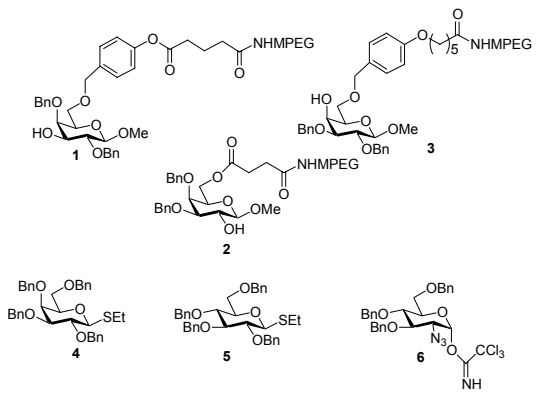
GALAL A. ELSAYED
Novel approaches for synthesizing oligosaccharides and glycoconjugates of biological importance
(Under the Direction of Dr. GEERT-JAN BOONS)

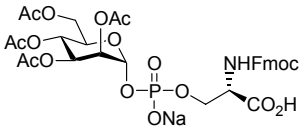
Glycoconjugates play a major role in inflammation, immune response, metastasis, fertilization, and embryogenesis. A major impediment to the rapidly growing field of molecular glycobiology is the lack of pure, structurally defined oligosaccharides and glycoconjugates. Besides the fact that these molecules are often found only in low concentrations in nature, the identification and isolation of complex carbohydrates from natural sources are greatly complicated by their microheterogeneity. Synthesis is an alternative way to have these molecules but the procurement of synthetic material presents a formidable challenge to synthetic chemists although the steady increasing in recent years for chemically defined oligosaccharides.

As an attempt to develop a methodology for the synthesis of oligosaccharide libraries, in this program, a novel approach to synthesize a library of disaccharides using soluble polymeric support is demonstrated. The resin-bound glycosyl acceptors **1**, **2**, and **3** were glycosylated with a range of glycosyl donors **4**, **5**, and **6**.




A library of 18 disaccharides was obtained, six disaccharides in each pot. Each disaccharide was released from the polymer support by selective cleavage of the linkers.

In this program, α-D-Mannopyranosylphosphate serine derivatives were conveniently synthesized by reaction of benzyl or cynaoethyl phosphochloroamidite with 2,3,4,6-tetra-*O*-acetyl-D-mannopyranose to give intermediate α-manopyranosyl phosphoramidites were successively reacted with properly protected serine (as carbamate or imine) derivatives in the presence of 1*H*-tetrazole to give phosphite triesters which could be oxidized to phosphotriesters using *t*-BuOOH. It has been shown that the new approach for coupling α-mannosyl phosphoramidite to a range of serine derivatives provides phosphotriester products in good yield and pure α-anomeric selectivity. The best yields were obtained when the imine-protected derivatives of serine were coupled to the phosphoramidite of α-mannose. This approach can be utilized for the phosphoglycosylation of different peptides containing hydroxyl amino acids such as, serine, threonine, tyrosine, or hydroxylysine. Also, it can be employed for the global glycophosohorylation of pre-assembled peptides. The building block approach can be also utilized to synthesize the same phosphoglycopeptide using the appropriate protected mannosyl phosphate serine derivatives such as building block **37**.




**37**

INDEX WORDS: MPEG, Glycosylation, Phosphorylation, Carbohydrates, Synthesis

# NOVEL APPROACHES FOR SYNTHESIZING OLIGOSACCHARIDES AND GLYCOCONJUGATES OF BIOLOGICAL IMPORTANCE

by

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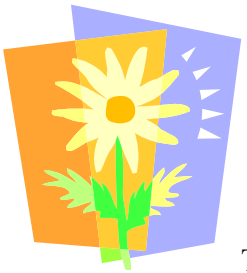

To respect, understanding and love, without which life would be worthless.

To Lamiaa, Raana, Raghad.

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TABLE OF CONTENTS

Page

LIST OF ABBREVIATIONS

Å.....Angstrom

Ac.....Acetyl

All ..... Allyl

Ar ..... Aromatic

Bn.....Benzyl

Boc ..... *tert*-Butyloxycarbonyl Bu

BSA…….. benzene sulfonic acid

Bu.....Butyl

Bz ..... Benzoyl

Cbz ..... Benzyloxycarbonyl

CSA.....(±)10-Camphorsulfonic acid

d.....Dublet

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

DCE..... 1,2-Dichloroethane

DCM ..... Methylene chloride

DDQ..... 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

DIPEA.....*N*,N-diisoprpyl ethyl amine

DMAP .....4-Dimethylaminopyridine

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

DMTST .....Dimethyl(methylthio) sulfonium trifluoromethanesulfonate

DTBMP.....2,6-di-*tert*-butyl-4-methylpyridine

Et.....Ethyl

FAB-MS.....Fast atom bombardment mass spectroscopy

Fmoc .....9-fluorene methyloxycarbonyl

gCOSY.....gradient Correlation Omonuclear Spectroscopy

gHSQC.....gradient Correlation Heteronuclear Spectroscopy

Glc..... Glucose

Gly.....Glycine

HIV .....Human immunodeficiency virus

HOBt..... 1-hydroxy benzotriazole

HPLC .....High performance liquid chromatography

Hz.....Hertz

IDCP .....Iodonium dicollidine perchlorate

m .....Multiplet

Man .....Mannose

MBz.....*p*-methoxybenzoyl

*m/z*.....Mass to charge ratio

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

Me .....Methyl

MS..... Molecular sieves

MPEG ..... monomethyl ether Polyethylene glycol

NBS.....N-bromo succinimide

Nbz.....*p*-nitro benzyl

NIS ..... *N*-iodosuccinimide

NMR ..... Nuclear magnetic resonance

Ph ..... Phenyl

Phe..... Phenyl alanine

Phth ..... Phthalimido

PMB ..... *p*-methoxy benzyl

ppm ..... Parts per million

PTSA..... *P*-toluene sulfonic acid

PyBOP.....[(1H-benzotriazol-1-yl)]tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate

q..... Quartet

$R_f$ ..... Retention factor

s ..... Singlet

Ser ..... Serine

$SLe^a$ ..... Sialyl Lewis a

$SLe^x$ ..... Sialyl Lewis x

t ..... Triplet

TE..... 2-(Trimethylsilyl)ethyl

TFA ..... Trifluoroacetic acid

Tf..... Trifluoromethanesulfonyl (triflate)

THF ..... Tetrahydrofuran

TLC ..... Thin layer chromatography

TMS ..... Trimethylsilyl

Ts..... *p*-Toluenesulfonyl

# CHAPTER 1

## INTRODUCTION AND LITERATURE REVIEW

Oligosaccharides of glycoproteins and glycolipids are the most functionally and structurally diverse molecules in nature. Nowadays it is well established that glycoconjugates play essential roles in many molecular processes impacting on eukaryotic biology and disease. For example, glycoconjugates are involved in fertilization, embryogenesis, neuronal development, hormone activities, proliferation of cells, cell-cell communications, cancer metathesis, microbial infections, and chronic inflammation[1]. Such diversity of biological activities has been attributed primarily to the oligosaccharide moiety of these glycoproteins, and glycolipids. Understanding the structure-function relationship of these molecules at a molecular level is a non trivial undertaking which is complicated by the chemical diversity of oligosaccharides. Obtaining sufficient quantities of these complexes glycans for probing biological functions has been a major challenge at the glycochemistry/glycobiology interface. Nonetheless, technological advances of the past decade have laid the foundation for exploiting the diagnostic and therapeutic potential of this class of biomolecules.

Advances in analytical techniques, NMR spectroscopy, and mass spectrometry expedited the isolation and structural determination of oligosaccharides.[1,2] While this made the chemical diversity of oligosaccharides rapidly accessible, it further highlighted

the urgent need for access to sufficient quantities of these molecules to understand the mechanism of actions at a molecular level. The lack of pure, structurally defined complex carbohydrates and glycoconjugates is a major impediment to the rapidly growing field of molecular biology. Besides the fact that these molecules are found only in low concentrations in nature, the identification and isolation of complex carbohydrates are greatly complicated by their microheterogenity. Synthesis of complex oligosaccharides and glycoconjugates is an alternative way to obtain them. However, the chemical complexity of these biomolecules makes them very challenging synthetic targets. These polyhydroxy compounds contain an array of monosaccharide units with a variety of glycosidic linkages among them. Each glycosidic linkage can exist as α- or β-anomer. Therefore, the synthesis of carbohydrates requires many orthogonal protection and deprotection schemes and involves difficult coupling reactions.[3] Nonetheless, many groups have risen to this challenge and several synthetic approaches leading to complex oligosaccharides and glycoconjugates have been reported.[4] Despite these advances, the synthesis of these biomolecules remains time consuming and expensive since there is no one general method that can be applied. This has fueled parallel developments in synthetic glycoconjugate mimics and inhibitors of oligosaccharide functions.[5] It is well established that despite the complexity of the oligosaccharide moieties of glyco-conjugates, the terminal sugars (two to four residues) and their conformations are critical for biological activities. This not only reduces the chemical complexity of the synthetic target(s), but also makes possible the use of revolutionary new synthetic strategies such as combinatorial chemistry for rapid access to potential carbohydrate mimics.

**1.1 Carbohydrates as Targets for Drug Design.**

Carbohydrates-containing biomolecules are found on all cell surfaces and because of their inherent structural diversity, many oligosaccharides are information carriers and recognition molecules through linkages with other component such as lipids and proteins. Many studies have revealed that carbohydrates provide signals for protein targeting and serve as receptors binding toxins, viruses and hormones. They control vital events in fertilization and early development, regulate many critical immune system recognition events and target aging cells for destruction. Cell-cell interactions, such as antigen-antibody interactions and virus-host interactions are classical examples of the aforementioned biochemical functions. Another extremely important and recent discovery in cell-cell adhesion in inflammatory responses is the role of sialyl Lewis$^x$, ($SLe^x$), a terminal tetrasaccharide of glycolipids. Sialyl Lewis$^x$ is displayed on the surface of white blood cells and is responsible for the repair of injured tissues. This particular discovery has significant potential in the development of new nonsteroidal antiinflamatory drugs, as well as anticancer drugs designed to prevent the spread on cancer cells: metastasis.[7,8]

In metastasis, cell surface carbohydrates change upon malignant transformation[6] and are responsible for the significant differences in surface properties between metastatic and nonmetastatic cells. It is also well documented that the total and neuraminidase-releasable sialic acid contents of tumor cell surfaces are closely related to the metastatic potential of the tumor cells.[9-13] These important new discoveries are excellent and logical leads as key steps for rational design of anti-cancer agents for the treatment of metastatic tumors. Particularly, the development of specific cancer vaccines

to induce an anticancer immune response now appears more feasible. This might also offer alternative treatments to chemotherapy and radiation therapy for metastatic cancers as well. The ultimate goal of a cancer vaccine design is the generation of antigen (carbohydrates as targets) specific vaccines (active specific immunotherapy ASI) by using chemically well-characterized synthetic antigens as active immunogens. The fact that particular antigens might be selective, ideally, or specific for cancer cells could be proven to be very useful in stimulating antibody production and promoting active immunity against cancers.

Recent advances in monoclonal antibody technology together with the rapid progress in synthetic and structural chemistry have identified and characterized a number of tumor-associated antigens.[14-18] These new important discoveries in molecular biology, immunology, and synthetic carbohydrate chemistry offer great potential for further development of new and diverse cancer vaccines. The immunological and clinical aspects for cancer vaccine development were reviewed.[19]

Presently, there are several established carbohydrate-based products with bio-pharmaceutical applications as well as other new products with potential applications in medicine. Recently, purified polysaccharides of bacterial origins, for example have been prepared for use as antigenic vaccine against pneumococcal and meningococcal infections. Due to the ability of certain polysaccharides to cross react with other antisera, they may also provide immunity against other infections. Other artificial antigens, glycosylated recombinant proteins and immunoadjuvants are emerging as new areas of interest and extensive research.

Other glycobiology-related areas such as neurobiology, neuropathology, and neuropharmacology have investigated the important function of carbohydrates in the understanding of neurobiology.

### 1.2 Functional Classification of Biologically Active Carbohydrates

Because of the multifunctionality of carbohydrates, it is useful to classify biologically active carbohydrates according to their therapeutic activity such as anti-inflammatory, anticancer, antidiabetic, anticonvulsant, antibiotics as well as antiviral. Traditional classification would divide the existing drugs or new analogues being developed as potential therapeutics into the following chemical classes of derivatives; mono- disaccharides, oligosaccharides, and polysaccharides.

#### 1.2.1 Anticonvulsant Agents

Tropiramate, a simple sugar with strong biological activity, is a prototype of an antiepileptic drug based on fructose (Figure 1.1). In late-phase clinical trials, Tropiramate has proven to be effective as an anti-epileptic drug[20] and through biological screening, the

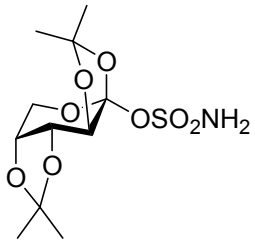



**Figure 1.1.** Chemical structure of Topiramate

anti-convulsant properties of Tropiramate were discovered. As an attempt to establish the relationship between the structure and the biological activity, recently a carboxylic analogue of Tropiramate was synthesized.[21]However, no biological activity was reported.

### 1.2.2 Antiviral agents

4-Guanidino-Neu5Ac2en, is a potent and selective inhibitor for *influenza neuraminidase* ($K_i$ viral=0.1nmol; $K_i$ human=100nmol) (Figure 1.2). When administrated intranasally, it inhibits the replication of both influenza A and B viruses on cell culture and in animal models. [22,23] Many synthetic derivatives were developed by von Itzstein and co-workers.[24-26]Most importantly, the design of this inhibitor is based on protein structure data from a complex of influenza sialidase with an inhibitor.[27]

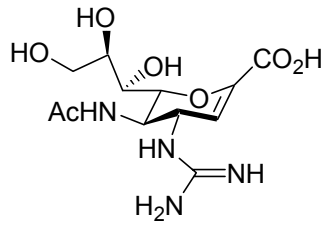



**Figure 1.2.** Chemical structure of 4-Guanidino-Neu5Ac2en

Sialic acid C-Glycoside analogues, previously synthesized by two research groups[28,29] have been recently tested together with new analogues of 4-Guanidino-Neu5Ac2en as potential influenza inhibitors.[30] Encouraging obtained results, opened the door for more research to be done to predict the active site of the receptor as well as to explore other biologically active conformations capable of receptor binding.

Azasugars class of simple monosaccharides with potentially, multiple biological activities, belongs to the polyhydroxypiperidine and polyhydroxypyrrolidine groups. These analogues are derivatives of well known family of specific glucosidase inhibitors originated from nojirimycin and its reduced form, 1-deoxynojirimycin. Both of them showed high potential as antidiabetics[31] and antitumor[32] agents. Since some derivatives of azasugars have exhibited activity against the human immunodeficiency virus (HIV),[33-36] a

tremendous effort has been there in the search for new azasugars and their analogues.[37-46] Deoxyfuconojirimycin[47,48] and α-Homofuconojirimycin[49] ( Figure 1.3) are two examples of this class. Both have $K_i$ of $10^{-8}$ mol $dm^{-3}$ or less against a number of fucosidases, so they are considered as the most potent fucosidase inhibitors yet reported.


**Figure 1.3.** Deoxyfuconojirimycin (left) and α-Homofuconojirimycin (right).

Two other representatives of this class are Castanospermine and Swainsonine[50-53] (Figure 1.4). They showed high potential for treating cancer and viral infections. Castanospermine is also a strong inhibitor of various intestinal glucosidases. Results from biological screening of various castanospermine derivatives showed that alterations of configuration in the piperidine ring as well as removal of the hydroxyl groups lead to significantly weaker α-and β-glycosidase inhibition. Swainsonine, another representative of bicyclic pyrrolidine derivatives, has a powerful inhibitory effect on α-D-mannosidases involved in the biosynthesis of glycoproteins[54] and it also effectively inhibits human B cell development, so it may also be utilized in cancer chemotherapy.[55,56] Surprisingly, swainsonine showed no inhibition for glucosidases.

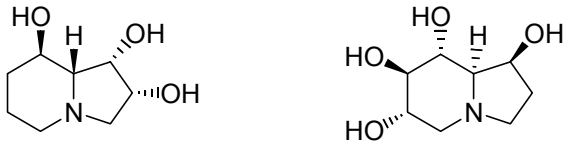



**Figure 1.4.** Chemical structures of (-) Swainsonine and (+) Castanospermine

### 1.2.3 Antidiabetic Agents

#### 1.2.3.1 Glucosidase Inhibitors

Some of the natural α-glycosidase inhibitors[57] originally isolated from various species of *Streptomyces* and strains of the genus *Bacillus*, are now synthesized. Miglitol is considered as one of the first synthesized compounds (Figure 1.5), a hydroxyethyl derivative of deoxynojiramycin, (1,5-dideoxy-1,5-[2-hydroxyethylimino]-D-glucitol. It is fully absorbed and totally excreted by the kidneys. This gives it an advantage over Acarbose, another α-glucosidase inhibitor from the pseudooligosaccharide family, is poorly absorbed. It is also distributed rapidly and heterogeneously to tissues and organs of rats. Also, no biotransformation of Miglitol was observed in rats or human. Emiglitate (1,5-dideoxy-1,5-[2-4-ethoxycarbonylphenoxy]ethylimino-Dglucitol), another analogue of Miglitol, is more lipophilic than Miglitol, but its inhibitory profile is not as useful.

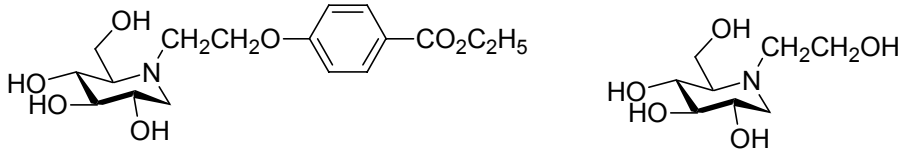
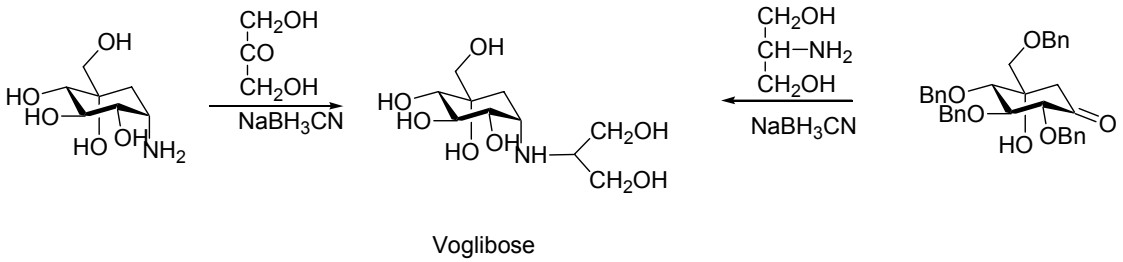



**Figure 1.5.** Chemical structures of Miglitol (right) and Emiglitate (left)

Voglibose, another α-glucosidase inhibitor, is obtained either by reductive alkylation of valiolamine with hydroxyacetone (Scheme 1.1) or by reductive amination of the inosose (synthetically available) using 2-aminopropane-1,3-diol, followed by de-benzylation. Voglibose, is an extremely potent sucrase ($IC_{50}$ $4.6x10^{-9}$ M) and maltase ($IC_{50}$ $1.5x10^{-8}$ M) inhibitor.




**Scheme 1.1.** Synthesis of Voglibose

MDL 73945, a C-disaccharide analogue of nojirimycin (Figure 1.6), shows high and selective inhibition for intestinal α-glucohydrolases, which effectively reduces glycemic and insulin responses to a carbohydrate load and has a long duration of action.[58]

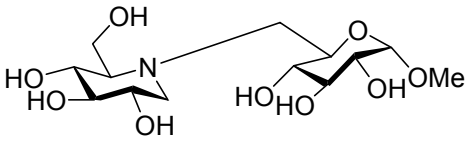
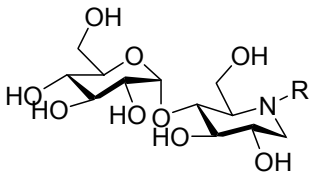



**Figure 1.6.** Chemical structure of MDL 73945

MDL 73945, as a new derivative, warrants further evaluation as a drug for reducing post-prandial hyperglycemia in both insulin-dependant and non insulin-dependant diabetes mellitus (NIDDM) because of its uniqueness among the known intestinal α-glucohydrolase inhibitors.

On the other hand, azadisaccharides, a nonclassified disaccharides class, shows high specificity in the inhibition of glucohydrolase and potential antidiabetic activity. Inhibitors of this class have relatively simple structures with nitrogen in the sugar ring as in glucopyranosyl moranolines[59] (amino-bridged disaccharides, Figure 1.7).




R= -H, -$CH_3$, -$C_2H_5$, -$C_3H_7$, -$C_4H_9$,$BrC_6H_4CH_2$-, $CH_3C_6H_4CH_2$-,

**Figure 1.7.** Chemical structures of Glucopyranosyl Moranolines

Acarbose, an oligosaccharide therapeutic (Figure 1.8), was the first of a new class of drugs for treating NIDDM. Acarbose is a complex oligosaccharide originally isolated from *Actinoplanes*. It is reportedly useful as an adjuvant therapy in diabetes.[60-62] By inhibiting α-glucosidase, Acarbose delays carbohydrates metabolism in the gastrointestinal tract and modulates changes in food induced blood sugar levels. Also it is

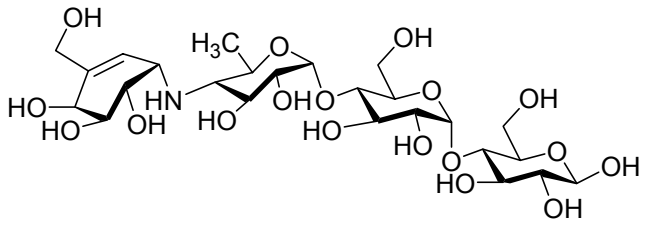



**Figure 1.8.** Chemical structure of Acarbose

potent and fully competitive inhibitor of glucoamylase, sucrase, maltase, and isomaltase activity in the small intestine of various species. Particularly, it reversibly and competitively inhibits two enzymes involved in carbohydrate digestion, the pancreatic α-amylase and membrane bound intestinal α-glucosidase. Pancreatic α-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine, whereas α-glucosidases hydrolyze oligosaccharides, tri, and disaccharides to glucose and other mnonsaccharides in the brush border of the small intestine. Most importantly, Acarbose does not inhibit lactase, so it is not expected to cause lactose intolerance. The evidence

from the clinical evaluation of α-glucosidase inhibitors, much of which was based on Acarbose, showed that they can be effective therapeutic agents in both type I and especially in type II diabetes. A number of β-glucosides of Acarbose have been prepared which are hardly more effective than Acarbose itself.

### 1.2.3.2 Carbohydrate Biguanides

Carbohydrate biguanides, an interesting group of potential antidiabetics activity, were developed by the R.W. Johnson Pharmaceutical Research Institute.[63,64] These monosaccharides containing a biguanide functionality exhibit a significant hypoglycemic activity. Two of the most active biguanides are glucose analogues with biguanidine group at C-6 in which the hydroxyls are substituted with alkyl moieties. This is a classical example of biologically active analogues resulting from incorporation of a guanidine moiety (Figure 1.9).

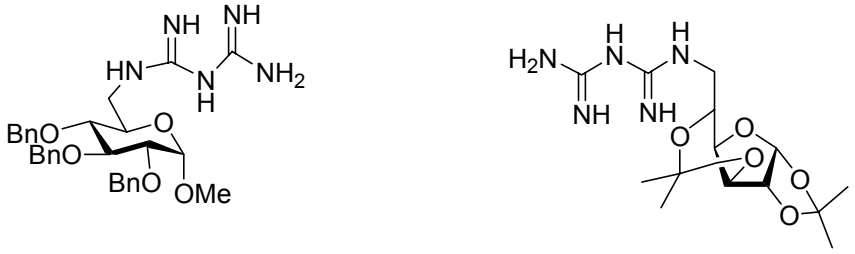



**Figure 1.9.** Chemical structures of new biguanidine carbohydrate antidiabetic agents

Interestingly, this group of natural and modified carbohydrates is one of the most important leads in the development of carbohydrates based drugs. Clinical studies are underway to verify and evaluate the expected pharmacological activities.

### 1.2.3.3 Inositol Phosphates

Recent advances in molecular and cellular biology have demonstrated that the receptor-controlled hydrolysis of phospholipids leading to intracellular second messengers is a fundamental and widespread mechanism for the transmission of signals across cell membranes such as signal transduction. Inositol phosphate esters play a key role in the linkage of large numbers of surface glycoproteins and cell membranes[65] through the glycophosphatidyl inositol (GPI) anchor molecule. Moreover, phospho-oligosaccharides containing an inositol phosphate moiety of similar structure to the GPI may have insulin mimetic activity and could be the intracellular second messengers of insulin,[66] possibly derived from the GPI. Also, inositol phosphate glycan fragments produced from GPI anchors can reproduce the actions of insulin and the insulin sensitive inostiol glycan on fat and liver cells. Considering that the exact structure of the GPI anchor is known, the design and synthesis of insulin-mimetic compounds are logical steps to exploit the above class of compounds. This discovery has stimulated research for antidiabetic drugs structurally related to GPI for the treatment of non-insulin dependant Diabetes Mellitus (NIDDM).

### 1.2.4 Antitumor Agents

One important growth signaling pathway also utilizes inositol phosphates to convey information inside the cell. When an oncogene is over-expressed or constitutively activated through mutation, the cell receives a continuous signal to grow. This mechanism suggests the possibility of controlling oncogenesis by inhibiting the signaling pathways and mediating the effects of activated oncognes. This is a new and

mechanistically based approach to develop new classes of drugs that possibly can control cancer cell growth.[67,68] The race is on to develop a simple analogue of inositol phosphate derivatives with the ability to prevent the formation and growth of cancer cells of any form. Interestingly, derivatives of *myo*-inositol substituted at the 3-position selectively inhibited the growth of cells in the absence of physiological concentration of *myo*-inositol.[69] Two potential inhibitors of cancer cell growth *in vitro,* 3-deoxy-3-fluoro-*myo*-inositol and 3-deoxy-3-fluoro-phosphatidylinositol[70] (Figure 1.10) clearly demonstrate this approach to the control of cell proliferation.

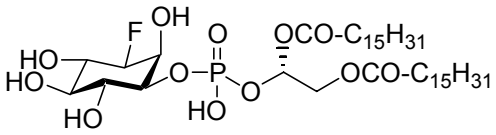



**Figure 1.10.** Chemical structure of 3-deoxy-3-fluoro-phosphatidyl inositol

Among the simple monosaccharides, Streptozotocin and Prumycin are two important aminosugars with antitumor activities (Figure 1.11).

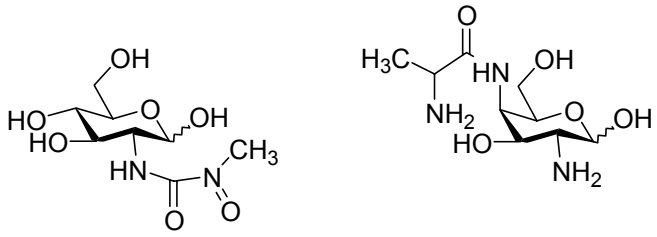



**Figure 1.11.** Chemical structures of Streptozotocin (left) and Prumycin (right)

Streptozotocin[71] is important only as an antitumor and diabetogenic agent, owing to its specific toxicity to the B-cells of the islets of Langerhans. It is clinically useful in the therapy of malignant insulinomas and Hodgkins' diseases. However, this anti-

neoplastic antibiotic is highly toxic when administered orally. On the other hand, Prumycin has antitumor activity, but less active than Streptozocin. Both antibiotics, however are used only in rare critical cases when alternative therapy can not be administered.

### 1.2.4.1 Sialyl Lewis$^x$ (sLe$^x$) mimics

The trisaccharide determinant 3-*O*-α-fucosyl-*N*-acetyllactosamine known as Lewis$^x$ antigen, has been found both in glycoprotein and in membrane glycolipids of the mammalian cells.[72] The glycan with an α-(2-3)-sialylated galactose moiety known as sialyl Lewis$^x$ (sLex) (Figure 1.12). It is a widely distributed ligand for glycoprotein receptors (lectins) known as selectins L, E, and P. The biochemical mechanism of the cell adhesion process involves recognition and binding of carbohydrate ligands like sLe$^x$. It is crucial to these new carbohydrate mimetics to determine the biologically active conformation of the carbohydrate ligand when bound to selectin. From reported data,[73] it seems that the structural elements of the sLe$^x$ unit which are essential for recognition by E selectin include the carboxylic function of the sialic acid, the two hydroxyl functions at C-4 and C-6 of the galactose residue, and the three hydroxyl functions of the fucose moiety. Interestingly, the methyl group at C-5 of this L-sugar unit is not essential for the activity and can be replaced by an arabinose unit.[73] This finding, together with the modification of the fucosyl moiety, have been explored by the synthesis of the carbocyclic analogue of GDP-fucose,[74] GMP fucose derivatives,[75] the C-trisaccharide[76] of Le$^x$, and the bicyclic α-L-homofucose.[77]

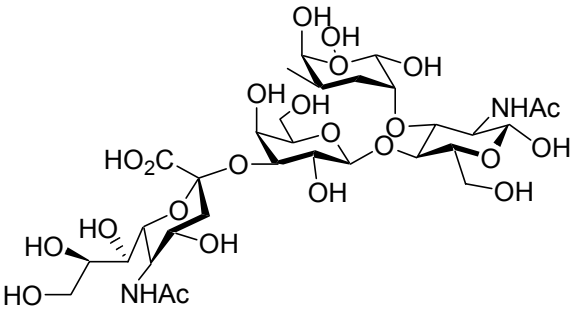


**Figure 1.12.** Structure of $sLe^x$

The thio analogue of L-fucose,[78] one of few very powerful inhibitors of α-L-fucosidase, is a promising target for further development of the above class of inhibitors. A class of (1-4)-linked, S-thiodisaccharides containing 5-thio-L-fucose and L-fucose units[79-81] (Figure 1.13) was synthesized and tested against L-fucosidase from bovine kidney and bovine epididymis revealing a mixed mode of inhibition at $K_i$=4.9 mM and $K_i$=3.59 mM respectively. Preliminary data[82] reported that the 5-thio analogue of (1-2)-linked thiodisaccharides showed a potent competitive inhibition of $K_i$=30 mM against α-L-fucosidase from bovine epididymis.

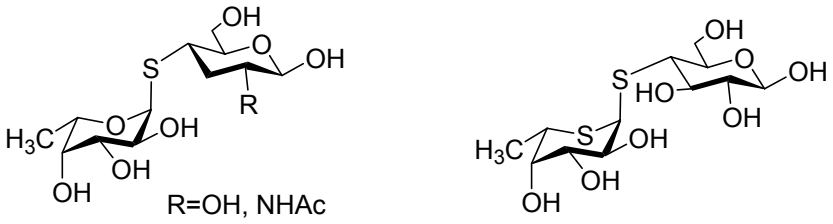



**Figure 1.13.** Chemical structures of new thio-disaccharides

### 1.2.4.2 Polysaccharides

Although polysaccharides are one of the most important classes of natural products, their significance as therapeutics is limited. Only some nontoxic antitumor polysaccharides, derived from bacteria, fungi, and algae have demonstrated important biological activity. For example, antitumor activity is observed in the case of (1-3)-β-D-glucans having an α-D-glucopyranosyl group linked (1-6) to every 3rd or 4th residue of the main chain. In some cases the (1-4) linkages also exist. Lichen glucans generally have β-D-(1-6) only. The molecular weights of the glucans vary from 10,000 to 1,000,000 with averages of 50,0000 to 100,000.

Some polysaccharides have antibacterial and/or antifungal activity. Generally, most of the polysaccharides of this group have host-mediated antitumor activities. This type of oligosaccharides does not affect the tumor cell directly, but after administration they stimulate the reticuloendothelial system of the host to produce antitumor substances resulting in a host-mediated action. Moreover, because of the lack of any cytotoxicity, they are nontoxic to the host.[83] Schizophylan[84] is an example of the above β-glucan family. It was introduced under the trade name Sonifilan. Schizophylan is an immuno-stimulant, isolated from the culture filtrate of *Schizophyllum commune*. It is useful in combination with other antineoplastic treatments in the management of carcinomas of the lungs, stomach, uterus, and breasts. Interestingly, branched (1-3)-β-D-glucans, related to Schizophyllan with D-arabinosyl or D-mannosyl branches exhibited high antitumor activity, while branched (1-4)-D-glucans with similar side chains showed no antitumor activity[85].

Among the most important *in vivo* effects of β-glucan is the stimulation of antitumor responses, cytokines, wound healing, resistance to microbial challenge, and hematopoesis.

Another class of complex carbohydrates is represented by Dextran with a molecular weight lower than 70,000. Dextran has been used extensively as a plasma expander. Plasma expanders are non-antigen and are degraded *in vivo* by means of a dextranase found in the liver. These aqueous polymer solutions are capable only of replacing the blood volume and can not carry oxygen.

An additional polysaccharide, starch transformed into the water-soluble hydroxyethyl starch (HES) derivative, has attracted interest as a plasma substitute, since it can have a significant intravascular half-time depending on the substitution ratio in hydroxyethyl groups that hampers α–amylase attack. HES has shown to be even better tolerated than Dextran. The other classical representative of the above class of polysaccharides is sodium celluose phosphate developed for the treatment of type I absorptive hypercalciuria.

### 1.2.5 Antibiotics

#### 1.2.5.1 Thio Antibiotics

Thio sugars, as potential therapeutics have gained attention due to their biological activities which include anthelmintic, antifungal, antiviral, antitumor, herbicidal, and insecticidal.[86,87] An interesting example of the thioglycoside moiety occurring in the linkosaminide family of antibiotics is represented by Lincomicin.[88] Linkosaminide

antibiotics[89] are characterized by an alkyl 6-amino-6,8-dideoxy-1-thio-α-D-galacto-octa-pyranoside joined with a proline ring by an amide linkage.

Clindamycin and Celesticetin are two other representatives of this class of molecules. Modification of the sugar moiety of Linkomycin by the introduction of a chloride atom at C-7 in the reaction with thionyl chloride produced the new analogue Clindamycin[90]. Celesticetin is the second analogue with a modified thiosugar moiety at C-1. Clindamycin is utilized in the treatment of common infections caused by gram-positive cocci also it highly effective in the treatment of anaerobic infections including actinomycosis. Natural thio sugars also include a new family of eneydiyne antibiotics. [91] This family consists of Esperamycin, Calichemicin, and Dynamycin A. Enediyne antibiotics are among the most potent antitumor agents with ng/ml level *in vitro* and/or *in vivo* activity.

**1.2.5.2 Aminoglycoside Antibiotics**

The aminoglycoside class of antibiotics is one of the most important and useful among traditionally recognized carbohydrate therapeutics.[92-94] The majority of the aminoglycosides are produced by *Actinomycetes*. A few new representative members from this class have been recently introduced to the market, among them: Arbekacin under the trade name Habekacin. Arbekacin is a semi-synthetic derivative of dibekacin (Figure 1.14) that used for the treatment of bacterial infections.[95-97]

Aminoglycosides are active against a broad spectrum of bacteria, including some of the gentamycin, kanamycin, and tobramycin resistant pathogens. Compared to well known Amikacin and Dibekacin, Abekacin has milder ototoxicity.

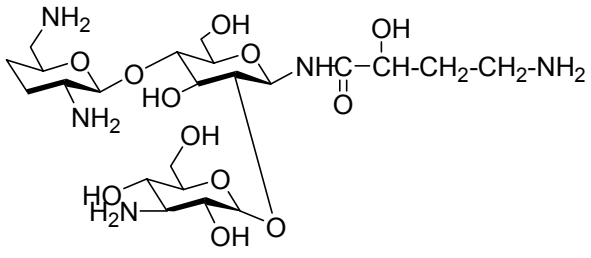


**Figure 1.14.** Chemical structure of Arbekacin (Habekacin)

### 1.2.5.3 Macrolide Antibiotics

Macrolide antibiotics are produced as secondary metabolites of soil micro-organisms. The majority of them have been produced by various strains of *Streptomyces*. Macrolide antibiotics are classified according to the size of the macrocyclic lactone ring forming the aglycone, either as 12-, 14-, or 16-membered ring macrolides. They are poly-functional molecules that at least contain one aminosugar moiety. One of the new macrolide antibiotics is Clarithrmycin (Figure 1.15). It was introduced under the trade name Klacid. It is an acid stable antibiotic used for the treatment of the skin, urinary, and respiratory tract infections.[98-100] Compared to Erythromycin, Klacid exhibits almost the same *in vitro* activity against conventional pathogens, but it is far better tolerated due to fewer gastrointestinal problems.

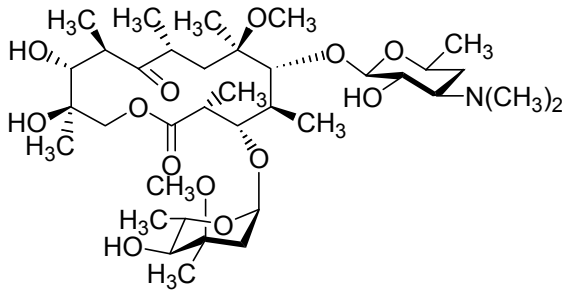


**Figure 1.15.** Chemical structure of Macrolide Antibiotic Clarithromycin

### 1.2.5.4 Avermectins Antibiotics

These types of natural products have excellent insecticidal activity.[101] The complex structural diversity of Avermectins is an important factor for further development of new analogues of this particular class of antibiotics[102] (Figure 1.16). Biological studies revealed that introducing of an amino or aminoethyl function in C-4'' of L-oleandrose significantly increased the insecticidal activity.

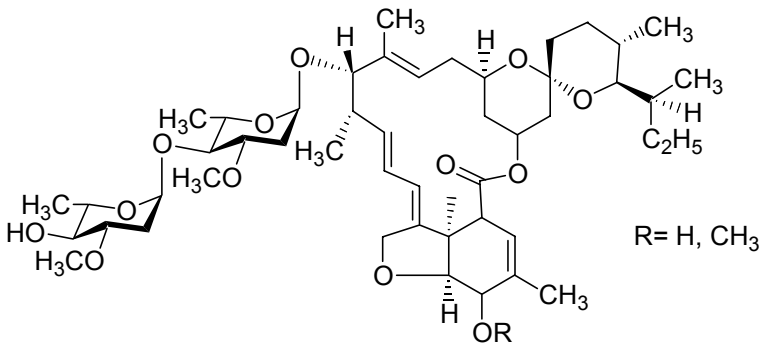



**Figure 1.16.** Chemical structure of Avermectins

### 1.2.5.5 Oligosaccharide Antibiotics

This class of carbohydrates with very complex structures and many asymmetric centers is classified into four categories: Everninomycins, Curamycins, Avilamycin, and Falmbamycins[103] (Figure 1.17). Everninomycins are the first among this class of antibiotics to have their precise structures determined. They are classified as B, C, D, and D1. The two main Everninomycins B and D oligosaccharides are produced by *Micromonospora carbonacea.* They are active against a variety of strains of *Staphylococcus, Streptococcus, Bacillus* and *Mycobacteria*, but they have no activity against *Enterobacteriace* or *Pseudomonas*. Flambamycins are produced by *Streptomyces*

*hygroscopicus.* The structure-activity relationships in this particular group of unusual antibiotics are not completely understood but the presence of the free phenolic hydroxyl group and the ortho ester linkages, however, are essential for antibiotics activity.

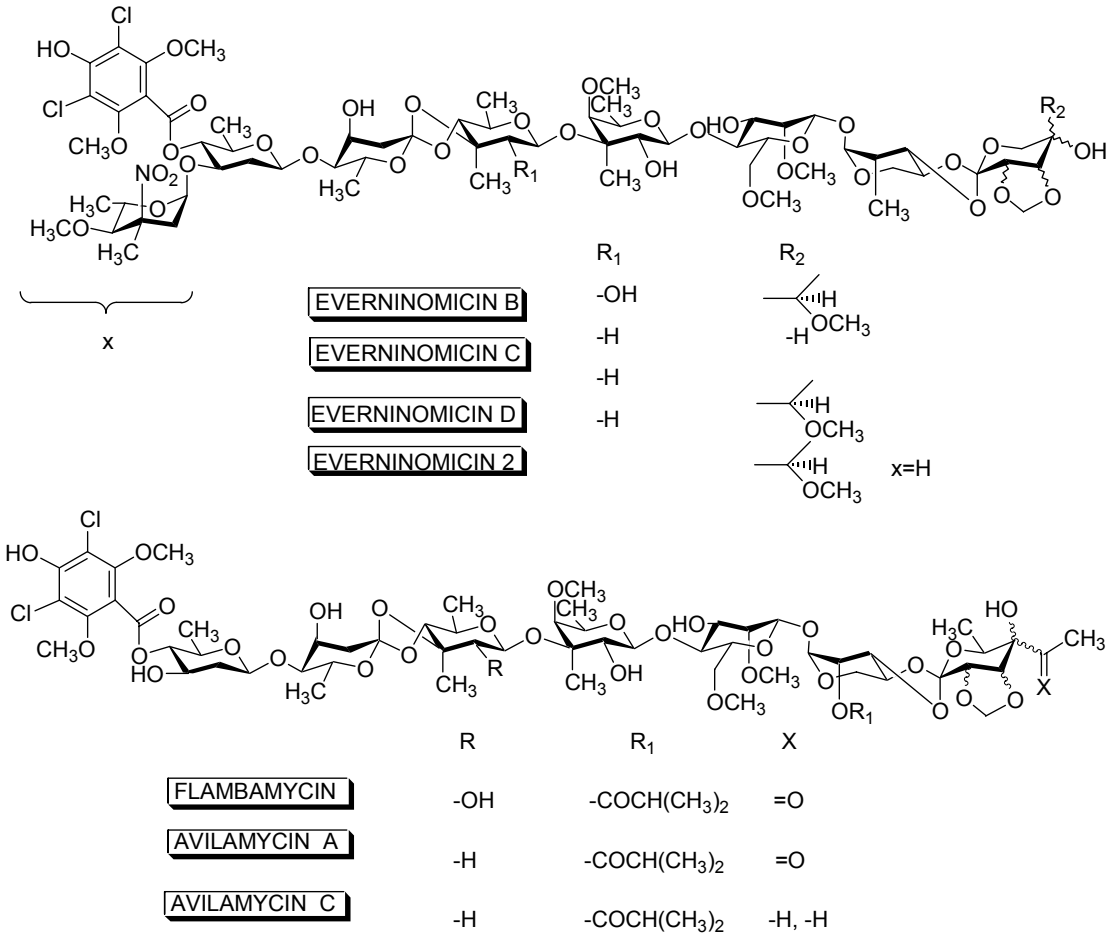



**Figure 1.17.** Chemical structures of oligosaccharide antibiotics: Avilamycins, Flambamycin, and Everninomicins.

### 1.2.6 Anticoagulant Agents (Heparin Analogues)

Anticoagulant agents are constant source of attention and research. Heparin (Figure 1.18) occurs in the tissue of vertebrates, especially in the lung, liver, intestine mucosa, thymus, spleen, heart, lymph, vascular endothelium, and plasma, where it is

stored exclusively in the granules of basophilic granulocytes and mast cells, respectively.[104] It forms complexes with histamine and proteins. Its turnover is very slow and it is released only after cell lysis. Most likely, heparin does not act as an anticoagulant in the organism, but possibly has an important function for cell protection by binding of proteinases.[105] Although, the biological role of endogenous heparin is not completely understood, its use for a variety of medical indications has not been precluded. Its use as an antithrombotic drug was the first and still the most important therapeutic application. For six decades, heparin has been the drug of choice in the prevention and treatment of arterial thromboembolic disorders[106] including pulmonary embolism, acute myocardial infraction, unstable angina pectoris, acute peripheral vascular occlusion, cerebral insult, and disseminated intravascular coagulation. Moreover, heparin modulates the immune system and it is able to regulate complement resulting in anti-inflammatory properties. Finally, the antiviral effect, especially the inhibition of herpes-simplex virus type 1, has to be mentioned.

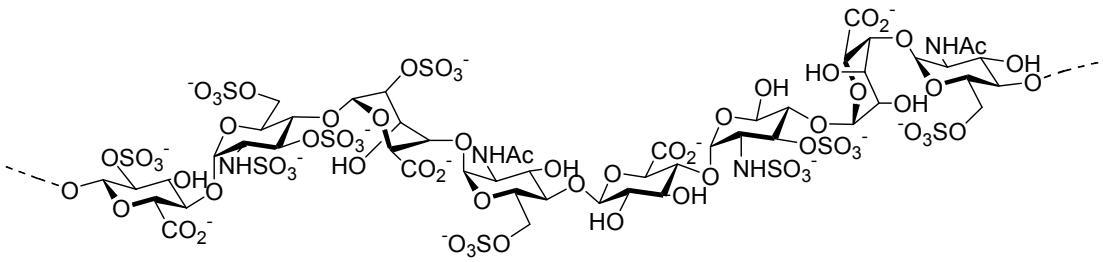


**Figure 1.18.** Hypothetical fragment of a Heparin molecule

### 1.2.7 Miscellaneous Agents

Among the large number of unclassified carbohydrate therapeutics (mainly mono- and disaccharides) with a wide spread of biological activities, one particular class of

antirheumatic agents should be mentioned. An interesting representative of this class of derivatives is Auranofin (Figure 1.19), used clinically for the treatment of chronic rheumatoid conditions such as rheumatoid arthritis. This compound was introduced under the trade name Ridaura.[107] It is the first orally effective derivative of gold to be marketed. Moreover, it is better tolerated and more convenient than gold sodium thiomalate which is administered intramuscularly. Other examples of synthetic carbohydrates are Amiprilose and its hydrochloride salt, Therafectin (Figure 1.19). Both are known to exhibit antiproliferative and anti-inflammatory activities.[108] Amiprilose acts as an immunomodulator[109] and therefore has a therapeutic effect on autoimmnune disorders such as arthritis, psoriasis, eczema, and systemic lupus erythematosus.[110-112] It also has low toxicity and no serious side effects[113,114] but it is required in large doses for effective therapy. This presents a problem, especially for oral administration because treatment of inflammatory or autoimmune disorders is often chronic.

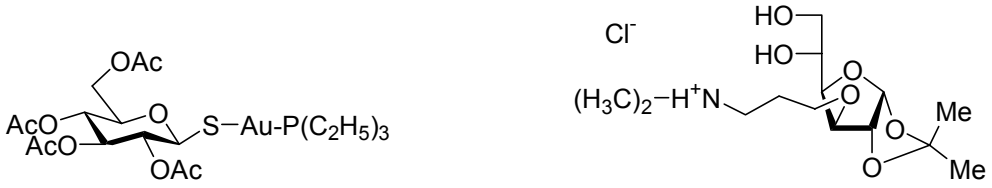



**Figure 1.19.** Chemical structure of Auranofin (left) and Therafectin (right)

The recent reports[115,116] on the synthesis of new 6-thio analogs of Amiprilose showed that they are approximately 5-1000 times more potent than the parent.

Sucralflate is another example from the above class of sugars (Figure 1.20). It is an aluminum hydroxide complex of sucrose sulfate currently used in the therapy of

duodenal ulcers.[117]It acts by selectively binding to necrotic ulcer tissues, thus counteracting further destruction to the mucosa.

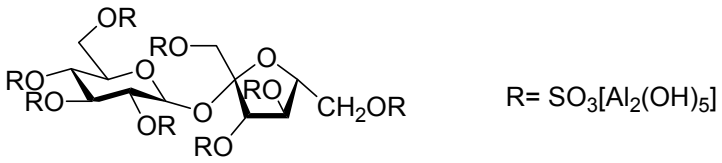



**Figure 1.20.** Chemical structure of sucralfate

### 1.3 Synthesis of Oligosaccharide Libraries

Oligosaccharides can be isolated from natural sources, but only small quantities can be obtained. Synthesis of oligosaccharides is an attractive alternative. Enzymes have been employed in oligosaccharide synthesis,[118] but this method is hampered by the lack of sufficient variety of commercially available glycosyltransferases and glycosidases. At the moment, the most convenient method to obtain oligosaccharides is based on chemical synthesis. This requires high yielding and stereoselective procedures for glycosidic bond formation, and in the past two decades new reagents and procedures that partly fulfill these requirements have been developed.

Combinatorial chemistry, a multi-dimensional strategy, has evolved to meet the growing demand for economical synthesis of large number of diverse chemical compounds in a relatively short time. In this approach, a large array of building blocks is chemically assembled to give all possible combinations, either in solution or more commonly, on a solid support. The collection of compounds can be generated using a "split-pool" or "parallel" synthetic strategy. This diverse collection of compounds, a chemical library, is then screened for biological activities. Combinatorial libraries have

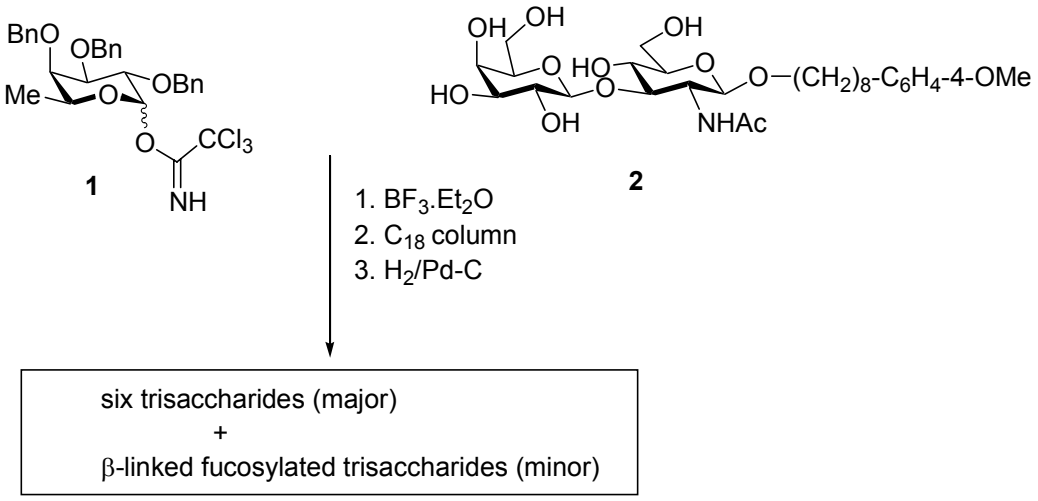
now been added to the repertoire of strategies used in the pharmaceutical sector for lead discovery and lead optimization, as many aspects of this evolving technology.

### 1.3.1 Combinatorial Oligosaccharide Synthesis

The polyvalent nature of carbohydrates and the lack of a general method to form glycosidic linkages have resulted in unique approaches for the generation of oligo-saccharide libraries.[120] The challenge to gain access to monosaccharide building blocks continues, which can be readily synthesized and assembled in a controlled combinatorial fashion. Having generated the library, purification and analysis are equally important. Both solution- and solid-phase strategies have been developed in the search for libraries of oligosaccharides for biological investigation.

#### 1.3.1.1 Random Glycosylation

The first synthesized oligosaccharide library consisted of di- and tri-saccharides was produced by a random glycosylation strategy in solution (Scheme 1.2). As reported by Hindsgaul *et al.*[121] This approach circumvented the need for numerous orthogonally protected monosaccharide building blocks. A fully benzylated trichloroacetimidate glycosyl donor **1** was coupled with disaccharide acceptor **2**, a *p*-methoxyphenoxyoctyl glycoside with six unprotected hydroxyl groups to give a mixture of all six possible trisaccharides in a single step. In this reaction only about 30% of the disaccharide acceptor was fucosylated and interestingly, all the OH groups showed similar reactivity. Chromatographic separation by HPLC and NMR confirmed the structures of the tri-saccharides.



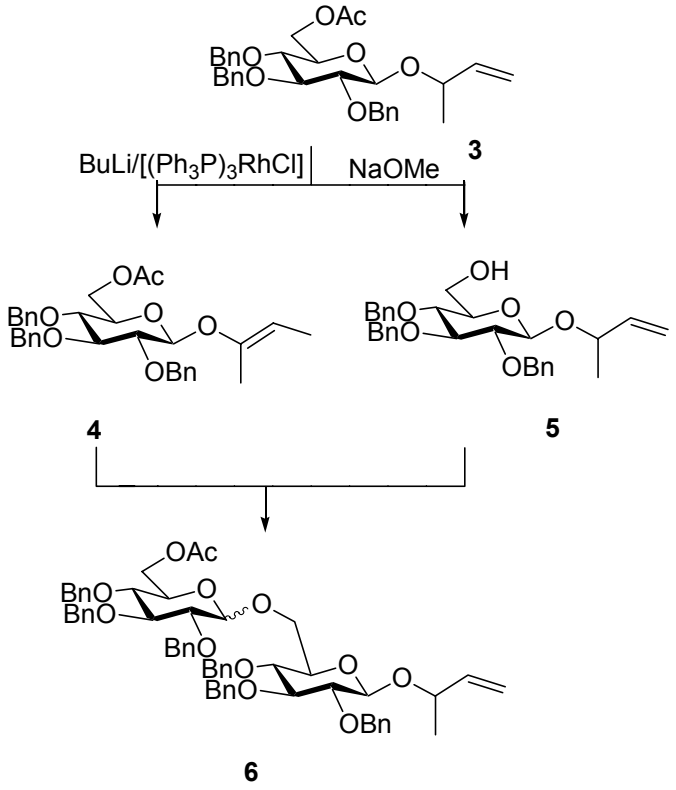

**Scheme 1.2.** Hindsgaul's random glycosylation

Using this strategy, Hindsgaul's group further investigated a fucosyl-transferase enzyme present in human milk using a disaccharide mixture in which active compounds were present in less than 5%.[122] However, the uncontrolled glycosylation reaction, low yield and the need for extensive purification limit the widespread applicability of this methodology.

### 1.3.1.2 Latent Active Glycosylation Strategy

An alternative solution phase combinatorial approach was developed by Boons *et al.*[123] In this novel latent-active glycosylation approach (Scheme 1.3) one major building block, 3-buten-2-yl glycoside **3** which can be converted into a glycosyl donor and acceptor was used.[124] Isomerization of compound **3** with BuLi/[$(Ph_3P)_3RhCl$] gave the glycosyl donor **4** whereas removal of the acetyl group of **3** gave the glycosyl acceptor **5**. Coupling of compounds **4** and **5** gave the disaccharide **6** in 89% yield as an anomeric

mixture. Using this methodology, building blocks containing other selectively removable groups such as *p*-methoxybenzyl ether were prepared and used for the solution-phase synthesis of mixtures of linear or branched trisaccharide libraries.[125] These libraries were readily purified by gel-filtration chromatography and contained over 80% of the expected products.

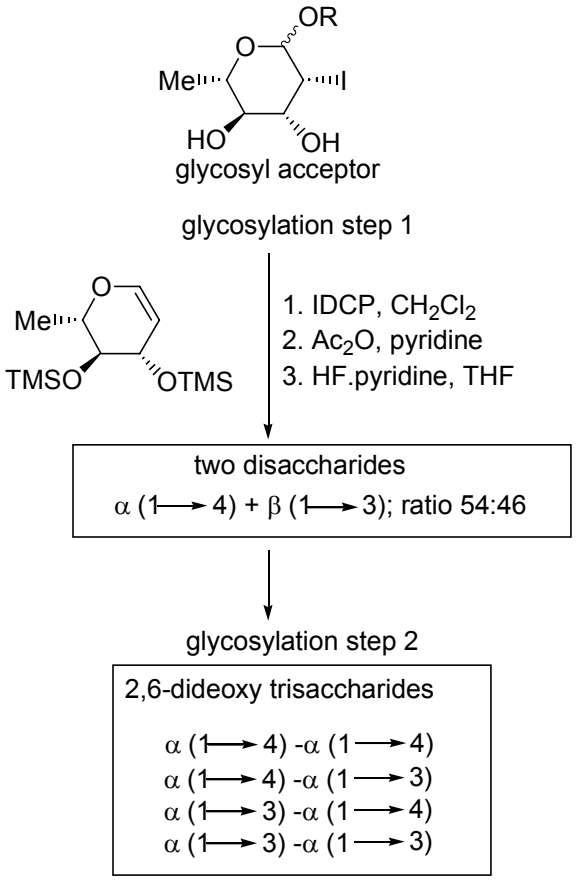



**Scheme 1.3.** Boon's latent-active glycosylation

### 1.3.1.3 Stereoselective and non-Stereoselective Glycosylation

Ichikawa's group has developed a ''stereoselective, yet non-regioselective'' glycolsylation approach towards solution-phase combinatorial oligosaccharide synthesis.[126] Only one monosaccharide building block, 6-deoxy-3,4-di-*O*-trimethylsilyl-L-glucal was utilized in the synthesis of a small library of 2,6-dideoxy trisaccharides in

the search for antitumor agents (Scheme 1.4). The stereoselectivity of the glycosidc linkage (α-anomer) was controlled by performing the glycosylation reaction under iodinium ion catalyzed conditions.



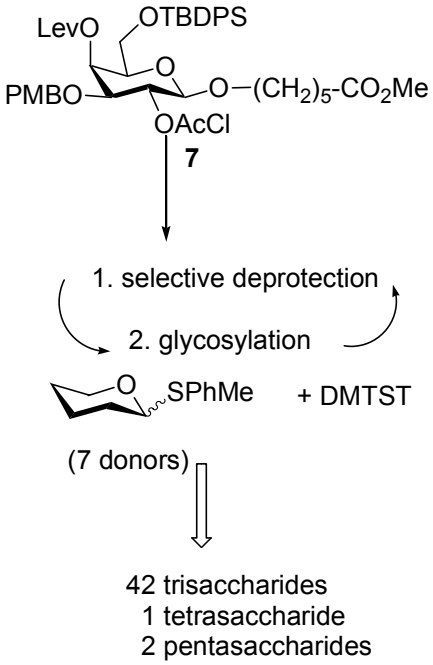

**Scheme 1.4.** Ichikawa's 2-6-dideoxy-based trisaccharide library synthesis

The glucal was first coupled to 6-trifluoroacetamidohexanol in the presence of iodinium di(*sym*-collidine)perchlorate (IDCP) to generate the α-glycoside and an iodo-group at the 2-position. Subsequently, the glycosyl acceptor having two free hydroxyl groups was obtained by removal of the silyl groups. After two cycles of glycosylation under IDCP catalysis, regioisomeric linear trisaccharides were obtained in 73% yields.

Since each glycosylation reaction generated an iodo-group at the 2-position, the mixture can undergo further modification.

### 1.3.1.4 Orthogonally Protected Carbohydrates

Wong and co-workers[127] have utilized a versatile central monosaccharide building block with four selectively removable protecting groups for the parallel combinatorial synthesis of a disaccharide library (Scheme 1.5).




**Scheme 1.5.** Wong's orthogonally protected building block approach.

The key compound is a monosaccharide glycosyl acceptor **7** with a chloroacetyl (ClAc), *p*-methoxybenzyl (PMB), levulinoyl (Lev), and *tert*-butyldiphenylsilyl (TBDPS) group, in which every protecting group can be removed selectively in high yields. In this synthesis, seven thioglycoside donors were coupled in the presence of (dimethyl-thio)methylsulfonium triflate (DMTST) with the selectively deblocked glycosyl acceptor.

They demonstrated efficient orthogonal protection-deprotection schemes in the parallel solution synthesis of a library of 45 oligosaccharides.

### 1.3.1.5 Split-Pool Approach

Lubineau and Bonaffe[128] developed a split-pool library approach for the synthesis of all sulfoforms of chondroitin sulfate (CS) disaccharide. An orthogonally protected disaccharide was central to the success of the synthesis (Figure 1.21).

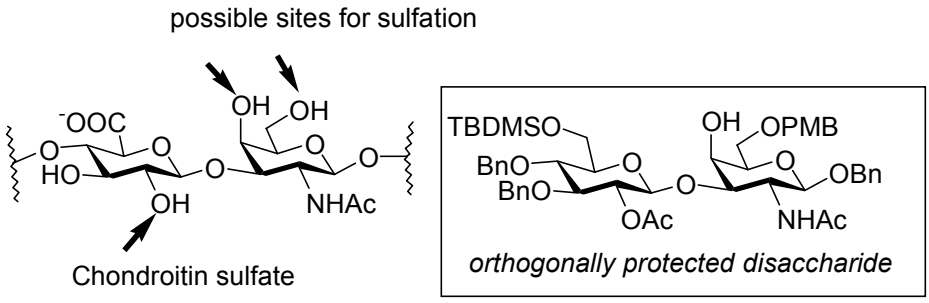



**Figure 1.21.** Bonnaffe's combinatorial approach to chondroitin sulfate disaccharide

Authors demonstrated that Sulfate ester protecting groups are effective in the crucial C-6 oxidation of a glucosyl to a glucuronyl. Since natural chondroitin sulfates of glycosaminoglycans are chemically modified upon enzymatic or chemical degradation, the synthesis of sulfated CS will undoubtedly contribute to exploring the biological functions of these glycoconjugates.

### 1.3.1.6 One-Pot Glycosylation

The reactivity of the *p*-methylphenylthioglycoside of different monosacchrides with different protecting groups (e.g. electron-donating or electron-withdrawing leaving groups) has been quantitatively evaluated by Wong's group in the search for a facile

strategy for oligosaccharide synthesis.[129] This has led to the development of a computerized database of anomeric reactivity values for orthogonally protected thioglycosides.[130] This database was then used for the selection of glycosyl donors and acceptors for the one-pot, parallel solution phase synthesis of a library of oligo-saccharides.

Takahashi's group has also synthesized a library of 72 trisaccharides by solution-phase one-pot glycosylation.[131] In this approach a combination of bromo glycosides, phenylthioglycosides and 2-bromoethyl glycosides of glucose, galactose, and mannose in the presence of selective activating agents were rapidly assembled on a QUEST 210 manual synthesizer in good yields (64% to 99%).

The above chemoselective one-pot glycosylation approach may prove to be very powerful strategy in the future generation of combinatorial oligosaccharide libraries. Furthermore, the use of Wong's Optimer database for selection of glycosyl donors and acceptors, and Takahashi's manual synthesizer approach would certainly rival solid-phase approaches for the rapid synthesis of oligosaccharide libraries. Standard work-up and purification for larger libraries may be more challenging for routine library synthesis. In this respect, solid-phase approaches may simplify product isolation and purification in the generation of larger oligosaccharide libraries. However, solid support oligosaccharide synthesis requires an initial investment in optimization steps for adapting solution synthesis to a solid support. This approach also embodies additional challenges. The resin, linker, and the screening techniques to be used must be considered in planning the library. [132] It is therefore not surprising that very few oligosaccharide libraries have been successfully synthesized on the solid support so far.

### 1.3.2 Solid Phase Oligosaccharide Synthesis

Despite many recent advances, solution based synthesis of complex oligosaccharides has still many problems. Many of the reactions performed for the synthesis of oligosaccharides, glycosylations in particular, are often incomplete and side reactions result in the formation of by-products. This makes purification, usually achieved by chromatography, necessary after each synthetic step. The whole process thus becomes tedious and time consuming. In order to overcome these limitations, considerable efforts have been directed to adopt solid phase synthesis for the preparation of oligosaccharides. In principle, large excess of reagents can be used to drive glycosylation reactions to completion and excess of the reagents can be easily removed by washing the solid support. Recent advances have indeed demonstrated that many efficient methodologies used for oligosaccharide synthesis in solution phase can be employed on solid support.

#### 1.3.2.1 Linkers for Solid Phase Organic Synthesis[133]

The attachment of a compound to a solid support is achieved through a cleavable linker. Linkers perform similar functions as protecting groups and many of the linkers developed in recent years are based on functional groups frequently used in solution phase synthesis. An ideal linker should be cheap and readily available. The attachment of the starting material should be readily achieved in high yield. The linker should be stable to the chemistry used during the synthesis and its cleavage should be efficient under conditions that do not damage the final product. One of the key challenges is to utilize cleavage reagents that are easily removed from the final cleaved product. Many linkers do not meet all of these criteria.

### 1.3.2.2 Polymers

The most commonly used polymer backbone in solid phase synthesis is polystyrene, crosslinked with 1 or 2% divinylbenzene.[134] These resins withstand a wide range of reaction conditions, and are compatible with a variety of polar and apolar solvents, (*e.g*. DMF, dichloromethane, THF, acetonitrile). These resins have to swell in the reaction solvent in order to make the polymeric network accessible to the reactants. The accessibility of the internal volume of the polymer for the substrate plays a decisive role. For the loading capacity of a polymer to reach an appreciable extent, the substrate must penetrate the internal volume of the beads. To achieve this, the polymer must swell efficiently, when solvents such as DCM or DMF are used, the resin swells well enough (3-6mL/g) to achieve good loadings. Upon swelling the polymer becomes very soft and flexible. Mixing can be achieved by employing shakers, or bubbling gas through the suspension, thus avoiding prolonged stirring that can cause mechanical damage to the resin. Higher degrees of cross-linking, up to *ca.* 5% provide more stable resins to physical damage, but the high degree of crosslinking reduces their swelling and results in lower loading capacity. A second approach to introduce mechanical stability consists of grafting an organic polymer on an inorganic macroporous support, such as glass or silica.[135] In contrast to the swellable resins, these supports show a permanent porosity, and no swelling is necessary. They are characterized by better mechanical and thermal stability, but their loading capacity is lower than polystyrene based support. An example of this type of support is controlled pore glass (CPG).[136] It is important to note that resin parameters like cross-linking, swelling properties, and bead size have a major effect on the outcome of a reaction that is performed on a support.

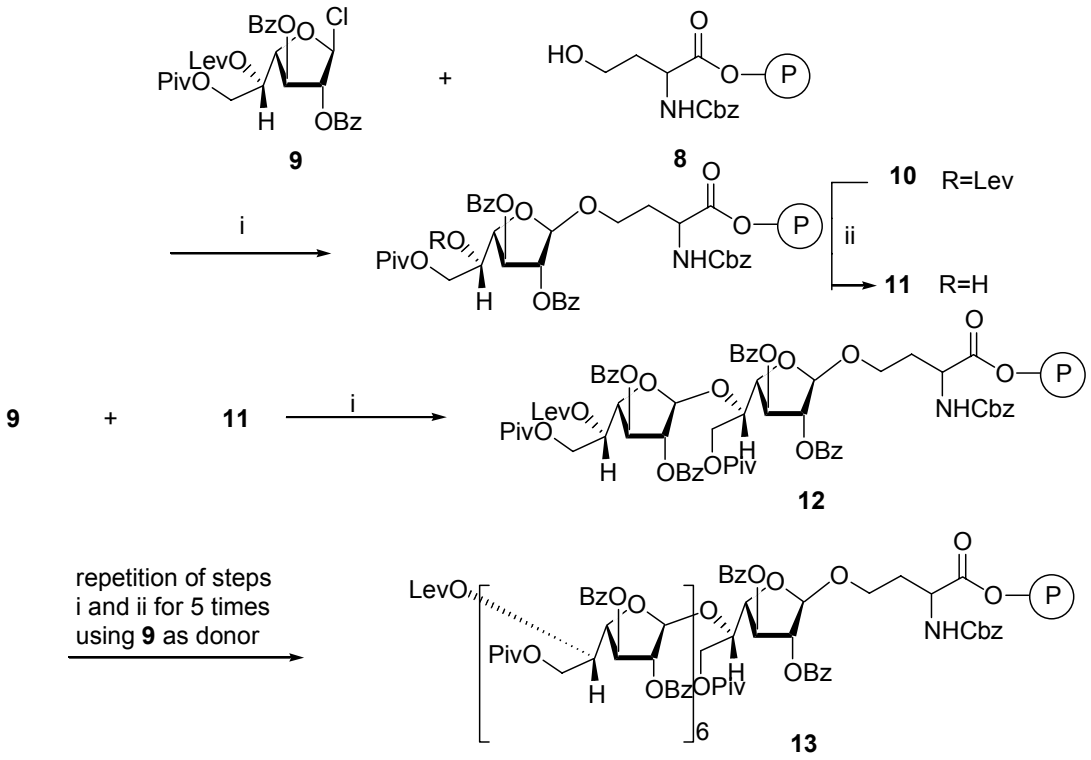
Another type of polymeric support has been obtained by grafting polyethylene-glycol (PEG) chains onto a polystyrene crosslinked resin.[137] The resin thus obtained (Tentagel), even though presenting a lower loading than crosslinked poly-styrene, is more effective than normal polystyrene for automated peptide synthesis, owing to improved swelling and mechanical properties. This PEG grafted polymer swells in all solvents that dissolve PEG, and conversely swelling is negligible in solvents which do not dissolve PEG, such as hydrocarbons or diethyl ether. The properties of Tentagel resin are dominated by the properties of PEG and not by the properties of the polystyrene backbone, and the reactive sites that are located at the end of the PEG chain behave as though they were in solution.[138] This is due to the flexibility and good solvation properties of the PEG tentacles.$^{13}$C NMR relaxation measurements indicate the high flexibility of the PEG chains. Indeed, when the resin is swollen, PEG tentacles are well solvated and highly flexible and high $T_1$ values are observed.[138] A major limitation of using solid support is the difficulty in characterizing the products while still on the resin. Analysis of the products can be performed by cleaving the product from a small portion of the polymer and analysis by TLC or MS. Recently, magic angle spinning (MAS) NMR has been used for observation of small molecules bound to a resin.[139]

An alternative to the use of a solid support is the use of soluble polymeric support.[140] In this approach, purification of the products is achieved by adding the polymer to a solvent, such as hydrocarbons that induces precipitation of the macro-molecular support. Analogous to solid phase synthesis, the resulting heterogeneous mixture is filtered to isolate the polymer-product conjugate while excess reagents and impurities are washed away. Soluble and functionalized PEG of molecular mass between

3000 and 20000 is soluble in many solvents and can be used as a soluble polymeric support, and it can be precipitated by addition of hexane or diethyl ether. Careful precipitation conditions or cooling in ethanol or methanol yields crystalline PEG.The kinetics of reactions for coupling of amino-acids supported on PEG has been shown to be of the same order of magnitude as the same coupling performed in solution.[140] Soluble polymeric supports allow following individual reaction using NMR and other techniques without the need to cleave a fraction of the product from the support itself.

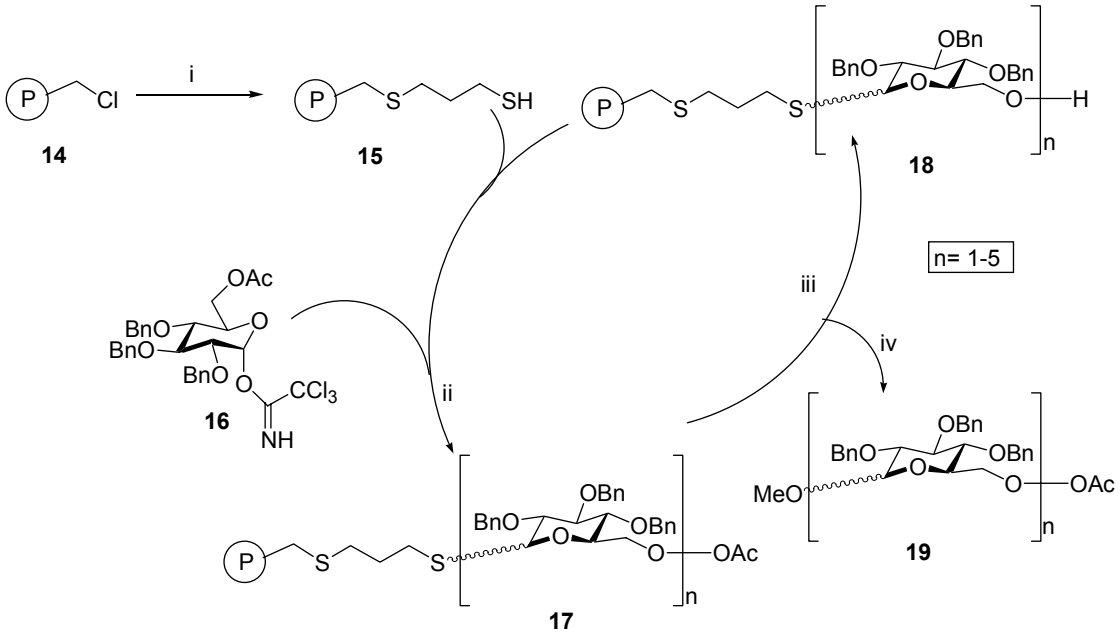
#### 1.3.2.3 Development of Solid Phase Oligosaccharide Synthesis

The first attempts to synthesize oligosaccharides on solid support were conducted in the early seventies,[141,142] and were only marginally successful, mostly due to the limited array of reagents and procedures available at that time for glycosylation. The progress in solution based oligosaccharide synthesis that occurred during the last two decades is making it possible to develop successful solid phase methodologies for the synthesis of oligosaccharides. In 1987, van Boom and co-workers.[143] reported the synthesis of β-(1→5)-linked D-galactofuranosyl heptamer on solid support. The anomeric center of the first sugar was linked to L-homoserine derivatized Merrifield polystyrene **8** and D-galactofuranosyl chloride **9** was employed as a glycosyl donor for chain elongation, using $Hg(CN)_2/HgBr_2$ as the promoter (Scheme 1.6). After each coupling step, it was necessary to cap the unreacted glycosyl donor, using a mixture of acetic anhydride, pyridine, and DMAP. Failure to do this caused a large amount of shorter single deletion fragments to be formed together with the expected product. Product **13** was obtained after six repeated glycosylations in a yield of 23%.



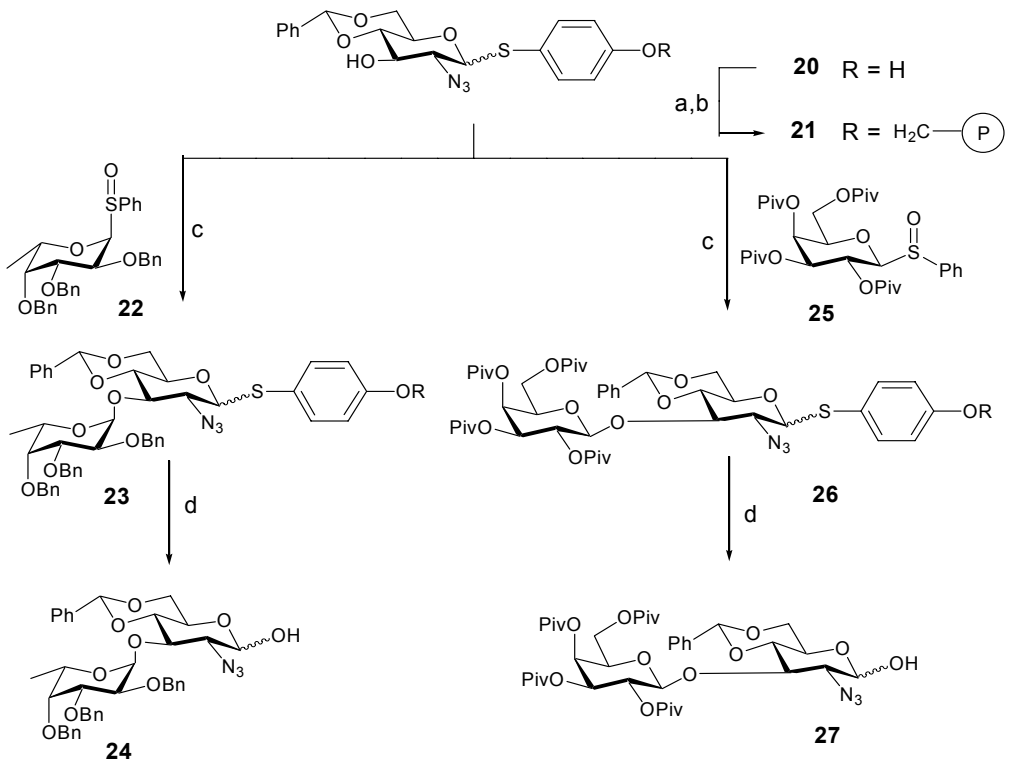

**Scheme 1.6:** Reagents and conditions: i) $Hg(CN)_2$ / $HgBr_2$; ii) $N_2H_4$.HOAc/Pyridine

Schmidt *et al.*[136,144] described the synthesis of oligosaccharides on solid support using trichloroacetimidates as glycosyl donors. Glycosyl donor **16** was attached to the solid support through a thiol linker that can be cleaved by reaction with NBS (Scheme 1.7). Synthesis of the linear pentasaccharide **19** (n=5) was achieved by cleaving the acetyl protecting group from compound **17** followed by glycosylation of the obtained acceptor **18** with the glycosyl donor **16** and a catalytic amount of TMSOTf as the promoter.




**Scheme 1.7.** conditions, i) propenedithiol, DBU ii) TMSOTf, DCM, r.t. iii) DCM / 0.5M NaOMe in MeOH iv) DMTSB, DIPEA, DCM / MeOH.

Kahne and co-workers[145] showed that anomeric sulfoxides are efficient glycosyl donors for solid phase oligosaccharide synthesis. Anomeric sulfoxides can be activated almost instantaneously by triflic anhydride at low temperature, and their reactivity is not dependant on the protecting groups of the donor. At low temperature, excellent anomeric control is obtained and side reactions are prevented. A coupling reaction can be repeated, and high yields can be thus obtained even when glycosylating unreactive or hindered secondary hydroxyls. For example, disaccharides **24** and **27** were obtained stereo-selectively in an overall yield of 67% and 64%, respectively after cleavage from the Merrifield resin used as a solid support (Scheme 1.8)

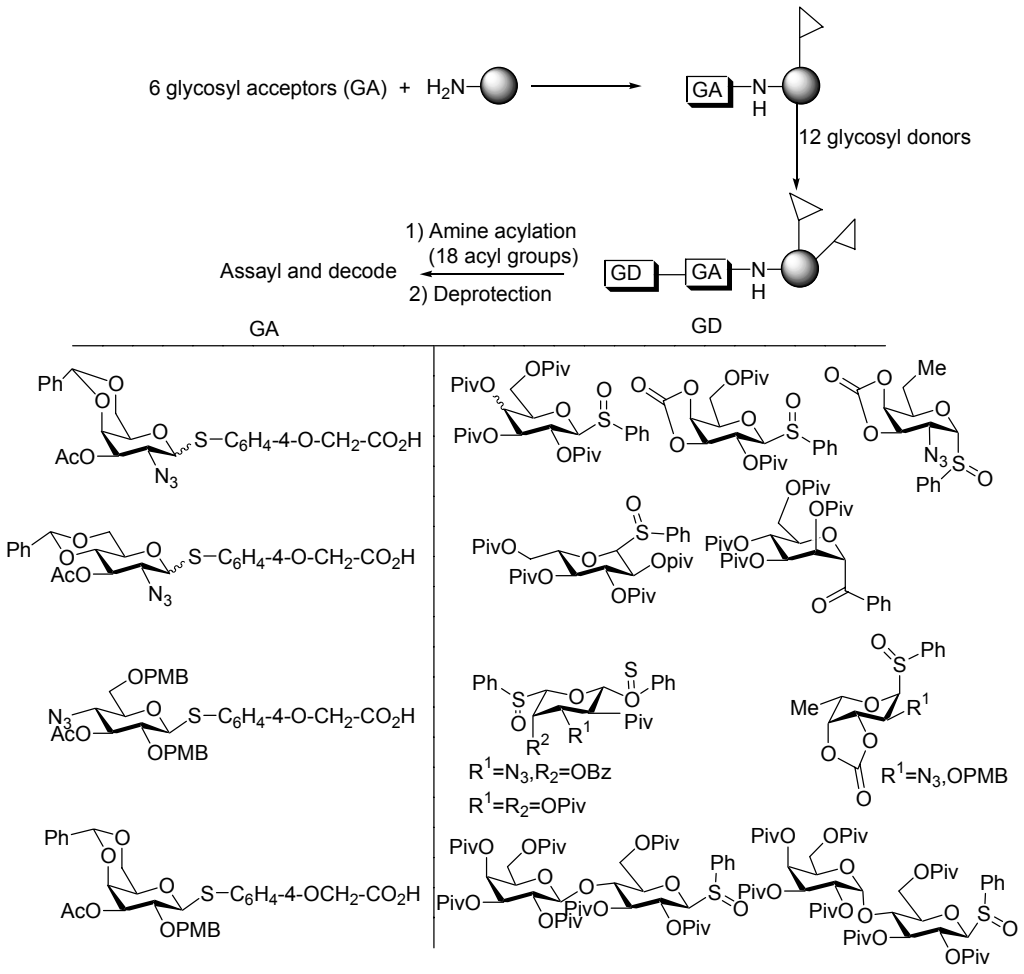



**Scheme 1.8.** Conditions: a) $Cs_2CO_3$, MeOH. b) P—$CH_2Cl$, N-methylpyrrolidine, 55°C c) $Tf_2O$, DTBMP, -60- -30°C, DCM; d) $Hg(OCOCF_3)_2$,DCM/$H_2O$

## 1.4 Combinatorial Synthesis of Carbohydrate Libraries Using Polymeric Support

### 1.4.1 Anomeric Sulfoxides

For a successful solid-phase synthesis, glycosylation reactions must be stereo-specific and high yielding. To achieve this, Kahne's group used anomeric sulfoxides as glycosyl donors.[146] Previous studies had demonstrated that these sulfoxides were readily activated at low temperatures regardless of the protecting groups of the glycosyl donor and acceptor pairs. Moreover, nearly quantitative yields (~90%) of the glycosylated products were obtained on solid support. This novel coupling procedure was used to produce a library of approximately 1300 di- and trisaccharides (Scheme 1.9) in only three steps.

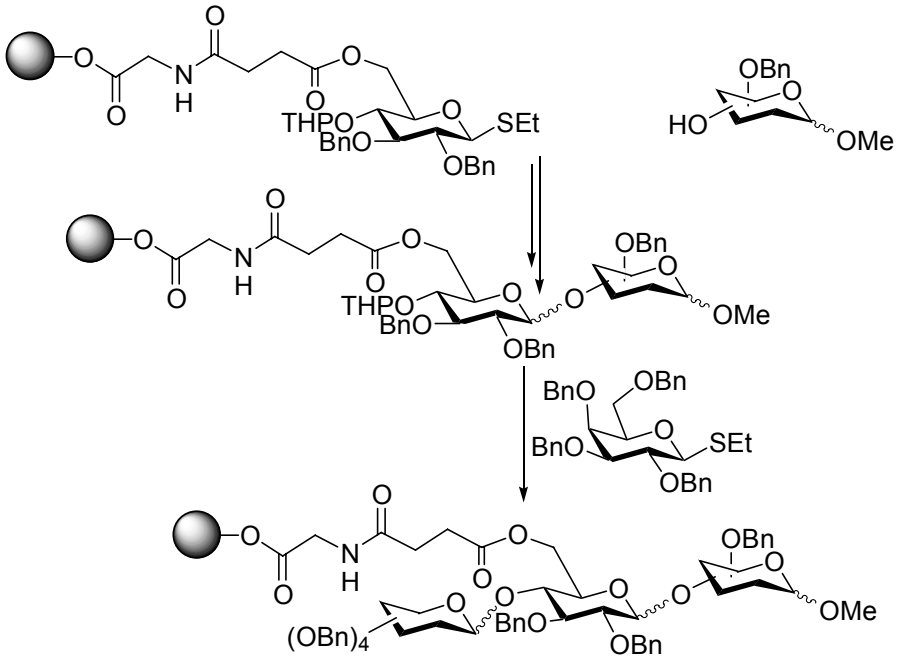



**Scheme 1.9.** Kahne's di- and trisaccharide libraries using split-mix synthesis and screening on the solid phase

The monomers used were appropriately protected to ensure diversity in glycosidic linkages. An encoded split-mix library approach on TentaGel resin was used. Six glycosyl acceptors were attached separately to the resin. This was pooled and divided into twelve parts, each of which was coupled separately with one of twelve glycosyl donors. Again, the beads were pooled, the azido group was reduced to amine and the beads were divided into eighteen parts. Each set of beads was *N*-acylated with different reagents. All the beads were combined again and fully deprotected.

### 1.4.2 Two-directional Solid-Phase Approach

Zhu and Boons[147] synthesized the second solid-phase library, a small trisaccharide library of 12 compounds (Scheme 1.10). In this synthesis a thioethyl glycoside that can act as a donor or acceptor was immobilized on solid support glycine-derivatized TentaGel resin through a succinimidyl linker. The key to this approach was the use of the tetrahydropyranyl group (THP) on the immobilized thioglycoside, which eliminated the formation of oligomeric side products during *N*-iodosuccinimide/trimethylsilyl trifluoro-methanesulfonate glycosylation.




**Scheme 1.10.** Boons' two directional approach for solid-phase synthesis of trisaccharide libraries

The immobilized thioglycoside was glycosylated separately with three different glycosyl acceptors, the resin was pooled, and the THP group was removed. The anomeric mixture of disaccharide acceptors was coupled with a perbenzylated thioglycoside donor

to give a mixture of trisaccharides. The trisaccharides were cleaved from the resin, purified by size exclusion chromatography, followed by full deprotection.

So far, only a few solid-phase oligosaccharide libraries have been reported. The challenges of well-planned orthogonal protecting groups and high yielding stereospecific glycosidic bond formation on solid support continue to stimulate chemists to devise novel approaches. A number of these innovative strategies would certainly impact future solid-phase oligosaccharide library generation. They include the following: the use of soluble polymer-based liquid phase glycosylation;[148] solid-supported chemical-enzymatic synthesis,[149] the widely applicable and high yielding trichlroracetimidate glycosylation;[150] novel linkers such as a new thiol linker for α-mannose and α-fucose glycosides[151] and a ring closing metathesis based linker that generates *O*-allyl glycosides upon cleavage from the resin;[152] the use of glycosylating agents such as *n*-pentenyl glycosides;[153] the synthesis of *β*-(1-4)-and *β*-(1-6)-linked oligosaccharides using glycosyl phosphate in combination with a versatile octenediol linkers;[154] the glycal assembly method for the synthesis of polymer bound thioethyl glycosyl donors for the synthesis of β-linked oligosaccharides;[155] the synthesis of thio-oligosaccharides by nucleophilic substitution of triflate activated glycosides by resin-bound sugar-1-thiolate containing unprotected hydroxyl groups;[156] and the use of a novel photocleavable aglycan linker[157] are very promising approaches for the rapid access to oligosaccharides.

### 1.5 Combinatorial Libraries Using Carbohydrate Scaffolds

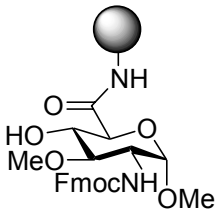
Oligosaccharide library synthesis has been hampered by the polyfunctional nature of carbohydrates. This same feature places carbohydrates in a distinctive class of

privileged template structures for displaying chemical diversities toward drug discovery efforts.[158] The advantageous use of the polyfunctional nature of carbohydrate units as scaffolds for displaying diversity represents a unique approach to combinatorial libraries that are not limited to glycoconjugate investigation. Previous work had demonstrated the validity of this approach in the design of nonpeptide somatostatin mimics.[159]

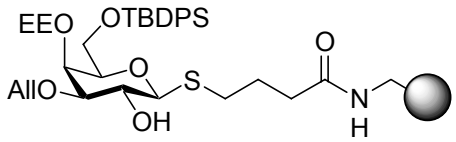
Sofia *et al*[160] reported the first such carbohydrate scaffold library containing three sites of diversity (Figure 1.22). The important features of the scaffold were the use of a functional triad that included a carboxylic acid moiety, a free hydroxyl group, and a protected amino functionality **28**. This derivatized monosaccharide was then coupled to an amino acid functionalized trityl TentaGel resin. The IRORI radiofrequency tagged split-pool methodology[161] was employed to form **28**, sixteen 48-member libraries from eight amino acids, six isocyanates, and eight carboxylic acids. The libraries were analyzed by LC/MS in greater than 80% purity. These libraries were referred to as 'universal pharmacophore mapping libraries'

Unlike Sofia's approach, Kunz *et al.*[162] initially used an orthogonally protected thioglucoside as a scaffold. The protecting groups included *tert*-butyldiphenylsilyl (TBDPS), 1-ethoxy ethyl (EE), and the propyl moiety. An important feature of the scaffold was the use of a functionalized thioglycoside, which are not only served as a glycosyl donor but also as a linker for immobilizing the compound on amino-methyl polystyrene resin. Diversity was introduced at position 2 and 6 after selective deprotection and alkylation. An anomeric mixture of methyl glycosides was obtained in yields of 30 to 80%. This combinatorial methodology was extended to a galactopyranose scaffold **29**, which contained five sites of diversity (Figure 1.22b).[163] Instead of the propyl

group at position 3, the *O*-allyl group was introduced. Using sequential deprotection and alkylation protocols, an array of structurally diverse compounds was successfully synthesized.

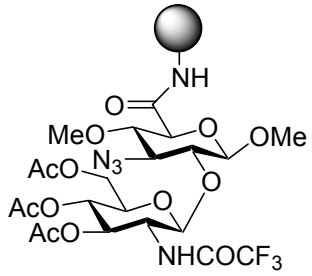



**28**




**29**

a) scaffold with a functional triad b) orthogonally protected scaffold




c) disaccharide scaffold

**Figure 1.22.** Carbohydrate-based scaffolds

Silva's group has recently reported the synthesis of a unique *β*-linked disaccharide scaffold that was employed in the solid-phase synthesis of a 48-member library (Figure1. 23c).[164] Central to this approach was the use of phenylsulfenyl 2-deoxy-2-trifluoro-acetamido glycopyranosides as glycosyl donors in the synthesis of the *β*-linked di-saccharide. These scaffolds may provide important small molecules for probing a variety of biological processes. No biological data have been presented. Other motifs have been investigated especially in the search for potent aminoglycoside mimics. An amino-glucopyranoside core containing a 1,3-hydroxyamine motif at the anomeric position has

also been used as a privileged template for design of RNA binders using a parallel solution phase approach.[165] Unlike Sofia's use of a scaffold with no a prior information.

Wong's use of this amino-glucopyranoside core represented a rational approach for small-molecule derivatives of aminoglycoside antibiotics based on available structural information. Small-molecule mimics of glycoconjugates are therapeutically more relevant than biologically active oligosaccharides for even aminoglycoside antibiotics since important pharmacokinetics and pharmacodynamic properties can be incorporated in the structure. Therefore, it is not surprising that combinatorial glycomimetics library generation is a very dynamic and rapidly expanding field.

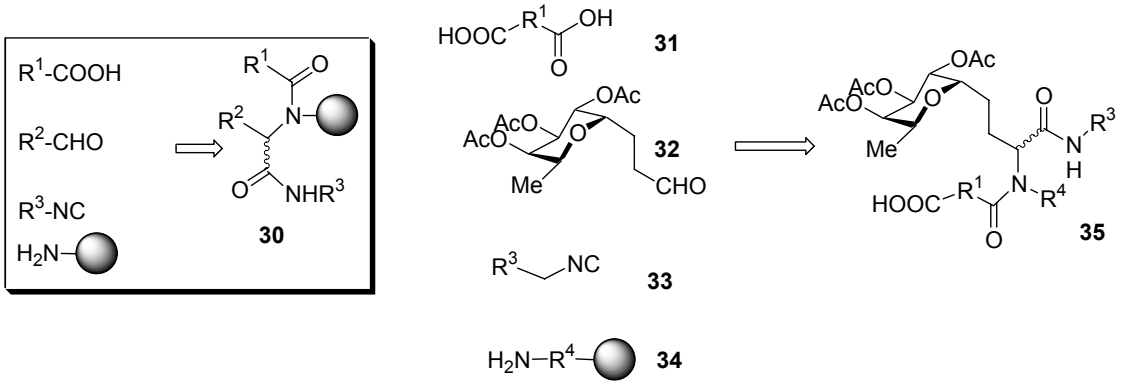
### 1.6 Combinatorial Glycomimetics Libraries

To overcome the many challenges of complex oligosaccharide libraries, small glycoconjugates including glycopeptides have been exploited as functional mimics of oligosaccharides. These glycomimetics, in addition to being more readily accessible, may contain diverse aglycon scaffolds with an array of hydrophobic and/or charged functionalities upon which pertinent sugar moieties are displayed. Furthermore, the glycoside moieties may be present in its native form (*O*-or *N*-linked) or as stable isoteres such as *C*-linked and *S*-linked glycosides. A number of conceptual aproaches have been successfully used for the rapid generation of libraries for biological studies.

#### 1.6.1 Multiple Component Reaction (MCR):

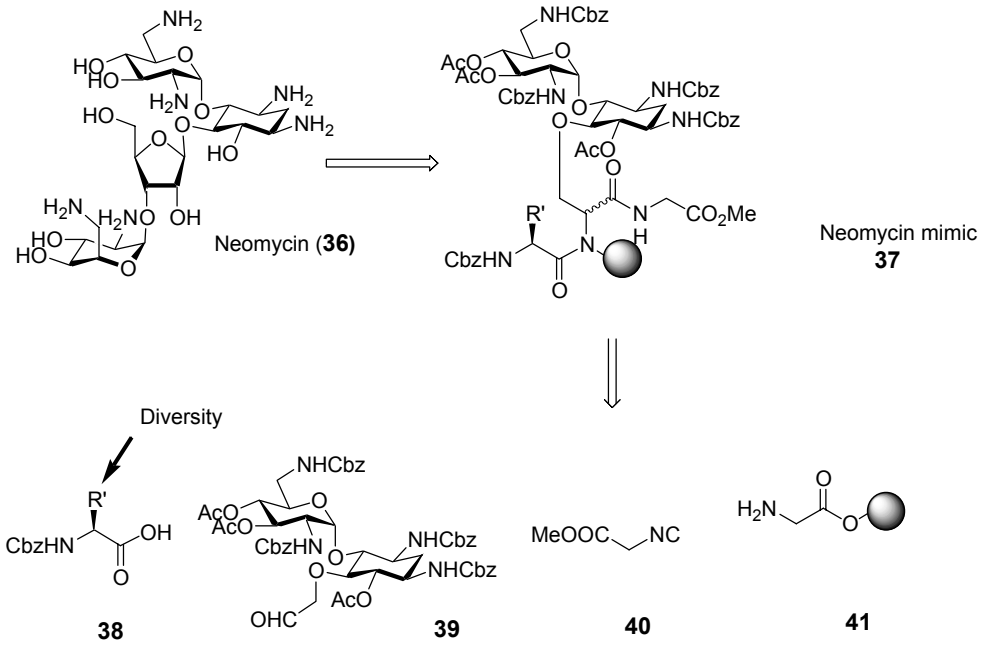
Ugi's novel four component condensation reaction of an amine, aldehyde, isocyanate, and carboxylic acid to give the glycomimietic **30** has been successfully

adapted to the solid phase.[166] This powerful strategy (Scheme 1.11) has been used to rapidly generate solid-phse combinatorial libraries of C-glycosides **35**.[167] Using eight diacids (**31)**, a C-fucose aldehyde (**32)**, two isocyanides (**33**), and Rink amine resin derivatized with five different amino acids (**34**). Armstrong's group synthesized a focussed library of sialyl Lewis x mimietics **35** with high purity.




**Scheme 1.11.** Armstrong's glycomimetics by Ugi four-component condensation

Wong's group has also used this methodology on a soluble polyethyleneglycol (PEG) polymer for the generation of mimietics of the aminoglycoside antibioltics **36** (Scheme 1.12).[168] In this library, the neamine moiety **39** (Cbz: benzyloxycarbonyl) which is critical for inhibition of HIV RNA transactivator protein was kept constant and diversity was introduced in the amino acid group **38**.



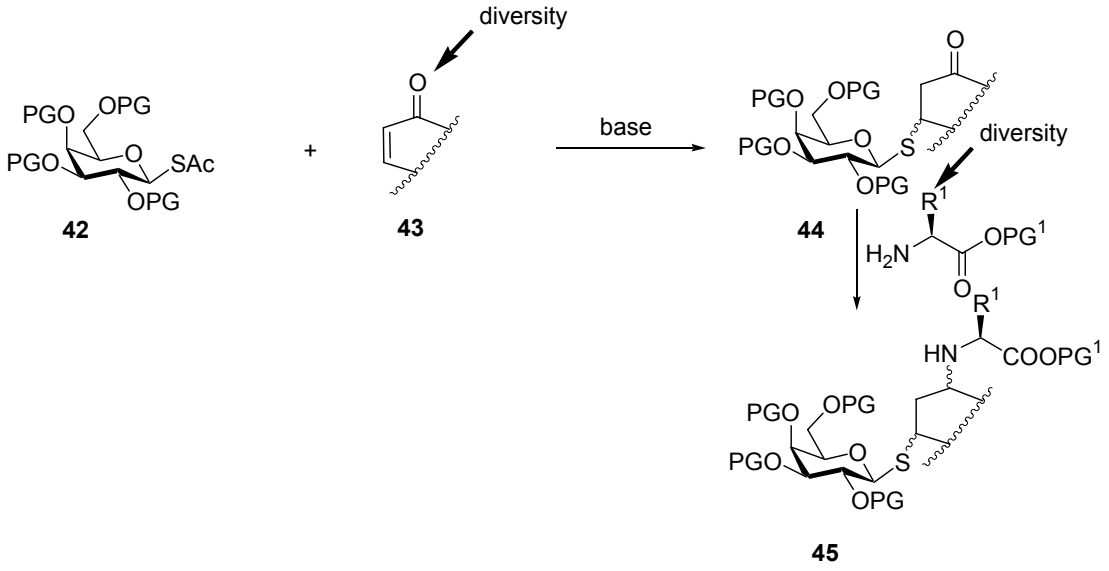

**Scheme 1.12.** Wong's neomycin mimics by Ugi four-component condesation.

Using Ugi's versatile approach, carbohydrate building blocks containing aldehyde, amino, carboxylic, and isocyanide groups can be readily incorporated into small glycomimetics and used as small probes for carbohydrate-receptor interaction as well as therapeutically useful lead compounds.[169]

### 1.6.2 Glycohybrids

In another approach, a 1-thio-*β*-D-galactopyranoside library was synthesized by solution using solid-phase extraction techniques for purification (Scheme 1.13). A building block such as **42** containing *O*-laurates (PG) as hydrophobic tags which facilitated reverse-phase C18 silica purification of the glycohybrids was used. This thio-glycoside building block underwent Michael addition reactions followed by derivatiztion of the carbonyl group with several amino acids. A library of an easily separable mixture of thirty compounds, **45,** each present as four diastereomers was produced. This library

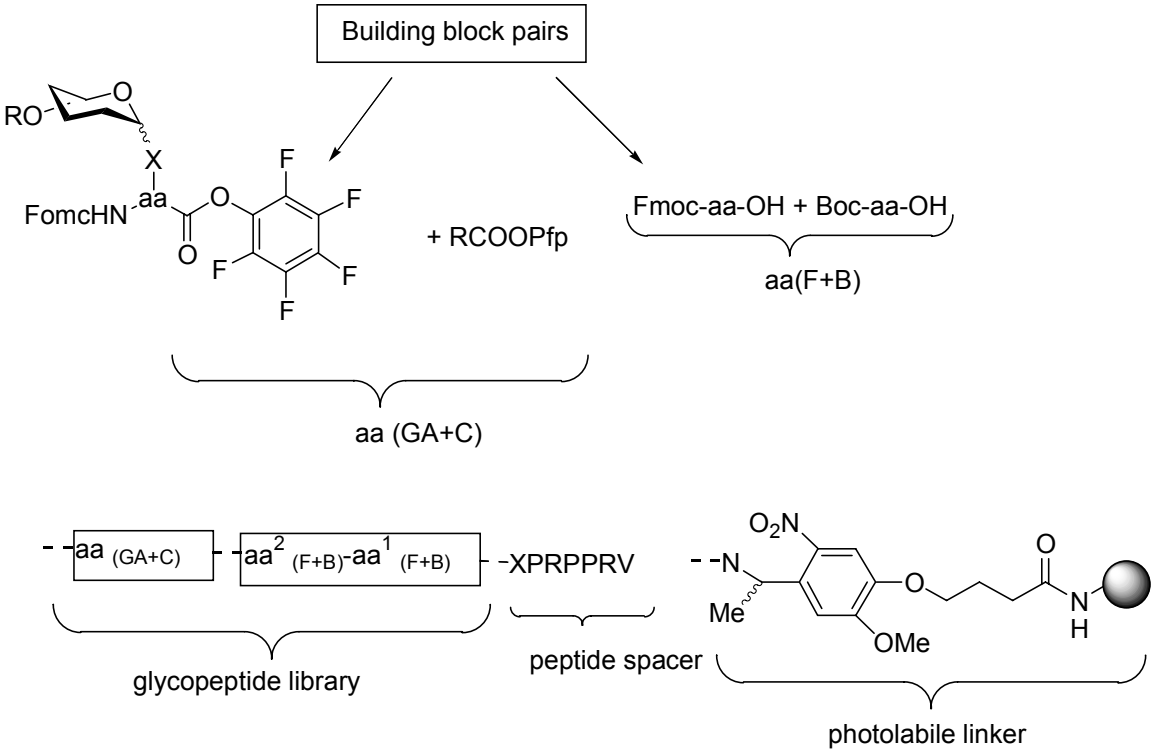
was screened for inhibitors of *β*-galactosidase from *E. coli.* One of the members was a better inhibitor than their reference compound.[170]




**Scheme 1.13.** Hindsgaul's glycohybrids

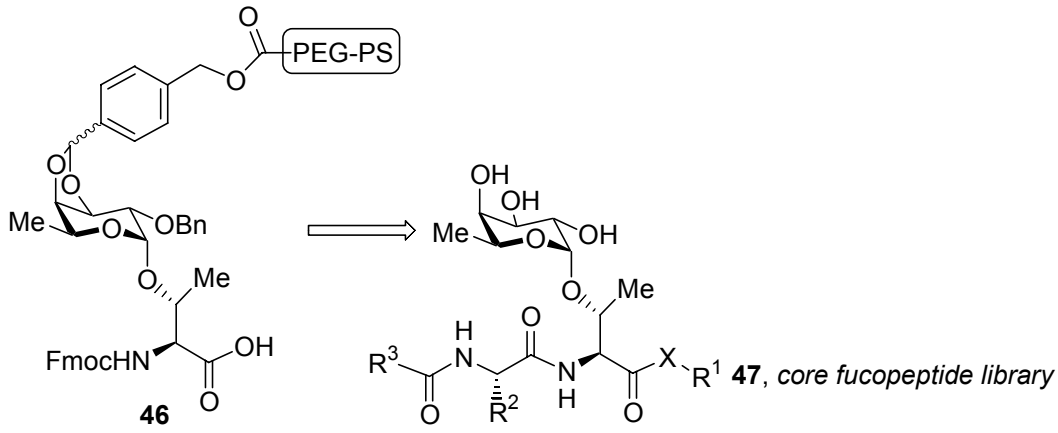
### 1.6.3 Glycosylated Amino Acid Building Blocks

The glycosylation of *N*-fluoren-9-yl-methoxycarbonyl (Fmoc) amino acid penta-fluorophenyl esters (Pfp) has provided a range of building blocks for assembly of glycopeptides by multiple column solid-phase peptide synthesis. A variety of solid supports have also been used to produce parallel arrays of glycopeptides with native and isosterically substituted glycosidic linkages.[171] St. Hilaire and Meldal have reported an elegant strategy for combinatorial glycopeptide libraries that afforded unambiguous characterization of active compounds (Scheme 1.14).[172] An encoded one-bead-one-compound heptaglycopeptide libraray consisting 300000 members were rapidly synthesized on a PEGA resin containing a photolabile linker by the split-mix technique




**Scheme 1.14.** Encoded glycopeptide library

Wong et al used a fucosylated amino acid building block approach (Scheme 1.15).[173]The fucose moiety of fucosylated threonine derivatives was immobilized through a *p*-(acyloxymethyl) benzylidene acetal (*p*-AMBA) on a carboxyl-functionalized PEG-PS resin **46**. This was used to generate a fucopeptide library of sialyl Lewis x mimetics using parallel synthesis. In this library, the critical hydroxyl groups of the fucose moiety required for recogintiion of sialyl Lewis x by E-selectin was invariant and structural diversity was introduced at both the *N*- and *C*-termini of the glycopeptide.

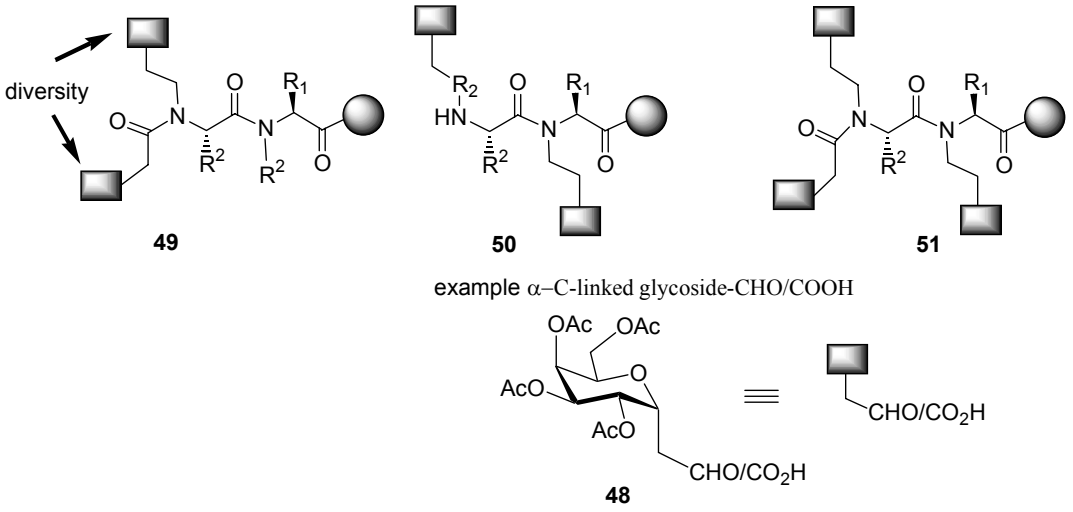



**Scheme 1.15.** Sialyl Lewis x mimic

### 1.6.4 Automated Multistep Approach to Neoglycopeptide Libraries

A versatile, fully automated multi-step solid-phase strategy has also been developed for the parallel synthesis of neoglycopeptide libraries (Scheme 1.16). Instead of using a glycosylated amino acid building block, which limits the choices of attached amino acids, C-glycoside **48** building blocks protected as acetates are used.[174] The C-glycoside can be in either the $\alpha$- or $\beta$-configuration or even as a mixture of anomers and conatin an aldehyde or carboxylic acid functionality. These building blocks can then be independently incorporated on a peptide/pseudo-peptide scaffold. Furthermore, the chain lengh of the *C*-glycoside can be varied and the carbohydrate moiety can be synthesized in either the pyranose or furanose form. In addition, these types of carbohydrate building blocks are not limited to monosaccharide derivatives since disaccharides can also be used. Using this approach, libraries of neoglycopeptides are readily synthesized for probing carbohydrate-protein interaction. A number of "working models" have been developed for these libraries which addresses the multivalent presentation of carbohydrates **49**, **50**, and **51** (Scheme

1.16) while the dipeptide scaffold may contribute to secondary interactions with the biological targets.[175]




**Scheme 1.16.** Programmed approach to neoglycopeptides.

Initially, the neoglycopeptides were synthesized by a convergent strategy on a peptide synthesizer.[176] Since then, the synthesis has been successfully transferred to a fully automated multiple organic synthesizer and has been further optimized.[177] This fully automated methodology involves coupling of an amino acid to an insoluble support such as Rink amide MBHA resin or TentaGel derictized Rink amide resin. After removal of the protecting groups on the amino acid, the sugar aldehyde undergoes reductive elimination (Scheme 1.16, models **51** and **52**) with the resin bound amino group followed by amide bond formation with a second amino acid. After the amino group is deprotected, it can undergo either reductive amination with any sugar aldehyde or coupling with any sugar acid or both. Using this approach, a parallel 96-compound library was recently synthesized usind 24-dipeptides and two sugars, α-C-linked mannose- and glucose aldehyde derivatives.[178]

# CHAPTER 2

## DEMIXING LIBRARIES OF SACCHARIDES USING A MULTI-LINKER APPROACH IN COMBINATION WITH A SOLUBLE POLYMERIC SUPPORT[1].

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[1]Galal A. Elsayed.; Tong Zhu.; Boons, G-J. *Tetrahedron Lett*.; **2002**, 43, 4691-4694.

## Abstract

A novel methodology for synthesis of oligosaccharide libraries has been developed using a multi-linker strategy in combination with soluble polymers. A range of glycosyl acceptors was immobilized on a soluble polymeric support monomethyl ether of polyethylene glycol (MPEG) via the formation of amide bonds between the amino-fuctionalized monomethyl ether of polyethylene glycol and the carboxylic acid moieties of different linkers (**1**, **2**, and **3**). The mixture of those polymer-bound glycosyl acceptors was glycosylated with various glycosyl donors to give a library of disaccharides.The obtained disaccharide libraries were deconvoluted by selective cleavage of the linkers to afford well-defined disaccharides.


## Introduction

Liquid polymer-, dendrimer and fluorous supported syntheses are emerging as attractive alternatives for solid-phase organic chemistry.[178] These synthetic approaches have the favorable properties of traditional solution phase chemistry, while the macromolecular or fluorous properties of the liquid supporting system facilitate product purification. For example, compounds that are linked to polyethylene glycol monomethyl ether (MPEG)[179] are soluble in many organic solvents. However, due to the helical structure of MPEG, it has a high propensity to crystallize in diethyl ether and ethanol and, thus, product purification by crystallization can be accomplished at each stage of the synthesis. Dendrimer supported synthesis exploits that compounds can be easily separated from excess reagents by size exclusion chromatography,[180] whereas fluorous supported synthesis relies on selective extraction in a fluorocarbon solvent.[181] In general,

these liquid polymer supported methods have similar reaction kinetics compared to traditional solution-phase chemistry; they do not require the use of a large excess of reagents, and they allow supported intermediates and final products to be easily characterized. Despite these attractive features, liquid polymer supported methods have rarely been used in combinatorial chemistry[182] and one of their main shortcomings is that there are no general methods for synthesizing mixtures of compound that at the end of a sequence of reactions can easily be separated to give individual compounds.[183,184] A notable exception is a method developed by Curran and co-workers[185] whereby four different compounds can be linked to four different fluorous tags of increasing fluorine content. The tagged compounds can then be mixed and after multiple reactions, demixed by fluorous chromatography to provide individual compounds.

Herein, we report a novel and general method for the demixing liquid phase supported compounds by an orchestrated release from the supporting system by selective cleavage of different linkers. Thus, in the proposed strategy, a series of substrates will be tagged with a series of selective cleavable linkers.These linker-tagged compounds can then be mixed, attached to a soluble support and used in a series of combinatorial reactions. At the end of the reaction sequence, individual compounds can be obtained by selective cleavage of the linkers. The new method was applied to the preparation of a library of 18 disaccharides[186-190] using glycosyl acceptor **1**-**3** (Figure 2.1) that are tagged by unique linkers, glycosyl donors **4-6**, and MPEG as the liquid supporting system.[191]

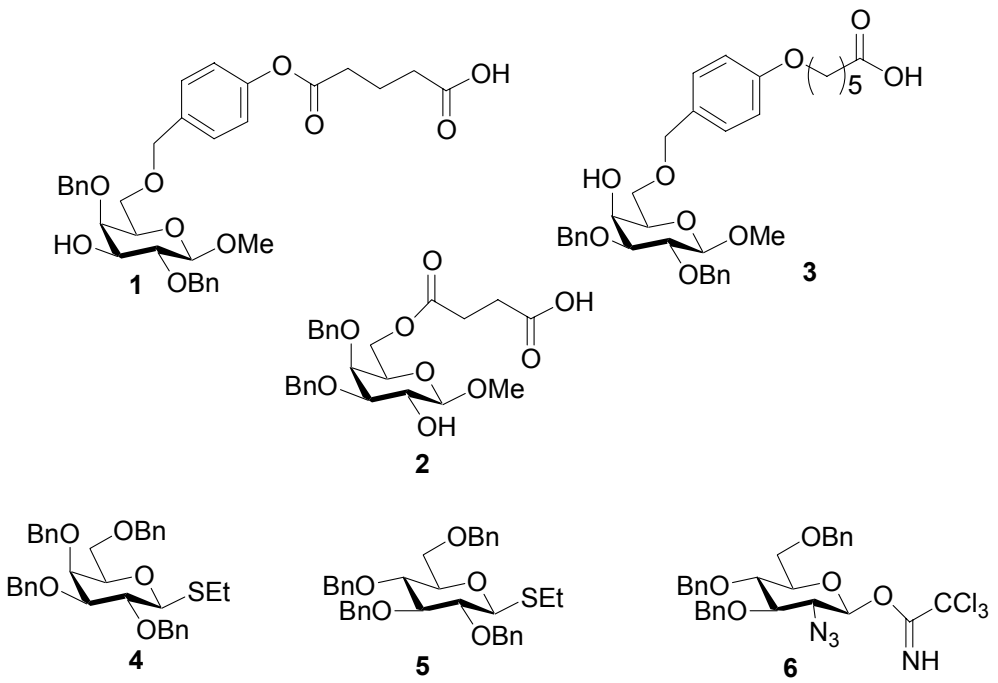


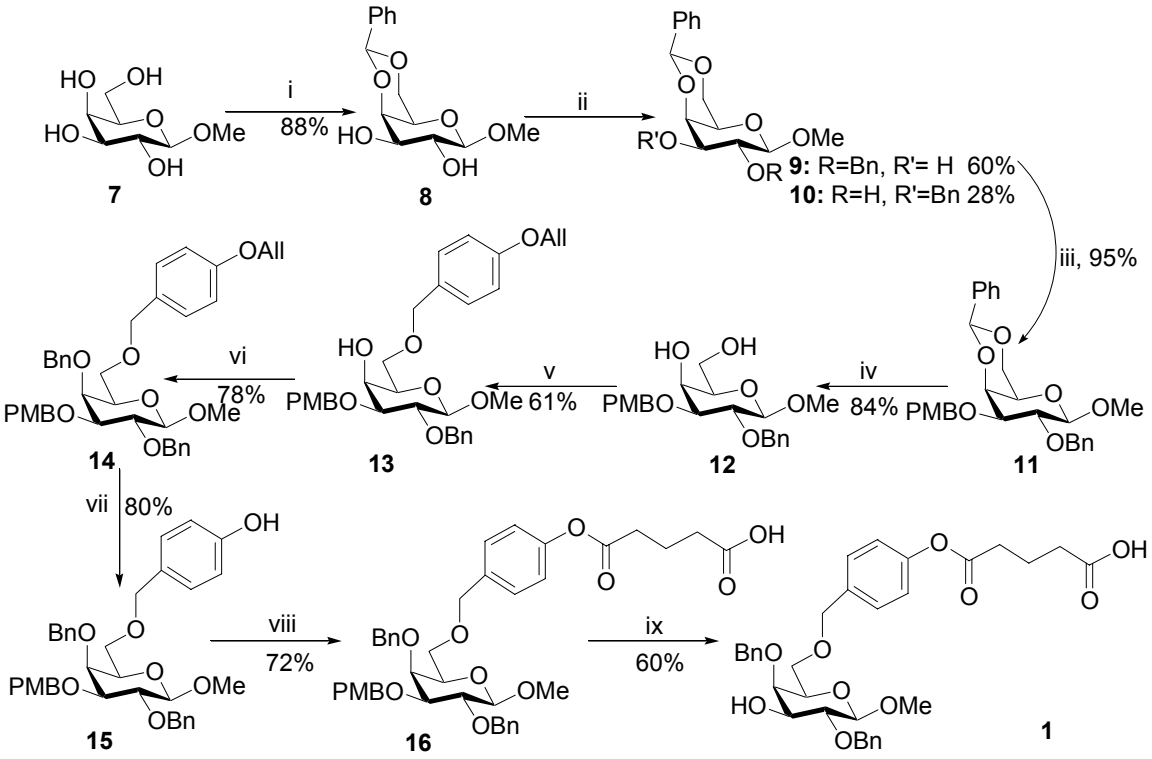

**Figure 2.1.** Building blocks for library synthesis

## Results and discussion

**Synthesis of building blocks.** The target compound **1** has a free C-3 hydroxyl group and is functionalized with a phenolic ester linker (Figure 2.1), which is stable towards Lewis acid conditions used in glycosylations but can be cleaved within minutes by treatment with $H_2O_2/Et_3N$.[192,193] After detachment, a *p*-hydroxyl benzyl ether will be obtained, which can easily be removed by oxidation with DDQ.[194] Compound **2** has a free C-2 hydroxyl group and is derivatized with a succinic ester linker.[195] This ester is substantially more stable than the phenolic ester linkage of **1** and requires treatment with sodium methoxide for cleavage. Glycosyl acceptor **3** has a free C-4 hydroxyl group and is derivatized with an acid sensitive *p*-alkoxybenzyl linker[196] which is orthogonal with the linkers of **1** and **2**. The linkers of **1-3** contain a carboxylic acid moiety, which will be utilized for the attachment to amino-functionalized MPEG.

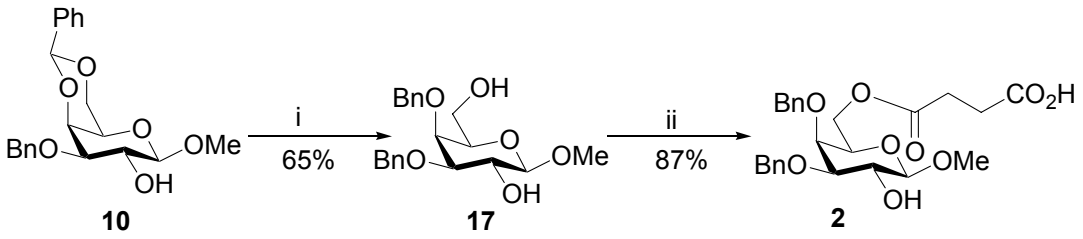
Spacer modified **1** was synthesized from methyl β-D-galactopyranoside **(7)**.[197] The C-4, C-6 diol of **7** was protected as benzylidene acetal by treating with benzaldehyde dimethyl acetal using camphorsulphonic acid as a catalyst in acetonitrile to give methyl 4,6-*O*-benzylidene acetal **8**. The C-2 hydroxyl of compound **8** was then selectively benzylated by using aqueous NaOH (10%) as the base and the phase-transfer catalyst ($Bu_4NHSO_4$) in DCM to give the monobenzylated product **9** in 60% yield (Scheme 2.1). The higher reactivity at the 2-position relative to that at 3-position probably arises from the higher acidity of 2-OH which in turn arises from its proximity to the anomeric center. Regioisomers **9** and **10** were separated by silica gel column chromatography. A fraction of regioisomer **9** was acetylated using acetic anhydride in pyridine. NMR analysis of the acetylated derivative showed a downfield shift from $\delta_{H\text{-}3}$=3.75 to $\delta_{H\text{-}3}$= 3.91 while $\delta_{H\text{-}2}$~ was unchanged. This observation confirmed that regioisomer **9** has the benzyl ether protecting group on C-2. The C-3 hydroxyl group of compound **9** was protected as temporary *p*-methoxybenzyl (PMB) ether by reaction with PMBCl and NaH in DMF to give the fully protected building block **11** in 95% yield. The benzylidene acetal of **11** was removed using aqueous acetic acid to give diol **12** in 84% yield. The C-6 hydroxyl of diol **12** was functionalized as a *p*-alkoxybenzyl ether by first formation of an intermediate 4,6-*O*-stannylene acetal[198] with $Bu_2Sn(OMe)_2$ which was regioselectively reacted with *p*-allyloxybenzyl chloride in the presence of $Bu_4N^+I^-$ to give **13** in 61% yield. Next, the remaining C-4 hydroxyl of **13** was benzylated under standard conditions to give **14** in 78% yield, which was treated with $Pd(PPh_3)_4$ in refluxing ethanol to remove the phenolic allyl ether to give **15** in 80% yield. Reaction of **15** with glutaric anhydride in the presence of DMAP in pyridine gave the linker modified building block **16** in 72% yield. Finally,

the target compound **1** was obtained by removal of the PMB ether by treating compound **16** with 10%TFA/DCM (60% yield).



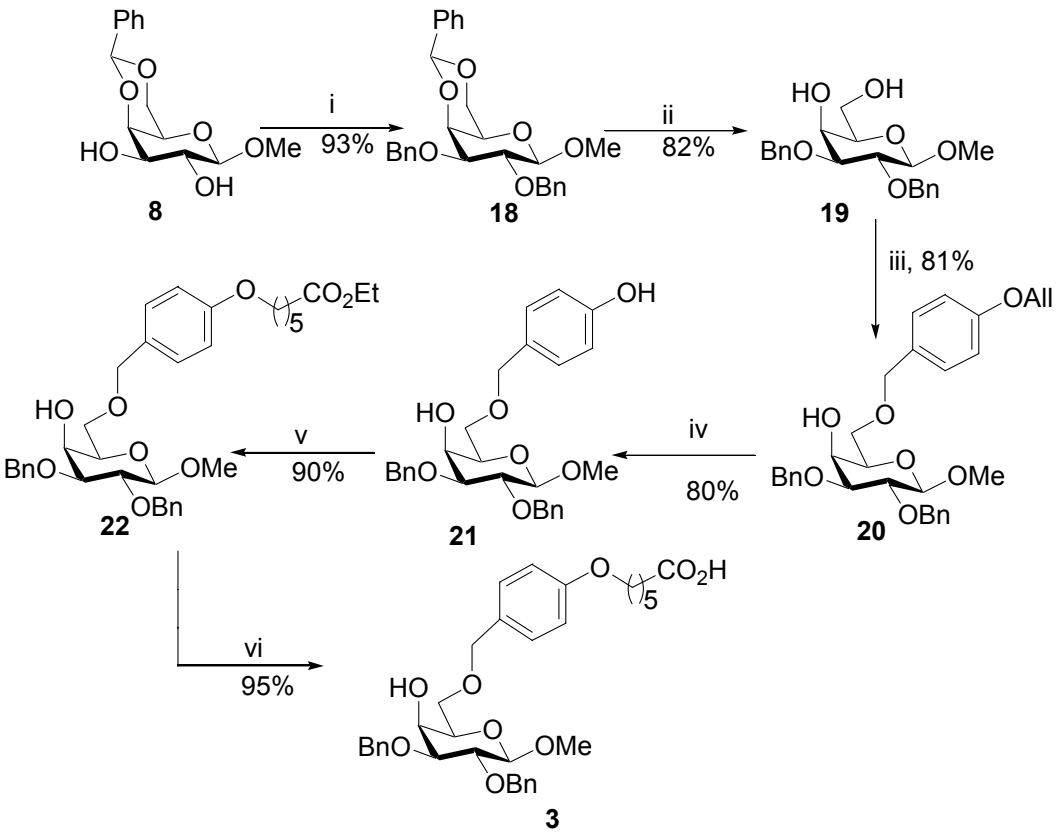

**Scheme 2.1.** Reagents and conditions: i) $PhCH(OMe)_2$, CSA, $CH_3CN$; ii) BnBr, aq. NaOH, $Bu_4N^+HSO_4^-$/DCM; iii) PMBCl/NaH/DMF; iv) 80% aq. AcOH, 50°C; v) $Bu_2Sn(OMe)_2$/ $Bu_4N^+I^-$ /*p*-allyloxy benzyl chloride, dry toluene; vi) BnBr/NaH/DMF; vii) $Pd(Ph_3P)_4$/EtOH.THF; viii) glutaric anhydride/pyridine/DMAP; ix) 10%TFA/DCM.

Target compound **2** was easily obtained by regioselective opening of the benzylidene acetal of the already synthesized building block **10** by treatment with $BH_3.Me_3N$[199] complex in the presence of $AlCl_3$ (Scheme 2.2) to give compound **17** in 65% yield. Selective acylation of C-6 hydroxyl group of compound **17** with succinic anhydride only proceeded with high regioselectivity when first an intermediate of tri-*n*-butyltin acetal was formed by reaction with $(Bu_3Sn)_2O$ to give comound 2 in 87% yield.




**Scheme 2.2.** Reagents and conditions: i) $BH_3.Me_3N/AlCl_3$/MS 4Å, dry toluene; ii) $(Bu_3Sn)_2O$, succininc anhydride/dry toluene.

Spacer modified **3** was prepared from building block **8**, which was benzylated (BnBr, NaH, DMF) to give compound **18** in 93% yield (Scheme 2.3). The benzylidene acetal of **18** was removed using aqueous acetic acid to afford the diol **19** in 82% yield. The C-6 hydroxyl group of diol **19**[200] was functionalized as a *p*-allyloxybenzyl ether by first formation of an intermediate 4,6-*O*-stannylene acetal[198] when heated under reflux with $Bu_2Sn(OMe)_2$ then coupled with *p*-allyloxybenzyl chloride in presence of n-$Bu_4N^+I^-$ in dry toluene to give compound **20** in 81% yield. The allyl ether protecting group of compound **20** was cleaved by treatment with $Pd(PPh_3)_4$ in refluxing ethanol to yield **21** in 80% yield. Alkylation of the phenolic hydroxyl group of compound of **21** with ethyl-6-bromo-hexanoate in the presence of $Cs_2CO_3$ gave ester **22** in 90% yield. The linker modified glucosyl acceptor **3** was obtained when the ester functionality in **22** was hydrolyzed by a methanolic solution of sodium hydroxide in 95% yield.

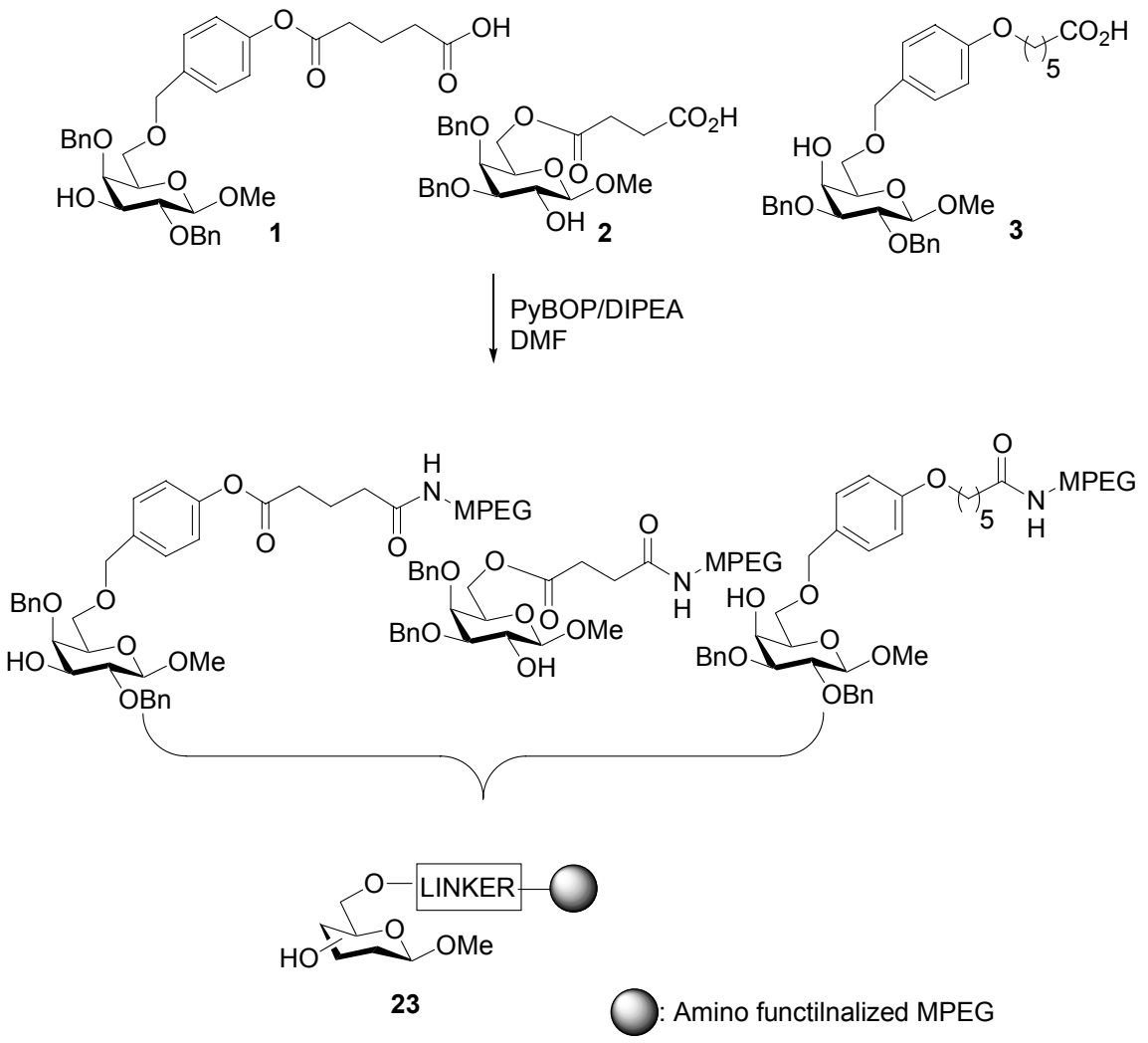



**Scheme 2.3.** Reagents and conditions: i) BnBr/NaH/DMF; ii) 80% aq. AcOH; iii) $Bu_2Sn(OMe)_2/Bu_4N^+I^-$/*p*-allyloxy benzyl chloride; iv) $Pd(Ph_3P)_4$/EtOH.THF, 70$^oC$; v) $Br(CH_2)_5CO_2Et/Cs_2CO_3$/DMF; vi) NaOH/MeOH.

**Immobilization of the galactosyl acceptors**

With ample quantities of the linker-modified acceptors in hand, attention was focused on the combinatorial synthesis of a library of eighteen disaccharides. Mixing of compounds **1-3** followed by attachment to amino-functionalized MPEG (Mw 5000) by using the standard amide bond formation conditions of PyBOP and DIPEA in DMF afforded library **23** (Scheme 2.4). The completion of the reaction was examined by Kaiser test. After being coupled to the resin, the mixture of the immobilized acceptors (**23**) was easily purified by precipitation with ice-cooled diethyl ether. Inspection of the

NMR spectrum of the resulting material indicated that the three monosaccharides were present in approximately equal molar quantities. The monosaccharides library **23** was dried in a vacuum line overnight and split into three pools for the purpose of combinatorial glycosylation with different common glycosyl donors.

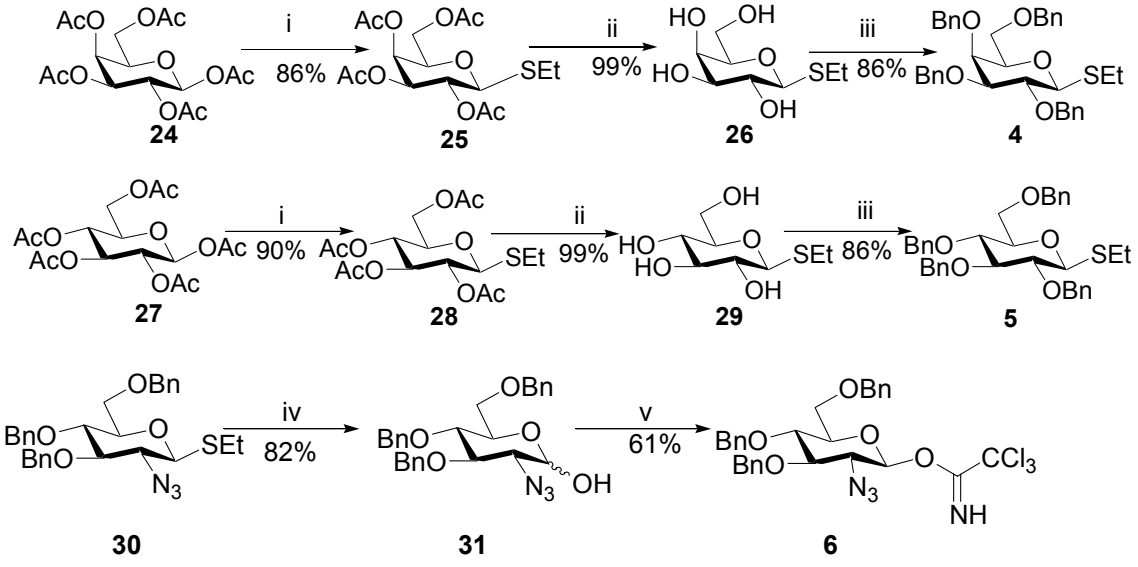



**Scheme 2.4:** Immobilization of the glycosyl acceptors on the polymeric support

**Synthesis of the glycosyl donors**

Galactoside **4** was synthesized from the commercially available starting material *β*-D-galactopyranose pentaacetate **24.** After introduction of the ethylthio function at the

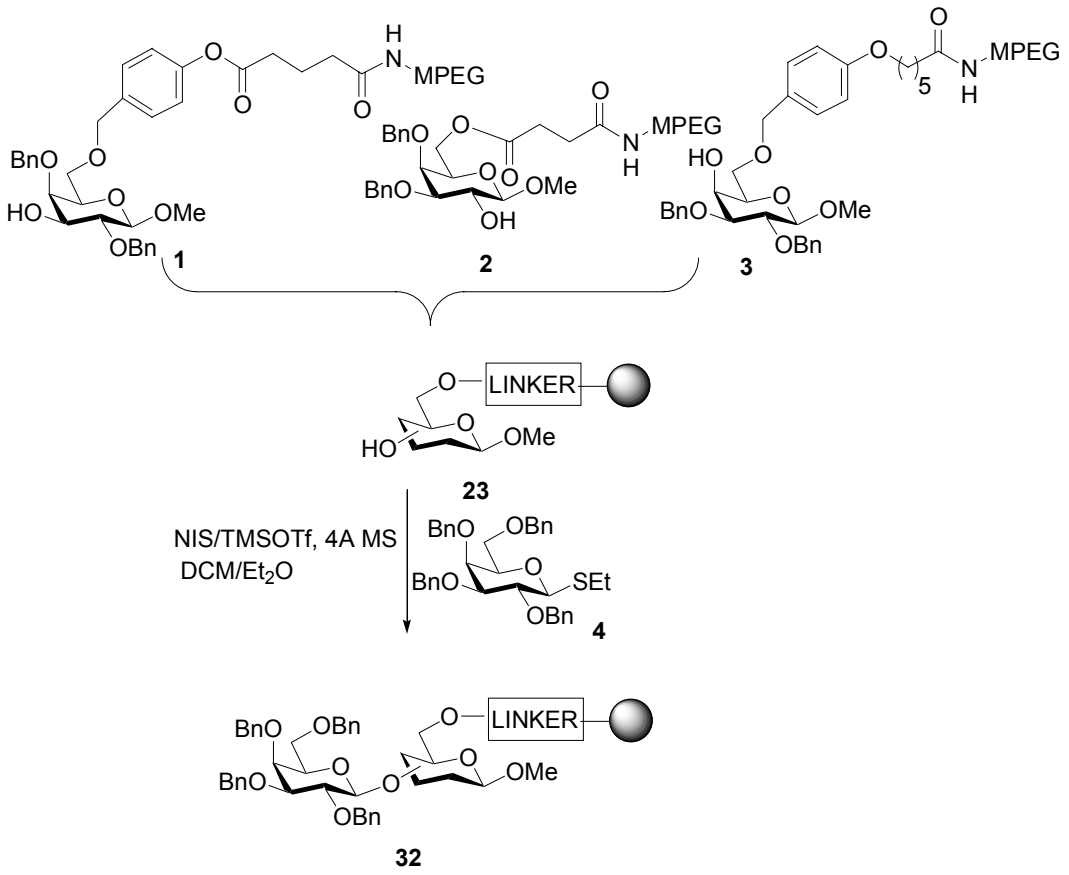
anomeric center using EtSH and $ZrCl_4$ in DCM to give compound **25** in 86% yield followed by deacetylation using NaOMe/MeOH, compound **26** was treated with benzyl bromide and sodium hydride in the presence of DMF as the solvent to give the fully benzylated thiogalactoside **4** in 86% yield (Scheme 2.5). By the same procedure, the thioglucoside **5** was synthesized from the commercially available *β*-D-glucopyranose pentaacetate **27.** After introduction of the ethylthio function at the anomeric center and deacetylation, compound **29** was treated with benzyl bromide and sodium hydride in the presence of DMF to give the fully benzylated thioglucoside **5** in 86% yield. On the other hand, the trichloroacetimidate donor **6** was synthesized from compound **30** (Scheme 2.5). The anomeric ethylthio group in compound **30** was removed by treatment with *N*-bromosuccinimide in acetone to give hemiacetal **31** 82% yield. In the next step, trichloroacetimidate functionality was introduced at the anomeric center of **31** by treatment with trichloroacetonitrile in the presence of DBU to give donor **6** in 61% yield.




**Scheme 2.5.** i) Reagents and Conditions: i) EtSH/$ZrCl_4$/DCM; ii) MeONa/MeOH; iii) BnBr/NaH/DMF; iv) NBS/acetone; v) $CCl_3CN$/DBU/DCM.

**Combinatorial glycosylations**

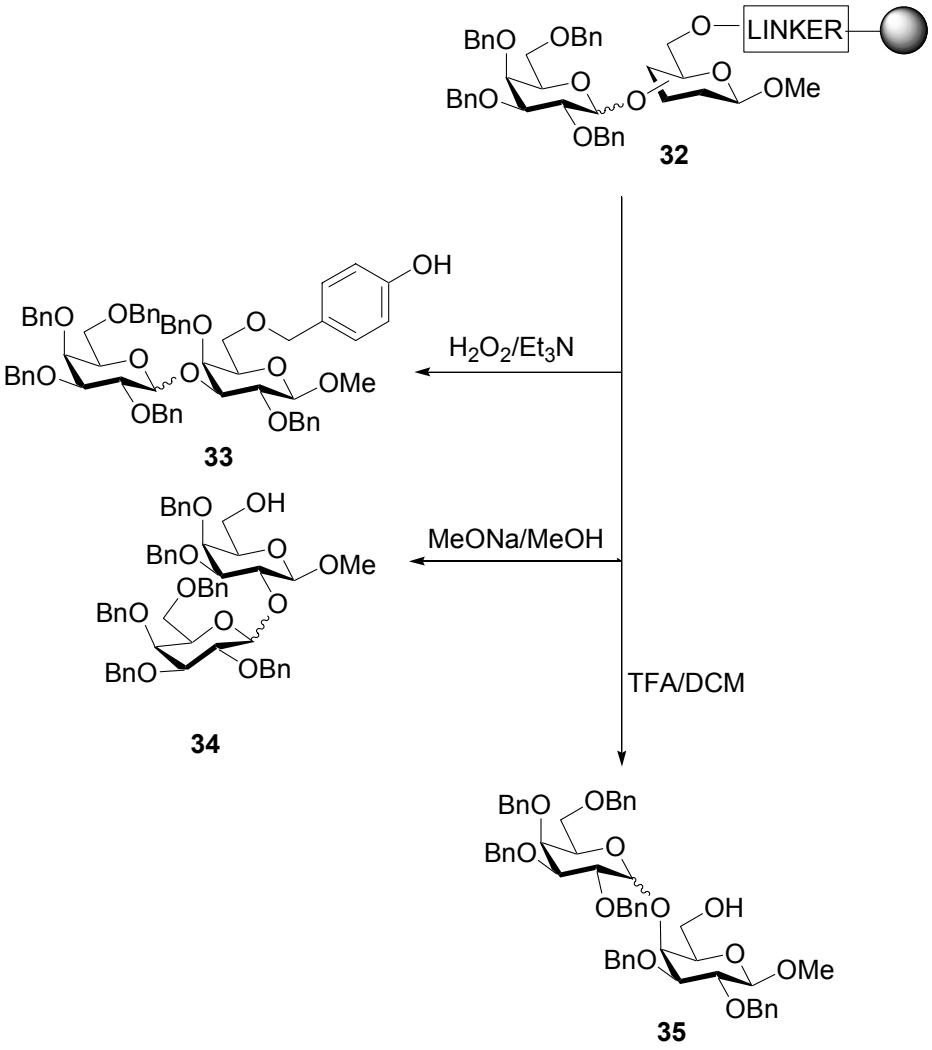
The first pool of the resin-bound monosaccharides library **23** was glycosylated with the thiogalactoside donor **4**[201] using NIS/TMSOTf[202] as the activator and dichloromethane/diethyl ether as the solvent mixture in the presence of 4Å molecular sieves (Scheme 2.6). The resulting MPEG-bound disaccharides **32** were easily purified by selective precipitation, filtration, and washing**.**




**Scheme 2.6:** Combinatorial glycosylation with common thiogalactoside donor **4**.

The next stage of the synthesis entailed demixing of the library **32** by selective cleavage of the linkers followed by precipitation of the MPEG-bound compounds (Scheme 2.7) to give the individual disaccharides **33**, **34**, and **35**. Thus, library **32** was

first treated with $H_2O_2$ and $Et_3N$ in dichloromethane. This oxidative mild basic condition only cleaved the *p*-phenolic ester linker in order to release the disaccharide **33**. Release of only disaccharide **33**, was confirmed by NMR and the MALDI-TOF MS analyses, which showed only the presence of disaccharide **33** and intact acceptor indicating that the cleavage was indeed selective and that the glycosylation had proceeded to completion. MPEG which contained the other disaccharides was precipitated from cold diethyl ether and collected by filtration. While the filtrate, which contained the released disaccharide **33** was kept for purification and deprotection, the precipitated MPEG was dissolved in MeOH and treated with catalytic amount of NaOMe to cleave the succinoyl linker in order to release the second disaccharide **34.** NMR and MALDI-TOF MS analyses also showed only the presence of **34**. The standard procedure of precipitation, filtration, and washing provided the filtrate which contained the released disaccharide **34** and the precipitated MPEG, which still contained the remaining third disaccharide. Next, the precipitated MPEG was treated with 25% TFA in dichloromethane to cleave the acid-labile *p*-alkoxybenzyl linker and release the third disaccharide **35**. NMR and MADLI-TOF MS analyses showed that the third disaccharide had been released. Also NMR analysis indicated that the precipitated MPEG did not contain any carbohydrate, which means that the cleavage of all the linker was complete.

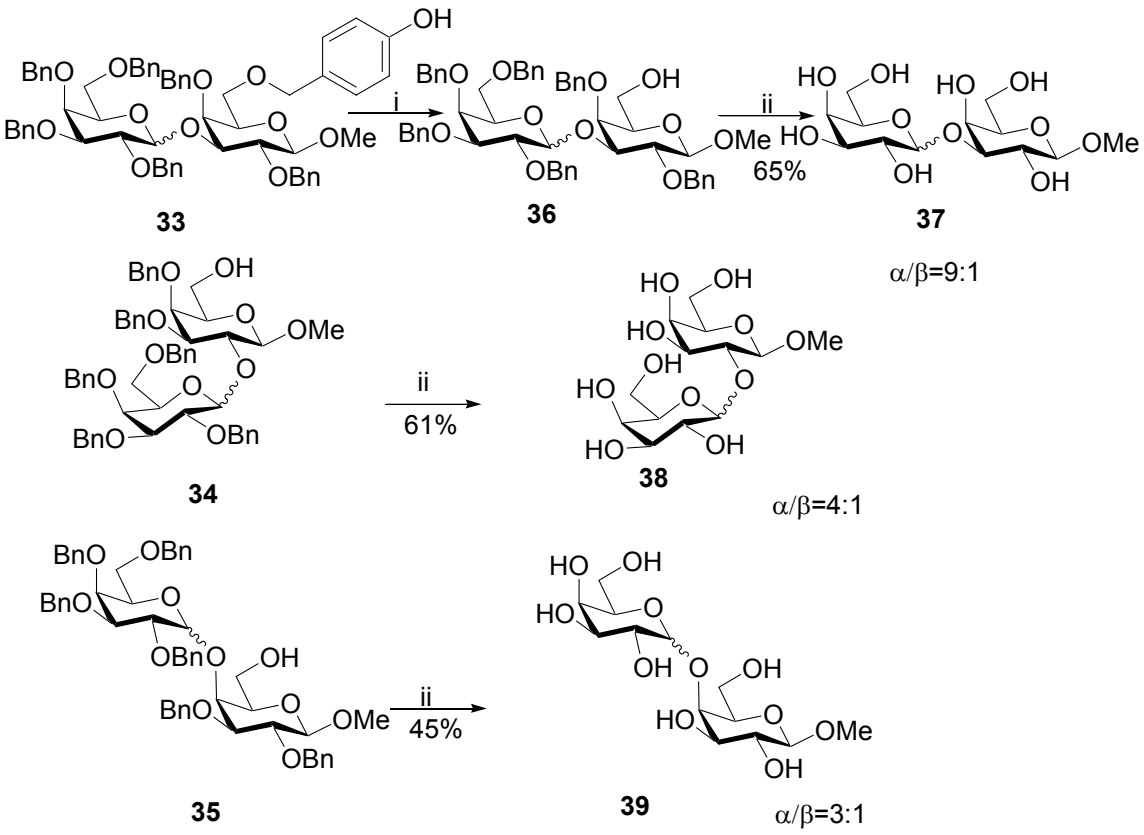



**Scheme 2.7:** Deconvolution of the disaccharides library

**Deprotection of the released disaccharides**

The *p*-hydroxyl benzyl ether protecting group at C-6 of compound **33,** which was obtained after detachment from the polymer, was removed by oxidation with DDQ in dichloromethane to give disaccharide **36** in 81% yield (Scheme 2.8). All the benzyl protecting groups in disaccharide **36** were cleaved by catalytic hydrogenation over Pd/C in ethanol to give fully deprotected disaccharide **37** in 65% yield mainly as α anomer (α/β=9/1). The second released disaccharide **34** was also debenzylated by catalytic

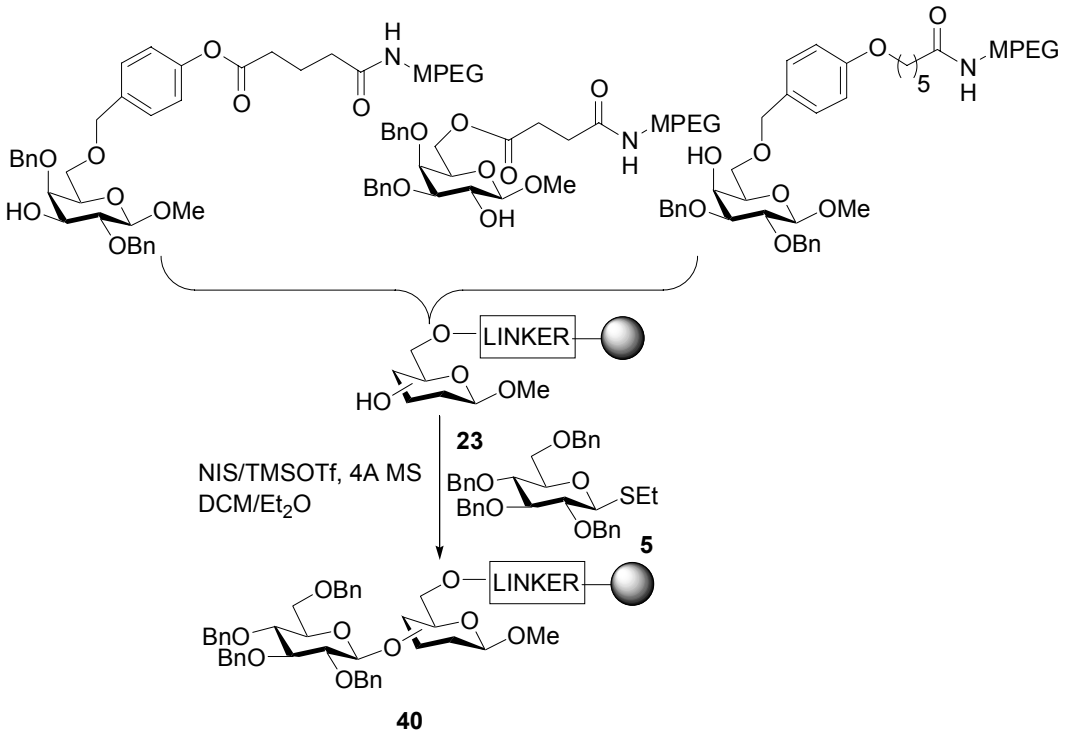
hydrogenation over Pd/C to give the fully deprotected disaccharide **38** in an acceptable yield of 61% (α/β=4/1). As the last step of this synthetic route, the released disaccharide **35** was subsequently treated with hydrogen over Pd/C in ethanol to give the fully deprotected sugar **39** in an overall yield of 45% (α/β=3/1).




**Scheme 2.8.** i) Reagents and conditions: DDQ/DCM; ii) $H_2$/Pd/C/EtOH

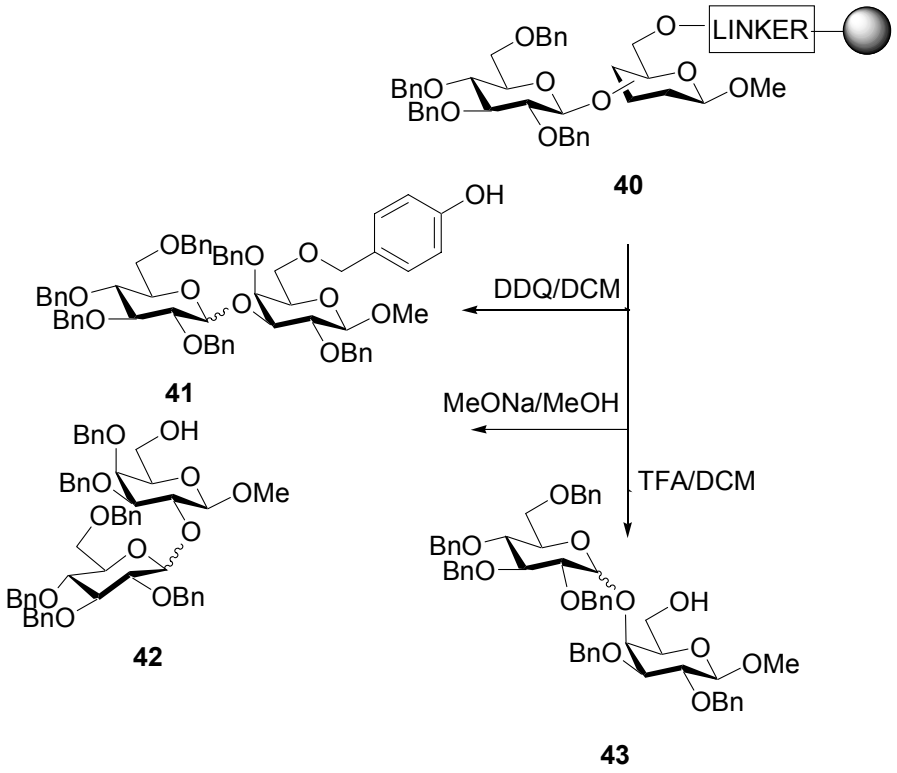
In two separate glycosylations, the second and the third pools of library **23** were coupled with two glucoside donors **5**[203] and **6**[204] were coupled with the glucosyl acceptors library **23** using NIS/TMSOTf[202] and TMSOTf[205] respectively, as the activators. First, as shown in Scheme 2.9, the immobilized galactosyl acceptors library **23** was treated with the thioglucoside donor **5** using NIS/TMSOTf as the activator at 0$^{o}$C in a dry mixture of

$Et_2O$/DCM in the presence of 4Å molecular sieves to give the MPEG-bound disaccharides library **40** which was easily purified by selective precipitation followed by filtration and washing.




**Scheme 2.9:** Combinatorial glycosylation with common thioglucoside donor **5**

The next stage of the synthesis was the demixing of library **40** by selective cleavage of the linkers followed by precipitation of the MPEG-bound disaccharides (Scheme 2.10) to give the individual disaccharides **41**, **42**, and **43**. Thus, library **41** was first treated with $H_2O_2/Et_3N$ in dichloromethane to cleave the phenolic ester linker and release the first disaccharide **41**. After precipitation of MPEG from diethyl ether at $0^oC$ and collected by filtration, the filtrate was examined by NMR and MALDI-TOF MS. The analysis showed only the presence of disaccharide **41** and the absence of any other disaccharide.

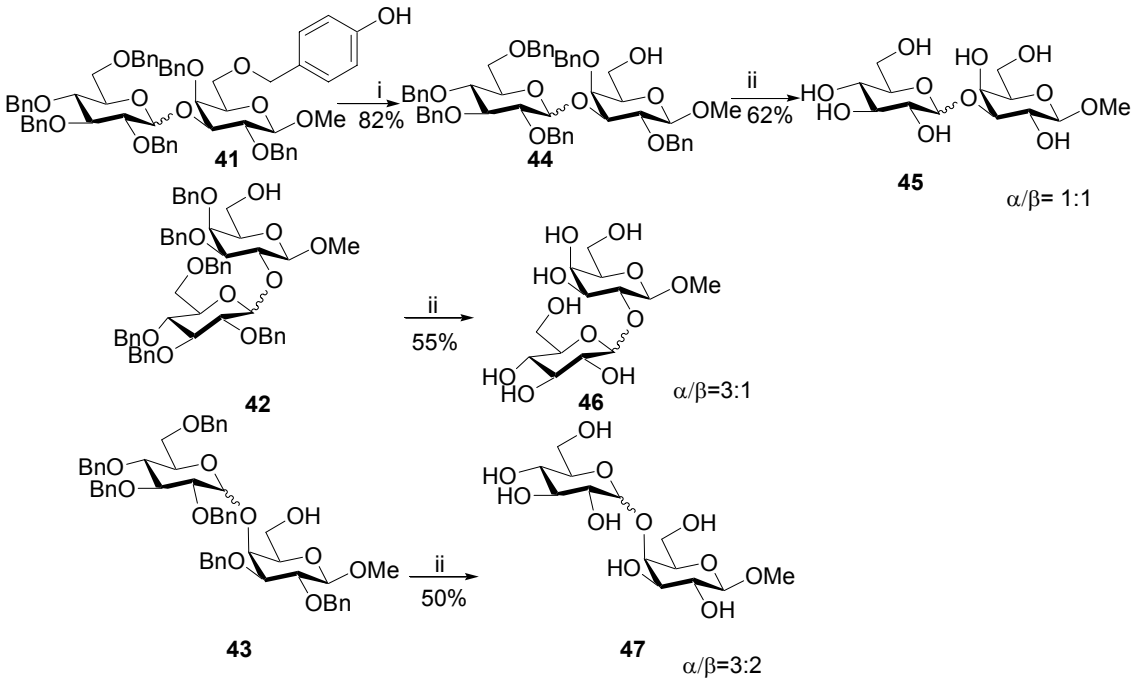



**Scheme 2.10:** Deconvolution of the disaccharides library.

For sequenced release of the other two disaccharides, the precipitated MPEG was dissolved in methanol and treated with catalytic amount of NaOMe. MALDI-TOF Ms analysis showed that disaccharide **42** was released from the polymer. After the typical isolation procedure of precipitation, filtration, and washing, disaccharide **42** was collected in the filtrate when the MPEG containing the third disaccharide was precipitated from diethyl ether at $0^{o}$C. Finally, the precipitated MPEG was treated with 25% TFA in dichloro-methane to cleave the acid-labile *p*-alkoxybenzyl linker in order to release the third disaccharide **43**.

**Deprotection of the released disaccharides**

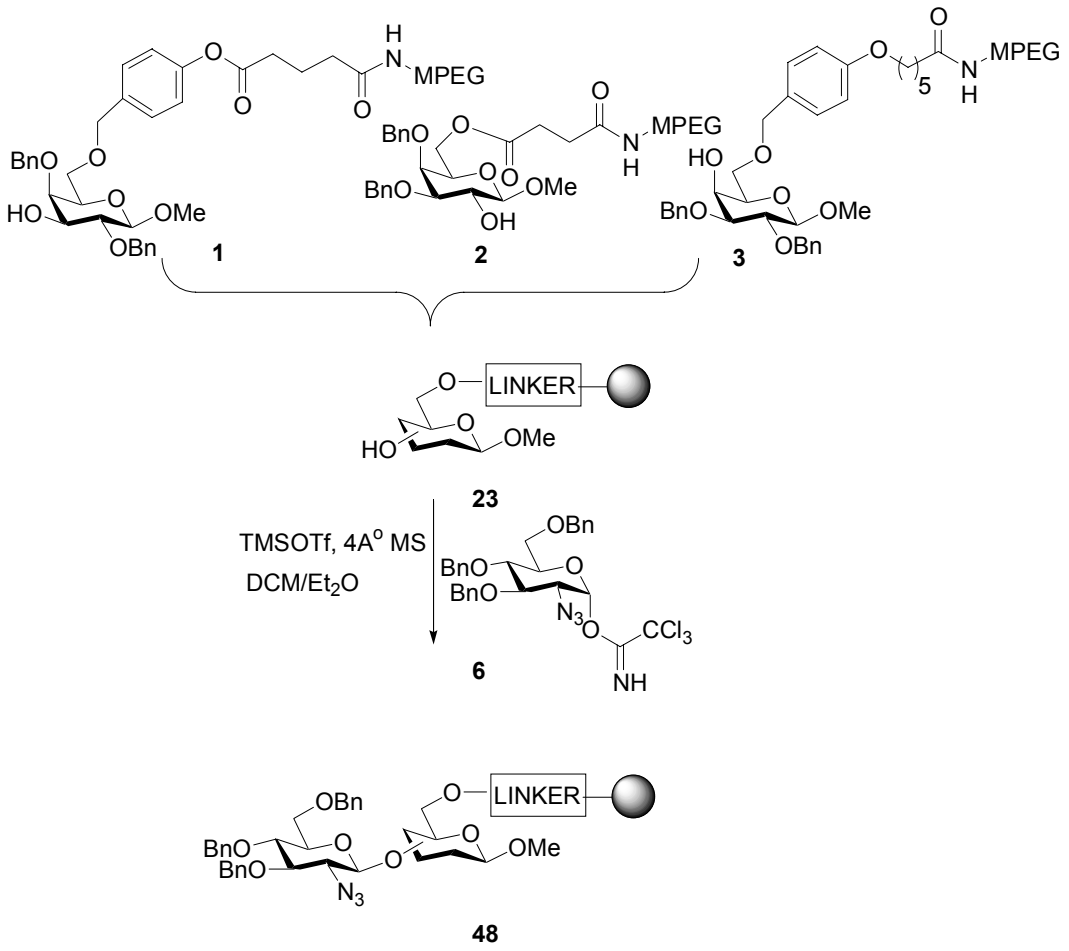
The released disaccharide **41** was treated with DDQ in dichloromethane to cleave the *p*-hydroxyl benzyl ether to give compound **44** in 82% yield. All the benzyl protecting groups of disaccharide **44** were cleaved by catalytic hydrogenation over Pd/C in ethanol to give disaccharide **45** in an overall yield of 62% (α/β=1/1). The released disaccharide **42** was debenzylated by catalytic hydrogenation over Pd/C to give the fully deprotected disaccharide **46** in 55% yield (α/β=3/1). As the last step of the synthetic route, the released disaccharide **43** was also treated with hydogen over Pd/C in ethanol (Scheme 2.11) to give the fully deprotected disaccharide **47** in 50% yield (α/β=3/2).




**Scheme 2.11:** i) DDQ/DCM; ii) $H_2$/Pd/C/EtOH.

The third pool of the library **23** was glycosylated with the trichloroacetimidate donor **6** (Scheme 2.12). TMSOTf-mediated coupling of **23** with 2-azido-3,4,6-tri-*O*-

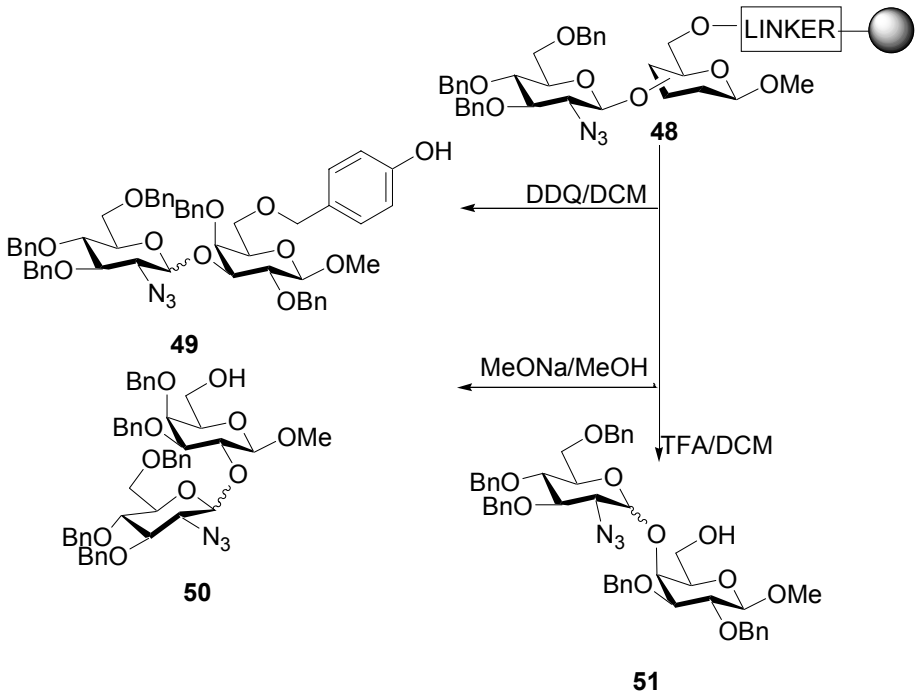
benzyl-2-deoxy-α-D-glucopyranosyl trichloroacetimidate **(6)** gave MPEG-bound disaccharides library **48** which was easily purified by selective precipitation, filtration, and washing.




**Scheme 2.12:** Combinatorial glycosylation with trichloroacetimidate donor **6**.

The disaccharides **49**, **50,** and **51** were demixed by sequential cleavage of the different linkers followed by precipitation, filtration, and washing of the resin after each step. First, library **48** was dissolved in dichloromethane and treated with $H_2O_2/Et_3N$ for 10 minutes to release the first disaccharide **49** after cleaving the *p*-phenolic ester linker (Scheme 2.13). MPEG resin, which contained the other two disaccharides, was

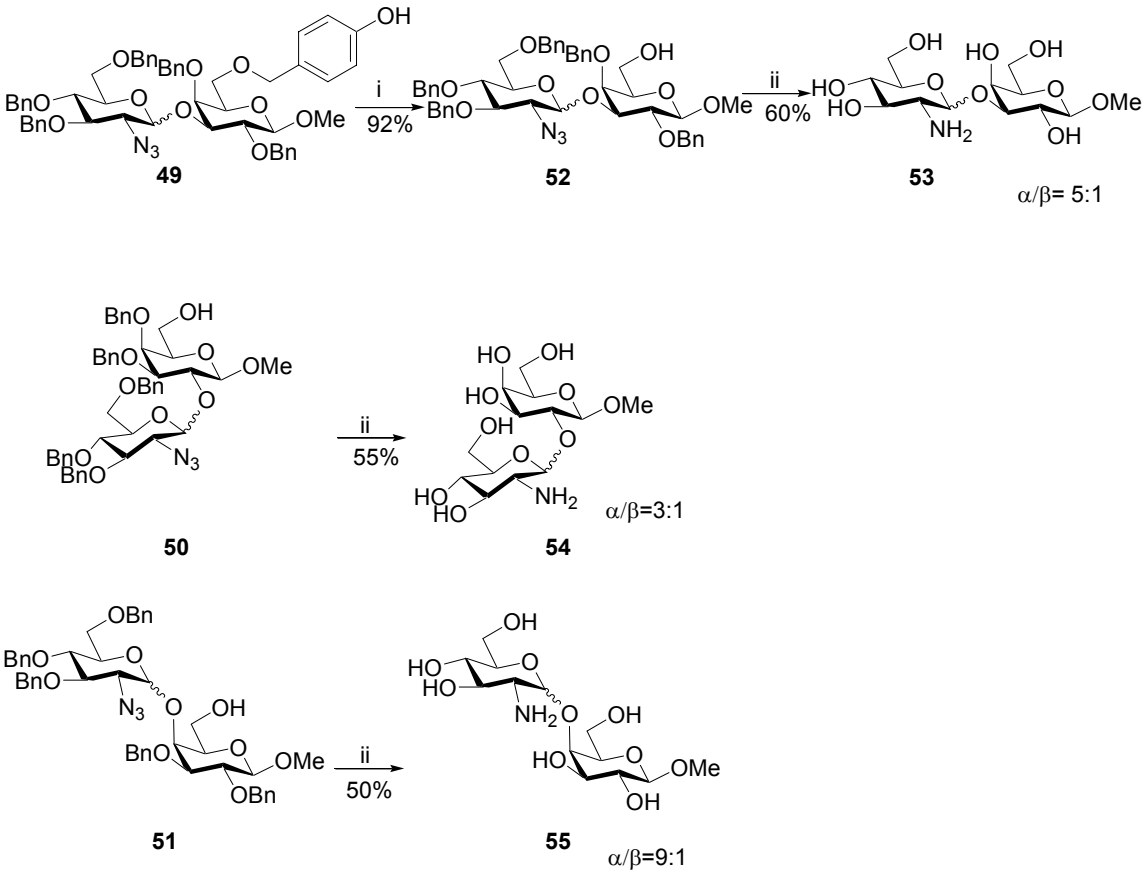
precipitated by adding diethyl ether at 0°C to the reaction mixture with vigorous stirring. The precipitated MPEG was collected by filtration after washing with excessive diethyl ether. The filtrate, which contained the released disaccharide **49**, was concentrated and analyzed by NMR and Maldi-TOF MS. The analysis showed only the presence of disaccharide **49** and the absence of any of the other disaccharides and intact acceptor indicating that the cleavage occurred selectively and the glycosylation had proceeded to completion. Next, the precipitated MPEG was dissolved in methanol and treated with catalytic amount of NaOMe. MALDI-TOF MS analysis showed that disaccharide **50** was released in the solution. After the typical isolation procedure of precipitation, filtration, and washing, disaccharide **50** was collected in the filtrate while the precipitated MPEG was treated with 25% TFA in dichloromethane to cleave the acid-labile *p*-alkoxybenzyl linker to release the third disaccharide **51**.




**Scheme 2.13:** deconvolution of the disaccharides library.

**Deprotection of the released disaccharides**

Full deprotection of the released disaccharide **49** started with cleavage of the *p*-hydroxyl benzyl ether using DDQ in dichloromethane (Scheme 2.14) to give compound **52**. Catalytic hydrogenation over Pd/C to remove all the benzyl protecting groups in compound **52** as well as conversion of the azido functionality into an amine afforded the amino disaccharide **53** in a yield of 60% (α/β=5/1). Both of the other two released disaccharides **50** and **51** were treated similarly to afford the amino disaccharides **54** and **55**, respectively. Disaccharide **54** was obtained in a yield of 55% (α/β=3/1) while disaccharide **55** had an overall yield of 50% (α/β=9/1).




**Scheme 2.14:** i) DDQ/DCM; ii) $H_2$/Pd/C/EtOH.

**Conclusion**

In conclusion, we have developed a new mehod for demixing libraries of compounds that are attached to a soluble polymeric support by tagging starting materials with selective cleavable linkers. Major attractions of the methodology are that libraries of the linker-tagged monosaccharides can repeatedly be used in glycosylations with different glycosyl donors to give a large number of oligosaccharide libraries. Each of these libraries can then be demixed by simple chemical manipulations to give well-defined products. Unlike deconvolution procedures based on tagging of beads, the method described here provides preparative quantities of material that can be characterized by conventional methods. This is important because oligosaccharide synthesis is prone to side-product formation and there are also no reliable strategies for deblocking oligosaccharides attached to the polymeric support. It is to be expected that the new methodology can be applied to other types of liquid supported synthesis and in particular the combination of selective cleavable linkers with fluorous tags will be attractive to demix a relatively large number of compounds. Currently, we are expanding the new methodology by developing several other linkers that will be compatible with the existing linkers and by employing temporary protecting groups to prepare larger oligosaccharides.

## Experimental section

**General.** All reactions were conducted under argon atmosphere. Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), size exclusion column chromatography was performed on Sephadex LH-20 (methanol/dichloromethane,

1/1, v/v). Reactions were monitored by TLC on Kieselgel 60 $F_{254}$ (EM Science) and the compounds were detected by examination under UV light and charring with 5% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40$^{o}$C. All organic solvents were distilled from the appropriate drying agents prior to use: acetonitrile, dichloromethane, diethyl ether, *N,N*-dimethylformamide, pyridine and toluene were distilled from $CaH_2$. Tetrahydrofuran was distilled from sodium directly prior to use. Methanol was dried by refluxing with magnesium methoxide, distilled and stored under argon. Molecular sieves (3Å and 4Å), were crushed and activated *in vacuo* at 390$^{o}$C for 3h prior to application. All NMR spectra were recorded on varian 300 MHz, 500 MHz and 600MHz spectrometers equipped with sun off-line editing workstations. Chemical shifts are reported in parts per million (ppm) using trimethylsilane as internal standard. Matrix-assisted Laser Desorption Ionization- Time-of-Flight (MALDI-TOF) mass spectrometry was performed using a HP MALDI-TOF spectrometer with gentisic acid as matrix. Optical rotations were measured on a Jasco P-1020 polarimeter, and $[\alpha]_D$ are given in units of deg $cm^3$ $g^{-1}$.

**Methyl 4,6-*O*-benzylidene-β-D-galactopyranoside (8).**

To a solution of methyl β-D-galactopyranoside **7** (5g, 25.74mmol) in dry acetonitrile (50mL) was added benzaldehyde dimethyl acetal (8.23mL, 30.88mmol) and catalytic amount of camphorsulfonic acid until pH=3. The mixture was stirred at room temperature overnight. TLC analysis (dichloromethane/methanol, 9/1, v/v) indicated that all the starting material had been consumed. Few drops of $Et_3N$ were added to neutralize the reaction and the reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/hexane, 4/1, v/v), to afford the product **8**

as a white solid (6.4g, 88%). $^{1}$H NMR (300MHz, $CDCl_3$) δ: 7.68-7.40 (m, 5H, Ar-*H*), 5.62 (s, 1H, PhC*H,* benzylidene), 4.43 (d, 1H, H-1, $J_{1,2}$=7.4Hz), 4.39 (dd, 1H, H-6b, $J_{5,6b}$=6.8Hz), 4.29 (dd, 1H, H-6a, $J_{5,6a}$=7.9Hz, $J_{6a,6b}$=11.2Hz), 3.84 (dd, 1H, H-3, $J_{2,3}$=9.6Hz, $J_{3,4}$=2.6Hz), 3.82-3.66 (m, 2H, H-2, H-5), 3.63 (s, 3H, OC$H_3$) 3.58 (s, 1H, H-4). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 136.11, 128.50, 128.43, 127.96, 127.65, 127.53, 101.51, 85.35, 80.13, 78.47, 77.32, 73.67, 68.84, 56.82. FAB-MS: *m/z* 305.26 $[M+Na]^+$. $[\alpha]_D$=-12.19 (c 0.55, $CH_2Cl_2$).

**Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (9) and Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (10).**

To a stirred solution of methyl 4,6-*O*-benzylidene-β-D-galactopyranoside **8** (4g, 14.1mmol) and tetrabutyl ammonium hydrogen sulfate (1.2g, 3.5mmol) in dichloro-methane (50mL) was added benzyl bromide (3.5mL, 21.2mmol) and aqueous sodium hydroxide solution (10%, 10mL). The reaction mixture was heated under reflux for 48h at 60$^{o}$C until TLC analysis (dichloromethane/ methanol, 1/9, v/v) indicated that the reaction is complete. The reaction mixture was cooled and both layers were separated. The organic layer was washed with water (5x50mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent: ethyl acetate/hexane 3/2, v/v) to give **9** as a white solid (2.81g, 60%). Further elution of the column with ethyl acetate gave **10** as a white solid (1.48g, 28%). $^{1}$H NMR (300MHz, $CDCl_3$) **9** δ: 7.33-7.52 (m, 10H, Ar-*H*), 5.56 (s, 1H, PhC*H*, benzylidene), 4.95, 4.86 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.3Hz), 4.34 (dd, 1H, H-6a, $J_{5,6a}$=5.4Hz, $J_{6a,6b}$=11.2Hz), 4.32 (d, 1H, H-1, $J_{1,2}$=7.6Hz), 4.22 (d, 1H, H-4, $J_{3,4}$=2.7Hz), 4.08 (dd, 1H, H-6b, $J_{5,6b}$=6.8Hz), 3.75 (dd, 1H, H-3, $J_{2,3}$=9.6Hz), 3.65 (dd, 1H, H-2), 3.59 (s, 3H, OC$H_3$), 3.35 (ddd, 1H, H-

5). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 136.41, 135.11, 128.54, 128.52, 128.11, 127.99, 127.68, 127.66, 127.63, 127.54, 127.51, 101.45, 85.33, 80.13, 78.47, 77.32, 73.67, 68.84, 55.34. FAB-MS: *m/z* 395.41 $[M+Na]^+$. *Anal* Calcd for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50; found: C, 67.71; H, 6.50. $[\alpha]_D$=-3.6 (c 0.3, $CH_2Cl_2$).

$^1$HNMR (300MHz, $CDCl_3$) **10** δ: 7.34-7.41 (m,10H, Ar-*H*), 5.50 (s, 1H, PhC*H*), 4.91, 4.70 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.3Hz), 4.62 (dd, 1H, H-3, $J_{2,3}$=6.7Hz, $J_{3,4}$=2.3Hz), 4.41 (d, 1H, H-1, $J_{1,2}$=7.5Hz), 4.25 (dd, 1H, H-6b, $J_{6a,6b}$=11.3Hz, $J_{5,6b}$=6.8Hz), 4.22 (dd, 1H, H-4, $J_{4,5}$=1.8Hz), 4.08 (dd, 1H, H-6a, $J_{5,6a}$=6.9Hz), 3.63 (dd, 1H, H-2), 3.59 (s, 3H, OC$H_3$), 3.42 (dd, 1H, H-5). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 135.66, 135.23, 129.54, 129.22, 129.08, 128.56, 128.23, 127.89, 127.36, 127.11, 126.78, 126.53, 101.95, 86.33, 83.13, 78.47, 77.32, 73.67, 68.84, 54.16. FAB-MS: *m/z* 395.21 $[M+Na]^+$. $[\alpha]_D$=-82.01 (c 0.5, $CH_2Cl_2$).

**Methyl 2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-4,6-*O*-benzylidene-β-D-galactopyranoside (11).**

A Solution of *p*-methoxybenzyl chloride (0.65mL, 4.8mmol) in DMF (5mL) was added dropwise to a cooled (0$^o$C) suspension of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside **9** (1.5g, 4.03mmol) and NaH (60% dispersion, 0.48 g, 12.09mmol) in DMF (15mL). After 1h, TLC analysis (ethyl acetate/hexane, 1/1, v/v) showed the completion of this reaction. The excess of NaH was decomposed by the addition of MeOH (1mL) and the resulting mixture was poured into ice-cold water (100mL) and extracted with diethyl ether (5x30mL). Ether layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was crystallized from hexane to afford **11** as a

white solid (1.8g, 95%). $^{1}$H NMR (300MHz, $CDCl_3$) δ: 7.57-7.54 (m, 2H, *o*-Ar-C*H*), 7.40-7.26 (m, 10H, Ar-*H*), 6.85-6.82 (dd, 2H, *m*-Ar-*H*, *J*=8.3Hz), 5.49 (s, 1H, PhC*H*, benzylidene), 4.91, 4.88 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=10.9Hz), 4.79, 4.75 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.2Hz), 4.69 (d, 1H, H-1, $J_{1,2}$=7.6Hz), 4.52 (dd, 1H, H-3, $J_{3,4}$=5.4Hz), 4.48 (dd, 1H, H-2, $J_{2,3}$=6.5Hz), 4.22 (d, 1H, H-4, $J_{4,5}$=1.9Hz), 4.04-4.01 (dd, 2H, H-6a,6b, $J_{6a,6b}$=11.0Hz, $J_{5,6a}$=6.9Hz), 3.79 (s, 3H, PhO*Me*), 3.53 (s, 3H, O*Me*), 3.33 (dd, 1H, H-5, $J_{5,6b}$=3.9Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 159.24, 139.64, 138.33, 129.35, 129.24, 128.95, 128.88, 128.64, 128.60, 128.55, 128.23, 127.98, 127.65, 127.11, 127.04, 126.67, 126.55, 101.60, 99.26, 78.82, 78.46, 75.19, 74.03, 71.65, 69.23, 66.46, 56.99, 55.26. FAB-MS: *m/z* 515.55 $[M+Na]^+$. *Anal.* Calcd for $C_{29}H_{32}O_7$: C, 70.71; H, 6.55; found: C, 70.69; H, 6.58. $[\alpha]_D$= 168.78 (c 0.3, $CH_2Cl_2$).

**Methyl 2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-β-D-galactopyranoside (12).**

A solution of methyl 2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-4,6-*O*-benzylidene-β-D-galactopyranoside **11** (1.87g, 3.81mmol) in 80% aqueous acetic acid (10mL) was heated under reflux at 50$^{o}$C. After 1h, TLC analysis (ethyl acetate/hexane, 4/1, v/v) showed completion of the reaction. The solvent was removed by co-evaporation with toluene and the residue was diluted with dichloromethane (30mL) and washed with saturated aqueous solution of $NaHCO_3$ (2x30mL). The organic layers were collected, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by a column of silica gel column chromatography (eluent: ethyl acetate/hexane, 2/1, v/v) to give **12** as a white solid (1.3g, 84%). $^{1}$H NMR (300MHz, $CDCl_3$) δ: 7.38-7.23 (m, 7H, Ar-*H*), 6.86-6.83 (dd, 2H, *m*-Ar-*H*, *J*=8.8Hz), 4.90, 4.86 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.4Hz), 4.73-4.69 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.4Hz), 4.28 (d, 1H, H-1, $J_{1,2}$=7.8Hz), 4.26 (dd, 1H, H-3, $J_{2,3}$=9.6Hz,

$J_{3,4}$=2.3Hz), 4.05 (m, 3H, H-4, H-6a,b), 3.98 (dd, 1H, H-5, $J_{5.6a}$=7.2Hz, $J_{5,6b}$=6.8Hz), 3.82 (s, 3H, PhO*Me*), 3.51 (s, 3H, O*Me*), 3.44 (dd, 1H, H-2). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 159.24, 139.22, 137.99, 129.55, 129.34, 129.17, 128.14, 127.98, 127.56, 127.45, 126.78, 126.55, 102.06, 98.11, 79.82, 78.66, 75.32, 74.13, 70.65, 69.62, 67.46, 56.99. FAB-MS: *m/z* 427.16 $[M+Na]^+$. *Anal*. Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98; found: C, 65.31; H, 6.96. $[\alpha]_D$= -55.34 (c 0.3, $CH_2Cl_2$).

**Methyl 6-*O-p-allyloxy*benzyl-2-*O-benzyl-3-O-p*-methoxybenzyl-β-D-galactopyranoside (13).**

To a stirred solution of methyl 2-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-β-D-galactopyranoside **12** (1.05g, 2.59mmol) in dry toluene (30mL) was added dibutyl tin dimethoxide (0.66mL, 2.86mmol) and the mixture was heated under reflux with continuous removal of the resulting MeOH using Dean-Stark conditions. After 2h, toluene (15mL) was removed by distillation. Next, *p*-allyloxybenzyl chloride (0.521g, 2.86mmol) and tetra-*n*-butylammonium iodide (1.44g, 3.9mmol) were added to the tin acetal intermediate and the reaction mixture was heated under reflux for 72h with vigorous stirring. When TLC analysis (ethyl acetate/hexane, 3/2, v/v) indicated completion of the reaction, the solvent was removed under reduced pressure and the residue was poured on ice-cold water and extracted with DCM (60mL). The organic layers were successively washed with aqueous solution of KF (1M, 2x30mL). The organic phase was dried over $MgSO_4$ and concentrated *in vacuo* to afford the crude product which was purified on silica gel column chromatography (eluent: ethyl acetate/hexane, 1/4, v/v) to give **13** as a yellowish syrup (870 mg, 61%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.38-7.24 (m, 9H, Ar-*H*), 6.90 (d, 2H, *m*-Ar-*H*, *p*-methoxyphenyl, *J*=8.9Hz), 6.85 (d, 2H, allyloxybenzyl, Ar-*H*,

*J*=7.0Hz), 6.08-6.04 (m, 1H, $OCH_2CH$=$CH_2$), 5.43-5.26 (dd, 2H, $OCH_2CH$=$CH_2$, *J*=7.1Hz, *J*=8.7Hz), 5.02 (d, 2H, $OCH_2CH$=$CH_2$, *J*=5.2Hz), 4.89-4.69 (ABq, 2H, $OCH_2Ph$, $J_{AB}$=10.9Hz), 4.63-4.51 (m, 4H, 2x$OCH_2Ph$), 4.26 (d, 1H, H-1, $J_{1,2}$=7.9Hz), 4.23 (dd, 1H, H-3, $J_{2,3}$=9.6Hz), 3.98 (s, 3H, ArO*Me*), 3.97 (d, 1H, H-4, $J_{3,4}$=2.6Hz, $J_{4,5}$=1.1Hz), 3.86 (dd, 1H, H-2), 3.77-3.63 (m, 3H, H-5, H-6a,b), 3.56 (s, 3H, O*Me*). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 158.5, 151.24, 149.10, 139.23, 138.96, 137.65, 137.52, 129.55, 129.45, 129.11, 128.37, 128.31, 128.02, 127.66, 127.54, 127.23, 127.13, 126.55, 126.34, 126.30, 102.63, 98.19, 79.82, 78.66, 75.32, 74.13, 70.65, 69.62, 67.46, 56.99, 55.26. FAB-MS: *m/z* 573.63 $[M+Na]^+$. *Anal*. Calcd for $C_{32}H_{38}O_8$: C, 69.80; H, 6.96; found: C, 69.83; H, 6.95. $[\alpha]_D$= -12.9 (c 1, $CH_2Cl_2$).

**Methyl 6-*O*-*p*-*allyloxy*benzyl-*3*-*O*-*p*-methoxybenzyl-2,4-di-*O*-benzyl-β-D-galacto-pyranoside (14)**.

A solution of benzyl bromide (0.23mL, 1.92mmol) in DMF (1mL) was added dropwise to a cooled (0$^o$C) suspension of methyl 6-*O*-*p*-*allyloxy*benzyl-2-*O*-*benzyl*-*3*-*O*-*p*-methoxybenzyl-β-D-galactopyranoside **13** (0.85g, 1.6mmol) and NaH (60% dispersion, 96mg, 2.4mmol) in DMF (10mL). After 2h, TLC analysis (ethyl acetate/hexane, 3/7, v/v) showed completion of the reaction and the excess of NaH was decomposed by the addition of MeOH (1mL). The resulting mixture was poured into ice-cold water (50mL) and extracted with diethyl ether (3x30mL). Ether layers were combined and dried over $MgSO_4$, and concentrated *in vacuo.* The resulting syrup was applied to a column of silica gel column chromatography (eluent: ethyl acetate/hexane, 1/4, v/v) to give **14** as a colorless syrup (0.77g, 78%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.38-7.16 (m, 14H, Ar-*H*), 6.88 (d, 2H, *m*-Ar-*H*, *p*-methoxyphenyl, *J*=5.5Hz), 6.83 (d, 2H, allyloxybenzyl, Ar-*H*,

*J*=8.1Hz), 6.06-6.00 (m, 1H, $OCH_2CH{=}CH_2$), 5.44-5.37 (dd, 2H, $CH_2{=}CHCH_2$, *J*=7.1Hz, *J*=10.3Hz), 5.30-5.26 (ABq, 2H, $OCH_2Ph$, $J_{AB}$=11.3Hz), 5.02 (d, 2H, $OCH_2CH{=}CH_2$, *J*=5.2Hz), 4.94-4.51 (m, 6H, 3x$OCH_2Ph$), 4.27 (d, 1H, H-1, $J_{1,2}$=7.9Hz), 3.91 (dd, 1H, H-6a, $J_{5,6a}$=6.9Hz, $J_{6a,6b}$=11.2Hz), 3.85 (d, 1H, H-4, $J_{3,4}$=2.3Hz), 3.79 (dd, 1H, H-6b, $J_{5,6b}$=6.8Hz), 3.77-3.74 (m, 2H, H-2, H-5), 3.69 (s, 3H, PhO*Me*), 3.35 (dd, 1H, H-3, $J_{2,3}$=9.5Hz), 3.25 (s, 3H, O*Me*). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 159.38, 158.57, 139.15, 136.98, 133.49, 130.88, 130.45, 129.72, 129.39, 129.34, 128.45, 128.34, 128.28, 127.67, 117.85, 114.90, 114.83, 113.98, 105.23, 82.09, 79.86, 75.33, 74.66, 73.76, 73.64, 73.40, 72.85, 69.06, 68.79, 57.19, 55.26. FAB-MS: *m/z* 663.75 $[M+Na]^+$. *Anal*. Calcd for $C_{39}H_{44}O_8$: C, 73.10; H, 6.92; found: C, 73.09; H, 6.93. $[\alpha]_D$= 14 (c 1, $CH_2Cl_2$).

**Methyl 2,4-di-*O*-benzyl-6-*O*-*p*-hydroxybenzyl-3-*O*-*p*-methoxybenzyl-β-D-galactopyranoside (15).**

To a stirred solution of methyl 6-*O*-*p*-allyloxybenzyl-3-*O*-*p*-methoxybenzyl-2,4-di-*O*-benzyl-β-D-galactopyranoside **14** (0.75g, 1.17mmol) in EtOH (5mL) with few drops of $H_2O$, was added Pd $(Ph_3P)_4$ (35mg, 0.03mmol). The reaction mixture was heated under reflux at 70$^o$C for 12h until all the starting material had been consumed (monitored by TLC, ethyl acetate/hexane, 1/1, v/v). The reaction mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (eluent: ethyl acetate/toluene 1/9, v/v) to afford **15** as a colorless syrup (0.55g, 80%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.38-7.11 (m, 10H, Ar-*H*), 7.14-7.11 (m, 6H, Ar-*H*), 6.85-6.75 (dd, 2H, Ar-*H*, *J*=3.1Hz), 4.94-4.56 (m, 6H, 3x$OCH_2Ph$), 4.35-4.21 (ABq, 2H, $OCH_2Ph$, $J_{AB}$=11.0Hz), 4.25 (d, 1H, H-1, $J_{1,2}$=7.9Hz), 3.84 (d, 1H, H-4, $J_{3,4}$=2.6Hz, $J_{4,5}$=1.7Hz), 3.78 (s, 3H, PhO*Me*), 3.76 (ddd, 1H, H-5, $J_{5,6a}$=7.0Hz, $J_{5,6b}$=7.9Hz), 3.73 ( d, 1H, H-2,

$J_{2,3}$=9.6Hz), 3.70 (m, 3H, H-3, H-6a,6b), 3.54 (s, 3H, O*Me*). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 159.37, 155.68, 139.11, 138.95, 130.86, 130.22, 129.97, 129.40, 128.46, 128.35, 128.29, 127.67, 115.51, 113.99, 105.23, 82.06, 79.85, 75.34, 74.66, 73.77, 73.65, 73.42, 72.85, 68.82, 57.26, 55.48. FAB-MS: *m/z* 610 $[M+Na]^+$. *Anal*. Calcd for $C_{35}H_{38}O_8$: C, 71.65; H, 6.53; found: C, 71.65; H, 6.55. $[\alpha]_D$=-22 (c 1, $CH_2Cl_2$).

**Methyl 2,4-di-*O*-benzyl-6-*O*-*p*-(5-carboxy-2-one)pentoxybenzyl-3-*O*-*p*-methoxybenzyl -β-D-galactopyranoside (16).**

To a stirred solution of methyl 2,4-di-*O*-benzyl-6-*O*-*p*-hydroxybenzyl-3-*O*-*p*-methoxybenzyl-β-D-galactopyranoside **15** (91mg, 0.15mmol) in pyridine (5mL) were added glutaric anhydride (0.17g, 1.5mmol) and DMAP (catalytic amount). The reaction mixture was stirred at room temperature until TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated completion of the reaction. The reaction mixture was poured into water (5mL) and extracted with dichloromethane (3x5mL). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude compound was purified by LH-20 size exclusion chromatography (eluent: dichloromethane/methanol, 1/1, v/v) to afford **16** as a white foam (80mg, 72%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.40-7.26 (m, 14H, Ar-*H*), 7.14-7.11 (d, 2H, Ar-*H*, *J*=7.9Hz), 6.85 (d, 2H, Ar-*H*, *J*=8.3Hz), 4.96-4.87 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=10.9Hz), 4.76, 4.58 (m, 4H, 2xOC$H_2$Ph), 4.44, 4.39 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.8Hz), 4.26 (d, 1H, H-1, $J_{1,2}$=7.8Hz), 3.83 (dd, 1H, H-3, $J_{2,3}$=9.7Hz, $J_{3,4}$=2.3Hz), 3.78 (s, 3H, PhO*Me*), 3.59 (ddd, 1H, H-5, $J_{5,6a}$=6.8Hz, $J_{5,6b}$=6.8Hz), 3.55 (m, 2H, H-2, H-6a), 3.52 (m, 2H, H-4, H-6b), 2.96 (s, 3H, O*Me*), 2.67 (t, 2H, C$H_2CO_2H$, *J*=3.4Hz), 2.49 (t, 2H, OCOC$H_2$, *J*=7.0Hz), 2.59 (m, 2H, $CH_2CH_2CH_2$). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 172.17, 170.08, 158.54, 139.53, 139.23, 138.25, 137.68, 137.56, 129.56, 129.11, 128.54,

128.22, 127.95, 127.56, 127.44, 126.90, 126.83, 126.55, 126.49, 126.40, 126.33, 102.34, 83.88, 79.82, 79.40, 77.22, 72.79, 70.65, 69.62, 67.46, 56.99, 55.26, 28.97, 28.78. FAB-MS: *m/z* 737.28 $[M+Na]^+$. *Anal*. Calcd for $C_{41}H_{46}O_{11}$: C, 68.89; H, 6.49; found: C, 68.88; H, 6.50. $[\alpha]_D$=-36 (c 1, $CH_2Cl_2$).

**Methyl 2,4-di-*O*-benzyl-6-*O-p-(5-carboxy-2-one)pentoxybenzyl*-β-D-galactopyranoside (1).**

Methyl 2,4-di-*O*-benzyl-6-*O-p*-(5-carboxy-2-one) pentoxy-benzyl-3-*O-p*-methoxybenzyl-β-D-galactopyranoside **16** (40mg, 0.05mmol) was stirred with 10% TFA/DCM (1mL, 1/9, v/v). After 1h, TLC analysis (methanol/dichloromethane 1/19, v/v) indicated completion of the reaction. The reaction mixture was poured into water (5mL) and extracted with dichloromethane (3x2mL). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude compound was purified by LH-20 size exclusion chromatography (eluent: dichloromethane/methanol, 1/1, v/v) to afford **1** as a white foam (20mg, 60%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.36-7.25 (m, 12H, Ar-*H*), 7.05-7.02 (d, 2H, Ar-*H*, *J*=7.9Hz), 4.95-4.78 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=11.3Hz), 4.60, 4.54 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=12.5Hz), 4.38, 4.36 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=11.8Hz), 4.18 (d, 1H, H-1, $J_{1,2}$=7.4Hz), 3.77 (s, 1H, H-4), 3.54 (ddd, 1H, H-6a, $J_{5,6a}$=7.0Hz, $J_{6a,6b}$=11.6Hz), 3.41 (m, 3H, H-2, H-3, H-6b), 3.45 (m, 1H, H-5), 3.49 (s, 3H, O*Me*), 2.60 (t, 2H, C*H*$_2$CO$_2$H, *J*=7.0Hz), 2.46 (t, 2H, OCOC*H*$_2$, *J*=7.0Hz), 2.01 (m, 2H, OCOCH$_2$C*H*$_2$CH$_2$CO$_2$H). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 171.77, 159.34, 138.73, 138.67, 135.80, 128.94, 128.68, 128.52, 128.31, 127.99, 127.87, 121.68, 104.90, 86.45, 83.22, 79.90, 75.71, 75.15, 74.82, 74.24, 73.79, 73.02, 69.08, 57.11, 33.55, 22.16, 20.26,

19.45. FAB-MS: *m/z* 617.29 $[M+Na]^+$. *Anal*. Calcd for $C_{33}H_{38}O_{10}$: C, 66.65; H, 6.44; found: C, 66.65; H, 6.48. $[\alpha]_D$= -32.88 (c 0.5, $CH_2Cl_2$).

**Methyl 3,4-di-*O*-benzyl-β-D-galactopyranoside (17).**

A solution of **10** (100mg, 0.26mmole), Borane.trimethyl amine complex (0.58g, 8mmol), and anhydrous $AlCl_3$ (106mg, 0.8mmol) in dry toluene (5mL) was stirred in the presence of molecular sieves (200mg, 4Å, powdered) for 1h at room temperature. When TLC analysis (ethyl acetate/toluene, 2/1, v/v) indicated that most of **10** had been consumed, the reaction was filtered off and the filtrate was treated with Dowex $50H^+$ resin. After removing the resin by filtration and concentration in *vacuo*, the residue was purified by silica gel column chromatography (eluent: ethyl acetate/toluene, 2/1, v/v) to give **17** as a white solid (66mg, 65%). $^1$HNMR (300MHz, $CDCl_3$) δ: 7.36-7.25 (m, 10H, Ar-*H*), 4.92, 4.90 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=12.0Hz), 4.80, 4.66 (ABq, 2H, OC*H*$_2$Ph, *J*=11.5Hz), 4.24 (d, 1H, H-1, $J_{1,2}$=7.5Hz), 4.0 (dd, H, H-6a, $J_{5,6a}$=6.9Hz, $J_{6a,6b}$=10.8Hz), 3.62 (dd, 1H, H-4, $J_{3,4}$=2.4Hz, $J_{4,5}$=1.8Hz), 3.73 (dd, 1H, H-3, $J_{2,3}$=9.3Hz), 3.58 (s, 3H, O*Me*), 3.55 (dd, 1H, H-2), 3.45 (ddd, 1H, H-5, $J_{5.6b}$=3.7Hz), 3.41 (s, 1H, H-6b). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 138.11, 138.04, 128.54, 128.34, 128.23, 128.10, 128.06, 127.95, 127.69, 127.58, 127.56, 127.53, 98.20, 80.13, 78.47, 77.32, 75.38, 73.67, 73.21, 68.84, 55.29. FAB-MS: *m/z* 397.20 $[M+Na]^+$. *Anal*. Calcd for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00; found: C, 67.35; H, 7.02. $[\alpha]_D$=-22.7 (c 6.4, $CHCl_3$).

**Methyl 3,4-di-*O*-benzyl-6-*O*-succinoyl-β-D-galactopyranoside (2).**

To a stirred solution of methyl 3,4-di-*O*-benzyl-β-D-galactopyranoside **17** (100mg, 0.26mmol) in dry toluene (5mL) was added bis(tri-n-butyltin)oxide (0.13mmol, 60μL)

and the mixture was heated under reflux with continuous removal of MeOH using Dean-Stark conditions. After 2h, toluene (2mL) was removed by distillation and the tin acetal solution was allowed to cool at room temperature. Next, succinic anhydride (26mg, 0.26mmol) was added to the reaction mixture. TLC analysis (acetic acid/ethyl acetate/diethyl ether, 0.05/1/4, v/v/v) showed completion of the reaction after stirring for 2h. After removal of all the solvent under reduced pressure, the residue was diluted with DCM (5mL) and washed successively with aqueous KF (1M, 2x5mL), aqueous solution of $NaHCO_3$ (15%, 2x5mL), and brine (2x5mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness *in vacuo*. The crude product was purified by silica gel column chromatography (eluent: acetic acid/ethyl acetate/diethyl ether, 0.01/1/4, v/v/v) to give **2** as a colorless foam (110mg, 87%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.42-7.22 (m, 10H, Ar-*H* , 2xBn), 4.95, 4.91 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=10.4Hz), 4.72, 4.68 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=10.4Hz) 4.48 (d, 1H, H-1, $J_{1,2}$=7.4Hz), 4.38 (dd, 1H, H-6a, $J_{5,6a}$=6.7Hz, $J_{6a,6b}$=12.4Hz), 4.81 (dd, 1H, H-4, $J_{3,4}$=3.3Hz, $J_{4,5}$=1.7Hz), 4.32 (dd, 1H, H-6b, $J_{5,6b}$=3.4Hz), 3.99 (m, 1H, H-3), 3.60-3.37 (m, 2H, H-2, H-5), 3.52 (s, 3H, O*Me*), 2.65-2.45 (m, 4H, C*H*$_2$C*H*$_2$COOH). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 172.11, 170.09, 138.36, 138.18, 128.77, 128.59, 128.53, 128.49, 128.41, 128.29, 128.12, 128.02, 127.99, 127.92, 82.35, 77.67, 77.25, 76.83, 75.38, 74.64, 73.65, 72.77, 63.12, 29.90, 28.11, 16.69. FAB-MS: *m/z* 497.21 $[M+Na]^+$. *Anal*. Calcd for $C_{25}H_{30}O_9$: C, 63.28; H, 6.37; found: C, 63.30; H, 6.39. $[\alpha]_D$=-41 (c 0.5, $CH_2Cl_2$).

**Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (18).**

A solution of methyl 4,6-*O*-benzylidene-β-D-galactopyranoside **8** (1g, 3.54mmol) in DMF (10mL) was added dropwise to a suspension of NaH (60% dispersion, 0.42g,

10.5mmol) in DMF (10mL) at 0°C and the mixture was stirred for 30 minutes at 0°C. Benzyl bromide (1mL, 8.42mmol) was added over a period of 30 minutes to the reaction which was left stirring for 1h until TLC analysis (methanol/dichloromethane, 1/9, v/v) indicated that all the starting material had been consumed. The excess of NaH was quenched by addition of MeOH (1mL) and the resulting mixture was poured into ice-cold water (100mL) and extracted with diethyl ether (5x20mL). The ether layers were collected, dried over $MgSO_4$, and concentrated under reduced pressure. The crude residue was purified by crystallization from hexane to afford **18** as white crystals (1.5g, 93%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.36-7.11 (m, 15H, Ar-*H*), 5.46 (s, 1H, PhC*H*, benzylidene), 4.77, 4.73 (ABq, 2H, OC$H_2$Ph $J_{AB}$=12.2Hz), 4.53 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.8Hz), 4.32 (d, 1H, H-1, $J_{1,2}$=7.6Hz), 4.14 (bd, 1H, H-4, $J_{3,4}$=2.3Hz), 4.08 (dd, 1H, H-6a, $J_{5,6a}$=7.3Hz, $J_{6a,6b}$=12.3Hz), 4.04 (dd, 1H, H-6b, $J_{5,6b}$=6.8Hz), 3.73 (dd, 1H, H-3, $J_{2,3}$=9.8Hz), 4.00 (dd, 1H, H-2), 3.57 (s, 3H, OC$H_3$), 3.38 (dd, 1H, H-5). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 138.77, 138.41, 138.33, 136.55, 128.51, 128.34, 128.32, 127.98, 127.66, 127.56, 127.34, 127.31, 127.08, 126.99, 126.73, 126.45, 126.34, 126.11, 101.55, 85.33, 84.13, 78.46, 77.33, 75.83, 74.37, 73.69, 73.66, 55.48. FAB-MS: *m/z* 485.53 $[M+Na]^+$. *Anal.* Calcd for $C_{28}H_{30}O_6$: C, 72.71; H, 6.54; found: C, 72.73; H, 6.55. $[\alpha]_D$= 50.5 (c 10, $CHCl_3$).

**Methyl 2,3-di-*O*-benzyl-β-D-galactopyranoside (19).**

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside **18** (1.63g, 3.5mmol) was stirred with 80% aqueous solution of acetic acid (20mL, 1/3, v/v) at 50°C. TLC analysis (ethyl acetate/hexane1/1, v/v) indicated completion of the reaction after stirring for 2h. The reaction mixture was poured into ice-cooled water (50mL) and extracted with

dichloromethane (3x30mL). The organic layers were washed successively with saturated aqueous solution of $NaHCO_3$ (3x30mL) and $H_2O$ (3x30mL). The organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (eluent: ethyl acetate) to afford **19** as colorless syrup (1.08g, 82%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.36-7.10 (m, 10H, Ar-*H*), 4.90, 4.75 (ABq, 2H, OC*H*$_2$Ph, $J_{A,B}$=11.3Hz), 4.79, 4.75 (ABq, 2H, OC*H*$_2$Ph, $J_{A,B}$=12.13Hz), 4.28 (d, 1H, H-1, $J_{1,2}$=7.9Hz), 3.85 ( s, 1H, H-4), 3.82 (m, 2H, H-6a,b) 3.78 (dd, 1H, H-3, $J_{2,3}$ =9.7Hz, $J_{3,4}$=2.6Hz), 3.60-3.29 (m, 2H, H-2, H-5), 3.58 (s, 3H, OC*H*$_3$). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 138.77, 138.41, 128.63, 128.56, 128.34, 128.21, 128.06, 127.99, 127.78, 127.72, 127.69, 127.66, 98.22, 81.49, 79.61, 75.83, 74.37, 69.47, 69.33, 55.30. FAB-MS: *m/z* 397.46 $[M+Na]^+$. *Anal*. Calcd for $C_{21}H_{26}O_6$: C, 67.36; H, 7.00; found: C, 67.38; H, 6.98. $[\alpha]_D$= 8 (c 10, $CHCl_3$).

**Methyl 6-*O*-*p*-Allyloxybenzyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (20).**

To a stirred solution of methyl 2,3-di-*O*-benzyl-β-D-galactopyranoside **19** (1.08g, 2.88mmol) in dry toluene (20mL) was added dibutyl tin dimethoxide (0.71mL, 3.42mmol) and the mixture was heated under reflux at 70$^o$C with continuous removal of the formed methanol using Dean-Stark conditions. After 2h of reflux, (10mL) of the toluene was removed under reduced pressure followed by adding *p*-allyloxybenzyl chloride (1.49g, 3.42mmol) and tetra-*n*-butylammonium iodide (4.18g, 4.32mmol). The reaction mixture was left stirring under reflux for 72h until TLC analysis (ethyl acetate) indicated that all the starting materials had been consumed. After removal of all the solvents under reduced pressure, the residue was poured on ice-cold water (50mL) and extracted with DCM (3x20mL). The organic layers were successively washed with

aqueous KF (1M, 2x30mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified on silica gel chromatography (eluent: ethyl acetate/hexane 1/4, v/v) to give **20** as a yellow syrup (1.2g, 81%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.38-7.24 (m, 12H, Ar-*H*), 6.88 (d, 2H, Ar-*H*, *J*=8.8Hz), 6.10-6.02 (m, 1H, $OCH_2CH$=$CH_2$), 5.44-5.26 (dd, 2H, $CH_2$=$CHCH_2O$, *J*=7.4Hz, *J*=6.7Hz), 5.01 (d, 2H, $CH_2$=$CHCH_2O$, *J*=5.5Hz), 4.90-4.71 (ABq, 2H, $OCH_2Ph$, $J_{AB}$=11.4Hz), 4.74-4.51 (m, 6H, 3x$OCH_2Ph$, $J_{AB}$=12.0Hz), 4.26 (d, 1H, H-1, $J_{1,2}$=7.4Hz), 4.01 (dd, 1H, H-4, $J_{3,4}$=2.6Hz, $J_{4,5}$=1.6Hz), 3.81 (dd, 1H, H-2, $J_{2,3}$=9.8Hz), 3.76 (dd, 1H, H-3), 3.67 (m, 3H, H-5, H-6a,b), 3.56 (s, 3H, O*Me*). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 151.11, 149.43, 139.45, 139.11, 138.23, 137.50, 129.55, 129.16, 128.96, 128.65, 128.45, 127.77, 127.71, 127.56 (2x), 126.54, 126.42, 126.18, 126.02, 103.76, 79.82, 78.66, 75.32, 74.13, 70.65, 69.62, 67.46, 56.99, 55.26. FAB-MS: *m/z* 543.30 $[M+Na]^+$. *Anal*. Calcd for $C_{31}H_{30}O_7$: C, 71.52; H, 6.97; found: C, 71.53; H, 6.96. $[\alpha]_D$= -3.6 (c 0.8, $CHCl_3$)

**Methyl 2,3-di-*O*-benzyl-6-*O*-*p*-hyhroxybenzyl-β-D-galactopyranoside (21).**

To a stirred solution of methyl 6-*O*-*p*-allyloxybenzyl-2,3-di-*O*-benzyl-β-D-galactopyranoside **20** (2.03g, 3.91mmol) in absolute ethanol (30mL), was added $(Ph_3P)_4Pd$ (370 mg, 0.32mmol). The reaction mixture was heated under reflux at 70$^o$C until **20** had been consumed (monitored by TLC, eluent: ethyl acetate/hexane, 1/3, v/v). The reaction mixture was concentrated *in vacuo* and the residue was subjected to silica gel column chromatography (eluent: ethyl acetate/toluene, 1/3, v/v) to afford **21** as a colorless syrup (1.52g, 80%). $^1$H-NMR (300MHz, $CDCl_3$) δ: 7.37-7.19 (m, 12H, Ar-*H*), 6.80-6.77 (d, 2H, Ar-*H*, *J*=8.8HZ), 4.90, 4.86 (ABq, 2H, $OCH_2Ph$, $J_{AB}$=11.02Hz), 4.86, 4.82 (ABq, 2H, $OCH_2Ph$, $J_{AB}$=11.6Hz) 4.50, 4.44 (ABq, 2H, $OCH_2Ph$, $J_{AB}$=12.3Hz), 4.26 (d, 1H, H-

1, $J_{1,2}$=7.4Hz), 4.13 (dd, 1H, H-4, $J_{3,4}$=2.5Hz, $J_{4,5}$=1.9Hz), 3.79 (dd, 1H, H-2, $J_{2,3}$=9.9Hz), 3.72-3.48 (m, 2H, H-6a,b), 3.62 (dd, 1H, H-3), 3.56 (s, 3H, O*Me*), 3.41 (dd, 1H, H-5, $J_{5,6a}$=6.9Hz, $J_{5,6b}$=7.9Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 139.87, 139.55, 138.34, 137.59, 129.78, 129.65, 129.44, 128.94, 128.88, 128.26, 128.14, 127.67, 127.34, 127.13, 126.98, 126.45, 126.42, 126.15, 101.31, 80.76, 78.66, 75.32, 74.13, 72.65, 69.62, 68.46, 59.99, 55.30. FAB-MS: *m/z* 503.56 $[M+Na]^+$. *Anal*. Calcd for $C_{28}H_{32}O_7$: C, 69.98; H, 6.71; found: C, 69.96; H, 6.70**.** $[\alpha]_D$= -13.2 (c 1, $CHCl_3$)

**Methyl 2,3-di-*O*-benzyl-6-*O-p-(5-ethoxycarbonyl)pentoxybenzyl*-β-D-galactopyranoside (22).**

To a stirred solution of methyl 2,3-di-*O*-benzyl-6-*O-p*-hydroxybenzyl-β-galactopyranoside **21 (**1.5g, 3.17mmol) in DMF (20mL) were added ethyl-6-bromohexanoate (0.67mL, 3.18mmol) and $Cs_2CO_3$ (1.55g, 4.75mmol). After stirring the mixture for 4h at room temperature, TLC analysis (ethyl acetate/hexane, 1/1, v/v) showed completion of the reaction. The reaction mixture was poured into water (50mL) and extracted with diethyl ether (4x20mL). The organic layers were washed with brine (2x30mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (eluent: ethyl acetate/hexane, 1/3, v/v) to afford **22** as a colorless syrup (1.77g, 90%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.31-7.19 (m, 12H, Ar-*H*), 6.80-6.78 (d, 2H, Ar-*H*, *J*=8.3Hz), 4.84-4.64 (m, 6H, 3xOC$H_2$Ph), 4.20 (d, 1H, H-1, $J_{1,2}$=7.6Hz), 4.09-3.74 (m, 2H, C$H_2$, aliphatic), 3.93 (d, 1H, H-3, $J_{2,3}$=9.6Hz, $J_{3,4}$=2.6Hz), 3.72 (dd, 1H, H-4, $J_{4,5}$=2.0Hz), 3.71-3.59 (m, 4H, H-2, H-5, H-6a,b), 3.56 (s, 3H, O*Me*), 1.98 (m, 2H, C$H_2$, aliphatic), 1.78-1.42 (m, 6H, 3xC$H_2$, aliphatic), 1.41-1.39 (m, 2H, $CO_2CH_2CH_3$), 1.37 (t, 3H, $CO_2CH_2CH_3$, *J*=2.9Hz), $^{13}$C NMR (125MHz, $CDCl_3$) δ:

173.62, 159.04, 138.73, 138.66, 136.59, 134.76, 129.70, 129.67, 129.56, 129.18, 128.67, 128.56, 127.88, 127.47, 127.23, 127.13, 126.96, 126.46, 126.44, 126.40, 103.67, 79.76, 79.40, 77.26, 74.82, 74.24, 73.79, 73.02, 70.10, 57.16, 34.26, 28.09, 25.60, 24.77, 14.26. FAB-MS: *m/z* 643.78 $[M+Na]^+$. *Anal*. Calcd for $C_{36}H_{46}O_9$ C, 71.59; H, 7.79; found: C, 71.58; H, 7.74. $[\alpha]_D$= -34.5 (c 0.6, $CHCl_3$)

**Methyl 2,3-di-*O*-benzyl-6-*O*-*p*-[(5-carboxy)pentoxy]benzyl-β-D-galactopyranoside (3).**

A methanolic solution of NaOH (saturated solution, 5mL) was added to a solution of **22** (1.8g, 2.9mmol) in methanol/tetrahydrofuran mixture (15mL, 1/1, v/v). The reaction mixture was stirred at room temperature for 30 minutes. After TLC analysis (ethyl acetate/hexane/acetic acid, 1/1/0.03) indicated that **22** had been completely consumed, the reaction mixture was neutralized by addition of Dowex $50H^+$ resin until pH=3. The resin was filtered and the solvent was evaporated to dryness under reduced pressure to afford **3** as a colorless syrup (1.65g, 95%) which was used for the next reaction without further purification. $^1$H NMR (300MHz, $CDCl_3$) δ: 7.31-7.17 (m, 12H, Ar-*H*), 6.81-6.78 (d, 2H, Ar-*H*, *J*=8.3Hz), 4.84-4.64 (m, 4H, 2xOC$H_2$Ph), 4.48 (ABq, 2H, OC$H_2$Ph, *J*=12.5Hz), 4.22 (d, 1H, H-1, $J_{1,2}$=7.4Hz), 3.94 (dd, 1H, H-2, $J_{2,3}$=9.5Hz), 3.82 (m, 2H, H-6a), 3.74 (s, 1H, H-4), 3.74-3.40 (m, 2H, H-3, H-5), 3.51 (s, 3H, O*Me*), 2.35-2.30 (t, 2H, C$H_2CO_2$H, *J*=7.6Hz), 1.79-1.43 (m, 8H, 4xC$H_2$, aliphatic). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 174.29, 159.50, 138.73, 138.67, 136.90, 134.66, 129.80, 129.77, 129.66, 129.53, 129.47, 128.91, 128.33, 128.21, 127.15, 127.04, 126.90, 126.88, 126.86 126.71, 104.89, 86.12, 82.15, 79.75, 79.45, 77.28, 74.62, 74.24, 73.99, 73.05, 71.10, 56.14, 34.26,

28.19, 25.73, 24.73, 14.28. FAB-MS: *m/z* 617.7 $[M+Na]^+$. *Anal*. Calcd for $C_{34}H_{42}O_9$: C, 68.67; H, 7.12; found: C, 68.65; H, 7.13. $[\alpha]_D$= 3.49 (c 0.5, $CH_2Cl_2$).

**Ethyl 2,3,4,6-tetra-*O*-Acetyl-1-thio-β-D-galactopyranoside (25).**

To a stirred solution of β-D-galactose pentaacetate **24** (5g, 12.8mmol) in dichloromethane (100mL) at 0°C was added ethanethiol (0.95mL, 12.8mmol) and $ZrCl_4$ (2.8g, 12mmol) and the resulting suspension was stirred for 1h. An additional equivalent of ethanethiol (0.95mL, 12.8mmol) was added and the mixture was stirred at 0°C for 1h. When TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated that all the starting material had been consumed, the reaction mixture was diluted with dichloromethane (100mL) and filtered. The filtrate was washed successively with ice-cold water (2x50mL), aqueous $NaHCO_3$ (15%, v/w, 2x50mL), and water (2x50mL). The organic layers were collected, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from ethanol to give **25** as colorless crystals (4.29g, 86%). $^1$H NMR (300MHz, $CDCl_3$) δ: 5.44 (d, IH, H-4, $J_{3,4}$=2.5Hz), 5.25 (d, 1H, H-1, $J_{1,2}$=7.5Hz), 5.11 (dd, 1H, H-2, $J_{2,3}$= 9.9Hz), 5.05 (dd, 1H, H-3, $J_{3,4}$=5.6Hz), 4.18 (dd, 1H, H-6a, $J_{5,6a}$= 6.9Hz, $J_{6a,6b}$=11.0Hz), 4.10 (dd, 1H, H-6b, $J_{5,6b}$=6.9Hz), 3.94 (ddd, 1H, H-5), 2.84-2.64 (m, 2H, SC$H_2$), 2.17, 2.09, 2.05, 2.00 ( 4s, each 3H, C$H_3$CO), 1.28 (t, 3H, $SCH_2CH_3$, *J*=7.3Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 170.59, 170.44, 170.29, 169.79, 84.24, 74.53, 72.08, 67.44, 67.36, 61.65, 24.56, 21.01, 20.86, 20.86, 20.78, 15.04. FAB-MS: *m/z* 415 $[M+Na]^+$. *Anal.* Calcd for $C_{16}H_{24}O_9S$: C, 48.97; H, 6.16; found: C, 49.01; H, 6.15. $[\alpha]_D$= -8.5 (c 10, $CHCl_3$).

**Ethyl 1-*thio*-β-D-galactopyranoside (26).**

A solution of NaOMe in methanol (1%, 5mL) was added (pH of solution: 11-12) to a solution of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **25** (4.0g, 10.19mmol) in methanol/DCM (30mL, 5/1, v/v). The mixture was stirred at room temperature for 30 min. When TLC analysis (MeOH/DCM, 1/4, v/v) indicated that all the starting material had been consumed, the reaction mixture was neutralized by addition of Dowex $50H^+$ resin and filtered. The filtrate was concentrated *in vacuo* to afford **26** as a white solid (2.26g, 99%) which was used without further purification for the next step. $^1$H NMR (300MHz, $D_2O$) δ: 4.86 (d, IH, O*H*, *J*=5.3Hz), 4.72 (d, 1H, O*H*, *J*=5.3Hz), 4.52 (t, 1H, 6-O*H*, $J_{6a,OH}$=$J_{6b,OH}$=5.3Hz), 4.34 (d, 1H, O*H*, *J*=4.4Hz), 4.20 (d, 1H, H-1, $J_{1,2}$=8.2Hz), 3.67 (m, 1H, H-3), 3.46 (m, 2H, H-6a, H-6b), 3.42-3.21(m, 3H, H-2, H-4, H-5), 2.78-2.45 ( m, 2*H*, SC*H*$_2$CH$_3$), 1.18 (t, 3H, SCH$_2$*CH*$_3$, *J*=7.1Hz), $^{13}$C NMR (125MHz, $D_2O$) δ: 85.29, 79.04, 74.64, 69.69, 68.35, 60.54, 22.98, 15.13. FAB-MS: *m/z* 247 $[M+Na]^+$. $[\alpha]_D$= -22.7 (c 10, $H_2O$).

**Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (4).**

A solution of ethyl 1-thio-β-D-galctopyranoside **26** (2.26g, 10.07mmol) in DMF (30mL) was added dropwise to a suspension of NaH (60% dispersion, 2.4g, 60.0mmol) in DMF (50mL) at 0$^o$C. The mixture was stirred for 30 minutes at 0$^o$C. Benzyl bromide (5.7mL, 48.0mmol) was added dropwise and the mixture was stirred at room temperature for 2h. When TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated that all the starting material had been consumed, the excess of NaH was quenched by addition of MeOH (2mL). The resulting mixture was poured into ice-cold water (100mL) and extracted with diethyl

ether (5x50mL). Ether layers were combined and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was crystallized from hexane to give **4** as a crystalline white solid (5.08g, 86%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.38-7.08 (m, 20H, Ar-*H*), 4.96, 4.83 (ABq, 2H, OC*H*$_2$Ph, $J_{A,B}$=10.3Hz), 4.78, 4.72 (ABq, 2H, OC*H*$_2$Ph, $J_{A,B}$=11.6Hz), 4.69 (s, 2H, OC*H*$_2$Ph), 4.63, 4.57 (ABq, 2H, OC*H*$_2$Ph $J_{A,B}$=11.8Hz), 4.45 (d, 1H, H-1, $J_{1,2}$=7.6Hz), 4.42 (dd, 1H, H-2, $J_{2,3}$=9.9Hz), 3.96 (d, 1H, H-4, $J_{3,4}$=2.7Hz), 3.77 (dd, 1H, H-6a, $J_{5,6a}$=6.9Hz, $J_{6a,6b}$=11.2Hz), 3.73-3.69 (m, 2H, H-3, H-6b), 3.46 (dd, 1H, H-5, $J_{5,6a}$=7.3Hz), 2.83-2.63 (q, 2H, SC*H*$_2$CH$_3$) 1.30 (t, 3H, SCH$_2$C*H*$_3$, *J*=7.4Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 138.78, 138.41, 138.33, 137.89, 128.50, 128.46, 128.44(2x), 127.96, 127.89, 127.55(3x), 127.23, 127.21, 127.11, 127.02(2x), 126.98(3x), 126.65, 126.53, 85.35, 84.13, 78.47, 77.22, 75.83, 74.47, 73.60, 73.57, 72.75, 68.84, 24.85, 15.13. FAB-MS: *m/z* 607.72 $[M+Na]^+$. $[\alpha]_D$= -6.4 (c 10, $CHCl_3$).

**Ethyl 2,3,4,6-tetra-O-Acetyl-1-*thio*-β-D-glucopyranoside (28).**

To a stirred solution of β-D-glucose pentaacetate **27** (5g, 12.8mmol) in dichloromethane (100mL) at 0°C was added ethanethiol (0.95mL, 12.8mmol) and $ZrCl_4$ (2.8g, 12mmol) and the resulting suspension was stirred for 1h. An additional quantity of ethanethiol (0.19mL, 2.6mmol) was added and the mixture was stirred at 0°C for a further 1h. When TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated that all the starting material had been consumed, the reaction mixture was diluted with dichloromethane (100mL) and filtered. The filtrate was washed successively with ice-cold water (2x50mL), aqueous $NaHCO_3$ (15%, v/w, 2x 50mL), water (2x50mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from ethanol to give **28** as a colorless crystalline solid (4.5g, 90.2%). $^1$H NMR (300MHz,

$CDCl_3$) δ: 5.23 (dd, 1H, H-3, $J_{2,3}$=8.4Hz, $J_{3,4}$=9.2Hz), 5.09 (dd, 1H, H-2, $J_{1,2}$=7.7Hz), 5.04 (dd, IH, H-4, $J_{4,5}$=9.9Hz), 4.50 (d, 1H, H-1), 4.27 (dd, 1H, H-6a, $J_{5,6a}$=5.1Hz, $J_{6a,6b}$=10.5Hz), 4.13 (dd, 1H, H-6b, $J_{5,6b}$=9.2Hz), 3.72 (ddd, 1H, H-5, $J_{5,6b}$=3.6Hz), 2.79-2.62 (m, 2H, SC$H_2$CH$_3$), 2.08, 2.06, 2.03, 2.01 (4s, 12H, 4xC$H_3$CO), 1.27 (t, 3H, SCH$_2$C$H_3$, *J*=7.0Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 170.68, 170.21, 169.79, 169.42, 83.53, 75.89, 69.85, 68.35, 73.93, 62.18, 24.19, 20.75, 20.63, 14.84. FAB-MS: *m/z* 415.54 $[M+Na]^+$. *Anal*. Calcd for $C_{16}H_{24}O_9S$: C, 48.97; H, 6.16; S, 8.17; found: C, 49.09; H, 6.15; S, 8.16. $[\alpha]_D$= -29.2 (c 10, $CHCl_3$).

**Ethyl 1-thio-β-D-glucopyranoside (29).**

A solution of NaOMe in methanol (1%, 5mL) was added (pH of solution: 11-12) to a solution of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **28** (4.0g, 10.2mmol) in MeOH/DCM mixture (30mL, 5/1, v/v). The solution was stirred at room temperature for 1h until TLC analysis (MeOH/DCM, 1/4, v/v) indicated that all the starting material had been consumed. The reaction mixture was neutralized by addition of Dowex $50H^+$ resin and filtered. The filtrate was concentrated *in vacuo* to afford **29** as a white solid (2.26g, 99%) which was used without further purification. $^1$H NMR (300MHz, $CDCl_3$) δ: 4.82 (d, 1H, O*H*, *J*=5.3Hz), 4.72 (d, 1H, O*H*, *J*=5.3Hz), 4.52 (t, 1H, 6-O*H* $J_{6a,OH}$= $J_{6b,OH}$=5.3Hz), 4.38 (d, 1H, O*H*, *J*=4.4Hz), 4.20 (d, 1H, H-1, $J_{1,2}$=7.9Hz) 3.65 (m, 1H, H-3), 3.42 (m, 4H, H-3, H-6a,b), 3.42-3.20 (m, 3H, H-2, H-4, H-5), 2.76-2.45 (m, 2H, SC$H_2$CH$_3$), 1.19 (t, 3H, SCH$_2$C$H_3$). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 85.26, 79.04, 74.66, 69.54, 68.34, 60.52, 22.86, 15.22. FAB-MS: *m/z* 247.26 $[M+Na]^+$. $[\alpha]_D$= -54 (c 1, $H_2O$).

**Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (5).**

A solution of ethyl 1-thio-β-D-glucopyranoside **29** (2.27g, 10.12mmol) in DMF (20mL) was added dropwise to a suspension of NaH (60% dispersion, 2.4g, 60.0mmol) in DMF (20mL) at 0°C and the mixture was stirred for 30 minutes at 0°C. Benzyl bromide (5.7mL, 48mmol) was added dropwise and the mixture was stirred at room temperature for 2h until TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated that all the starting material had been consumed. The excess of NaH was quenched by addition of MeOH (2mL). The resulting mixture was poured into ice-cold water (100mL) and extracted with diethyl ether (5x50mL). The ether layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was crystallized from hexane to give **5** as a white solid (5.08g, 86%). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.40-7.10 (m, 20H, Ar-*H*), 4.96, 4.94 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=10.6Hz), 4.88, 4.83 (ABq, 2H, O*CH*$_2$Ph, $J_{AB}$=10.0Hz), 4.77, 4.69 (ABq, 2H, OC*H*$_2$Ph $J_{AB}$= 9.8Hz), 4.57, 4.46 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=12.2Hz), 4.47 (d, 1H, H-1, $J_{1,2}$=7.6Hz), 3.75 (dd, 1H, H-6a, $J_{5,6a}$=5.1Hz, $J_{6a,6b}$=11.6Hz), 3.72-3.62 (m, 2H, H-6b, H-4), 3.60 (dd, 1H, H-3, $J_{2,3}$=8.3Hz $J_{3,4}$=9.2Hz), 3.48 (m, 1H, H-5), 3.44 (dd, 1H, H-2), 2.68-2.86 (m, 2H, SC*H*$_2$CH$_3$), 1.32 (t, 3H, SCH$_2$C*H*$_3$, *J*=7.3Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 138.55, 138.26, 138.07, 128.54, 128.48, 128.17(3x), 128.12, 127.96, 127.90 (2x), 127.88, 127.56, 127.44, 127.32 (3x), 127.12 (2x), 127.10, 126.87, 126.08, 101.05, 88.73, 85.12, 81.83, 79.16, 78.04, 75.84, 75.58, 75.14, 73.50, 69.18, 25.10, 15.27. FAB-MS: *m/z* 607.78 $[M+Na]^+$. *Anal* Calcd for $C_{36}H_{40}O_5S$: C, 73.94; H, 6.89; S, 5.48; found: C, 74.96; H, 6.88; S, 5.47. $[\alpha]_D$= 3.4 (c 10, $CHCl_3$).

**2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (31).**

A mixture of ethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-β-D-glucopyranoside **30** (1.0g, 1.92mmol**)** and *N*-bromosuccinimide (0.34g, 1.92mmol) in acetone (25mL) was stirred at room temperature overnight. When TLC analysis (ethyl acetate/hexane, 3/2, v/v) indicated completion of the reaction, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1/1) to give **31** as a white solid (0.9g, 82%). $^{1}$H NMR (300MHz, $CDCl_3$) δ: 7.58-7.16 (m, 15H, 3xBn, Ar-*H*), 4.87, 4.52 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=10.0Hz), 4.72, 4.66 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=11.6Hz), 4.48, 4.42 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=11.7Hz), 4.40 (d, 1H, H-1, $J_{1,2}$=7.9Hz), 3.94 (dd, 1H, H-4, $J_{3,4}$=8.8Hz, $J_{4,5}$=9.6Hz), 3.84 (dd, 1H, H-2, $J_{2,3}$=8.3Hz), 3.66-3.54 (m, 3H, H-5, H-6a,b), 3.42 (dd, 1H, H-3). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 139.16-135.85 (*C*q, Ar), 128.51-126.37 (C*H*, Ar), 96.22, 92.11, 83.27, 77.72, 74.91, 73.45, 72.15, 60.12. FAB-MS: *m/z* 498.23 $[M+Na]^+$. *Anal*. Calcd for $C_{27}H_{29}N_3O_5$: C, 68.19; H, 6.15; N, 8.84; found: C, 68.21; H, 6.15; N, 8.81, $[\alpha]_D$=18.1 (c 1, $CHCl_3$).

**2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl trichloroacetimidate (6).**

To a solution of 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose **31** (1.0g, 2.1mmol) in $CH_2Cl_2$ (20mL), were added trichloroacetonitrile (3.17mL, 31.54mmol) and catalytic amount of DBU. The reaction mixture was stirred at room temperature for 4h. After TLC analysis (ethyl acetate/hexane, 1/1, v/v) showed that all the starting material had been consumed, the reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: hexane/ethyl

acetate/ triethylamine, 7/3/0.5, v/v/v) to give **6** as a colorless foam (0.8g, 61%). $^{1}$H NMR (300MHz, $CDCl_3$) δ: 8.75 (s, 1H, N*H*), 7.35-7.15 (m, 15H, Ar-*H*), 5.62 (d, 1H, H-$J_{1,2}$=7.9Hz), 4.94, 4.90 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=10.2Hz), 4.86, 4.82 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=12.2Hz), 4.79, 4.74 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=9.93Hz), 3.83-3.54 (m, 4H, H-3, H-5, H-6a, H-6b), 3.69 (dd, 1H, H-2, $J_{2,3}$=8.4Hz), 3.35 (dd, 1H, H-4, $J_{3,4}$=8.9Hz, $J_{4,5}$=9.5Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 138.34, 138.15, 138.11, 128.51, 128.23, 128.16 (3x), 127.88, 127.65(2x), 127.62, 127.60(2x), 127.58(2x), 127.53, 127.50, 96.33, 84.13, 78.46, 77.33, 75.83, 74.37, 73.69, 73.66. FAB-MS: *m/z* 642.90 $[M+Na]^+$. *Anal.* Calcd for $C_{29}H_{29}Cl_3N_4O_5$: C, 56.19; H, 4.72; Cl, 17.16; N, 9.04; found: C, 56.20; H, 4.41; Cl, 17.16; N, 9.11. $[\alpha]_D$=-3 (c 1.15, $CHCl_3$).

**General Procedure for Immobilization of the Glycosyl Acceptors.**

300 mg of the amino-functionalized MPEG (loading capacity 0.2mmole/g, Mw =5000) was placed in a round bottom flask and dissolved in *N,N*-dimethylformamide (~2mL). A mixture of 1, 2, and 3 (2 equivalents each, 0.02mmol: 11.9mg of 1, 9.5mg of 2, and 11.9 mg, of 3), PyBOP (0.12mmol, 124.2mg), and DIPEA, (0.12mmol, 46.2μL) were added to the polymer. The reaction mixture was stirred at room temperature under Argon atmosphere for 2h and monitored by Kaiser's test. When Kaiser's test indicated completion of the reaction, the reaction mixture was cooled to 0$^{o}$C in an ice bath and diethyl ether (150mL) was added gradually under vigorous stirring to precipitate all the resin-bound glycosyl acceptors mixture 23. The precipitated library 23 was filtered and kept dry in vacuum line for the further reactions.

**General Procedure for NIS/TMSOTf Mediated Glycosylation on Polymer Support**. The mixture of the immobilized acceptors **23** (300mg) was divided into three pools. Each pool was glycosylated with the glycosyl donors **4**, **5**, and **6** in separate flasks. The first pool (100mg) was dissolved in dry dichloromethane/diethyl ether mixture (2mL, 1/1, v/v) and stirred with methyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside **4** (70mg, 0.12 mmol) in the presence of molecular sieves (100mg, 4Å, powdered) for 30minutes. The mixture was cooled ($0^oC$, ice bath) and NIS (27mg, 0.12mmol) and TMSOTf (1.80 μL, 0.012mmol) were added, and the mixture was stirred for 2h under Argon atmosphere. The reaction was monitored by TLC (ethyl acetate/hexane, 3/7, v/v) until it showed complete consumption of donor **4**. The reaction mixture was filtered and the filtrate was diluted with dichloromethane (20mL) and washed with aqueous sodium thiosulfate (20%, 2x15mL). The organic layer was concentrated and cooled ($0^oC$, ice bath). Excessive amount of diethyl ether (200mL) was added to the residue with vigorous stirring for 2h until all of resin-bound disaccharides library **32** was precipitated, filtered, and kept dry in a vacuum line for the next steps. The same procedure mentioned above was applied for the glycosylation of the second pool of **23** (100mg) with the thioglucoside donor **5** (70mg, 0.12mmol) using NIS/TMSOTf as the activator to give the resin-bound disaccharides library **40**. TMSOTf-mediated coupling of the trichloroacetimidate donor **6** (74.39mg, 0.12mmol) with the third pool of **23** afforded the disaccharides library **48**.

**Methyl 2,4-di-*O*-benzyl-6-*O*-(*p*-hydroxybenzyl)-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)-β-D-galactopyranoside (33).**

The resin-bound disaccharides mixture **32** (300mg) was dissolved in tetrahydrofuran (5mL). Few drops of triethylamine were added until the medium became basic (pH=9)

followed by addition of few drops of aqueous hydrogen peroxide solution (50μL). The reaction mixture was stirred at room temperature for 2h until TLC analysis (ethyl acetate/hexane, 1/1, v/v) showed the release of disaccharide **33**. The solvent was removed under reduced pressure and the resulting residue was dissolved in dichloromethane (0.5mL) and cooled to 0°C in an ice bath. Diethyl ether (150mL) was added gradually with vigorous stirring for 1h until all the resin was precipitated. The precipitate was collected by filtration and the filtrate was concentrated *in vacuo.* The residue obtained from the concentration of the filtrate was purified by silica gel column chromatography (ethyl acetate/hexane, 3/7, v/v) to afford disaccharide **33** as a colorless syrup (15mg). $^{1}$H NMR (500 MHz, $CDCl_3$) δ: 7.38 (d, 2H, Ar-*H*), 7.30 (d, 2H, Ar-*H*), 7.35-7.13 (m, 64H, Ar-*H*), 5.52 (d, 1H, H-1'α, $J_{1',2'}$=3.8Hz), 4.93, 4.91 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.0Hz), 4.87 (d, 1H, H-1'β, $J_{1',2'}$=7.9Hz), 4.85-4.78 (m, 14H, 7xOC$H_2$Ph), 4.67, 4.62 (ABq, 2H, OC$H_2$Ph, *J*=11.8Hz), 4.60-4.48 (m, 10H, 5xOC$H_2$Ph), 4.39 (d, 1H, H-1β, $J_{1,2}$=7.4Hz), 4.18 (d, 1H, H-3'α, $J_{3',4'}$=2.6Hz), 4.15 (d, 1H, H-4'α), 4.42-3.90 (m, 5H, H-2', H-6'a,b, H-5'), 3.90-3.77 (m, 4H, H-2, H-3, H-6a,b), 3.75 (d, 1H, H-4β, $J_{3,4}$=2.9Hz), 3.67 (t, 1H, *J*=7.7Hz), 3.65-3.44 (m, 2H, H-5α, H-3α), 3.55 (s, 3H, OC$H_3$α), 3.51 (s, 3H, OC$H_3$β), 3.41-3.39 (m, 1H), 3.11 (dd, 1H, *J*=2.5Hz, *J*=10.4Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ 138.96-131.67 (*C*q, Ar), 127.46-125.17 (*C*H, Ar) 104.77, 102.53 (2x), 95.10, 89.18, 83.66, 82.90, 79.76, 78.89 (3x), 77.81 (3x), 76.74 (3x), 75.22, 75.17 (2x), 74.67 (2x), 73.00, 72.56 (2x), 72.35, 72.21, 70.66, 69.78, 68.37, 66.90, 66.46, 62.75, 57.99, 57.89. FAB-MS: *m/z* 1026.19 $[M+Na]^+$ . *Anal.*Calcd for $C_{62}H_{66}O_{12}$: C, 74.23; H, 6.63; found: C, 74.21; H, 6.65.

**Methyl 3,4-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)-β-D-galactopyranoside (34).**

(150mg) of the remaining resin-bound disaccharide which contained the disaccharides **34** and **35** was dissolved in methanol (2mL) and treated with catalytic amount of sodium methoxide (20mg). After 1h, TLC analysis (DCM/MeOH, 9/1, v/v) indicated the release of the disaccharide **34**. The reaction mixture was neutralized with Dowex $50H^+$ resin until pH=3. After removal of the resin by filtration and concentration of the solvent under reduced pressure, the residue was dissolved into DCM (0.5mL) and cooled to $0^oC$ in an ice-bath followed by gradual addition of diethyl ether (150mL) to precipitate all the remaining resin-bound disaccharide. The precipitate was collected by filtration and the filtrate which contained the released disaccharide **34** was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (ethyl acetate/hexane, 2/8, v/v) to afford the disaccharide **34** as a colorless syrup (7mg). $^1$H NMR (500 MHz, $CDCl_3$) δ: 7.45-7.10 (m, 60H, Ar-*H*), 5.53 (d, 1H, H-1'α, $J_{1',2'}$=3.7Hz), 4.96 (d, 1H, OC$H_2$Ph, *J*=11.2Hz), 4.93 (d, 1H, OC$H_2$Ph, *J*=11.0Hz), 4.91 (d, 1H, H-1'β, $J_{1',2'}$=7.9Hz), 4.87-4.78 (m, 12H, 6xOC$H_2$Ph), 4.69 (d, 1H, OC$H_2$Ph, *J*=11.8Hz), 4.63-4.49 (m, 8H, OC$H_2$Ph), 4.44 (d, 1H, OC$H_2$Ph, *J*=12.0Hz), 4.40 (d, 1H, H-1β, $J_{1,2}$=7.5Hz), 4.26 (d, 1H, OC$H_2$Ph, *J*=12.7Hz), 4.20 (d, 1H, H-3'α, $J_{2,3}$=9.6Hz, $J_{3,4}$=2.5Hz), 4.42-3.93 (m, 6H, H-2, H-3, H-5, H-4, H-6a,b), 3.91 (dd, 1H, H-4β, $J_{4,5}$=1.8Hz), 3.89 (dd, 1H, H-6'a, $J_{6a,6b}$=11.3Hz, $J_{5,6a}$=6.5Hz), 3.84 (dd, 1H, *J*=9.9Hz, *J*=4.3Hz), 3.65-3.61 (m, 2H), 3.52 (s, 3H, OC$H_3$α), 3.49 (s, 3H, OC$H_3$β). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 138.96-136.76 (*C*q, Ar), 128.93-127.43 (*C*H, Ar), 104.82, 102.85, 96.15, 85.16, 83.67, 82.93 (2x), 79.74, 78.22 (3x), 75.85 (3x), 75.18 (3x), 74.32, 73.85 (2x), 73.76 (2x), 73.22, 72.64 (2x), 72.55,

72.48, 70.26, 69.16, 68.37, 66.76, 66.41, 62.70, 57.44, 56.62. FAB-MS: *m/z* 920.10 $[M+Na]^+$. *Anal.* Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74; found: C, 73.65; H, 6.76.

**Methyl 2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)-β-D-galactopyranoside (35).**

The precipitated resin-bound disaccharide (65mg) from the previous step was treated with 25% TFA/DCM (0.5mL, 1/3, v/v). The reaction mixture was left stirring for 2h. TLC analysis (ethyl acetate/hexane, 3/7, v/v) indicated the release of the disaccharide **35**. After co-evaporation with toluene under reduced pressure, the residue was dissolved in dichloromethane (0.2mL) and cooled to $0^oC$ in an ice-bath. Diethyl ether (100mL) was added gradually with vigorous stirring until all the free resin was precipitated. After filtration using Celite and concentration, the filtrate was concentrated *in vacuo* and the resulting residue was purified by silica gel column chromatography (ethyl acetate/hexane, 2/8, v/v) to afford disaccharide **35** as a colorless syrup (10mg). $^1$H NMR (500MHz, $CDCl_3$) δ: 7.37-7.13 (60H, m, Ar-*H*), 5.04 (d, 1H, H-1'α, $J_{1',2'}$=2.9Hz), 4.97, 4.94 (ABq, 2H, OC$H_2$Ph, *J*=11.6Hz), 4.86, 4.82 (ABq, 2H, OC$H_2$Ph, *J*=11.3Hz), 4.81, 4.79 (ABq, 2H, OC$H_2$Ph, *J*=11.9Hz), 4.79, 4.77 (ABq, 2H, OC$H_2$Ph, *J*=11.6Hz), 4.70-4.33 (m, 16H, 8xOC$H_2$Ph), 4.23 (d, 1H, H-β, $J_{1,2}$=7.7Hz), 3.93 (bd, 1H, H-4', $J_{4,5}$=1.6Hz), 3.88-3.74 (m, 4H), 3.71-3.65 (m, 2H), 3.62 (dd, 1H, H-2α, $J_{2,3}$=9.9Hz), 3.54 (s, 3H, OC$H_3$α), 3.51 (s, 3H, OC$H_3$β), 3.46-3.42 ( (m, 4H, H-5, H-6a,b, H-2'), 3.33 (dd, 1H, *J*=4.3Hz, *J*=4.2Hz), 1.67 (t, 1H, O*H*, *J*=4.4Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 139.4-136.75 (*C*q, Ar), 127.46-127.03 (*C*H, Ar), 103.25, 98.36, 82.77, 80.19, 80.11, 79.29, 77.13, 75.45, 75.38, 74.73, 74.20, 73.77, 73.62, 73.47, 73.21, 72.76, 70.78, 68.29, 61.15, 55.27. FAB-

MS: *m/z* 920.13 $[M+Na]^+$. *Anal.* Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74; found: C, 73.67; H, 6.72.

**Methyl 2,4-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)-β-D-galactopyranoside (36).**

To a stirred solution of methyl 2,4-di-*O*-benzyl-6-*O*-(*p*-hydroxybenzyl)-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)-β-D-galactopyran-side 33 (18mg, 0.017mmol) in dichlorormethane (1mL) was added DDQ (6.7mg, 0.03mmol). The reaction mixture was stirred under Argon atmosphere at room temperature for 2h until TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated that all the starting material had been consumed. The solvent was removed under reduced pressure and the remaining residue was purified by silica gel column chromatography (eluent: ethyl acetate/hexane, 3/7, v/v) to afford 36 as a colorless syrup (13mg, 81%). $^1$H NMR (500MHz, $CDCl_3$) δ: 7.37-7.13 (m, 60H, Ar-*H*), 5.55 (d, 1H, H-1'α, $J_{1',2'}$=3.5Hz), 5.04 (ABq, 2H, OC*H*$_2$Ph, *J*=10.7Hz), 4.88 (d, 1H, H-6a, $J_{6a,6b}$=9.9Hz), 4.84 (m, 3H), 4.81-4.79 (m, 4H, 2xOC*H*$_2$Ph), 4.68-4.63 (m, 4H, 2xOC*H*$_2$Ph), 4.61 (d, 1H, H-1'β, $J_{1'\beta,2'\beta}$=7.9Hz), 4.50, 4.42 (ABq, 2H, OC*H*$_2$Ph, *J*=11.8Hz), 4.25 (d, 1H, H-1β, $J_{1\beta,2\beta}$ =7.7Hz), 4.21, 4.11 (ABq, 2H, OC*H*$_2$Ph, *J*=11.9Hz), 3.93 (dd 1H, H-4'β, $J_{3,4}$=2.6Hz, $J_{4,5}$=1.7Hz), 3.71-3.65 (m, 2H, H-6a,b), 3.54-3.46 (m, 5H), 3.37 (s, 3H, OC*H*$_3$α), 3.34 (s, 3H, OC*H*$_3$β), 3.33 (dd, 1H, *J*=4.3, 8.2Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 139.6-137.12 (*C*q, Ar), 128.36-127.04 (*C*H, Ar), 101.22, 100.19, 98.15, 96.34, 95.17, 89.77, 89.34, 88.12, 87.45, 87.18, 83.34, 83.13, 81.34, 79.12, 78.29, 77.96, 77.34, 76.81, 75.64, 69.29, 69.12, 61.33, 57.18, 57.02. FAB-MS: *m/z* 920.08 $[M+Na]^+$. *Anal.* Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74; found: C, 73.62; H, 6.73.

**Methyl 3-*O*-(α/β-D-galactopyranosyl)-β-D-galactopyranoside (37).**

10% Palladium on charcoal (20mg) was added to a solution of methyl 2,4-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)-β-D-galactopyranoside **36** (5mg, 0.005mmol) in ethanol (0.2mL). The mixture was vigorously stirred under hydrogen atmosphere for 18h. When TLC analysis (chloroform/methanol, 9/1, v/v) indicated completion of the reaction, the mixture was filtered using Celite and concentrated *in vacuo*. The crude material was purified by chromatography (Iatrobeads: chloroform/methanol/water, 65/33/2, v/v/v) to afford compound **37** as a white solid (4.65mg, α/β=9:1, 65% overall yield). $^{1}$H NMR (500MHz, $D_2O$) δ: 5.13 (d, 1H, H-1'α, $J_{1',2'}$=3.7Hz), 4.43 (d, 1H, H-1'β, $J_{1',2'}$=7.7Hz), 4.14 (d, 1H, H-1β, $J_{1,2}$=7.9Hz), 4.22 (d, 1H, *J*=7.8Hz), 4.02-3.99 (m, 2H), 3.85 (d, 1H, H-4β, $J_{3,4}$=2.9Hz), 3.90 (dd, 1H, H-2, $J_{2,3}$=9.8Hz), 3.81 (dd, 1H, H-3), 3.80 (d, 1H, H-4'β, $J_{3',4'}$=3.1Hz), 3.78 (m, 2H, H-5, H-5'), 3.68-3.60 (m, 4H, H-6a,b, H-6'a,b), 3.52 (s, 3H, O*Me*), 3.50 (dd, 1H, H-2'β), 3.40 (s, 3H, O*Me*). $^{13}$C NMR (125MHz, $D_2O$) δ: 102.18, 101.31, 76.98, 75.37, 75.11, 72.53, 71.04, 69.55, 68.63, 68.24, 63.81, 57.41, 56.86 (2x). FAB-MS: *m/z* 379.22 $[M+Na]^+$. *Anal.* Calcd for $C_{13}H_{24}O_{11}$: C, 3.82; H, 6.79; found: C, 43.80; H, 6.77.

**Methyl 2-*O*-(α/β-D-galactopyranosyl)-β-D-galactopyranoside (38).**

10% Palladium on charcoal (20mg) was added to a solution of methyl 3,4-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)-β-D-galactopyranoside **34** (5mg, 0.005mmol) in ethanol (0.5mL). The mixture was vigorously stirred under hydrogen atmosphere for 18h. When TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction, the mixture was filtered using Celite and concentrated *in vacuo*. The crude

residue was purified by chromatography (Iatrobeads, chloroform/methanol/water, 65/33/2, v/v/v) to afford **38** as a white solid (4.4mg, α/β=4:1, 61% overall yield). $^{1}$H NMR (500MHz, $D_2O$) δ: 5.22 (d, 1H, H-1'α, $J_{1,2}$=2.9Hz), 4.41 (d, 1H, H-1'β, $J_{1',2'}$=7.7Hz), 4.23 (d, 1H, H-1β, $J$=7.9Hz), 4.20 (dd, 1H, H-4β, $J_{3,4}$=2.7Hz, $J_{4,5}$=1.9Hz), 4.06-3.92 (m, 2H), 3.90 (d, 1H, 1H, H-4'α, $J_{3',4'}$=2.6Hz, $J_{4',5'}$=1.7Hz), 3.88 (dd, 1H, H-2, $J_{2,3}$=9.7Hz), 3.83 (dd, 1H, H-3, $J_{3,4}$=3.2Hz), 3.80 (d, 1H, H-4'β, $J_{3',4'}$=6.1Hz, $J_{4',5'}$=1.7Hz), 3.69-3.56 (m, 4H), 3.52 (m, 3H, H-5' α, H-5'β), 3.49 (dd, 1H, H-2'α, $J_{2',3'}$=9.8Hz), 3.39 (dd, 1H, $J$=7.7Hz, $J$=6.5Hz). $^{13}$C NMR (125MHz, $D_2O$) δ: 104.93, 104.36, 93.45, 92.11, 89.12, 88.76, 81.77, 75.33, 75.17, 74.34, 73.56, 72.98, 72.36, 71.09, 69.54, 68.72, 68.44, 66.45, 65.34, 64.11, 62.45, 62.11, 60.55, 57.03, 56.52. FAB-MS: *m/z* 379.34 $[M+Na]^+$. *Anal.* Calcd for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79; found: C, 43.81; H, 6.80.

**Methyl 4-*O*-(α/β-D-galactopyranosyl)-β-D-galactopyranoside (39).**

10% Palladium on charcoal (20mg) was added to a solution of methyl 3,4-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)-β-D-galactopyranoside **35** (4.36mg, 0.005mmol) in ethanol (0.5mL). The mixture was vigorously stirred under hydrogen atmosphere for 18h. When TLC anaylsis (chloroform/methanol, 9/1, v/v) indicated completion of the reaction, the mixture was filtered using Celite and concentrated *in vacuo* to afford the crude material which was purified by chromatography (Iatrobeads, chloroform/methanol/water, 74/24/2, v/v/v) to give **39** as a white solid (3.93mg, α/β=3:1, 55% overall yield). $^{1}$HNMR (500MHz, $D_2O$) δ: 4.88 (d, 1H, H-1'α, $J_{1',2'}$ =3.9Hz), 4.50 (d, 1H, H-1'β, $J_{1',2'}$=7.7Hz), 4.29 (d, 1H, H-1β, $J_{1,2}$=7.9Hz), 4.25 (d, 1H, H-4β, $J_{3,4}$=2.9Hz), 4.09 (dd, 1H, H-4'β, $J_{3',4'}$=2.8Hz, $J_{4',5'}$=1.8Hz), 3.86-3.80 (m, 7H, H-2', H-3, H-3', H-6a,b, H-6'a,b), 3.77-3.62 (m, 3H, H-2, H-5, H-5'), 3.55 (OC$H_3$α), 3.52 (OC$H_3$β). $^{13}$C

NMR (125MHz, $D_2O$) δ: 106.55, 106.23, 96.28, 79.91, 79.82, 79.50, 78.45, 78.11, 77.95, 77.88, 77.65, 77.02, 76.54, 75.54, 74.29, 74.13, 73.19, 71.40, 69.67, 68.12, 63.79, 63.29, 61.22, 60.90, 60.35, 59.45, 56.43. FAB-MS: *m/z* 379.41 $[M+Na]^+$. *Anal.*Calcd for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79; found: C, 43.80; H, 6.82.

**Methyl 2,4-di-*O*-benzyl-6-*O*-(*p*-hydroxybenzyl)-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopyranoside (41).**

To a stirred solution of the resin-bound disaccharide mixture **40** (300mg) in tetrahydrofuran (2mL) was added few drops triethylamine until the medium became basic (pH=9) followed by addition of few drops of aqueous hydrogen peroxide solution (50μL). When TLC analysis (ethyl acetate/hexane, 1/1, v/v) showed the release of the disaccharide **41** after 2h, the solvent was removed under reduced pressure and the resulting residue was dissolved in dichloromethane (0.5mL) and cooled to $0^oC$ in an ice-bath. Diethyl ether (150mL) was added gradually with vigorous stirring until all the resin-bound disaccharides were precipitated. The precipitate was collected by filtration using Celite and the filtrate was concentrated *in vacuo* to give the crude disaccharide **41**. Purification of the released disaccharide by silica gel column chromatography (ethyl acetate/hexane, 3/7, v/v) afforded the disaccharide **41** as a colorless syrup (18mg). $^1H$ NMR (500 MHz, $CDCl_3$) δ: 7.36 (d, 2H, Ar-*H*), 7.32 (d, 2H, Ar-*H*), 7.30-7.10 (m, 64H, Ar-*H*), 5.46 (d, 1H, H-1'α, $J_{1',2'}$=3.8Hz), 4.95, 4.93 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=12.3Hz), 4.90 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.0Hz), 4.88 (d, 1H, H-1'β, $J_{1,2}$=7.9Hz), 4.85-4.78 (m, 12H, 6xOC$H_2$Ph), 4.67 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.8Hz), 4.65-4.48 (m, 6H, 3xOC$H_2$Ph), 4.39 (d, 1H, H-1β, $J_{1,2}$=7.4Hz), 4.18 (dd, 1H, H-3'α, $J_{2',3'}$=9.6Hz, $J_{3',4'}$=9.5Hz), 4.15 (d, 1H, H-4α, $J_{3,4}$=3.8Hz), 4.02 (dd, 1H, *J*=10.0Hz, *J*=10.0Hz), 3.85

(dd, 1H, *J*=11.4Hz, *J*=5.7Hz), 3.80 (dd, 1H, H-3α, $J_{2,3}$=9.7Hz), 3.75 (dd, 1H, H-4β), 3.52-3.44 (m, 4H), 3.55 (s, 3H, OC$H_3$α), 3.51 (s, 3H, OC$H_3$β), 3.01 (dd, 1H, H-5, $J_{5,6a}$=6.9Hz, $J_{5,6b}$=3.5Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 138.96-136.75 (*C*q, Ar), 129.97-127.46 (*C*H, Ar), 104.77, 102.53, 102.51, 95.22, 91.16, 89.22, 83.69, 82.95, 82.91, 79.77, 78.89(2x), 77.90, 77.89(2x), 76.74, 76.70, 76.68, 75.91, 75.22, 75.17(2x), 75.11, 74.66, (3x), 73.02, 72.56 (2x), 72.39, 72.21, 71.64, 69.78, 68.39, 66.99, 66.67, 62.75, 57.89, 55.23. FAB-MS: *m/z* 1026.16 $[M+Na]^+$. *Anal.* Calcd for $C_{62}H_{66}O_{12}$: C, 74.23; H, 6.63; found: C, 74.28; H, 6.61.

**Methyl 3,4-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopyranoside (42).**

To a stirred solution of the remaining resin-bound disaccharide mixture (150mg) in methanol (2mL) was added a catalytic amount of sodium methoxide (20mg). When TLC analysis (DCM/MeOH: 9/1, v/v) indicated the release of disaccharide **42** after 1h, the reaction mixture was neutralized with Dowex $50H^+$ resin until pH=3. After removal of resin by filtration and evaporation of the solvent, the residue was dissolved in DCM (0.5mL) and cooled to 0$^o$C in an ice bath. Diethyl ether (150mL) was added gradually with vigorous stirring until the all the remaining resin-bound disaccharide was precipitated. The precipitate was collected by filtration using Celite and the filtrate was concentrated *in vacuo* to give disaccharide **42** (6mg) as a white material which was washed with hexane and used for the next step without further purification. $^1$H NMR (500 MHz, $CDCl_3$) δ: 7.50-7.00 (m, 60H, Ar-*H*), 5.47 (d, 1H, H-1'α, $J_{1',2'}$=3.7Hz), 5.32 (d, 1H, H-1β, $J_{1,2}$=7.9Hz), 5.01, 4.98 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.0Hz), 4.96, 4.94 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.4Hz), 4.90 (d, 1H, H-1'β, $J_{1',2'}$=7.9Hz), 4.85-4.80 (m, 6H,

3xOC*H*$_2$Ph), 4.69, 4.67 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=11.8Hz), 4.65-4.52 (m, 6H, 3xOC*H*$_2$Ph), 4.44, 4.42 (ABq, 2H, OC*H*$_2$Ph, $J_{AB}$=12.5Hz), 4.33 (m, 4H, 2xOC*H*$_2$Ph), 4.19-4.12 (m, 3H, H-3'α, H-3α, H-5β), 4.10 (d, 1H, H-4α, $J_{3,4}$=3.5Hz), 4.06-3.93 (m, 6H), 3.98 (dd, 1H, *J*=9.8Hz, *J*=10.0Hz), 3.89 (ddd, 1H, H-5 $J_{5,6a}$=7.1Hz, $J_{5.6b}$=4.1Hz), 3.77 (d, 1H, H-4β, $J_{3,4}$=2.3Hz), 3.67 (dd, 1H, *J*=10.5Hz, *J*=5.6Hz), 3.65-3.46 (m, 3H), 3.49 (s, 3H, OC*H*$_3$α), 3.22 (s, 3H, OC*H*$_3$β). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 138.96-136.75 (*C*q, Ar), 128.80-127.46 (*C*H, Ar), 104.82, 102.86, 102.86, 96.10, 85.18, 83.01, 82.99 (2x), 79.75, 78.06 (3x), 75.81 75.79, 75.77, 75.08 75.06, 75.04, 74.20, 73.90, 73.81, 73.55, 73.01, 72.96, 72.64, 72.60, 72.45, 72.22, 70.12, 69.06, 68.38, 66.96, 66.77, 62.71, 57.78, 56.60. FAB-MS: *m/z* 920.08 $[M+Na]^+$. *Anal*. Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74; found: C, 73.62; H, 6.74.

**Methyl 2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopyranoside (43).**

The remaining resin-bound disaccharide (70mg) was reacted with 25% TFA/DCM (1mL, 1/3, v/v) and stirred at room temperature for 2h. When TLC analysis (ethyl acetate/hexane, 3/7, v/v) indicated the release of the disaccharide **43**, the solvent was removed by co-evaporation with toluene under reduced pressure. The remaining residue was dissolved in dichloromethane (0.5mL) and cooled to 0$^o$C in an ice-bath. Diethyl ether (100mL) was added gradually with vigorous stirring until all the free resin was precipitated and collected by filtration using Celite. The filtrate was evaporated *in vacuo* to give the crude disaccharide **43** which was purified by silica gel column chromatography (ethyl acetate/hexane, 2/8, v/v) to afford **43** (8mg) as a colorless syrup. $^1$H NMR (500MHz, $CDCl_3$) δ: 7.44-7.10 (m, 60H, Ar-*H*), 5.24, 5.16 (ABq, 2H, OC*H*$_2$Ph,

$J_{AB}$=11.3Hz), 5.09 (d, 1H, H-1'α, $J_{1',2'}$=3.5Hz), 5.02 (d, 1H, H-1'β, $J_{1',2'}$=7.9Hz), 4.98, 4.92 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.2Hz), 4.90 (d, 1H, H-1β, $J_{1,2}$=7.4Hz), 4.86 (1H, d, H-2β, $J_{2,3}$=9.7Hz), 4.83 (d, 1H, $J$=11.0Hz), 4.80, 4.64 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=10.7Hz), 4.79, 2.67 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.8Hz), 4.65-4.52 (m, 4H, 2xOC$H_2$Ph), 4.50 (dd, H-3β, 1H, $J_{3,4}$=2.3Hz), 4.48-4.38 (m, 12H, 6xOC$H_2$Ph), 4.30-4.00 (m, 5H, H-3, H-5', H-5, H-6a,b), 3.73-3.59 (m, 4H, H-3', H-4', H-6'a,b), 3.47 (s, 3H, OC$H_3$ α), 3.45 (s, 3H, OC$H_3$β). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 139.69-138.25 (*C*q, Ar), 128.35-124.99 (*C*H, Ar), 102.87, 98.38, 96.11, 94.23, 89.11, 85.08, 82.86, 80.15, 79.24, 78.14, 76.72, 75.65, 75.28, 75.26, 75.23, 75.13, 74.81, 73.69, 73.36, 70.83, 69.07, 68.27, 66.78, 61.11, 56.34, 55.34. FAB-MS: *m/z* 920.11 $[M+Na]^+$. *Anal*. Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74; found: C, 73.65; H, 6.77.

**Methyl 2,4-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopyranoside (44).**

To a stirred solution of methyl 2,4-di-*O*-benzyl-6-*O*-(*p*-hydroxybenzyl)-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopranoside **41** (15mg, 0.014mmol) in DCM (0.2mL) was added DDQ (6.7mg, 0.029mmol). The reaction mixture was stirred under Argon atmosphere at room temperature for 2h until TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated that all the starting material had been consumed. The solvent was removed under reduced pressure and the remaining residue was purified by silica gel column chromatography (eluent: ethyl acetate/hexane, 3/7, v/v) to give **44** as a colorless syrup (11mg, 82%). $^1$H NMR (500 MHz, $CDCl_3$) δ: 7.57-7.06 (m, 60H, Ar-*H*), 5.23 (d, 1H, H-1'α, $J_{1',2'}$=3.8Hz), 4.98, 4.76 (ABq, 2H, OC$H_2$Bn, $J_{AB}$=11.0Hz), 4.89, 4.84 (ABq, 2H, OC$H_2$Bn, $J_{AB}$=11.4Hz), 4.82 (d, 1H, H-1'β, $J_{1',2'}$=7.8Hz), 4.82-4.70 (m,

8, 4xOC$H_2$Bn), 4.67, 4.63 (ABq, 2H, OC$H_2$Bn, $J_{AB}$=11.2Hz), 4.63-4.50 (m, 10H, 5xOC$H_2$Bn), 4.47 (d, 1H, H-1β, $J_{1\beta,\ 2\beta}$=7.9Hz), 4.22 (dd, 1H, H-3'α, $J_{2',3'}$=8.4Hz, $J_{3',4'}$=8.8Hz), 4.19 (d, 1H, H-4α, $J_{3,4}$=2.5Hz), 4.12-3.90 (m, 3H, H-5, H-6a, H-2), 3.88 (dd, 1H, H-3'β $J_{2',3'}$=9.7Hz, $J_{3',4'}$=9.8Hz), 3.85-3.65 (m, 6H, H-4, H-5, H-6a,b, H-6'-a,b), 3.52 (s, 3H, OC$H_3$α), 3.46 (s, 3H, OC$H_3$β). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 138.96-136.70 (*C*q, Ar), 129.67-127.98 (*C*H, Ar), 103.56, 102.32 (2x), 100.29, 89.56, 87.08, 85.09, 80.67, 78.89, 78.05, 77.83, 77.81, 77.15, 76.08(2x), 76.04, 74.20(2x),74.11, 73.58, 73.40, 73.22, 73.06, 72.66, 72.42, 72.35, 70.23, 69.06, 68.38, 67.11, 66.91, 65.78, 57.13, 56.22. FAB-MS: *m/z* 920.16 $[M+Na]^+$. *Anal*. Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74; found: C, 73.60; H, 6.76.

**Methyl 3-*O*-(α/β-D-glucopyranosyl)-β-D-galactopyranoside (45).**

10% Palladium on charcoal (20 mg) was added to a solution of methyl 2,4-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopyranoside **44** (14mg, 0.015mmol) in ethanol (0.2mL). The mixture was vigorously stirred under hydrogen atmosphere for 24h. When TLC (chloroform/methanol, 9/1, v/v) indicated that all the starting material **44** had been consumed, the reaction mixture was filtered using Celite and concentrated *in vacuo*. The crude material was purified by chromatography (Iatrobeads, chloroform/methanol/water, 65/33/2, v/v/v) to afford **45** as a white solid (4.45mg, α/β=1:1, 62% overall yield). $^1$H NMR (500MHz, $D_2O$) δ: 5.01 (d, 1H, H-1'α, $J_{1',2'}$=3.3Hz), 4.57 (d, 1H, H-1'β, $J_{1',2'}$=7.7Hz), 4.28 (d, 1H, H-1β, $J_{1,2}$=7.9Hz), 4.07 (dd, 1H, H-4', $J_{3',4'}$=8.9Hz, $J_{4',5'}$=9.5Hz), 3.64-3.82 (m, 3H, H-3, H-5, H-6'b), 3.74-3.57 (m, 3H, H-4, H-6a, H-5'), 3.54-3.49 (m, 3H, H-2, H-2', H-5'), 3.42 (s, 3H, OC$H_3$α), 3.39 (s, 3H, OC$H_3$β). $^{13}$C NMR (125MHz, $D_2O$) δ: 105.94, 104.35, 102.96, 101.17, 96.10, 85.18,

83.01, 81.86, 81.11, 78.09, 78.02, 75.30, 75.12, 72.59, 72.22, 71.03, 70.08, 70.04, 69.78, 69.05, 68.67, 68.20, 66.96, 66.47, 62.77, 57.01, 56.83. FAB-MS: *m/z* 378.02 $[M+Na]^+$. *Anal.* Calcd for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79; found: C, 43.78; H, 6.80.

**Methyl 2-*O*-(α/β-D-glucopyranosyl)-β-D-galactopyranoside (46).**

10% Palladium on charcoal (20mg) was added to a solution of methyl 3,4-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopyranoside **42** (7mg, 0.007mmol) in ethanol (0.5mL). The mixture was vigorously stirred under hydrogen atmosphere for 20h. When TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction, the reaction mixture was filtered using Celite and concentrated *in vacuo* followed by purification of the product by using chromatography (Iatrobeads, chloroform/methanol/water, 69/30/12, v/v/v) to afford **46** as a white solid (1.5mg, α/β=3:1, 55% overall yield). $^1$H NMR (500MHz, $D_2O$) δ: 5.20 (d, 1H, H-1'α, $J_{1,2}$=3.5Hz), 4.45 (d, 1H, H-1'β, $J_{1',2'}$=7.7Hz), 4.36 (d, 1H, H-1β, $J_{1,2}$=7.3Hz), 4.22 (dd, 1H, H-4β', $J_{3',4'}$=9.5Hz, $J_{4',5'}$=9.6Hz), 4.20-3.92 (m, 6H, H-2, H-6a,b , H-6'a,b, H-3'), 3.94 (d, 1H, H-4α, $J_{3,4}$=2.3Hz), 3.84 (d, 1H, H-3α, $J_{2,3}$=9.2Hz), 3.72 (d, 1H, *J*=6.4Hz), 3.69-3.65 (m, 2H), 3.48 (s, 3H, OC$H_3$α), 3.42 (s, 3H, OC$H_3$β), 3.39-3.34 (m, 2H, H-5, H-5'). $^{13}$C NMR (125MHz, $D_2O$) δ: 104.22, 102.93, 102.36, 101.23, 98.54, 88.56, 85.65, 85.61, 83.22, 82.33, 82.13, 81.75, 80.23, 80.11, 79.45, 79.23, 78.11, 77.65, 75.30, 75.11, 74.34, 72.54, 71.04, 69.76, 68.79, 68.43, 67.57, 66.60, 57.37, 55.67. FAB-MS: *m/z* 378.01 $[M+Na]^+$. *Anal.* Calcd for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79; found: C, 43.83; H, 6.81.

**Methyl 4-*O*-(α/β-D-glucopyranosyl)-β-D-galactopyranoside (47).**

10% Palladium on charcoal (20 mg) was added to a solution of methyl 3,4-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopyranoside 43 (11mg, 0.01mmol) in ethanol (0.5mL). The mixture was vigorously stirred under hydrogen atmosphere for 18h. When TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction, the mixture was filtered using Celite and concentrated *in vacuo*. The crude product was purified by chromatography (Iatrobeads, chloroform/methanol/water, 65/33/2, v/v/v) to afford 47 as a white solid (3.56mg, α/β=3:2, 50% overall yield). $^{1}$H NMR (500MHz, $D_2O$) δ: 5.31 (d, 1H, H-1'α, $J_{1',2'}$=2.9Hz), 4.45 (d, 1H, H-1'β, $J_{1',2'}$=7.7Hz), 4.33 (d, 1H, H-1β, $J_{1,2}$=7.9Hz), 4.19 (dd, 1H, H-3'α, $J_{2',3'}$=8.4Hz, $J_{3',4'}$=9.4Hz), 4.14 (m, 2H, H-4, H-4'), 3.92 (dd, 1H, H-2'), 3.88-3.82 (m, 2H, H-3, H-5'), 3.80 (dd, 1H, H-2, $J_{2,3}$=9.2Hz), 3.70 (ddd, 1H, H-5α, $J_{5,6a}$=6.9Hz, $J_{5,6b}$=3.3Hz ), 3.69-3.60 (m, 4H, H-6a,b, H-6'a,b), 3.49 (s, 3H, OC$H_3$α), 3.46 (s, 3H, OC$H_3$β). $^{13}$C NMR (125MHz, $D_2O$) δ: 104.73, 103.98, 101.79, 101.22, 89.45, 88.61, 86.23, 86.11, 84.23, 83.99, 83.79, 82.71, 81.30, 77.32, 76.45, 75.23, 75.16, 74.58, 74.07, 73.89, 73.20, 72.90, 72.75, 69.60, 57.52, 56.89. FAB-MS: *m/z* 378.03 $[M+Na]^+$. *Anal.* Calcd for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79; found: C, 43.80; H, 6.80.

**Methyl 2,4-di-*O*-benzyl-6-*O*-(*p*-hydroxybenzyl)-3-*O*-(2-azido-2-deoxy-,3,4,6-tri-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopyranoside (49**).

To a stirred solution of the resin-bound disaccharide mixture **48** (300mg) in THF (2mL) was added few drops of triethylamine until the medium became basic (pH=9) followed by addition of few drops of aqueous hydrogen peroxide solution (50μL). When TLC

analysis (ethyl acetate/hexane, 1/1, v/v) showed the release of the disaccharide **49** after 30 minutes stirring, the solvent was removed under reduced pressure and the resulting residue was dissolved in dichloromethane (0.5mL) and cooled to 0$^{o}$C in an ice bath. Diethyl ether (150mL) was added gradually with vigorous stirring for 2h until all the MPEG-resin bound disaccharide was precipitated. The precipitate was collected by filtration using Celite and the filtrate was concentrated *in vacuo* to afford the crude disaccharide **49**. Purification of the crude product by silica gel column chromatography (eluent: ethyl acetate/hexane, 3/7, v/v) afforded the disaccharide **49** as a colorless syrup (17mg). $^{1}$H NMR (500 MHz, $CDCl_3$) δ: 7.32-7.11 (m, 8H, Ar-*H*), 7.35-7.13 (m, 50H, Ar-*H*), 5.52 (d, 1H, H-1'α, $J_{1',2'}$=3.8Hz), 4.95, 4.93 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=12.6Hz), 4.92, 4.89 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=12.6Hz), 4.88 (d, 1H, H-1'β, $J_{1',2'}$=7.9Hz), 4.85-4.78 (m, 12H, 6xOC$H_2$Ph), 4.65-4.48 (m, 8H, 4xOC$H_2$Ph), 4.39 (d, 1H, H-1β, $J_{1,2}$=7.4Hz), 4.15 (dd, 1H, H-4α, $J_{3,4}$=2.7Hz, $J_{4,5}$=1.7Hz), 4.42-3.90 (m, 6H, H-6b, H-6'a,b, H-5, H-5', H-2'), 3.85 (dd, 1H, H-6a, $J_{5,6a}$=7.2Hz, $J_{6a,6b}$=11.7Hz), 3.75 (d, 1H, H-4β, $J_{3,4}$=6.3Hz, $J_{4,5}$=1.6Hz), 3.67 (t, 1H, *J*=10.2Hz), 3.53 (s, 3H, OC$H_3$α), 3.51 (s, 3H, OC$H_3$β), 3.23-3.19 (m, 2H). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 138.96-132.77 (*C*q, Ar), 128.56-125.54 (*C*H, Ar), 102.53, 101.12, 98.45, 98.17, 95.10, 89.18, 83.66, 82.90, 82.88, 79.76, 78.89, 78.87, 78.32, 77.81, 76.74, 75.22, 75.17, 74.67, 73.04, 72.56, 72.35, 72.21, 70.66, 69.78, 68.37, 66.90, 66.46, 62.75, 57.99, 57.89, 56.11. FAB-MS: *m/z* 961.05 $[M+Na]^+$. *Anal.* Calcd for $C_{55}H_{59}N_3O_{11}$: C, 70.42; H, 6.34; N, 4.48; found: C, 70.41; H, 6.34; N, 4.51.

**Methyl 3,4-di-*O*-benzyl-2-*O*-(2-azido-2-deoxy-3,4,6-tri-*O*-benzyl-α/β-D-glucopyran-osyl)-β-D-galactopyranoside (50**).

To a stirred solution of the precipitated resin-bound disaccharide (100mg) in methanol (2mL) was added a catalytic amount of sodium methoxide (20mg). When TLC analysis (DCM/MeOH: 9/1, v/v) showed the completion of this reaction after 2h, the mixture was neutralized with Dowex $50H^+$ resin until pH=3. After removing the resin by filtration, the solvent was removed under reduced pressure and the residue was dissolved in DCM (0.5mL) and cooled to $0^oC$ in an ice bath. Diethyl ether (100mL) was added gradually with vigorous stirring until all the remaining resin-bound disaccharide was precipitated. The precipitate was collected by filtration using Celite and the filtrate was evaporated *in vacuo* to give the crude disaccharide which was purified by gel column chromatography (eluent: ethyl acetate/hexane, 1/3, v/v) to afford **50** (5mg) as a colorless syrup. $^1$H NMR (500MHz, $CDCl_3$) δ: 7.57-7.24 (m, 50H, Ar-*H*) 5.29 (d, 1H, H-1'α, $J_{1',2'}$=3.5Hz), 4.98, 4.96 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.0Hz), 4.93, 4.91 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.0Hz), 4.82 (d, 1H, H-1'β, $J_{1',2'}$=7.9Hz), 4.80-4.63 (m, 4H, H-2, H-2', H-4, H-4'), 4.28 (d, 1H, H-1β, $J_{1,2}$=8.1Hz), 4.26-4.18 (m, 8H, 4xOC$H_2$Ph), 4.12 (d, 1H, H-4α $J_{3,4}$=3.3Hz), 4.16-4.02 (m, 8H, 4xOC$H_2$Ph), 4.00 (dd, 1H, H-3, $J_{2,3}$=9.2Hz), 3.85 (m, 4H, H-6a,b, H-6'a,b), 3.70 (s, 3H, OC$H_3$α), 3.69 (ddd, 1H, H-5, $J_{5,6a}$=1.8Hz, $J_{5,6b}$=7.9Hz), 3.66 (s, 3H, OC$H_3$β) 3.59 (dd, 1H, H-6'a, $J_{5',6'a}$=5.12Hz, $J_{6'a,6'b}$=10.6Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 139.5-133.34 (*C*q, Ar), 129.45-126.12 (*C*H, Ar), 101.33, 103.66, 96.10, 85.18, 83.11, 82.38, 79.74, 79.33, 78.66, 78.56, 78.02, 76.94, 75.81, 75.56, 74.89, 74.70, 72.64, 71.34, 69.67, 66.96, 66.47, 62.78, 61.37, 57.41, 56.89. FAB-MS: *m/z* 854.35 $[M+Na]^+$. *Anal.* Calcd for $C_{48}H_{53}N_3O_{10}$: C, 69.30; H, 6.42; N, 5.05; found: C, 69.30; H, 6.44; N, 5.03.

**Methyl 2,3-di-*O*-benzyl-4-*O*-(2-azido-2-deoxy-3,4,6-tri-*O*-benzyl-α/β-D-glucopyran-osyl)-β-D-galactopyranoside (51).**

The resin-bound disaccharide (65mg) was stirred with 25% TFA/DCM (1mL, 1/3, v/v) at room temperature for 2h. When TLC analysis (ethyl acetate/hexane, 3/7, v/v) indicated the release of the disaccharide **51**, the solvent was removed by co-evaporation with toluene under reduced pressure. The residue was dissolved in dichloromethane (0.5mL) and cooled to $0^{o}$C in an ice bath. Diethyl ether (150mL) was added gradually with vigorous stirring until all the free resin was precipitated. The precipitated resin was filtered using Celite and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/hexane, 2/8, v/v) to yield disaccharide **51** as a colorless syrup (10mg). $^{1}$H NMR (500MHz, $CDCl_3$) δ: 7.57-7.25 (m, 50H, Ar-*H*) 5.54 (d, 1H, H-1'α, $J_{1',2'}$ =3.2Hz), 5.21 (dd, 1H, H-2α, $J_{2,3}$=9.8Hz), 4.99, 4.96 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.6Hz), 4.83, 4.79 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.6Hz), 4.52 (d, 1H, H-1'β, $J_{1',2'}$=7.9Hz), 4.42-4.30 (m, 6H, 3xOC$H_2$Ph), 4.28 (d, 1H, H-1β $J_{1,2}$=8.2Hz), 4.20-4.10 (m, 10H, 5xOC$H_2$Ph), 4.12 (dd, 1H, H-4α, $J_{3,4}$=3.1Hz, $J_{4,5}$=1.9Hz), 3.96 (dd, 1H, H-6a $J_{5,6a}$=5.7Hz, $J_{6a,6b}$=11.8Hz), 3.77-3.72 (m, 5H, H-6b, H-6'a,b, H-5, H-5'), 3.69 (dd, 1H, H-3', $J_{2',3'}$=8.4Hz, $J_{3',4'}$=9.2Hz), 3.48 (s, 3H, O*Me*), 3.46 (s, 3H, O*Me*), $^{13}$C NMR (125MHz, $CDCl_3$) δ: 139.5-137.34 (*C*q, Ar), 129.45-126.12 (*C*H, Ar), 102.45, 102.14, 98.11, 96.45, 96.24, 96.11, 96.07, 85.18, 83.01, 82.30, 79.74, 78.31, 78.02, 76.94, 75.81, 75.57, 74.89, 74.70, 72.64, 69.67, 66.96, 66.47, 62.78, 61.37, 56.98, 53.11. FAB-MS: *m/z* 855 $[M+Na]^+$. *Anal.* Calcd for $C_{48}H_{53}N_3O_{10}$: C, 69.30; H, 6.42; N, 5.05; found: C, 69.34; H, 6.45; N, 5.02.

**Methyl 2,4-di-*O*-benzyl-3-*O*-(2-azido-2-deoxy-3,4,6-tri-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopyranoside (52).**

To a stirred solution of methyl 2,4-di-*O*-benzyl-6-*O*-(*p*-hydroxybenzyl)-3-*O*-(2-azido-2-deoxy-3,4,6-tri-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopyranoside **49** (15mg, 0.01mmol) in dichloromethane (0.2mL) was added DDQ (6.7mg, 0.02mmol). The reaction mixture was stirred under Argon atmosphere at room temperature for 3h until TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated that all the starting material had been consumed. The reaction mixture was concentrated under reduced pressure and the remaining residue was purified by silica gel column chromatography (eluent: ethyl acetate/hexane, 2/3, v/v) to give **52** as a colorless syrup (12mg, 92%). $^{1}$H NMR (500MHz, $CDCl_3$) δ: 7.57-7.24 (m, 50H, Ar-H) 5.29 (d, 1H, H-1'α, $J_{1',2'}$=3.5Hz), 4.98, 4.96 (ABq, 2H, OC$H_2$Ph, $J$=11.8Hz), 4.93, 4.91 (ABq, 2H, OC$H_2$Ph, $J$=12.3Hz), 4.82 (d, 1H, H-1'β, $J_{1',2'}$=8.1Hz), 4.80-4.63 (m, 6H, 3xOC$H_2$Ph), 4.28 (d, 1H, H-1β, $J_{1,2}$=7.7Hz), 4.26-4.18 (m, 6H, 3xOC$H_2$Ph), 4.12 (d, 1H, H-4α, $J_{3,4}$=2.5Hz, $J_{4,5}$=1.0 Hz), 4.00-3.82 (m, 8H, H-3, H-3', H-5, H-5', H-2, H-2', H-4', H-6b), 3.90 (d, 1H, H-6a, $J_{5,6a}$=7.5Hz, $J_{6a,6b}$=11.8Hz), 3.82, 3.79 (ABq, 2H, OC$H_2$Ph, $J$=11.4Hz), 3.77, 3.69 (ABq, 2H, OC$H_2$Ph, $J$=12.5Hz), 3.75 (dd, 1H, H-3', $J_{2',3'}$=9.5Hz, $J_{3',4'}$=9.5Hz), 3.67 (s, 3H, OC$H_3$α), 3.62 (s, 3H, OC$H_3$β), 3.55 (m, 2H, H-6'a, H-6'b). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 139.5-133.34 (*C*q, Ar), 129.45-126.12 (*C*H, Ar), 102.67, 102.33, 103.66, 96.10, 85.18, 83.11, 82.38, 79.74, 79.33, 78.66, 78.56, 78.02, 76.94, 75.81, 75.56, 74.89, 74.70, 72.64, 69.67, 66.96, 66.47, 66.34, 65.34, 62.78, 61.37, 57.41, 56.89. FAB-MS: *m/z* 854.39 $[M+Na]^+$. *Anal.* Calcd for $C_{48}H_{53}N_3O_{10}$: C, 69.30; H, 6.42; N, 5.05; found: C, 69.29; H, 6.43; N, 5.07.

**Methyl 3-*O*-(2-amino-2-deoxy-α/β-D-glucopyranosyl)-β-D-galactopyranoside (53).**

10% Palladium on charcoal (20mg) was added to a solution of methyl 2,4-di-*O*-benzyl-3-*O*-(2-azido-2-deoxy-2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)-β-D- galactopyranoside **52** (12mg, 0.014 mmol) in ethanol (0.3mL). The mixture was vigorously stirred under hydrogen atmosphere at room temperature for 18h. When TLC (chloroform/methanol, 9/1, v/v) indicated that reaction is complete, the reaction mixture was filtered using Celite and concentrated *in vacuo*. The crude product was purified by chromatography (Iatrobeads, chloroform/methanol/water, 65/33/2, v/v/v) to afford **53** as a colorless syrup (4.3mg, α/β=5:1, 60% overall yield). $^{1}$H NMR (500MHz, $D_2O$) δ: 4.92 (1H, d, H-1'α, $J_{1',2'}$=3.3Hz), 4.33 (1H, d, H-1β, $J_{1,2}$=7.7Hz), 4.19 (dd, 1H, H-3'β, $J_{2',3'}$=8.5Hz, $J_{3',4'}$=9.1Hz), 4.17-4.02 (m, 2H, H-4'β, H-4β), 3.94 (t, 1H, *J*=9.9Hz), 3.92 (bd, H-6'a, $J_{6'a,6'b}$=12.2Hz), 3.88-3.67 (m, 7H, H-2, H-2', H-5, H-5', H-6a,b), 3.46 (s, 3H, OC$H_3$α), 3.44 (s, 3H, C$H_3$β). $^{13}$C NMR (125MHz, $D_2O$) δ: 104.67, 103.22, 101.23, 98.16, 97.18, 96.55, 96.45, 85.20, 83.03, 82.30, 79.78, 78.42, 77.97, 75.58, 74.86, 74.69, 73.93, 72.74, 70.43, 69.67, 66.96, 66.51, 65.77, 61.57, 57.28, 55.57. FAB-MS: *m/z* 378.35 $[M+Na]^+$. *Anal*. Calcd for $C_{13}H_{25}NO_{10}$: C, 43.94; H, 7.09; N, 3.94; found C, 43.92; H, 7.11; N, 3.94.

**Methyl 2-*O*-(2-amino-2-deoxy-α/β-D-glucopyranosyl)-β-D-galactopyranoside (54).**

10% Palladium on charcoal (20 mg) was added to a solution of methyl 3,4-di-*O*-benzyl-2-*O*-(2-azido-2-deoxy-2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)-β-D-galactopyranoside **50** (5mg, 0.0006mmol) in ethanol (0.2mL). The mixture was vigorously stirred under hydrogen atmosphere at room temperature for 18h. When TLC analysis

(chloroform/methanol, 9/1, v/v) indicated that reaction is complete, the mixture was filtered by using Celite and concentrated *in vacuo*. The crude product was purified by chromatography (Iatrobeads, chloroform/methanol/ water, 65/33/2, v/v/v) to afford **54** as a colorless syrup (3.92mg, α/β=3:1, overall yield 55%). $^{1}$H NMR (500MHz, $D_2O$) δ: 5.33 (d, 1H, H-1'α, $J_{1',2'}$=3.5Hz), 4.31 (d, 1H, H-1'β $J_{1',2'}$=7.7Hz), 4.33 (d, 1H, H-1β, $J_{1,2}$=9.6Hz), 4.12 (dd, 1H, 1H, H-4, $J_{3,4}$=2.3Hz, $J_{4,5}$=1.2Hz), 3.94 (dd, 1H, H-3, $J_{2,3}$ =9.6Hz), 3.90 (bd, 1H, H-2', $J_{2',3'}$=8.4Hz), 3.77-3.60 (m, 6H, H-2, H-4', H-6a,b, H-6'a,b), 3.45 (s, 3H, OC$H_3$α), 3.43 (s, 3H, OC$H_3$β), 3.27-3.20 (m, 2H, H-5', H-5'). $^{13}$C NMR (125MHz, $D_2O$) δ: 103.67, 101.21, 96.71, 95.11, 89.23, 88.56, 86.34, 85.91, 85.16, 84.66, 82.34, 81.68, 81.56, 79.65, 78.45, 78.38, 78.36, 77.94, 75.58, 74.86, 74.69, 72.74, 69.67, 68.34, 66.96, 62.78, 61.57, 58.11, 55.55. FAB-MS: *m/z* 380 $[M+Na]^+$. *Anal*. Calcd for $C_{13}H_{25}NO_{10}$: C, 43.94; H, 7.09; N, 3.94; found: C, 43.88; H, 7.13; N, 3.98.

**Methyl 4-*O*-(2-amino-2-deoxy-α/β-D-glucopyranosyl)-β-D-galactopyranoside (55).**

10% Palladium on charcoal (20mg) was added to a solution of methyl 2,3-di-*O*-benzyl-4-*O*-(2-azido-2-deoxy-3,4,6-tri-*O*-benzyl-α/β-D-galactopyranosyl)-β-D-galactopyranoside **51** (10mg, 0.012mmol) in ethanol (0.2mL). The mixture was vigorously stirred under hydrogen atmosphere at room temperature for 18h. When TLC analysis (chloroform/methanol, 9/1, v/v) indicated that reaction is complete, the reaction mixture was filtered by using Celite and concentrated *in vacuo*. The crude product was purified by chromatography (Iatrobeads, chloroform/methanol/water, 65/33/2, v/v/v) to afford **55** as a white solid (3.55 mg, α/β=9/1, 50% overall yield). $^{1}$HNMR (500 MHz, $D_2O$) δ: 5.32 (d, 1H, H-1'α, $J_{1,2}$=3.3Hz), 4.94 (d, 1H, H-1'β, $J_{1,2}$=7.9Hz), 4.39 (d, 1H, H-1β, $J_{1,2}$=7.7Hz), 3.97 (dd, 1H, H-3'α, $J_{2',3'}$=8.4Hz, $J_{3',4'}$=9.4Hz), 3.90 (dd, 1H, H-4'α,

$J_{4',5'}$=9.5Hz), 3.80-3.72 (m, 6H, H-3, H-4, H-6a,b, H-6'a,b), 3.68 (ddd, 1H, H-5, $J_{5,6a}$=7.9Hz, $J_{5,6b}$=6.8Hz), 3.59-3.52 (m, 3H, H-2, H-2', H-5'), 3.46 (s, 3H, OC$H_3$α), 3.41 (s, 3H, OC$H_3$β). $^{13}$C NMR (125MHz, $D_2O$) δ: 103.02, 102.33 (2x), 98.17, 97.11, 96.17, 96.10, 96.02, 92.11, 89.11, 82.45, 81.45, 78.31, 77.87, 76.94, 76.22, 75.57, 75.22, 74.89, 74.70, 72.64, 69.56, 62.67, 61.37, 56.89, 55.24. FAB-MS: *m/z* 379 $[M+Na]^+$. *Anal*. Calcd for $C_{13}H_{25}NO_{10}$: C, 43.94; H, 7.09; N, 3.94; found: C, 43.88; H, 7.11; N, 3.94.

# CHAPTER 3

## SYNTHESIS OF PHOSPHOGLYCOSYLATED SERINE DERIVATIVES: A NEW CLASS OF SYNTHETIC GLYCOPEPTIDES

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## Abstract

α-D-Mannopyranosylphosphate serine derivatives were conveniently synthesized by reaction of benzyl or cyanoethyl phosphochloroamidite with 2,3,4,6-tetra-*O*-acetyl-D-mannopyranose to give intermediate α-manopyranosyl phosphoramidites (**5**,**6**), which were successively reacted with properly protected serine (as carbamate or imine) derivatives in the presence of 1*H*-tetrazole to give phosphite triesters which could be oxidized to phosphotriesters using *t*-BuOOH.


## Introduction

The majority of proteins contain oligosaccharide side chains. This post transitional modification is referred to as glycoprotein. It has become evident that glycoproteins play an important role in many different cellular recognition processes;[206] such as intercellular and intracellular transport of the gene products,[207] the alteration of peptide backbone conformation,[208] control of membrane permeability, and molecular recognition.[209] In glycoproteins, the saccharide residues are covalently linked to the protein backbone through either *N*-glycosidically via the side chain of asparagines or *O*-glycosidically via the hydroxyl of serine, threonine, tyrosine or hydroxylysine.[210,211] Recently, a new class of *O*-glycoprotein modified by phosphodiester linkage was discovered.[212] The first reported example of a protein modified by phosphoglycosylation was an endopeptidase isolated from the cellular slime mold of *Dictyostelium discoideum* known as Preotinase I.[213] Its structure was shown to contain GlcNAc-1-$PO_4$ linked to

hydroxyl of serine. Two other cysteine proteinases from *D.discoideum*, known as cprD and cprE, have also been shown to carry GLcNAc-1-$PO_4$ (Figure 3.1). [214]

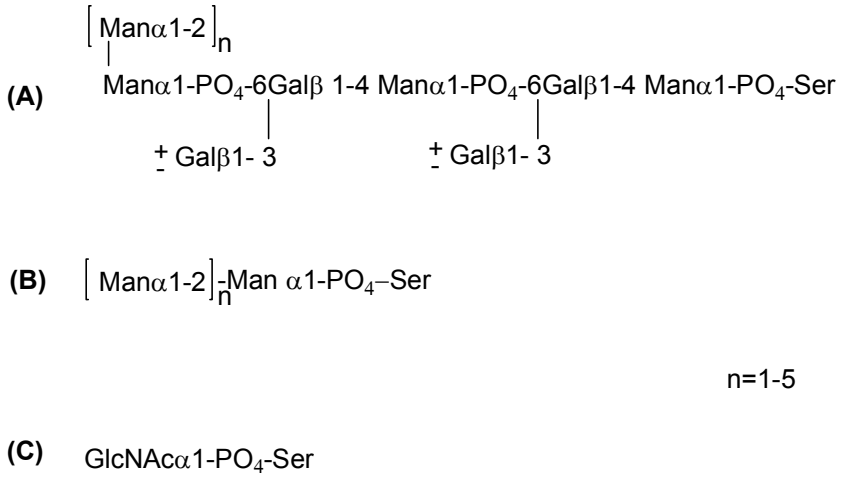



**Figure 3.1:** Structures of oligosaccharides released from three major phosphoglycosylated proteins. (A, B) Glycans isolated from *L. Mexicana* secreted acid phophatase, (C) Glycan isolated from *D. discoideum* Proteinase I.

Protozoan parasites of the genus *Leishmania* infect two groups of host organisms, the vector sandflies and mammals. In sandflies the parasites colonize the lumen of the digestive system, whereas in the mammalian host they reside in the phagolysosomal compartment of macrophages. Different *Leishmania* species cause several diseases in humans ranging from relatively benign cutaneous ulcers to fatal visceral spread of the parasites.[215] The amastigotes of this species have indicated that complex glycoconjugates may play a major role in the infection process. Among these glycoconjugates, a family of phosphorylated glycoproteins, collectively termed as proteophosphoglycans (PPGs) have been identified. [216,217] This family consists of unconjugated phosphoglycan (PG),[218,219] lipid-linked lipo-phosphoglycans (LPG),[220,221] and several protein-linked phospho-glycans. In the case of PPGs, phosphosaccharide repeating units and capping glycans are

linked to a protein backbone *via* phosphodiester linkages to the side chain hydroxyl at serine. This unusual form of protein glycosylation is termed as phosphoglycosylation.[222-225] PPGs consist primarily of secreted parasite products such as acid phosphatase (SAP),[222,226-229] filamentous proteophosphoglycans from promastigotes[230] (fPPG), and a non-filamentous proteophosphoglycan from amastigotes (aPPG).[231] The precise functions of most of these PPGs for the parasite remains illusive; however, recent studies have provided some clues that fPGG appears to be important for efficient transmission of *Leishmania* promastigotes from the sandfly to the mammalian host.[232]

There is a great demand to develop a methodology to synthesize fragments of natural proteophosphoglycans for biological experiments.These experiments may provide more detailed understanding of the functions of these proteophosphoglycans (PPGs). In general, the synthesis of *O*-glycoproteins is complicated by the acid lability of the glycosidic bond, and base lability of the amino residue. Additional complications arise due to the poor reactivity in Koenigs-Knorr type reactions of the typical *N*-acylated (*e.g.* Boc, Cbz, protection) of peptides which contain serine and threonine. Due to this poor reactivity, harsh conditions are required for an effective glycosidic bond formation, which may lead to poor yields as well as low anomeric selectivity.[233] The poor reactivity is most likely due to the unfavorable intramolecular hydrogen bonding between amide–type protecting groups and hydroxyl group.[234] This type of hydrogen bonding decreases the nucleophilicity of the hydroxyl group. Replacement of the amide (H-bond donor) by an imine (H-bond acceptor) protecting group increases the nucleophilicity of the hydroxyl (Figure 3.2).

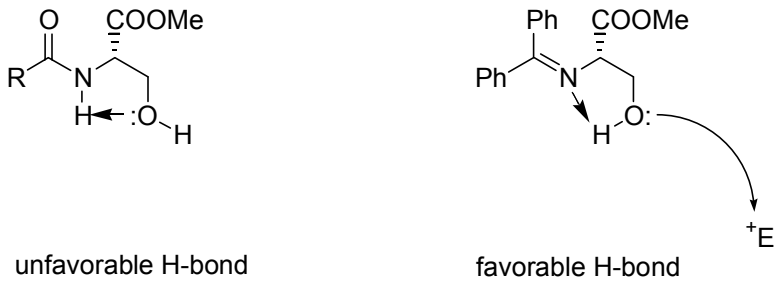
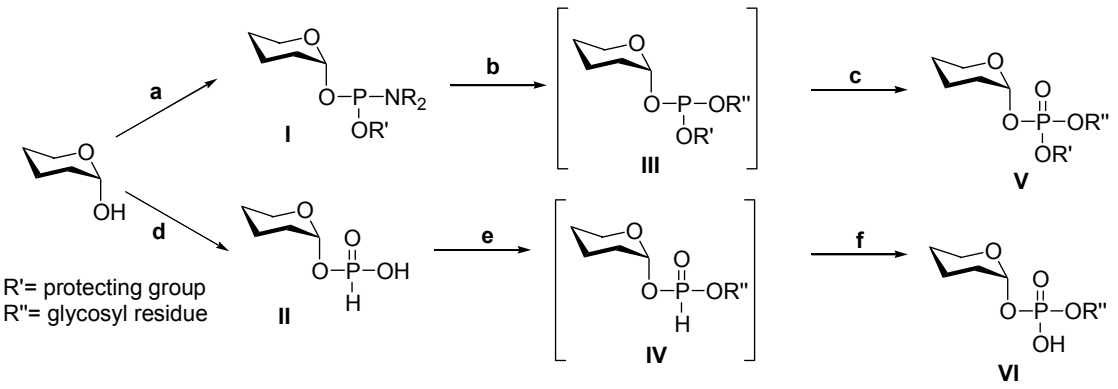



**Figure 3.2.** Amide-type (left) and imine-type (right) intramolecular hydrogen bonding in serine derivatives.

A number of different synthetic methodologies for the synthesis of interglycosidic phosphodiester linkages have been developed and these include the phosphodiester,[235] phosphotriester,[236] phosphoramidite,[237] and hydrogenphosphonate (H-phosphonate)[238] methodologies. The first two methods use P(V) compounds and nowadays are rarely used due to long reaction times, low yields, and complexity in their reaction mixture. On the other hand, the last two mentioned methods have been widely used during the past decade in carbohydrate chemistry, especially the H-phosphonate approach.

Both of the phosphoramidite and the H-phosphonate procedures involve a three step reaction sequence (Scheme 3.1). First, the phosphoramidite **I** and H-phosphonate monoester **II** are formed. Subsequent coupling to a suitably protected alcohol to yield the intermediates phosphite **III** and H-phosphonate diester **IV** which are then oxidized to produce the phosphotriester **V** and phosphodiester **VI**, respectively.



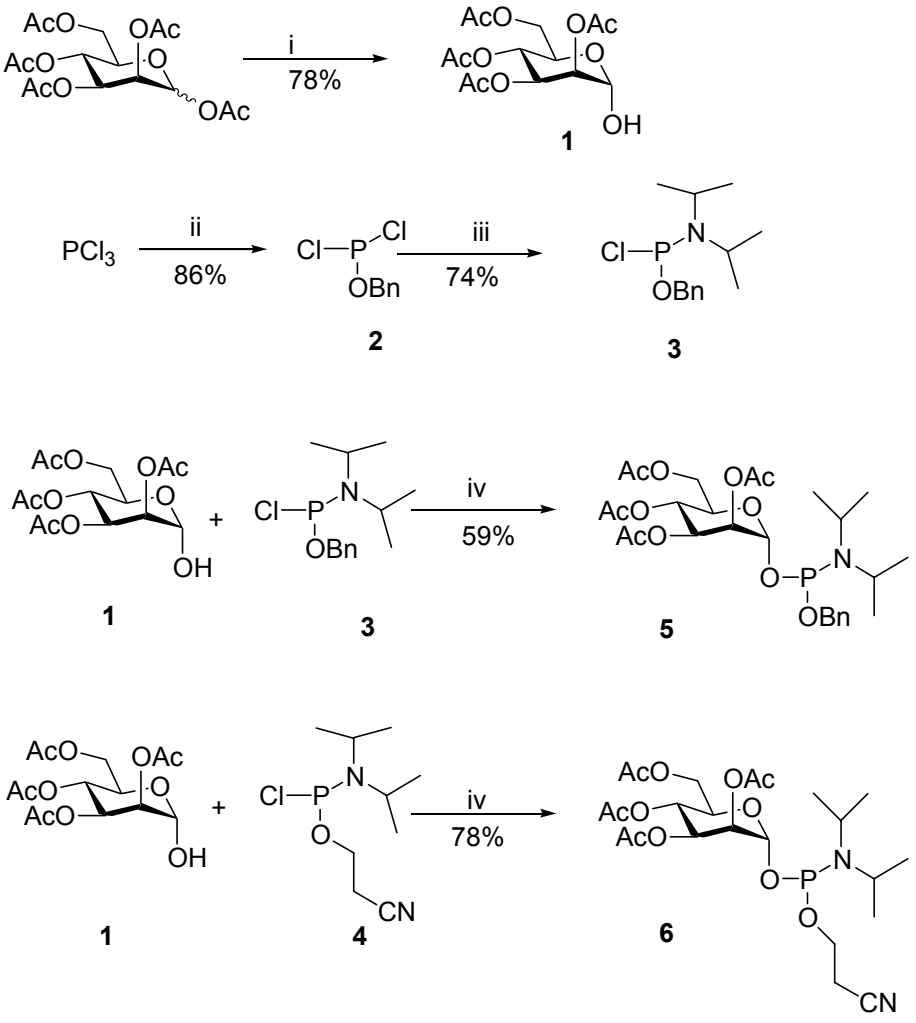

**Scheme 3.1:** The phosphoramidite and H-phosphonate method a) $R_2NPCl(OR')$; b) R''OH, 1*H*-tetrazole; c) *m*-CPBA; d) $PCl_3$, imidazole; e) R''OH, PivCl, pyridine; f) $I_2$, pyridine.

Herein we report, for the first time, a convenient synthetic approach for the preparation of a range of properly protected α-D-mannosylphosphate serine derivatives which can be used for the synthesis of phosphoglycopeptides. A wide range of serine derivatives was utilized to optimize the reaction conditions and to study the behavior of serine derivatives as glycosyl acceptors during the formation of the phosphotriester linkage using the phosphoramidite approach.

**Results and discussion**

Two monofunctional phosphitylating reagents **3** and **4**[239] were utilized for the synthesis of anomeric phosphates. Reagent **4** is commercially available while reagent **3** was synthesized from phosphorus trichloride by treatment with benzyl alcohol in freshly distilled diethyl ether at -78$^{o}$C to form benzyldichlorophosphite **2**, which was reacted with diisopropylamine[240] in dichloromethane at -20$^{o}$C to give **3** in 74% yield after distillation in a short-path apparatus under reduced pressure (Scheme3.2). Hemiacetal **1** was obtained by treatment of β-D-mannose pentaacetate with hydrazine acetate in DMF

to afford **1** in 78% yield. Anomeric phosphitylation of hemiacetal **1** was performed by treatment of **1** with **3** and **4** in dichloromethane in the presence of the hindered base diisopropylethylamine to afford **5** (59%) and **6** (78%), respectively (Scheme3.2).



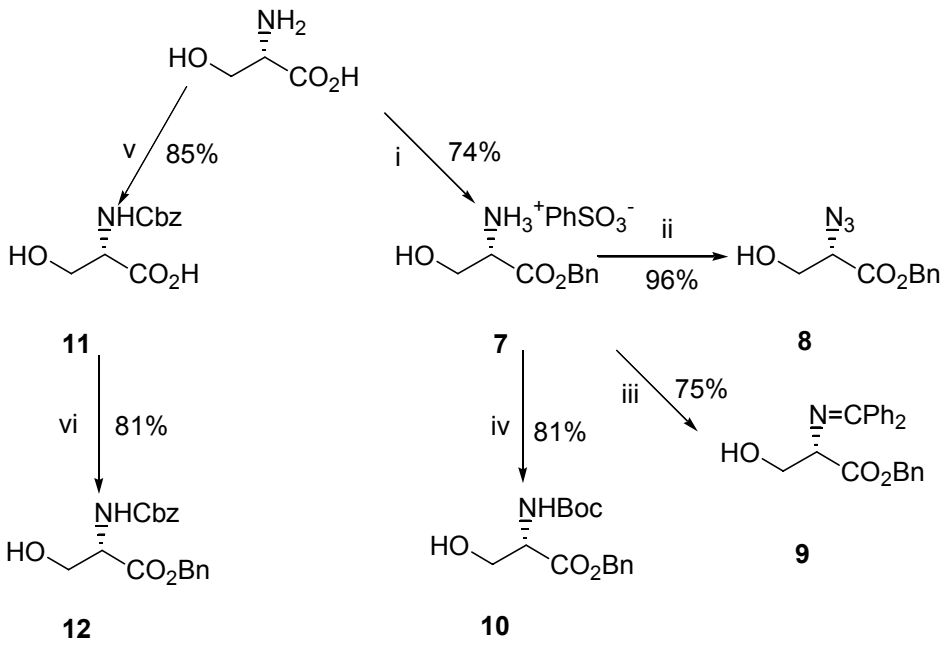

**Scheme 3.2.** Reagents and conditions: i) $N_2H_4$.HOAc/ DMF; ii) BnOH/$Et_2O$, -78°C; iii) *i*$Pr_2NH$, $CH_2Cl_2$, -20°C; iv) DIPEA, $CH_2Cl_2$.

The anomeric configuration of **5** and **6** were ascertained by $^1H$ NMR spectrometry, where the anomeric proton appeared at 5.8 ppm ($^1H$-$^{31}P$ coupling constant of~6.7Hz) and both H-3 and H-5, which are 1,3-*syn* diaxial to the phosphite group, are deshielded by 0.2 ppm compared to hemiacetal **1**. Since reagent **4** gave a higher yield,

attention was focused on using it for coupling with different serine derivatives. In general, phosphoramidites are highly reactive in a 1*H*-tetrazole promoted coupling, therefore, it was anticipated that the reaction between phosphoramidite **5** and **6** and the primary alcohol group of the glycosyl acceptors (serine derivatives) would proceed smoothly.

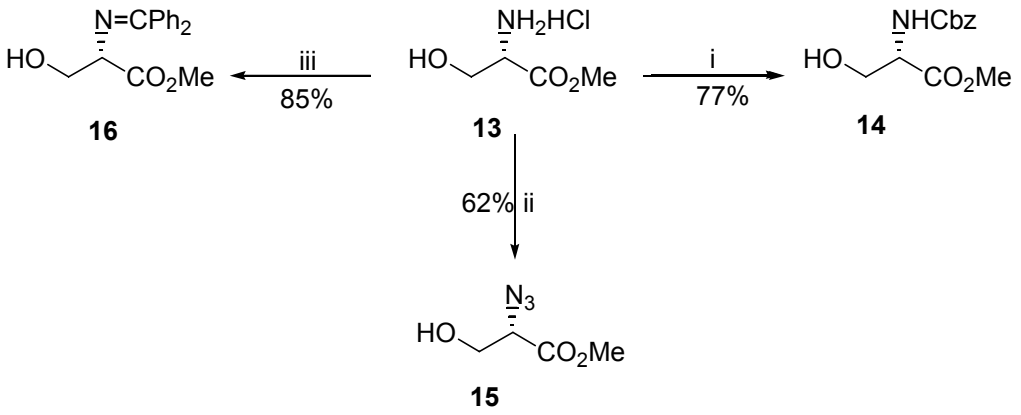
**Synthesis of the serine derivatives**

A wide range of serine derivatives were synthesized and used as the glycosyl acceptor for reaction with phosphoramidites **5** and **6**. Several serine derivatives were synthesized as benzopheonone Schiff base esters (imine-type) while others contained carbamate protecting groups. As can be seen in (Scheme 3.3), four different serine derivatives **8**, **9**, **10**, and **12** were synthesized from L-serine. The carboxyl group of L-serine was protected as a benzyl ester by the reaction with benzyl alcohol in the presence of benzene sulfonic acid[241] to give the benzyl serinate benzene sulfonate **7** in 74% yield. Ester **7** was subsequently treated with triflic azide[242] and DMAP in dichloromethane to yield **8** in 96% yield. Derivative **9** was obtained when compound **7** was treated with benzophenone imine[243] in dry DCM to give **9** in 75% yield. The Boc protected **10** was obtained in a yield of 81% when **7** was dissolved in THF and treated with $(Boc)_2O$ in the presence of an aqueous solution of sodium hydroxide.[244] In a different approach, the amino group of L-serine was protected as Cbz-amide when L-serine was treated with benzylchloroformate[245] in the presence of aqueous sodium bicarbonate to afford the serine derivative **11** in 85% yield. Subsequent, benzyl ester formation of derivative **11** using $BnBr/Cs_2CO_3$/ DMF conditions[246] afforded derivative **12** in 81% yield.



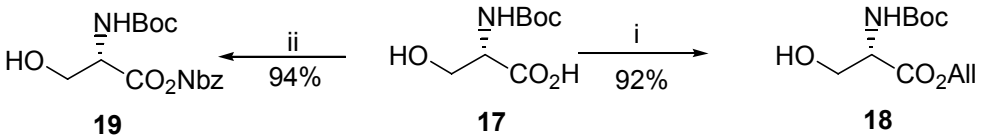

**Scheme 3.3.** Reagents and conditions: i) BnOH, BSA, $CCl_4$; ii) $TfN_3$, DMAP, $CH_3CN$, 0°C; iii) $Ph_2CNH$, $CH_2Cl_2$; iv) $(Boc)_2O$, THF, aq. NaOH; v) $PhCH_2OCOCl$, aq. $NaHCO_3$; vi) BnBr, $Cs_2CO_3$, DMF.

Methyl ester derivatives of serine were synthesized from methyl L-serinate hydrochloride **13** (Scheme 3.4). The amino group of compound **13** was protected as benzyloxycarbonyl by treatment with benzyl chloroformate and triethyl amine in DCM to give the serine derivative **14** in a yield of 77%. When methyl serinate **13** was treated with triflic azide and DMAP in acetonitrile, compound **15** was obtained in 62% yield. Schiff's base **16** was synthesized in 85% yield by treatment of compound **13** with benzophenone imine in DCM.



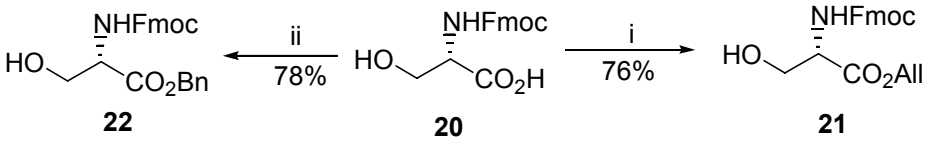

**Scheme 3.4.** Reagents and conditions: i) $PhCH_2OCOCl$, $Et_3N$, $CH_2Cl_2$, ii) $TfN_3$, DMAP, $CH_3CN$, $0^oC$; iii) $Ph_2CNH$, $CH_2Cl_2$.

Another range of L-serine derivatives was synthesized from N-(*t*-butoxy-carbonyl) L-serine **17 (**Scheme 3.5). The Boc-serine derivative **17** was treated with allyl bromide in the presence of DIPEA/DMF[247] to give the allyl serinate derivative **18** in a yield of 92%. On the other hand, when **17** was treated with *p*-nitrobenzyl bromide in the presence of DIPEA[248] as the base in *N,N*-dimethyl formamide, derivative **19** was obtained in 94% yield.




**Scheme 3.5:** Reagents and conditions; i) AllBr, DIPEA, DMF; ii) *p*-$NO_2PhCH_2Br$, DIPEA, DMF.

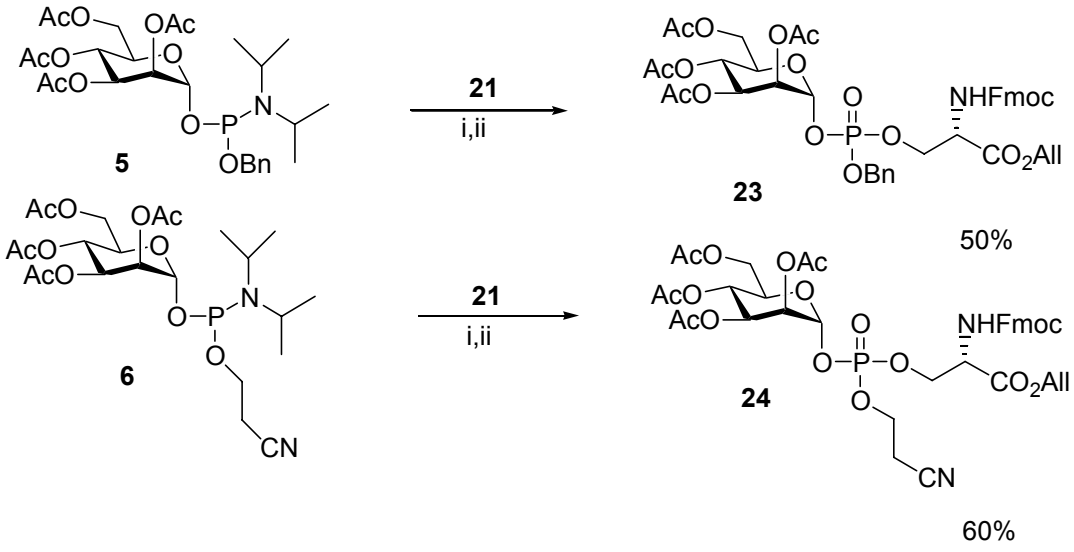
Finally, serine derivatives **21** and **22** were obtained by treatment of compound **20** with AllBr/DIPEA/DMF to give serine derivative **21** in 76% yield. Treatment of **20** with $BnBr/Cs_2CO_3$/DCM afforded derivative **22** in 78% yield (Scheme 3.6).




**Scheme 3.6:** Reagents and conditions: i) AllBr, DIPEA, DMF; ii) BnBr, $Cs_2CO_3$, DCM.

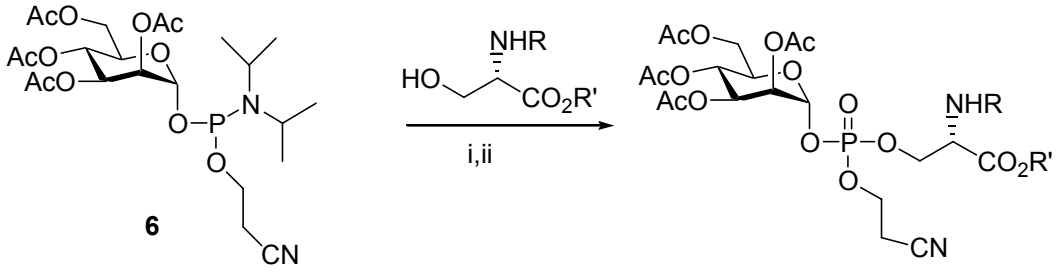
**Synthesis of the anomeric phosphotriester.**

The α-mannosyl phosphoramidites **5** and **6** were coupled to a wide range of serine derivatives to obtain a library of Manα-1-$PO_4$-Ser derivatives. As a preliminary observation, when phosphoramidites **5** and **6** were coupled with the hydroxyl group of the serine derivative **21** in a 1*H*-tetrazole promoted reaction, the use of phosphoramidite reagent **6** gave higher yields than the other reagent **5** (Scheme 3.7). Therefore, reagent **6** was the reagent of choice for the phosphorylation. Oxidation of the obtained phosphite intermediates was accomplished by using the oxidizing reagent *t*-BuOOH[249] at -40°C to give the phosphor-triesters **23** and **24** in 50% and 60% yields, respectively.




**Scheme 3.7.** Reagents and conditions: i) 1*H*-tetrazole, $CH_3CN$; ii) *t*-BuOOH, -40°C.

Phosphoramidite **6** was reacted with several carbamate protected serine derivatives (**14**, **18**, **12**, **10**, **19**, **22**) using 1H-tetrazole as the activator followed by oxidation with *t*-BuOOH to give the phosphotriesters **25**, **26**, **27**, **28**, **29**, **30,** respectively (Scheme 3.8)

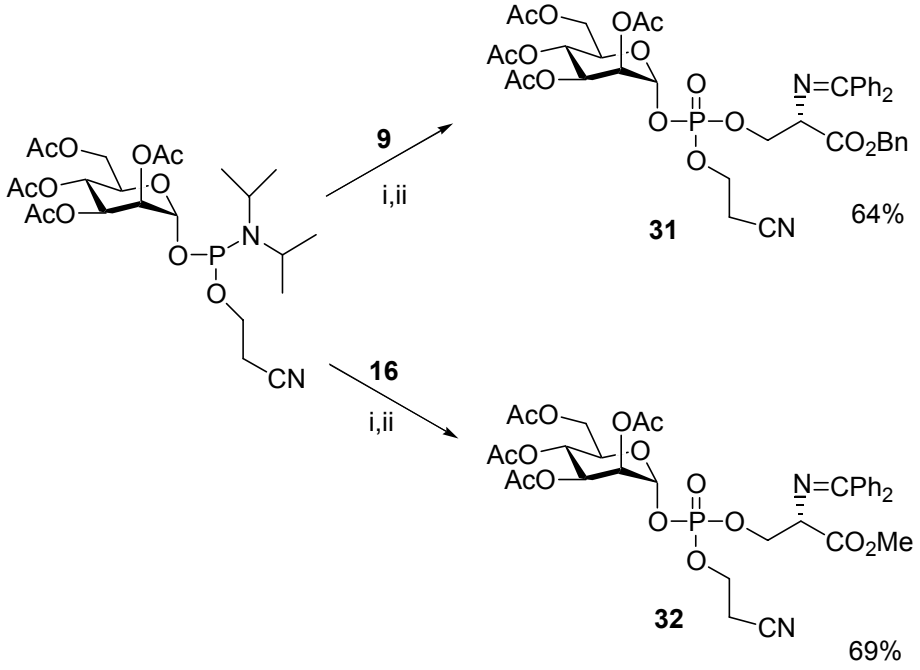



**25:** R= Cbz, R'=Me, (53%)
**26:** R= Boc, R'=All, (53%)
**27:** R= Cbz, R'=Bn, (52%)
**28:** R= Boc, R'=Bn, (48%)
**29:** R= Boc, R'=Nbz, (53%)
**30:** R= Fmoc, R'=Bn, (50%)

**Scheme 3.8.** Reagents and conditions: i) 1*H*-tetrazole, $CH_3CN$; ii) *t*-BuOOH, -40$^{o}$C.

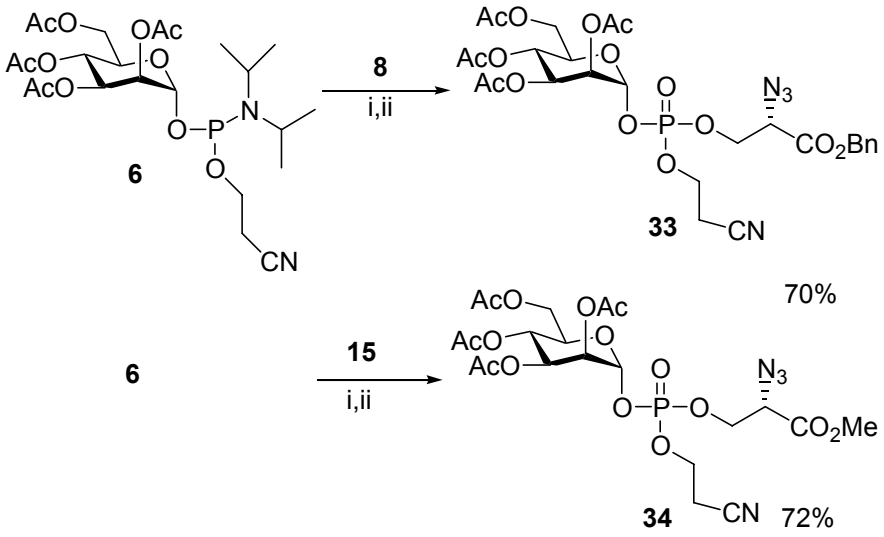
To optimize the yield of the phosphorylation, attention was focused on serine derivatives that are more reactive. Since serine derivatives with imine-type H-bonding pattern have enhanced nucleophilicities, and this increase in the nucleophilicity may lead to a higher reaction rate during the phosphorylation. Therefore, crystalline benzophenone Schiff base esters **9** and **16** were coupled with phosphoramidite reagent **6** using 1*H*-tetrazole as the mediator followed by oxidation with *t*-BuOOH to afford the triesters **31** and **32** in 64% and 69% yields, respectively (Scheme 3.9). The relatively high yields here compared to those of serine derivatives with amide-type protecting groups confirmed the

importance of intramolecular hydrogen bond to increase the nucleophilicity of the lone pair.




**Scheme 3.9.** Reagents and conditions: i) 1*H*-tetrazole, $CH_3CN$; ii) *t*-BuOOH, -40°C.

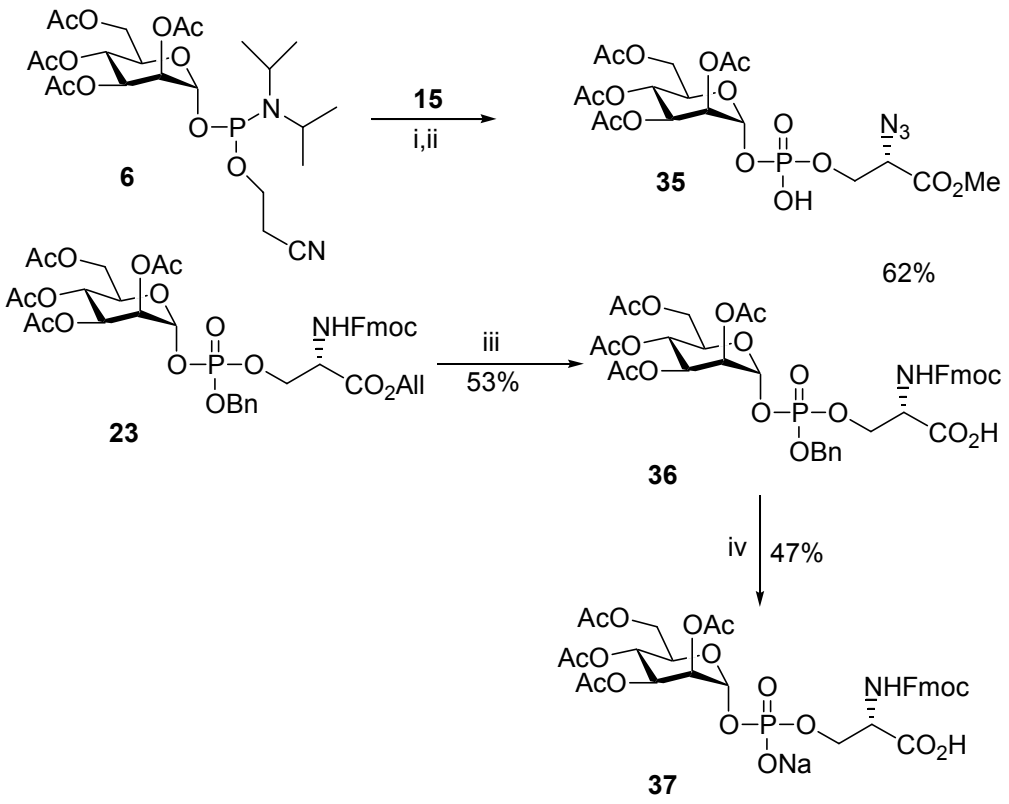
A third group of serine derivatives that contains an azido functionality as amino-masking group was also examined. So, by Treatment of the azido derivatives **8** and **15** with phosphoramidite **6** using the standard two-step procedure of 1*H*-tetrazole mediated coupling and *t*-BuOOH as the oxidizing agent afforded the phosphotriesters **33** and **34**, in 70% and 72% yields, respectively (Scheme 3.10). The yields in this case were even higher than those obtained with Schiff's base derivatives. It is believed to be because of the high electron density on the azido group that makes the lone pair of the hydroxyl group more available for nucleophilic substitution.




**Scheme 3.10.** Reagents and conditions: i) 1*H*-tetrazole, $CH_3CN$; ii) *t*-BuOOH, -40°C.

**Synthesis of anomeric phosphodiesters**

After establishing a convenient method to synthesize phosphotriesters, two of the previously synthesized phosphotriesters were converted into the corresponding phosphodiesters as models. Therefore, phosphoramidite **6** was coupled to serine derivative **15** in the presence of 1*H*-tetrazole to form phosphite intermediate. In the oxidation step, a mixture of *t*-BuOOH and $Et_3N$ was used. While *t*-BuOOH oxidizied the intermediate P(III) to P(V), $Et_3N$ cleaved the 2-cyanoethyl protecting group to give phosphodiester **35** in 62% yield (Scheme 3.11). In the case of the other benzyl-protected phosphotriester **23**, its allyl protecting group was cleaved by treatment with $Pd(PPh_3)_4$, acetic acid, and $Bu_3SnH$[250] to give acid **36** in 53% yield. In the next step, triester **36** was debenzylated by using two equivalents of NaI in $CH_3CN$ to give the diester **37** in 47% yield.[242]

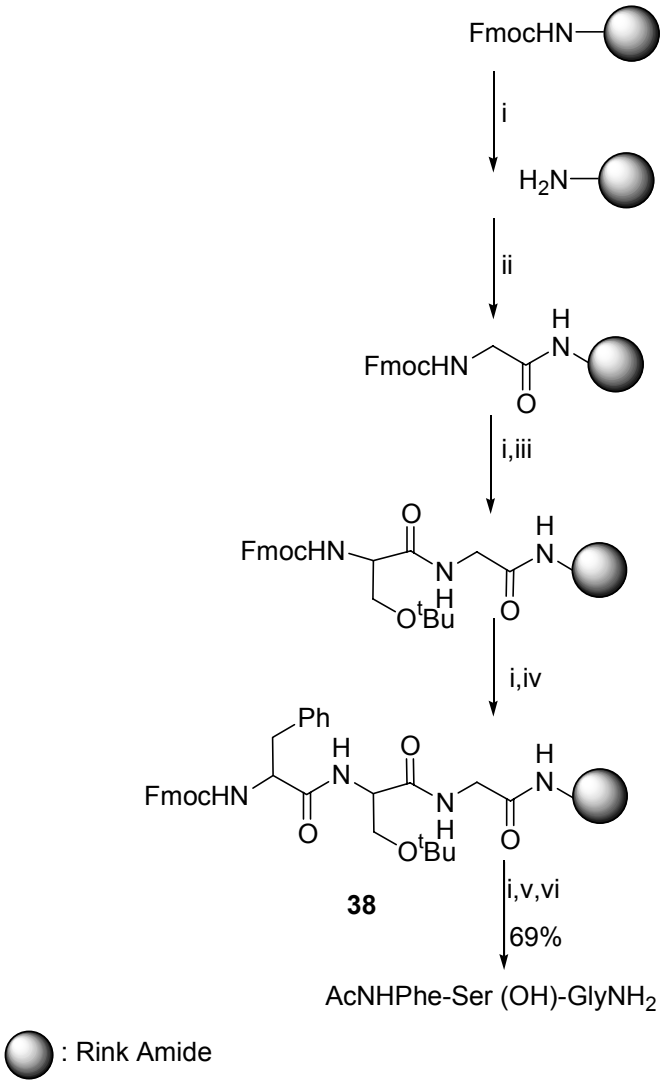



**Scheme 3.11.** Reagents and conditions: i) 1*H*-tetrazole, $CH_3CN$; ii) *t*-BuOOH/$Et_3N$,-40°C; iii) $Pd(Ph_3P)_4$, $Bu_3SnH$, AcOH/THF; iv) NaI, $CH_3CN$.

**Global glycophosphorylation of a peptide-containing serine chain**

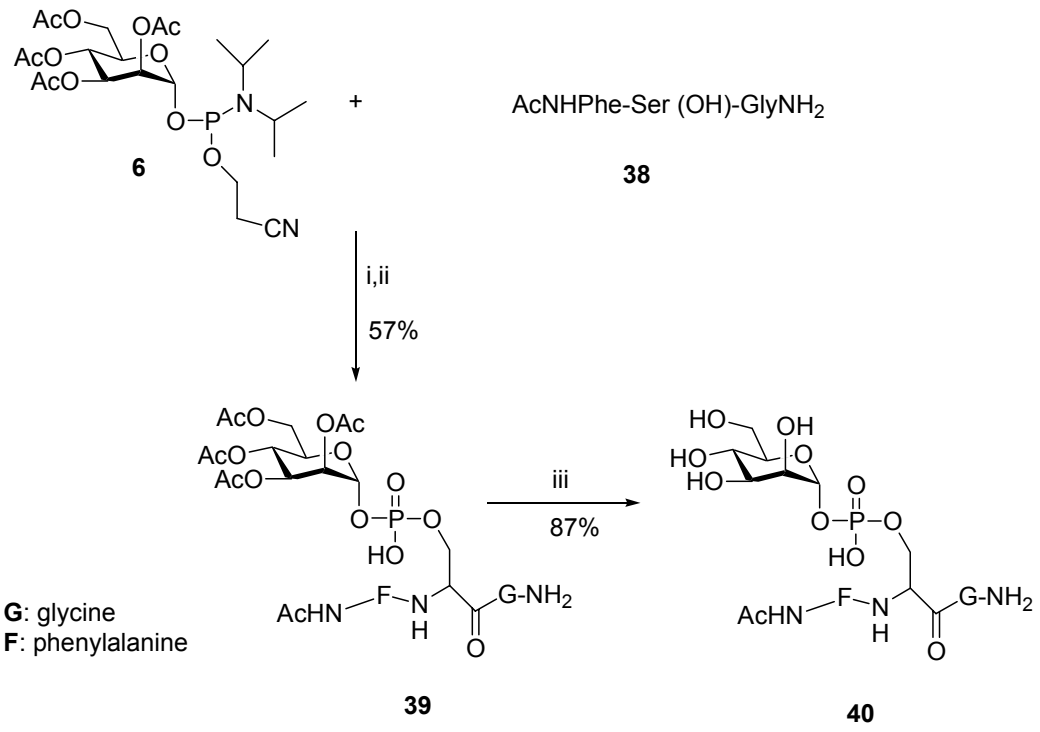
As previously discussed, coupling of an anomeric hydroxyl group of a monosaccharide with the hydroxyl side chain of an amino acid *via* a phosphodiester linkage using the phosphoramidite methodology provides a reliable approach for synthesizing phosphoglycosylated serine derivatives. For expanding the applications of this methodology, phosphoramidite **6** was coupled with the hydroxyl side chain of serine residue in the tripeptide (Gly-Ser-Phe). First, This tripeptide was synthesized by the solid phase approach,[251] using the Fmoc continous-flow strategy.[252] Manual synthesis of peptide **38** was performed on the Fmoc-amino protected resin (Rink Amide). The resin was allowed to be swelled by shaking it with DMF using a continous flow of nitrogen gas

followed by cleavage of the Fmoc protecting group of the resin by treatment with 20% piperidine in DMF. The first amino acid (Fmoc-Gly-OH) was coupled with the resulting amino free resin by using the coupling standard conditions of PyBOP/HOBt/ DIPEA[253] in DMF for amide linkage formation. The completion of the reaction was monitored by Kaiser's test[254] which indicated the absence of the free amino group. After loading the first amino acid on the resin, all the excess regents were removed by washing the resin-containing glycine with DMF followed by cleavage of the Fmoc-protecting group of glycine by using 20% piperidine/DMF. The completion of this reaction was also monitored by Kaiser's test which indicated the presence of the free amino group. The second amino acid Fmoc-serine (OtBu)-OH was coupled through its free carboxylic group with the free amino group of glycine located on the resin using the same conditions of PyBOP/HOBt/DIPEA. Repeating the same cycle of Fmoc-cleavage and introduction of the amino acid residues, the third amino acid Fmoc-Phe-OH was introduced to the resin. Finally, the Fmoc-protecting group of the terminal amino acid residue (Fmoc-Phe) was cleaved by treatment with 20% piperidine in DMF. The resulting free amino group was reprotected as acetate by the reaction with $Ac_2O$/DMF. To deprotect the hydroxyl protecting group of serine and to cleave the peptide from the resin at the same time, the protected peptide resin was treated with 55% TFA in DCM in the presence of triisopropyl silane as the scavenger to release tripeptide **38** which was precipitated with diethyl ether at -6$^{o}$C in 69% yield (Scheme 3.12).




**Scheme 3.12.** Reagents and conditions i) 20%piperidine/DMF; ii) FmocNHGly-OH/PyBOP/DIPEA/DMF; iii) FmocNHSer(O$^t$Bu)-OH/PyBOP/HOBt /DIPEA/DMF; iv) FmocNHPhe-OH/PyBOP/HOBt/DIPEA/DMF; v) $Ac_2O$/DMF; vi) 55%TFA/DCM.

Coupling of the tripeptide **38** with phosphoramidite **6** was activated by 1*H*-tetrazole followed by adding a mixture of *t*-BuOOH/$Et_3N$ to give the phosphordiester **39** in 57% yield. This compound was fully deacetylated by using a methanolic solution of sodium methoxide to give phosphodiester **40** in 87% yield (Scheme 3.13).




**Scheme 3.13.** Reagents and conditions: i) 1*H*-tetrazole/$CH_3CN$; ii) *t*-BuOOH/$Et_3N$; iii) NaOMe/MeOH.

## Conclusion

It has been shown that the new approach for coupling α-mannosyl phosphoramidite to a range of serine derivatives provides phosphotriester products in good yield and pure α-anomeric selectivity. The best yields were obtained when the imine-protected derivatives of serine were coupled to the phosphoramidite of α-mannose. The high yields of the phosphoglycosylation probably result from the high nucleophilicity of the serine hydroxyl group due to the favorable intramolecular hydrogen bonding. The favorable property of this approach is the ability to directly synthesize the stable form 'the phosphodiesters' through the phosphotriester intermediate. This approach can be also utilized for the incorporation of different peptides containing hydroxyl amino acids such as, serine, threonine, tyrosine, or hydroxylysine. The

methodology presented here can be employed for the global glycolphosohorylation of pre-assembled peptides as well as by the building block phosphorylation strategy to synthesize the same phosphoglycopeptide using the appropriate glycophosphorylated serine derivative such as building block **37**.

## Experimental section

**General.** All reactions were conducted under argon atmosphere. Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), size exclusion column chromatography was performed on Sephadex LH-20 (methanol/dichloromethane, 1/1, v/v). Reactions were monitored by TLC on Kieselgel 60 $F_{254}$ (EM Science) and the compounds were detected by examination under UV light and charring with 5% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40$^{o}$C. All organic solvents were distilled from the appropriate drying agents prior to use: acetonitrile, dichloromethane, diethyl ether, *N,N*-dimethylformamide, pyridine, and toluene were distilled from $CaH_2$. Tetrahydrofuran was distilled from sodium directly prior to use. Methanol was dried by refluxing with magnesium methoxide, distilled, and stored under argon. Molecular sieves (3Å and 4Å), were crushed and activated *in vacuo* at 390$^{o}$C for 3h prior to application.

$^{1}$H NMR, $^{13}$C NMR, and $^{31}$P NMR (external 85% $H_3PO_4$) spectra were recorded on varian 300 MHz, 500 MHz and 600MHz spectrometers equipped with sun off-line editing workstations. Chemical shifts are reported in parts per million (ppm) using trimethylsilane as internal standard. Matrix-assisted Laser Desorption Ionization- Time-

of-Flight (MALDI-TOF) mass spectrometry was performed using a HP MALDI-TOF spectrometer with gentisic acid as matrix.

**2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose. (1).**

To a stirred solution of D-mannose pentaacetate (5g, 12.8mmol) in DMF (50mL) was added hydrazine acetate (2.49g, 27mmol) and the reaction mixture was stirred at room temperature for 48h. When TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated that all the starting material had been consumed, the reaction mixture was poured into water (200mL) and extracted with ethyl acetate (4x30mL). The organic layers were combined, dried over $MgSO_4$, filtered, and the filtrate evaporated *in vacuo*. The residue was purified by silica gel column chromatography (eluent: ethyl acetate/hexane, 1/9, v/v) to give **1** as a colorless syrup (3.5g, 78%). $^1$H NMR (300MHz, $CDCl_3$) δ: 5.43 (dd, 1H, H-3, $J_{2,3}$=2.9Hz $J_{3,4}$=10.0Hz), 5.31 (dd, 1H, H-4, $J_{4,5}$=10.0Hz), 5.28 (dd, 1H, H-2, $J_{1,2}$=1.8Hz), 5.23 (br d, 1H, H-1), 4.29-4.23 (m, 2H, H-5, H-6a), 4.16-4.12 (m, 1H, H-6b), 2.17-2.00 (4s, 12H, 4xC$H_3$CO). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 170.23, 169.85, 169.83, 169.79, 92.11, 70.02, 68.86, 68.45, 66.16, 62.50, 20.83, 20.77, 20.65, 20.44.

**Benzyl *N,N*-diisopropylchlorophosphoramidite (3).**

To a solution of phosphorus trichloride (2.62mL, 30.0mmol) in dry ether (25mL) was added dropwise a solution of benzyl alcohol (2.38mL, 23.0mmol) in dry ether (45mL) at –78$^o$C over a period of 1h. After stirring for 2h, the mixture was warmed to room temperature and distilled under reduced pressure to give benzyl phosphodichloridite **2** as an oil. Crude benzyl phosphodichloridite **2** was dissolved in dichloromethane (40mL) and cooled to -20$^o$C. To this solution, diisopropylamine (1.6mL, 46mmol) was added dropwise and the reaction mixture was stirred overnight. The slurry mixture was filtered

off and the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane and washed with aqueous $NaHCO_3$ (1M, 2x25mL), dried over $MgSO_4$. After filtration and concentration under reduced pressure, crude **3** was centrifuged to remove additional precipitate and distillated under reduced pressure to give **3** as a colorless oil (3g, 73.5 %). $^1$H NMR (300MHz, $CDCN_3$) δ: 7.35 (m, 5H, Ph), 4.89 (s, 2H, OC*H*$_2$Ph), 3.83 (m, 2H, 2xC*H*$(CH_3)_2$), 1.22 (d, 12H, 2xCH(C*H*$_3)_2$). $^{31}$P NMR ($CD_3CN$) δ: 181.1 (br s).

**(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl) benzyl *N,N*-diisopropyl phosphoramidite (5).**

To a stirred solution of **1** (0.55g, 1.43mmol) and DIPEA (0.52mL, 2.86 mmol) in $CH_2Cl_2$ (5mL) was added benzyl *N,N*-diisopropylchlorophosphoramidite **3** (0.3g, 1.4mmol). The reaction mixture was stirred at room temperature for 2h. When TLC analysis (ethyl acetate/hexane 2/1, v/v) showed completion of the reaction, the mixture was diluted with $CH_2Cl_2$ (30mL) and subsequently washed with ice-cold 10% aqueous $NaHCO_3$ (10mL), brine (10mL), and water (15mL). The organic layer was collected and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, compound **5** was obtained as a colorless oil (0.5g, 59%). $^{31}$P NMR (δ: 14.9, 15.2, 2xs, two diastereomers). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.22-7.16 (m, 5H, Ar-*H*), 5.84 (dd, 1H, H-1, $J_{1,2}$=1.6Hz, $J_{1,P}$=8.0Hz), 5.61 (s, 2H, OC*H*$_2$Ph), 5.38 (dd, 1H, H-3, $J_{2,3}$=3.6Hz $J_{3,4}$=9.5Hz), 5.11 (t, 1H, H-4, $J_{4,5}$=10.0Hz), 5.09 (dd, 1H, H-2), 4.93 (dd, 1H, H-6a, $J_{5,6a}$=5.0Hz $J_{6a,6b}$=12.4Hz), 4.27 (ddd, 1H, H-5, $J_{5,6b}$=2.1Hz), 3.96 (dd, 1H, H-6b), 2.97-2.53 (m, 2H, 2xC*H*$(CH_3)_2$), 2.11-1.65 (4s, 12H, 4xCOC*H*$_3$), 1.63-1.59 (d, 12H, 2xCH(C*H*$_3)_2$). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 172.13, 171.54, 170.19, 169.23, 139.14, 127.23, 126.78,

125.11, 123.45, 101.23, 98.34, 87.23, 77.12, 76.82, 72.14, 69.14, 69.11, 67.56, 58.33, 53.61, 44.16, 42.65, 41.86, 29.87, 24.56, 23.11, 22.13, 20.93, 20.84, 20.67, 20.55, 20.33, 19.89. FAB-MS: *m/z* 608.26.

**(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl) 2-cyanoethyl *N,N*-diisopropyl phosphoramidite (6).**

To a stirred solution of **1** (0.55g, 1.43mmol) and DIPEA (0.52mL, 2.86 mmol) in $CH_2Cl_2$ (5mL) was added **4** (0.54g, 1.4mmol). After 1h, TLC analysis (ethyl acetate/hexane, 2/1, v/v) showed completion of the reaction. The reaction mixture was diluted with $CH_2Cl_2$ (20mL) and subsequently washed with ice-cold 10% aqueous $NaHCO_3$ (2x10mL), brine (2x10mL), water (2x15mL), followed by drying over $MgSO_4$. After evaporation of the solvent, compound **6** was obtained as a colorless oil (0.6g, 78%). $^{31}$P NMR (δ: 15.36, 15.01, 2xs, two diastereomers). $^{1}$H NMR (300MHz, $CDCl_3$) δ: 5.56 (dd, 1H, H-1, $J_{1,2}$=1.6Hz, $J_{1,P}$=6.6Hz), 5.39 (dd, 1H, H-3, $J_{2,3}$=3.6Hz, $J_{3,4}$=9.6Hz), 5.21 (dd, 1H, H-2), 4.55 (dd, 1H, H-4, $J_{4,5}$=10.2Hz), 4.39 (dd, 1H, H-6a, $J_{5,6a}$=5.02Hz, $J_{6a,6b}$=12.7Hz), 4.26 (ddd, 1H, H-5, $J_{5,6b}$=2.0Hz), 2.72 (m, 2H, 2xC*H*$(CH_3)_2$), 2.66 (t, 2H, OC$H_2$$CH_2$CN), 2.62 (t, 2H, $OCH_2C$$H_2$CN), 2.02 (4s, 12H, 4xC$H_3$CO), 1.95 (d, 12H, 2xCH(C$H_3$)$_2$). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 170.81, 170.77, 170.29, 170.27, 170.23, 170.17, 170.09, 169.90, 169.87, 117.61, 117.47, 93.29, 93.15, 93.03, 92.85, 77.17, 76.89, 72.12, 69.12, 58.33, 53.61, 44.14, 43.97, 43.86, 29.87, 24.56, 23.11, 21.07, 20.95, 20.84, 20.75, 20.61, 20.57, 20.50, 20.43, 20.25. FAB-MS: *m/z* 571.56 $[M+Na]^+$ .

**Serine benzyl ester benzenesulfonate (7).**

A mixture of L-serine (5.25g, 50.0mmol), technical-grade benzenesulfonic acid (11.25g, 90%, 62.5mmol), and benzyl alcohol (25mL) in carbon tetrachloride (100mL) was

distilled gently for 8h until no more water was formed. Carbon tetrachloride was added periodically to maintain the solvent level. After removal of the remaining solvent by distillation under reduced pressure, diethyl ether (100mL) was added to the reaction mixture with vigorous stirring. Storage of the resulting oil at 4$^{o}$C for 24h gave a solid product, which was collected, washed with cold ether, and dried in vacuum line. Recrystallization from 2-propanol/anhydrous diethyl ether yielded **7** as a white powder (13.07g, 74%): mp 97-98$^{o}$C; $[\alpha]_D$ =–2.0 (c 1.0, EtOH). $^1$H NMR (300MHz, $CDCl_3$) δ: 8.04 (b s, 3H, N*H*$_3^+$), 7.76 (d,, 2H, *o*-PhSO$_3^-$, *J*=7.5Hz), 7.11-7.02 (m, 8H, Ar-*H*), 4.99 (1H, d, OC*H*$_2$Ph, *J*=12.5Hz), 4.91 (1H, d, OC*H*$_2$Ph, *J*=12.5Hz), 4.09 (dd, 1H, α-C*H,* *J*=4.4Hz, *J*=8.8Hz), 3.92 (dd, 1H, β-C*H*H, *J*=5.4Hz, *J*=9.7Hz), 3.82 (dd, 1H, β-CH*H*, *J*=7.7Hz, *J*=9.8Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 172.70, 169.16, 135.10, 128.97, 128.90, 128.87, 128.82, 128.77, 128.62, 127.35, 68.22, 63.74, 62.91.

**Benzyl (2*S*)-2-azido-3-hydroxypropanoate (8).**

To a solution of $NaN_3$ (16.6g, 0.25mol) in $H_2O/CH_2Cl_2$ mixture (100mL, 2/3, v/v) was added $Tf_2O$ (16.6g, 57mmol) with vigorous stirring at 0$^{o}$C for 4h. The organic layer was separated, and the aqueous layer was extracted with $CH_2Cl_2$ (2x50mL). The combined organic layers were washed with saturated $NaHCO_3$ (80mL), $H_2O$ (80mL) and dried over $MgSO_4$. To a solution of benzyl L-serine benzenesulfonate **7** (2g, 5.64mmol) and DMAP (3.15g, 25.8mmol) in acetonitrile (30mL) was added $Tf_3N$ solution in dichloromethane (30mL, as prepared above) at 0$^{o}$C, and stirring was continued at 0$^{o}$C for 2h. When TLC analysis (methanol/dichloromethane, 1/9, v/v) indicated the complete consumption of the starting material, the reaction mixture was diluted with dichloroethane (40mL), concentrated at 30$^{o}$C *in vacuo* to ca. 40 mL, and filtered. The mother liquid was subjected

to silica gel column chromatography (hexane/ethyl acetate, 3/2, v/v) to afford **8** as a pale yellow oil (1.2g, 96%). $^{1}$H NMR (300MHz, $CDCl_3$) δ: 7.38-7.34 (m, 5H, Ar-*H*), 5.24 (s, 2H, OC*H*$_2$Ph), 4.11-4.08 (t, 1H, α-C*H*, *J*=6.8Hz), 3.93-3.91 (t, 2H, β-C*H*$_2$, *J*=7.7Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 172.70, 169.16, 135.10, 128.97, 128.93, 128.82, 128.73, 128.60, 127.39, 68.12, 63.74, 62.91. FAB-MS: *m/z* 244.21 $[M+Na]^+$. *Anal*. Calcd for $C_{10}H_{11}N_3O_3$: C, 54.29; H, 5.01; N, 19.00; found: C, 54.28; H, 5.02; N, 19.02.

**Benzyl *N*-(diphenylmethylene)-L-serinate (9).**

A mixture of L-serine benzyl ester benzenesulfonate **7** (1.95g, 5.52mmol), and benzophenoneimine (1.00g, 5.52mmol) in dry dichloromethane (20mL) was stirred at room temperature for 24h with the exclusion of moisture ($CaCl_2$ tube). When TLC analysis (DCM/MeOH, 9/1, v/v) indicated completion of the reaction, the reaction mixture was filtered to remove $NH_4Cl$ and washed successively with 1% aqueous $NaHCO_3$ (3x30mL) and water (3x30mL) to remove any remained $NH_4Cl$. The organic layers were collected and concentrated to dryness under reduced pressure. The residue was taken up in 20mL of diethyl ether, filtered, washed with 20mL of water, and dried over $MgSO_4$. Filtration and concentration of the filtrate followed by recrystallization (cyclohexane) afforded **9** as a white powder (1.5g, 75%). mp 90-91$^{o}$C; $[\alpha]_D$ =–4.0 (c 1.0, EtOH). $^{1}$H NMR (300MHz, $CDCl_3$) δ: 7.82-7.12 (m, 15H, Ar-*H*), 5.18 (s, 2H, OC*H*$_2$Ph), 4.50 (dd, 1H, α-C*H*, *J*=6.8Hz, *J*=7.7Hz), 3.96 (t, 2H, β-C*H*$_2$, *J*=7.2Hz). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 172.65, 170.63, 139.34, 136.46, 136.01, 135.23, 128.72, 128.01, 127.61, 126.99, 55.56, 51.60. FAB-MS: *m/z* 382.30 $[M+Na]^+$. *Anal*. Calcd for $C_{23}H_{21}NO_3$: C, 76.86; H, 5.89; N, 3.90; found; C, 76.80; H, 5.90; N, 3.87.

**Benzyl *N*-(*t*-butoxycarbonyl)-L-serinate (10).**

Di-*tert*-butyl percarbonate (7.20g, 33mmol) was added to an ice-cooled solution of **7** (1.06g, 30.00mmol) in THF (60mL) and aqueous sodium hydroxide (30mL; 1M). The resulting solution was stirred at room temperature for 1h, during which time there was significant $CO_2$ evolution. When TLC analysis (methanol/dichloromethane, 1/9, v/v) showed no remaining benzene-sulfonate salt, the solution was concentrated under reduced pressure, cooled in an ice bath, covered with a layer of ethyl acetate (90mL), and washed with distilled water (3x30mL). The organic layers were combined, dried over $MgSO_4$ and concentrated under reduced pressure to give a residue which solidified on standing. Recrystallization from ethyl acetate/hexane (2/8, v/v) afforded **10** as a white crystalline solid (7.14g, 81%): mp 70-71$^oC$; $[\alpha]_D$ –13.5 (c 1.0 in EtOH). $^1H$ NMR (300MHz, $CDCl_3$) δ: 7.35 (s, 5H, Ar-*H*), 5.42 (br s, 1H, N*H*), 5.21 (s, 2H, OC$H_2$Ph), 4.42 (br s, 1H, α-C*H*), 3.98 (dd, 1H, β-CH*H*, *J*=4.3Hz, *J*=9.7Hz), 3.90 (dd, 1H, β-C*H*H, *J*=8.2Hz, *J*=9.7Hz), 2.11 (br s, 1H, O*H*), 1.44 (s, 9H, OC($CH_3$)$_3$). $^{13}C$ NMR (125MHz, $CDCl_3$) δ: 173.12, 172.66, 139.63, 138.22, 133.54, 128.91, 128.01, 127.64, 55.55, 51.60, 28.66, 26.22, 24.87. FAB-MS: *m/z* 318 $[M+Na]^+$. *Anal*. Calcd for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74; found; C, 61.02; H, 7.19; N, 4.70.

***N*-(benzyloxycarbonyl)-L-serine (11).**

To a solution of L-serine (5g, 0.04mol) in aqueous sodium bicarbonate solution (50mL, 10%, w/v) was added benzylchloroformate dropwise (8.03mL, 0.05mol) at room temperature. The reaction mixture was left stirring for 4h until TLC analysis (methanol/dichloromethane, 1/4, v/v) indicated completion of the reaction. The mixture was extracted with diethyl ether (5x25mL) and the aqueous phase was cooled in ice bath

and acidified with hydrochloric acid (1M) until (pH=3). The resulting milky solution was extracted with ethyl acetate (3x25mL) and the combined organic layers were washed with saturated brine solution (3x25mL) and dried over $MgSO_4$. Filtration followed by evaporation of the solvent *in vacuo* gave **11** as a white solid (9.74g, 85%) which was used for the next step without further purification: m.p. 115-116$^oC$; $[\alpha]_D$=2.45 (c 1, $CHCl_3$). $^1H$ NMR (300MHz, $CD_4O$) δ: 7.33-7.26 (m, 5H, Ar-*H*), 5.08 (s, 2H, OC$H_2$Ph), 4.31 (m, 1H, α-C*H*), 3.89-3.85 (dd, 2H, β-C$H_2$, *J*=8.8Hz, *J*=9.6Hz). $^{13}C$ NMR (125MHz, $CD_4O$) δ: 172.70, 157.39, 136.89, 128.35, 128.06, 127.91, 127.74, 66.67, 62.00, 56.53. FAB-MS: *m/z* 262.14 $[M+Na]^+$. *Anal*. Calcd for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.86; found: C, 55.22; H, 5.49; N, 5.87.

**Benzyl *N*-(benzyloxycarbonyl)-L-serinate (12).**

A solution of *N*-benzyloxycarbonyl-L-serine **11** (4.64g, 19.39mmol) in 80% aqueous ethanol (20mL) was titrated with a solution of $Cs_2CO_3$ (0.9g, 19.39mmol) dissolved in the minimum amount of water, until pH=6.8 using a pHmeter. The reaction mixture was stirred for 3h at pH<7.0. Ethanol was completely evaporated from the solution under reduced pressure. The remaining aqueous solution was freeze dried overnight and kept at vacuum pump for 24h over $P_2O_5$. The resulting cesium salt was dissolved in DMF (20mL) and cooled to 0$^oC$ under nitrogen atmosphere. Benzyl bromide (2.76mL, 23.22mmol) was added dropwise to the cesium salt over period of 10 minutes and the reaction mixture was left stirring at room temperature for 3h. When TLC indicated the completion of the reaction (ethyl acetate/hexane 1/1, v/v), the reaction mixture was filtered and the residue was suspended in water (100mL). The aqueous layer was extracted with dichloromethane (3x25mL) and the organic layers were collected and

concentrated *in vacuo* to give **12** as white crystals. The crude product washed successively with hexane (5x25mL) and used for the next step without further purification (5.19g, 81%), m.p. 83-83.5°C, $[\alpha]_D$= -5.6 (c 4, $CHCl_3$). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.33 (s, 10H, Ar-*H*), 5.86 (d, 1H, N*H*, *J*=8.0Hz), 5.19 (s, 2H, OC$H_2$Ph), 5.10 (s, 2H, OC$H_2$Ph), 4.48 (m, 1H, α-C*H*), 4.00-3.87 (dd, 2H, β-C$H_2$, *J*=7.7Hz, *J*=9.8Hz), 2.63 (br s, 1H, O*H*). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 170.70, 156.52, 136.30, 135.39, 128.86, 128.76, 128.72, 128.45, 128.39, 128.31, 67.68, 67.44, 63.42, 56.48. FAB-MS: *m/z* 352.32 $[M+Na]^+$. *Anal*. Calcd for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.81; N, 4.25; found: C, 65.60; H, 5.79; N, 4.27.

**Methyl *N*-(benzyloxycarbonyl)-L-serinate (14).**

To a suspension of methyl serinate hydrochloride **13** (2g, 12.85mmol) in dry dichloromethane (25mL) at 0°C, a few drops of $Et_3N$ were added followed by careful addition of benzyl chloroformate (1.83mL, 12.85mmol) over a period of 10 minutes. The reaction mixture was left for stirring for 16h at room temperature. When TLC analysis (methanol/dichloromethane, 1/4, v/v) indicated that all the starting material had been consumed, the reaction mixture was diluted with dichloromethane (20mL) and washed successively with 0.5N solution of hydrochloric acid (3x30mL) and water (3x30mL). The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo* to give **14** as a white solid (2.5g, 77%) which was used for the next step without further purification. m.p: 80-82°C; $[\alpha]_D$=-3.1 (c 1.0, EtOH). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.82-7.31 (m, 5H, Ar-*H*), 5.69 (br s, 1H, N*H*), 5.21 (s, 2H, OC$H_2$Ph), 4.49 (t, 1H, α-C*H*, *J*=8.5Hz), 4.01-3.95 (dd, 2H, β-C$H_2$, *J*=7.3Hz, *J*=9.7Hz), 3.77 (s, 3H, $CO_2CH_3$). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 173.12, 171.08, 139.38, 130.45, 129.97, 129.56, 127.78, 126.62, 55.56, 51.60,

45.67, 45.59. FAB-MS: *m/z* 276.18 $[M+Na]^+$. *Anal*. Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53; found: C, 56.85; H, 5.55; N, 5.54.

**Methyl (2*S*)-2-azido-3-hydroxypropanoate (15).**

To a mixture of $NaN_3$ (66.7g, 1.0mol) in $H_2O$ (160mL) and $CH_2Cl_2$ (240mL), $Tf_2O$ (66.7g, 0.23mol) was added with vigorous stirring at 0°C. The mixture was stirred for 4h at 0°C. The organic layer was separated, and the aqueous layer was extracted with $CH_2Cl_2$ (2x70mL). The combined organic layers were washed successively with saturated $NaHCO_3$ (120mL), $H_2O$ (120mL), and dried over $MgSO_4$. To a solution of methyl serinate **13** (6.45g, 41.4mmol) and DMAP (28g, 0.23mol) in MeCN (100mL), was added $Tf_3N$ solution (62.5mL, as prepared above) at 0°C and the reaction mixture was left stirring at 0°C for 6h. When TLC analysis (ethyl acetate/hexane, 1/1, v/v) showed the completion of the reaction, the reaction mixture was diluted with 1,2-dichloroethane (100mL), concentrated at 30°C *in vacuo* to ca. 50mL, and filtered. After removing the solvent under reduced pressure, the residue was purified by a column of silica gel chromatography (eluent: hexane/ethyl acetate, 1/4, v/v) to afford **15** (3.75g, 62%) as a pale yellow oil: $[\alpha]_D$=-92.2 (c 1.0, $CDCl_3$). $^1$H NMR (300MHz, $CDCl_3$) δ: 4.11 (dd, 1H, α-C*H*, *J*=4.4Hz, *J*=8.4Hz), 3.94 (d, 1H, β-C*H*H, *J*=9.7Hz), 3.90 (d, 1H, β-CH*H*, *J*=8.2Hz) 3.85 (s, 3H, $CO_2CH_3$), 2.11 (br s, 1H, O*H*). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 169.01, 63.42, 62.77, 52.98. FAB-MS: *m/z* 168.05 $[M+Na]^+$. *Anal*. Calcd for $C_4H_7N_3O_3$: C, 33.11; H, 4.86; N, 28.96; found: C, 33.13; H, 4.87; N, 28.99.

**Methyl *N*-(diphenylmethylene)-L-serinate (16).**

A mixture of methyl L-serinate hydrochloride **13** (0.77g, 4.94mmol) and benzophenone imine (1.00g, 5.52mmol) in dry dichloromethane (20mL) was stirred at room temperature for 24h with the exclusion of moisture ($CaCl_2$ tube). The reaction mixture was filtered to remove $NH_4Cl$ and washed successively with 1% aqueous $NaHCO_3$ (2x20mL) and water (2x20mL) to remove any remained $NH_4Cl$. The organic layers were collected and evaporated to dryness under reduced pressure. The residue was taken up in diethyl ether (20mL) and filtered. The filtrate was washed with $H_2O$ (3x20mL) and the organic layers were dried over $MgSO_4$. Filtration and solvent removal followed by recrystallization (diethyl ether/hexane) afforded **16** as a white powder (1.2g, 85%), mp 88-89$^oC$; $[\alpha]_D$=3.6 (c 1.0, EtOH). $^1H$ NMR (300MHz, $CDCl_3$) δ: 7.67-7.22 (m, 10H, Ar-*H*), 3.94 (t, 1H, α-C*H*, *J*=6.7Hz*)*, 3.75 (s, 3H, $CO_2CH_3$), 3.72 (dd, 2H, β-$CH_2$, *J*=6.2Hz, *J*=9.7Hz). $^{13}C$ NMR (125MHz, $CDCl_3$) δ: 171.43, 139.37, 136.60, 130.54, 128.67, 128.10, 127.36, 55.25, 51.60. FAB-MS: *m/z* 306.16 $[M+Na]^+$. *Anal*. Calcd for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94; found: C, 72.03; H, 6.03; N, 4.88.

**Allyl *N*-(*t*-butyloxycarbonyl)-L-serinate (18).**

To a solution of *N*-(*t*-butyloxycarbonyl)-L-serine **17** (2.0g, 9.74mmol) and diisopropylethylamine (1.77mL, 9.74mmol) in $CH_3CN$ (20mL) was added allyl bromide (0.84mL, 9.74mmol). The reaction mixture was stirred at room temperature for 20h. When TLC [$CHCl_3$/MeOH/HOAc, 95:5:3, v/v/v] indicated that the esterification is complete, the reaction mixture was diluted with ethyl acetate (20mL) and washed successively with 10% $NaHCO_3$ (3x25mL), saturated aqueous NaCl (3x25mL), and dried over $MgSO_4$. Filtration followed by evaporation of the solvent *in vacuo* afforded **18** as a

yellow oil (2.2g, 92%) which was used for the next step without further purification. $[\alpha]_D$=-5.71 (c 1.4, $CHCl_3$). $^1H$ NMR (300MHz, $CDCl_3$) δ: 5.90 (m, 1H, $OCH_2CH$=$CH_2$), 5.48 (broad s, 1H, N*H*), 5.35 (ddt, 1H, OCH*H*CH=$CH_2$, *J*=17.7Hz, *J*=10.6Hz, *J*=1.4Hz), 5.27 (ddt, 1H, OC*H*HCH=$CH_2$, *J*=17.2Hz, *J*=9.8Hz, *J*=1.4Hz), 4.70 (dd, 1H, $OCH_2CH$=C*H*H, *J*=2.5Hz, *J*=1.4Hz), 4.67 (dd, 1H, $OCH_2CH$=CH*H*, *J*=2.7Hz, *J*=1.4Hz), 4.40 (t, 1H, α-C*H*, *J*=8.2Hz), 4.00 (dd, 2H, β-$CH_2$, *J*=8.2Hz, *J*=9.8Hz), 3.91 (t, 1H, O*H*, *J*=6.2Hz), 1.46 (s, 9H, $OC(CH_3)_3$). $^{13}C$ NMR (125MHz, $CDCl_3$) δ: 166.12, 151.26, 126.98, 114.08, 75.62, 61.51, 58.58, 55.63, 51.28, 23.71. FAB-MS: *m/z* 268.16 $[M+Na]^+$. *Anal*. Calcd for $C_{11}H_{19}NO_5$: C, 53.87, H, 7.81; N, 5.71; found: C, 53.85, H, 7.76; N, 5.71.

**_N_-(_t_-butyloxycarbonyl)-L-serine _p_-nitrobenzyl ester (19).**

To a solution of *N*-(*t*-butyloxycarbonyl)-L-serine **17** (2.0g, 9.7mmol) and diisopropylethylamine (1.7mL, 9.7mmol) in DMF (20mL) was added *p*-nitrobenzyl bromide (2.1g, 9.74mmol) over a period of 30 minutes. The mixture was stirred at room temperature for 18h. When TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated completion of the reaction, the mixture was poured into $H_2O$ (150 mL) and extracted with diethyl ether (5x30mL). The organic layer were collected and washed successively with 1M HCl (2x25mL), 5% aqueous $NaHCO_3$ (2x25mL), and dried over $MgSO_4$. After filtration and evaporation of the solvent *in vacuo*, the residue was recrystallized from (ethyl acetate/pentane, 1/4) to give **19** as a creamy colored needles (2.**7**g**,** 94%). m.p. 102-103$^{o,}$ $[\alpha]_D$= -5.71 (c 1.4, $CHCl_3$). $^1H$ NMR (300MHz, $CDCl_3$) δ: 8.23 (d, 3H, Ar-*H,* *J*=8.8Hz), 7.53 (d, 2H, Ar-*H*, *J*=8.8Hz), 5.45 (d, 1H, N*H*, *J*=7.6Hz), 5.32 (s, 2H, OC*H*$_2$Ph), 4.31-4.53 (t, 1H, α-C*H, J*=4.6Hz, *J*=7.7Hz), 3.93-4.07 (dd, 2H, β-C*H*$_2$, *J*=7.7Hz, *J*=9.7Hz), 2.25 (t, 1H, O*H*, *J*=5.8Hz), 1.45 (s, 9H, $OC(CH_3)_3$). $^{13}C$ NMR

(125MHz, $CDCl_3$) δ: 170.71, 155.82, 147.73, 142.63, 128.29, 123.81, 80.66, 65.63, 63.33, 55.91, 28.35. FAB-MS: *m/z* 333.11 $[M+Na]^+$. *Anal*. Calcd for $C_{15}H_{20}N_2O_7$: C, 52.94; H, 5.92; N, 8.23; found; C, 52.92; H, 5.89; N, 8.19.

**Allyl *N*-(9-Fluorenylmethoxycarbonyl)-L-serinate (21).**

To a solution of *N*-(9-Fluorenylmethoxycarbonyl)-L-serine **20** (6.99g, 21.4mmol) and DIPEA (4.45mL, 24.8mmol) in DMF (30mL) was added allyl bromide (2.04mL, 23.5mmol) in DMF (30mL) was added DIPEA (4.45mL, 24.8mmol). The reaction mixture was stirred under Argon atmosphere for overnight. When TLC analysis (methanol/dichloromethane, 1/5, v/v) indicated that all the starting material had been consumed, the reaction was diluted with $H_2O$ (100mL) and extracted with $CH_2Cl_2$ (5x30mL). The combined organic layers were successively washed with 1M HCl (2x25mL), 5% aqueous $NaHCO_3$ (3x25mL), and dried over $MgSO_4$. After filtration and evaporation of the solvent under reduced pressure, the crude solid residue was recrystallized from (DCM/hexane, 2/8, v/v)) to afford **21** as a white needle crystals (6.00g, 76%), m.p 82.5-84$^o$C, $[\alpha]_D$= 0.3 (c 7.5, EtOAc). $^1$H NMR (300MHz, $CDCl_3$) δ: 7.76 (d, 2H, Ar-*H*, *J*=7.6Hz), 7.60 (d, 2H, Ar-*H,* *J*=7.1Hz), 7.43-7.25 (m, 4H, Ar-*H*), 5.97-5.84 (m, 1H, $OCH_2CH$=$CH_2$), 5.70 (d, 1H, N*H*, *J*=7.4Hz), 5.37-5.25 (dd, 2H, $OCH_2CH$=C$H_2$, *J*=0.8Hz, *J*=17.2Hz), 4.69 (d, 2H, O$CH_2$CH=C$H_2$, *J*=5.2Hz), 4.43 (dd, 3H, α-C*H*, C$H_2$O (Fmoc), *J*=4.5Hz, *J*=6.8Hz), 4.25-4.20 (t, 1H, H-9 (Fmoc)), *J*=6.6Hz), 4.04-3.93 (dd, 2H, β-C$H_2$, *J*=8.2Hz, *J*=9.9Hz), 2.11 (br s, 1H, O*H*). $^{13}$C NMR (125MHz, $CDCl_3$) δ: 170.21, 156.23, 143.72, 141.31, 131.29, 127.74, 127.06, 125.07, 119.99, 119.02, 67.19, 66.35, 63.26, 56.05, 47.08. FAB-MS: *m/z* 390.14 $[M+Na]^+$. *Anal*. Calcd for $C_{21}H_{21}O_5N$: C, 68.65; H, 5.76; N, 3.81, found: C, 68.62; H, 5.74; N, 3.82.

**Benzyl *N*-(9-Fluorenylmethoxycarbonyl)-L-serinate 22).**

A solution of *N*-(9-Fluorenylmethoxycarbonyl)-L-serine **20** (2.0g, 6.11mmol) in 80% aqueous ethanol (20mL) was titrated with a solution of $Cs_2CO_3$ (0.92g, 6.18mmol) dissolved in the minimum amount of water, until pH 6.8 using a pHmeter. The reaction mixture was stirred for 3h at room temperature. Ethanol was evaporated under reduced pressure and the remaining aqueous solution was freeze dried overnight. The residue was further dried at the vacuum pump for 24h over $P_2O_5$. The resulting cesium salt was suspended in DMF (20mL) and cooled to $0^oC$ under nitrogen atmosphere. Benzyl bromide (1.08mL, 9.16mmol) was added dropwise to the cesium salt over a period of 10 minutes and stirred at room temperature for 3h. When TLC analysis (methanol/dichloromethane, 1/4, v/v) indicated completion of the reaction, the mixture was filtered and the filtrate was suspended in water. The suspension was extracted with dichloromethane (3x25mL) and the organic layers were collected, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was recrystalized from dichloromethane/hexane mixture (100mL, 1/4, v/v) to afford **22** as a white crystals (2g, 78%) m.p. 98-100$^oC$, $[\alpha]_D$= 3.2 (c 4, $CHCl_3$). $^1H$ NMR (300MHz, $CDCl_3$) δ: 7.75 (d, 2H, Ar-*H*, *J*=7.7Hz), 7.59 (d, 2H, Ar-*H*, *J*=7.1Hz), 7.42-7.25 (m, 9H, Ar-*H*), 5.77 (d, 1H, N*H*, *J*=5.8Hz), 5.21 (s, 2H, OC$H_2$Ph), 4.47-4.41 (m, 3H, α-C*H*, C$H_2$O (Fmoc)), 4.22-4.18 (t, 1H, H-9 (Fmoc), *J*=7.7Hz), 3.99-3.94 (dd, 2H, β-C$H_2$, *J*=7.9Hz *J*=9.7Hz), 2.4 (broad, s, 1H, O*H*). $^{13}C$ NMR (125MHz, $CDCl_3$) δ: 170.70, 156.57, 144.07, 143.39, 141.57, 141.54, 128.88, 128.74, 127.98, 127.34, 125.33, 120.23, 67.74, 67.50, 63.42, 56.46, 47.36, 34.61. FAB-MS: *m/z* 440.43 $[M+Na]^+$. *Anal*. Calcd for $C_{25}H_{23}O_5N$: C, 71.93; H, 5.55; N, 3.36; found: C, 71.90; H, 5.57; N, 3.38.

**Benzyl-{{[(*S*)-2-allyloxycarbonyl-2-(9-Fluorenylmethoxycarbonylamino)] ethyl}-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (23).**

To a stirred mixture of phosphoramidite **5** (100mg, 0.17mmol) and amino acid **21** (62.4mg, 0.17mmol) in acetonitrile (1.5mL) was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile). The mixture was stirred for 3h at room temperature until TLC analysis (ethyl acetate/hexane, 2/3, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (77μL, 5.0-6.0M) was added at -40°C and the mixture was left stirring at -40°C for 4h. When TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated completion of the reaction, the solvent was evaporated *in vacuo* and the residue was purified by a column of Sephadex LH-20 (eluent: $CH_2Cl_2$/MeOH, 2/1, v/v) to afford **23** as a colorless syrup (70mg, 50%). $^{31}$P NMR (δP: -3.19, -3.29, two diastereomers). $^{1}$H NMR (500MHz, DMSO) δ: 8.10 (dd, 1H, N*H*, *J*=9.7Hz, *J*=8.7Hz), 7.87 (d, 2H, Ar-*H*, *J*=7.3Hz), 7.71 (d, 2H, Ar-*H*, *J*=6.8Hz), 7.40-7.30 (m, 9H, Ar-*H*), 5.90-5.85 (m, 1H, $OCH_2CH$=$CH_2$), 5.68 (dd, 1H, H-1, $J_{1,2}$=1.5Hz, $J_{1,P}$=7.3Hz), 5.32-5.04 (m, 7H, H-2, H-3, $OCH_2$Ph, H-9 (Fmoc), $OCH_2$-CH=C$H_2$), 4.61-4.55 (m, 3H, $OCH_2CH$=$CH_2$, α-C*H*), 4.35-3.94 (m, 8H, H-4, H-5, H-6a,b, β-$CH_2$, $CH_2O$ (Fmoc)), 2.10-1.94 (4s, 12H, 4x$CH_3CO$). $^{13}$C NMR (125MHz, DMSO) δ: 170.63, 170.15, 170.02, 169.56, 132.81, 118.69, 118.50, 95.55, 79.47, 70.60, 68.84, 68.69, 68.39, 67.45, 65.98, 65.26, 63.59, 62.12, 28.74, 21.19, 21.12, 21.05, 19.69, 19.59. FAB-MS: *m/z* 730.99 $[M+Na]^+$. *Anal*. Calcd for $C_{42}H_{46}NO_{17}P$: C, 58.13; H, 5.34; N, 1.61; P, 3.57; found: C, 57.96; H, 5.26; N, 1.61; P, 3.58.

**2-cyanoethyl {{[(*S*)-2-allyloxycarbonyl-2-(9-Fluorenylmethoxycarbonylamino)] ethyl}-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (24).**

To a stirred mixture of phosphoramidite **6 (**100mg, 0.16mmol) and the amino acid **21** (58.78mg, 0.16mmol) in acetonitrile (1mL) was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile). The mixture was stirred for 2h at room temperature until TLC analysis (ethyl acetate/hexane, 2/3, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (77μL, 5.0-6.0M) was added at -40$^{o}$C and the mixture was left stirring at -40$^{o}$C for 4h. When TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated completion of the reaction, the solvent was evaporated *in vacuo* and the residue was purified by a column of Sephadex LH-20 (eluent: $CH_2Cl_2$/MeOH, 2/1, v/v) to afford **24** as a colorless syrup (90mg, 60%). $^{31}$P NMR (δP: -3.13, -3.22, two diastereomers). $^{1}$H NMR (500MHz, DMSO) δ: 8.02 (t, 1H, N*H*, *J*=7.9Hz), 7.87 (d, 2H, Ar-*H*, *J*=7.4Hz), 7.70 (d, 2H, Ar-*H*, *J*=7.1Hz), 7.42-7.29 (m, 4H, Ar-*H*), 5.92-5.81 (m, 1H, $OCH_2CH$=$CH_2$), 5.60 (dd, 1H, H-1, $J_{1,P}$=4.6Hz, $J_{1,2}$=1.3Hz), 5.33-5.18 (m, 4H, H-2, H-3, $OCH_2CH$=$CH_2$), 4.62-4.51 (m, 3H, $OCH_2CH$=$CH_2$, H-9 (Fmoc)), 4.44 (br s, 1H, α-C*H*), 4.35-3.97 (m, 10H, H-4, H-5, H-6a,b, $CH_2O$ (Fmoc), $OCH_2CH_2CN$, β-$CH_2$), 3.22 (t, 2H, $OCH_2CH_2CN$, *J*=0.02Hz), 2.23-2.02 (4s, 12H, 4x$CH_3CO$). $^{13}$C NMR (125MHz, DMSO) δ: 170.65, 170.24, 170.18, 170.05, 170.01, 169.98, 169.37, 169.31, 166.71, 144.44, 144.38, 141.41, 132.74, 128.35, 127.76, 125.89, 118.62, 95.56, 70.61, 68.85, 68.69, 68.38, 67.37, 66.72, 66,21, 65.26, 63.66, 63.59, 62.12, 54.74, 54.62, 47.24, 21.42, 21.18, 21.12, 21.03, 19.71, 19.61. FAB-MS: *m/z* 853.69 $[M+Na]^+$. *Anal*. Calcd for $C_{38}H_{43}N_2O_{17}P$: C, 54.94; H, 5.22, N, 3.37, P 3.73, found: C, 54.95; H, 5.24; N, 3.33, P 3.71.

**2-cyanoethyl-{{[(*S*)-2-methoxycarbonyl-2-(benzyloxycarbonylamino)] ethyl}-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (25).**

To a stirred mixture of phosphoramidite **6** (100mg, 0.16mmol) and the amino acid **14** (37.6mg, 0.16mmol) in acetonitrile (2.5mL) was added was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile). The mixture was stirred for 2h at room temperature until TLC analysis (ethyl acetate/hexane, 2/3, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (90μL, 5.0-6.0M) was added at -40$^{o}$C and the mixture was left stirring at -40$^{o}$C for 2h. When TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated completion of the reaction, the solvent was evaporated *in vacuo* and the residue was purified by a column of Sephadex LH-20 (eluent: $CH_2Cl_2$/MeOH, 2/1, v/v) to afford **25** as a colorless syrup (70mg, 53%). $^{31}$P NMR (δP: -3.14, -3.02, two diastereomers). $^{1}$H NMR (500MHz, DMSO) δ: 8.02 (t, 1H, N*H*, *J*=7.9Hz), 7.33-7.25 (m, 5H, Ar-*H*), 5.84 (dd, 1H, H-1, $J_{1,P}$=5.1Hz, $J_{1,2}$=1.3Hz), 5.32-5.16 (m, 2H, H-2, H-3), 5.11, 5.04 (ABq, 2H, OC$H_2$Ph, *J*=12.1Hz), 4.41 (m, 1H, α-C*H*), 4.32-4.16 (m, 6H, H-4, H-5, H-6a,b, β-C$H_2$), 4.11-3.99 (t, 2H, OC$H_2$C$H_2$CN, *J*=7.2Hz), 3.37 (s, 3H, $CO_2CH_3$), 2.96-2.88 (t, 2H, $OCH_2CH_2$CN, *J*=5.2Hz), 2.11-1.90 (4s, 12H, 4x$CH_3$CO). $^{13}$C NMR (125MHz, DMSO) δ: 172.55, 172.13, 171.23, 169.33, 138.11, 137.49, 136.34, 135.22, 128.11, 128.07, 127.65, 126.11, 96.15, 94.23, 77.11, 71.23, 68.45, 68.27, 28.17, 28.03, 26.11, 22.56, 19.18. FAB-MS: *m/z* 740 $[M+Na]^+$. *Anal.* Calcd for $C_{29}H_{37}N_2O_{17}P$: C, 48.61; H, 5.20; N, 3.91; P, 4.32; found: C, 48.59; H, 5.22; N, 3.89; P, 4.31.

**2-cyanoethyl-{{[(*S*)-2-allyloxycarbonyl-2-(*t*-butoxycarbonylamino)]ethyl}-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (26).**

To a stirred mixture of phosphoramidite **6** (100mg, 0.16mmol) and amino acid **18** (40.52mg, 0.16mmol) in acetonitrile (2mL) was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile). The mixture was stirred for 2h at room temperature until TLC analysis (ethyl acetate/hexane, 2/3, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (80μL, 5.0-6.0M) was added at -40°C and the mixture was left stirring at -40°C for 5h. When TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated completion of the reaction, the solvent was evaporated *in vacuo* and the residue was purified by a column of Sephadex LH-20 (eluent: $CH_2Cl_2$/MeOH, 2/1, v/v) to afford **26** as a colorless syrup (70mg, 53%). $^{31}$P NMR (δP: -3.19, -3.29, two diastereomers). $^{1}$H NMR (500MHz, DMSO) δ: 8.06 (t, 1H, N*H*, *J*=7.9Hz), 5.92-5.81 (m, 1H, $OCH_2CH$=$CH_2$), 5.68 (dd, 1H, H-1, $J_{1,2}$=1.3Hz, $J_{1,P}$=4.6Hz), 5.34-5.18 (m, 4H, H-2, H-3, $OCH_2CH$=$CH_2$), 4.60 (d, 2H, $OCH_2CH$=$CH_2$, *J*=4.6Hz), 4.43 (m, 1H, α-C*H*), 4.33-4.13 (m, 6H, H-4, H-5, H-6a,b, β-$CH_2$), 4.11-3.99 (t, 2H, $OCH_2CH_2CN$, *J*=7.1Hz), 2.91 (t, 2H, $OCH_2CH_2CN$, *J*=5.3Hz), 2.10-1.93 (4s, 12H, 4x$CH_3CO$), 1.37 (s, 9H, $OC(CH_3)_3$). $^{13}$C NMR (125MHz, DMSO) δ: 173.17, 171.34, 170.32, 170.11, 127.45, 126.34, 125.34, 101.23, 99.34, 95.55, 79.47, 70.60, 68.84, 68.69, 68.39, 67.45, 65.98, 65.26, 63.59, 62.12, 28.74, 21.19, 21.12, 21.05, 19.69, 19.59. FAB-MS: *m/z* 730.99 $[M+Na]^+$. *Anal*. Calcd for $C_{28}H_{41}N_2O_{17}P$: C, 47.46; H, 5.83, N, 3.95, P 4.37; found: C, 47.42; H, 5.84, N, 3.97; P 4.35.

**2-cyanoethyl{{[(*S*)-2-benzyloxcarbonyl-2-[*N*-benzyloxycarbonylamino]] ethyl}-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (27).**

To a stirred mixture of phosphoramidite **6** (100mg, 0.16mmol) and amino acid **12** (52.6mg, 0.16mmol) in acetonitrile (2.5mL) was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile). The mixture was stirred for 4h at room temperature until TLC analysis (ethyl acetate/hexane, 2/3, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (77μL, 5.0-6.0M) was added at -40°C and the mixture was left stirring at -40°C for 5h. When TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated completion of the reaction, the solvent was evaporated *in vacuo* and the residue was purified by a column of Sephadex LH-20 (eluent: $CH_2Cl_2$/MeOH, 2/1, v/v) to afford **27** as a colorless syrup (75mg, 52%). $^{31}$P NMR (δP: -3.06, -3.33, two diastereomers). $^{1}$H NMR (500MHz, DMSO) δ: 7.52-7.21 (m, 10H, Ar-*H*), 5.99 (br s, 1H, H-1), 5.42, (dd, 1H, H-2, $J_{2,3}$=3.5Hz), 5.40-5.11 (m, 5H, H-3, 2xOC$H_2$Ph), 4.22 (t, 2H, α-C*H, J*=4.2Hz), 3.79-3.45 (m, 8H, H-4, H-5, H-6a,b, β-C$H_2$, OC$H_2$CH$_2$CN), 3.01 (t, 2H, OCH$_2$C$H_2$CN, *J*=5.8Hz), 2.01-1.96 (4s, 12H, 4xC$H_3$CO). $^{13}$C NMR (125MHz, DMSO) δ: 170.70, 169.83, 169.73, 138.16, 136.54, 128.90, 128.76, 128.68, 128.46, 128.29, 96.08, 71.00, 68.74, 68.27, 67.46, 65.40, 62.79, 62.12, 29.89, 23.15, 20.81, 20.87, 19.73. FAB-MS: *m/z* 815.72 $[M+Na]^+$. *Anal*. Calcd for $C_{35}H_{41}N_2O_{17}P$: C, 53.03; H, 5.21; N, 3.53; P, 3.91 found: C, 53.04; H, 5.23; N, 3.51; P, 3.90.

**2-cyanoethyl-{{[(*S*)-2-benzyloxycarbonyl-2-(*t*-butoxycarbonylamino)] ethyl}-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (28).**

To a stirred mixture of phosphoramidite **6** (100mg, 0.16mmol) and amino acid **10** (47.2mg, 0.16mmol) in acetonitrile (2.5mL) was added 1*H*-tetrazole (0.72mL of 3wt. %

solution in acetonitrile). The mixture was stirred for 2h at room temperature until TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (77μL, 5.0-6.0M) was added at -40°C and the mixture was left stirring at -40°C for 3h. When TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated completion of the reaction, the solvent was evaporated *in vacuo* and the residue was purified by a column of Sephadex LH-20 (eluent: $CH_2Cl_2$/MeOH, 1/1, v/v) to afford **28** as a colorless syrup (66mg, 48%). $^{31}$P NMR (δP: -3.27, -3.21, two diastereomers. $^{1}$H NMR (500MHz, DMSO) δ: 7.83-7.49 (m, 5H, Ar-*H*), 5.66 (dd, 1H, H-1, $J_{1,P}$=4.4Hz, $J_{1,2}$=1.8Hz), 5.29 (s, 2H, OC$H_2$Ph), 5.22-5.15 (m, 2H, H-2, H-3), 4.35 (t, 1H, α-C*H,* *J*=5.5Hz), 4.33 (dd, 2H, β-C$H_2$, *J*=4.3Hz, *J*=6.8Hz), 4.30-4.15 (m, 4H, H-4, H-5, H-6a,b), 4.02 (t, 2H, OC$H_2$CH$_2$CN, *J*=7.1Hz), 2.90 (t, 2H, OC$H_2$CH$_2$CN, *J*=5.7Hz), 2.29-2.11 (4s, 12H, 4xC$H_3$CO), 1.42 (s, 9H, OC(CH$_3$)$_3$) $^{13}$C NMR (125MHz, DMSO) δ: 172.16, 171.14, 170.45, 170.42, 169.18, 169.83, 169.71, 128.15, 128.14, 128.02, 101.14, 96.04, 89.41, 68.42, 67.42, 66.34, 65.12, 62.54, 61.79, 29.13, 28.11, 20.15, 19.18. FAM MS: *m/z* 781.25 [M+Na]$^+$. *Anal*. Calcd for $C_{32}H_{34}N_2O_{17}P$: C, 50.66; H, 5.71; N, 3.69; P, 4.08; found: C, 50.63; H, 5.73; N, 3.65; P. 4.09.

**2-cyanoethyl-{{[(*S*)-2-*p*-nitrobenzyloxycarbonyl-2-(*t*-butoxycarbonylamino)] ethyl}-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (29).**

To a stirred mixture of phosphoramidite **6** (100mg, 0.16mmol) and amino acid **19** (54.42mg, 0.16mmol) in acetonitrile (2mL) was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile). The mixture was stirred for 1h at room temperature until TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (85μL, 5.0-6.0M) was added at -40°C and the mixture was left

stirring at -40°C for 5h. When TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated completion of the reaction, the solvent was evaporated *in vacuo* and the residue was purified by a column of Sephadex LH-20 (eluent: $CH_2Cl_2$/MeOH, 1/1, v/v) to afford **29** as a colorless syrup (78mg, 53%). $^{31}P$ NMR (δP: -3.16, -3.26, two diastereomers). $^{1}H$ NMR (500MHz, DMSO) δ: 8.17 (t, 1H, N*H*, *J* =8.2Hz), 8.21 (d, 2H, Ar-*H*, *J*=8.2Hz), 7.81 (d, 2H, Ar-*H*, *J*=7.7Hz), 5.66 (d, 1H, H-1, $J_{1.P}$=4.4Hz), 5.29 (s, 2H, OC$H_2$Ph), 5.22-5.17 (m, 3H, H-2, H-3, α-C*H*), 4.33 (t, 2H, β-$CH_2$, *J*=6.5Hz), 4.29-4.11 (m, 4H, H-4, H-5, H-6a,b), 4.08 (t, 2H, OC$H_2$CH$_2$CN, *J*=7.1Hz), 2.94 (t, 2H, OCH$_2$C$H_2$CN, *J*=6.1Hz), 2.10-1.92 (4s, 12H, 4xC$H_3$CO), 1.35 (s, 9H, OC(C$H_3$)$_3$). $^{13}C$ NMR (125MHz, DMSO) δ: 172.14, 171.43, 170.41, 170.25, 170.21, 170.16, 170.05, 170.02, 169.99, 169.95, 169.77, 156.02, 148.50, 138.65, 134.94, 130.66, 123.64, 123.15, 123.11, 118.68, 118.61, 95.64, 95.56, 95.45, 79.52, 72.36, 70.60, 70.27, 68.93, 68.84, 68.78, 68.69, 68.39, 67.32, 65.90, 65.77, 65.23, 65.17, 64.15, 63.80, 63.72, 63.64, 63.57, 62.11, 62.02, 60.41, 54.45, 54.35, 28.67, 21.40, 21.16, 21.09, 21.06, 21.02, 20.98, 19.68, 19.58, 14.73. FAB-MS: *m/z* 826.70 $[M+Na]^+$. *Anal*. Calcd for $C_{32}H_{42}N_3O_{19}P$: C, 47.82; H, 5.27; N, 5.23; P, 3.85; found: C, 47.81; H, 5.25; N, 5.25; P 3.81.

**2-cyanoethyl-{{[(*S*)-2-benzyloxycarbonyl-2-(9-Fluorenylmethoxycarbonylamino] ethyl}-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (30).**

To a stirred mixture of phosphoramidite **6** (100mg, 0.16mmol) and amino acid **22** (66.7mg, 0.16mmol) in acetonitrile (2.5mL) was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile). The mixture was stirred for 1h at room temperature until TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (85μL, 5.0-6.0M) was added at -40°C and the mixture was left

stirring at -40°C for 4h. When TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated completion of the reaction, the solvent was evaporated *in vacuo* and the residue was purified by a column of Sephadex LH-20 (eluent: $CH_2Cl_2$/MeOH, 2/1, v/v) to afford **30** as a colorless syrup (80mg, 50%). $^{31}P$ NMR (δP: -3.10, -3.26, two diastereomers). $^{1}H$ NMR (500MHz, DMSO) δ: 7.66-7.51 (m, 4H, Ar-*H*), 7.26-7.16 (m, 9H, Ar-*H*), 5.48 (dr d, 1H, H-1), 5.22 (s, 2H, OC*H*$_2$Ph), 5.19 (m, 2H, H-2, H-3), 4.61-4.22 (m, 2H, α-C*H*, H-9 (Fmoc)), 4.21 (t, C*H*$_2$O (Fmoc), *J*=7.5Hz), 4.11 (t, 2H, OC*H*$_2$CH$_2$CN, *J*=6.7Hz), 2.96 (t, 2H, OCH$_2$C*H*$_2$CN, *J*=6.7Hz), 2.23-1.97 (4s, 12H, 4xC*H*$_3$CO). $^{13}C$ NMR (125MHz, DMSO) δ: 170.67, 170.12, 169.92, 169.73, 168.78, 144.02 141.50, 135.17, 128.89, 128.70, 127.97, 127.33, 125.44, 124.12, 122.13, 120.20, 96.01, 71.04, 68.90, 68.77, 68.47, 68.31, 68.25, 67.72, 65.41, 65.32, 62.93, 62.16, 60.60, 54.74, 47.74, 47.26, 29.89, 20.88, 20.80, 19.80, 19.70. FAB-MS: *m/z* 903.76 $[M+Na]^+$. *Anal.* Calcd for $C_{42}H_{45}N_2O_{17}P$: C, 57.27; H, 5.15, N, 3.18, P 3.52. Found: C, 57.34; H, 5.17; N, 3.19; P 3.53.

**2-cyanoethyl-{{[(*S*)-2-benzyloxycarbonyl-2-[*N*-(diphenylmethylene) amino]] ethyl}-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (31).**

To a stirred mixture of phosphoramidite **6** (100mg, 0.16mmol) and amino acid **9** (57mg, 0.16mmol) in acetonitrile (2.5mL) was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile). The mixture was stirred for 2h at room temperature until TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (85μL, 5.0-6.0M) was added at -40°C and the mixture was left stirring at -40°C for 3h. When TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated completion of the reaction, the solvent was evaporated *in vacuo* and the residue was purified by a

column of Sephadex LH-20 (eluent: $CH_2Cl_2$/MeOH, 1/1, v/v) to afford **31** as a colorless syrup (95mg, 64%). $^{31}$P NMR (δP: -3.10, -3.26, two diastereomers). $^{1}$H NMR (500MHz, DMSO) δ: 7.53-7.31 (m, 13*H*, Ar-*H*), 7.12 (m, 2H, Ar-*H*), 5.72 (s, 2H, OC$H_2$Ph), 5.63 (d, 1H, H-1, $J_{1,2}$=1.8Hz), 5.21-5.13 (m, 2H, H-2, H-3), 4.45 (t, 2H, β-C$H_2$, *J*=6.5Hz), 4.35 (t, 1H, α-C*H*, *J*=4.2Hz), 4.18-4.03 (m, 6H, H-4, H-5, H-6a,b, OC$H_2$C$H_2$CN), 2.85 (t, 2H, OCH$_2$C$H_2$CN, *J*=5.8Hz), 2.10-1.91 (4s, 12H, 4xC$H_3$CO). $^{13}$C NMR (125MHz, DMSO) δ: 173.04, 172.98, 170.61, 170.53, 170.01, 139.27, 139.21, 136.29, 135.84, 131.61, 130.27, 129.68, 129.37, 129.26, 129.11, 128.98, 128.83, 28.55, 128.39, 128.14, 118.66, 118.53, 95.47, 70.61, 68.89, 68.74, 68.33, 67.07, 65.35, 65.23, 63.63, 63.55, 63.45, 62.05, 61.56, 55.57, 44.74, 21.15, 21.06, 21.03, 19.68, 19.61, 19.51. FAB-MS: *m/z* 845.72 $[M+Na]^+$. *Anal*. Calcd for $C_{40}H_{43}N_2O_{15}P$: C, 58.39; H, 5.27; N, 3.40; P, 3.76; found: C, 58.41; H, 5.25; N, 3.31; P 3.77.

**2-cyanoethyl-{{[(*S*)-2-methoxycarbonyl-2-[*N*-(diphenylmethylene) amino]]ethyl }-2,3 ,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (32**).

To a stirred mixture of phosphoramidite **6** (100mg, 0.16mmol) and serine derivative **16** (45.33mg, 0.16mmol) in acetonitrile (2mL) was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile). The mixture was stirred for 1h at room temperature until TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (85μL, 5.0-6.0M) was added at -40$^{o}$C and the mixture was left stirring at -40$^{o}$C for 3h. When TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated that the reaction is complete, the solvent was removed *in vacuo* and the residue was purified by a column of Sephadex LH-20 (eluent: $CH_2Cl_2$/ MeOH, 2:1, v/v) to afford **32** as a colorless syrup (95 mg, 69%). $^{31}$P NMR (δP: -3.43, -2.34, two diastereomers). $^{1}$H NMR

(500MHz, DMSO) δ: 7.55-7.22 (m, 10H, Ar-*H*), 5.61 (dd, 1H, H-1, $J_{1,P}$=5.8Hz, $J_{1,2}$=1.9Hz), 5.19-513 (m, 2H, H-2, H-3), 4.42 (t, 2H, β-C$H_2$, *J*=6.6Hz), 4.37 (t, 1H, α-C*H*, *J*=4.2Hz), 4.20-4.11 (m, 6H, H-4, H-5, H-6a,b, OC$H_2$C$H_2$CN), 3.35 (s, 3H, $CO_2CH_3$), 2.92 (t, 2H, $OCH_2CH_2CN$, *J*=5.9Hz), 2.13-2.06 (4s, 12H, 4x$CH_3CO$). $^{13}$C NMR (125MHz, DMSO) δ: 173.04, 172.98, 170.65, 170.55, 170.12, 170.01, 168.92, 139.27, 136.11, 129.31, 129.21, 129.15, 128.95, 128.81, 95.42, 70.66, 68.81, 66.13, 65.43, 65.12, 55.14, 55.13, 44.23, 42.12, 21.45, 21.33, 20.13, 16.45. FAB-MS: *m/z* 769.55 $[M+Na]^+$. *Anal*. Calcd for $C_{34}H_{39}N_2O_{15}P$: C, 54.69; H, 5.26; N, 3.75; P, 4.15; found: C, 54.67; H, 5.27; N, 3.75; P, 4.13.

**2-cyanoethyl-{{[(*S*)-2-benzyloxycarbonyl-2-azido]ethyl}-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (33).**

To a stirred mixture of phosphoramidite **6** (100mg, 0.16mmol) and **8** (35.3mg, 0.16mmol) in acetonitrile (2.5mL) was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile). The mixture was stirred for 2h at room temperature until TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (77μL, 5.0-6.0M) was added at -40$^o$C and the mixture was left stirring at -40$^o$C for 4h. When TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated that the reaction is complete, the solvent was removed *in vacuo* and the residue was purified by a column of Sephadex LH-20 (eluent: $CH_2Cl_2$/MeOH, 2:1, v/v) to afford **33** as a colorless syrup (90mg, 70%). $^{31}$P NMR (δP: -3.19, -2.67, two diastereomers). $^1$H NMR (500MHz, DMSO) δ: 7.38 (s, 5H, Ar-*H*), 5.72, 6.98 (ABq, 2H, OC$H_2$Ph, $J_{AB}$=11.8Hz), 5.17 (dd, 1H, H-1, $J_{1,P}$=4.9Hz, $J_{1.2}$=1.7Hz), 4.82 (dd, 1H, H-3, $J_{2,3}$=3.5Hz, $J_{3,4}$=9.8Hz), 4.42 (dd, 1H, H-2), 4.41 (t, 1H, α-C*H*, *J*=4.9Hz), 4.21-4.07 (m, 8H, H-4, H-5, H6a,b, β-C$H_2$,

OC*H*$_2$CH$_2$CN), 2.91 (t, 2H, OCH$_2$C*H*$_2$CN, *J*=7.6Hz), 2.11-1.93 (4s, 12H, 4xC*H*$_3$CO). $^{13}$C NMR (125MHz, DMSO) δ: 172.11, 171.65, 170.14, 170.02, 136.33, 128.12, 127.56, 126.34, 101.22, 98.13, 83.56, 82.11, 77.14, 76.34, 72.11, 68.11, 66.45, 65.12, 46.43, 22.99, 22.78, 22.71, 22.55, 19.11. FAB-MS: *m/z* 707.51 [M+Na]$^+$. *Anal.* Calcd for $C_{27}H_{33}N_4O_{15}P$: C, 47.37; H, 4.86; N, 8.18; P, 4.52; found: C, 47.32; H, 5.01; N, 8.23; P, 4.63.

**2-cyanoethyl-{{[(*S*)-2-methoxycarbonyl-2-azido]ethyl}-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl} phosphate (34).**

To a stirred mixture of phosphoramidite **6** (100mg, 0.16mmol) and **15** (25.78mg, 0.16mmol) in acetonitrile (2.5mL) was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile). The mixture was stirred for 2h at room temperature until TLC analysis (ethyl acetate/hexane, 1/1, v/v) indicated completion of the reaction. A solution of *t*-BuOOH in decane (90μL, 5.0-6.0M) was added at -40$^o$C and the mixture was left stirring at -40$^o$C for 3h. When TLC analysis (ethyl acetate/hexane, 2/1, v/v) indicated that the reaction is complete, the solvent was removed *in vacuo* and the residue was purified by a column of Sephadex LH-20 (eluent: $CH_2Cl_2$/MeOH, 2:1, v/v) to afford **34** as a colorless syrup (80mg, 72%). $^{31}$P NMR (δP: -3.36, -3.32, two diastereomers). $^1$H NMR (500MHz, DMSO) δ: 5.20 (dd, 1H, H-1, $J_{1,P}$=4.9Hz, $J_{1,2}$=1.7Hz), 4.82-4.42 (m, 2H, H-2, H-3), 4.41 (t, 1H, α-C*H*, *J*=4.8Hz), 4.22-4.11 (m, 8H, H-4, H-5, H-6a,b, β-C*H*$_2$, OC*H*$_2$CH$_2$CN), 3.37 (s, 3H, CO$_2$C*H*$_3$), 2.88 (t, 2H, OCH$_2$C*H*$_2$CN, *J*=7.6Hz), 2.10-1.93 (4s, 12H, 4xC*H*$_3$CO). $^{13}$C NMR (125MHz, DMSO) δ: 170.62, 170.25, 170.17, 170.03, 167.95, 101.34, 95.66, 95.59, 92.11, 86.11, 70.66, 70.32, 68.83, 67.91, 62.32, 62.11, 61.48, 61.38, 55.56, 54.14, 22.78, 22.71, 22.16, 19.98. FAB-MS: *m/z* 631.42 [M+Na]$^+$. *Anal*. Calcd for

$C_{21}H_{29}N_4O_{15}P$: C, 41.45; H, 4.80; N, 9.21; P, 5.09; found: C, 41.47; H, 4.78; N, 9.23; P, 5.09.

**{[(*S*)-2-methoxycarbonyl-2-azido] ethyl}(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranos-yl) phosphate (35).**

To a stirred mixture of phosphoramidite **6** (100mg, 0.16mmol) and amino acid **15** (23.2mg, 0.16mmol) in acetonitrile (2.5mL) was added 1*H*-tetrazole (0.72mL of 3wt. % solution in acetonitrile) at room temperature. The mixture was stirred at room temperature for 2h until TLC analysis (ethyl acetate/hexane, 2/3, v/v) showed the completion of this reaction. A mixture of *t*-BuOOH/$Et_3N$ (0.1mL/1mL) was added at -40$^o$C and stirring was continued at -40$^o$C for 3h until TLC analysis (ethyl acetate/hexane, 4/1, v/v) indicated that all of PIII intermediate was converted into diester **35**. The reaction mixture was concentrated *in vacuo* to a small volume and the residue was purified by a column of Sephadex LH-20 eluted with ($CH_2Cl_2$/MeOH, 2:1, v/v), to afford **35** as a colorless syrup (80mg, 62%). $^{31}$P NMR (δP=-1.26). $^1$H NMR (500MHz, DMSO) δ: 5.18 (dd, 1H, H-1, $J_{1,P}$=6.3Hz, $J_{1,2}$=1.9Hz), 4.63 (dd, 1H, H-2, $J_{2,3}$=3.6Hz), 4.46 (dd, 1H, H-3, $J_{3,4}$=9.6Hz) 4.40 (t, 1H, α-C*H*, *J*=5.2Hz), 4.26-4.21 (m, 4H, H-4, H-6a, β-C$H_2$), 4.18 (m, 2H, H-5, H-6b), 3.35 (s, 3H, $CO_2CH_3$), 2.12-1.95 (4s, 12H, 4x$CH_3$CO). $^{13}$C NMR (125MHz, DMSO) δ: 171.61, 170.55, 170.27, 170.13, 170.01, 167.98, 101.34, 95.67, 93.43, 93.22, 75.34, 74.17, 73.11, 72.45, 70.54, 70.13, 68.01(2x), 66.69, 64.45, 62.13, 55.69, 54.33, 51.11, 21.24. FAB-MS: *m/z* 578.32 $[M+Na]^+$. *Anal*. Calcd for $C_{18}H_{26}N_3O_{15}P$: C, 38.93; H, 4.72; N, 7.57; P, 5.58; found: C, 38.91; H, 4.68; N, 7.51; P, 5.56.

**Benzyl {[(*S*)-2-carboxy-2-(9-Fluorenylmethoxycarbonylamino)]ethyl} (2,3,4,6,tetra-*O*-acetyl-α-D-mannopyranosyl) phosphate (36).**

To a stirred solution of **23** (40mg, 0.04mmol) in dry DCM (0.5mL) was added a mixture of $Pd(PPh_3)_4$ (2mg, 0.001mmol), $Bu_3SnH$ (24.74μL, 0.09mmol), and AcOH (6μL, 0.11mmol). The mixture was stirred at room temperature for 48h. When TLC (DCM/MeOH, 1/10, v/v) analysis indicated completion of the reaction, the solvent was removed by co-evaporation with toluene under reduced pressure and the crude product was purified by LH-20 column chromatography (MeOH/DCM, 3/1, v/v) to give **36** as a colorless syrup (15mg, 53%). $^{31}$P NMR (δP: -3.39, -2.43, two diastereomers). $^{1}$H NMR (500MHz, DMSO) δ: 7.74-7.23 (m, 13H, Ar-*H*), 5.62 (br d, 1H, $J_{1,2}$=1.5Hz), 5.50, 5.46 (ABq, 2H, OC$H_2$Ph, *J*=11.3Hz), 5.38-5.02 (m, 6H, α-C*H*, H-2, H-3, H-9 (Fmoc), C$H_2$O (Fmoc)), 4.42-3.94 (m, 6H, H-4, H-5, H-6a,b, β-C$H_2$), 2.21-2.18 (4s, 12H, 4xC$H_3$CO). δ: $^{13}$C NMR (125MHz, DMSO) δ: 170.66, 170.58, 170.22, 170.15, 170.01, 169.94, 144.61, 144.52, 141.37, 136.49, 136.39, 129.15, 128.87, 128.67, 128.54, 127.73, 125.85, 120.67, 95.16, 70.14, 69.78, 69.70, 69.63, 68.96, 68.82, 68.56, 68.47, 66.41, 65.29, 65.09, 62.03, 56.66, 47.34, 41.06, 40.78, 40.50, 40.22, 39.94, 39.67, 39.39, 21.18, 21.10, 21.02. FAB-MS: *m/z* 873.63 $[M+2Na]^+$. *Anal.* Calcd for $C_{39}H_{42}NO_{17}P$: C, 56.59; H, 5.11; N, 1.69; P, 3.74; found: C, 56.52; H, 5.03; N, 1.67, P; 3.73.

**{[(*S*)-2-carboxy-2-(9-Fluorenylmethoxycarbonylamino)]ethyl} (2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl) sodium phosphate (37).**

To a stirred solution of **36** (110mg, 0.13mmol) in acetonitrile (1mL) was added NaI (38.9mg, 0.26mmol). After stirring at room temperature for 4h, the solid product started

to precipitate while stirring. TLC analysis (MeOH/DCM, 1/9, v/v) showed the absence of the starting material in the mother liquid. The solid was filtered using Celite and washed several times with acetonitrile to give **37** as a white solid (50mg, 47%). $^{31}$P NMR (δP: -1.93). $^{1}$H NMR (500MHz, DMSO) δ: 7.77 (d, 2H, Ar-*H*, *J*=5.5Hz), 7.59 (s, 2H, Ar-*H*), 7.36-7.30 (m, 4H, Ar-*H*) 5.34 (br d, 1H, H-1), 5.18-5.08 (m, 2H, H-2, H-3), 4.29-4.22 (m, 3H, C*H*$_2$O (Fmoc), α-C*H*), 4.17-4.03 (m, 5H, H-4, H-5, H-6a,b, H-9 (Fmoc)), 3.92 (t, 2H, β-C*H*$_2$, *J*=9.3Hz), 2.02-1.75 (4s, 12H, 4xC*H*$_3$CO). $^{13}$C NMR (125MHz, DMSO) δ: 175.85, 173.75, 173.20, 172.77, 172.67, 157.82, 144.07, 143.92, 141.07, 128.27, 127.73, 125.47, 120.36, 93.57, 69.97, 69.54, 69.23, 66.95, 66.64, 65.44, 61.93, 46.94, 20.17, 20.04. FAB-MS: *m/z* 805.15 $[M+2Na]^+$. *Anal*. Calcd for $C_{32}H_{35}N\ NaO_{17}P$: C, 50.60; H, 4.64; N, 1.84; P, 4.08; found: C, 50.55; H, 4.63; N, 1.82; P, 4.06.

**$_L$-glycyl-$_L$-seryl-$_L$–phenylalanine (38).**

After swelling the Fmoc-protected Rink Amide resin (100mg) by shaking it with DMF (5mL) for 30 minutes, 2mL of 20% piperidine/DMF was added and shaking was continued for 30 min. When the full cleavage of the Fmoc group was confirmed by Kaiser's test, the resin was washed with DMF (4x5mL) and coupled with Fmoc-Gly-OH (29.13mg, 0.098mmol) in the presence of PyBOP (50.9mg, 0.098mmol), HOBt (13.23mg, 0.098mmol), and DIPEA (34.2μL, 0.19mmol) in DMF (3mL). The reaction mixture was left shaking for 2h and the completion of the reaction was monitored by Kaisier's test. The resin was washed with DMF (4x5mL) and shaken with 20% piperidine in DMF (3mL). The cleavage of the Fmoc protecting group of the first amino acid was monitored by Kaiser's test. After washing with DMF (4x5mL), the resin was coupled with Fmoc-Ser(O-$^t$Bu)-OH (37.5mg, 0.098mmol) using the same quantities of PyBOP,

HOBt, and DIPEA in DMF (2mL) as was mentioned for the coupling of the first amino acid. After repeating the same cycle of washing and cleavage, the third amino acid was attached to the polymer when Fmoc-Phe-OH (37.9mg, 0.098mmol) reacted with the resin-bound dipeptide using the same activating mixture of (PyBOP, HOBt, DIPEA). After the coupling step, the Fmoc-protecting group of the phenylalanine amino acid residue was removed by treatment with 20% piperidine in DMF. The resin was washed with DMF (4x5mL) and treated with 50% $AC_2O$/DMF (5mL) and left shaken overnight. The reaction was monitored by Kaiser's test and the resin was subjected to the following cycle of washing: I) DMF (4x10mL), MeOH (4x10mL), and DCM (4x10mL) followed by shaking with 50% TFA/DCM (10mL) containing 2mL of thioanisole for 60 minutes. After filtration and rinsing in 50% TFA solution in DCM, the filtrate was concentrated, dissolved in 20mL of TFA solution containing 2mL of thioanisole, and magnetically stirred for 2h at room temperature. The solvent was removed *in vacuo* to give an oily residue which was triturated in ether (30mL) and left overnight at -4$^{o}$C to precipitate **38**. The precipitated tripeptide was filtered using Celite and washed with diethyl ether (4x20mL) to give **38**. Purification by silica flash column chromatography (eluent: MeOH/DCM, 1/4, v/v) afforded **38** as a white solid (12mg, 69%). $^{1}$H NMR (500MHz, DMSO) δ: 7.43-7.36 (m, 5H, Ar-*H*), 4.40 (t, 1H, α-C*H*(Phe), *J*=4.9Hz), 3.91-3.79 (m, 4H, β-C$H_2$(Ser), β-C$H_2$(Phe)), 3.23-3.17 (dd, 1H, α-C*H*(Ser), *J*=5.5Hz, *J*=5.0Hz), 3.0-3.92 (m, 1H, O*H*), 2.70 (s, 2H, $CH_2$-Gly), 1.97 (s, 3H, COC$H_3$). $^{13}$C NMR (125MHz, DMSO) δ: 173.50, 173.48, 171.83, 137.40, 129.78, 129.26, 127.64, 61.55, 56.02, 55.38, 37.60, 22.56. FAB-MS: *m/z* 373.41 $[M+Na]^+$. *Anal*. Calcd for $C_{16}H_{22}N_4O_5$: C, 54.85; H, 6.37; N, 15.99; found: C, 54.86; H, 6.33; N, 16.02.

**L-*N*-phenylalanylacetate-L-seryl-2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl phosphate)-L-glycinamide (39).**

To a stirred mixture of peptide **38** (30mg, 0.08mmol) and phosphoramidite **6** (50mg, 0.08mmol) in acetonitrile (2.5mL) at room temperature was added 1*H*-tetrazole (0.36mL of 3wt. % solution in acetonitrile). The reaction was left stirring for 3h until TLC analysis (ethyl acetate/hexane, 4/1, v/v) showed completion of the reaction. A mixture of *t*-BuOOH/$Et_3N$ (0.1mL/1mL) was added at -40$^oC$ and stirring was continued for 3h at the same temperature. After TLC analysis (ethyl acetate) indicated completion of the reaction, the reaction mixture was concentrated *in vacuo* and the residue was purified by a column of Sephadex LH-20 eluted with ($CH_2Cl_2$/MeOH, 2:1, v/v), to afford **39** as a colorless syrup (40mg, 57%). $^{31}P$ NMR (δP=-3.22). $^1H$ NMR (500MHz, DMSO) δ: 7.38-7.30 (m, 5H, Ar-*H*), 5.21 (dd, 1H, H-1, $J_{1,P}$=6.3Hz, $J_{1,2}$=1.7Hz), 5.18-4.87 (m, 6H, H-2, H-3, 2xα-C*H*(Ser, Phe), C$H_2$(Gly)), 4.84-3.99 (m, 3H), 3.52-3.37 (m, 3H), 3.24 (s, 3H, COC$H_3$), 3.16 (d, 1H, *J*=4.1Hz), 2.10-1.92 (4s, 12H, 4xC$H_3$CO). $^{13}C$ NMR (125MHz, DMSO) δ: 170.70, 170.53, 170.43, 170.26, 170.21, 170.18, 170.12, 137.75, 137.66, 129.18, 129.08, 129.03, 128.58, 128.44, 127.92, 91.69 (2x), 70.84, 70.58 (2x), 70.30, 69.26, 68.70, 65.55, 46.94, 45.34, 37.60, 22.56. FAB-MS: *m/z* 783.39 $[M+Na]^+$. *Anal*. Calcd for $C_{30}H_{41}N_4O_{17}P$: C, 47.37; H, 5.43; N, 7.37; P, 4.07; found: C, 47.38; H, 5.41; N, 7.38; P, 4.07.

**L-*N*-phenylalanylacetate-L-seryl-2-(α-D-mannopyranosylphosphate)-L-glycinamide (40)**.

A solution of NaOMe in methanol (1%, 3mL) was added (pH of solution: 11-12) to a solution of **39** (30mg, 0.03mmol) in methanol 9 (1mL). The reaction mixture was stirred

at room temperature for 10 minutes. When TLC analysis (MeOH/DCM, 1/4, v/v) indicated that the reaction is complete, the reaction mixture was neutralized by addition of Dowex $50H^+$ resin and filtered. The filtrate was concentrated *in vacuo* to afford **40** as a colorless syrup (20mg, 87%). $^{31}$P NMR (δP=3.23, 3.11). $^{1}$H NMR (500MHz, DMSO) δ: 7.36-7.31 (m, 5H, Ar-*H*), 5.24 (dd, 1H, H-1, $J_{1,P}$=4.5Hz, $J_{1,2}$=1.8Hz), 5.16-4.89 (m, 6H, H-2, H-3, 2xα-C*H*(Ser, Phe), C$H_2$(Ser)), 4.11-3.55 (m, 8H, H-4, H-5, H-6a,b, C$H_2$(Gly), C$H_2$(Phe)), 3.52-3.37 (m, 3H), 3.21 (s, 3H, COC$H_3$). $^{13}$C NMR (125MHz, DMSO) δ: 136.64, 129.51, 129.06, 127.49, 126.11, 125.09, 94.24, 93.91, 72.69, 71.49, 71.00, 70.49, 67.09, 66.84, 65.13, 61.22, 55.69, 55.37, 54.36, 42.35, 37.62, 22.01. FAB-MS: *m/z* 615.51 $[M+Na]^+$. *Anal*. Calcd for $C_{22}H_{33}N_4O_{13}P$: C, 44.60; H, 5.61; N, 9.46; P, 5.23; found: C, 44.58; H, 5.61; N, 9.47; P, 5.24.

# CHAPTER 4

# CONCLUSIONS AND FUTURE WORK

The abundance of carbohydrates in nature and their diverse roles in biological systems make them attractive as subjects for chemical and biological research. They are found as monomers, oligomers, polymers, or as components of biopolymers such as glyco-conjugates. As domains of natural products, they play important roles in conferring certain physical, chemical, and biological properties to their carrier molecules. Furthermore, they have been implicated in many cellular processes, including cell-cell recognition, cellular transport, and adhesion: they appear in cells in some form or another, for example, as peptide-and proteoglycans, glycoproteins, nucleic acids, lipo-polysaccharides, or glycolipids.

Detailed biophysical and biochemical studies of carbohydrates require sufficient quantities of defined oligosaccharides. Oligosaccharides are often found only in low concentration in nature and identification and isolation of them from natural sources is greatly complicated by their microheterogenity. Chemical synthesis is the alternative way to have well-defined oligosaccharides. Unfortunately, the preparation of biologically important oligosaccharides typically requires mutli-step transformations involving iterative protection-glycosylation-deprotection reactions with chromatographic purification of intermediates at each stage of the synthesis. Such preparations would

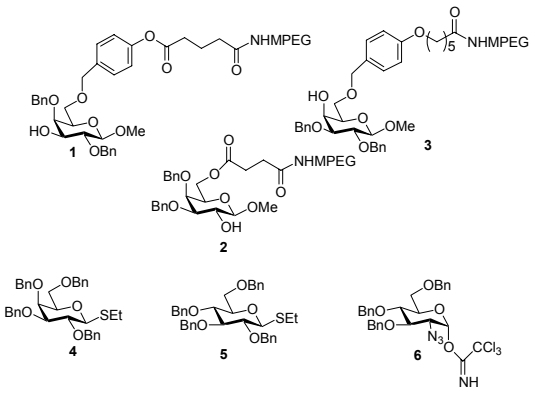
greatly benefit from development in polymer supported oligosaccharide synthetic strategies.

The last two decades witnessed a burst of activity in the development of Polymer-supported synthetic methodologies which offer many advantages over solution phase chemistry. These advantages include increased yield, due to the ability to add excess reagents to drive the reaction to completion, and increased speed of synthesis, due to the elimination of the purifications steps. Two main strategies can be envisaged for solid-phase oligosaccharide synthesis entailing either attachment of the acceptor or of the donor to the solid support. In the former strategy, an acceptor is bound to the solid support, usually at the anomeric position, and a solution based donor and promoter are administrated for the coupling step. In the second approach, glycosyl donors are bound to the solid support by a suitable hydroxyl group and then reacted with solution phase acceptors.

Polymeric supports can be either soluble or insoluble support. Soluble polymer supports such as polyethyleneglycol monomethyl ether (MPEG) combines advantages of the solution phase regime with the easy workup of solid phase synthesis. While all chemical transformations are carried out in homogeneous solution, the polymer is precipitated out after each step to ensure the removal of any excess reagents by simple filtration. A potential drawback of using soluble polymeric support is the loss of material during the precipitation step after each coupling.

In this study, a library of disaccharides was synthesized on the polymeric support (MPEG) by introducing the polymer support to three different glycosyl acceptors **1**, **2**, and **3**, and a solution based donor such as **4**, **5**, and **6**) and promoter were administered for

the coupling step. The individual disaccharides were demixed by selective cleavage the orthogonal linkers to give 6 different disaccharides in each one-pot reaction.




As an expansion of the applications of this methodology, more orthogonal linkers can be introduced to that set of linkers, such as a photolabile linker. In this case, upon coupling with a common glycosyl donor, a larger library of disaccharide will be obtained. Furthermore, this methodology can be utilized for synthesizing of more complex oligosaccharides, such as trisaccharides by coupling the resin-modified glycosyl acceptors **1**, **2**, and **3** with a common disaccharidedonors. Using common trisaccharide donors can also afford a library of different tetrasaccharides. In general as major attractions of this methodology, the library of the linker-tagged monosaccharides **1**, **2**, and **3** can repeatedly be used in glycosylations with different glycosyl donors to give a large number of oligosaccharide libraries.

As a part of this program, attention was diverted into studying a new class of glycoproteins; called proteophosphoglycans (PPGs) which has emerged in the last decade. In this kind of protein, phosphosaccharide repeats and cap glycans linked to a protein backbone via a phosphodiester linkage to the side chain hydroxyl at serine were discovered. In order to synthesize this kind of linkage, the phosphoramidite strategy was

applied to establish a reliable approach to prepare this kind of linkage. Coupling of different serine derivatives with pure α-mannosyl phosphoramidite gave phosphotriester products in acceptable yields. It is expected that this strategy can be applied to couple the glycosyl phosphoramidite moiety to the hydroxyl group of hydroxy amno acid such as serine, threonine, and hydroxylysine residue in a peptide chain either by the global or building block strategy.

# REFERENCES

1) Varki, A. *Glycobiology* **1993**, *3*, 97-130.

2) a) Duus, J.; Hilaire, P. M. St.; Meldal, M.; Bock, K. *Pure Appl. Chem.* **1999**, *71*, 755; b) Mischnick, P. *Angew. Chem.* **2000**, *112*, 1274; *Angew. Chem . Int. Ed*. **2000**, *39*, 1222.

3) a) Benjamin, G.; Davis, J.; *J. Chem. Soc. Perkin Trans. 1*, **2000**, 2137; b) Toshima, K.; Tatsuta, K. *Chem. Rev*. **1993**, *93*, 1503.

4) a) Danishefsky, S. J.; Roberge, J. Y. *Pure. Appl. Chem*. **1995**, *67*, 1647; C. b) Gege, C.; Vogel, J.; Bendas, G.; Rothe, U.; Schmidt, R. R. *Chem. Eur. J.* **2000**, *6*, 111; c) Baeschlin, D.K.; Chaperon, A. R.; Green, L. G.; Hahn, M. H.; Ince, S. J.; Ley, S. V. *Chem. Eur. J*. **2000,** *6*, 172.

5) Hilaire, P. M.; Meldal, M. *Angew. Chem*. *Int. Ed*. **2000**, *112*, 1210; *Angew. Chem*. *Int. Ed*. **2000**, *39*, 1162.

6) Alhadeff, J. A. Malignant Cell Glycoproteins and Glycolipids. In: *Crit. Rev. Onc Hemt*. **1989**; 37-58.

7) Matsusako, T.; Muramatsu, H.; Shirahama, T; Ohi, Y. *Biophys. Res. Commun*. **1991**, *181*, 1218-1222.

8) Bosmann, H.B.; Bieber, G.F.; Brown, A.E.; Case, K.R.; Gersten, D.M.; Kimmerer, T.W.; Lione, A. *Nature*. **1983**, *246*, 487-489.

9) Yogeeswaran, G.; Stein, B.S.; Sebastian, H. *Cancer Res*. **1978**, *38*, 1336-1344.

10) Tao, T.W.; Burger, M. M. *Nature*. **1977**, *270*, 437-438.

11) Yogeeswaran, G.; Salk, P. *Science*. **1981**, *212*, 1514-1516.

12) Matumoto, H.; Muramatsu, H.; Muramatsu, T.; Shimazu, H. *Cancer* **1992**, *69*, 2084-2090.

13) Miyake, M.; Taki, T.; Hitomi, S.; Hakomori, S.; *N Engl. J. Med*. **1992**, *327*, 14-18.

14) Muramatsu, T. *Glycobiology*, **1993**, 294-296.

15) Fukuda, M. *Biorg. Med. Chem*, **1995**, *3*, 207-215.

16) Lowe, J. B.; Fukuda, M.; Hindsgaul, O. *IRL Press. Oxford*, **1994**, 163-2-5.

17) Hakomori, S. *Adv. Cancer. Res*. **1989**, *52*, 257-331.

18) Toyokuni, T.; Singhal, A. K. *Chem. Soc. Rev*. **1995**, 231-242.

19) MacLean, G. D.; Longenecker, B. M. *Can. J. Oncol*. **1994**, *4*, 249-258.

20) Maryanoff, B.E.; Nortey, S.O.; Gardocki, J. F.; Shank, R. P.; Dodgson, S. P. *J. Med. Chem.* **1987**, *30*, 880-887.

21) McComsey, D. F.; Maryanoff, B. E. *J. Org. Chem*. **1994**, 59, 2652-2654.

22) von Itzestin, M.; Wu, W. Y.; Kok, G. B.; Peg, M. S.; Dyson, J.C.; Jin, B.; Van Phan, T.; Smythe, M. L.; White, H. F.; Oliver, S.W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotman, V. J.; Cameron, J. M.; Penn, C. R. *Nature*. **1993**, *363*, 418-423.

23) Von Itzstein, M; Wu, W-Y.; Jin, B. *Cabohydr. Res*. **1994**, *259*, 301-305.

24) Holzer, C.T.; Von Itzstein, M., Jin, B.; Pegg, M.S.; Stewart, W.P.; Wu, W-Y. *Glycoconj. J*. **1993**, *10*, 40-44.

25) Chong, A. K. J.; Pegg, M. S.; Von Itzstein, M. *Biochem.Biophys. Acta.* **1991**, *1077*, 65-71.

26) Chong, A. K. J.; Pegg, M. S.; Taylor, N. T.; Von Itzstein, M. *Eur. J. Biochem*. **1992**,

*207*, 335-343.

27) Unverzagt, C. *Angew. Chem. Int. Ed. Engl*. **1993**, *32*, 1691-1693.

28) Paulsen, H.; Matschulat, P. *Liebigs. Ann. Chem*. **1991**, 487-495.

29) Walliman, K.; Vasella, A. *Helv. Chim. Acta*. **1991**, *74*, 1520-1532.

30) Nagy, J.O.; Wang, P.; Gilbert, J. H.; Schaefer, M. E.; Hill, T.G.; Callstrom, M. R.; Bednarski, M. D. *J. Med. Chem*. **1992**, *35*, 4501-4502.

31) Rhinehart, L.; Robinson, K. M.; King, C.H.; Liu, P.S. *Biochem. Pharmacol*. **1990**, *39*, 1537-1543.

32) Sunkara, P. S.; Bowlin, T. L.; Liu, P. S.; Sojerdsma, A. *Biochem.Biophys. Res. Commun.* **1987**, *148*, 200-206.

33) Gruters, R. A.; Neefjes, J. J.; Teresmette, M.; De Goede, R.; Tulp, A.; Huisman, H. G.; Miedema, F.; Plogh, H. L. *Nature*, **1987**, *330*, 74-77.

34) Fleet, G. W. J.; Karpas, A.; Dwek, R. A.; Fellows, L. E.; Tyms, A. S.; Peturson, S.; Namgoong, S. K.; Ramsden, P. W.; Smith, J. C.; Son, F.; Wilson, D. R.;Jacob, G. S; Rademacher, ThW. *Febs. Lett*. **1988**, *237*, 128-132.

35) Karpas, A.; Fleet, G. W. J.; Dwek, R. A.; Peturson, S.; Namgoong, K.; Ramsden, N. G.; Jacob G. S.; Rademacher, ThW. *Proc. Natl Acad. Sci*. **1988**, *85*, 9229-9233.

36) Tyms, A. S.; Taylor, D. L., Sincere, P. S.; Kang, M. S.; *De Clerq E, ed. Elsevier, Amsterdam,* **1990**, 257-318.

37) Casiraghi, G.; Rassu, G.; Spanu, P.; Pinna, L.; Ulgheri, F. *J. Org. Chem*. **1992**, *57*, 3760-3763.

38) Ina, H.; Kibayashi, C. *J. Org. Chem*. **1993**, *58*, 52-61.

39) Casiraghi, G.; Rassu, G.; Spanu, P.; Pinna, L.; Ulgheri, F. *J. Org. Chem*. **1993**, *58*, 3397-3400.

40) Zhou, P.; Mohd, S.; Hpnek, J. *J. Org. Chem*. **1993**, *58*, 264-266.

41) Hudlicky, T.; Rouden, J.; Luna, H. *J. Org. Chem*. **1993**, *58*, 985-987.

42) Arnone, A.; Bravo, P.; Donadelli, A.; Resnatii, G. *J. Chem. Soc. Chem. Commun.***1993**, 984-985.

43) Furneaux, R. H.; Lynch, G. P.; Way, G.; Winchester, B. *Tetrahedron Lett.* **1993**, 3477-3480.

44) Kefalas, P.; Grierson, D.S. *Tetrahedron Lett.* **1993**, 3555-3558.

45) Bruce, I.; Fleet, G.W.J.; Cenci Di Bello, I.; Winchester, B. *Tetrahedron.* **1992**, *48*, 10191-10200.

46) Dondoni, A.; Merino, P.; Perrone, D. *Tetrahedron.* **1993**, *49*, 2939-2956.

47) Fleet, G. W.; Petrusson, S.; Campbell, A. L.; Mueller, R. A.; Behling, J. R.; Babiak, K. A.; Ng, J. S.; Scaros, M. G. *J. Chem. Soc Perkin Trans*. 1, **1989**, 665-666.

48) Hughes, A. M.; Rudge, A. J. *Natural Product Rep*. **1994**, 135-162.

49) Andreews, D. M.; Bird, M. I.; Cunningham, M. M.; Ward, P. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 2533-2536.

50) Burgess, K.; Hemderson, I. *Tetrahedron.* **1992**, *48*, 4045-4066.

51) Humphries, M. J.; Matsumoto, K.; White, S.L.; Olden, K. *Cancer Res*. **1986**, *46*, 5215-5222.

52) Johnson, V. A.; Walker, B.D.; Barlow, M. A.; Paradis, T.J.; Chou, T.C.; Hirsch, M. S.; *Antimicrobial Agents and Chemother*. **1989**, *33*, 53-57.

53) Tulsiana, D. R. P.; Broquist, H..P.; Touster, O. *Arch. Biochem. Biophy*. **1985**, *236*, 427-434.

54) Dennis, J. W. *Cancer Res*. **1986**, *46*, 5131-5136.

55)Hino, M.; Nakayama, O.; Tsurumi, K.; Adechi, K.; Shibata, T.; Terano, H.; Kohasaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1985**, *38*, 926-935.

56) Kino, T.; Inamura, N.; Nakahara, K.; Kiyoto, S.; Goto, Y.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H.; *J. Antibiot.* **1985**, *38*, 936-940.

57) Truscheit, E.; Frommer, W.; Junge, B.; Muller, L.; Schmidt, D.D.; Wingender.; W. *Angew. Int. Ed. Engl.* **1981**, *20*, 744-761.

58) Robinson, K. M.; Begovic, M. E.; Rhinehart, B. L.; Heineke, E. W.; Duccep, J. B.; Kastner, P. R.; Marshall, F. N.; Danzin, C. *Diabetes*. **1991**, *40*, 825-830.

59) Yoshikuni, Y.; Ezure, Y.; Seto, T.; Mori, K.; Watanabe, K.; Enomoto, H. *Chem. Pharm. Bull*. **1989**, *37*, 106-109.

60) Jubert, P. H.; Venter, H. L.; Foukarids, G. N. *Br. J. Clin. Pharmacol*. **1990**, *30*, 391-396.

61) Junge, B.; Boshagen, H.; Stoltefuz, J.; Muller, L. *Verlag Chemie Weinheim.* **1980**, 123-137.

62) Chakrabarti, S.; Cherian, P.V.; Sima, A.A.F. *Diabetes Res. Clin. Pract*. **1993**, *20*, 123-128.

63) Reitz, A. B.; Tuman, R. W. In: New Antidiabetic Drugs, Bailey, C. J.; Flatt, PR, *Eds*., *Smith-Grdon*, **1990**, 171-176.

64) Reitz, A. B.; Tuman, R. W.; Marchione, C.S.; Jordan, Jr., A. D.; Bowden, C. R.; Maryanoff, B. E. *J. Med. Chem*. **1989**, *32*, 2110-2116.

65) Ferguson, M. A. J.; Williams, A. F. *Ann. Rev. Biochem*. **1988**, *57*, 265-320.

66) Saltiel, A. R.; Cuatrecasas, P. *Proc.Nat. Acad. Sci,* **1986**, *83*, 5793-5797.

67) Powis, G. *Trends Pharmacol. Sci*. **1991**, *12*, 188-198.

68) Powis, G.; Kozikowski, A. P., *Eds. CRC Press; Boca Raton*, **1992**.

69) Kozikowski, A. P.; Faug, A. H.; Malaska, M. J.; Tuckmantel, W.; Ognyanov, V. I.; Powis, G. *Curr. Med. Chem*. **1994**, *1*, 1-12.

70) Kozikowski, A. P.; Tuckmantel, W.; Powis, G. *Angew. Chem. Intl. Ed. Engl.* **1992**, *31*, 1379-1381.

71) Wiley, P. F.; Jahnke, H. K.; Chidester, C. G.; Mizsak, S. A.; Spaulding, L.B.; Argoudelis, A. D. *J. Org. Chem*. **1979**, *44*, 9-16.

72) Ichikawa, Y.; Halcomb, R. I.; Wong, C.H. *Chem. Brit*. **1994**, 117-121.

73) DeFrees, S. A.; Gaeta, F.; Lin, Y-C.; Ichikawa, Y.; Wong, C-H. *J. Am. Chem. Soc.* **1993**, *115*, 7549-7550.

74) Cai, S.; Stroud, M. R.; Hakomori, S.; Toyokuni, T. *J. Org. Chem*. **1992**, *57*, 6693-6696.

75) Schmidt, R. R.; Frische, K. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1747-1750.

76) Witczak, Z. J. *XVII International Carbohydrate Symposium.Ottawa, Ontario* Jul 17-22, **1994**.

77) Beacham, A. R.; Biggadike, K.; Taylor, H. E.; Hackert, L.; Winchester, B.G.; Watkin, D. J.; Fleet, G. W. J. *J. Chem. Soc. Chem. Commun*. **1994**, 2001-2002.

78) Scheffler, K.; Ernst, B.; Katopodis, A.; Magnani, J. L.; Wang, W-T.; Weiseemann, R.; Perets, T. *Angew. Chem. Intl. Ed. Engl.* **1995**, *34*, 1841-1844.

79) Hashimoto, H.; Izumi ,M. *Tetrahedron Lett*. **1993**, *34*, 4949-4952.

80) Hashimoto, H.; Shimada, K.; Horito, S. *Tetrahedron Asymmetry*. **1994**, *5*, 2351-2366.

81) Witczak, Z. J.; Sun, J.; Mielgui, R. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 2169-2174.

82) Hashimoto, H.; Shimada, K.; Horito, S. *Tetrahedron Asymmetry*. **1994**, *5*, 2351-2366.

83) Witczak, Z. J.; Whistler, R. L. *Gordon& Breach*. **1987**, 157-173.

84) Misaki, A.; Kishida, E.; Kakuta, M.; Tabata, K. *ATL Press*, **1993**, 116-129.

85) Matsuzaki, K.; Sato, T.; Enomoto, K.; Yamamoto, I.; Ohshima, R.; Hatanaka, K.; Uryu, T.; Eaku, H.; Sone, Y.; Misaki, A. *Carbohydr. Res*. **1986**, *157*, 171-182.

86) Garner, P. *Elsevier Amsterdam,* **1988**, part A Vol. 1. p. 397.

87) Knapp, S. *Chem. Rev*. **1995**, *95*, 1859-1876.

88) Mason, D. J.; Dietz, A.; Deboer, C. *Antimicrob. Agents Chemother*. **1962**, 554-559.

89) Birkenmeyer, R. D.; Kagan, F. *J. Med. Chem*. **1970**, *13*, 616-619.

90) Fass, R. J.; Saalaw, S. *Am. J. Med. Chem*. **1972**, *263*, 369-382.

91) Borders, D. B.; Doyle, T. W. *Marcel Dekker Inc. New York*, **1995**.

92) Berdy, J.; Aszalos, A.; Bostian, M.; McNitt, K. Handbook of Antibiotic Compounds, Vol 1, Carbohydrate Antibiotics, *CRC Press, Boca Raton*, **1980**.

93)Umezawa, H.; Hoeper, I.R., eds. Handbook of Experimental Pharm. Vol 80. Aminoglycoside Antibiotics, *Springer Verlag, Berlin*, **1982**.

94) Ristuccia ,A. M. Aminoglycosides. In: Antimicrobial Therapy. Ristuccia AM. Cunha BA, eds. *Raven Press, New York* **1984,** pp 305-327.

95) Fillastre, J. P.; Leroy, A.; Humbert, G.; Moulin, H. B.; Beruadet, P.; Josse, S. *Antimicrob. Agents Chemother*. **1987**, *31*, 575-577.

96) Hill, C.D.; Counts, G.W.; Turck, M. *Antimicrob. Agents Chemother*. **1981**, 1190-192.

97) Leroy, A.; Humbert, G.; Fillastre, J.P. *J. Antimicrob. Chemother*. **1980**, *6*, 113-120.

98) Fernandes, P. B.; Bailer, R.; Swanson, R.; Hanson, C. W.; Mcdonald, E.; Ramer,N.; Hardy, D.; Shipkowitz, N.; Bower, R. R.; Gade, E. *Antimicrob. Agents Chemother*. **1986**, *30*, 865-873.

99) Ferero, J. I.; Bopp, B.A.; Marsh, K. G.; Quigley, S.C.; Johnson, M. J.; Anderson, D.L.; Lamm, J. E.; Tolman, K. G.; Sanders, S. W.; Cavanaugh, J. H.. *Drug Metab. Dispos*. **1990**, *18*, 441-446.

100) Hodinka, R. L.; Jack-Wait, K.; Gilligan, P. H. *Eur. J. Clin. Microbiol.* **1987**, *6*, 103-108.

101) Blizzard, T.; Fisher, M. H.; Mrozik, H.; Shih, T. L., e*ds., Springer-Verlag, Berlin,* **1990**, pp.66-83.

102) Krohn, K.; Kirst, H.A.; Maag, H., *eds. Antibiotics and antiviral Compunds Chemical Synthesis and Modification. VCH, Weinheim,* **1993.**

103) Ganguly, A. K.; Sarre, O. Z.; Greeves, D.; Motron, J. *J. Am. Chem. Soc*. **1975**, *97*, 198-1985.

104) Conrad, H. E. *Pure&Appl. Chem*.**1993**, *65*(4): 787-791.

105) Nader, H. B.; Dietrich, C.P., eds Heparin*, Chemical and Biological properties, Clinical Applications, London:Edward Arnold*, **1989**, 81-96.

106) Ofosu, F. A. *Semin Thromb hemost*, **1993**, *19* (suppl 1): 29-30.

107) Brown, H. D.; Smith, W. E. *Chem. Soc. Rev*. **1980**, *9*, 217-240.

108) Linhardt, R. J.; Baenziger, N. C.; Ronson, B. *J. Pharm. Sci*. **1990**, *79*,158-162.

109) Harris, E. D.; Sporn, M.B. *Am. J. Medicine*. **1981**, *70*, 1231-1236.

110) Brickerhoff, C. E. *Agents Actions*, **1990**, *30*, 322-328.

111) Morrison, C. J.; Gordon, P.; Hashomoto, T. *Antimicrob. Agents. Chemother*. **1984**, *26*, 74-77.

112) Kieval, R. I.; Young, C.T.; Prohazka, D.; Brickerhoff, C. E.; Trentham, D. E. *J. Rheumatol*. **1989**, *16*, 74-77.

113) Garrett, E. R.; Van Peer, A. *J. Pharm. Sci.* **1983**, *72*, 1045-1057.

114) Weinblatt, M. E.; Fraser, P. A.; Anderson, R.; Coblyan, J. S.; Trentham, D.E. *J. Rheumatol.* **1987**, *4*, 859-860.

115) Arora, S. K.; Whistler, R. L.; Thomas, A.V. *WO Patent.* **1992**, *92*/04359; *Chem. Abstr*, **1992**, *117*: 90700q.

116) Ronsen, B.; Arora, S. K.; Thomas, A.V. *EP* P*atent.* **1990**; 379,379; *Chem. Abstr***. 1991**; *114*: 247656t.

117) Ronsen, B.; Arora, S. K.; Thomas, A.V. *EP* P*atent.* **1990**; *404*, 36; *Chem. Abstr***. 1991**, *114*: 247656t.

118) Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C-H. *Chem. Rev.* **1996**, *96*, 443-73.

119) a) Thompson, L. A.; Ellman, J. A. *Chem. Rev*. **1996**, *95*, 555. b) Guillier, F. Orian, D. Bradley, M. Bradley, *Chem. Rev*, **2000**, 100, 2091.

120) Wang, Z, G.; Hindsgaul, O. *Glycoimmunology* **1998**, *2*, 219; F. Schweizer, F.; Hindsgaul O. *Curr. Opin. Chem. Biol*. **1999**, *3*, 291.

121) Kanie ,O.; Berresi, F.; Ding, Y.; lab, J.; Otter, E.; Forsberg, A.; Ernst, L. S. B.; Hindsgaul, O. *Angew. Chem*. **1995**, *107*, 2912;.

122) Ding ,Y.; Labbe ,J.; Kaine ,O.; Hindsgaul ,O. *Bioorg. Med. Chem.* **1996**, 4, 683.

123) Boons, G-J.; Heskamp, B.; Hout, F. *Angew. Chem*. **1996** *108*, 3053; *Angew. Chem*. Int. Ed. Engl.**1996**, *35*, 2845.

124) Boons, G-J.; Isles, S. *J. Org. Chem*. **1996**, *61*, 4262.

125) Johnson, M.; Arles, C.; Boons, G-J. *Tetrahedron Lett*. **1998**, *39*, 9801.

126) Izumi, M.; Ichikawa, Y. *Tetrahedron Lett*. **1998**, *39*, 2079.

127) Wong ,C-H.; Ye ,X-S.; Zhang, Z. *J. Am. Chem. Soc*. **1998**, *39*, 9801.

128) Lubineau , A.; Bonnaffe, D. *Eur. J. Org. Chem*. **1999**, 2523.

129) Zhang, Z.; Ollmann, I. R.; Ye, X-S.; Wischnat, R.; Bassov, T. V.; Wong, C.-H. *J. Am. Chem. Soc*. **1999**, *121*, 734.

130) Ye, X-S.; Wong, C.-H. *J. Org. Chem*. **2000**, *65*, 2410.

131) a) Yamada, H.; Kato, T.; Takahashi, T. *Tetrahedron Lett*. **1999**, *40*, 4581. b) T. Takahashi, Adachi, M.; Matsuda, A.; Doi, T. *Tetrahedron Lett*. **2000**, *41*, 2599.

132) Osborn, H. M. I.; Khan, T. H. *Tetrahedron* **1999,** *55*, 1807.

133) James, I. W. *Tetrahedron* **1999,** *55*, 4855-4946.

134) Merrifield, R. B. *J. Am. Chem. Soc*. **1963**. *85*, 2149-2152.

135) Small, P. W.; Sherrington, D. C. *J. Chem. Soc. Chem. Commun.* **1989**, 589-1591.

136) Heckel, A.; Mross, E.; Jung, K. H.; Rademann, J.; Schmidt, R. R. *Synlett* **1998**, 171-173.

137) Bayer, E. *Angew. Chem. Int. Ed. Engl*. **1991**, *30*, 113-129.

138) Bayer, E.; Albert, K.; Willisch, H.; Rapp, W.; Hemmasi, B. *Macromolecules* **1990**, *23*, 1937-1940.

139) Look, G. C.; Holmes, C. P.; Chinn, J. P.; Gallop, M. A. *J. Org. Chem*. **1994**, *59*, 7588-7590.

140) Gravert, D. J.; janda, K. D. *Chem. Rev*. **1997**, *97*, 489-509.

141) Frechet, J. M.; Schurech, C. *J. Am. Chem. Soc*. **1971**, *93*, 492-496.

142) Frechet, J. M.; Schurech, *Carbohydr. Res*. 1**972**, *22*, 399-412.

143) Veeneman, G. H.; Notermans, S.; Liskamp, R. M. J.; Van der Marel, G. A. van Boom, J. H. V. *Tetrahedron Lett*. **1987**, *28*, 6695-6698.

144) Rademann, J.; Schmidt, R. R. *Tetrahedron Lett*. **1996**, *37*, 3989-3990.

145) Yan, L.; Taylor, C. M.; Goodnow, Jr., R.; Kahne, D. *J. Am. Chem. Soc*. **1994**, *116*, 6953-6954.

146) Horan, N.; Yan, L.; Isobe, H.; Whitesides, G. M.; Kahne, D. *Proc. Natl. Acad. Sci. USA,* **1999**, *96*, 11782.

147) Zhu, T.; Boons ,G-J. *Angew. Chem*. **1998**, 110, 2000; Angew. *Chem. Int. Ed. Engl.* **1998**, *37*, 1898.

148) Ito, Y.; Kanie, O.; Ogawa, T. *Angew. Chem*. **1996**, *35*, 2150.

149) Schuster, M.; Wang, P.; Paulson, J. C.; Wong, C-H. *J. Am. Chem. Soc*. **1994**, *116*, 1135.

150) Schmidt, R. R. *Angew. Chem. Int. Ed. Engl*. **1986**, *25*, 212; Rademann, J.; Schmidt, R. R. *Tetrahedron Lett*. **1996**, *37*, 3989.

151) Rademann, J.; Schmidt, R. R. *J. Org. Chem*. **1997**, *62*, 3650.

152) Knerr,L.; Schmidt, R. R. *Synlett.* **1999**,1802.

153) Anilkumar, G.; Nair, L. G.; Fraser-Reid, B. *Org. Lett*. **2000**, *2*, 2587.

154) Andrade, R.; Plante, O. J.; Melean, L. G.; Seeberger, P. H. *Org. Lett*. **1999**, *1*, 1811.

155) Zheng, C.; Seeberger, P. H; Danishefsky, S. J. *J. Org. Chem*. **1998**, *63*, 1126.

156) Hummel, G.; Hindsgaul, O. *Angew. Chem*. **1999**, 111, 1900; *Angew. Chem. Int. Ed. Engl.* **1999,** *38*, 1782.

157) Nicolaou, K. C.; Watanabe, N.; Li, J.; Pastor, J.; Winssinger, N. *Angew. Chem*. **1998**, *110*, 1636; *Angew. Chem. Int. Ed. Engl.* **1998,** *37*, 1559.

158) Sofia, M. J. *Med. Chem. Res*. **1998***, 8*, 362.

159) Hirschmann, R.; Yao, W.; Cascieri, M. A; Strader, C. D.; Maechler, L.; Cichy-Knight, M. A.; Hynes, J. Jr., D. van Rijn, P. A. Sprengler, A. B. *J. Med. Chem.* **1996**, *39*, 2441.

160) Sofia, M. J.; Hunter R.; Chqn, T. Y.; Vaughan, A.; Dulina, R.; Wang, H.; Gange, D. *J. Org. Chem.* **1998**, *63*, 2802.

161) Nicolaou, K. C.; Xiao, X. Y.; Parandoosh, Z.; Senyei, A.; Nova, M. P. *Angew. Chem*. **1995**, *107*, 2476; *Angew. Chem. Int. Ed. Engl.* **1995,** *34*, 2289.

162) Wunberg, T.; Kallus, C.; Opatz, T.; Henke, S.; Schmidt, W.; Kunz, H. *Angew. Chem*. **1998**, *110*, 2620; *Angew. Chem. Int. Ed. Engl.* **1998,** *37*, 2503.

163) Kallus, C.; Opatz, T.; Wunberg, T.; Henke, S.; Schmidt, W.; Kunz, H. *Tetrahedron Lett*. **1999**, *40*, 7783.

164) Silva, D. J.; Wang, H.; Allanson, N.M.; Jain, R. K.; Sofia, M. J. *J. Org. Chem*. **1999**, *64*, 5926.

165) Wong, C-H.; Hendrix, M.; Manning, D. D.; Rosenbohm, C.; Greenberg, W. A. *J. Am. Chem. Soc.* **1998**, *120*, 8319.

166) Ugi, I. *Angew. Chem*. **1982**, *94*, 826; *Angew. Chem. Int. Ed. Engl.* **1982,** *21*, 810.

167) Sutherlin, D. P.; Stark, T. M.; Hughes, R.; Armstrong, R W. *J. Org. Chem.* **1996**, *61*, 8350.

168) Park, W. K. C.; Auer, M.; Jaksche, H.; Wong, C-H. *J. Am. Chem. Soc.* **1996**, *118*, 10150.

169) Lockhoff, O. *Angew. Chem*. **1988**, *110*, 3634; *Angew. Chem. Int. Ed. Engl.* **1998,** *37*, 3436.

170) Nilsson, U. J.; Fournier, E. J-L.; Hindsgau,O. l, *Bioorg. Med. Chem.* **1998**, *6*, 1563.

171) Meldal, M.; St. Hilaire, P. M. *Curr. Opin. Chem. Biol*. **1997**, *1*, 552.

172) St. Hilaire, P. M.; Lowary, T. L.; Meldal, M.; Bock, K. *J. Am. Chem. Soc.* **1998**, *120*, 13312.

173) Lampe, T. F. J.; Weitz-Schmidt, G.; Wong, C.-H. *Angew. Chem*. **1998**, *110*, 761; *Angew. Chem. Int. Ed. Engl.* **1998,** *37*, 1707.

174) Arya, P.; Dion, S.; Shimizu, G. K. H. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1537.

175) Arya, P.; Kutterer, K. M. K.; Qin H.; Roby, J.; Barnes, M. L.; Lin, S.; Lingwood, C. A.; Peter, M. G. *Bioorg. Med. Chem. Lett*. **1999**, *7*, 2823; Arya, P.; Ben, R. N.; Kutterer, K. M. K., *Organic synthesis Highlights*, *Vol. IV*, Wiley-VCH, Weinheim, **2000**, p.337.

176) Kutterer, K. M. K.; Barnes, M. L.; Arya, P. *J. Comb. Chem.* **1999**, *1*, 28.

177) Arya, P.; Kutterer, K. M. K.; Barkley, A. *J. Comb. Chem.* **2000**,

179) Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489-509.

180) Kim, R. M.; Manna, M.; Hutchins, S. M.; Griffin, P. R.; Yates, N. A.; Bernick, A. M.; Chapman, K. T. *Proc. Nat. Acad. Sciences.* **1996**, *93*, 10012-10017.

181) Studer, A.; Hadida, S.; Ferritto, R.; Kim, S. Y.; Jeger, P.; Wipf, P.; Curran, D. P. *Science.* **1997**, *275*, 823-826.

182) Han, H. S.; Wolfe, M. M.; Brenner, S.; Janda, K. D. *Proc. Nat. Acad. Sciences.* **1995**, *92*, 6419-6423.

183) Barnes, C.; Balasubramanian, S. *Current Opinion in Chemical Biology.* **2000**, *4*, 346-350.

184) Wentworth, P.; Janda, K. D. *Current Opinion in Biotechnology* **1998**, *9*, 109-115.

185) Luo, Z. Y.; Zhang, Q. S.; Oderaotoshi, Y.; Curran, D. P. *Science* **2001**, *291*, 1766-1769.

186) Barkley, A.; Arya, P. *Chem. Eur. J.* **2001**, *7*, 555-563.

187) Seeberger, P. H.; Haase, W. C. *Chem. Rev.* **2000**, *100*, 4349-4393.

188) St. Hilaire, P. M.; Meldal, M. *Angew. Chem. Int. Ed.* **2000**, *39*, 1162-1179.

189) Kanemitsu, T.; Kanie, O. *Trends in Glycoscience and Glycotechnology* **1999**, *11*, 267-276.

190) Schweizer, F.; Hindsgaul, O. *Current Opinion in Chemical Biology* **1999**, *3*, 291-298.

191) Douglas, S. P.; Whitfield, D. M.; Krepinsky, J. J. *J. Am. Chem. Soc.* **1991**, *113*, 5095-5097.

192) Zhu, T.; Boons, G. J. *J. Am. Chem. Soc.* **2000**, *122*, 10222-10223.

193) Zhu, T.; Boons, G. J. *Chem., Eur. J.* **2001**, *7*, 2382-2389.

194) Jobron, L.; Hindsgaul, O. *J. Am. Chem. Soc.* **1999**, *121*, 5835-5836.

195) Krepinsky, J. J. Khan, S. H.; O'Neil, R. A. *eds.; Harwood Academic Publisher* **1996**.

196) Ito, Y.; Kanie, O.; Ogawa, T. *Angew. Chem. Int. Ed.* **1996**, *35*, 2510-2512.

197) Maddali, U. B.; Ray, A. K.; Roy, N. *Carbohydr. Res.* **1990**, *208*, 59-66.

198) David, S.; Hanessian, S. *Tetrahedron.* **1985**, *41*, 643.

199) Ray, A. K.; Maddali, U. B.; Roy, A.; Roy, N. *Carbohydr. Res.* **1990**, *197*, 93-100.

200) Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97-102.

201) Sarkar, A. K.; Ray, A. K.; Roy, N. *Carbohydr. Res.* **1989**, 190, 181-189

202) (a) Veeneman, G. H.; Vanleeuwen, S. H.; Vanboom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331-1334. (b) Konradson, P.; Udodong, U. K.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313-4316.

203) Dasgupta, F.; Garegg, P. J. *Acta. Chem. Sacand.* **1989,** *43***,** 471-475.

204) Kinzy, W.; Schmidt, R. R. *Liebigs Ann. Chem.* **1985**, *8*, 1537-1545.

205) Schmidt, R. R. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212-235.

206) Montreuil, J. *Adv. Carbohydr. Chem. Biochem*., **1980**, *37*, 157-223.

207) Eylar, H. *J. Theor. Biol*. **1965**, 89-112.

208) Dill, K.; Hardy, R. E.; Damna, M. E.; Lacombe, J.M.. *Carbohydr. Res*. **1982**, *108*, 31-40.

209) Winterburn, P. J.; Phelps, C. F. *Nature* **1972**, *236*, 147-151.

210) Arsequell, G.; Valencia., G. *Tetrahedron Asymmetry* **1997**, *8*, 2839.

211) Lis, H.; Sharon, N. *Eur. J. Biochem*. **1993**, *218*, 1.

212) Paul A. H. *Glycobiology* **1997**, *2*, 1-5.

213) Gustason, G.L.; Milner, L. A. *J. Biol. Chem*. **1980**, *255*, 7208-7210.

214) Souza, G. M.; hairai, J.; Mehta, D. P.; Freeze, H. H. *J. Biol. Chem*. **1995**, *270*, 28938-28945.

215) Alexander, J.; Russell, D.G. *Adv. Parasitol*. **1992**, *31*, 175-254.

216) Ilg, T.; Handman, E.; Ng, K.; Stierhof, Y,-D.; Bacic, A. Trends Glycosci. Glycotech. **1999**, *11*, 1-19.

217) Ilg, T.; Handman, E.; Ng, K.; Stierhof, Y-D. *Biochem.Soc.Trans*., **1999**, *27*, 48-55.

218) Handman, E.; Greenblatt, C. L.; Goding, J. W. *EMBO. J*. **1984**, *3*, 2301-2306.

219) Greis, K. D.; Turco, S. J.; Thomas, J. R.; McConville, M.J.; Homans, S. W.; Ferguson, M. A. J. *J. Biol. Chem,* **1992***, 267*, 5876-5881.

220) Turco, S. J.; Descotteaux, A. *Annu. Rev. Microbiol*. **1992**, *46*, 65-94.

221) McConville, M. J.; Ferguson, M. A. J. *Biochem. J*. **1993**, *294*, 305-324.

222) Ilg, T.; Overath, P.; McConville, M. J.; Ferguson, M. A. J.; Rutherford, T.; Campbell, D. G. *J. Biol. Chem*. **1994**, *269*, 24073-24081.

223) Ilg, T.; Stierhof, Y-D.; Craik, D.; Simpson, R.; Handman, E.; Bacic, A. *J. Biol. Chem*. **1996**, *271*, 21583-21596.

224) Ilg, T.; Craik, D.; Currie, G.; Muthaup, G.; Bacic, A. *J. Biol. Chem*. **1998**, 13509-13523.

225) Mehta, D. P.; Ichikawa, M.; Salimath, P. V.; Etchison, J. R.; Haak, P.; Manzi, A.; Freeze, H. H. *J. Biol. Chem.* **1996**, *271*, 10897-10903.

226) Jaffe, C. L.; Perez, L.; Schnur, F. *Mol. Biochem. Parasitol*. **1990**, *41*, 233-240.

227) Bates, P. A.; Hermes, I.; Dwyer, D. M. *Mol. Biochem. Parasitol,* **1990**. *39*, 247-255.

228) Ilg, T.; Menz, B.; Winter, G.; Russell, D. G.; Etges, R.; Schell, D.;Overath, P. *J. Cell. Sci*. **1991**, *99*, 175-180.

229) Ilg, T.; Stierhof, Y. D.; Etges, R.; Adrian, M.; Harbecke, D.; Overath, P. *Proc. Natl. Acad. Sci*. *USA*. **1991**, *88*, 8774-8778.

230) Stierhof, Y-D.; Ilg, T.; Russell, D. G.; Hohenberg, H.; Overath, P. *J. Cell. Biol*. **1994**, *125*, 21-331.

231) Ilg, T.; Stierhof, Y-D.; McConville, M. J.; Overath, P. *Eur. J. Cell. Biol*. **1995,** *66*, 205-215.

232) Stierhof, Y-D.; Bates, P. A.; Jacobson, R.; Schlein, Y.; Rogers, M.; Handman, E.; Ilg, T. *Eur.J. Cell. Biol*. **1999**, *78*, 675-689.

233) (a) Wakabayashi, K.; Pigman, W. *Carbohydr. Res*. **1974**, 35, 3-14. (b) Lacombe, J.M.; Pavia, A. A.. *J. Org. Chem*. **1983**, *48*, 2557-2563.

234) Higashi, K.; Nakayama, K.; Soga, T.; Kouichi, U.; Kusama, T. *Chem. Pharm. Bull.,* **1990**, *38*, 3280-3282.

235) Khorana, H. G.; Tener, G. M.; Moffat, J. G. *Pol. Chem. Ind*. **1956**, 1523.

236) Michelson, A. M.; Todd, A. R. *J. chem. Soc*. **1995**, 2632-2638.

237) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859-1862.

238) a) Corby, N. S.; Kenner, G.; Todd, A. R. *J. Chem. Soc*. **1952**, 3669-3675.

b) Garegg, P. J.; Regberg, T.; Stwinski, J.; Stromberg, P. *Chem. Scripta*. **1985**, *25*, 280-282.

239) Sinha, N. D.; Biernat, J.; MaManus, J.; , Koster, H. *Nucl. Acids Res*. **1984**, *12*, 4539.

240) Freese , S. J.; . Freese, Vann , W. F. *Carbohydr. Res*. **1996**, *281*, 313-319.

241) Jackson R. F. W.; Wishart, N. A.; Wood, K.; James, K.; Wythes, M. J. Wythes, *J. Org. Chem.* 57, *12*, **1992**, 3397-3404.

242) Manabe, S.; Sakamoto, K.; Nakahara, Y.; Sisido, M.; Hohsaka, T.; Ito, Y. *Bioorg. Med. Chem*. **2002**, *10*, 573-581.

243) O'Donnell , M. J.; Plot, R. L. *J. Org. Chem.* **1982**, *47*, 2663-2666.

244) Pope, B. M.; Yamamoto, Y.; Tarbell, D. S. *Org. Synth. Coll. VI* **1988**, *418*.

245) Bergman, M; Zervas, L. *Ber. Dtssch. Chem. Ges.* **1932**, *65*, 1192.

246) Chang, C-D.; M. Waki, M.; Ahimad, M.; Meinhoffer, J.; Lunderr, E. O.; Lundell.; Haug, J. D. *Int. J. Pept. Protein. Res.* **1980**, *15*, 59-66.

247) Alsina, J.; Rabanal, E.; Giralt, F. *Tetrahedron*. **1998**, *54*, 10125-10152.

248) Alewood, P. F.; Perich, J. W.; Johns, R. B. *Synth. Commun*. **1982**, *12*, 10, 821-828.

249) Engles, J.; Jager, A. *Angew. Chem. Suppl*., **1982**.

250) Vorherr, T.; Bannwarth, W. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 22, 2661-2664.

251) Merrifield, R. B. *J. Chem. Soc*. **1963**, *85*, 2149-2154.

252) Athreton, E.; Shepard, R. C. Eds. I. R. L. Press, Oxford, **1989**.

253) Caste, J.; Le-Ngugen, D.; Castro, B. *Tetrahedron Lett*. **1990**, *31*, 205.

254) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem*. **1970**, *34*, 595.