-supported synthesis can accommodate most of the difficulties. Since a linear polymer support [usually high molecular weight poly(ethylene glycol) monomethyl ether] is completely soluble in the reaction solution, the glycosylation kinetics and anomericity control are very much similar to those observed in classical solution chemistry. In principle, the conventional solution-phase coupling methods can directly be applied onto a polymer-supported synthesis. No large excess of glycosyl donors or acceptors are necessary and subsequently it will reduce the cost and labor of oligosaccharide syntheses. Also, there is no need to worry about the overcrowding of the reactive sites. The selection of the molecular weight of the polymer is based on the consideration that the polymer-bound oligosaccharide must remain soluble in the glycosylation step but can be precipitated readily from the solution without contamination of the reagents.[30]

1.2.1 Krepinsky's Polymer-supported Solution Synthesis of A D-Mannopentose.

After their pioneering work of using poly(ethyleneglycol) monomethyl ether (PEG, average molecular weight 5000) as a polymer support for the preparation of

disaccharides,[30] Krepinsky and co-workers reported the synthesis of a more complex D-mannopentose using this methodology. The PEG strategy requires the polymer-saccharide conjugates to be soluble under reaction conditions of glycosylation or deprotection and to be insoluble during the workup while all impurities are soluble. When the reaction is completed, the PEG-bound oligosaccharide is precipitated from reaction solution with *tert*-butyl methyl ether and then is recrystallized from ethanol. Therefore, the difficult and time-consuming purification by column chromatography at each step is avoided. The solubility of the reactants enables reaction kinectics and α/β selectivity similar to those observed in conventional solution glycosylation. In addition, progress of the synthesis can be monitored with the standard spectroscopic techniques.

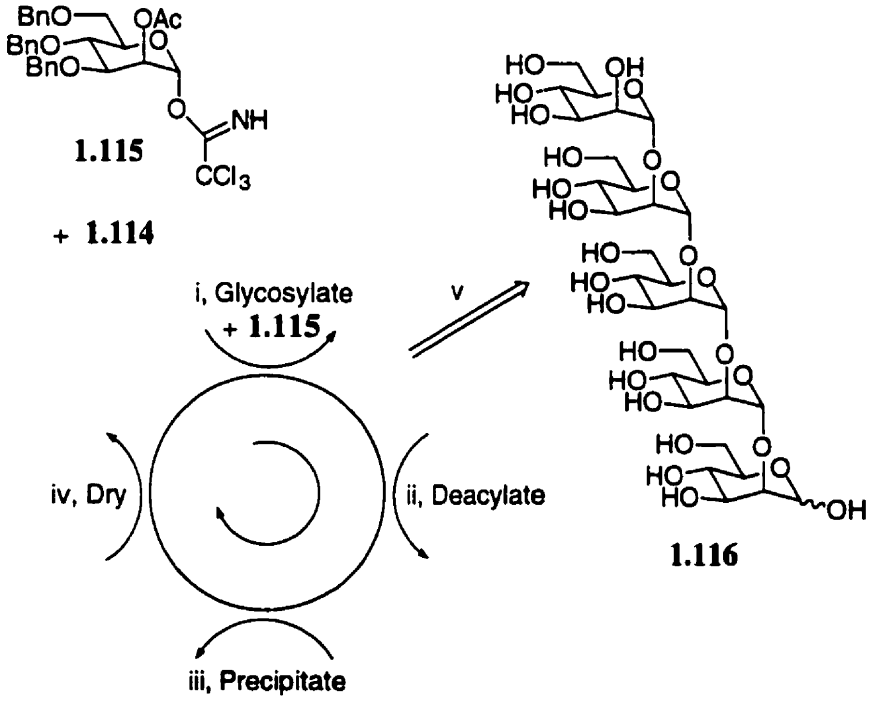


Reagents and conditions: (i) NaH, NaI, 30 equiv of xyleneCl$_2$, THF, 96 h; (ii) 10% aqueous Na_2CO_3, 70 °C, 16 h.

Scheme 1.18

Krepinsky and co-workers chose α,α'-dioxyxylyl diether, -$CH_2C_6H_4CH_2O$-, as the linker (Scheme 1.18),[31] which was synthesized from the poly(ethyleneglycol) monomethyl ether (PEG) and a large excess of α,α'-dichloro-*p*-xylene by Williamson ether synthesis followed by hydrolysis. The alcohol **1.114** was glycosylated with 2-*O*-

acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl 2,2,2-trichloroacetimidate (**1.115**) and a catalytic amount of triethylsilyl trifluoromethansulfonate (TESOTf) (Scheme 1.19). The acetyl group at 2-position of the mannosyl donor **1.115** accounts for the stereocontrol via neighbouring group participation and is easily removed by treatment with DBU and methanol to furnish a glycosyl acceptor, which after precipitation with ether and drying under high vacumm, is ready for the next coupling reaction. Repetition of the glycosylation with the same donor **1.115** and hydrolysis steps afforded the fully protected pentamannose, which after hydrogenation, acetylation, purification, and deacetylation, yielded the free pentamannopyranoside **1.116**.

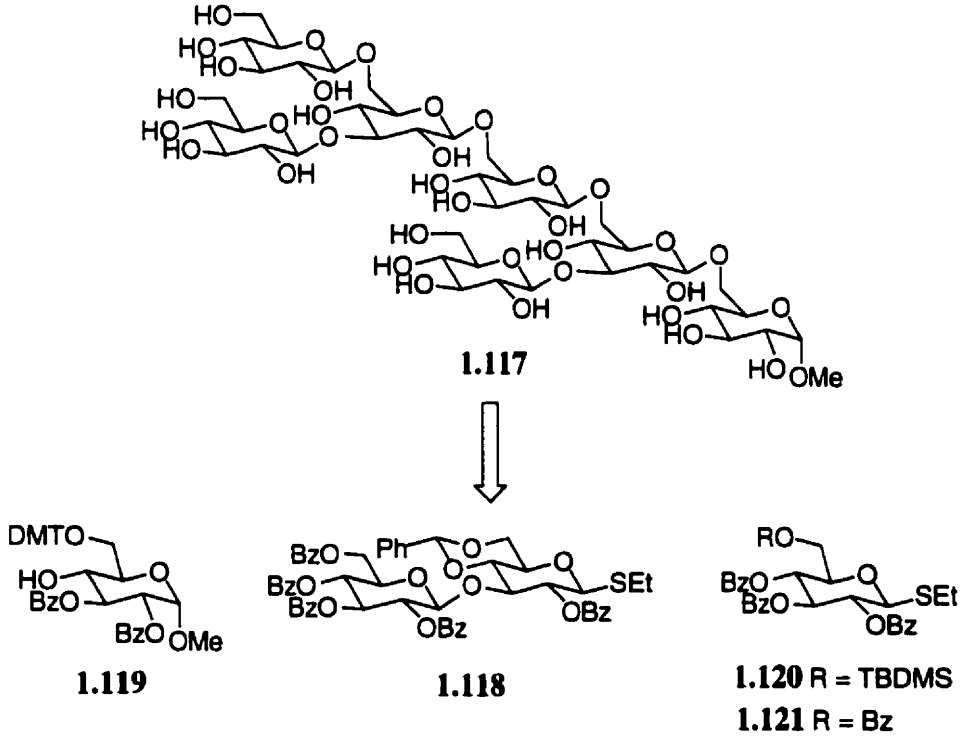


Reagents and conditions: (i) **1.115**, TESOTf, 4Å MS, CH_2Cl_2, 0-5 °C, 4 h; (ii) DBU, CH3OH, 16 h; (iii) MTBE, then EtOH reprecipitation; (iv) high vacuum; (v) Raney nickel W2, EtOH, reflux 16 h.

Scheme 1.19

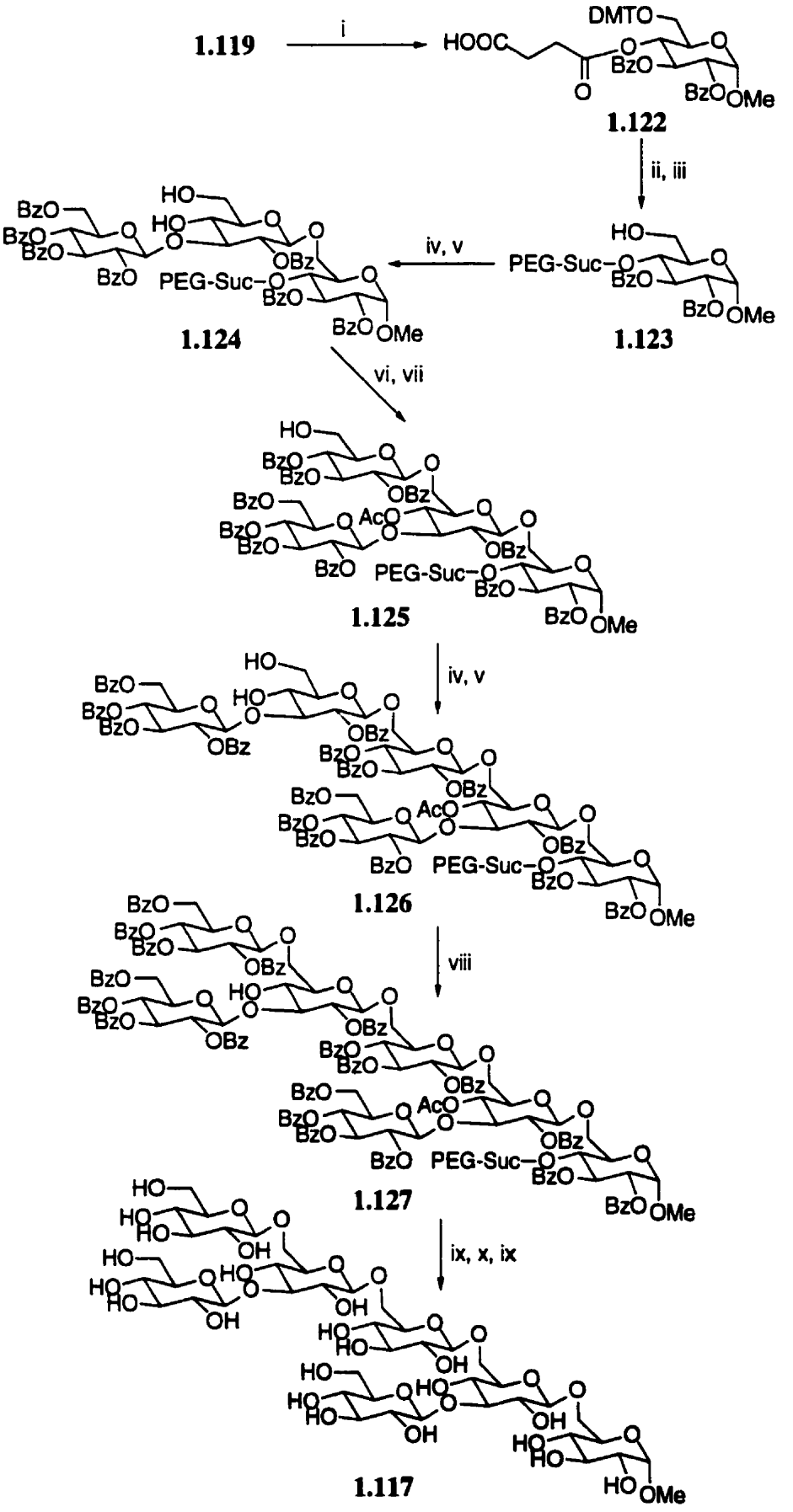
1.2.2 van Boom's Polymer-Supported Solution Synthesis of A Heptaglucoside Having Phytoalexin Elicitor Activity.

Adopting the PEG-methodology[30] and their successful solution synthetic route[32] to the target molecule **1.117**, van Boom's group reported the synthesis of the heptaglucoside **1.117** using poly(ethyleneglycol) monomethyl ether (PEG, average molecular weight 5000) as a polymer support.[33] Scheme 1.20 illustrates their retrosynthetic plan, which requires one disaccharide building block (**1.118**) and three monosaccharide building blocks (**1.119**, **1.120**, **1.121**).



Scheme 1.20

The assembly of **1.117** begins with the immobilization of **1.119** to poly(ethyleneglycol) monomethyl ether (PEG) (Scheme 1.21). Treatment of **1.119** with succinic anhydride in the presence of DMAP gave the acid **1.122**, which was attached to


1.119
i
HOOC
DMTO
BzO
BzOOMe
1.122
ii, iii
HO
BzO
PEG-Suc-O
iv, v
1.124
1.123
vi, vii
AcO
1.125
1.126
viii
1.127
ix, x, ix
OH
HO
1.117
OMe

Reagents and conditions: (i) Suc_2O, DMAP, Py; (ii) DCC, PEG-5000 monomethyl ether, DMAP, CH_2Cl_2; (iii) $PhSO_3H$, CH_3OH, CH_2Cl_2; (iv) **1.118**, NIS, catalytic TfOH, 4Å MS, Et_2O, $ClCH_2CH_2Cl$, 0 °C; (v) HCl, CH_3OH, CH_2Cl_2; (vi) **1.120**, NIS, catalytic TfOH, 4Å MS, Et_2O, $ClCH_2CH_2Cl$, 0 °C; (vii) Ac_2O, Py, then HCl, CH_3OH, CH_2Cl_2; (viii) **1.121**, NIS, catalytic TfOH, 4Å MS, Et_2O, $ClCH_2CH_2Cl$, 0 °C; (ix) $NaOCH_3$, CH_3OH; (x) Ac_2O, Py.

Scheme 1.21

the PEG by esterification effected with DCC. After capping of the unreacted PEG followed by removal of the trityl group, the PEG-bound monosaccharide acceptor **1.123** was precipitated and washed with diethyl ether. Glycosylation of **1.123** with the disaccharide donor **1.118** was achieved using N-iodosuccinimide (NIS) and a catalytic amount of triflic acid (TfOH) as the promoter. Debenzylidenation and precipitation yielded the trisaccharide diol **1.124**, which was regioselectively coupled with the monosaccharide building block **1.120** using the same promoter system. Again, capping of the remaining 4-hydroxyl group with acetic anhydride and pyridine was followed by the removal of the *tert*-butyldimethylsilyl group, which furnished the PEG-bound tetramer **1.125**. Finally, regioselective elongation of the hexamer **1.126**, obtained by glycosylation of **1.125** with the donor **1.118** and subsequent debenzylidenation, afforded the protected PEG-bound heptamer **1.127**. After PEG was cleaved off under Zemplen conditions, purification gave homogeneous **1.117** in 18% overall yield based on **1.123**. Purification between each step was performed by repeated precipitation of the polymer solution with diethyl ether.

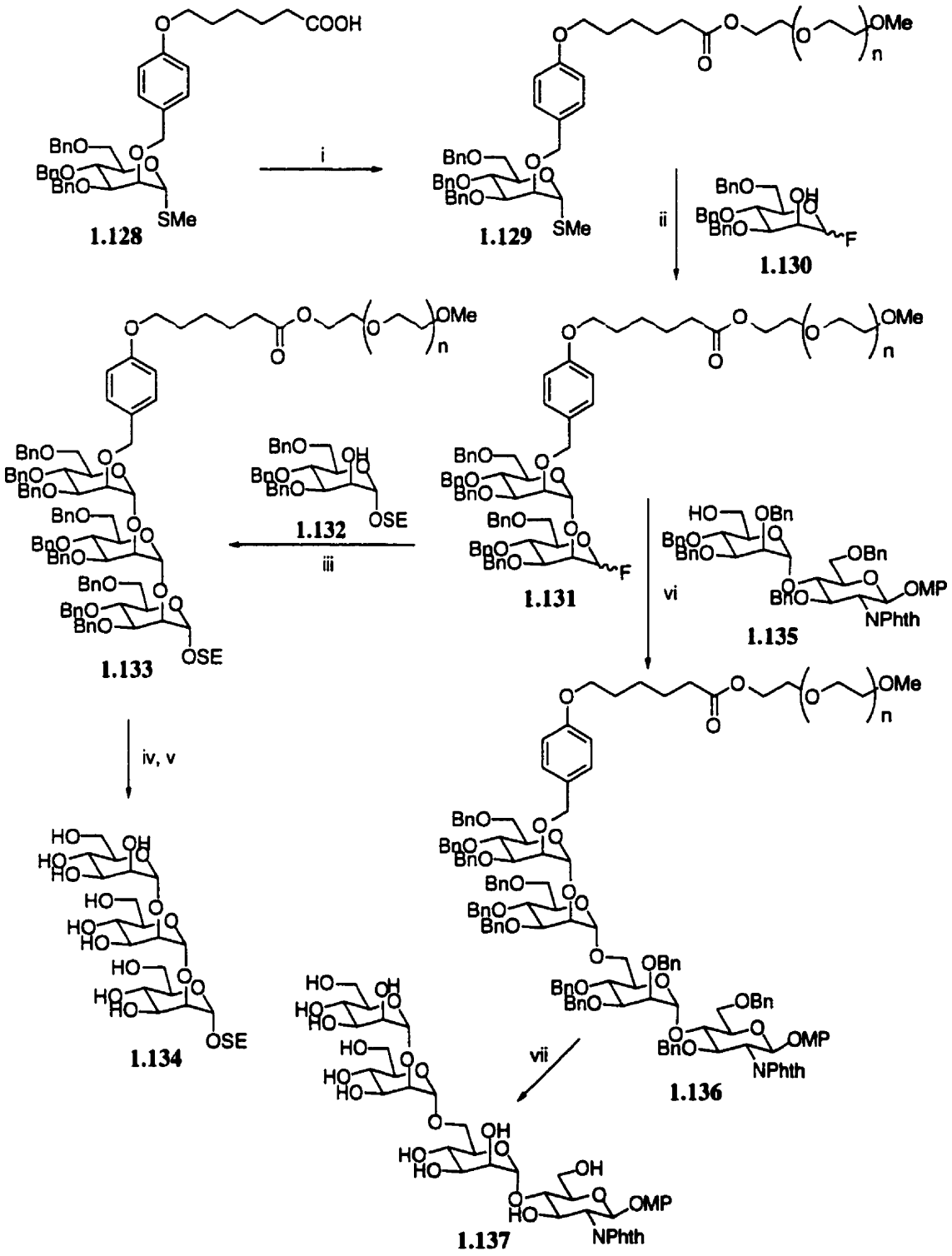
1.2.3 Ito's Orthogonal Glycosylation Strategy for Rapid Assembly of Oligosaccharides on A Polymer Support.

The Japanese group at RIKEN recently reported a novel strategy for the synthesis of oligosaccharides on polymer supports based on the concept of orthogonal

glycosylation.[34] Since thioglycoside and glycosyl fluoride were demonstrated to be a competent set of orthogonal glycosyl donors,[35] they were employed in combination. In order to avoid potential difficulties in isolating the final product, a hydrophobic tag was to be attached to the anomeric position of the last glycosyl donor after the oligosaccharide had been assembled. Therefore, the target oligosaccharide should be easily separated from all the other side-products by using reversed-phase silica gel chromatography.

Their synthesis started with carboxylic acid **1.128** (Scheme 1.22), which was attached to PEG monomethyl ether (Average molecular weight 5000) to furnish the polymer-bound thiomannoside **1.129**. Coupling of this polymeric donor **1.129** with the fluoride acceptor **1.130** was carried out in the presence of $MeOSO_2CF_3$ and MeSSMe in CH_2Cl_2 to give the PEG-bound disaccharide **1.131**. The next glycosylation reaction of **1.131** with the 2-(trimethylsilyl)ethyl (SE) mannoside **1.132** was performed under Suzuki conditions to afford the the trisaccharide-PEG conjugate **1.133**. Here the SE group served as a hydrophobic tag. After the PEG was cleaved off under basic conditions, the crude product was hydrogenated over Pearlman's catalyst. The desired trisaccharide **1.134** was seperated from the reaction mixture on a short column of C_{18} reversed-phase silica gel in 40% overall yield based on **1.128**.

To synthesize the partially protected tetrasaccharide **1.137** of the common carbohydrate structure of the glycosyl phosphatidyl inositol (GPI) anchor, the PEG-bound disaccharide donor **1.31** was coupled with the disaccharide acceptor **1.135** in the presence of $Cp_2HfCl_2/AgOSO_2CF_3$ in benzene. The resulting PEG-bound tetrasaccharide **1.136** was subjected to catalytic hydrogenation. Since the *p*-methoxyphenyl and phthalimide groups are present in the molecular structure, the desired product is hydrophobic enough to seperate from the side-products on a C_{18} column. In a similar manner, reversed-phase chromatographic separation afforded tetrasaccharide **1.137** in 42% yield from **1.131**.



Reagents and conditions: (i) DEAD, Ph_3P, PEG-5000 monomethyl ether, THF, CH_2Cl_2; (ii) **1.130**, $MeOSO_2CF_3$, MeSSMe, 4Å MS, CH_2Cl_2, rt; (iii) **1.132**, $[Cp_2HfCl_2)$, $AgOSO_2CF_3$, 4Å MS, CH_2Cl_2, 0 °C to rt; (iv) NaOMe, MeOH; (v) H_2, $Pd(OH)_2$, MeOH,

EtOAc; (vi) **1.135**, $MeOSO_2CF_3$, MeSSMe, 4Å MS, $ClCH_2CH_2Cl$, rt; (vii) H_2, $Pd(OH)_2$, MeOH.

Scheme 1.22

1.2.4 Other Approaches to Polymer-supported Solution-phase Oligasaccharide Synthesis.

Wang, Voelter and co-workers[36] described the synthesis of gentiobiose using a baselabile linker coupled to poly(ethyleneglycol) monomethyl ether (PEG, average molecular weight 5000). Nishimura, Lee and co-workers[37] reported the chemoenzymatic synthesis of N-acetyl-lactosamine on a linear polyacrylamide resin.

1.3 Proposed Research

Despite recent dramatic progress in the solid-phase and polymer-supported solution-phase synthesis of oligosaccharides, many problems remain to be solved. Our intention is to develop a method which can enable the synthesis of complex oligosaccharides in large scale. We believe that the polymer-supported solution-phase oligosaccharide synthesis offers more advantages than the solid-phase strategy does. However, incomplete precipitation between each step causes great loss of the intermediates and products. The overall yield is still not satisfactory. The question is how to improve the recovery of PEG-bound sugar intermediates during each precipitation step. It is a common experience that if one tries to separate two compounds with very different polarities on a silica gel column, the chromatographic separation can be very easy. Sometimes, it is just like a filtration on the silica gel column. It is also well known that poly(ethylene glycol) has fair strong affinity toward silica gel. Unfortunately, high molecular weight poly(ethylene glycol) (M_w > 4000) is too polar to be washed off a silica gel column even by methanol. We then turned our attention to the low molecular weight polyethylene glycol (M_n = about 550), which is polar enough to stay on the baseline of a silica gel column during a neat ethyl acetate wash and can be readily washed off the column by the mixture of ethyl acetate-methanol (4:1).

Since Schmidt's trichloroacetimidate is one of the most popular glycosyl donors in oligosaccharide syntheses, they are chosen to be tested on this strategy. Based on that (a) the common solid-phase peptide synthesis linker, 4-hydroxymethyl benzoic acid (HMB), can be coupled to a glycosyl donor through a glycosidic bond, (b) it is stable under either basic or acidic conditions and (c) it can be readily removed at the end of a synthesis by hydrogenolysis, the HMB linker is therefore the choice. The sugars that interest us are the high mannose type oligosaccharides present on the envelope glycoprotein gp 120 of the human immunodeficiency virus (HIV). *The chemical synthesis of this binding region is of*

interest for structure determination, for studies of glycoprotein function, and for determination of the specificity of biosynthetic addition and removal of sugar residues.[38] It is the subject of the present study to synthesize these structures using the low molecular weight polyethylene glycol (M_n = about 550) as an oligomer support.

Chapter 2 describes a preliminary study towards the oligomer-supported solution synthesis of oligosaccharides using low molecular weight polyethylene glycol. A model linear mannotetraose was synthesized in a 10% overall yield.

In order to synthesize the highly branched mannononaose by our new approach, two key monosaccharide building blocks were needed. Although preparation of both donors were known, the synthesis of B was quite time-consuming and difficult, so there were still needs for better ways of preparing it. **Chapter 3** deals with the regioselectivity in the acylation of hexopyranosides with pivaloyl chloride in pyridine. Manno- and galato-pyranosides were regioselectively acylated to give the 3-*O*-pivaloylated compounds in good yields. This regioselective pivaloylation gave an easy access of the building block B. On the other hand, **Chapter 4** demonstrates that BH_3/Bu_2OTf is an effective reagent to reductively cleave 4,6-*O*-benzylidene acetals of various hexopyranosides to the corresponding 4-*O*-benzyl ethers. 4,6-*O*-isopropylidene acetals can be similarly cleaved. Common protecting groups are stable to the reaction conditions. This regioselective reductive ring-opening reaction provided another easy way of making the same building block.

Chapter 5 illustrates a concise synthesis of the mannan residue (**5.1**) of a highly branched mannose type oligosaccharide present on the viral coat of HIV-1 from only two monosaccharide building blocks (**5.2** and **5.3**). The use of low molecular weight MPEG aided considerably in the purification of the intermediates and in the analyses of the progress of the assembly by using conventional spectroscopic techniques. This synthesis demonstrates that oligomer-supported solution synthesis using a low molecular weight

polyethylene glycol is a powerful tool for the rapid and efficient preparation of complex oligosaccharides.

Chapter 6 describes the synthesis of a heptaglucoside phytoalexin elicitor (HPE) by the oligomer-supported solution-phase synthesis methodology. Even though the synthesis of HPE (**6.1**) presented problems at the last step, it shown that adjustment of the length of the poly(ethylene glycol) oligomer support could result in better overall yields. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC NMR spectroscopies are extremely useful in monitoring the progress of oligosaccharide construction. The regioselective reductive ring opening of 4,6-*O*-benzylidene acetals of hexopyranosides has been exploited for the concise syntheses of two building blocks of HPE.

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CHAPTER 2

Use of Low Molecular Weight Polyethylene Glycol Linker for Oligomer-supported Solution Synthesis of Oligosaccharides

2.0 Introduction.

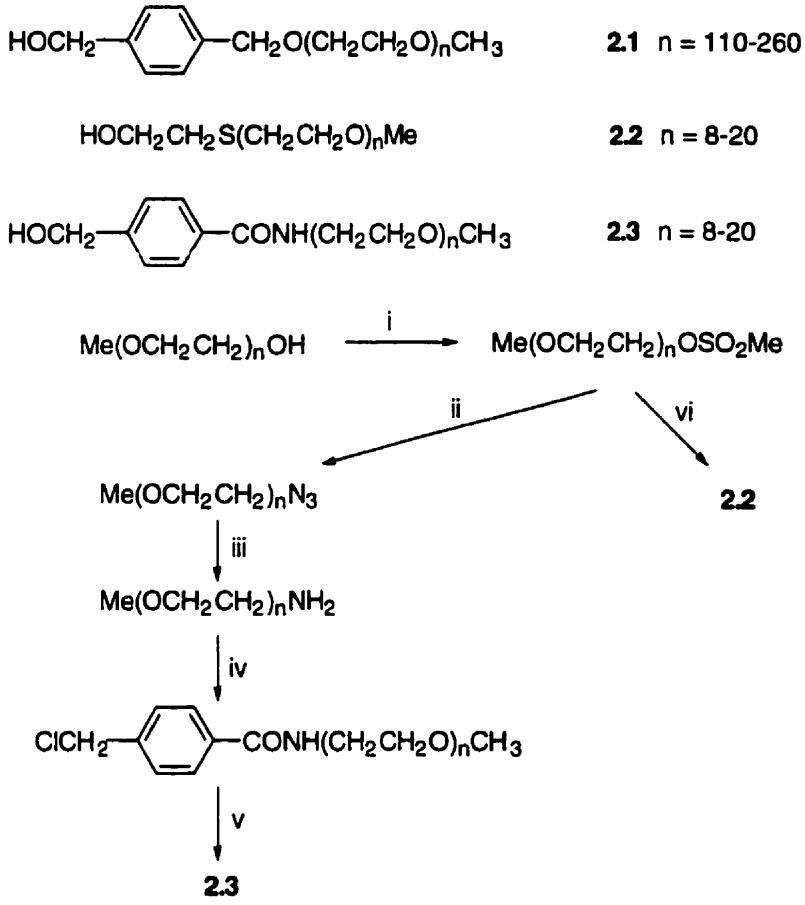
Due to the emerging recognition of the important role of oligosaccharides in many biological processes, the efficient synthesis of oligosaccharides has become a subject of much current interest. In analogy to the oligopeptide and oligonucleotide fields, solid-phase synthesis of oligosaccharides on polystyrene polymers[1] or on silica[2] have been explored. The stepwise synthesis on solid support has many attractive features, such as (a) the growing molecule is covalently bonded to an insoluble support, making purification simpler at each step by filtering and washing; (b) the reaction rates can be increased by using a large excess of reagents which can be easily separated after the completion; and (c) automation of the synthesis is feasible. Based on significant improvements on solution-phase glycosylation methods in recent years, newly developed solid-phase strategies do improve the coupling yields and simplify the purification procedures in many cases with respect to the classical oligosaccharide synthesis. However, improved yields result from the use of a large excess of glycosyl donors or acceptors, which generally are quite difficult and expensive to prepare. In addition, the solution-phase coupling methods have to be adapted to solid-phase methodologies. Since overcrowding of the reactive sites can cause internal deletions in an oligosaccharide sequence, the loading of most solid supports is generally low and therefore, only a small amount of final products can be prepared. Furthermore, even the crosslinking degree of most solid supports (usually polystyrene)

used is quite low, elongation of the large or branched oligosaccharide chains could still be sterically hindered.

Alternatively, polymer-supported solution synthesis of oligosaccharides using the polyethylene glycol linker **2.1** has been used.[3] Solution-phase synthesis is believed to offer advantages over solid-phase synthesis because of the greater efficiency of the glycosylation reactions in solution. Since a linear high molecular weight poly(ethylene glycol) monomethyl ether is completely soluble in the reaction solution, the glycosylation kinetics and anomericity control are very much similar to those observed in classical solution chemistry. In principle, the conventional solution-phase coupling methods can directly be applied onto a polymer-supported synthesis. No large excess of glycosyl donors or acceptors are necessary and consequently it will reduce the material and labour cost of oligosaccharide syntheses. Also, there is no need to worry about the overcrowding of the reactive sites. The number of repeating ethylene glycol units in **2.1** is about 110 or about 260. The selection of the molecular weight of the polymer is based on the consideration that the polymer-bound oligosaccharide must remain soluble in the glycosylation step but can be precipitated readily from the solution without contamination of the reagents.[3] However, incomplete precipitation between each step causes great loss of the intermediates and products, resulting in an unsatisfactory overall yield.

It is a common experience that if one tries to separate two compounds with very different polarities on a silica gel column, the chromatographic separation can be very easy. Sometimes it is just like a filtration on the silica gel column. It is also well known that poly(ethylene glycol) has fair strong affinity toward silica gel. Unfortunately, high molecular weight poly(ethylene glycol) (M_w > 4000) is too polar to be washed off a silica gel column even by methanol. Our attention was then turned to the low molecular weight polyethylene glycol (M_w = *ca.* 550), which is polar enough to stay on the baseline of a silica gel column during a neat ethyl acetate wash and can be readily washed off the column by the mixture of ethyl acetate-methanol (4:1).

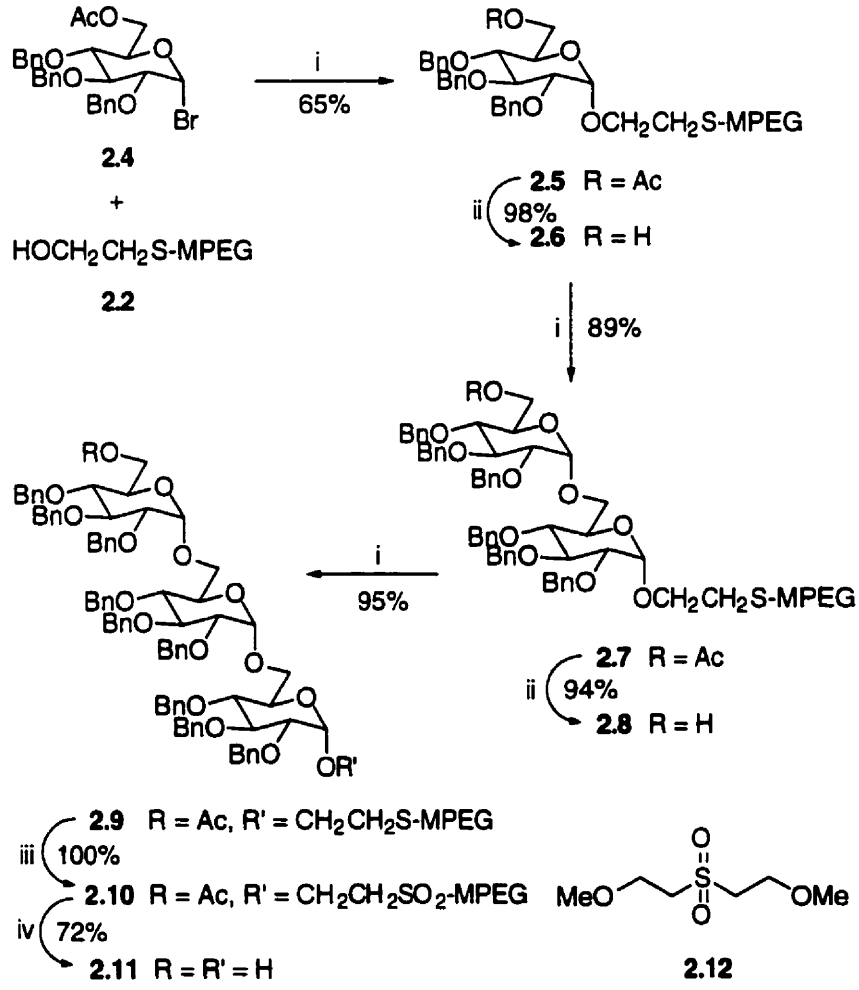
2.1 Results and discussion.



Scheme 2.1 *Reagents and conditions*: (i) Et_3N, 2.0 equiv. of $MeSO_2Cl$, CH_2Cl_2, 0 °C to rt, 2 h, 98.5%; (ii) 3.0 equiv. of NaN_3, DMF, 80 °C, 5 h, 95%; (iii) 40 psi H_2, 10% Pd-C, MeOH, 3 h, quantitative; (iv) 1.5 equiv. of 4-chloromethylbenzoic acid, 1.5 equiv. of DCC, DMAP, CH_2Cl_2, 12 h, 96%; (v) 1.0 equiv. of Ag_2CO_3, 0.1 equiv. of $AgClO_4$, 48 h, 95%; (vi) 2.0 equiv. of $HOCH_2CH_2SH$, Et_3N, 24 h, 93%.

The use of low molecular weight poly(ethylene glycol) ω-monomethyl ether (MPEG, average M_w 550, n = *ca.* 8-20) as the supporting polymer is described here. Two novel linkers, **2.2** and **2.3**, have been developed and their syntheses are outlined in Scheme 2.1. Poly(ethylene glycol) ω-monomethyl ether (MPEG, average M_w 550, n = *ca.*

8-20) was first mesylated with methanesulfonyl chloride in the presence of triethylamine. Displacement of the mesylate with mercaptoethanol under basic conditions smoothly afforded **2.2**. On the other hand, catalytic hydrogenation of MPEGazide made from the displacement of the same mesylate gave MPEGamine, which was coupled with 4-(chloromethyl)benzoic acid in the presence of DCC and DMAP to furnish, after hydrolysis of the benzyl chloride, linker **2.3**.



Scheme 2.2 *Reagents and conditions*: (i) 2.0 equiv. of **2.4**, 2.0 equiv. of Hunig base, CH_2Cl_2, 35 °C, 24 h; (ii) K_2CO_3, wet MeOH, 4 h; (iii) 2.5 equiv. of dimethyldioxirane, -78 °C; (iv) 3.0 equiv. of NaOMe, MeOH-THF, 0 °C.

The utility of **2.2** as a linker is demonstrated by the synthesis of the trisaccharide **2.11** using a sequence of reactions (Scheme 2.2, Dr. Richard C. Hartley's work) patterned after the chemistry previously developed by our group.[4] The MPEG-thioethanol **2.2** was glycosylated with **2.4** to give the MPEG-monosaccharide **2.5** in 65% yield. Deacetylayion of **2.5** afforded the alcohol **2.6** in 98% yield. A second glycosylation furnished the MPEG-disaccharide **2.7** in 89% yield. Deacetylation of **2.7** went in 94% yield to give alcohol **2.8** which was in turn glycosylated to afford the MPEG-trisaccharide **2.9** in 95 % yield. The trisaccharide can be detached from the polymer linker by first quantitative oxidation of **2.9** with dimethyldioxirane at -78 °C to the corresponding sulfone **2.10**. Treatment of the sulfone **2.10** with three equivalent of 1 M sodium methoxide in methanol to a cooled (<0 °C) dilute (0.006 M) solution of the sulfone in THF released the trisaccharide **2.11** in 72 % yield. However, one problem in the use of **2.2** is that in the final cleavage step of the trisaccharide **2.11** from the MPEG support, compound **2.12** was obtained as the side product and was only separated from **2.11** with difficulty. Since alcoholysis glycosylation is not very effective toward the secondary alcohol, and it is not commonly used in oligosaccharide syntheses any more, it was felt that there was need for a better glycosylation method and a different linker.

The use of low molecular weight MPEG as the oligomer support retains the normal advantages of polymer-supported solution synthesis of oligosaccharides, *viz*: (1) **2.2** and its supported synthons are completely soluble in the normal reaction solvents and the efficiency of the various steps follows that expected of solution chemistry; and (2) purification of **2.2** and its supported synthons by fast column chromatography on silica gel 60 (230-400 mesh, E. Merck) is simplified by the fact that even in neat ethyl acetate, the MPEG derivatives remain on the baseline while the by-products move rapidly through the column. Changing the eluent to dichloromethane-methanol (4:1) or to ethyl acetate-methanol (4:1) allows quick elution of the MPEG derivatives. Another advantage of using low molecular weight MPEG is that the reaction can be monitored readily by the

conventional arsenal of spectroscopic techniques. Thus, in Scheme 2.2, in each of the coupling steps, the MPEG derivatives **2.5**, **2.7** and **2.9** can be analyzed by electrospray mass spectrometry to show clearly the incorporation of the additional saccharide unit. The use of ^{1}H NMR to monitor the progress of the reactions is also facilitated by the use of the methyl group of MPEG as an internal reference.

Based on that (a) benzyl is the most common permanent protecting group in oligosaccharide syntheses, (b) it is stable under either basic or acidic conditions and (c) it can be readily removed at the end of a synthesis by hydrogenolysis, 4-hydroxymethyl benzoic acid (HMB), the common solid-phase peptide synthesis linker, was therefore the choice of the linker for our oligomer-supported oligosaccharide synthesis. And since Schmidt's trichloroacetimidate is one of the most popular glycosyl donors in oligosaccharide syntheses, it was chosen to be tested on this strategy.

The application of linker **2.3** was demonstrated by the synthesis of the mannotetraose **2.13** according to Scheme 2.3. In this case, the polymer **2.3** was glycosylated with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate **2.14** using Schmidt's protocol.[5] To avoid the formation of 1,2-orthoacetate and destruction of the molecule, the amount of Lewis acid (TMSOTf) used in the glycosylation had to be optimized and 0.8 equivalent was found to be the best one, which allowed the fast formation of the α glycosidic bond instead of the orthoester. Meanwhile, it was found that the trichloroacetimidate glycosyl donor **2.14** was quite sensitive to the heat existing in the process of removing water by co-distillation with toluene and not very stable to store at room temperature. The acetyl group of the MPEG-monosaccharide **2.15** was readily removed by base treatment to yield alcohol **2.16**, while completion of the deacetylation was indicated by the disappearance of the acetyl peak (δ = 2.12 ppm) on ^{1}H NMR. Thus, the deacetylation with K_2CO_3 and the glycosylation with imidate donor **2.14** became a set of standard procedures for elongation of the glycan chain and the progress of each successive coupling was easily monitored by ^{1}H NMR. The product of each coupling

reaction also showed a new anomeric peak between 4.9 and 5.2 ppm which was visible just downfield from the benzyl CH_2 shifts. The chemical shifts of new anomeric protons and C-2 protons were readily derived from the ^1H-^1H COSY NMR spectrum. Repetition of the glycosylation and hydrolysis steps three times gave the protected MPEG-mannotetraose **2.20** through the formation of MPEG-bound monosaccharides **2.15** and **2.16**, disaccharides **2.17** and **2.18** as well as trisaccharide **2.19**. In the MPEG-supported intermediate **2.20** each of the four anomeric peaks could be assigned by analyses of the ^1H-^1H COSY NMR spectrum which revealed fully the extent of coupling, as well as the stereochemistries at the anomeric centers of the tetrasaccharide. Hydrogenolysis of **2.20** over 10% Pd-C in methanol released the mannotetraose **2.13** from the oligomer. Purification and characterisation of **2.13** were achieved by conversion to the peracetylated derivative **2.21** which was predominately the α-anomer. Compound **2.21** was characterized by electrospray mass spectrometry (Figure 2.1), ^1H NMR (anomeric hydrogens at δ 6.23, 5.11, 5.08 and 4.95) and ^{13}C NMR (anomeric carbons at δ 91.61, 99.16, 99.57 and 99.96). As showed in Figure 2.1, the isotopic abundances at all ions in the ($M+Na^+$) cluster closely match the theoretically predicted. The ^1H-^{13}C HMQC NMR spectrum of **2.21** is also in full agreement with the structure, displaying the presence of four anomeric centers' cross peaks (Figure 2.2). The overall yield of the per-*O*-acetyl-tetramannopyranoside **2.21** was found to be 10% based on the starting polymer **2.3**.

In conclusion, oligomer-supported solution synthesis of oligosaccharides is clearly demonstrated by this preliminary study. The use of low molecular weight MPEG aided considerably in the purification of the MPEG-bound intermediates by simple flash column chromatography and the analyses of the progress of oligosaccharide assembly by using conventional spectroscopic techniques. Synthesis of more complex oligosaccharides is under way in our laboratory.

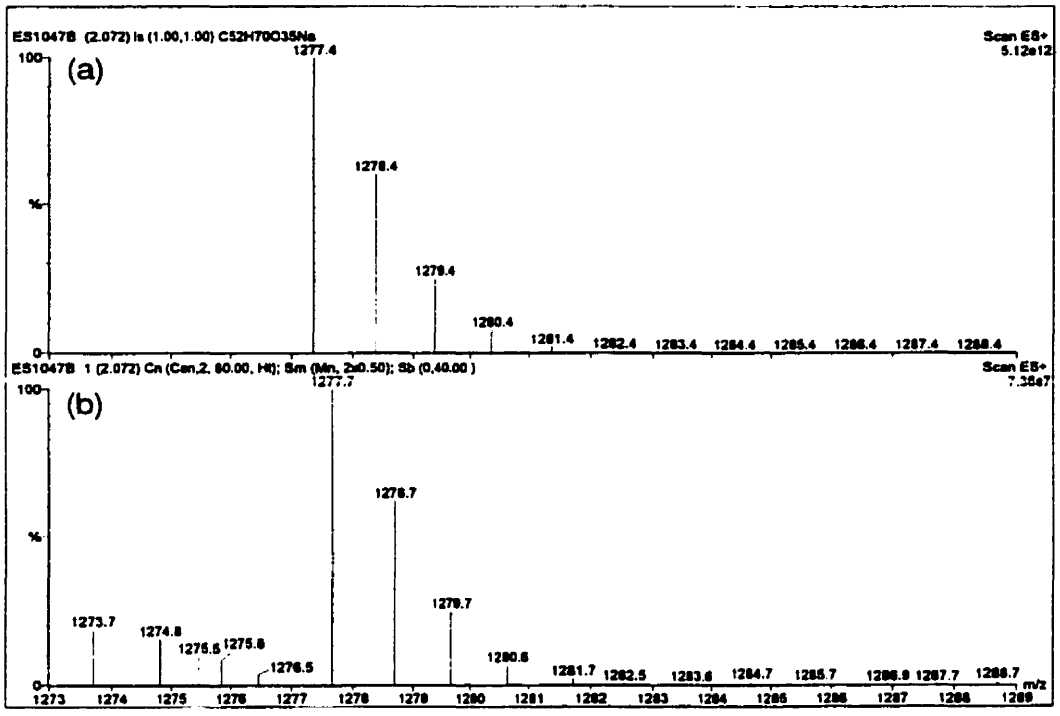


Figure 2.1. (a) Theoretical spectrum created by isotopic modeling for a sodium adduct of **2.21**; (b) experimental spectrum of **2.21**.

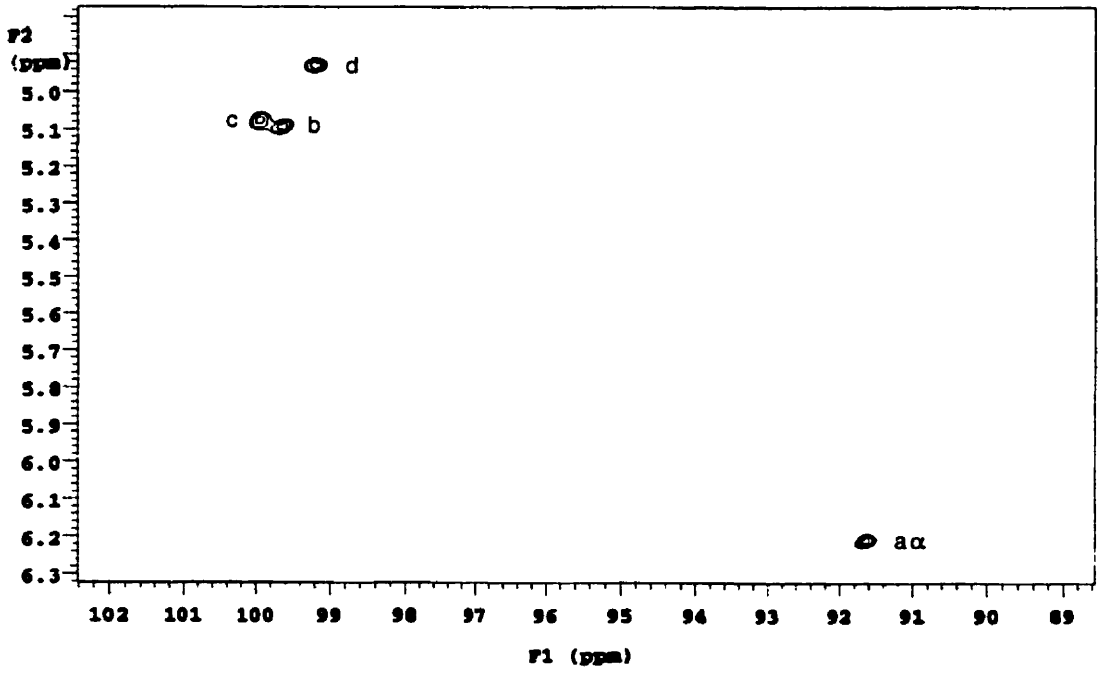
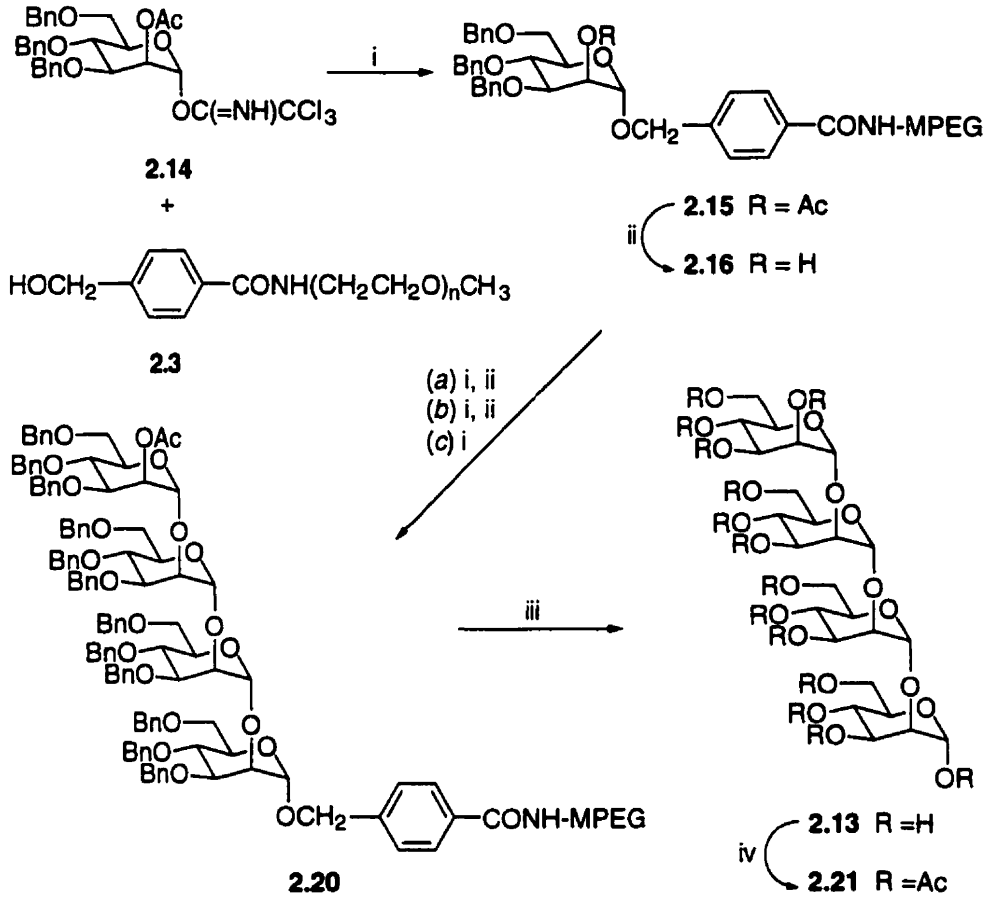


Figure 2.2. Anomeric region of the ^{1}H-^{13}C HMQC NMR spectrum of **2.21**.



Scheme 2.3 *Reagents and conditions*: (i) 2.0 equiv. of imidate **2.14**, 0.8 equiv. of TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C, 2 h; (ii) K_2CO_3, wet MeOH, 4 h; (iii) 40 psi H_2, 10% Pd-C, MeOH, AcOH, 48 h; (iv) Ac_2O, Py, DMAP, 24 h.

2.2 Experimental Section.

General. See Chapter 3, section **3.2.**

MPEG Functionalizations: MPEGOMs550. After poly(ethylene glycol) methyl ether 550 (12.54 g, 20.0 mmol OH) was dissolved in CH_2Cl_2 (20 mL) and NEt_3 (10 mL), methanesulfonyl chloride (8.02 g, 5.5 mL, 70 mmol) was added at 0 °C. Upon removal of the ice bath right after completion of the addition, the reaction mixture was allowed to warm up to room temperature slowly and kept for 4 hours. Chloroform and an

aqueous $NaHCO_3$ solution were then added to quench the reaction. After washed with an aqueous 5% HCl solution, an aqueous $NaHCO_3$ solution and brine, the organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. A clear light yellow liquid (13.9 g, 98.5%) was obtained after dried on a high vacuum pump. ^1H NMR (500 MHz, $CDCl_3$) δ 4.36 (2H, t, 4.5 Hz, CH_2OSO_2), 3.74 (2H, t, 4.5 Hz, $CH_2CH_2OSO_2$), 3.64-3.61 (48H, m, PEG), 3.54-3,51 (2H, m), 3.36 (3H, s, CH_3O), 3.06 (3H, s, CH_3SO_3).

MPEGAzide550. The MPEGOMs550 (13.9g) and sodium azide (4.55g, 70 mmol) were dissolved in DMF (50 mL) and the reaction mixture was heated at 80 °C for 5 hours. Water was added after DMF being removed under reduced pressure. Following the extraction from the aqueous solution with chloroform (3*50 mL), the organic layer was washed with brine and then dried over anhydrous Na_2SO_4. Evaporation of the organic layer to dryness under reduced pressure left a darker liquid (12.2g, 95%). ^1H NMR (500 MHz, $CDCl_3$) δ 3.66-3.61 (50H, m, PEG), 3.53 (2H, t, 2.4 Hz, $OCH_2CH_2N_3$), 3.36 (2H, t, 2.4 Hz, $OCH_2CH_2N_3$), 3.35 (3H, s, CH_3O).

MPEGAmine550. A mixture of the PEGazide550 (12.2g), 10% Pd/C (0.8g) and methanol (40 mL) was stirred under 40 psi H_2 for 3 hours at room temperature. The solution was filtered and the Pd-C washed with methanol. The filtrate was evaporated to dryness under reduced pressure to give a clear light reddish liquid (11.7g, 100%). ^1H NMR (500 MHz, $CDCl_3$) δ 3.62-3.58 (48H, m. PEG), 3.50 (2H, m), 3.47 (2H, t, 5.2 Hz, $OCH_2CH_2NH_2$), 3.34 (3H, s, CH_3O), 2.81 (2H, t, 5.5 Hz, $OCH_2CH_2NH_2$).

MPEGNHCOPhCH$_2$Cl. A mixture of the PEGamine550 (5.5g), DCC (3.1g, 15mmol), 4-chloromethylbenzoic acid (2.2g, 13mmol), CH_2Cl_2 (20 mL) and a catalytic amount of DMAP was stirred at room temperature overnight. Water was added after removing CH_2Cl_2 under reduced pressure. The precipitate dicyclohexylurea was filtered out and the aqueous solution was extracted with chloroform (3*20 mL). The organic layer was washed with an aqueous $NaHCO_3$ solution, brine and then dried over anhydrous Na_2SO_4.

Removing solvent left a sticky light yellow liquid (6.6g, 96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (2H, d, 7.6 Hz, Ar-*H*), 7.43 (2H, d, 7.6 Hz, Ar-*H*), 6.90 [1H, s (br), PhCON*H*], 4.59 (2H, s, ClC*H*$_2$Ph), 3.70-3.50 (54H, m, PEG), 3.36 (3H, s, C*H*$_3$O).

MPEGNHCOPhCH$_2$OH (2.3). A mixture of the PEGNHCOPhCH$_2$Cl (4.3g), Ag_2CO_3 (1.6g, 5.8mmol), $AgClO_4$ (0.1g) and water (25mL) were stirred at room temperature for two days. The reaction mixture was first filtered through celite, and then extracted with chloroform (3*20 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to give a dark syrup, which still contained silver salts. The resulting syrup was dissolved in a small quantity of ethyl acetate and added onto the top of a silica gel column for flash column chromatography. Elution with ethyl acetate/methanol (4:1 and 3:1, 150 mL each) gave **2.3** (4.0g, 95%) as a sticky light yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (2H, d, 7.6 Hz, Ar-*H*), 7.39 (2H, d, 7.6 Hz, Ar-*H*), 7.06 [1H, s (br), PhCON*H*], 4.70 (2H, d, 4.1 Hz, PhC*H*$_2$OH), 3.70-3.40 (54H, m, PEG), 3.35 (3H, s, C*H*$_3$O), 2.61 [1H, s (br), CH$_2$O*H*].

Protocol for the Solution-phase Glycosylations. After donor imidate **2.14** and a MPEG-acceptor (1 mmol) were co-distillated with toluene 5 times under reduced pressure, dry 4 Å molecular sieve powder (1 g/g reactant) and dichloromethane (2.5 mL/g reactant) were added into the flask. The mixture was stirred for 10-15 minutes under Argon and then cooled to 0 °C. The color of the reaction mixture turned pink immediately as 2M solution of TMSOTf (0.8 equiv.) in dichloromethane was syringed into the flask slowly. Upon removal of the ice bath right after completion of the addition, the reaction mixture was allowed to warmed up to room temperature slowly and kept for 2 hours. Chloroform and an aqueous $NaHCO_3$ solution were added to quench the reaction, followed by filtration of the mixture through a pad of celite to remove the molecular sieve powder. After washed with brine, the organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The resulting syrup was dissolved in a small amount of ethyl acetate and put onto the top of a silica gel column (15 times weight of the syrup) for flash

column chromatography. Gradient elution with hexanes/ethyl acetate (2:1, 1:1, 1:2, 1:4 and neat EtOAc, 50 mL each) removed the side-products and further elution with ethyl acetate/methanol (4:1 and 3:1, 150 mL each) gave the MPEG-sugar conjugate.

Protocol for the Deacetylation with K_2CO_3. Potassium carbonate was added to a stirred solution of MPEG-sugar in wet methanol and the reaction was monitored by ^1H NMR. Upon completion of the deacetylation indicated by the disappearance of the acetyl group on ^1H NMR, methanol was evaporated under reduced pressure and brine was added. After the aqueous solution was extracted with chloroform (3*50 mL), the organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. Purification of the MPEG-bound products by chromatographic separation procedure was the same as above.

Protocol for the Debenzylation of MPEG-mannotetraose 2.20. A mixture of **2.20** and 10% Pd-C (1g) in a mixture of methanol and acetic acid (2:1, 50 mL) was stirred under 40 psi H_2 for 48 hours at room temperature. The solution was filtered and the Pd-C washed with methanol. After the filtrate was evaporated under reduced pressure to give a syrup, acetic anhydride, pyrydine and a catalytic amount of DMAP were added. The mixture was stirred at room temperature for two days and usual work-up gave a glassy solid, which was subject to flash column chromatography over silica gel (30 g, EtOAc/Hexanes 2:1) to afford the peracetylated mannotetraose **2.21** (predominately the α-anomers) in 10.3% yield (129 mg).

MPEG-man2OAc (2.15). ^1H NMR (500 MHz, $CDCl_3$) δ 7.76 (2H, d, 7.1 Hz, Ar-*H*), 7.40-7.12 (17H, m, Ar-*H*), 6.78 [1H, s (br), PhCON*H*], 5.38 (1H, dd, 1.7 and 2.6 Hz, *H*-2), 4.90 (1H, d, 1.0 Hz, *H*-1), 4.83 (1H, d, 10.7 Hz, PhC*H*), 4.70 (1H, d, 11.7 Hz, PhC*H*), 4.66 (1H, d, 11.7 Hz, PhC*H*), 4.54-4.49 (4H, m, PhC*H*), 4.44 (1H, d, 10.7 Hz, PhC*H*), 3.99 (1H, dd, 3.2 and 9.0 Hz, *H*-3), 3.88 (1H, dd, 9.3 and 9.3 Hz, *H*-4), 3.81-3.76 (2H, m, *H*-5 and *H*-6a), 3.68-3.51 (m, *H*-6b and PEG), 3.35 (3H, s, OCH_3), 2.12 (3H, s, CH_3CO).

MPEG-man2OH (2.16).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (2H, d, 7.1 Hz, Ar-*H*), 7.43-7.13 (m, Ar-*H*), 6.90 [1H, s (br), PhCON*H*], 4.96 (1H, d, 1.0 Hz, *H*-1), 4.80 (1H, d, 10.7 Hz, PhC*H*), 4.72 (1H, d, 12.4 Hz, PhC*H*), 4.68-4.67 (2H, m, PhC*H*), 4.64 (1H, d, 12.2 Hz, PhC*H*), 4.52 (1H, d, 12.2 Hz, PhC*H*), 4.51 (1H, d, 12.5 Hz, PhC*H*), 4.48(1H, d, 10.7 Hz, PhC*H*), 4.05 (1H, dd, 1.7 and 3.2 Hz, *H*-2), 3.90 (1H, dd, 3.2 and 9.4 Hz, *H*-3), 3.85 (1H, dd, 8.8 and 9.4 Hz, *H*-4), 3.81-3.79 (1H, m, *H*-5), 3.73 (1H, dd, 10.8 and 10.8 Hz, *H*-6a), 3.70-3.51 (m, *H*-6b and PEG), 3.35 (3H, s, OCH_3).

MPEG-Diman2OAc (2.17).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (2H, d, 7.1 Hz, Ar-*H*), 7.40-7.12 (m, Ar-*H*), 6.72 [1H, s (br), PhCON*H*], 5.51 (1H, dd, 1.9 and 3.4 Hz, *H*-2a), 5.03 (1H, d, 1.9 Hz, *H*-1a), 4.95 (1H, d, 1.9 Hz, *H*-1b), 4.84-4.31 (m, $PhCH_2$), 3.99 (1H, dd, 2.0 and 2.9 Hz, *H*-2b), 3.93 (1H, dd, 3.2 and 9.3 Hz, *H*-3a), 3.91 (1H, dd, 2.7 and 8.8 Hz, *H*-3b), 3.82-3.51 (m), 3.35 (3H, s, OCH_3), 2.03 (3H, s, CH_3CO).

MPEG-Diman2OH (2.18).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (2H, d, 7.3 Hz, Ar-*H*), 7.35-7.14 (m, Ar-*H*), 6.75 [1H, s (br), PhCON*H*], 5.11 (1H, d, 1.5 Hz, *H*-1a), 5.01 (1H, d, 2.0 Hz, *H*-1b), 4.83-4.29 (m, $PhCH_2$), 4.11 (1H, dd, 1.7 and 3.4 Hz, *H*-2a), 4.04 (1H, dd, 1.5 and 2.7 Hz, *H*-2b), 3.91-3.52 (m), 3.35 (3H, s, OCH_3).

MPEG-Triman2OAc (2.19).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (2H, d, 7.3 Hz, Ar-*H*), 7.35-7.14 (m, Ar-*H*), 6.78 [1H, s (br), PhCON*H*], 5.53 (1H, dd, 1.7 and 3.2 Hz, *H*-2a), 5.18 (1H, d, 1.9 Hz, *H*-1a), 5.05 (1H, d, 1.7 Hz, *H*-1b), 5.04 (1H, d, 1.9 Hz, *H*-1c), 4.84-4.24 (m, $PhCH_2$), 3.99-3.51 (m), 3.35 (3H, s, OCH_3), 2.05 (3H, s, CH_3CO).

MPEG-Tetraman2OAc (2.20).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (2H, d, 7.1 Hz, Ar-*H*), 7.40-7.02 (m, Ar-*H*), 6.80 [1H, s (br), PhCON*H*], 5.52 (1H, dd, 2.0 and 3.2 Hz, *H*-2a), 5.19 (1H, d, 1.7 Hz, *H*-1a), 5.15 (1H, d, 1.6 Hz, *H*-1b), 5.05 (1H, d,

1.7 Hz, *H*-1c), 5.00 (1H, d, 1.7 Hz, *H*-1d), 4.85-4.20 (m, PhCH_2), 3.98-3.50 (m), 3.35 (3H, s, OCH_3), 2.05 (3H, s, CH_3CO).

MannotetraoseAcetate (2.21): colorless glassy solid; R_f 0.45 (5:1 EtOAc/Hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.23 (1H, d, 2.4 Hz, *H*-1a), 5.39-5.21 (m), 5.11 (1H, d, 2.2 Hz, *H*-1b), 5.08 (1H, d, 2.7 Hz, *H*-1c), 4.95 (1H, d, 2.0 Hz, *H*-1d), 4.24-3.98 (m), 2.14 (3H, s, CH_3CO), 2.13 (3H, s, CH_3CO), 2.11 (3H, s, CH_3CO), 2.10 (3H, s, CH_3CO), 2.09 (3H, s, CH_3CO), 2.08 (3H, s, CH_3CO), 2.07 (3H, s, CH_3CO), 2.06 (3H, s, CH_3CO), 2.05 (3H, s, CH_3CO), 2.04 (3H, s, CH_3CO), 2.02 (3H, s, CH_3CO), 2.01 (6H, s, CH_3CO), 2.00 (3H, s, CH_3CO), 1.98 (3H, s, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8 (2C), 170.7, 170.5, 170.1, 170.0, 169.7, 169.7, 169.6, 169.5, 169.4, 169.3, 169.1, 168.2, 100.0, 99.6, 99.2, 91.6, 76.3, 75.3, 70.7, 69.9, 69.7, 69.6 (2C), 69.5, 69.4, 68.3, 66.4, 66.3, 66.1, 65.5, 62.4, 62.2, 62.1, 61.7, 20.9, 20.8, 20.7, 20.6, 20.6, 20.5.

2.3 References.

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CHAPTER 3

Regioselective Acylation of Hexopyranosides with Pivaloyl Chloride

3.0 Introduction.

Selective acylation of carbohydrates is one of the most valuable reactions in carbohydrate synthesis.[1] The most widely studied is selective benzoylation. A number of benzoylating reagents, such as benzoyl cyanide,[2] benzoyl imidazole,[3] and 1-(benzoyloxy)benzotriazole[4] in addition to benzoyl chloride,[5] have been examined. More recently, activation through partial stannylation of carbohydrate with $(Bu_3Sn)_2O$ or Bu_2SnO followed by electrophilic attack with benzoyl chloride[6] has been used. However, the selectivity of benzoylation has not always been high, and the separation of various regioisomers of benzoylated carbohydrate derivatives, often by chromatography, can be tedious. Selective acylation of carbohydrates with pivaloyl chloride to give the corresponding pivalate has been used, however, mainly as a selective method of esterifying the primary hydroxy group in the presence of secondary hydroxyl groups.[7] There have been few systematic studies in the selective pivaloylation of secondary hydroxy groups in various mono- or oligosaccharides. Treatment of methyl α-D-glucopyranoside (**3.1**) in ether with pivaloyl chloride and pyridine at 4 °C gave almost exclusively the 2,6-di-*O*-pivalate (**3.2**) (Table 3.1).[8] Similarly, methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**3.3**) gave the corresponding 2-*O*-pivalate **3.4** as the major product. It thus appears that the C_2-OH is the most reactive among all the secondary hydroxy functions in α-D-glucopyranosides. As far as we are aware, no investigation of pivaloylation of other monosaccharides has been reported.[9] Because the pivaloylated sugars are usually

crystalline compounds and easily characterized by ^{1}H NMR spectroscopy, we became interested in the selective esterification of other hexopyranosides.

Table 3.1 Selective Acylation of Hexopyranosides with Pivaloyl Chloride in Pyridine at 0 °C.

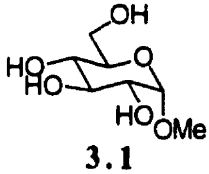
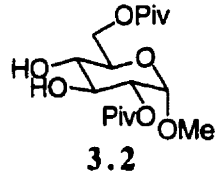
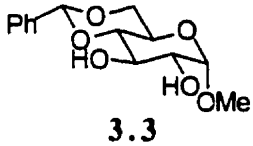
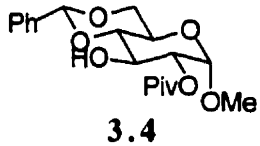
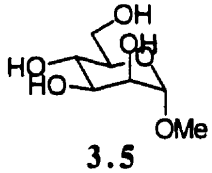
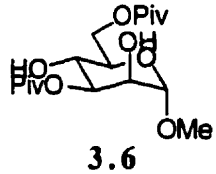
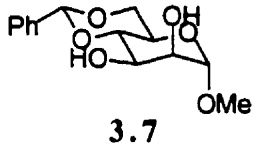
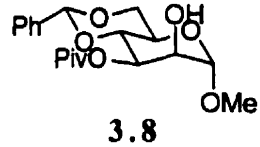
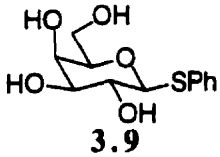
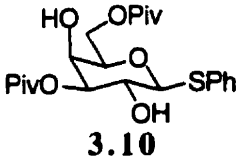
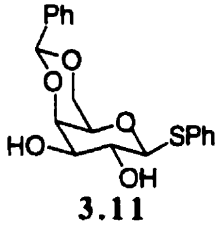
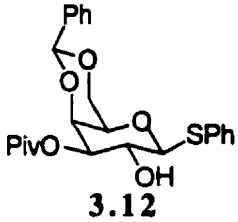
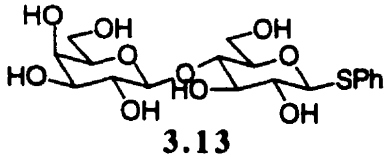
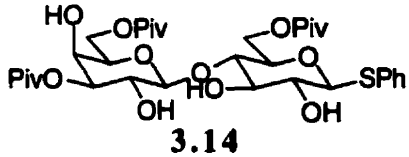
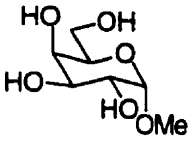
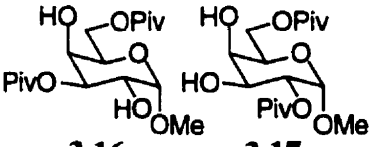
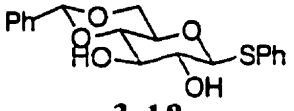
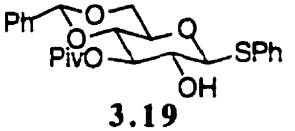
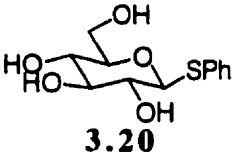
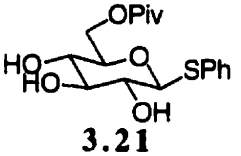
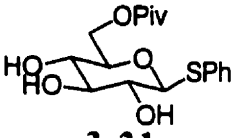
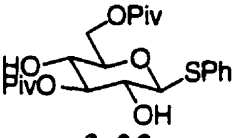
Entry	Substrate	Product	Isolated Yield (%)
1	 **3.1**	 **3.2**	83
2	 **3.3**	 **3.4**	79
3	 **3.5**	 **3.6**	91
4	 **3.7**	 **3.8**	89
5	 **3.9**	 **3.10**	78
6	 **3.11**	 **3.12**	75
7	 **3.13**	 **3.14**	73

Table 3.1 (continued)

8	 **3.15**	 **3.16** **3.17**	88 (1:1)
9	 **3.18**	 **3.19**	79
10	 **3.20**	 **3.21**	77
11	 **3.21**	 **3.22**	74

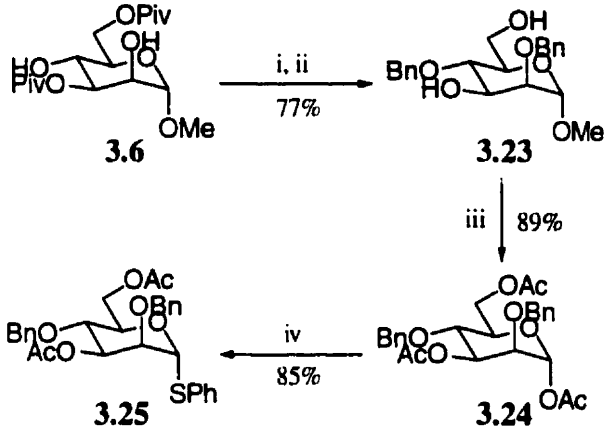
3.1 Results and discussion.

It is found that the use of pivaloyl chloride in pyridine is very effective in the selective acylation of carbohydrates under mild conditions. As reported previously with ether as the solvent, **3.1** was converted selectively to **3.2** in 83% yield, and **3.3** to **3.4** in 79% yield. On the other hand, treatment of methyl α-D-mannopyranoside (**3.5**) with pivaloyl chloride in pyridine gave selectively methyl 3,6-di-*O*-pivaloyl-α-D-mannopyranoside (**3.6**) in 91% isolated yield as a crystalline solid. Similarly, methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**3.7**) was regioselectively converted to methyl 4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranoside (**3.8**) in 89% yield. The higher reactivity of the C_3-OH group in mannopyranosides is not unexpected since the C_2-OH is in the sterically hindered axial position, and the C_4-OH is generally known to be the least reactive. On the other hand, treatment of phenyl 1-thio-β-D-galactopyranoside (**3.9**) with

pivalolyl chloride in pyridine at 0°C gave the 3,6-di-*O*-pivalate **3.10** in 78% yield. The selective acylation of C_3-OH persisted in the reaction of phenyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**3.11**) under the same conditions. The 3-*O*-pivalate **3.12** was obtained in 75% yield. The greater reactivity of the C_3-OH in the galactopyranosides is more difficult to explain. It may be caused by the lower reactivity of the C_2-OH which is due to the presence of the β-substituent at the anomeric position, or the enhanced reactivity of the C_3-OH is due to the presence of the adjacent axial function at the C_4-position. It is likely that the latter explanation is more plausible.[10] When the thiophenyl lactose-pyranoside **3.13** was treated with pivaloyl chloride in pyridine, the 3',6',6-tri-*O*-pivalate **3.14** was obtained in 73% yield. It is the C_3-OH with the adjacent axial function (at C_4-OH) rather than the C_3-OH that has been esterified selectively after the primary hydroxyl groups. This is in agreement with the observation that methyl α-D-galactopyranoside (**3.15**) gave a 1:1 mixture of 3,6-di-*O*-pivalate **3.16** and 2,6-di-*O*-pivalate **3.17** in 88% yield under the same pivaloylation conditions. It may be due to the similar reactivities of the C_2- and C_3-OH which are both adjacent to an axial function at C_1- and C_4-positions. Finally, it should be noted that it is not possible to selectively pivaloylate the primary C_6-OH over one of the secondary hydroxyl groups in compouds **3.1**, **3.5**, **3.9**, **3.13**, and **3.15**. This suggests that the reactivity of a secondary hydroxyl group with an adjacent axial function is almost as high as that of the primary C_6-OH group toward pivaloylation.[10]

In the absence of the adjacent axial alkoxy group as in the case of the phenyl 1-thio-β-D-glucopyranoside **3.20**, the compound can be selectively pivaloylated to afford the 6-*O*-pivaloyl-β-D-glucopyranoside **3.21** in 77% yield together with 10% of the 3,6-di-*O*-pivaloyl-β-D-glucopyranoside **3.22**. Furthermore, compound **3.22** can be obtained in high yield (entry 11) from **3.21** under the same reaction conditions. This tends to suggest that in the absence of an adjacent axial function, the secondary C_3-OH may be more reactive than the secondary C_2-OH toward pivaloylation. Similarly, high selectivity was

observed on the pivaloylation of phenyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**3.18**) which gave the 3-*O*-pivalate **3.19** in 79% yield.



Scheme 3.1 *Reagents and Conditions*: (i) 4.0 equiv of benzyl 2,2,2-trichloroacetimidate, 0.05 equiv of CF_3SO_3H, 4:1 cyclohexane/CH_2Cl_2, rt, 4 h; (ii) KOH, CH_3OH, reflux for 3 h; (iii) CH_3COOH, $(CH_3CO)_2O$, catalytic H_2SO_4, 4 h; (iv) 1.2 equiv of thiophenol, 0.8 equiv of $Me_3SiOSO_2CF_3$, 4Å molecular sieves, CH_2Cl_2, 2 h.

These partially protected carbohydrates are very useful intermediates for oligosaccharide synthesis. For example, the mannose di-*O*-pivalate derivative **3.6** can be used readily as precursor for the synthesis of polymannans (Scheme 3.1).[11] Compound **3.6** was easily benzylated with benzyl 2,2,2-trichloroacetimidate under acidic conditions[12] and the pivaloyl groups can be readily removed with KOH in CH_3OH to give the 3,6-diol **3.23** which can serve as the glycosyl acceptor in the synthesis of 3,6-branched polymannans.[11a] Alternatively, compound **3.23** can undergo acetolysis with acetic anhydride/acetic acid with sulfuric acid as a catalyst to give the 1,3,6-tri-*O*-acetate **3.24**.[11b] The anomeric acetate group in **3.24** can be exchanged with thiophenol under acidic conditions to give the phenyl 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-1-thio-α-D-

mannopyranoside **3.25** which is an important glycosyl donor frequently used in the synthesis of branched high mannans.

3.2 Experimental section.

General. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, and the chemical shifts are reported in parts per million on the δ scale relative to $CDCl_3$ (δ 7.24 ppm) for ^{1}H NMR and $CDCl_3$ (δ 77.0 ppm) for ^{13}C NMR. The site of pivaloylation is determined by 2D COSY ^{1}H NMR spectroscopy. Specific rotations were determined with a Jasco DIP-140 digital polarimeter at 20 °C. Melting points are uncorrected. Solvents and reagents were used as received from commercial sources. Analytical thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F-254 polyester-backed plates (250 μm thick). Benzylidene acetals were prepared according to the known procedure.[13]

General Procedure for the Pivaloylation. To a stirred solution of the carbohydrate compound (10 mmol) in pyridine (15 mL) at 0 °C was slowly added pivaloyl chloride (20-50 mmol). The reaction was monitored by TLC, and the addition of pivaloyl chloride was stopped when the starting material or the intermediate disappeared. The reaction mixture was then diluted with ethyl acetate and washed with a dilute HCl solution, a saturated $NaHCO_3$ solution, and then brine. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness. The crude product was subject to flash column chromatography (E. Merck silica gel 60, 230-400 mesh ASTM) using hexanes and ethyl acetate (2:1, v/v) as an eluant to afford the pure product. It can also be crystallized from the 10:1 (v/v) mixture of hexanes and ethyl acetate with a slightly lower yield.

Methyl 2,6-di-*O*-pivaloyl-α-D-glucopyranoside (3.2):[8] colorless crystals; mp 84-85 °C (lit.[8] mp 82-84 °C); $[\alpha]^{20}_{D}$+87.7° (*c* 1.03, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.89 (1H, d, 3.7 Hz, *H*-1), 4.60 (1H, dd, 3.7 and 10.0 Hz, *H*-2), 4.52 (1H,

dd, 4.4 and 12.2 Hz, *H*-6a), 4.25 (1H, dd, 2.0 and 12.2 Hz, *H*-6b), 3.98 (1H, dd, 9.5 and 9.5 Hz, *H*-3), 3.76 (1H, ddd, 2.2, 4.1, and 10.1 Hz, *H*-5), 3.37 (3H, s, OCH_3), 3.34 (1H, dd, 9.5 and 9.5 Hz, *H*-4), 3.06 [1H, s (br), O*H*], 2.41 [1H, s (br), O*H*], 1.24 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.4, 178.3, 96.9, 72.9, 71.2, 70.4, 69.4, 62.8, 55.3, 38.8, 38.7, 27.0, 26.9.

Methyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-α-D-glucopyranoside (3.4):[8] colorless crystals; mp 150-152 °C (lit.[8] mp 149-151 °C); $[\alpha]^{20}_D$+105° (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.50-7.37 (5H, m, Ar-*H*), 5.56 (1H, s, PhC*H*), 4.94 (1H, d, 3.7 Hz, *H*-1), 4.74 (1H, dd, 3.7 and 9.5 Hz, *H*-2), 4.30 (1H, dd, 4.6 and 10.3 Hz, H-6a), 4.20 (1H, dd, 9.5 and 9.5 Hz, *H*-3), 3.86 (1H, ddd, 4.6, 9.9, and 10.0 Hz, *H*-5), 3.77 (1H, dd, 10.0 and 10.3 Hz, *H*-6b), 3.57 (1H, dd, 9.4 and 9.4 Hz, *H*-4), 3.39 (3H, s, OCH_3), 2.31 [1H, s (br), C_3-O*H*], 1.24 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.2, 137.0, 129.3, 128.3, 126.3, 102.0, 97.6, 81.3, 73.4, 68.9, 68.8, 62.0, 55.6, 38.9, 27.0.

Methyl 3,6-di-*O*-pivaloyl-α-D-mannopyranoside (3.6): colorless crystals; mp 93-94 °C; R_f = 0.28 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+56.3° (*c* 0.71, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.04-5.01 (1H, m, *H*-3), 4.70 (1H, d, 1.7 Hz, *H*-1), 4.41-4.35 (2H, m, *H*-6a, *H*-6b), 3.98 [1H, s (br), *H*-2], 3.81-3.75 (2H, m, *H*-4, *H*-5), 3.38 (3H, s, OCH_3), 2.62 (1H, d, 2.2 Hz, C_4-O*H*), 1.86 (1H, d, 2.9 Hz, C_2-O*H*), 1.23 [9H, s, $COC(CH_3)_3$], 1.22 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.9, 178.8, 100.4, 74.2, 70.9, 69.4, 66.3, 63.2, 54.9, 39.0, 38.7, 27.2, 27.1; FAB HRMS calcd for $C_{17}H_{31}O_8$ ($M+H^+$) 363.2019, found 363.2018. Anal. Calcd for $C_{17}H_{30}O_8$: C, 56.34; H, 8.34. Found: C, 56.72; H, 8.34.

Methyl 4,6-*O*-benzylidene-3-*O*-pivaloyl-α-D-mannopyranoside (3.8): colorless crystals; mp 98-100 °C; R_f = 0.27 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+28.9° (*c* 0.58, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.44-7.32 (5H, m, Ar-*H*), 5.57 (1H, s, PhC*H*), 5.33 (1H, dd, 3.4 and 10.3 Hz, *H*-3), 4.76 (1H, d, 1.7 Hz, *H*-1), 4.30 (1H, dd, 4.2 and

9.8 Hz, *H*-6a), 4.13 (1H, dd, 1.5 and 3.2 Hz, *H*-2), 4.10 (1H, dd, 9.3 and 10.3 Hz, *H*-4), 3.92 (1H, ddd, 4.4, 9.3, and 10.3 *H*-5), 3.85 (1H, dd, 10.0 and 10.3 Hz, *H*-6b), 3.41 (3H, s, OCH_3), 2.04 [1H, s (br), C_2-O*H*], 1.23 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.2, 137.3, 128.8, 128.2, 125.9, 101.4, 101.3, 76.3, 70.4, 70.0, 68.9, 63.6, 55.1, 39.0, 27.2; FAB HRMS calcd for $C_{19}H_{27}O_7$ ($M+H^+$) 367.1757, found 367.1755. Anal. Calcd for $C_{19}H_{26}O_7$: C, 62.27; H, 7.16. Found: C, 62.24; H, 7.25.

Phenyl 3,6-di-*O*-pivaloyl-1-thio-β-D-galactopyranoside (3.10): colorless cryatals; mp 107-108 °C; R_f = 0.38 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+11.5° (*c* 0.75, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.57-7.31 (5H, m, Ar-*H*), 4.88 (1H, dd, 3.2 and 9.5 Hz, *H*-3), 4.61 (1H, d, 9.8 Hz, *H*-1), 4.35 (1H, dd, 5.5 and 11.6 Hz, *H*-6a), 4.28 (1H, dd, 6.8 and 11.6 Hz, *H*-6b), 4.01 [1H, s (br), *H*-4], 3.88 [1H, dd (br), 9.5 and 9.8 Hz, *H*-2], 3.80 [1H, dd (br), 6.1 and 6.1 Hz, *H*-5], 2.37 [1H, s (br), C_2-O*H*], 2.06 [1H, s (br), C_4-O*H*], 1.25 [9H, s, $COC(CH_3)_3$], 1.20 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.4, 178.0, 132.4, 132.3, 129.1, 128.1, 89.6, 76.2, 75.6, 67.6 (2C), 62.8, 39.1, 38.8, 27.2, 27.1; FAB HRMS calcd for $C_{22}H_{33}O_7S$ ($M+H^+$) 441.1947, found 441.1949. Anal. Calcd for $C_{22}H_{32}O_7S$: C, 59.97; H, 7.33. Found: C, 59.77; H, 7.54.

Phenyl 4,6-*O*-benzylidene-3-*O*-pivaloyl-1-thio-β-D-galactopyranoside (3.12): colorless cryatals; mp 178-179 °C; R_f = 0.36 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+46.7° (*c* 0.61, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.67-7.20 (10H, m, Ar-*H*), 5.50 (1H, s, PhC*H*), 4.86 (1H, dd, 3.5 and 9.6 Hz, *H*-3), 4.60 (1H, d, 9.5 Hz, *H*-1), 4.40-4.37 (2H, m, *H*-4, *H*-6a), 4.05-4.01 (2H, m, *H*-6b, *H*-2), 3.61 [1H, s (br), *H*-5], 2.29 (1H, d, 2.7 Hz, C_2-O*H*), 1.19 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.4, 137.8, 133.0, 131.1, 129.0, 128.9, 128.1, 128.0, 126.1, 100.5, 88.0, 74.6, 73.4, 69.9, 69.2, 65.9, 39.0, 27.0; FAB HRMS calcd for $C_{24}H_{29}O_6S$ ($M+H^+$) 445.1685, found 445.1686. Anal. Calcd for $C_{24}H_{28}O_6S$: C, 64.84; H, 6.35. Found: C, 64.90; H, 6.29.

Phenyl 4-*O*-(3,6-di-*O*-pivaloyl-β-D-galactopyranosyl)-6-*O*-pivaloyl-1-thio-β-D-glucopyranoside (3.14): colorless crystals; mp 101-103 °C; R_f = 0.40 (1:2 hexanes/EtOAc); $[\alpha]^{20}_{D}$+10.8° (*c* 0.58, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57-7.27 (5H, m, Ar-*H*), 4.83 (1H, dd, 3.3 and 10.1 Hz, *H*-3 Gal), 4.67 (1H, dd, 1.5 and 12.0 Hz, *H*-6a Glc), 4.58 (1H, d, 9.8 Hz, *H*-1 Glc), 4.38 (1H, dd, 4.9 and 11.9 Hz, *H*-6a Gal), 4.35 (1H, d, 7.6 Hz, *H*-1 Gal), 4.23 (1H, dd, 7.3 and 11.7 Hz, *H*-6b Gal), 4.21 (1H, d, 1.5 Hz, C_3-O*H* Glc), 4.12 (1H, dd, 5.9 and 12.3 Hz, *H*-6b Glc), 3.99 [1H, dd (br), 4.2 and 4.4 Hz, *H*-4 Gal], 3.92 (1H, ddd, 3.9, 7.8, and 10.3 Hz, *H*-2 Gal), 3.82 [1H, dd (br), 4.9 and 7.6 Hz, *H*-5 Gal], 3.64 (1H, ddd, 1.2, 8.6, and 8.8 Hz, *H*-3 Glc), 3.55 (1H, ddd, 1.5, 6.1, and 10.0 Hz, *H*-5 Glc), 3.39 (1H, ddd, 1.7, 8.8, and 9.8 Hz, *H*-2 Glc), 3.32 [1H, s (br), C_2-O*H* Gal], 3.31 (1H, dd, 8.4 and 9.8 Hz, *H*-4 Glc), 2.79 [1H, s (br), C_2-O*H* Glc], 2.42 [1H, s (br), C_4-O*H* Gal], 1.25 [9H, s, COC(CH_3)$_3$], 1.20 [9H, s, COC(CH_3)$_3$], 1.19 [9H, s, COC(CH_3)$_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.8, 178.5, 178.0, 132.5, 132.2, 128.9, 128.0, 104.3, 87.2, 80.9, 77.3, 76.1, 74.2, 73.1, 71.6, 69.0, 67.2, 63.4, 62.8, 39.0, 38.9, 38.8, 27.2, 27.1, 27.0; FAB HRMS calcd for $C_{33}H_{50}O_{13}SNa$ (M+Na$^+$) 709.2870, found 709.2868. Anal. Calcd for $C_{33}H_{50}O_{13}S\cdot 2H_2O$ C, 54.82; H, 7.53. Found: C, 55.16; H, 7.68.

Mixture of methyl 3,6-di-*O*-pivaloyl-α-D-galactopyranoside (3.16) and methyl 2,6-di-*O*-pivaloyl-α-D-galactopyranoside (3.17): colorless foam; R_f = 0.30 (2:1 hexanes /EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.05 (1H, dd, 3.2 and 10.3 Hz), 4.93 (1H, dd, 3.7 and 10.0 Hz), 4.89 (1H, d, 3.7 Hz), 4.84 (1H, d, 3.9Hz), 4.43 (1H, dd,6.3 and 11.5 Hz), 4.33 (1H, dd, 5.4 and 11.5 Hz), 4.24 (1H, dd, 7.1 and 11.5 Hz), 4.20 (1H, dd, 6.6 and 11.5 Hz), 4.04-3.95 (5H, m), 3.92 [1H, dd (br), 3.2 and 3.4 Hz], 3.45 (3H, s, OCH_3), 3.37 (3H, s, OCH_3), 2.70 (1H, d, 3.7 Hz, O*H*), 2.56 (1H, d, 6.8 Hz, O*H*), 2.06 (1H, d, 1.9 Hz, O*H*), 1.88 (1H, d, 11.2 Hz, O*H*), 1.26 [9H, s, COC(CH_3)$_3$], 1.24 [9H, s, COC(CH_3)$_3$], 1.22 [9H, s, COC(CH_3)$_3$], 1.21 [9H, s, COC(CH_3)$_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.0, 178.7, 178.4, 178.3, 99.6, 97.4,

72.7, 71.4, 69.4, 68.3, 68.2, 68.0, 67.6, 67.3, 63.1, 63.0, 55.4 (2C), 39.0, 38.9, 38.8 (2C), 27.1 (2C), 27.0 (2C).

Phenyl 4,6-*O*-benzylidene-3-*O*-pivaloyl-1-thio-β-D-glucopyranoside (3.19): colorless cryatals; mp 131-132 °C; R_f = 0.51 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$-71.2° (*c* 0.68, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.56-7.33 (10H, m, Ar-*H*), 5.52 (1H, s, PhC*H*), 5.19 (1H, dd, 9.1 and 9.5 Hz, *H*-3), 4.71 (1H, d, 9.8 Hz, *H*-1), 4.40 (1H, dd, 5.0 and 10.6 Hz, *H*-6a), 3.79 (1H, dd, 10.3 and 10.3 Hz, *H*-6b), 3.66 (1H, dd, 9.5 and 9.5 Hz, *H*-4), 3.59-3.54 (2H, m, *H*-2, *H*-5), 2.88 (1H, d, 2.9 Hz, C_2-O*H*), 1.22 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.9, 136.9, 133.0, 131.6, 129.1, 128.9, 128.4, 128.2, 125.9, 101.1, 89.6, 78.2, 75.0, 71.9, 70.7, 68.5, 39.0, 27.1; FAB HRMS calcd for $C_{24}H_{29}O_6S$ ($M+H^+$) 445.1685, found 445.1686. Anal. Calcd for $C_{24}H_{28}O_6S$: C, 64.84; H, 6.35. Found: C, 64.83; H, 6.60.

Phenyl 6-*O*-pivaloyl-1-thio-β-D-glucopyranoside (3.21): colorless foam; R_f = 0.20 (1:3 hexanes/EtOAc); $[\alpha]^{20}_D$-32.5° (*c* 0.75, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$) δ 7.50-7.18 (5H, m, Ar-*H*), 4.58 (1H, d, 9.8 Hz, *H*-1), 4.48 [s (br), H_2O], 4.43 (1H, dd, 1.8 and 12.1 Hz, *H*-6a), 4.14 (1H, dd, 6.9 and 12.1 Hz, *H*-6b), 3.56 (1H, dd, 8.8 and 9.0 Hz, *H*-3), 3.51 (1H, ddd, 1.8, 7.2, and 9.7 Hz, *H*-5), 3.37 (1H, dd, 9.3 and 9.3 Hz, *H*-2), 3.33 (1H, dd, 9.5 and 9.5 Hz, *H*-4), 1.16 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.1, 132.8, 131.8, 128.9, 127.6, 87.6, 77.7, 77.6, 71.9, 70.1, 64.0, 38.8, 27.1; FAB HRMS calcd for $C_{17}H_{25}O_6S$ ($M+H^+$) 357.1372, found 357.1370. Anal. Calcd for $C_{17}H_{24}O_6S{\cdot}H_2O$: C, 54.53; H, 7.00. Found: C,54.89; H, 6.70.

Phenyl 3,6-di-*O*-pivaloyl-1-thio-β-D-glucopyranoside (3.22): colorless cryatals; mp 70-71 °C; R_f = 0.36 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$-34.8° (*c* 0.56, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$) δ 7.59-7.29 (5H, m, Ar-*H*), 4.89 (1H, dd, 9.2 and 9.2 Hz, *H*-3), 4.58 (1H, d, 9.8 Hz, *H*-1), 4.43 (1H, dd, 2.3 and 12.1 Hz, *H*-6a), 4.34 (1H, dd, 5.5 and 12.1 Hz, *H*-6b), 3.59 (1H, ddd, 2.3, 5.5, and 9.8 Hz, *H*-5), 3.46 (1H, dd, 9.4 and 9.4 Hz, *H*-2), 3.44 (1H, dd, 9.5 and 9.5 Hz, *H*-4), 3.02 [1H, s (br), O*H*], 2.58 [1H, s

(br), O*H*], 1.24 [9H, s, COC(C*H*$_3$)$_3$], 1.22 [9H, s, COC(C*H*$_3$)$_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.3, 178.8, 132.8, 131.7, 129.0, 128.2, 88.4, 79.0, 78.5, 70.4, 69.2, 63.4, 39.1, 38.9, 27.2, 27.1; FAB HRMS calcd for $C_{22}H_{33}O_7S$ ($M+H^+$) 441.1947, found 441.1949. Anal. Calcd for $C_{22}H_{32}O_7S \cdot 0.75H_2O$: C, 58.19; H, 7.44. Found: C, 58.31; H, 7.27.

Methyl 2,4-di-*O*-benzyl-α-D-mannopyranoside (3.23):[11a] colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.25 (10H, m, Ar-*H*), 4.91 (1H, d, 11.2 Hz, PhC*H*$_2$), 4.75 (1H, d, 1.7 Hz, *H*-1), 4.72 (1H, d, 11.7 Hz, PhC*H*$_2$), 4.66 (1H, d, 11.2 Hz, PhC*H*$_2$), 4.60 (1H, d, 11.7 Hz, PhC*H*$_2$), 3.98 (1H, dd, 3.9 and 9.0 Hz, *H*-3), 3.86 (1H, dd, 2.9 and 11.7 Hz,*H*-6a), 3.79 (1H, dd, 4.4 and 11.7 Hz,*H*-6b), 3.73 (1H, dd, 1.7 and 3.7 Hz, *H*-2), 3.67 (1H, dd, 9.5 and 9.5 Hz, *H*-4), 3.59 (1H, ddd, 2.9, 4.4, and 9.8 Hz, *H*-5), 3.31 (3H, s, OC*H*$_3$), 2.27 [1H, s (br), C_2-O*H*], 1.93 [1H, s (br), C_6-O*H*]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3, 137.6, 128.6, 128.5, 128.1, 128.0, 127.8, 127.7, 98.1, 78.3, 76.5, 74.9, 73.1, 71.7, 71.1, 62.3, 54.8.

1,3,6-Tri-*O*-acetyl-2,4-di-*O*-benzyl-α-D-mannopyranose (3.24):[11a] colorless syrup; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.36-7.25 (10H, m, Ar-*H*), 6.15 (1H, d, 2.2 Hz, *H*-1), 5.19 (1H, dd, 3.4 and 9.3 Hz, *H*-3), 4.71 (1H, d, 12.2 Hz, PhC*H*$_2$), 4.69 (1H, d, 11.2 Hz, PhC*H*$_2$), 4.57 (1H, d, 11.2 Hz, PhC*H*$_2$), 4.53 (1H, d, 12.2 Hz, PhC*H*$_2$), 4.31 (1H, dd, 2.2 and 12.2 Hz,*H*-6a), 4.27 (1H, dd, 4.4 and 12.2 Hz,*H*-6b), 3.99 (1H, dd, 9.5 and 9.8 Hz, *H*-4), 3.93 (1H, ddd, 2.2, 4.4, and 9.8 Hz, *H*-5), 3.84 (1H, dd, 2.2 and 3.4 Hz, *H*-2), 2.08 (3H, s, COC*H*$_3$), 2.05 (3H, s, COC*H*$_3$), 1.96 (3H, s, COC*H*$_3$).

Phenyl 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (3.25): colorless foam; R_f = 0.42 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+64.9° (*c* 0.50, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.25 (15H, m, Ar-*H*), 5.54 (1H, d, 2.0 Hz *H*-1), 5.20 (1H, dd, 3.3 and 9.4 Hz, *H*-3), 4.71 (1H, d, 11.0 Hz, C*H*$_2$Ph), 4.67 (1H, d, 12.2 Hz, C*H*$_2$Ph), 4.58 (1H, d, 11.2 Hz, C*H*$_2$Ph), 4.48 (1H, d,

12.2 Hz, CH_2Ph), 4.38 (1H, ddd, 3.2, 4.7, and 9.8 Hz, *H*-5), 4.34-4.28 (2H, m, *H*-6a, *H*-6b), 4.11 (1H, dd, 2.0 and 3.2 Hz, *H*-2), 3.98 (1H, dd, 9.5 and 9.5 Hz, *H*-4), 2.02 (3H, s, $COCH_3$), 1.98 (3H, s, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.0, 137.7, 137.4, 133.7, 131.8, 129.0, 128.5, 128.4, 127.9 (2C), 127.8, 127.7, 127.6, 85.1, 76.9, 74.7, 73.8, 73.5, 72.2, 70.6, 63.3, 20.9, 20.8; FAB HRMS calcd for $C_{24}H_{27}O_7$ (MH^+-C_6H_6S) 427.1757, found 427.1756. Anal. Calcd for $C_{30}H_{32}O_7S$: C, 67.14; H, 6.01. Found: C, 66.93; H, 5.91.

3.3 Reference and notes.

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9) For selective pivaloylation of sucrose, see Hough, L.; Chowdhary, M. S.; Richardson, A. C. *J. Chem. Soc., Chem. Comm.*, **1978**, 664.

10) The origin of the selectivity is not clear. Since pivaloyl chloride is relatively bulky, it is likely that the reagent would approach the hydroxyl group from the side and not from the top or bottom of the ring system. The reaction is therefore more subject to steric hindrance of the adjacent equatorial substituents.

11) (a) Ogawa, T.; Katano, K.; Sasajima, K.; Matsui, M.; *Tetrahedron*, **1981**, *37*, 2779. (b) Ogawa, T.; Sasajima, K. *Tetrahedron*, **1981**, *37*, 2787. (c) Merritt, J. R.; Naiseng, E.; Fraser-Reid, B. *J. Org. Chem.*, **1994**, *59*, 4443.

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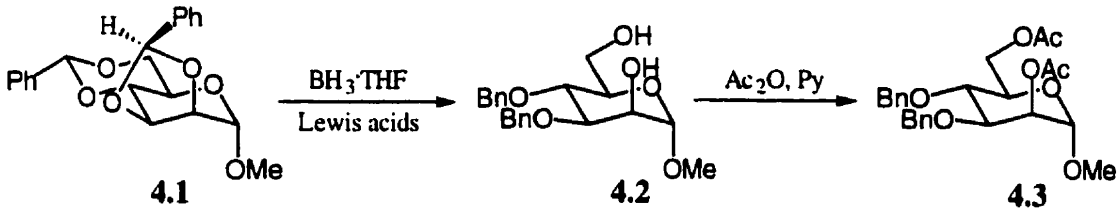
CHAPTER 4

Borane/Bu_2BOTf: A Mild Reagent for the Regioselective Reductive Ring Opening of Benzylidene Acetals in Carbohydrates

4.0 Introduction.

Selective protection of different hydroxyl groups in a carbohydrate molecule is a key step in the chemical synthesis of complex carbohydrates. In this connection, the reductive ring opening of cyclic benzylidene acetals to the corresponding *O*-benzyl ethers, in a regioselective manner, is a useful strategy because of the ease of formation of the acetal as well as the well-established nature of the benzyl ether protection.[1] The regioselectivity in the reductive ring opening of 4,6-*O*-benzylidene acetals of hexopyranosides varies with the reagents and the solvents. For the preparation of the 6-*O*-benzyl derivatives, $NaBH_3CN$-HCl in THF gives the best results.[2] Other reagents such as Et_3SiH-trifluoroacetic acid,[3] $Me_3N{\bullet}BH_3$-$AlCl_3$ in THF[4] and $Me_2NH{\bullet}BH_3$-$BF_3{\bullet}OEt_2$ in CH_3CN[5] are also effective. On the other hand, there is a need for a mild and effective reagent for the regioselective reductive cleavage of 4,6-*O*-benzylidene acetals to the corresponding 4-*O*-benzyl ethers with the 6-hydroxy unsubstituted. The reagents so far reported in the literature for that purpose are either not chemoselective ($LiAlH_4$-$AlCl_3$ in THF),[1] not sufficiently regioselective ($Me_2NH{\bullet}BH_3$-$BF_3{\bullet}OEt_2$ in dichloromethane)[5] or give modest yield of the desired products ($Me_3N{\bullet}BH_3$-$AlCl_3$ in toluene).[4]



Scheme 4.1

Table 4.1. Reductive Opening of Benzylidene Acetal **4.1** with BH_3•THF.

Entry	Lewis Acid	Quantity (equiv.)	Temp. (°C)	Reaction Time (h)	Isolated Yield (%) of **4.3**
1	BF_3•OEt_2	10	25	12	complex mixtures
2	1M Bu_2BOTf in CH_2Cl_2	1	25	1/12	83
3	1M Bu_2BOTf in CH_2Cl_2	0.5	0	1	97
4	2M TMSOTf in CH_2Cl_2	0.5	0	1	85
5	TfOH	0.5	0	1	75

4.1 Results and discussion.

Although it is well known that simple acetals and ketals are reductively cleaved with BH_3•THF alone under mild conditions, benzylidene acetals of hexopyranosides are resistant to it. Using compound **4.1** as the common substrate,[6] we found that BH_3•THF in conjunction with Lewis acid is effective in reductive ring opening of such benzylidene acetals (Scheme 4.1). The regioselectivity and the efficacy of the opening depend on the

type of Lewis acid and the solvent (Table 4.1). As previously observed, BF_3 etherate in THF (entry 1, Table 4.1) gave a complex mixture of products due to non-regioselective opening. Triflic acid (entry 5) gave regioselectively, but with modest yield, the 3,4-di-*O*-benzyl derivative **4.2** which, for ease of characterisation, was converted to the 2,6-di-*O*-

Table 4.2. Reductive Openings of Benzylidene and Isopropylidene Acetals of Hexopyranosides with BH_3·THF and Bu_2BOTf at 0 °C.

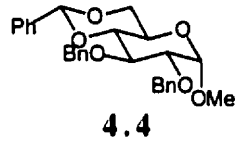
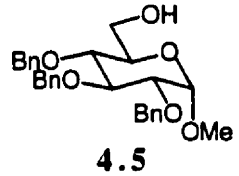
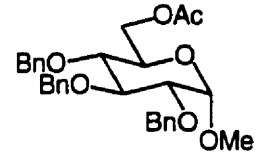
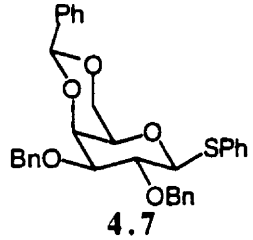
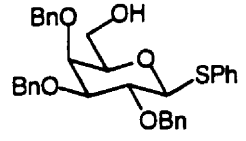
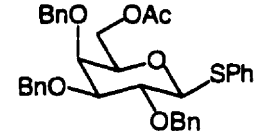
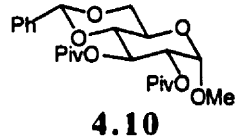
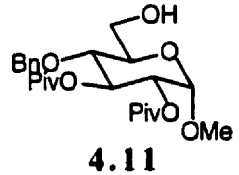
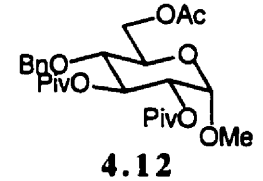
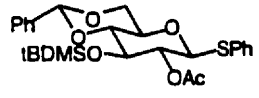
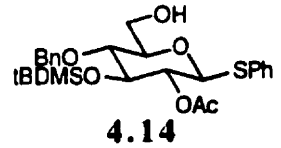
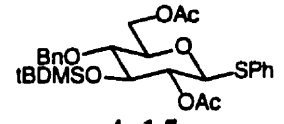
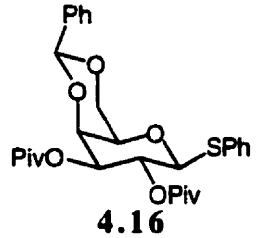
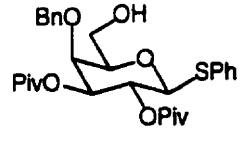
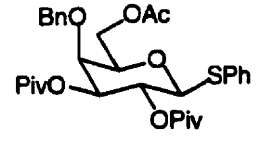
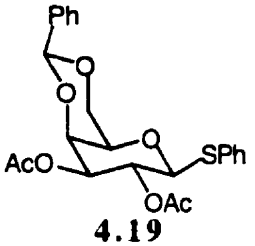
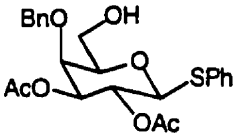
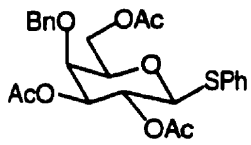
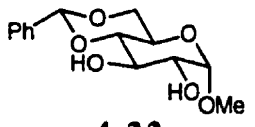
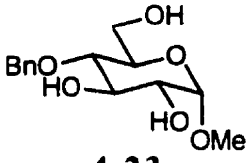
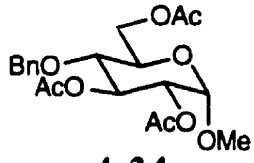
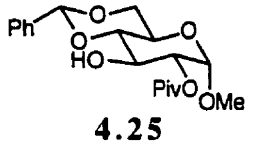
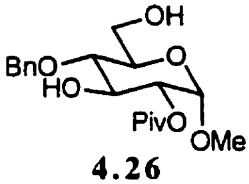
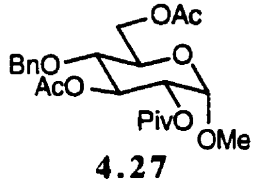
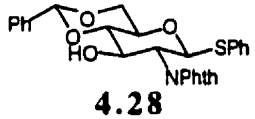
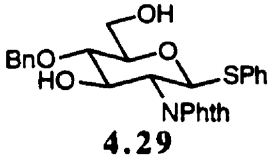
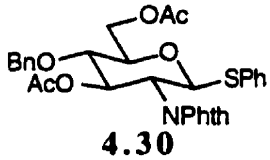
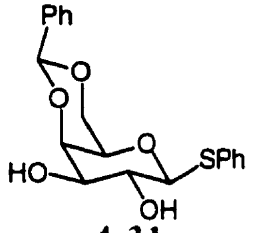
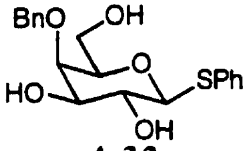
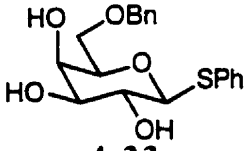
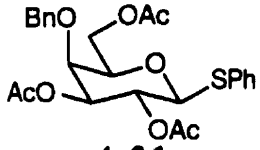
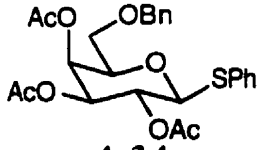
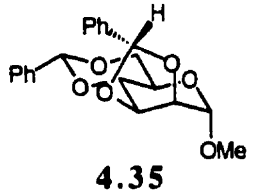
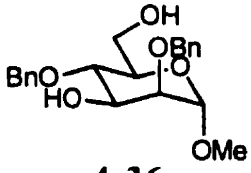
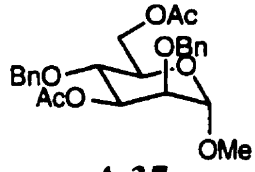
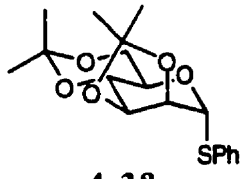
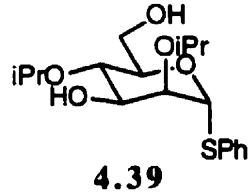
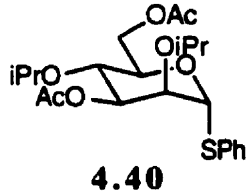
Entry	Substrate	Reduced Product	Acetylated Product	Isolated Yield(%)
1	 **4.4**	 **4.5**	 **4.6**	87
2	 **4.7**	 **4.8**	 **4.9**	95
3	 **4.10**	 **4.11**	 **4.12**	88
4	 **4.13**	 **4.14**	 **4.15**	81
5	 **4.16**	 **4.17**	 **4.18**	90

Table 4.2 (continued)

6	 **4.19**	 **4.20**	 **4.21**	70
7	 **4.22**	 **4.23**	 **4.24**	91
8	 **4.25**	 **4.26**	 **4.27**	86
9	 **4.28**	 **4.29**	 **4.30**	72
10	 **4.31**	 **4.32**  **4.33**	 **4.21**  **4.34**	89 (1:1)
11	 **4.35**	 **4.36**	 **4.37**	88
12	 **4.38**	 **4.39**	 **4.40**	77

acetate **4.3**. Trimethylsilyl triflate (entry 4) was similarly regioselective and gave a better yield of **4.3** (85%). Among the Lewis acids tested by us, dibutylboron triflate gave the best result, with **4.3** as the exclusive product in 97% isolated yield. Since the reagent, 1M dibutylboron triflate in dichloromethane, is commercially available and gave the best result, we have examined further the scope of this Lewis acid in conjunction with BH_3•THF for the reductive ring opening of other cyclic acetals.

It seems that BH_3•THF-Bu_2BOTf is a mild and effective reagent for the regioselective reductive ring opening of 4,6-*O*-benzylidene acetals of hexopyranosides to the corresponding 4-*O*-benzyl ethers with the C_6-OH unsubstituted. Thus, methyl 4,6-*O*-benzylidene-2,3-di-*O*-benzyl-α-D-glucopyranoside (**4.4**) (entry 1, Table 4.2) was converted efficiently to the corresponding 2,3,4-tri-*O*-benzyl derivative **4.5** (again characterized as the acetylated product **4.6**) in 87% isolated yield. Similarly, phenyl 4,6-*O*-benzylidene-2,3-di-*O*-benzyl-1-thio-β-D-galactopyranoside (**4.7**) (entry 2, Table 4.2) was reduced to the 2,3,4-tri-*O*-benzyl compound **4.8**. In this case, the anomeric thiophenyl function was inert to the reductive conditions. Other commonly used protecting groups in carbohydrate chemistry are also inert to this reagent. Methyl 4,6-*O*-benzylidene-2,3-di-*O*-pivaloyl-α-D-glucopyranoside (**4.10**) (entry 3, Table 4.2) was reduced to the 4-*O*-benzyl ether with the pivaloyl protection intact. Acetyl and *t*-butyldimethylsilyl protection are similarly compatible to the reaction conditions (entries 4, 5 and 6). Also, the phthalimide group (entry 9) which may have been sensitive to borane reduction survived the reaction conditions unchanged. Hydrolysis of the benzylidene acetals during reaction sometimes accounted for the low yield of reaction when $NaBH_3CN$-HCl in THF was used. Such undesirable hydrolysis would not occur in the present case since BH_3•THF reacts with water readily. On the other hand, the presence of free hydroxyl groups in the molecule may affect the regioselectivity of the reaction. In the case of 4,6-*O*-benzylidene acetal of glucopyranoside, the presence of one (entry 8 and 9) or even two (entry 7) free hydroxyl groups at the C_2, C_3- positions appeared not to affect the yield or regioselectivity of the

reductive ring opening. For the 4,6-*O*-benzylidene acetal of galactopyranoside, however, the presence of the two hydroxyl groups in **4.31** (entry 10) led to a loss of regioselectivity in the reduction, giving a 1:1 mixture of 4- and 6-*O*-benzyl ethers.

The reagent is also effective in the reductive ring opening of 2,3-*O*-benzylidene dioxolanes of mannosides. However, the regioselectivity of the ring opening depends on the stereochemistry of the phenyl substituent as observed by many others previously.[1] The exo-isomer **4.1** gave regioselectively the 3-*O*-benzyl ether **4.2** (entry 3, Table 4.1) whereas the endo isomer **4.35** gave the 2-*O*-benzyl ether (entry 11, Table 4.2) in 88%. It appears that the dependence of regioselectivity of the ring opening on the stereochemistry of the phenyl substituent is independent of the reducing reagent. We note, however, that when stereochemistry of the dioxolane ring is not at issue as in the case of methyl 2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranoside (**4.38**) (entry 12, Table 4.2), reductive ring opening of the dioxolane occured regioselectively to give the 2-*O*-isopropyl (as well as the 4-*O*-isopropyl) ether.

In conclusion, the BH_3•THF-Bu_2BOTf reductive system regioselectively cleaves benzylidene and isopropylidene acetals rapidly under mild conditions. Benzyl, silyl, ester and phthaloyl protecting groups are compatible with the reaction conditions. This protection protocol should have utility in oligosaccharide synthesis.

4.2 Experimental Section.

General. See Chapter 3, section **3.2.**

General Procedure for the Reduction. A solution of 1M BH_3 in THF (10 mL) was added to a 50 ml dry flask containing 1 mmol of compound **4.1** at 0 °C and the solution was stirred for 5 minutes. A solution of 1M Bu_2BOTf in CH_2Cl_2 (1 mL) was then added to the clear solution slowly. After 1 hour at 0 °C, TLC showed that the starting material had disappeared. Triethylamine (0.5 mL) was then added to the reaction flask

followed by careful addition of methanol until the evolution of H_2 had ceased. The reaction mixture was codistilled with methanol three times before being put on the silica gel column. Elution with 1:1 hexanes and ethyl acetate gave the pure 3,4-dibenzyl derivative **4.2** which was converted to the 2,6-di-*O*-acetate **4.3** by acetic anhydride and pyridine. In the case of the free hydroxy groups present in the substrates, 7 mL of 1M BH_3 in THF was used for every mmol of the substrate. For larger reaction scale (e.g. 10 mmol), the amount of borane required can be reduced to two fold excess.

Methyl 2,6-di-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranoside (4.3): colorless syrup; R_f = 0.27 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+28.5° (*c* 0.49, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.26 (10H, m, Ar-*H*), 5.36 (1H, dd, 1.7 and 3.4 Hz, *H*-2), 4.90 (1H, d, 10.7 Hz, PhCH_2), 4.70 (1H, d, 1.7 Hz *H*-1), 4.69 (1H, d, 11.0 Hz, PhCH_2), 4.55 (1H, d, 10.7 Hz, PhCH_2), 4.52 (1H, d, 11.0 Hz, PhCH_2), 4.37-4.31 (2H, m, *H*-6a, *H*-6b), 3.99 (1H, dd, 3.4 and 9.3 Hz, *H*-3), 3.82 (1H, ddd, 2.7, 4.6, and 10.0 Hz, *H*-5), 3.73 (1H, dd, 9.3 and 9.5 Hz, *H*-4), 3.35 (3H, s, OCH_3), 2.15 (3H, s, COCH_3), 2.07 (3H, s, COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.3, 138.0, 137.7, 128.4 (2C), 128.1, 128.0 (2C), 127.8, 98.7, 78.1, 75.2, 74.0, 71.7, 69.5, 68.4, 63.4, 55.0, 21.1, 20.9.

Methyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (4.6): colorless syrup; R_f = 0.36 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+38.5° (*c* 0.18, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.26 (15H, m, Ar-*H*), 5.00 (1H, d, 10.7 Hz, PhCH_2), 4.87 (1H, d, 11.0 Hz, PhCH_2), 4.82 (1H, d, 12.0 Hz, PhCH_2), 4.80 (1H, d, 12.7 Hz, PhCH_2), 4.66 (1H, d, 12.2 Hz, PhCH_2), 4.59 (1H, d, 3.7 Hz, *H*-1), 4.55 (1H, d, 10.7 Hz, PhCH_2), 4.29-4.22 (2H, m, *H*-6a, *H*-6b), 4.01 (1H, dd, 9.2 and 9.2 Hz, *H*-3), 3.82-3.79 (1H, m, *H*-5), 3.53 (1H, dd, 3.7 and 9.8 Hz, *H*-2), 3.47 (1H, dd, 9.8 and 9.8 Hz, *H*-4), 3.37 (3H, s, OCH_3), 2.02 (3H, s, COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 138.6, 138.0, 137.8, 128.5 (2C), 128.4, 128.1 (2C), 128.0 (2C), 127.9, 127.7, 98.0, 82.0, 79.8, 77.2, 75.8, 75.0, 73.4, 68.5, 63.0, 55.2, 20.8.

Phenyl 6-*O*-acetyl-1-thio-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (4.9): colorless crystals; mp 86-88 °C; R_f = 0.48 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+2.53° (*c* 0.14, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.58-7.22 (20H, m, Ar-*H*), 4.99 (1H, d, 11.7 Hz, PhCH_2), 4.82 (1H, d, 10.0 Hz, PhCH_2), 4.79-4.74 (3H, m, PhCH_2), 4.64 (1H, d, 11.5 Hz, PhCH_2), 4.62 (1H, d, 9.8 Hz *H*-1), 4.26 (1H, dd, 7.1 and 11.4 Hz, *H*-6a), 4.10 (1H, dd, 5.4 and 11.2 Hz, *H*-6b), 3.95 (1H, dd, 9.5 and 9.5 Hz, *H*-2), 3.84 (1H, dd, 1.0 and 2.7 Hz, *H*-4), 3.61-3.58 (2H, m, *H*-3, *H*-5), 1.99 (3H, s, COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 138.3, 138.2, 138.1, 134.0, 131.7, 128.7, 128.5, 128.3 (2C), 128.1 (2C), 127.8, 127.7 (2C), 127.6, 127.2, 87.8, 84.2, 77.3, 76.0, 75.7, 74.3, 73.3, 73.1, 63.4, 20.8.

Methyl 6-*O*-acetyl-4-*O*-benzyl-2,3-di-*O*-pivaloyl-α-D-glucopyranoside (4.12): colorless crystals; mp 88-89 °C; R_f = 0.64 (1:1 hexanes/EtOAc); $[\alpha]^{20}_D$+106.6° (*c* 0.10, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.34-7.24 (5H, m, Ar-*H*), 5.67 (1H, dd, 9.8 and 9.8 Hz, *H*-3), 4.88 (1H, d, 3.7 Hz *H*-1), 4.77 (1H, dd, 3.7 and 10.0 Hz, *H*-2), 4.67 (1H, d, 11.0 Hz, PhCH_2), 4.49 (1H, d, 11.0 Hz, PhCH_2), 4.33 (1H, dd, 2.2 and 12.0 Hz, *H*-6a), 4.23 (1H, dd, 4.6 and 12.0 Hz, *H*-6b), 3.94 (1H, ddd, 2.2, 4.4, and 10.0 Hz *H*-5), 3.63 (1H, dd, 9.8 and 9.8 Hz, *H*-4), 3.36 (3H, s, OCH_3), 2.08 (3H, s, COCH_3), 1.18 [9H, s, COC(CH_3)$_3$], 1.17 [9H, s, COC(CH_3)$_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.9, 176.8, 170.7, 137.3, 128.5, 127.9, 127.6, 96.7, 76.1, 74.2, 71.6, 71.4, 68.1, 62.8, 55.4, 38.8, 30.9, 27.2, 27.0, 20.8.

Phenyl 4-*O*-benzyl-3-*O*-(t-butyldimethyl)silyl-2,6-di-*O*-acetyl-1-thio-β-D-glucopyranoside (4.15): colorless foam; R_f = 0.49 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+10.8° (*c* 0.18, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.48-7.26 (10H, m, Ar-*H*), 4.94 (1H, dd, 9.3 and 9.8 Hz, *H*-2), 4.84 (1H, d, 11.5 Hz, PhCH_2), 4.59 (1H, d, 10.0 Hz *H*-1), 4.52 (1H, d, 11.5 Hz, PhCH_2), 4.39 (1H, dd, 2.2 and 11.7 Hz, *H*-6a), 4.05 (1H, dd, 5.4 and 11.9 Hz, *H*-6b), 3.82 (1H, dd, 8.8 and 8.8 Hz, *H*-3), 3.55 (1H, ddd, 2.2, 5.4, and 9.8 Hz *H*-5), 3.45 (1H, dd, 8.9 and 9.5 Hz, *H*-4), 2.15 (3H, s, COCH_3),

2.03 (3H, s, $COCH_3$), 0.89 [9H, s, $SiC(CH_3)_3$], 0.07 (3H, s, $SiCH_3$), 0.06 (3H, s, $SiCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 169.6, 137.4, 133.5, 131.9, 128.8, 128.4, 127.9, 127.7, 127.6, 86.5, 78.5, 77.0, 76.6, 75.2, 72.4, 63.1, 25.7, 21.5, 20.8, 17.8, -4.0, -4.4.

Phenyl 6-*O*-acetyl-4-*O*-benzyl-2,3-di-*O*-pivaloyl-1-thio-β-D-galactopyranoside (4.18): colorless crystals; mp 113-114 °C; R_f = 0.67 (1:1 hexanes/EtOAc); $[\alpha]^{20}_D$-18.5° (*c* 0.10, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.49-7.23 (10H, m, Ar-*H*), 5.48 (1H, dd, 10.0 and 10.0 Hz, *H*-2), 5.07 (1H, dd, 2.8 and 10.0 Hz, *H*-3), 4.82 (1H, d, 11.2 Hz, $PhCH_2$), 4.68 (1H, d, 10.0 Hz *H*-1), 4.47 (1H, d, 11.2 Hz, $PhCH_2$), 4.28 (1H, dd, 6.6 and 11.2 Hz, *H*-6a), 4.07 (1H, dd, 6.1 and 11.2 Hz, *H*-6b), 3.95 (1H, d, 2.7 Hz, *H*-4), 3.78 (1H, dd, 6.4 and 6.6 Hz *H*-5), 2.01 (3H, s, $COCH_3$), 1.22 [9H, s, $COC(CH_3)_3$], 1.19 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 176.4, 170.4, 137.4, 132.9, 132.3, 128.8, 128.4, 127.9, 127.8, 127.7, 86.8, 75.9, 75.0, 74.9, 74.2, 67.3, 62.6, 38.9, 38.7, 27.2, 27.1, 20.7.

Phenyl 4-*O*-benzyl-1-thio-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (4.21): colorless crystals; mp 76-77 °C; R_f = 0.16 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+23.0° (*c* 0.10, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.51-7.26 (10H, m, Ar-*H*), 5.42 (1H, dd, 9.8 and 10.0 Hz, *H*-2), 5.00 (1H, dd, 2.9 and 9.8 Hz, *H*-3), 4.75 (1H, d, 11.5 Hz, $PhCH_2$), 4.68 (1H, d, 10.0 Hz, *H*-1), 4.53 (1H, d, 11.6 Hz, $PhCH_2$), 4.29 (1H, dd, 6.8 and 11.2 Hz, *H*-6a), 4.07 (1H, dd, 6.1 and 11.2 Hz, *H*-6b), 3.94 (1H, d, 2.9 Hz, *H*-4), 3.75 (1H, dd, 6.2 and 6.6 Hz, *H*-5), 2.08 (3H, s, $COCH_3$), 2.02(3H, s, $COCH_3$), 2.01 (3H, s, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.3, 169.4, 137.4, 132.8, 132.3, 128.8, 128.4, 128.1, 128.0, 127.8, 86.4, 75.9, 75.0, 74.9, 73.6, 67.9, 62.6, 20.9, 20.8, 20.7.

Methyl 4-*O*-benzyl-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside (4.24): colorless syrup; R_f = 0.40 (1:1 hexanes/EtOAc); $[\alpha]^{20}_D$+90.1° (*c* 0.34, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.35-7.25 (5H, m, Ar-*H*), 5.56 (1H, dd, 9.3 and 9.3 Hz, *H*-3),

4.87-4.83 (2H, m, *H*-1, *H*-2), 4.60 (1H, d, 11.2 Hz, PhCH_2), 4.55 (1H, d, 11.2 Hz, PhCH_2), 4.33 (1H, dd, 2.4 and 12.0 Hz, *H*-6a), 4.25 (1H, dd, 4.4 and 12.0 Hz, *H*-6b), 3.91 (1H, ddd, 2.4, 4.4, and 10.0 Hz *H*-5), 3.63 (1H, dd, 9.3 and 9.8 Hz, *H*-4), 3.38 (3H, s, OCH_3), 2.08 (3H, s, COCH_3), 2.07 (3H, s, COCH_3), 1.99 (3H, s, COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.4, 169.8, 137.2, 128.6, 128.1, 128.0, 96.8, 75.8, 74.6, 72.1, 71.2, 68.3, 62.6, 55.2, 20.9, 20.8 (2C).

Methyl 4-*O*-benzyl-3,6-di-*O*-acetyl-2-*O*-pivaloyl-α-D-glucopyranoside (4.27): colorless crystals; mp 64-65 °C; R_f = 0.60 (1:1 hexanes/EtOAc); $[\alpha]^{20}_D$+108.5° (*c* 0.19, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.25 (5H, m, Ar-*H*), 5.62 (1H, dd, 9.8 and 9.8 Hz, *H*-3), 4.88 (1H, d, 3.7 Hz, *H*-1), 4.72 (1H, dd, 3.7 and 10.3 Hz, *H*-2), 4.60 (1H, d, 11.2 Hz, PhCH_2), 4.55 (1H, d, 11.2 Hz, PhCH_2), 4.33 (1H, dd, 2.2 and 12.0 Hz, *H*-6a), 4.25 (1H, dd, 4.4 and 12.0 Hz, *H*-6b), 3.91 (1H, ddd, 2.2, 4.4, and 10.0 Hz *H*-5), 3.61 (1H, dd, 9.5 and 9.8 Hz, *H*-4), 3.37 (3H, s, OCH_3), 2.08 (3H, s, COCH_3), 1.96 (3H, s, COCH_3), 1.17 [9H, s, COC(CH_3)$_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.0, 170.6, 169.6, 137.4, 128.5, 128.1, 128.0, 96.8, 75.8, 74.4, 71.8, 71.2, 68.4, 62.7, 55.4, 38.8, 20.9, 20.8.

Phenyl 4-*O*-benzyl-2-deoxy-2-phthalimido-3,6-di-*O*-acetyl-1-thio-β-D-glucopyranoside (4.30): colorless crystals; mp 115-116 °C; R_f = 0.40 (1:1 hexanes/EtOAc); $[\alpha]^{20}_D$+40.0° (*c* 0.22, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87-7.22 (14H, m, Ar-*H*), 5.81 (1H, dd, 8.9 and 10.1 Hz, *H*-3), 5.73 (1H, d, 10.5 Hz, *H*-1), 4.62 (1H, d, 11.5 Hz, PhCH_2), 4.58 (1H, d, 11.5 Hz, PhCH_2), 4.45 (1H, dd, 2.0 and 12.0 Hz, *H*-6a), 4.25 (1H, dd, 10.4 and 10.4 Hz, *H*-2), 4.24 (1H, dd, 5.1 and 12.0 Hz, *H*-6b), 3.82 (1H, ddd, 2.2, 5.1, and 10.0 Hz *H*-5), 3.69 (1H, dd, 8.9 and 9.9 Hz, *H*-4), 2.08 (3H, s, COCH_3), 1.77 (3H, s, COCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.0, 162.0, 137.3, 134.4, 134.2, 133.1, 131.3, 128.8, 128.6, 128.5, 128.2, 128.1, 127.8, 123.7, 123.6, 82.8, 77.0, 76.3, 74.7, 74.1, 62.9, 54.0, 20.9, 20.5.

Mixture of phenyl 4-*O*-benzyl-1-thio-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (4.21) and Phenyl 6-*O*-benzyl-1-thio-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (4.34): colorless syrup; R_f = 0.17 (2:1 hexanes/EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51-7.26 (20H, m, Ar-*H*), 5.49 (1H, d, 3.2 Hz), 5.42 (1H, dd, 10.0 and 10.0 Hz), 5.24 (1H, dd, 9.8 and 10.0 Hz), 5.05 (1H, dd, 3.3 and 9.9 Hz), 5.00 (1H, dd, 2.8 and 9.9 Hz), 4.74 (1H, d, 11.7 Hz), 4.73 (1H, d, 10.0 Hz), 4.67 (1H, d, 10.0 Hz), 4.54 (1H, d, 11.7 Hz), 4.53 (1H, d, 11.7 Hz), 4.42 (1H, d, 12.0 Hz), 4.29 (1H, dd, 6.8 and 11.2 Hz), 4.07 (1H, dd, 6.1 and 11.2 Hz), 3.94 (1H, d, 2.7 Hz), 3.90 (1H, dd, 6.2 and 6.2 Hz), 3.75 (1H, dd, 6.3 and 6.3 Hz), 3.60 (1H, dd, 6.1 and 6.3 Hz), 3.50 (1H, dd, 6.4 and 6.6 Hz), 2.08 (6H, s, $COCH_3$), 2.04(3H, s, $COCH_3$), 2.02(3H, s, $COCH_3$), 2.00 (3H, s, $COCH_3$), 1.97 (3H, s, $COCH_3$),; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.3, 170.2, 170.0, 169.5, 169.4, 137.5, 137.4, 132.9, 132.8, 132.3, 132.1, 128.9, 128.8, 128.4 (2C), 128.1, 128.0, 127.9 (2C), 127.8 (2C), 86.7, 86.4, 76.0, 75.9, 75.0, 74.9, 73.6, 73.5, 72.2, 67.9, 67.7 (2C), 67.5, 62.6, 20.9 (2C), 20.8 (2C), 20.7, 20.6.

Methyl 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (4.38): colorless syrup; R_f = 0.32 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+11.3° (*c* 0.25, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.26 (10H, m, Ar-*H*), 5.22 (1H, dd, 3.4 and 9.3 Hz, *H*-3), 4.73 (1H, d, 2.0 Hz *H*-1), 4.69 (1H, d, 11.2 Hz, $PhCH_2$), 4.66 (1H, d, 12.2 Hz, $PhCH_2$), 4.58 (1H, d, 11.2 Hz, $PhCH_2$), 4.56 (1H, d, 12.2 Hz, $PhCH_2$), 4.35 (1H, dd, 2.3 and 11.8 Hz, *H*-6a), 4.30 (1H, dd, 4.9 and 12.0 Hz, *H*-6b), 3.93 (1H, dd, 9.5 and 9.8 Hz, *H*-4), 3.86 (1H, dd, 2.0 and 3.4 Hz, *H*-2), 3.84 (1H, ddd, 2.3, 4.9, and 9.9 Hz, *H*-5), 3.36 (3H, s, OCH_3), 2.08 (3H, s, $COCH_3$), 1.98 (3H, s, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.1, 137.8 (2C), 128.5, 128.4, 127.8, 127.7 (3C), 98.7, 75.7, 74.7, 73.9, 73.2, 72.9, 69.6, 63.4, 54.9, 21.1, 20.9.

Phenyl 3,6-di-*O*-acetyl-2,4-di-*O*-isopropyl-1-thio-α-D-mannopyranoside (4.41): colorless crystals; mp 72-73 °C; R_f = 0.51 (2:1

hexanes/EtOAc); $[\alpha]^{20}_{D}$+122.3° (*c* 0.69, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.51-7.25 (5H, m, Ar-*H*), 5.43 (1H, d, 2.0 Hz *H*-1), 5.03 (1H, dd, 3.4 and 9.3 Hz, *H*-3), 4.35-4.24 (3H, m, *H*-5, *H*-6a, *H*-6b), 4.02 (1H, dd, 2.0 and 3.2 Hz, *H*-2), 3.78 (1H, dd, 9.3 and 9.6 Hz, *H*-4), 3.76 [1H, heptet, 6.1 Hz, $OCH(CH_3)_2$], 3.62 [1H, heptet, 6.1 Hz, $OCH(CH_3)_2$], 2.11 (3H, s, $COCH_3$), 2.05 (3H, s, $COCH_3$), 1.16 [3H, d, 6.1 Hz, $CH(CH_3)_2$], 1.15 [3H, d, 6.1 Hz, $CH(CH_3)_2$], 1.13 [3H, d, 6.1 Hz, $CH(CH_3)_2$], 1.12 [3H, d, 6.1 Hz, $CH(CH_3)_2$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.1, 134.1, 131.7, 128.9, 127.4, 86.5, 77.2, 75.3, 74.0, 73.4, 72.1, 71.3, 63.4, 23.0, 22.9, 22.3, 21.8, 21.0, 20.9.

4.3 Reference and notes.

1) (a) Bhattacharjee, S. S.; Gorin, P. A. *Can. J. Chem.* **1969**, *47*, 1195; (b) Liptak, A.; Imre, J.; Harangi, J.; Nanasi, P. *Tetrahedron* **1982**, *38*, 3721; (c) Gejas, J. *Adv. Carbohydr. Chem. Biochem.* **1981**, *39*, 71; (d) Garegg, P. J. *Pure App. Chem.* **1984**, *56*, 845; (e) Johansson, R.; Samuelsson, B. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2371.

2) Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97.

3) DeNinno, M. P.; Etienne, J. B.; Duplantier, K. C. *Tetrahedron Lett.* **1995**, *36*, 669.

4) Ek, M.; Garegg, P. J.; Hultberg, H.; Oscarson, S. *J. Carbohydr. Chem.* **1983**, *2*, 305.

5) Okawa, M.; Liu, W. C.; Nakai, Y.; Koshida, S.; Fukase, K.; Kusumoto, S. *Synlett.* **1996**, 1179.

6) Benzylidene and isopropylidene acetals were prepared according to the procedures described in: Ferro, V.; Mocerino, M.; Stick, R. V.; Tilbrook, M. G. *Aust. J. Chem.* **1988**, *41*, 813.

CHAPTER 5

Oligomer-supported Solution Synthesis of the Mannononaose Residue of High Mannose Glycoproteins

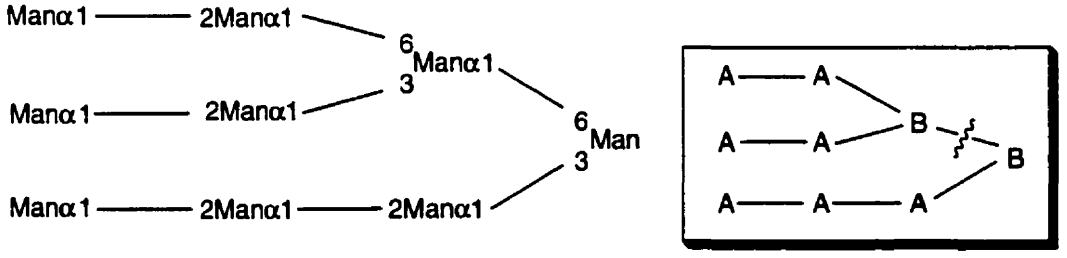
5.0 Introduction.

The branched mannononaose **5.1**, Man_9, plays an important role in the biosynthesis and processing of N-linked glycoproteins. Through the dolichol pathway, the oligosaccharide core with the composition $GlcNAc_2Man_9Glc_3$ is transferred to a polypeptide chain. The glucose and some of the mannose residues are then trimmed and other carbohydrate residues are added by various processing enzymes to give diverse N-linked glycoproteins.[1] The completed glycoproteins are targeted for secretion, incorporation into cellular organelles, or insertion into cell membranes. For membrane-bound glycoproteins, the carbohydrate moieties have been implicated in many cell-cell recognition processes such as adhesion and infection.[2] In this connection, it is interesting to note that mannononaose **5.1** is present on the conserved V3 loop of glycoprotein (gp) 120 of the viral coat of HIV-1.[3] Gp120 plays an important role in the HIV infection of cells, being responsible for the attachment and penetration of cells to be infected, and is thus a target for immunotherapy or vaccine development. Indeed, polyclonal antibodies directed against the mannose residues of HIV-1 glycoprotein Gp120 *in vitro* have been developed.[4]

For these considerations, mannononaose **5.1** presents itself as an attractive synthetic target. So far, only the syntheses of a highly protected mannononaose,[5] its methyl and 8-(methoxycarbonyl)octyl derivatives[6] have been reported and are based on the more classical solution synthesis. Both Fraser-Reid's and Ley's groups used a highly convergent block synthesis approach (Scheme 5.1). In Fraser-Reid's synthesis,[5] although only two n-

pentenyl glycosyl monosaccharide donors were needed for construction of the highly protected mannononaose, there were many protection and deprotection manipulations on the building blocks' double bonds, involved in the synthesis. On the other hand, in Ley's synthesis,[6] although the number of steps to prepare the protected mannononaose from the monosaccharide building blocks could be reduced to five with the help of so-called one-pot procedures, six monosaccharide glycosyl donors with carefully tuned reactivities were necessary for the chemoselective synthesis of the target nonasaccharides. Even though both groups employed very different, elegant protection and glycosylation methodologies, the key assembly reactions in their syntheses were very similar in the sense that the glycosylations were between a pentasaccharide donor and a tetrasaccharide acceptor. Furthermore, both syntheses are quite labor-intensive.

It is generally recognized that the synthesis of complex oligosaccharides is a matter of considerable challenge, and a subject of extensive contemporary research attention. Some of the current methods developed for the synthesis of oligosaccharides have involved the use of solid-phase[7] or high molecular weight polymer-supported solution phase synthesis.[8] The mannononaose residue **5.1** is sufficiently complicated to properly test some of these strategies for reaching complex branched oligosaccharides.

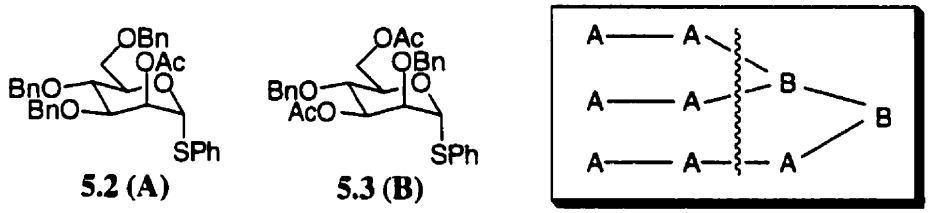


5.1

Scheme 5.1 General strategy of the convergent block synthesis of a complex branched mannononaose.

5.1 Results and discussion

Our synthetic plan for **5.1** is based on the use of low molecular weight poly(ethylene glycol) methyl ether (MPEG, average M_w550, n = *ca.* 8-20) linkers for oligomer-supported solution synthesis of oligosaccharides.[9] The use of low molecular weight MPEG as the oligomer support retains the normal advantages of polymer-supported solution synthesis of oligosaccharides, such as: (1) the MPEG-linker and its supported synthons are completely soluble in the normal reaction solvents and the efficiency of the various steps follows that expected of solution chemistry; and (2) purification of the MPEG-linker and its supported synthons by fast column chromatography on silica gel 60 (230-400 mesh, E. Merck) is simplified by the fact that even in neat ethyl acetate, the MPEG derivatives remain on the baseline while the by-products move rapidly through the column. Changing the eluent to dichloromethane-methanol (4:1) or to ethyl acetate-methanol (4:1) allows quick elution of the MPEG derivatives. Another advantage of using low molecular weight MPEG is that the reaction can be monitored readily by the conventional arsenal of spectroscopic techniques.



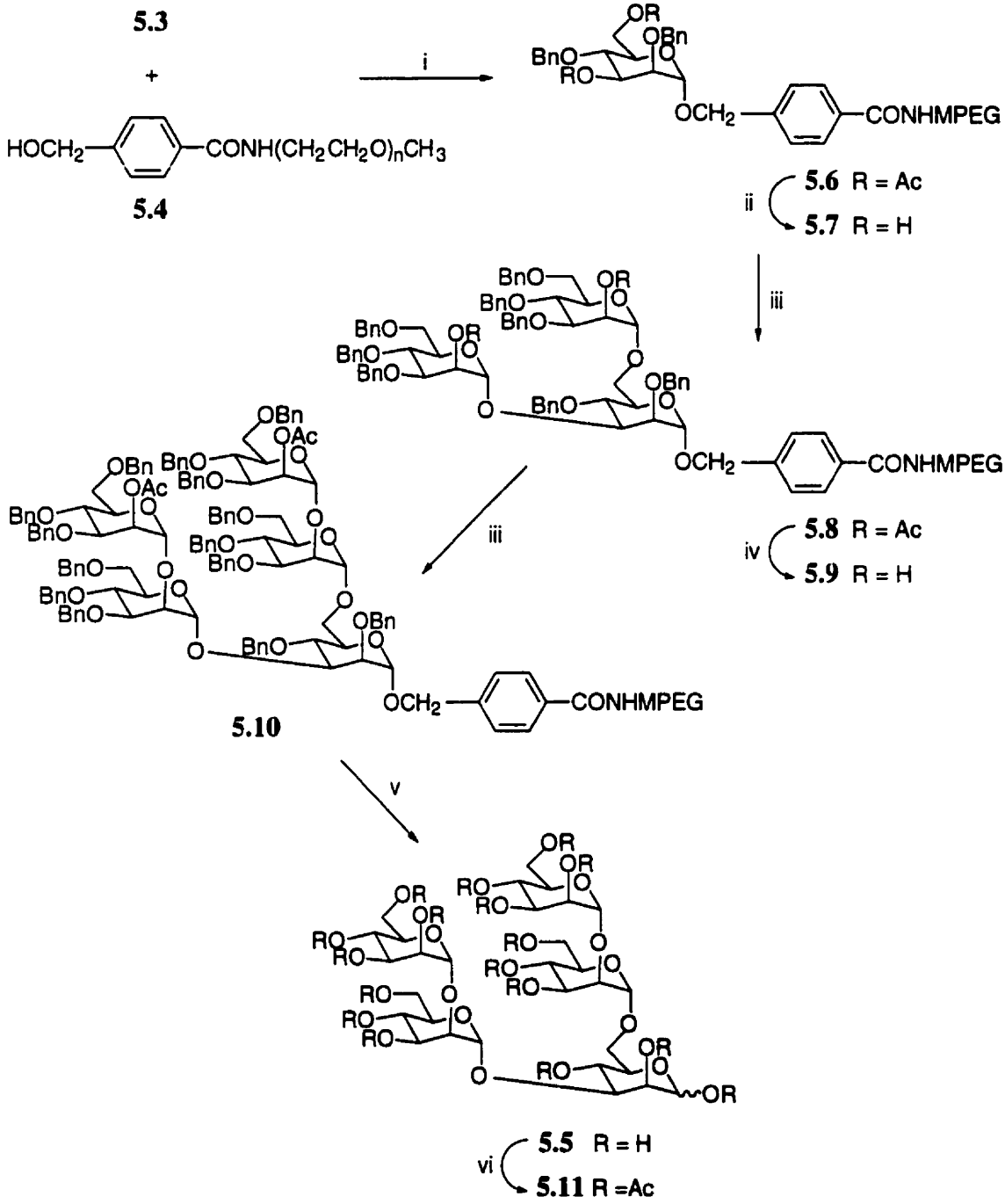
Scheme 5.2 Building blocks A and B for the synthesis of mannononaose **5.1**.

Now that trichloroacetimidate glycosyl donors are not very stable for storage at room temperature and quite sensitive to the heat generated during the removal of water by co-distillation with toluene,[9] it was determined to use thioglycosly donors, which are also easier to prepare in most cases, for the construction of the nonasaccharide **5.1**. Since the phenylthio moiety may easily be installed early in the glycosyl donor and can survive many types of protecting group manipulations, phenyl thiomannopyranosides **5.2**[10] and **5.3**[11]

were chosen as our only two monosaccharide building blocks (Scheme 5.2). It is our anticipation that *once the trisaccharide core is reached, only two more triple glycosylations with donor **5.2** are needed for the construction of mannononaose **5.1*** and therefore, it will greatly simplify the synthesis of **5.1** and save much of the effort on the bench.

In order to test these two glycosyl donors (**5.2** and **5.3**) on the polyethylene glycol linker **5.4** and the double glycosylation for the expected triple glycosylation, mannopentaose **5.5** was prepared according to Scheme 5.3. Oligomer **5.4** was glycosylated with phenyl 3,6-di-*O*-acetyl-2,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (**5.3**) using the combination of N-iodosuccinimide and trimethylsilyl triflate (NIS/Me_3SiOTf) as the coupling promoter.[12] The coupling reaction went smoothly and gave the MPEG-monosaccharide **5.6** in almost quantitative yield. Purification of the product employing flash column chromatography was greatly facilitated by the use of low molecular weight poly(ethylene glycol) methyl ether as the oligomer support as the excess monomeric reagents can be initially readily removed from the column. The two acetyl groups of **5.6** were readily removed by base treatment to yield diol **5.7** and the double glycosylation of **5.7** with 3.0 equivalents of donor **5.2** was carried out with NIS/TMSOTf as the promoter to afford the MPEG-trisaccharides **5.8**. The amount of the Lewis acid (TMSOTf) used in the glycosylation was quite critical, such that less than 0.8 equivalent of TMSOTf employed in the coupling reaction would lead to the formation of 1,2-orthoacetates. The success of the double glycosylation was evidenced by the fact that the ^1H NMR spectrum showed chemical shifts of 5.19 and 4.97 ppm for the two new anomeric protons, 5.49 and 5.47 ppm for the two new C-2 protons as well as 2.14 and 2.08 for the two acetate protons. The chemical shifts of the two new anomeric protons and new C-2 protons were derived from the ^1H-^1H COSY NMR spectrum. Incorporation of the two new mannopyranoside units was also confirmed by the ^1H-^{13}C HMQC NMR spectrum revealing the existence of three anomeric cross peaks. Repetition of the deacetylation step

and double glycosylation with glycosyl donor **5.2** gave the protected MPEG-pentasaccharide **5.10** through the formation of MPEG-trisaccharide **5.9**.



Scheme 5.3 *Reagents and conditions*: (i) 2.0 equiv. of thiomannoside **5.3**, 2.2 equiv. of NIS, 0.8 equiv. of TMSOTf, 4 Å molecular sieves, CH_2Cl_2, 0 °C to rt, 4 h; (ii) K_2CO_3, wet MeOH, 4 h; (iii) 3.0 equiv. of thiomannoside **5.2**, 3.3 equiv. of NIS, 0.8 equiv. of TMSOTf, 4 Å molecular sieves, CH_2Cl_2, 0

°C to rt, 4 h; (iv) NaOMe, MeOH, CH_2Cl_2, 12 h; (v) 50 psi H_2, 10 % Pd-C, CH_3COOH, 5 days; (vi) Ac_2O, Py, DMAP, 48 h.

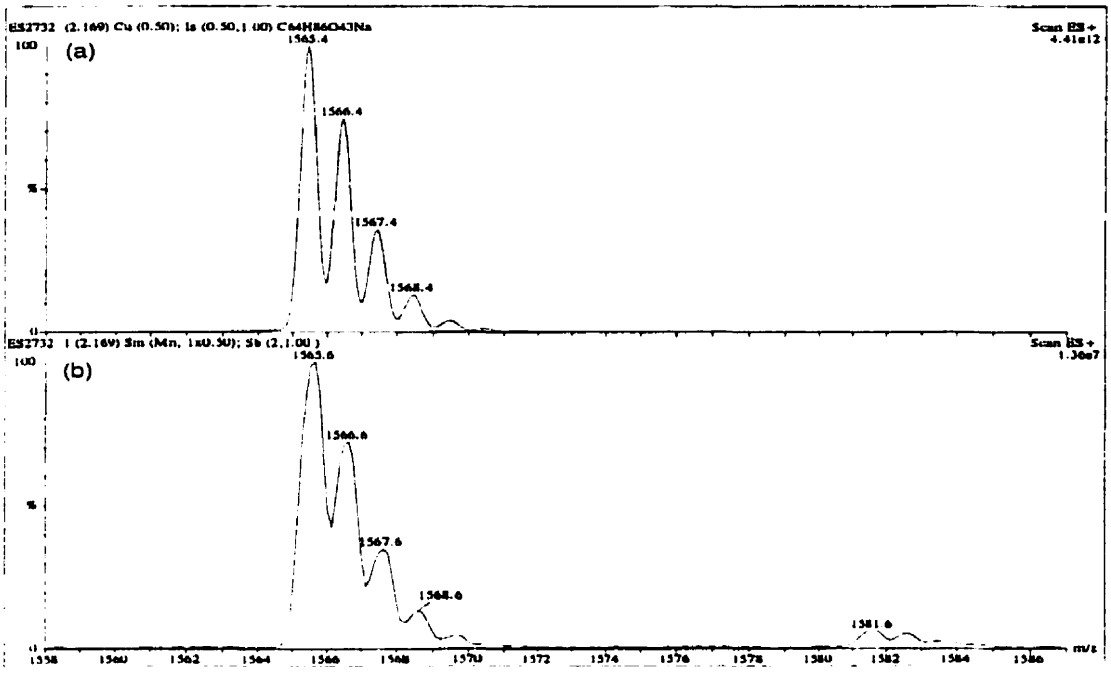


Figure 5.1 (a) Theoretical spectrum created by isotopic modeling for a sodium adduct of **5.11**; (b) experimental spectrum of **5.11**.

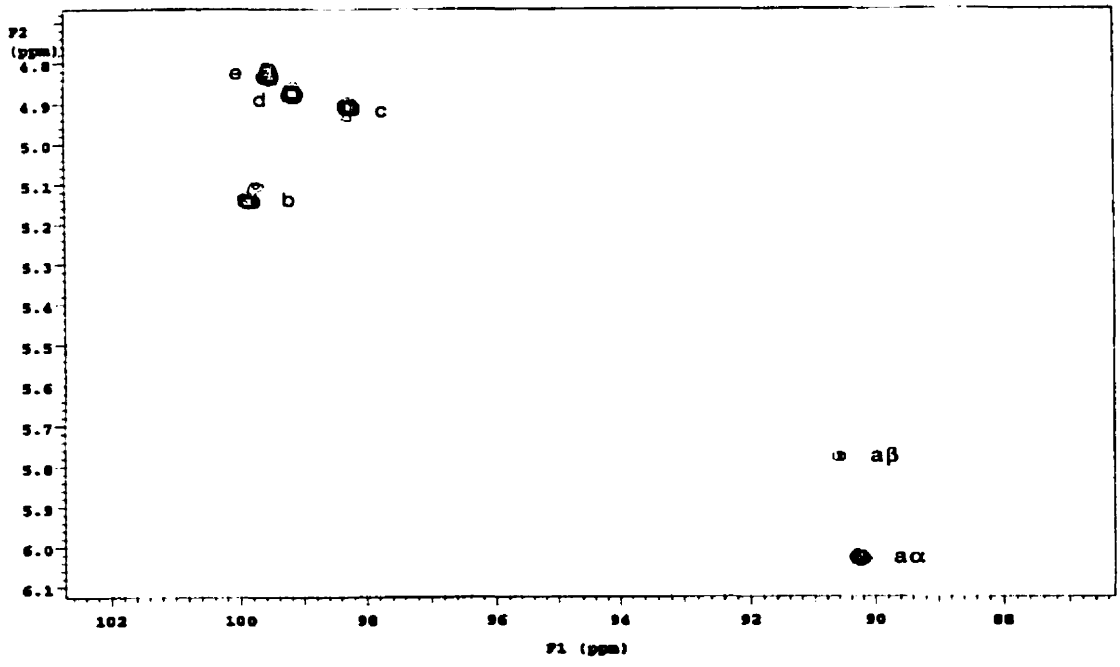


Figure 5.2 Anomeric region of the ^1H-^{13}C HMQC NMR spectrum of **5.11**.

Progress of the assembly was readily monitored by ^{1}H NMR spectroscopy, and the chemical shifts of protons and carbons can be assigned by 2D ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC NMR spectroscopies even though the proton peaks were broadened significantly along with the incorporation of more carbohydrate units. Hydrogenolysis of **5.10** over Pd-C in acetic acid released the pentasaccharide **5.5** from the oligomer smoothly. Purification and characterization of **5.5** were achieved by conversion to the peracetyl derivative **5.11** (as a mixture of α- and β-anomers). The overall yield of the peracetylpentamannopyranoside **5.11** was found to be 19% based on the starting polymer **5.4**. Compound **5.11** was characterized by electrospray mass spectrometry (Figure 5.1), ^{1}H NMR and ^{13}C NMR. As showed in Figure 5.1, the isotopic abundances at all ions in the ($M+Na^+$) cluster closely match the theoretically predicted. The ^{1}H-^{13}C HMQC NMR spectrum of **5.11** is also in full agreement with the structure, displaying the presence of five anomeric centers' cross peaks (Figure 5.2).

Having established the feasibility and efficiency of the solution phase chemistry, attention was then turned to the construction of the complex mannononaose residue **5.1** (Scheme 5.4). The primary hydroxyl group in the MPEG-monosaccharide diol **5.7** was selectively protected by the *tert*-butyldiphenylsilyl (TBDPS) group and then the partially protected **5.12** was glycosylated with phenyl 2-*O*-acetyl-1-thio-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**5.2**) to yield the MPEG-disaccharide **5.13**. Removal of the silicon protecting group gave the oligomer-bound glycosyl acceptor **5.14** which was glycosylated with donor **5.3** to form MPEG-trisaccharide **5.15**. To avoid using TBAF whose ammonium cation may cause problems during chromatographic separation, dilute HF was employed in the desilylation, which proceeded very slowly. The standard deacetylation went readily during the course of the synthesis. As anticipated, the triple glycosylation of MPEG-trisaccharide triol **5.16** with 4.5 equivalent of thiomannoside **5.2** went smoothly to furnish the MPEG-bound mannohexaose **5.17** whose ^{1}H NMR spectrum exhibited the chemical shifts of 2.11, 2.09 and 2.02 ppm for the three acetate protons as well as 5.49

ppm for the three new C-2 protons. The ^{1}H-^{13}C HMQC NMR spectrum of **5.17** also revealed the presence of six anomeric cross peaks. Reiteration of the deacetylation and the triple glycosylation steps gave the protected MPEG-nonasaccharide **5.18**. It is worth of mentioning that the MPEG-bound mannohexaoses and mannononaose can be more easily washed out from a silica gel column than the MPEG-bound lower mannans, probably due to the fact that hydrophobic portions of the MPEG-bound higher mannans affect the affinity of the MPEG-mannan conjugates toward silica gel more.

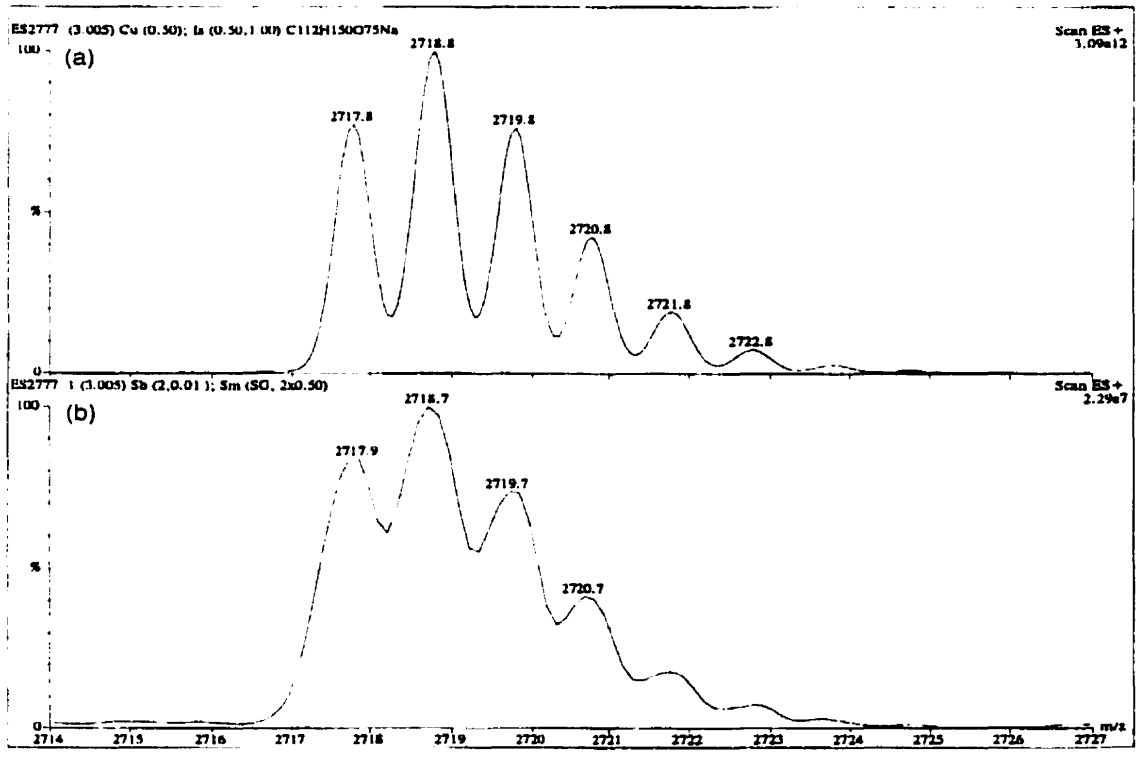


Figure 5.3 (a) Theoretical spectrum created by isotopic modeling for a sodium adduct of **5.19**; (b) experimental spectrum of **5.19**.

However, the final debenzylation turned out to be troublesome, in that hydrogenolysis over Pd/C in various media did not proceed even after prolonged exposure. Eventually, hydrogenolysis of **5.18** over Pearlman's catalyst in methanol under high

pressure (1000 psi) for seven days released the nonasaccharide **5.1** from the polymer. Acetylation of **5.1** furnished a mixture of fully acetylated α- and β-nonasaccharides (**5.19**, *ca.* 12% overall yield from **5.4**), whose electrospray mass spectrum was consistent with the expected structure. The experimental spectrum matches the theoretical spectrum created by isotopic modeling (Figure 5.3). Furthermore, the ^{1}H-^{13}C HMQC experiment also provided confirmation of the proposed structure of **5.19** by revealing the presence of nine anomeric centers' cross peaks. It should be noted that besides the advantages mentioned above, this poly(ethylene glycol) oligomer-supported oligosaccharide synthesis methodology also gives access to large scale synthesis. In this case, over 300 mg of final product **5.19** was obtained from only two easily prepared monosaccharide building blocks, **5.2** and **5.3**.

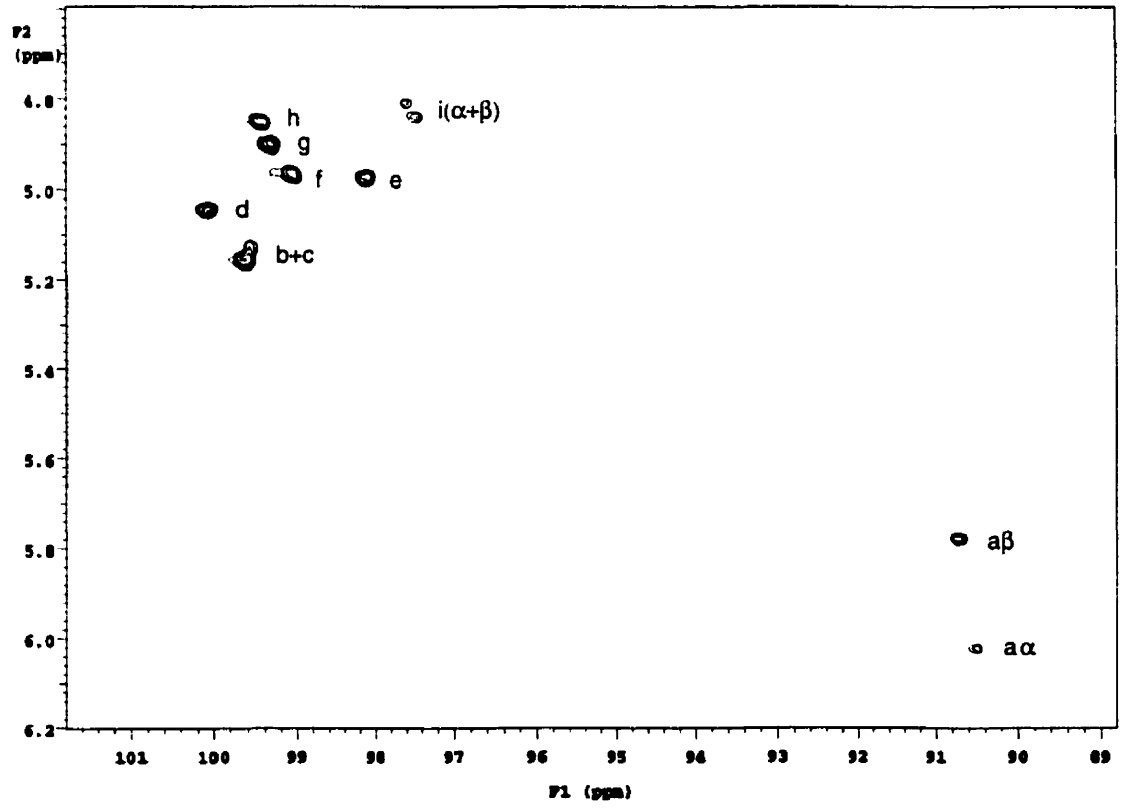
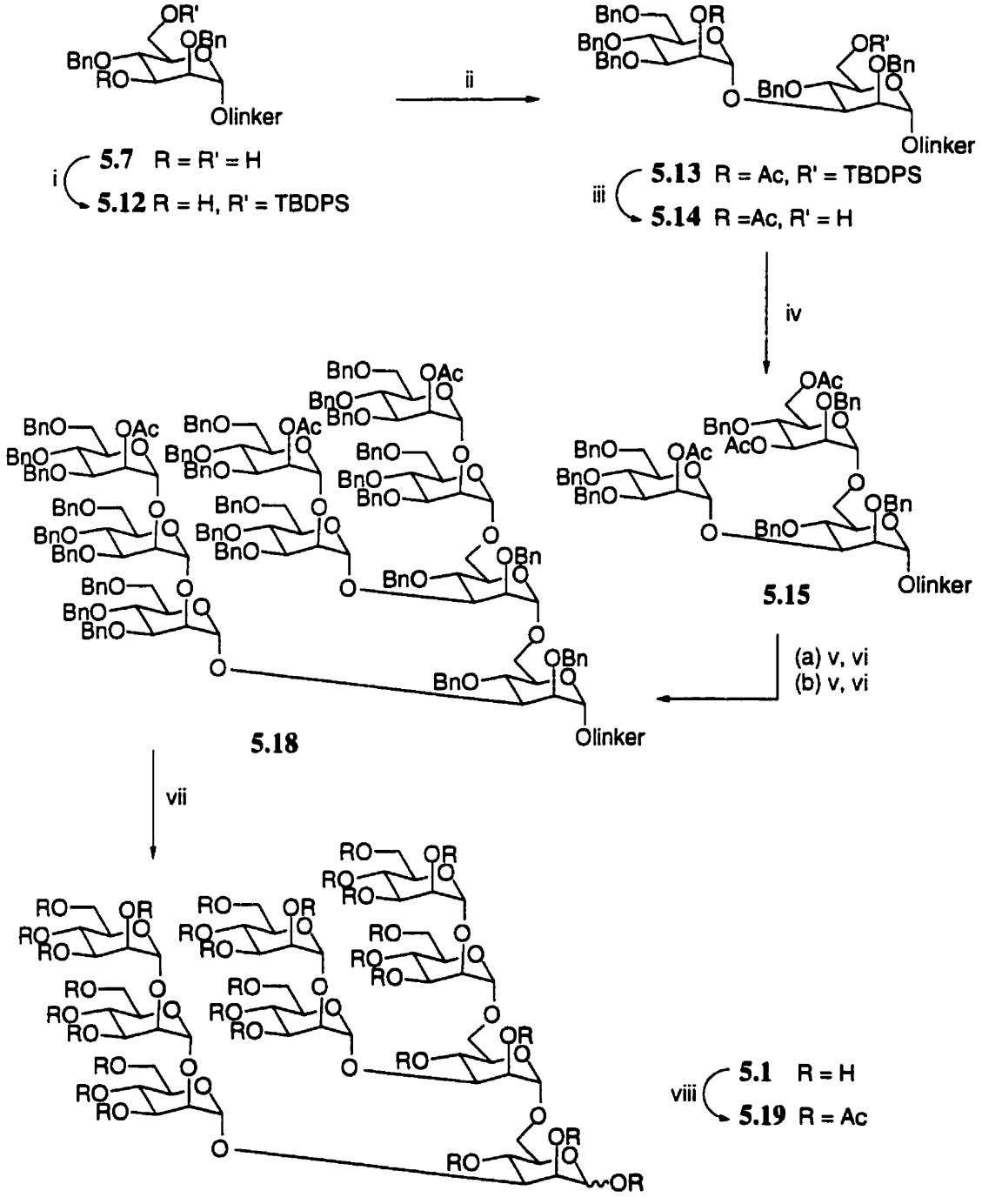


Figure 5.4 Anomeric region of the ^{1}H-^{13}C HMQC NMR spectrum of **5.19**.



Scheme 5.4 *Reagents and conditions*: (i) 2.0 equiv. of *t*-$BuPh_2SiCl$, 2.0 equiv. of DMAP, CH_2Cl_2, rt, 24 h; (ii) 2.0 equiv. of thiomannoside **5.2**, 2.2 equiv. of NIS, 0.8 equiv. of TMSOTf, 4 Å molecular sieves, CH_2Cl_2, 0 °C to rt, 4 h; (iii) 5% HF in MeOH, 3 days; (iv) 2.0 equiv. of thiomannoside **5.3**, 2.2 equiv. of NIS, 0.8 equiv. of TMSOTf, 4 Å molecular sieves, CH_2Cl_2, 0 °C to rt, 4 h; (v) NaOMe, MeOH, CH_2Cl_2, 12 h; (vi) 4.5 equiv. of thiomannoside **5.2**, 4.8 equiv. of NIS, 0.8 equiv. of TMSOTf, 4 Å molecular sieves, CH_2Cl_2, 0 °C to rt, 4 h; (vii) 1000 psi H_2, 20% $Pd(OH)_2$-C, MeOH, 7 days; (viii) Ac_2O, Py, DMAP, 48 h.

In summary, a concise synthesis of the mannan residue of a highly branched mannose type oligosaccharide present on the viral coat of HIV-1 from only two monosaccharide building blocks (**5.2** and **5.3**) has been achieved. The use of low molecular weight MPEG aided considerably in the purification of the intermediates and in the analyses of the progress of the assembly by using conventional spectroscopic techniques. This synthesis demonstrates that oligomer-supported solution synthesis using a low molecular weight polyethylene glycol is a powerful tool for the rapid and efficient preparation of complex oligosaccharides. The ability to introduce two or three saccharide units in one operation is particularly remarkable.

5.2 Experimental Section.

General. See Chapter 3, section **3.2**.

Protocol for the Solution-phase Glycosylations. After thiomannoside **5.2** (or **5.3**) (2.0 mmol) and a MPEG-acceptor (1 mmol) were co-distillated with toluene 5 times under reduced pressure, dry 4 Å molecular sieve powder (1 g/g reactant) and dichloromethane (2.5 mL/g reactant) were added into the flask. The mixture was stirred for 10-15 minutes under Ar and then cooled to 0 °C, followed by the addition of NIS. The color of the reaction mixture turned pink immediately as 2M solution of TMSOTf (0.8 mmol) in dichloromethane was syringed into the flask slowly. Upon removal of the ice bath right after completion of the addition, the reaction mixture was allowed to warm up to room temperature slowly and kept for 4 hours. Chloroform and an aqueous $NaHCO_3$ solution were added to quench the reaction, followed by filtration of the mixture through a pad of celite to remove the molecular sieve powder. After having been washed with an aqueous $Na_2S_2O_3$ solution and brine, the organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The resulting syrup was dissolved in a small amount of ethyl acetate and put onto the top of a silica gel column (15 times weight of

the syrup) for flash column chromatography. Gradient elution with hexanes/ethyl acetate (2:1, 1:1, 1:2, 1:4 and neat EtOAc, 50 mL each) removed the side-products and further elution with ethyl acetate/methanol (4:1 and 3:1, 150 mL each) gave the MPEG-sugar conjugate.

Protocol for the Deacetylation with K_2CO_3. Potassium carbonate was added to a stirred solution of MPEG-sugar in wet methanol and the reaction was monitored by ^1H NMR spectroscopy. Upon completion of the deacetylation indicated by the disappearance of the acetyl peak (or peaks) in the ^1H NMR spectrum, the methanol was evaporated under reduced pressure and brine was added. After the aqueous solution was extracted with chloroform (3*50 mL), the organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. Purification of the MPEG-bound products by chromatographic separation procedure was the same as described above.

Protocol for the Deacetylation with NaOMe. The procedure was essentially the same as above, except that sodium methoxide was used as the base and methanol/dichloromethane (1:2) was the solvent.

Protocol for the Debenzylation of MPEG-mannopentaose 5.8. A mixture of **5.8** and 10% Pd-C in acetic acid was stirred under 50 psi H_2 for 5 days at room temperature. The solution was filtered and the Pd-C washed with methanol. After the filtrate was evaporated under reduced pressure to afford a syrup, acetic anhydride, pyridine and a catalytic amount of DMAP were added. The mixture was stirred at room temperature for two days and usual work-up gave a glassy solid, which was subject to flash column chromatography over silica gel (40 g, EtOAc/Hexanes 2:1) to afford the peracetylated mannopentaose **5.9** (a mixture of α- and β-anomers) in 18.5% yield (285 mg).

Protocol for the Debenzylation of MPEG-mannononaose 5.14. A mixture of **5.14** and 20% $Pd(OH)_2$-C (Pearlman's catalyst) in methanol was stirred under 1000 psi H_2 for 7 days at room temperature. The usual work-up and acetylation sequence gave a glassy solid, which was subject to dry flash column chromatography over TLC

standard grade silica gel (Ave. particle size 2-25 μ, 25 g, EtOAc) to afford the peracetylated mannononaose **5.19** (a mixture of α- and β-anomers) in 12% yield (322 mg).

MPEG-Man36OAc (5.6): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (2H, d, 7.1 Hz, Ar-*H*), 7.57-7.27 (12H, m, Ar-*H*), 6.78 [1H, s (br), PhCON*H*], 5.26 (1H, dd, 3.4 and 9.0 Hz, *H*-3), 4.86 (1H, d, 2.0 Hz, *H*-1), 4.71 (1H, d, 12.4 Hz, PhCH_2), 4.67 (1H, d, 11.2 Hz, PhCH_2), 4.60 (1H, d, 12.2 Hz, PhCH_2), 4.55 (1H, d, 11.2 Hz, PhCH_2), 4.52 (1H, d, 12.2 Hz, PhCH_2), 4.51 (1H, d, 12.4 Hz, PhCH_2), 4.29 (2H, m, *H*-6a, *H*-6b), 3.93 (1H, dd, 9.3 and 9.8 Hz, *H*-4), 3.89 (1H, dd, 2.0 and 3.4 Hz, *H*-2), 3.70-3.46 (m, *H*-5, *H*-6, and PEG), 3.35 (1H, s, OCH_3), 2.06 (3H, s, CH_3CO), 1.97 (3H, s, CH_3CO).

MPEG-Man36OH (5.7): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (2H, d, 8.3 Hz, Ar-*H*), 7.36-7.30 (12H, m, Ar-*H*), 6.84 [1H, s (br), PhCON*H*], 4.91 (1H, d, 1.0 Hz, *H*-1), 4.90 (1H, d, 12.4 Hz, PhCH_2), 4.72 (1H, d, 12.0 Hz, PhCH_2), 4.69 (1H, d, 12.2 Hz, PhCH_2), 4.67 (1H, d, 11.0 Hz, PhCH_2), 4.59 (1H, d, 11.8 Hz, PhCH_2), 4.49 (1H, d, 12.0 Hz, PhCH_2), 4.04 (1H, m, *H*-3), 3.57-3.52 (m, *H*-2, *H*-4, *H*-5, *H*-6a, *H*-6b and PEG), 3.37 (3H, s, OCH_3), 2.34 (1H, d, 9.0 Hz, C_3-O*H*), 1.96 [1H, s (br), C_6-O*H*].

MPEG-TrimandiOAc (5.8): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 [2H, s (br), Ar-*H*], 7.38-7.20 (m, Ar-*H*), 6.78 [1H, s (br), PhCON*H*], 5.49 [1H, m (br), *H*-2a], 5.47 [1H, m (br), *H*-2b], 5.19 [1H, s (br), *H*-1a], 4.97 [1H, s (br), *H*-1b], 4.86 (2H, d, 10.7Hz, PhCH_2), 4.83 [1H, s (br), *H*-1c], 4.74 (1H, d, 11.0 Hz, PhCH_2), 4.66-4.58 (m, PhCH_2), 4.48-4.36 (m, PhCH_2), 4.18-3.34 (m), 2.14 (3H, s, CH_3CO), 2.08 (3H, s, CH_3CO).

MPEG-TrimandiOH (5.9): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (2H, d, 7.8 Hz, Ar-*H*), 7.34-7.12 (m, Ar-*H*), 6.77 [1H, s (br), PhCON*H*], 5.23 [1H, s (br), *H*-1a], 5.06 [1H, s (br), *H*-1b], 4.83 (1H, d, 10.8 Hz, PhCH_2), 4.82 (1H, d, 12.2 Hz, PhCH_2), 4.80 [1H, s (br), *H*-1c], 4.70-4.38 (m, PhCH_2), 4.16 (1H, dd, 1.0 and

9.0 Hz, *H*-3c), 4.08 [1H, s (br), *H*-2b], 4.02 [1H, s (br), *H*-2a], 3.99-3.48 (m), 3.37 (3H, s, OCH_3), 2.42 [1H, s (br), C_{2a}-O*H*], 2.38 [1H, s (br), C_{2b}-O*H*].

MPEG-PentamandiOAc (5.10): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 [2H, s (br), Ar-*H*], 7.38-7.08 (m, Ar-*H*), 6.55 [1H, s (br), PhCON*H*], 5.50 [2H, m (br), *H*-2a and *H*-2b], 5.19 [1H, s (br), *H*-1a], 5.05 [1H, s (br), *H*-1b], 5.03 [1H, s (br), *H*-1c], 4.93 [1H, s (br), *H*-1d], 4.86-4.22 (m, *H*-1e and PhCH_2), 4.06-3.46 (m), 3.36 (3H, s, OCH_3), 2.10 (3H, s, CH_3CO), 2.08 (3H, s, CH_3CO).

Mannopentaose Peracetate (5.11): colorless glassy solid; R_f 0.30 (5:2 EtOAc/Hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.01 (d, 1.7 Hz, *H*-1aα), 5.76 (d, 1.0 Hz, *H*-1aβ), 5.46 (dd, 1.0 and 2.1 Hz, *H*-2aβ), 5.38-5.15 (m), 5.12 (d, 1.7 Hz, *H*-1bα), 5.10-5.07 (m), 4.92 (d, 1.0 Hz, *H*-1cβ), 4.89 (d, 1.5 Hz, *H*-1cα), 4.88 (d, 1.0 Hz, *H*-1dβ), 4.86 (d, 1.5 Hz, *H*-1dα), 4.82 (d, 1.5 Hz, *H*-1eα), 4.80 (d, 1.0 Hz, *H*-1eβ), 4.22-3.51 (m), 2.19 (s, CH_3CO), 2.14 (s, CH_3CO), 2.13 (s, CH_3CO), 2.12 (s, CH_3CO), 2.11 (s, CH_3CO), 2.10 (s, CH_3CO), 2.09 (s, CH_3CO), 2.08 (s, CH_3CO), 2.05 (s, CH_3CO), 2.04 (s, CH_3CO), 2.03 (s, CH_3CO), 2.02 (s, CH_3CO), 2.01 (s, CH_3CO), 1.97 (s, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.6, 170.4, 170.2, 170.1, 170.0, 169.9, 169.7, 169.6, 169.5, 169.4, 169.3, 169.2, 167.7, 99.7, 99.6, 99.4, 99.0, 98.2, 90.5, 90.2, 77.8, 74.3, 71.0, 69.6, 69.5, 69.4, 69.3, 69.2, 69.0, 68.4, 68.2, 68.1, 66.8, 66.1, 66.0, 65.6, 65.3, 62.3, 62.1, 61.9, 61.8, 21.1, 20.9, 20.8, 20.7, 20.6, 20.5, 20.4, 20.3; ES-MS calcd for $C_{64}H_{86}O_{43}Na$ ($M+Na^+$) 1565.4, found 1565.6, the experimental spectrum matches the theoretical spectrum created by isotopic modeling.

MPEG-Man3OH6OTBDPS (5.12): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (2H, d, 7.1 Hz, Ar-*H*), 7.74 (2H, d, 7.1 Hz, Ar-*H*), 7.71 (2H, d, 7.1 Hz, Ar-*H*), 7.44-7.19 (18H, m, Ar-*H*), 6.83 [1H, s (br), PhCON*H*], 4.97 (1H, d, <1.0 Hz, *H*-1), 4.88 (1H, d, 11.0 Hz, PhCH_2), 4.73 (1H, d, 12.4 Hz, PhCH_2), 4.71 (1H, d, 11.8 Hz, PhCH_2), 4.61 (1H, d, 11.9 Hz, PhCH_2), 4.58 (1H, d, 11.0 Hz, PhCH_2), 4.48 (1H, d, 12.4 Hz, PhCH_2), 4.05 (1H, ddd, 3.4, 9.0, and 9.5 Hz, *H*-3), 3.94 (2H, m, *H*-6a and

H-6b), 3.80-3.77 (2H, m, *H*-2, and *H*-4), 3.70-3.52 (m, *H*-5 and PEG), 3.37 (3H, s, OCH_3), 2.43 (1H, d, 9.5 Hz, C_3-O*H*), 1.06 [9H, s, (CH_3)$_3$CPh_2Si].

MPEG-Diman6OTBDPS (5.13): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (4H, d, 7.8 Hz, Ar-*H*), 7.67 (2H, d, 8.1 Hz, Ar-*H*), 7.42-7.12 (m, Ar-*H*), 6.83 [1H, s (br), PhCON*H*], 5.48 (1H, dd, 1.0 and 1.9 Hz, *H*-2a), 5.18 (1H, d, <1.0 Hz, *H*-1a), 4.85 (1H, d, <1.0 Hz, *H*-1b), 4.84 (1H, d, 11.0 Hz, PhCH_2), 4.72 (1H, d, 10.5 Hz, PhCH_2), 4.66-4.56 (m, PhCH_2), 4.53 (1H, d, 11.0 Hz, PhCH_2), 4.46-4.40 (m, PhCH_2), 4.16 (1H, dd, 3.0 and 9.3 Hz, *H*-3b), 4.06-3.79 (m), 3.70-3.50 (m, PEG), 3.35 (3H, s, OCH_3), 2.08 (3H, s, CH_3CO), 1.03 [9H, s, (CH_3)$_3$CPh_2Si].

MPEG-Diman6OH (5.14): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (2H, d, 8.3 Hz, Ar-*H*), 7.35-7.14 (m, Ar-*H*), 6.76 [1H, s (br), PhCON*H*], 5.49 (1H, dd, 1.0 and 1.9 Hz, *H*-2a), 5.22 (1H, d, <1.0 Hz, *H*-1a), 4.87 (1H, d, 11.0 Hz, PhCH_2), 4.82 (1H, d, <1.0 Hz, *H*-1b), 4.80 (1H, d, 11.0 Hz, PhCH_2), 4.67-4.55 (m, PhCH_2), 4.48-4.43 (m, PhC*H*), 4.20 (1H, dd, 2.7 and 9.3 Hz, *H*-3b), 4.01-3.53 (m), 3.37 (3H, s, OCH_3), 2.10 (3H, s, CH_3CO).

MPEG-TrimantriOAc (5.15): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (2H, d, 8.1 Hz, Ar-*H*), 7.34-7.13 (m, Ar-*H*), 6.68 [1H, s (br), PhCON*H*], 5.49 (1H, dd, 1.0 and 1.9 Hz, *H*-2a), 5.32 (1H, dd, 2.0 and 8.0 Hz, *H*-3b), 5.21 (1H, d, 1.0 Hz, *H*-1a), 5.07 (1H, d, 1.0 Hz, *H*-1b), 4.86 (1H, d, 11.0 Hz, PhCH_2), 4.83 (1H, d, 10.6 Hz, PhCH_2), 4.75 (1H, d, 1.0 Hz, *H*-1c), 4.70-4.38 (m, PhCH_2), 4.36-3.52 (m), 3.37 (3H, s, OCH_3), 2.08 (3H, s, CH_3CO), 2.03 (3H, s, CH_3CO), 1.98 (3H, s, CH_3CO).

MPEG-TrimantriOH (5.16): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (2H, d, 8.1 Hz, Ar-*H*), 7.36-7.16 (m, Ar-*H*), 6.70 [1H, s (br), PhCON*H*], 5.24 (1H, d, <1.0 Hz, *H*-1a), 5.08 (1H, d, <1.0 Hz, *H*-1b), 4.92 (1H, d, 11.2 Hz, PhCH_2), 4.82 (1H, d, 11.0 Hz, PhCH_2), 4.78 (1H, d, <1.0 Hz, *H*-1c), 4.73 (1H, d, 11.0 Hz,

PhCH_2), 4.66-4.38 (m, PhCH_2), 4.18 (1H, dd, 2.9 and 9.5 Hz, *H*-3c), 4.02-3.52 (m), 3.37 (3H, s, OCH_3), 2.34 [1H, s (br), RO*H*], 1.94 [1H, t (br), C_6-O*H*].

MPEG-HexamantriOAc (5.17): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (2H, d, 7.6 Hz, Ar-*H*), 7.34-7.16 (m, Ar-*H*), 6.63 [1H, s (br), PhCON*H*], 5.49 [3H, m (br), *H*-2a, *H*-2b, and *H*-2c], 5.22 (1H, d, <1.0 Hz, *H*-1a), 5.13 (1H, d, <1.0 Hz, *H*-1b), 5.02 (1H, d, <1.0 Hz, *H*-1c), 4.98 (1H, d, <1.0 Hz, *H*-1d), 4.95 (1H, d, <1.0 Hz, *H*-1e), 4.86-4.34 (m, *H*-1f and PhCH_2), 4.28-3.49 (m), 3.37 (3H, s, OCH_3), 2.11 (3H, s, CH_3CO), 2.09 (3H, s, CH_3CO), 2.02 (3H, s, CH_3CO).

MPEG-NonamantriOAc (5.18): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (2H, d, 8.1 Hz, Ar-*H*), 7.36-7.05 (m, Ar-*H*), 6.45 [1H, s (br), PhCON*H*], 5.53 [1H, m (br), *H*-2a], 5.51 [1H, m (br), *H*-2b], 5.48 [1H, m (br), *H*-2c], 5.25 (1H, d, <1.0 Hz, *H*-1a), 5.18 (1H, d, <1.0 Hz, *H*-1b), 5.14 (1H, d, <1.0 Hz, *H*-1c), 5.09 (1H, d, <1.0 Hz, *H*-1d), 5.04 [2H, m (br), *H*-1e and *H*-1f], 4.97 (1H, d, <1.0 Hz, *H*-1g), 4.94 (1H, d, <1.0 Hz, *H*-1h), 4.86-3.52 (m, *H*-1i, PhCH_2, and PEG), 3.37 (3H, s, OCH_3), 2.10 (6H, s, CH_3CO), 2.05 (3H, s, CH_3CO).

Mannononaose Peracetate (5.19):colorless glassy solid; R_f 0.59 (neat EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.02 (d, 2.0 Hz, *H*-1aα), 5.78 (d, 1.2 Hz, *H*-1aβ), 5.49 (dd, 1.2 and 3.4 Hz, *H*-2aβ), 5.41-5.21 (m), 5.16-5.12 (m), 5.04 (d, 1.7 Hz, d, 1.7 Hz, *H*-1dα and *H*-1dβ), 4.97 (d, 1.7 Hz, *H*-1e), 4.96 (d, 1.7 Hz, d, 2.0 Hz, *H*-1fα and *H*-1fβ), 4.90 (d, 2.0 Hz, d, 2.2 Hz, *H*-1gα and *H*-1gβ), 4.85 (d, 2.2 Hz, d, 2.4 Hz, *H*-1hα and *H*-1hβ), 4.84 and 4.81 (d, 2.0 Hz, d, 2.0 Hz, *H*-1iα and *H*-1iβ), 4.24-3.47 (m), 2.24 (s, CH_3CO), 2.20 (s, CH_3CO), 2.15 (s, CH_3CO), 2.14 (s, CH_3CO), 2.13 (s, CH_3CO), 2.12 (s, CH_3CO), 2.11 (s, CH_3CO), 2.10 (s, CH_3CO), 2.09 (s, CH_3CO), 2.08 (s, CH_3CO), 2.07 (s, CH_3CO), 2.06 (s, CH_3CO), 2.05 (s, CH_3CO), 2.04 (s, CH_3CO), 2.03 (s, CH_3CO), 2.02 (s, CH_3CO), 2.01 (s, CH_3CO), 2.00 (s, CH_3CO), 1.99 (s, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.8, 170.7, 170.6, 170.5, 170.4, 170.3, 170.2, 169.9, 169.8, 169.7, 169.6, 169.5, 169.4, 169.3, 168.4, 167.9, 100.1,

99.6, 99.5, 99.4, 99.3, 99.1, 98.1, 97.6, 97.5, 90.7, 90.5, 78.0, 77.8, 77.7, 77.6, 77.5, 77.4, 77.2, 77.1, 75.6, 74.2, 74.0, 73.2, 71.7, 70.5, 70.4, 70.1, 70.0, 69.7, 69.6, 69.5, 69.4, 69.3, 69.2, 69.1, 68.6, 68.5, 68.3, 68.2, 67.4, 67.3, 66.4, 66.3, 66.2, 66.1, 65.8, 65.7, 65.5, 65.4, 65.3, 65.2, 62.4, 62.3, 62.2, 62.1, 62.0, 61.9, 61.8, 61.6, 61.5, 20.8, 20.7, 20.6, 20.5, 20.4, 20.3; ES-MS calcd for $C_{112}H_{150}O_{75}Na$ ($M+Na^+$) 2717.8, found 2717.8, the experimental spectrum matches the theoretical spectrum created by isotopic modeling.

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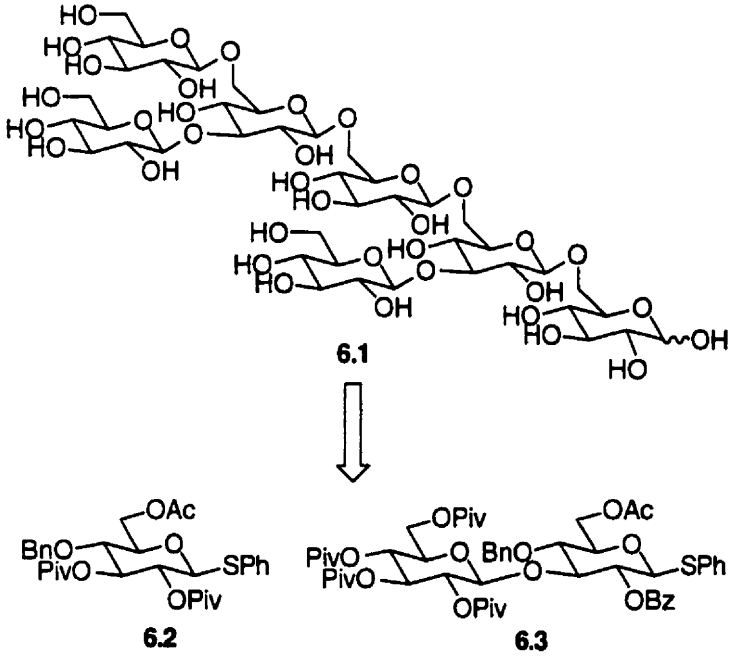
CHAPTER 6

Attempted Synthesis of A Heptasaccharide Phytoalexin Elicitor (HPE) by Oligomer-supported Solution Chemistry

6.0 Introduction.

Biological study of glycoproteins on the cell surface demands efficient syntheses of oligosaccharides. Because of significant improvements on glycosylation methodologies in the past two decades, there have been spectacular advances in solid-phase[1] and polymer-supported solution synthesis[2] of oligosaccharides recently. Of special interest to glycobiology and medicinal sciences is practical methodology for the construction of oligosaccharides of high complexity. Our oligomer-supported oligosaccharide synthesis using poly(ethylene glycol) monomethyl ether has been demonstrated by the syntheses of a linear tetramannan,[3a] a branched pentamannan and a complex nonamannan.[3b] Although the overall yield of the nonamannan had been improved significantly compared to the linear tetramannan, it was believed that there was still room for improvement on the overall yield of final products. Since the main reason for the decrease the overall yield was suspected to be the loss of less polar components of MPEG-olisaccharide conjugates during the chromatographic separation, it was natural to think about using the longer poly(ethylene glycol) monomethyl ether which has stronger affinity towards silica gel as the oligomer support to minimize the loss. Poly(ethylene glycol) monomethyl ether with molecular weight of 750 was chosen for such a purpose as it was difficult to wash off the MPEG with molecular weight of higher than 1,500 by the mixture of ethyl acetate-methanol (4:1). The solvent system more polar than this would elute a significant amount of silica gel from the column.

To validate our hypothesis, heptaglucoside **6.1** (HPE) was chosen to be the synthetic target using poly(ethylene glycol) monomethyl ether (MPEG, average M_n750) as the oligomer support. Although its total synthesis has been done by many groups not only by classic solution chemistry[4] but also on a solid support[1e] and a soluble polymer support,[2b] compound **6.1** is still an interesting target because of its phytoalexin elicitor activity in soybean.[5] The synthesis can also be used to demonstrate the usefulness of the regioselective reductive ring opening of 4,6-*O*-benzylidene acetals of hexopyranosides to the corresponding 4-*O*-benzyl ethers with the C_6-OH unsubstituted.[6]

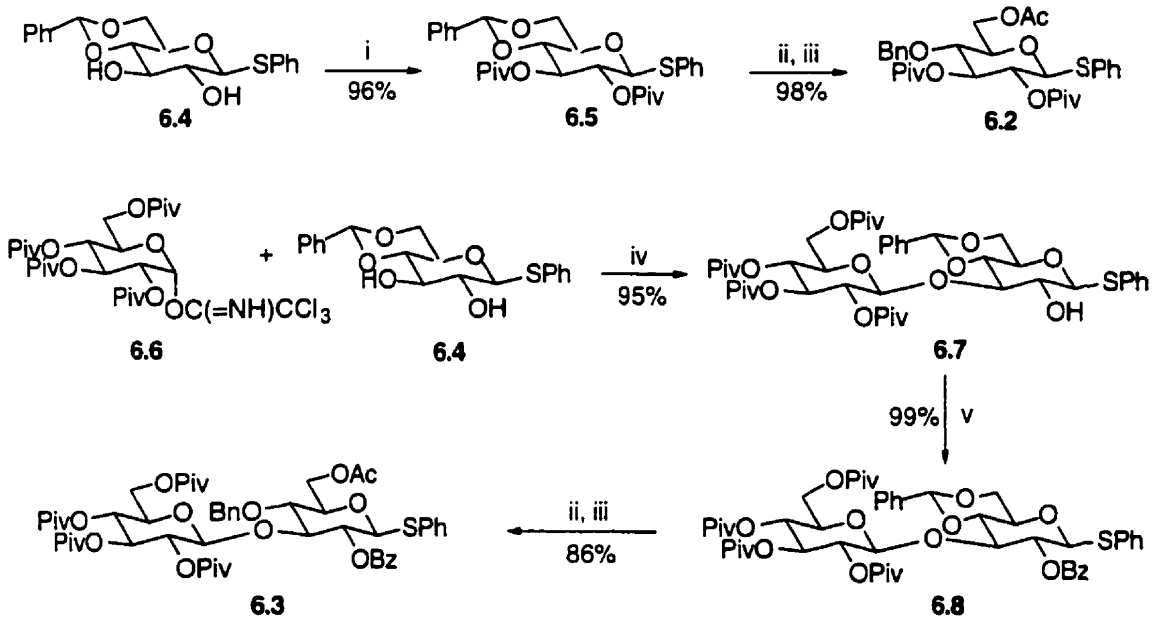


Scheme 6.1 Building blocks (**6.2** and **6.3**) for the synthesis of heptaglucoside **6.1**.

6.1 Results and discussion.

Our synthetic plan for **6.1** is shown in Scheme 6.1 which is similar to the one used by van Boom's group.[2b, 4e] Thioglycosyl donors **6.2** and **6.3** were chosen as two building blocks. In both donors, the acetyl groups were used as temporary protecting groups while

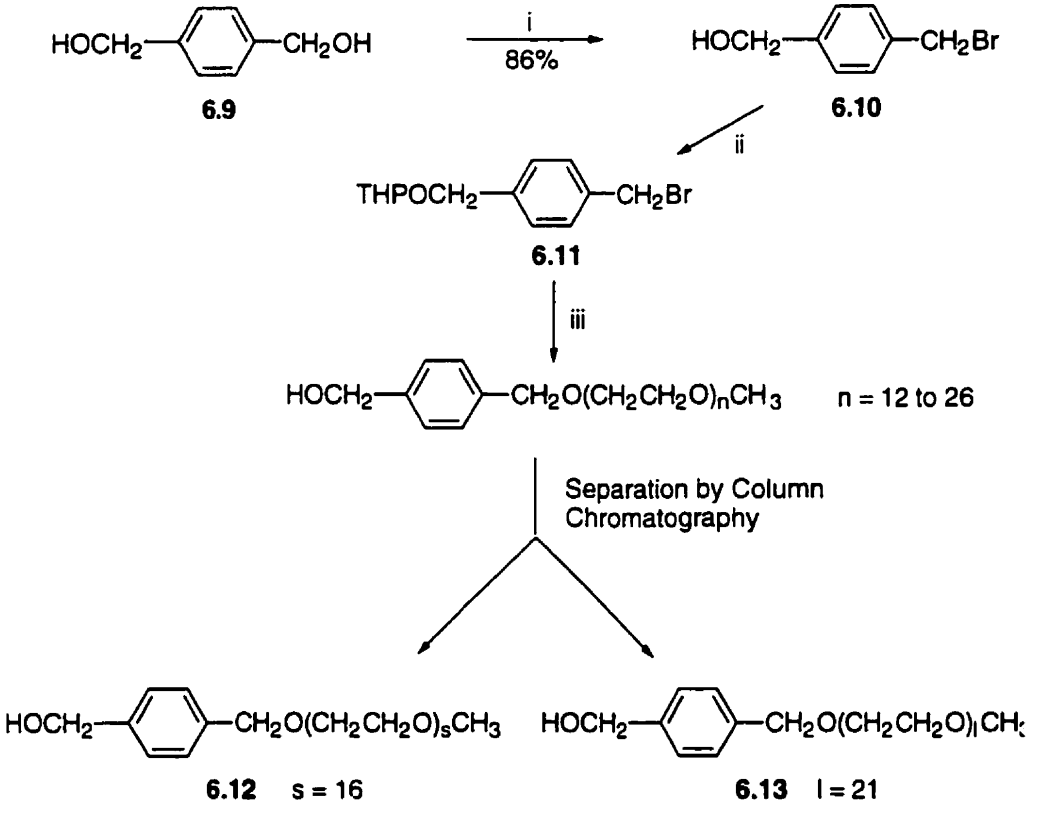
the benzyl, pivaloyl and benzoyl groups served as permanent protecting groups. Their syntheses are outlined in Scheme 6.2. Pivaloylation of diol **6.4**[7] at 100 °C gave compound **6.5**, which was regioselectively reduced to donor **6.2** in an almost quantitative yield. Preparation of donor **6.3** started from the regioselective glycosylation of diol **6.4** with the known trichloroacetimidate derivative **6.6**[8] in the presence of TMSOTf which afforded disaccharide **6.7** in an excellent yield. Regioselective reductive ring opening of the 4,6-*O*-benzylidene acetal of **6.8**, prepared from the benzoylation of **6.7**, and acetylation furnished the building block **6.3**.



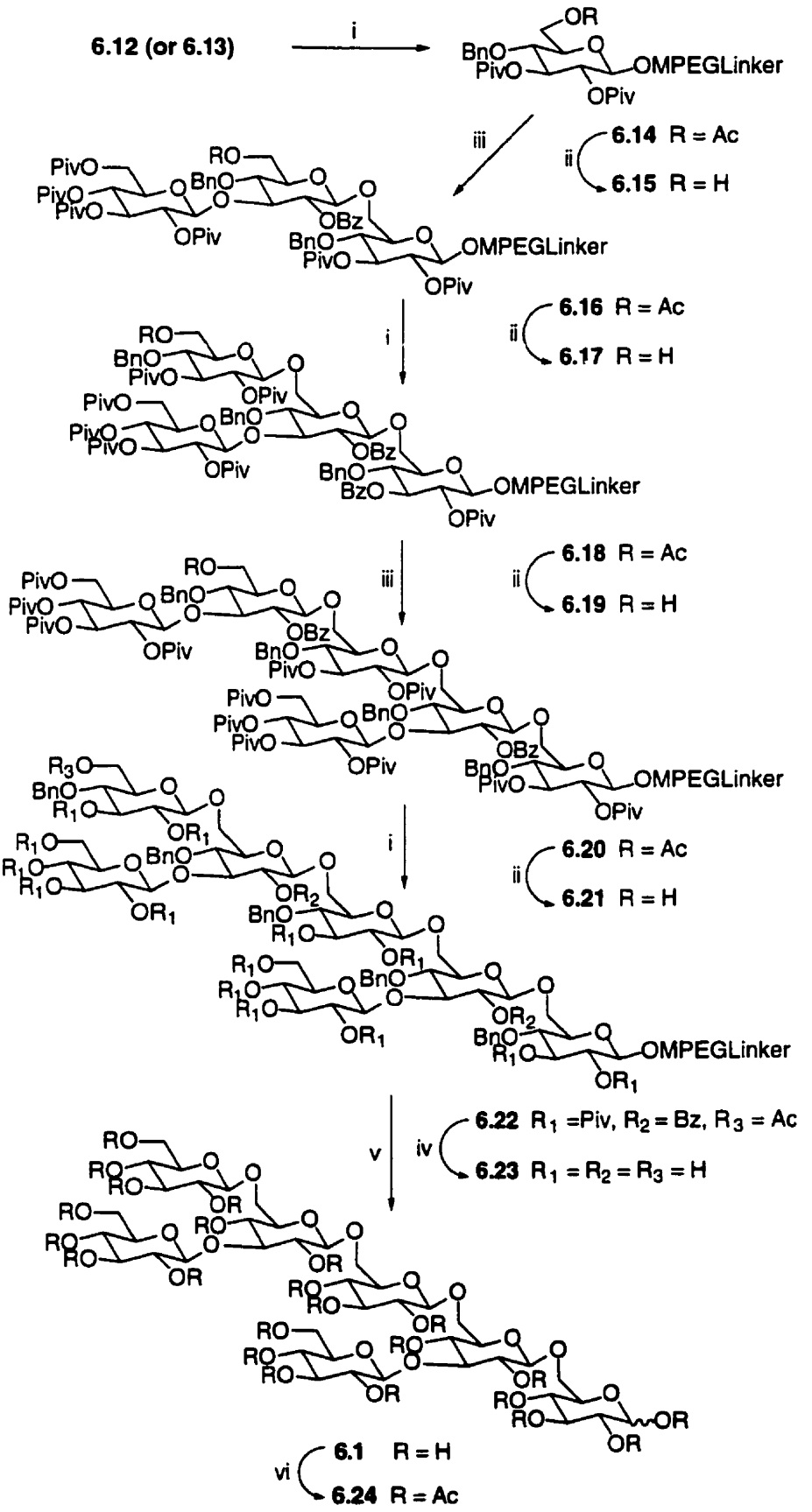
Scheme 6.2 *Reagents and Conditions*: (i) 4.0 equiv. of PivCl, DMAP, Py, 100°C, 4 h; (ii) 1.2 equiv. of BH_3•THF, 0.8 equiv. of Bu_2BOTf, 0°C, 1 h; (iii) Ac_2O, Py, DMAP, 0.5 h; (iv) 1.2 equiv. of **6.6**, 1.0 equiv. of **6.4**, 0.1 equiv. of $Me_3SiOSO_2CF_3$, 4Å molecular sieves, CH_2Cl_2, -10°C to rt, 2 h; (v) BzCl, DMAP, Py, rt, 12 h.

It was also our intention to compare the α, α'-dioxyxylyl diether linker used by Krepinsky's group with the HMB linker[3] developed by us in terms of their practicality. Scheme 6.3 shows the preparation of the MPEG-linkers **6.12** and **6.13**.

Monobromination of 1,4-benzenedimethanol (**6.9**) and subsequent THP protection of **6.10** gave the benzyl bromide derivative **6.11**. Reaction of poly(ethylene glycol) monomethyl ether (MPEG, average M_w750) and excess **6.11** under basic conditions furnished the derivatized MPEG which, after the deprotection of THP and separation by column chromatography on silica gel, afforded the MPEG-linkers **6.12** and **6.13** with average repeating units of 16 and 21, respectively. The faster moving component on silica gel, **6.12** which has shorter length of poly(ethylene glycol) chain, turns out to be a clear colorless liquid at room temperature while the slower one, **6.13**, is a white solid.



Scheme 6.3 *Reagents and Conditions*: (i) 1.5 equiv. of 48% HBr, benzene, rt, 2 h; (ii) 1.5 equiv. of 3,4-dihydro-2*H*-pyran, catalytic CSA, rt, 1 h; (iii) 2.0 equiv. of **6.11**, 1.5 equiv. of NaH, 1.0 equiv. of MPEG750, DMF, 12 h; then AcOH, MeOH, 60 °C, 1 h.


6.12 (or 6.13)
i
OMPEGLinker
iii
ii
6.14 R = Ac
6.15 R = H
6.16 R = Ac
6.17 R = H
6.18 R = Ac
6.19 R = H
6.20 R = Ac
6.21 R = H
v
iv
6.22 R1 =Piv, R2 = Bz, R3 = Ac
6.23 R1 = R2 = R3 = H
vi
6.1 R = H
6.24 R = Ac

Scheme 6.4 *Reagents and Conditions*: (i) 1.5 equiv. of thioglucoside **6.2**, 1.6 equiv. of NIS, 0.8 equiv. of TMSOTf, 4 Å molecular sieves, CH_2Cl_2, 0 °C to rt, 2 h; (ii) TEA, MeOH, 60 °C, 15 h; (iii) 1.5 equiv. of thioglucoside **6.3**, 1.6 equiv. of NIS, 0.8 equiv. of TMSOTf, 4 Å molecular sieves, CH_2Cl_2, 0 °C to rt, 2 h; (iv) NaOMe, MeOH, rt, 5 h; (v) 10% Pd/C, 50 psi H_2, NaOAC, AcOH, Formic acid, pH 2, 7 days; (vi) Ac_2O, Py, DMAP, rt, 24 h.

With both building blocks and the MPEG-linkers (**6.12** and **6.13**) readily available, the synthesis of heptasaccharide **6.1** was started with the coupling of **6.12** or **6.13** with donor **6.2** using the combination of N-iodosuccinimide and trimethylsilyl triflate (NIS/Me_3SiOTf) as the coupling promoter.[9] The iodonium ion mediated glycosylation went smoothly and gave the MPEG-monosaccharide **6.14** in almost quantitative yield. The difference in length of the two MPEG-linkers appeared to make no difference in the ease of purification of the product by flash column chromatography on silica gel at this stage. There were almost no impact on the ^1H, ^1H-^1H COSY and ^1H-^{13}C HMQC NMR spectrum except the different integration value of the PEG region (around δ 3.6 ppm). The acetyl group of **6.14** was readily removed by base treatment to yield **6.15** and subsequent iodonium ion mediated coupling with donor **6.3** provided the MPEG-triglucoside **6.16**. The success of the coupling reaction was confirmed by the fact that ^1H-^1H COSY and ^1H-^{13}C HMQC NMR spectrum of **6.16** showed the existence of three anomeric centers (Figure 6.1 and 6.2). The acetyl group also served as a marker which indicated the unmasking of the growing point, C_6-OH as the incorporation of the new glucoside units. Repetition of the deacetylation step and coupling with donor **6.2** again furnished the MPEG-tetrasaccharide **6.18** (Figure 6.3 and 6.4) which, after standard deacetylation, gave **6.19**. At this stage, ^1H-^1H COSY and ^1H-^{13}C HMQC NMR spectroscopies could still help to monitor the progress of the assembly, revealing the presence of four anomeric centers. However, the difference in length of the two MPEG-linkers started to make a difference in the purification of the product by flash column

chromatography on silica gel. Progressively, the weight gain of the shorter MPEG-linker became less than the weight gain of the longer one probably due to the shorter MPEG's weaker affinity towards silica gel during chromatographic separation.

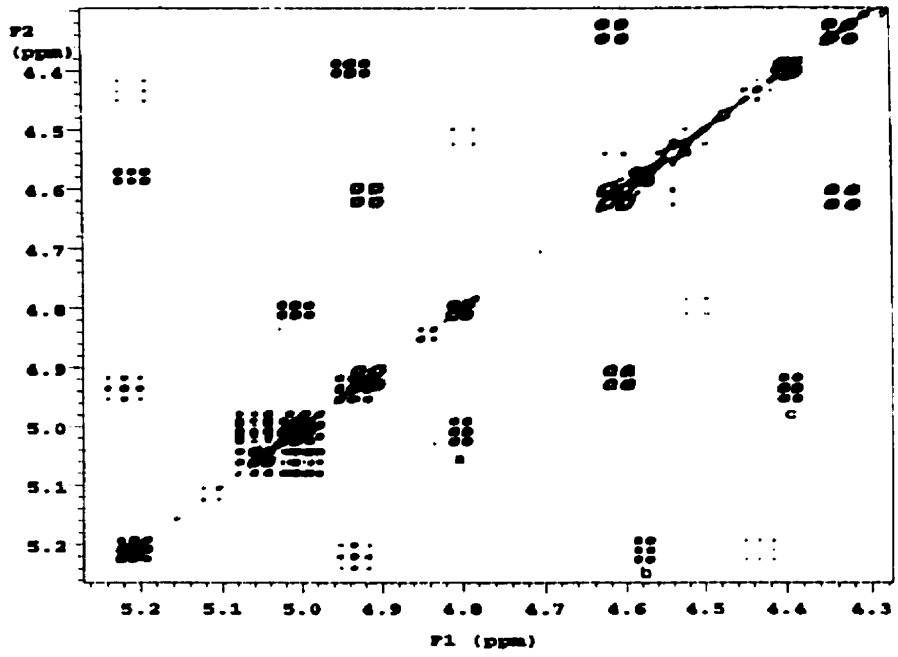


Figure 6.1 Anomeric region of the ^{1}H-^{1}H COSY NMR spectrum of **6.16**.

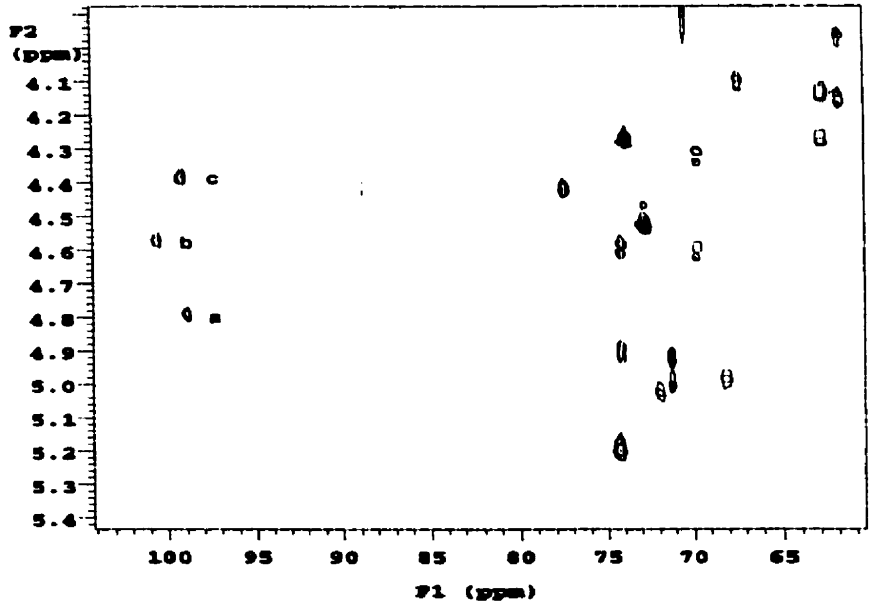


Figure 6.2 Anomeric region of the ^{1}H-^{13}C HMQC NMR spectrum of **6.16**.

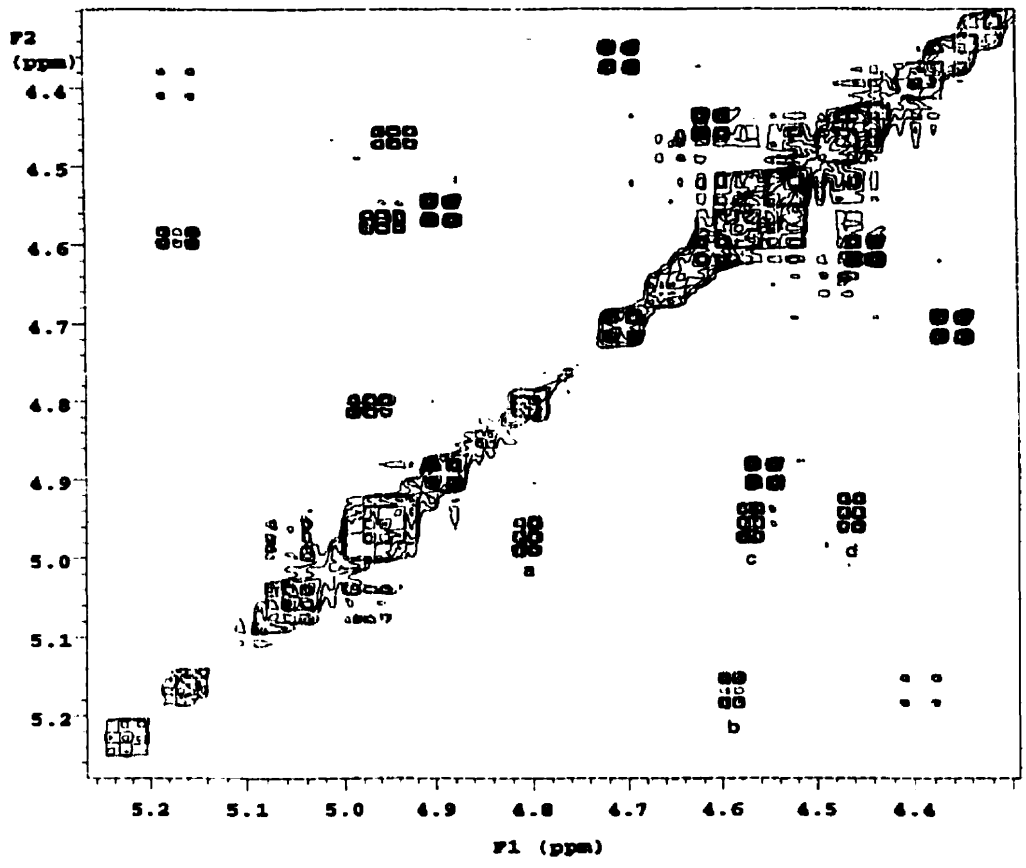


Figure 6.3 Anomeric region of the ^{1}H-^{1}H COSY NMR spectrum of **6.18**.

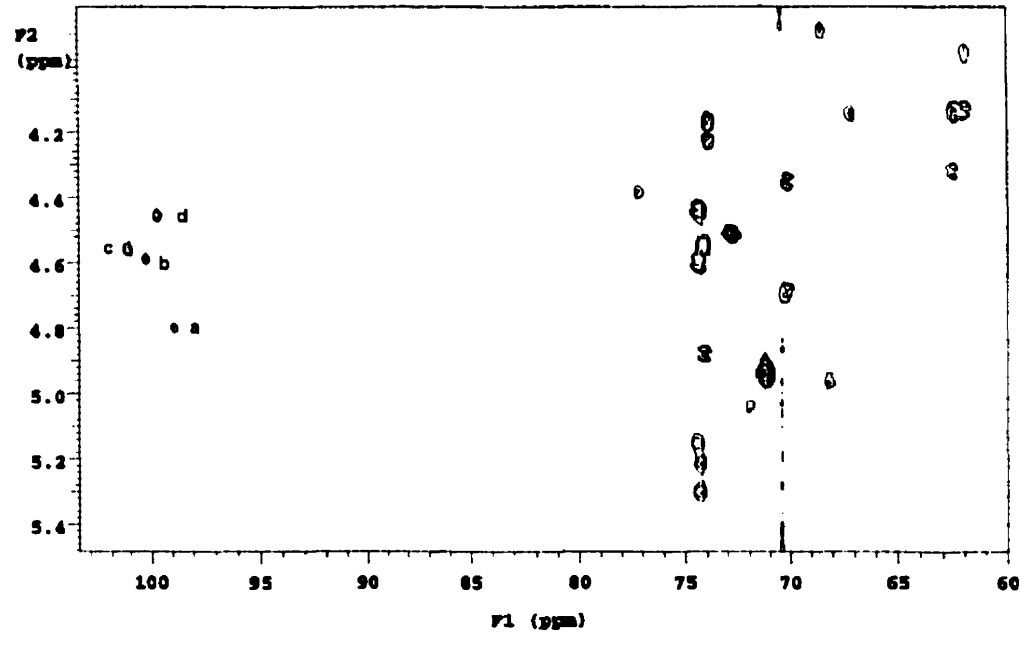


Figure 6.4 Anomeric region of the ^{1}H-^{13}C HMQC NMR spectrum of **6.18**.

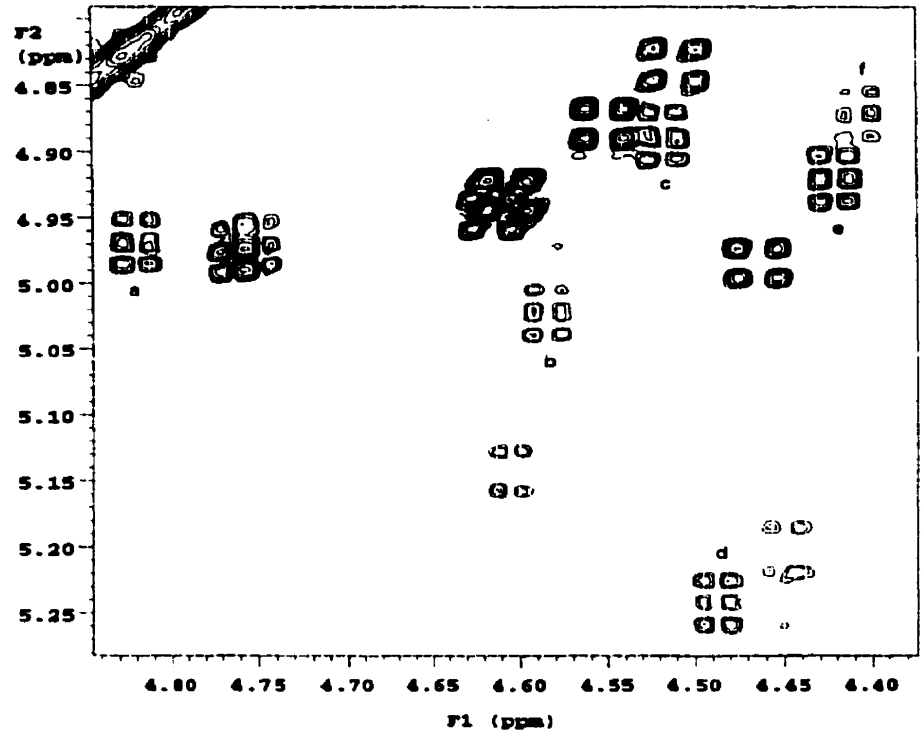


Figure 6.5 Anomeric region of the ^{1}H-^{1}H COSY NMR spectrum of **6.21**.

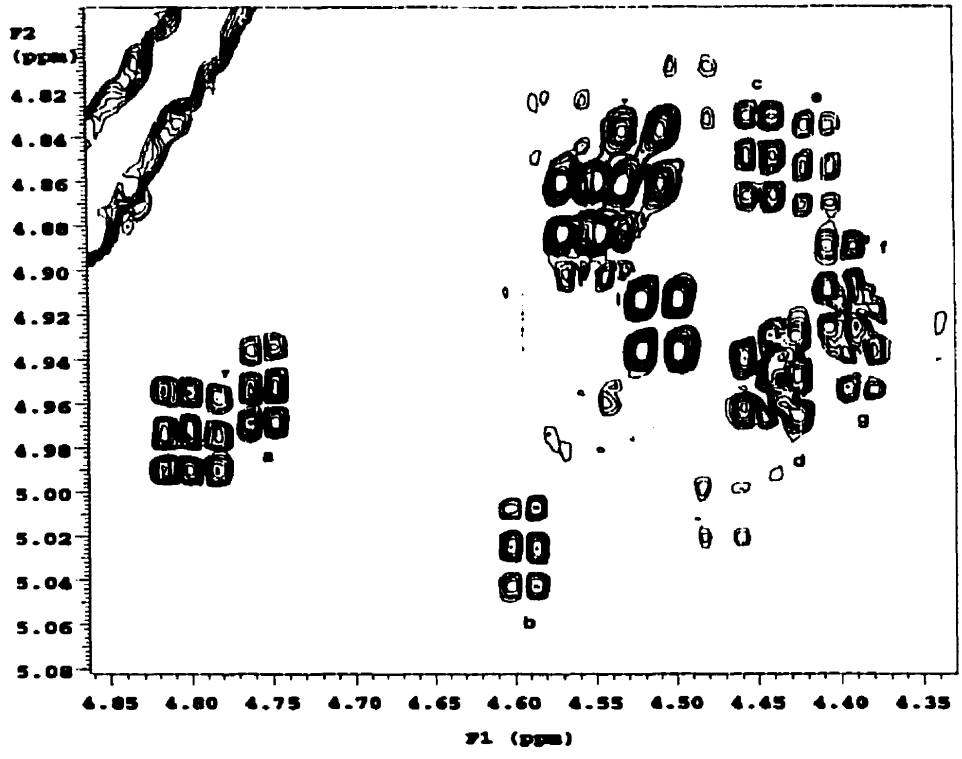


Figure 6.6 Anomeric region of the ^{1}H-^{1}H COSY NMR spectrum of **6.22**.

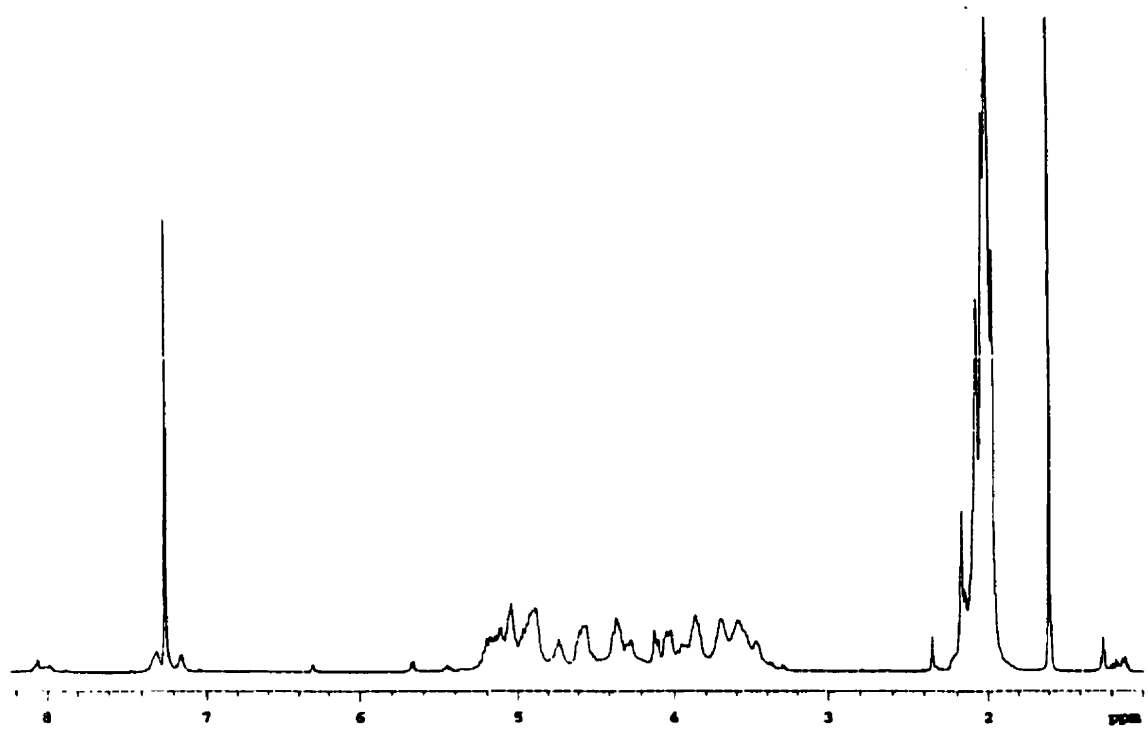


Figure 6.7 ^{1}H NMR spectrum of impure **6.24**.

Iodonium ion (NIS/TMSOTf) mediated elongation of **6.19** with thiolaminaribioside donor **6.3** furnished, after deacetylation of the coupling product **6.20**, the MPEG-hexaglucoside **6.21**. The proton peaks were broadened significantly along with the incorporation of more carbohydrate units at this point and to our surprise, ^{1}H-^{13}C HMQC NMR spectroscopy could not be helpful any more because of the poor resolution at the anomeric region. Fortunately, ^{1}H-^{1}H COSY NMR spectroscopy could still help to identify the presence of six anomeric protons in **6.21** (Figure 6.5). Final coupling of **6.21** with thioglucoside donor **6.2** gave the expected MPEG-heptaglucoside **6.22**, whose seven anomeric protons were determined by ^{1}H-^{1}H COSY NMR spectroscopy (Figure 6.6). However, the final debenzylation of **6.22** turned out to be a hurdle. Even the highly forced condition used in the successful debenzylation of a highly benzyl protected branched nonamannan[3b] failed to remove any benzyl group. Removal of acyl group prior to hydrogenation did not help either. Eventually, hydrogenation of the hydrolyzed **6.23** did

proceed in the presence of formic acid at pH 2 using 10% Pd-C as the catalyst. However, it seemed that the reduction of benzene rings also occurred leading to a mixture of products including **6.1**. Acetylation of the impure **6.1** gave the fully acetylated **6.24**, whose ^{1}H NMR spectrum and electrospray mass spectrum are shown in Figure 6.7 and 6.8, respetively.

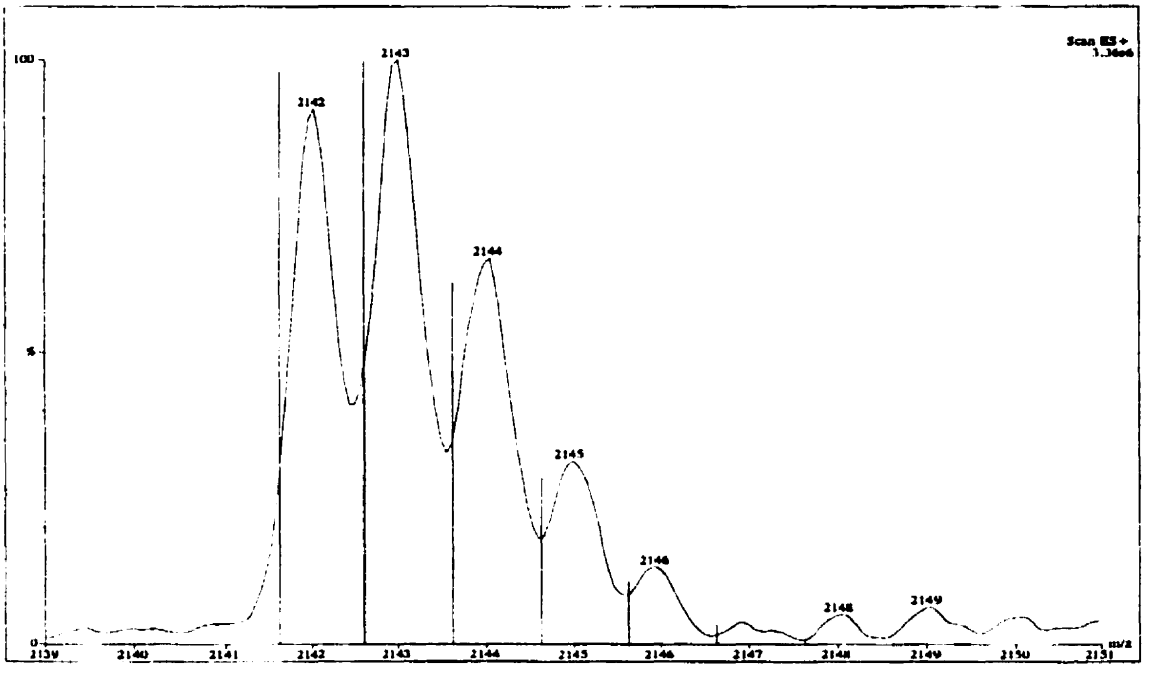


Figure 6.8 Vertical lines: theoretical spectrum created by isotopic modeling for a sodium adduct of **6.24**; gaussian peaks: experimental spectrum of **6.24**.

On the bright side, using weight gain of the oligomer support as a measurement of overall yield, it can be concluded that the longer MPEG (average repeating unit number is 21) gave much better overall yield than the shorter MPEG (average repeating unit number is 16) did. Both are still better than the one used (average repeating unit number is 12) in the branched nonamannan synthesis.[3b] It also appeared that the linker used in this HPE (**6.1**) synthesis and the HMB linker used in the branched nonamannan (**5.1**) synthesis[3b] did not make much difference on efficiency of the coupling reactions and monitoring of the

assembly of oligosaccharides by NMR spectroscopy. Another lesson learned is that special attention should be paid to designing appropriately protected building blocks whose protecting groups can not only be easily removed but also be helpful for the purification of final products. In some cases, the benzyl group appears not to be the best choice for permanent protection.

In conclusion, even though the synthesis of HPE (**6.1**) has encounted some problem at the last step, it shows that adjustment of the length of the poly(ethylene glycol) oligomer support could result in better overall yields. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC NMR spectroscopies are extremely useful in monitoring the progress of oligosaccharide construction. The regioselective reductive ring opening of 4,6-*O*-benzylidene acetals of hexopyranosides has been demonstrated by the concise syntheses of two building blocks (**6.2** and **6.3**) of HPE (**6.1**).

6.2 Experimental Section.

General. See Chapter 3, section **3.2**.

Phenyl 4,6-*O*-benzylidene-2,3-di-*O*-pivaloyl-1-thio-β-D-gluco-pyranoside (6.5). A stirred solution of phenyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside **6.4** (10.0 g, 27.8 mmol), pivaloyl chloride (13.4 g, 13.7 mL, 111.1 mmol) and a catalytic amount of DMAP in pyridine (150 mL) was heated at 100°C for 4 hours. After the solution was cooled to room temperature, excess pivaloyl chloride was destroyed with water and then the reaction mixture was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (200 mL) and washed with a dilute HCl solution, a saturated $NaHCO_3$ solution, and then brine. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness. Crystallization from neat ethyl acetate gave **6.5** as colorless crystals (14.1 g, 96%): mp 143-145 °C; R_f = 0.62 (2:1 hexanes/EtOAc); $[\alpha]^{20}_{D}$-53.1° (*c* 0.11, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48-7.33 (10H, m, Ar-*H*), 5.52 (1H, s,

PhC*H*), 5.38 (1H, dd, 9.4 and 9.4 Hz, *H*-3), 5.08 (1H, dd, 9.9 and 10.0 Hz, *H*-2), 4.82 (1H, d, 10.3 Hz, *H*-1), 4.41 (1H, dd, 5.0 and 10.6 Hz, H-6a), 3.81 (1H, dd, 10.3 and 10.3 Hz, *H*-6b), 3.71 (1H, dd, 9.5 and 9.5 Hz, *H*-4), 3.58 (1H, ddd, 4.9, 9.5, and 9.8 Hz, *H*-5), 1.22 [9H, s, $COC(CH_3)_3$], 1.15 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.1, 176.5, 162.0, 136.8, 132.8, 132.2, 129.0, 128.9, 128.3, 128.2, 125.8, 101.1, 87.2, 78.5, 72.3, 70.6, 69.8, 68.5, 38.8, 38.7, 27.1, 27.0.

Phenyl 6-*O*-acetyl-4-*O*-benzyl-2,3-di-*O*-pivaloyl-1-thio-β-D-gluco-pyranoside (6.2). A solution of 1M BH_3 in THF (31 mL) and **6.5** (13.5 g, 25.6 mmol) was stirred at 0 °C for 5 minutes. To this clear solution was then slowly added 1M Bu_2BOTf in CH_2Cl_2 (20.5 mL). After 1 hour at 0 °C, TLC showed that the starting material had disappeared. Triethylamine (5 mL) was then added to the reaction flask, followed by careful addition of methanol until the evolution of H_2 had ceased. The reaction mixture was co-distilled with methanol three times and then evaporated to dryness. The resulting syrup was dissolved in pyridine (50 mL) with a catalytic amount of DMAP, followed by the addition of acetic anhydride (10 mL). After the solution was left at room temperature for 30 minutes, excess acetic anhydride was destroyed with water and then the reaction mixture was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (150 mL) and washed with a dilute HCl solution, a saturated $NaHCO_3$ solution, and then brine. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness. Crystallization from ethyl acetate/hexanes gave **6.2** as colorless crystals (14.3 g, 98%): mp 151-152.5 °C; R_f = 0.61 (1:1 hexanes/EtOAc); $[\alpha]^{20}_D$-17.8° (*c* 0.14, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.48-7.21 (10H, m, Ar-*H*), 5.40 (1H, dd, 9.0 and 9.0 Hz, *H*-3), 5.00 (1H, dd, 9.7 and 9.8 Hz, *H*-2), 4.70 (1H, d, 10.0 Hz, *H*-1), 4.66 (1H, d, 11.0 Hz, PhCH_2), 4.49 (1H, d, 11.0 Hz, PhCH_2), 4.41 (1H, dd, 1.8 and 11.8 Hz, H-6a), 4.17 (1H, dd, 4.9 and 11.9 Hz, *H*-6b), 3.68-3.62 (2H, m, *H*-4 and *H*-5), 2.07 (3H, s, $COCH_3$), 1.22 [9H, s, $COC(CH_3)_3$], 1.16 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.0, 176.7,

170.5, 137.1, 132.7, 132.6, 128.9, 128.5, 128.1, 128.0, 127.5, 86.4, 76.8, 76.0, 75.5, 74.5, 69.9, 62.9, 38.8, 38.7, 27.2, 27.1, 20.9.

Phenyl 3-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (6.7). A solution of 2 M TMSOTf in CH_2Cl_2 (0.9 mL) was added to a mixture of **6.4** (6.0 g, 16.7 mmol), **6.6** (13.2 g, 20 mmol) and 4Å molecular sieves in dry CH_2Cl_2 (300 mL) at -10°C under argon. After 1 hour at -10°C, the reaction mixture was slowly warmed up to room temperature, neutralized with triethylamine (2 mL), filtered and then washed with a dilute HCl solution, a saturated $NaHCO_3$ solution, and brine. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness. Crystallization from hexanes gave **6.7** as colorless crystals (13.6 g, 95%): mp >170 °C (dec.); R_f = 0.69 (1:1 hexanes/EtOAc); $[\alpha]^{20}_D$-34.3° (*c* 0.14, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53-7.32 (10H, m, Ar-*H*), 5.52 (1H, s, PhC*H*), 5.26 (1H, dd, 9.5 and 9.5 Hz, *H*-3), 5.09 (1H, dd, 9.8 and 9.8 Hz, *H*-4), 5.00 (1H, dd, 8.8 and 9.0 Hz, *H*-2), 4.85 (1H, d, 8.1 Hz, *H*-1), 4.56 (1H, d, 9.8 Hz, *H*-1'), 4.35 (1H, dd, 5.0 and 10.4 Hz, *H*-6a'), 4.00 (1H, dd, 1.8 and 12.2 Hz, *H*-6a), 3.83 (1H, dd, 4.2 and 7.9 Hz, *H*-6b), 3.81-3.76 (2H, m, *H*-3' and *H*-6b'), 3.58 (1H, dd, 9.3 and 9.3 Hz, *H*-4'), 3.48-3.41 (3H, m, *H*-5, *H*-5' and *H*-2'), 2.55 (1H, d, 2.0 Hz, C_3'-O*H*), 1.15 [9H, s, $COC(CH_3)_3$], 1.12 [9H, s, $COC(CH_3)_3$], 1.09 [9H, s, $COC(CH_3)_3$], 1.08 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.1, 177.2, 177.1, 176.2, 137.0, 133.5, 130.5, 129.2, 129.1, 128.6, 128.3, 126.1, 101.4, 100.7, 87.8, 81.3, 78.9, 72.3, 71.9, 71.7, 71.0, 68.5, 67.7, 38.8 (2C), 38.7 (2C), 27.1 (3C), 27.0.

Phenyl 3-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (6.8). Benzoyl chloride (3.1 mL, 26.7 mmol) was added to a solution of **6.7** (12.9 g, 15.0 mmol) and a catalytic amount of DMAP in pyridine (80 mL). After 12 hours at room temperature, excess benzoyl chloride was destroyed with water and then the reaction mixture was concentrated *in vacuo*. The residue was then dissolved in ethyl acetate (150 mL), washed with a dilute

HCl solution, a saturated $NaHCO_3$ solution, and brine. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness. Crystallization from hexanes gave **6.8** as colorless crystals (14.3 g, 99%): mp 110-112 °C; R_f = 0.70 (1:1 hexanes/EtOAc); $[\alpha]^{20}_D$+4.4° (*c* 0.21, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (2H, d, 8.5 Hz, Ar-*H*), 7.61-7.27 (13H, m, Ar-*H*), 5.58 (1H, s, PhC*H*), 5.24 (1H, dd, 9.3 and 9.3 Hz, *H*-2'), 5.07-5.02 (2H, m, *H*-3 and *H*-4), 4.95-4.92 (1H, m, *H*-2), 4.89 (1H, d, 10.0 Hz, *H*-1'), 4.70 (1H, d, 7.8 Hz, *H*-1), 4.38 (1H, dd, 4.9 and 10.5 Hz, *H*-6a'), 4.24 (1H, dd, 8.7 and 8.8 Hz, *H*-3'), 4.07 (1H, dd, 1.9 and 12.3 Hz, *H*-6a), 3.86 (1H, dd, 10.3 and 10.3 Hz, *H*-6b'), 3.83 (1H, dd, 9.3 and 9.3 Hz, *H*-4'), 3.77 (1H, dd, 4.7 and 12.3 Hz, *H*-6b), 3.56 (1H, ddd, 4.9, 9.8 and 9.8 Hz, *H*-5'), 3.29 (1H, ddd, 3.2, 5.1 and 5.1 Hz, *H*-5), 1.20 [9H, s, $COC(CH_3)_3$], 1.06 [9H, s, $COC(CH_3)_3$], 1.03 [9H, s, $COC(CH_3)_3$], 0.89 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.2, 177.2, 176.2, 176.1, 164.8, 136.9, 133.4, 132.9, 132.2, 130.0, 129.7, 129.3, 129.0, 128.6, 128.4, 128.2, 126.2, 101.6, 99.3, 87.0, 79.1, 77.8, 72.6, 71.8, 71.4, 70.6, 68.6, 67.7, 61.6, 38.9, 38.6 (2C), 38.4, 27.2, 27.1, 26.9, 26.8.

Phenyl 3-*O*-(2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosyl)-6-*O*-acetyl-2-*O*-benzoyl-4-*O*-benzyl-1-thio-β-D-glucopyranoside (6.3). Prepared as described above for the conversion of **6.5** into **6.2**, starting from **6.8** (4.8 g, 5.0 mmol). Purification of the crude product by flash column chromatography on silica gel (180 g, 3:1 hexanes/EtOAc) afforded **6.3** as colorless foam (4.3 g, 86%): R_f = 0.45 (2:1 hexanes/EtOAc); $[\alpha]^{20}_D$+22.6° (*c* 0.31, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (2H, d, 8.1 Hz, Ar-*H*), 7.70-7.23 (13H, m, Ar-*H*), 5.10 [1H, dd (br), 8.9 and 8.9 Hz, *H*-2'], 5.05-4.96 (3H, m, *H*-2, *H*-3 and *H*-4), 4.94 (1H, d, 11.2 Hz, PhCH_2), 4.76 (1H, d, 10.7 Hz, *H*-1'), 4.74 (1H, d, 7.7 Hz, *H*-1), 4.61 (1H, d, 11.5 Hz, PhCH_2), 4.48 (1H, dd, 8.2 and 8.3 Hz, *H*-3'), 4.30 (1H, dd, 2.1 and 11.9 Hz, *H*-6a'), 4.14 (1H, dd, < 1.0 and 12.2 Hz, *H*-6a), 4.10 (1H, dd, 5.8 and 11.9 Hz, *H*-6b'), 3.96 (1H, dd, 6.4 and 12.2 Hz, *H*-6b), 3.58 (1H, ddd, 2.2, 7.1 and 7.1 Hz, *H*-5'), 3.53-3.41 (2H, m, *H*-4' and *H*-5), 1.99

(3H, s, $COCH_3$), 1.19 [9H, s, $COC(CH_3)_3$], 1.16 [9H, s, $COC(CH_3)_3$], 1.10 [9H, s, $COC(CH_3)_3$], 1.07 [9H, s, $COC(CH_3)_3$]; ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.7, 176.9, 176.7, 176.3, 170.4, 164.7, 137.6, 133.7, 133.0, 132.0, 129.6, 129.2, 128.7, 128.6, 128.2 (2C), 127.7, 127.6, 98.8, 85.9, 78.7, 76.9, 74.7, 74.4, 73.4, 72.2, 71.8, 71.2, 68.0, 63.1, 61.9, 38.7, 38.6, 38.5 (2C), 26.9 (3C), 26.8, 20.7.

4-(Bromomethyl)benzyl alcohol (6.10). A slurry of 1,4-benzenedimethanol (**6.9**, 13.8 g, 0.1 mol), 48% HBr (17 mL) and benzene (80 mL) was stirred at room temperature until the solid 1,4-benzenedimethanol (**6.9**) disappeared (about 2 hours). The reaction mixture was diluted with ethyl acetate (100 mL) and the organic phase was washed with a saturated $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. Crystallization from hexanes/ethyl acetate gave **6.10** as colorless crystals (17.3 g, 86%): mp 67-70 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 7.39 (2H, d, 8.3 Hz, Ar-*H*), 7.35 (2H, d, 8.2 Hz, Ar-*H*), 4.70 (2H, s, $PhCH_2OH$), 4.50 (2H, s, $PhCH_2Br$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.0, 129.1, 127.7, 127.2, 64.7, 33.1.

4-(Bromomethyl)benzyl THP ether (6.11). A solution of **6.10** (8.0 g, 40 mmol) , 3,4-dihydro-2*H*-pyran (5.0 g, 5.5 mL, 60 mmol), dry THF (50 mL) and a catalytic amount of camphorsulfonic acid was tired at room temperature until TLC analysis shown the disappearance of **6.10**. Triethylamine (0.5 mL) was added to neutralize the acid and the reaction mixture was evaporated to dryness under reduced pressure. The residue was dried further under high vacuum and used for the next step without purification.

MPEG-Linkers (6.12 and 6.13). 60% NaH (1.44 g, 36 mmol) in oil was added to a solution of poly(ethylene glycol) monomethyl ether (MPEG, average M_n750, 15.0 g, 20 mmol) in dry DMF (100 mL) at 0 °C with caution. The mixture was stirred for 15 minutes and until the evolution of H_2 had ceased, a solution of **6.10** in THF (30 mL) was syringed into the flask slowly. The reaction was left at room temperature over night and methanol was added slowly to destroy the excess NaH. Water was then added and then the reaction mixture was concentrated *in vacuo*. The residue was dissolved in acetic acid

(30 mL) and methanol (50 mL), heated at 60 °C for 1 hour and evaporated to dryness under reduced pressure. Again, the residue was dissolved in chloroform (200 mL) and washed with a saturated $NaHCO_3$ solution and brine. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. The crude product was dissolved in a small amount of ethyl acetate and put onto the top of a silica gel column (300 g) for flash column chromatography. Elution with neat ethyl acetate removed the side-products and further elution with ethyl acetate/methanol (4:1 and 3:1) gave colorless liquid **6.12** (6.8 g) and white solid **6.13** (9.3 g): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (4H, s, Ar-*H*), 4.68 (2H, s, PhC*H*$_2$OH), 4.56 (2H, s, PhC*H*$_2$OCH$_2$), 3.67-3.61 (m, PEG), 3.55-3.53 (m), 3.37 (3H, s, OC*H*$_3$), 1.71 [1H, s (br), PhCH$_2$O*H*].

Protocol for the Solution-phase Glycosylations. After thioglucoside **6.2** (or thiolaminariboside **6.3**) (1.5 mmol) and a MPEG-acceptor (1 mmol) were co-distillated with toluene 5 times under reduced pressure, dry 4 Å molecular sieve powder (1 g/g reactant) and dichloromethane (2.5 mL/g reactant) were added into the flask. The mixture was stirred for 10-15 minutes under argon and then cooled to 0 °C, followed by the addition of NIS (1.6 mmol). The color of the reaction mixture turned pink immediately as 2M solution of TMSOTf (0.8 mmol) in dichloromethane was syringed into the flask slowly. Upon removal of the ice bath right after completion of the addition, the reaction mixture was allowed to warm up to room temperature slowly and kept for 2 hours. Chloroform and an aqueous $NaHCO_3$ solution were added to quench the reaction, followed by filtration of the mixture through a pad of celite to remove the molecular sieve powder. After having been washed with an aqueous $Na_2S_2O_3$ solution and brine, the organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The resulting syrup was dissolved in a small amount of ethyl acetate and put onto the top of a silica gel column (15 times weight of the syrup) for flash column chromatography. Gradient elution with hexanes/ethyl acetate (2:1, 1:1, 1:2, 1:4 and neat EtOAc, 50 mL each)

removed the side-products and further elution with ethyl acetate/methanol (4:1 and 3:1, 150 mL each) gave the MPEG-sugar conjugate.

Protocol for the Deacetylation with TEA. Triethylamine (5 mL) was added to a stirred solution of MPEG-sugar in methanol (30 mL) and the reaction mixture was heated at 60 °C for at least 12 hours. The reaction was monitored by ^{1}H NMR spectroscopy and upon completion of the deacetylation indicated by the disappearance of the acetyl peak in the ^{1}H NMR spectrum, the methanol and triethylamine were evaporated under reduced pressure. The residue was therefore ready for the next coupling.

MPEG-Glc6OAc (6.14): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34-7.22 (9H, m, Ar-*H*), 5.35 (1H, dd, 9.1 and 9.5 Hz, *H*-3), 5.04 (1H, dd, 8.1 and 9.5 Hz, *H*-2), 4.83 (1H, d, 11.7 Hz, PhCH_2), 4.66 (1H, d, 11.0 Hz, PhCH_2), 4.58-4.55 (3H, m, PhCH_2), 4.53 (1H, d, 8.1 Hz, *H*-1), 4.49 (1H, d, 11.0 Hz, PhCH_2), 4.41 (1H, dd, 2.1 and 12.1 Hz, *H*-6a), 4.21 (1H, dd, 4.8 and 12.1 Hz, *H*-6b), 3.72-3.54 (m, *H*-4, *H*-5, and PEG), 3.38 (3H, s, OCH_3), 2.09 (3H, s, CH_3CO), 1.16 [9H, s, COC(CH_3)$_3$], 1.12 [9H, s, COC(CH_3)$_3$].

MPEG-Glc6OH (6.15): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47-7.24 (9H, m, Ar-*H*), 5.36 (1H, dd, 9.1 and 9.5 Hz, *H*-3), 5.02 (1H, dd, 8.1 and 9.5 Hz, *H*-2), 4.82 (1H, d, 12.0 Hz, PhCH_2), 4.67 (1H, d, 11.0 Hz, PhCH_2), 4.62-4.56 (3H, m, *H*-1 and PhCH_2), 4.54 (2H, m, PhCH_2), 3.89 (1H, ddd, 2.5, 4.6, and 12.0 Hz, *H*-5), 3.76 (1H, dd, 9.4 and 9.4 Hz, *H*-4), 3.74-3.44 (m, *H*-6a, *H*-6b, and PEG), 3.38 (3H, s, OCH_3), 1.89 [1H, s (br), C_6-O*H*], 1.15 [9H, s, COC(CH_3)$_3$], 1.12 [9H, s, COC(CH_3)$_3$].

MPEG-Triglc6OAc (6.16): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (2H, d, 7.6 Hz, Ar-*H*), 7.58-7.00 (17H, m, Ar-*H*), 5.22-5.19 (m), 5.06-4.91 (m), 4.80 (1H, dd, 7.8 Hz, *H*-1a), 4.61 (1H, d, 11.0 Hz, PhCH_2), 4.58 (1H, dd, 7.6 Hz, *H*-1b), 4.54 (2H, m, PhCH_2), 4.43-4.38 (m, *H*-1c), 4.33 (1H, d, 12.0 Hz, PhCH_2), 4.28-4.26 (m), 4.18-4.11 (m), 3.99 (1H, dd, 6.6 and 12.1 Hz, *H*-6), 3.68-3.50 (m, PEG), 3.38 (3H, s, OCH_3), 1.94 (3H, s, CH_3CO), 1.18 [9H, s, COC(CH_3)$_3$], 1.15 [9H, s,

$COC(CH_3)_3$], 1.11 [9H, s, $COC(CH_3)_3$], 1.06 [18H, s, $COC(CH_3)_3$], 1.05 [9H, s, $COC(CH_3)_3$].

MPEG-Triglc6OH (6.17): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (2H, d, 7.1 Hz, Ar-*H*), 7.59-7.00 (17H, m, Ar-*H*), 5.24-5.19 (m), 5.07-4.93 (m), 4.79 (1H, dd, 7.6 Hz, *H*-1a), 4.64 (1H, d, 12.0 Hz, $PhCH_2$), 4.63 (1H, d, 11.5 Hz, $PhCH_2$), 4.58-4.54 (m), 4.45-4.43 (m), 4.41 (1H, d, 8.1 Hz, *H*-1c), 4.37-4.29 (m), 4.18-4.16 (m), 4.07-3.98 (m), 3.79-3.46 (m, PEG), 3.38 (3H, s, OCH_3), 1.94 [1H, s (br), C_6-O*H*], 1.19 [9H, s, $COC(CH_3)_3$], 1.14 [9H, s, $COC(CH_3)_3$], 1.11 [9H, s, $COC(CH_3)_3$], 1.07 [9H, s, $COC(CH_3)_3$], 1.06 [18H, s, $COC(CH_3)_3$].

MPEG-Tetraglc6OAc (6.18): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (2H, d, 7.6 Hz, Ar-*H*), 7.58-6.94 (22H, m, Ar-*H*), 5.32 (1H, dd, 9.3 and 9.5 Hz), 5.25-5.15 (m), 5.08-4.92 (m), 4.89 (1H, d, 11.5 Hz, $PhCH_2$), 4.81 (1H, dd, 7.8 Hz, *H*-1a), 4.71 (1H, d, 12.0 Hz, $PhCH_2$), 4.64-4.32 (m), 4.25-4.13 (m), 3.99-3.77 (m), 3.66-3.48 (m, PEG), 3.38 (3H, s, OCH_3), 2.02 (3H, s, CH_3CO), 1.19-1.02 [s (m), $COC(CH_3)_3$].

MPEG-Tetraglc6OH (6.19): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (2H, d, 8.5 Hz, Ar-*H*), 7.55-6.99 (22H, m, Ar-*H*), 5.37-5.14 (m), 5.08-4.93 (m), 4.82-4.73 (m), 4.69-4.32 (m), 4.15-4.03 (m), 3.90-3.42 (m, PEG), 3.38 (3H, s, OCH_3), 1.18-1.01 [s (m), $COC(CH_3)_3$].

MPEG-Hexaglc6OAc (6.20): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02–7.99 (m, Ar-*H*), 7.57-6.83 (m, Ar-*H*), 5.23-5.13 (m), 5.04-4.76 (m), 4.67-3.89 (m), 3.78-3.48 (m, PEG), 3.38 (3H, s, OCH_3), 1.96 (3H, s, CH_3CO), 1.19-1.00 [s (m), $COC(CH_3)_3$].

MPEG-Hexaglc6OH (6.21): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02–7.99 (m, Ar-*H*), 7.57-6.82 (m, Ar-*H*), 5.34-4.74 (m), 4.76-3.86 (m), 3.78-3.48 (m, PEG), 3.38 (3H, s, OCH_3), 1.21-0.98 [s (m), $COC(CH_3)_3$].

MPEG-Heptaglc6OAc (6.22): colorless syrup; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01–7.99 (m, Ar-*H*), 7.57-6.86 (m, Ar-*H*), 5.36-5.28 (m), 5.21-4.75 (m), 4.65-3.82 (m), 3.80-3.47 (m, PEG), 3.38 (3H, s, OCH_3), 1.99 (3H, s, CH_3CO), 1.19-0.98 [s (m), $COC(CH_3)_3$].

Protocol for the Debenzylation of MPEG-heptaglucoside 6.22 by Pearlman's catalyst. A mixture of **6.22** and 20% $Pd(OH)_2$-C (Pearlman's catalyst) in methanol (50 mL) was stirred under 1000 psi H_2 for 7 days at room temperature and no debenzylation occurred. Removal of acyl group prior to hydrogenation also failed to help.

Protocol for the Deacetylation of MPEG-heptaglucoside 6.22 to 6.23. Sodium (0.5 g) was added to a solution of **6.22** in methanol (100 mL). After 5 hours at room temperature, Dowex 50WX2-100 ion-exchange resin was added to neutralize the base. Filtration and evaporation of methanol yielded the crude **6.23**, which was used directly for the next step.

Protocol for the Debenzylation of MPEG-heptaglucoside 6.23 by 10% Pd-C in the presence of Formic Acid. A mixture of **6.23** and 10% Pd-C in methanol (80 mL), acetic acid/NaOAc and formic acid at pH 2 was stirred under 50 psi H_2 for 7 days at room temperature. The solution was filtered and the Pd-C washed with methanol. After the filtrate was evaporated under reduced pressure to afford a syrup, acetic anhydride, pyridine and a catalytic amount of DMAP were added. The mixture was stirred at room temperature for 24 hours and usual work-up gave a glassy solid, which was subject to flash column chromatography over silica gel (40 g, neat EtOAc) to afford the impure peracetylated pentamannan **6.24** (a mixture of α- and β-anomers) (291 mg).

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CHAPTER 7

Conclusions and Future Perspectives

7.0 Introduction.

In the course of the research on the subject of oligosaccharide synthesis described in the proceeding chapters, several methods on selective protection of hydroxyl groups in a carbohydrate molecule and oligomer-supported solution-phase synthesis of oligosaccharides have been developed. The following sections deal briefly with the main conclusions and some suggestions for future work based on current results.

7.1 Regioselectivity in the pivaloylation of hexopyranosides.

Although pivaloylation of carbohydrates is not new, the study presented in **Chapter 3** sheds light on the regioselectivity of the esterification which is very useful in carbohydrate synthesis.[1] The simplicity of the procedure offers great advantages for the preparation of glycosyl donors and acceptors. Pivaloylation of various hexopyranosides was studied. Manno- and galacto-pyranosides were regioselectively acylated to give the 3-*O*-pivaloylated compounds in good yields. The enhanced reactivity of the C_3-OH is probably due to the presence of the adjacent axial function at the C_2- or C_4-position. It is also very interesting that the reactivity of a secondary hydroxyl group with an adjacent axial function is almost as high as that of the primary C_6-OH group toward pivaloylation. In the absence of an adjacent axial function as in the case of the phenyl 1-thio-β-D-

glucopyranoside, the secondary C_3-OH is more reactive than the secondary C_2-OH toward pivaloylation. The reaction has been utilized to provide suitably protected mannoside donors and acceptors for polymannan synthesis in a very concise way.

In our opinion, the origin of the selectivity of pivaloylation is purely steric effect. Since pivaloyl chloride is relatively bulky, it is likely that the reagent would approach the hydroxyl group from the side and not from the top or bottom of the ring system. The reaction is therefore more subject to steric hindrance of the adjacent equatorial substituents. For mechanistic consideration, pivaloylation could help to determine the relative reactivities of hydroxyl groups towards acylation in carbohydrates. Analysis of the relative yields of the acylation products could give the ratio of second-order rate-constants. *A knowledge of the relative reactivities of hydroxyl groups in carbohydrates is fundamental to a thorough understanding of carbohydrate chemistry.*[1]

7.2 Regioselective reductive ring opening of benzylidene acetals in carbohydrates.

Selective protection of different hydroxyl groups in a sugar molecule is very important in the chemical synthesis of complex carbohydrates. Because of the ease of formation of the acetal as well as the well-established nature of the benzyl ether protection, the regioselective reductive ring opening of cyclic benzylidene acetals to the corresponding *O*-benzyl ethers is a useful strategy. [2] The existing reductive systems for the regioselective reductive cleavage of 4,6-*O*-benzylidene acetals to the corresponding 4-*O*-benzyl ethers with the C_6-OH unsubstituted are still unsatisfactory. In this connection, **Chapter 4** describes the development of a mild and effective reagent, BH_3•THF-Bu_2BOTf, for the regioselective reductive ring opening of 4,6-*O*-benzylidene acetals of hexopyranosides to the corresponding 4-*O*-benzyl ethers with the C_6-OH unsubstituted. The reduction of 4,6-

O-benzylidene acetals of glucosides, galactosides and mannosides with various protecting group patterns was carefully investigated. Both the regioselectivities and yields of the ring opening reactions are excellent and benzyl, silyl, ester and phthaloyl protecting groups are compatible with the reaction conditions. The reagent is also effective in the reductive ring opening of the 4,6-*O*-isopropylidene acetal, 2,3-*O*-isopropylidene dioxolane and 2,3-*O*-benzylidene dioxolanes of mannosides. However, the regioselectivity of the ring opening of dioxolanes depends on the stereochemistry of the phenyl and methyl substituents as observed by many others previously.[2] The dependence of regioselectivity of the ring opening on the stereochemistry of the phenyl substituent is independent of the reducing reagent. This regioselective reductive ring-opening reaction can provide an easy access of the important 2,6-, 3,6-mannoside branch points as well as many other oligosaccharide building blocks.

This reaction could be extended to the reduction of 1,2-orthoesters of various pyranosides (e.g. galactopyranosides and glucopyranosides) to yield the corresponding α-pyranosides which, in some cases, are difficult to obtain using Fischer glycosylation procedure.[3] For simple primary alcohols, it could offer more advantages over the various coupling reactions. On the other hand, without Lewis acids, BH_3•THF could reduce 1,2-orthoesters of various pyranosides to yield the corresponding 1,2-acetals which are very useful intermediates in the preparation of complex carbohydrates.

7.3 Oligomer-supported solution-phase synthesis of oligosaccharides.

Chapter 2 describes the preliminary study of oligomer-supported solution synthesis of oligosaccharides. Model linear tetramannan was first synthesized using low molecular weight poly(ethylene glycol) monomethyl ether as the oligomer support. The use

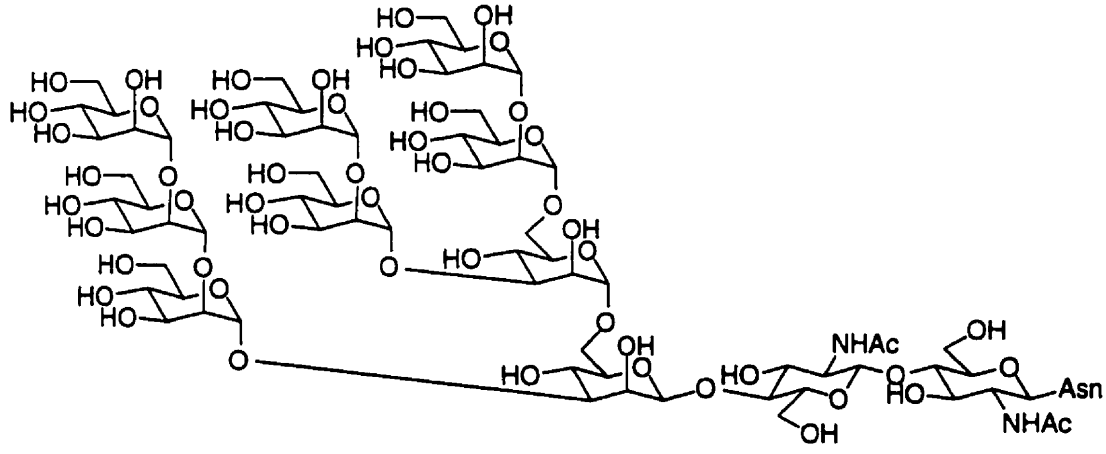
of this MPEG-linker as the oligomer support retains the normal advantages of polymer-supported solution synthesis of oligosaccharides. Purification of the supported synthons by flash column chromatography on silica gel is greatly simplified. Another advantage of this approach is that the reaction can be monitored readily by the usual arsenal of spectroscopic techniques. This study also reveals to us that trichloroacetimidate glycosyl donors are not very stable for storage at room temperature and are quite sensitive to the heat generated during the removal of water by co-distillation with toluene.

These results led us to utilize thioglycosyl donors, which are also easier to be prepared in most cases, for the construction of the nonamannan in **Chapter 5**. Instead of the classical block synthesis used by other groups,[4,5] the stepwise approach with two *triple glycosylations* was employed in our total synthesis of the nonasaccharide. This resulted in a concise synthesis of the mannan residue (**5.1**) of a highly branched mannose type oligosaccharide present on the viral coat of HIV-1 from only two monosaccharide building blocks. The use of low molecular weight MPEG (M_n = 550) aided considerably in the purification of the intermediates and in the analyses of the progress of the assembly by using conventional spectroscopic techniques. This synthesis demonstrates that oligomer-supported solution synthesis using a low molecular weight polyethylene glycol is a powerful tool for the rapid and efficient preparation of complex oligosaccharides. The ability to introduce three saccharide units in one operation is particularly remarkable.

To minimize the loss of less polar components of MPEG-oligosaccharide conjugates during the chromatographic separation and therefore, to improve the overall yield of final products further, a longer oligomer of poly(ethylene glycol) monomethyl ether (M_n = 750) which has stronger affinity towards silica gel was chosen for the synthesis of a heptaglucoside phytoalexin elicitor (HPE) (**Chapter 6**). Even though the last step of the synthesis of HPE was not completely successful, it showed that adjustment of the length of the poly(ethylene glycol) oligomer support could result in better overall yields. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC NMR spectroscopies are extremely useful in

monitoring the progress of oligosaccharide construction. The regioselective reductive ring opening of 4,6-*O*-benzylidene acetals of hexopyranosides has also been demonstrated by the concise syntheses of two building blocks of HPE. It is apparent to us that special attention should be paid to designing appropriately protected building blocks whose protecting groups can be helpful for the purification of final products and be easily removed afterwards. In some cases, the benzyl group appears not to be the best choice for permanent protection.

It is logical to think that more complex and challenging oligosaccharides could be synthesized by this methodology. For example, the undecamer (**7.1**, Figure 7.1) related to the nonamannan (**5.1**) could be synthesized by our methodology with the aid of the oligomer support. This could be very useful for immunotherapy or vaccine development.



Scheme 7.1 High mannose type oligosaccharide **7.1**.

If deprotection of temporary protecting groups does not require basic conditions, the MPEG-thioethanol linker used in our group earlier and even MPEG-bound ester linkages could be utilized in oligomer-supported solution-phase synthesis of oligosaccharides as well. On the other hand, linear polystyrene (M_w = 5,000 to 10,000) with one or more linkers on a single chain could be used for polymer-supported solution-phase synthesis of oligosaccharides, which provides many advantages over high molecular

weight poly(ethylene glycol) monomethyl ether, such as (a) polystyrene can be very well precipitated by methanol that dissolves all the impurities, making the recovery of the polymer support high; (b) polystyrene is extremely hydrophobic and soluble in many organic solvents very well, making the loss during work-up (especially the washing procedures) low; (c) unlike poly(ethylene glycol), polystyrene does not absorb moisture very much, making the drying procedures easier; (c) polystyrene-bound oligosaccharides can also be purified by column chromatography on silica gel or other media if necessary. This could address many issues presented in the current polymer-supported solution-phase synthesis of oligosaccharides using high molecular weight poly(ethylene glycol) monomethyl ether.

7.4 References.

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