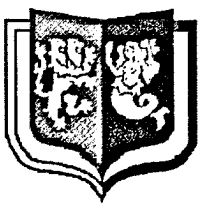

Boronate Esters in Oligosaccharide Synthesis

by

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SYNOPSIS

This thesis describes novel applications of aryl boronic acid to the synthesis of oligosaccharides in solution and on solid phase. In solution phase synthesis, phenylboronic acid is a competent protecting group for both glycosyl donors and acceptors during glycosylation reactions. Polystyrylboronic acid, the polymeric counterpart of phenylboronic acid, is a useful solid support for the synthesis of oligosaccharides on solid phase. Disaccharides and trisaccharides can be synthesised in good yield and manipulation of protecting groups on solid phase can be performed. Ease of loading and ease of cleavage of the oligosaccharide moiety and its potential reusability are the most interesting features of this resin. These properties also allow the development of a strategy that overcomes the problem of controlling the stereochemical outcome of every glycosylation during the synthesis of a complex oligosaccharide conducted on solid support. An anomeric mixture of oligosaccharides is easily cleaved from the support and, after separation of the anomers and reloading of only one anomer on the resin, the synthetic sequence is continued. A trisaccharide was synthesised by employing this methodology.

The second aim of this thesis is to investigate some aspects of the structure of boronate esters of carbohydrates.

CONTENTS

ABBREVIATIONS

Ac	acetyl
All	allyl
Bn	benzyl
Bu	butyl
Bz	benzoyl
cat.	Catalytic
°C	Degree Celsius
Cp	Cyclopentadienyl
CSA	camphorsulfonic acid
δ	chemical shifts in parts per million relative to trimethylsilane
d	doublet
dd	double doublet
DAST	diethylaminosulphur trifluoride
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMTST	dimethyl (thiomethyl) sulphonium triflate
DVB	divinylbenzene
Et	ethyl
FAB	fast-atom bombardment
IDCP	iodonium dicollidine perchlorate
m	multiplet
MALDI-TOF	matrix-assisted laser-desorption ionisation time-of-flight

Me	methyl
MS	mass spectrometry
m/z	mass to charge ratio
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
PEG	polyethylene glycol
Pent	*n*-pentenyl
Ph	phenyl
Pht	phthaloyl
ppm	parts per million
Pr	propyl
Py	pyridine
q	quartet
s	singlet
Su	succinoyl
t	triplet
TBDMS	*tert*-butyldimethylsilyl
Tf	trifluoromethanesulphonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
Tr	trityl
Ts	*p*-toluenesulfonyl

Chapter I
General Introduction

Carbohydrates play vital roles in many different biological processes. They constitute structural materials and energy sources and are critical to many complex processes of cellular recognition.[1,2] In the pharmaceutical industry, many carbohydrate based derivatives have been considered as leads for drug development, hence it is necessary to have at hand efficient methods for obtaining quantities of such substances sufficient for evaluation of bioactivity.

Oligosaccharides can be isolated from natural sources, but only small quantities can be obtained, and the materials thus obtained are often impure. Synthesis of oligosaccharide is an attractive alternative. Enzymes have been employed in oligosaccharide synthesis,[3] but this method is hampered by the lack of a sufficient variety of commercially available glycosyltransferases and glycosidases. At the moment, the most convenient method to obtain oligosaccharides is solution based chemical synthesis. This requires high yielding and stereoselective procedures for the formation the glycosidic bond, and in the past two decades new reagents and procedures that partly fulfill these requirements have been developed.

The success of solid phase synthesis of oligopeptides and oligonucleotides prompted synthetic chemists to employ solid phase methodologies for the synthesis of oligosaccharides. The main advantage offered by solid supported synthesis over traditional synthesis in solution is that large excess of reagents can be used to drive a reaction to completion and that time-consuming work-up procedures and chromatographic purifications can be avoided.

However, the synthesis of oligosaccharides on solid support is complicated by several factors. Many glycosylations result in formation of mixtures of anomers and after several coupling steps a complex mixture of products will be produced. In addition, the reactivities of glycosyl donor and acceptor are often significantly reduced after their immobilisation on a solid support. There is currently a great need for the development of novel polymers and linkers that could overcome the drawbacks and limitations associated with the methods currently available.

1.1 Chemical Oligosaccharide Synthesis

The conventional method for the formation of the glycosidic bond involves the coupling of a fully protected glycosyl donor with a suitably protected glycosyl acceptor. The glycosyl donor carries an anomeric leaving group that can be activated by a suitable promoter, while the glycosyl acceptor contains often only one free hydroxyl group.

1.1.1 Glycosyl donors

Traditionally, the most widely used glycosyl donors are the glycosyl halides.[4,5] The last two decades have seen a dramatic increase in the number of new glycosyl donors and procedures that can be used. New donors include thioglycosides, trichloroacetimidates, phosphites, fluorides, sulfoxides, ortho-esters, acetates, n-pentenyl glycosides, thiocyanates, selenoglycosides, 1,2-epoxides and glycals.[6,7] These donors constitute a new and important array of tools for the assembly of oligosaccharides. In the following discussion, attention will be focused only on the chemistry of the glycosyl halides, trichloroacetimidates and thioglycosides, these being the most widely used glycosyl donors.

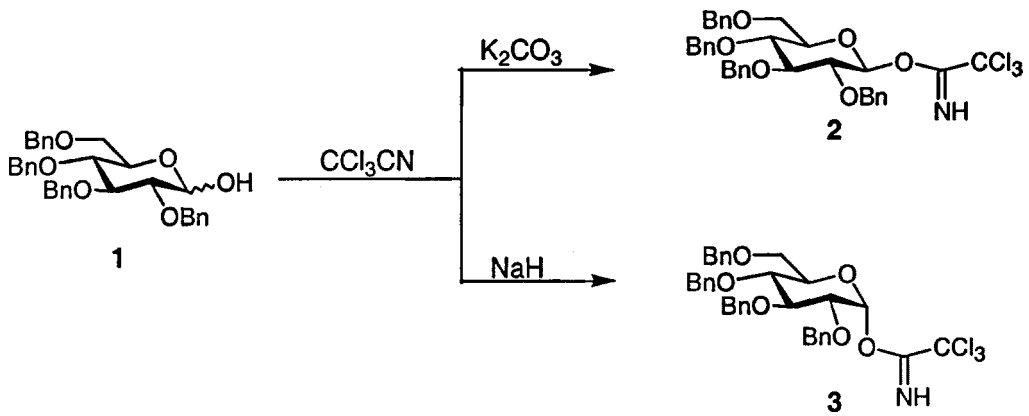
1.1.1.1 Glycosyl Halides

Traditionally, glycosyl halides were the most commonly used glycosyl donors. Typically glycosyl chlorides or glycosyl bromides were employed with activation by heavy metal salts such as Ag_2O, Ag_2CO_3,[4], $HgBr_2$, $Hg(CN)_2$[8] and AgOTf.[9] With careful selection of protecting groups and activators, chloride and bromide glycosyl donors allow to form a glycosidic linkage in high yield and with good α/β selectivity. However, these halides are often unstable and consequently do not have a long shelf-life. Furthermore, they often require relatively drastic conditions for their preparation. These unfavorable characteristics limit the use of glycosyl halides to linear glycosylation strategies.

Glycosyl fluorides, being more stable that chlorides and bromides, are easier to handle, can be purified by silica gel column chromatography, do not suffer from the drawbacks associated with their more reactive counterparts and have therefore found widespread use as glycosyl donors. Many powerful promoters for glycosyl fluorides have been reported, including: $BF_3.Et_2O$,[10,11] TMSOTf,[12] SiF_4,[12] TiF_4,[13] $SnCl_2/AgClO_4$,[14] $CpHfCl_2/AgOTf$,[15] Cp_2ZrCl_2/AgX (X= $^-ClO_4$,[16,17] ^-OTf,[18] $^-BF_4$[18]), Tf_2O,[19] Me_2GaCl,[20,21] $LiClO_4$[22] and $La(ClO_4)_3$[23]. Furthermore, glycosyl fluorides can be prepared efficiently by different approaches. Usually, an anomeric mixture can be prepared by treatment of a glycosyl acetate with HF in pyridine[24] or by treatment of a corresponding hemiacetal with DAST.[25,26] The latter method has proven to be convenient and highly yielding. Alternatively, glycosyl fluorides can be obtained by reaction of thioglycosides (*e.g.* SEt, SPh) with NBS/DAST.[27] Due to their high stability, glycosyl fluorides are widely used in convergent syntheses of oligosaccharides.

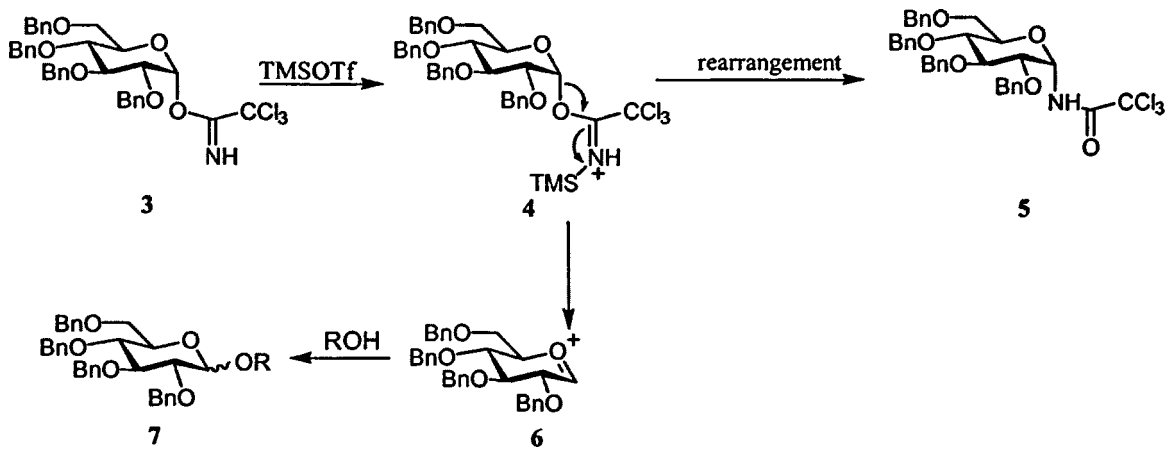
1.1.1.2 Trichloroacetimidates

Anomeric trichloroacetimidates were first reported by Schmidt and co-workers[28,29] in 1980, and they have proven to be highly effective donors to form glycosidic bonds due to their high reactivity under mild acidic conditions. The requisite anomeric trichloroacetimidates can be readily prepared from the corresponding hemiacetal. For example, the β-trichloroacetimidate of glucose **2** can be readily prepared in excellent yield from the corresponding hemiacetal **1** by treatment with trichloroacetonitrile and potassium carbonate[30] whereas in the presence of NaH,[28] DBU,[31] Cs_2CO_3[32] or *aq.* KOH,[33] the α-anomer **3** is formed exclusively (**Scheme 1.1**).



Scheme 1.1

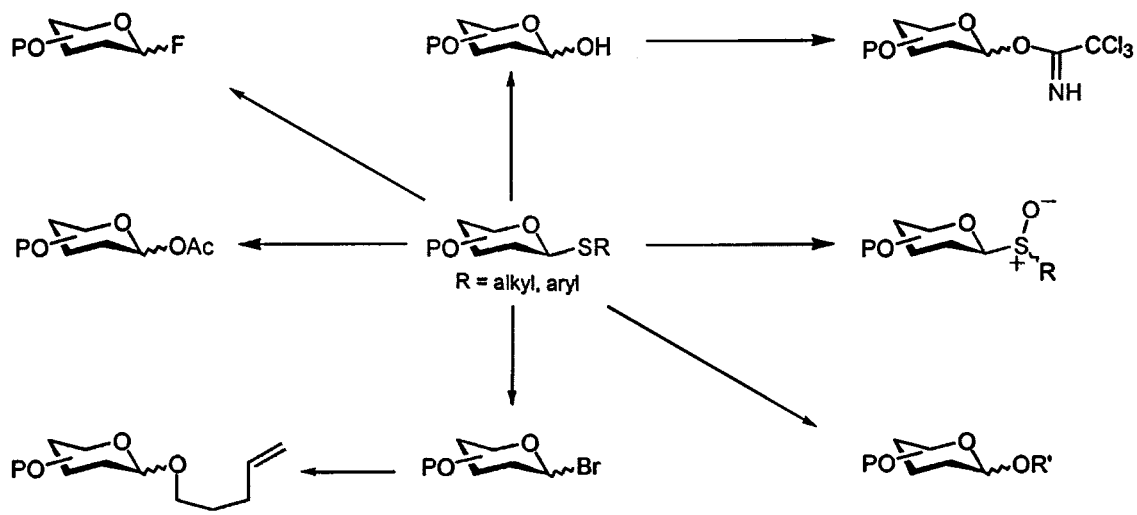
Glycosylations with trichloroacetimidates can be smoothly promoted by various mild promoters, such as catalytic amounts of $BF_3.Et_2O$,[29] TMSOTf,[29] $ZnBr_2$,[32] CCl_3CHO,[34] AgOTf,[35] and PPTS.[36] In general, glycosylations with trichloroacetimidates give good yields. However, if the reactivity of a glycosyl acceptor is very low, a significant amount of trichloroacetamide (*e.g.* **5**) can result from the rearrangement of **3** therefore decreasing the yield of a glycosylation (**Scheme 1.2**).[37]



Scheme 1.2

1.1.1.3 Thioglycosides

Alkyl and aryl thioglycosides have found widespread use as glycosyl donors due to their ability to offer effective temporary protection of the anomeric centre, while functioning as excellent anomeric leaving groups upon activation with suitable promoters. Furthermore, thioglycosides are also appropriate intermediates for conversion into other commonly used glycosyl donors (**Scheme 1.3**).



Scheme 1.3

The present popularity of thioglycosides is partly due to their ease of activation by a range of thiophilic promoters. Different promoters towards different types of alkyl- and arylthio groups are summarised in **Table 1.1**.

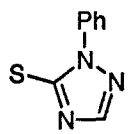
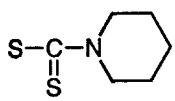
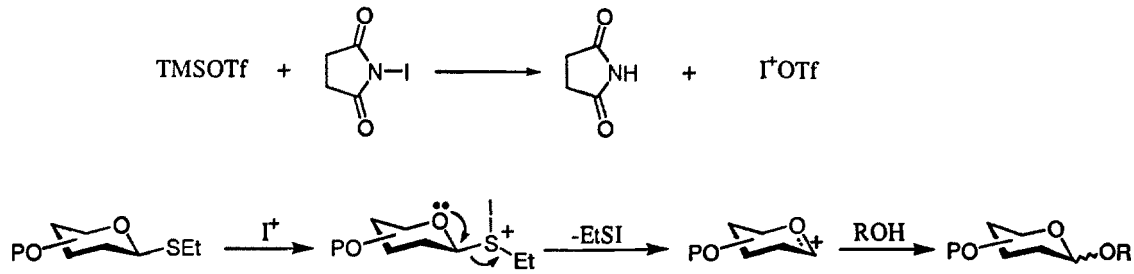
Promoter	SR	Reference
MeOTf	SMe, SEt, SPh	38,39
DMTST	SMe, SEt, SPh	40
$NOBF_4$	SMe, SEt, SPh	41
$TrClO_4$	SCN (ROTr as acceptor)	42
MeSOTf, MeSBr	SMe, SEt, SPh	43
PhSeOTf	SMe	44
MeI	SPy	45
NIS-TfOH	SMe, SEt, SPh	46
NIS-TESOTf (AgOTf)	SMe, SEt, SPh	47
$CuBr_2$-Bu_4NBr-AgOTf	SMe, SEt	48
NBS	SPh	49
I_2	SMe, SEt	50
IDCP	SEt	51
AgOTf		52
TBPA	SEt, SPh	53
DMTST, AgOTf $SnCl_4$, $FeCl_3$		54

Table 1.1

Among these reagents, DMTST and NIS-TfOH (TMSOTf or AgOTf) seem to be most promising and versatile, producing rapid reaction and giving satisfactory yields in most of thioglycoside based glycosylations. The mechanism of activation is outlined in **Scheme 1.4.**

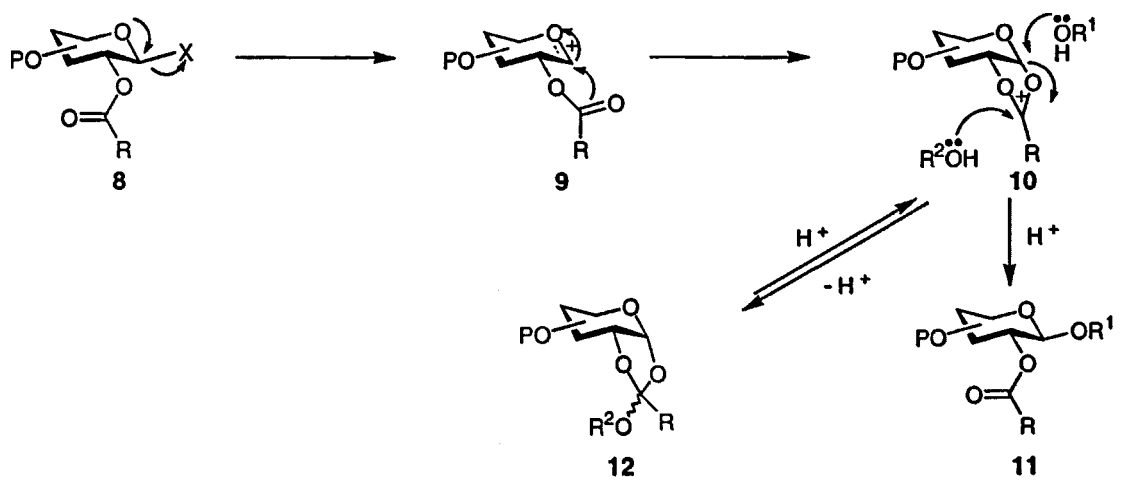


Scheme 1.4

1.1.2 Strategies for stereoselective glycosylations

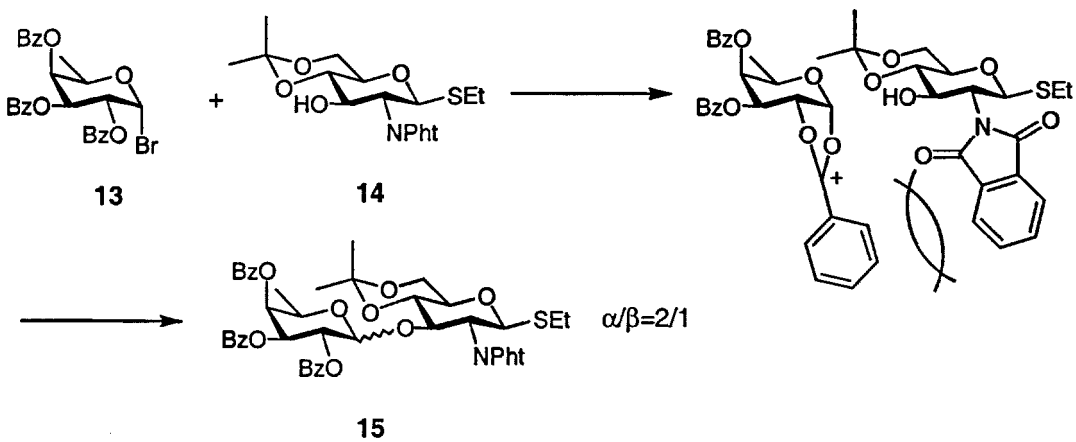
1.1.2.1 Neighbouring group participation[5]

In general, 1,2-*trans* glycosidic linkages can be reliably obtained when the C-2 position of a glycosyl donor is protected by an ester functionality (acetyl, benzoyl, pivaloyl, *etc.*). As can be seen in **Scheme 1.5**, after the formation of oxycarbonium ion **9**, the more stable acyloxonium ion **10** is formed *via* the participation of the ester group at C-2. Opening the ring by nucleophilic attack at the anomeric centre results in the formation of a 1,2-*trans* glycosidic bond. The transient acyloxonium ion **10** can be trapped by an alcohol nucleophile, resulting in the formation of the 1,2-orthoester **12**. With the usual acidic catalysts employed in glycoside synthesis, the formed orthoester **12** can rearrange to the β-glycoside **11** *via* the intermediate **10**, but it may be isolated as the main product under basic condition (*e.g.* excessive collidine).[37]



Scheme 1.5

Orthoester formation is also dependent on the acyl protecting group. Acetates are more susceptible then benzoates to form orthoesters, presumably due to electronic and steric factors. In fact, C-2 benzoates often give higher yields than C-2 acetates in glycosylation reactions.[37] Neighbouring group participation offers a highly reliable approach for the formation of 1,2-*trans* glycosidic linkages. However, some exceptions have been reported[55] where anomeric mixtures were obtained. For example, the coupling of glycosyl bromide **13** with **14** in the presence of AgOTf at -50°C gave disaccharide **15** as an anomeric mixture (α/β = 2/1)(**Scheme 1.6**). It was rationalised that the predominant formation of 1,2-*cis* linkage was due to the severe steric hindrance in the transition state caused by the bulky phtalimido group, leading to the formation of the β-anomer.

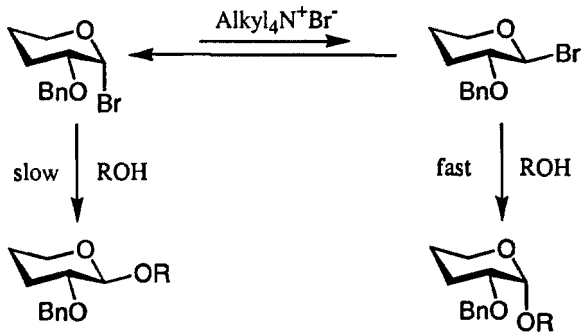


Scheme 1.6

1.1.2.2 *In-situ* anomerisation

α-Glycopyranosides from D-glucose, D-galactose and L-fucose have 1,2-*cis* arrangements and one of the approaches for their preparation involves the use of β-glycosyl halides which have a non-participating group at C-2, such as a benzyl ether. When a β-glycosyl halide is treated with an alcohol in solvents of low polarity in the presence of an active catalyst, displacement tends to occur with inversion of configuration and therefore mainly the α-glycoside is formed.

Unfortunately, β-anomers of glycosyl halides are very labile and difficult to handle. This difficulty has been ingeniously circumvented by Lemieux's *in situ* anomerisation method.[56]



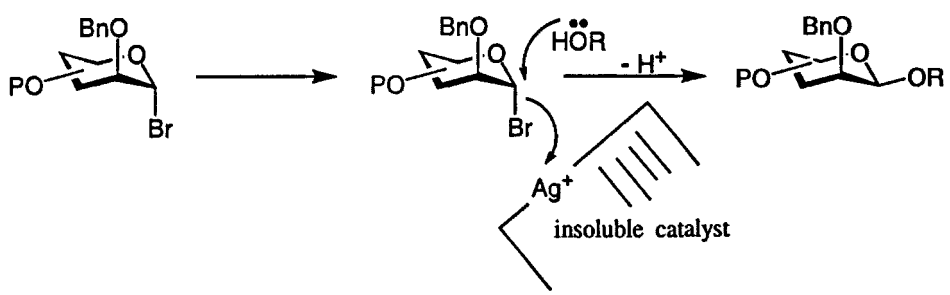
Scheme 1.7

In the presence of tetraalkylammonium bromide, the α- and the β-anomers of a glycosyl bromide are in fast equilibrium (**Scheme 1.7**). The equilibrium is shifted strongly towards the α–bromide since this anomer is stabilised by the anomeric effect and is therefore less reactive. As the β–anomer is more reactive, glycosylation will mainly occur with β–anomer *via* a S_N2 reaction and the α–linked product will be formed. Provided that the rate of equilibration between α- and β-anomer is sufficiently faster than that of glycosylation, the α–linked glycoside will be predominantly formed.

The concept of *in situ* anomerisation has also been applied to other glycosyl donors (*e.g.* fluoride, thioglycoside and trichloroacetimidate). With careful control of reaction conditions (solvents, activator, the reactivity of donor and acceptor, *etc.*), this method provides in some cases an efficient method for the preparation of α–glycosidic linkages with high anomeric selectivity.

1.1.2.3 Glycosylation with inversion of configuration

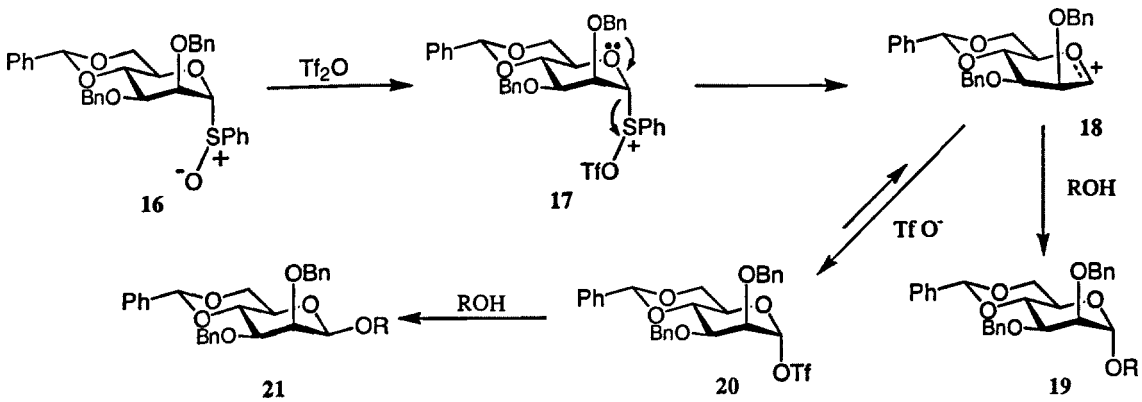
The formation of β-manno and β-rhamnosidic bonds, which cannot be formed by neighbouring group participation or *in situ* anomerisation, is one of the most challenging aspect of oligosaccharide synthesis. However, this difficulty could be solved by converting a relatively stable α–glycosyl halide to a β-glycoside *via* inversion of anomeric configuration. While the *in situ* anomerisation procedure requires a fast equilibration between the α- and the β-anomer, a procedure with inversion of configuration requires that anomerisation of the α-anomer is prevented. This can be achieved by using an insoluble promoter. The surface of the promoter can complex the α–anomer of a glycosyl halide, and the absence of nucleophiles in solution, other than the glycosyl acceptor, restricts the possibility of anomerisation. However, a very reactive halide and an hydroxyl group of sufficient reactivity need to be used in order to achieve α-selectivity. With unreactive hydroxyl groups the proportion of α-glycoside product increases. Several insoluble catalysts (*e.g.* silver oxide, silver silicate[5,57]) have been reported. The essence of this method is outlined in **Scheme 1.8.**



Scheme 1.8

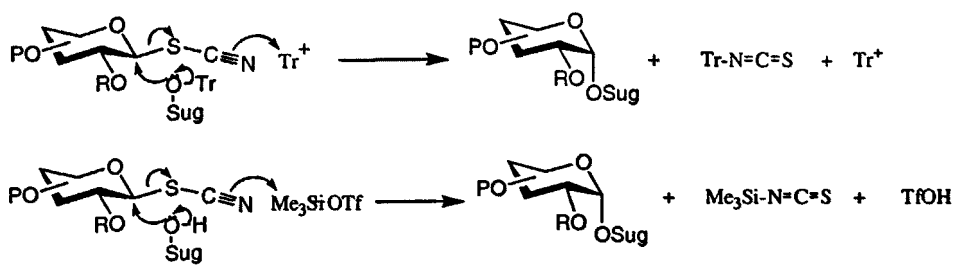
Crich *et al.*[58,59] reported a direct synthesis of β-mannopyranosides using glycosyl sulfoxide methodology. As can be seen in **Scheme 1.9**, after the activation of sulfoxide **16** with Tf_2O, the oxycarbenium cation **18** is formed which is trapped directly by the glycosyl acceptor to give an α-mannoside. However, if the sulfoxide is activated with Tf_2O for 5 minutes in absence of a glycosyl acceptor, **18** is trapped axially by a triflate anion to give the glycosyl

triflate **20**, which after addition of a glycosyl acceptor undergoes a S_N2-like substitution to give β-mannoside **21**. In this approach, glycosyl triflate **20** served as a glycosyl donor and was generated from the corresponding sulfoxide *in situ*. By selecting appropriate solvent and protecting groups, this method provides an attractive way to synthesise β-mannosides.



Scheme 1.9

Kochetkov and co-workers[42,60,61] reported an efficient method to synthesise 1,2-*cis* pyranosides by employing 1,2-*trans* glycosyl thiocyanates as glycosyl donors. $TrClO_4$[42,60] was used as promoter for the glycosylation of tritylated ether acceptors whereas TMSOTf[61] was used for acceptors containing a free hydroxyl group. The reaction proceeds by an S_N2 mechanism, with inversion of configuration. This is illustrated in **Scheme 1.10**. In case of the $TrClO_4$ promoted glycosylation, the tryphenylmethylium cation is attacked by the nitrogen of the thiocyanate group while the oxygen of the trityl ether attacks the anomeric carbon in a concerted push-pull process.

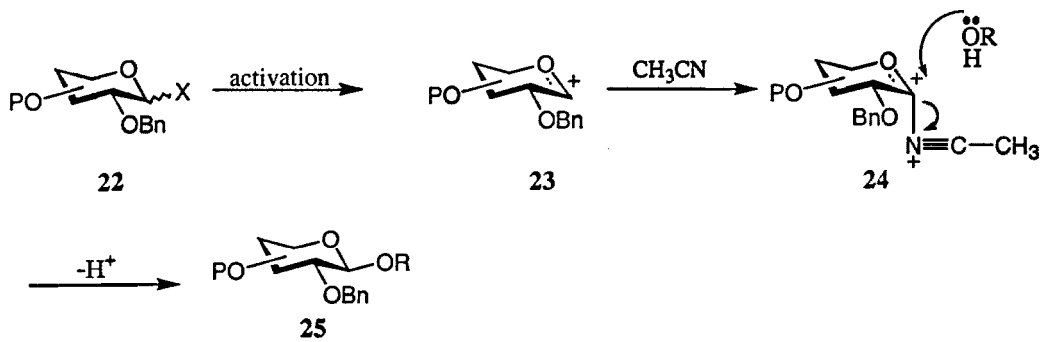


Scheme 1.10

1.1.2.4 Solvent participation

It is well established that solvents play an important role in the stereochemical outcome of glycosylations. When glycosyl donors with no participating group at the C-2 position are used in a glycosylation, different ratios of anomeric mixtures are obtained when different solvents are used. Furthermore, some solvents can react with the oxycarbenium ion and consequently affect the orientation of the incoming nucleophile.

Acetonitrile[62-65] is the most studied participating solvent which in many cases leads to the formation of β-glycosidic linkages (**Scheme 1.11**).



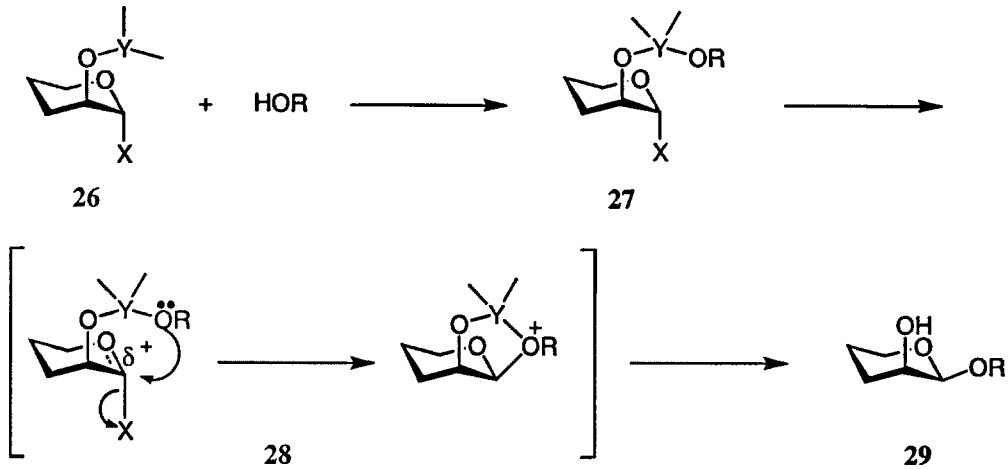
Scheme 1.11

It has been proposed that glycosylations in acetonitrile proceed through the formation of α-nitrilium ion intermediate (*e.g.* **24**) followed by the nucleophilic substitution by a glycosyl acceptor to give β-glycoside **25**.

1.1.2.5 Intramolecular aglycon delivery

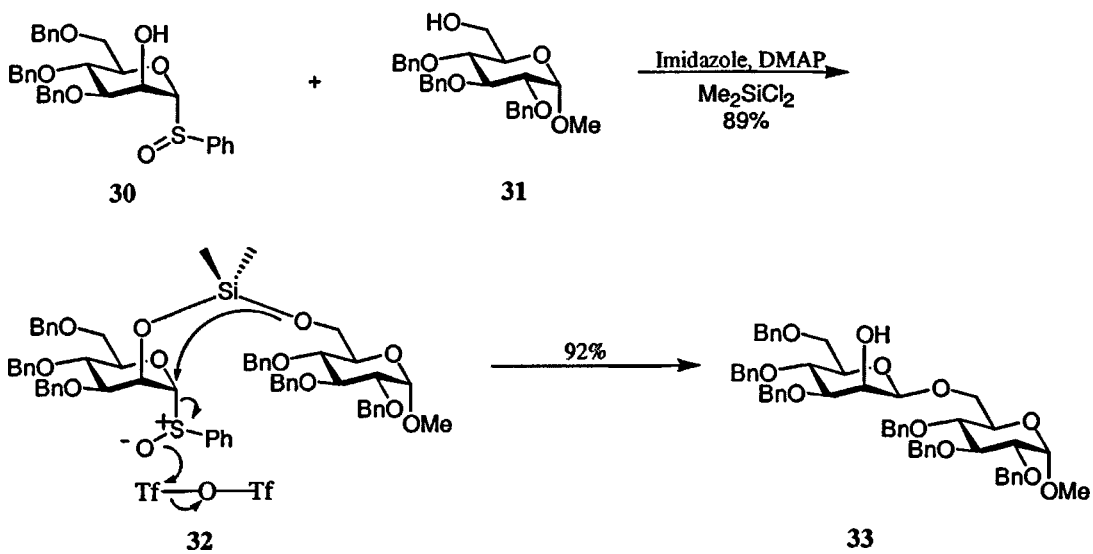
For the purpose of β-mannoside synthesis, the concept of intramolecular glycosylation was introduced independently by Stork and Hindsgaul.[66-68] A temporary tether was utilised to link the glycosyl acceptor to the C-2 position of a mannosyl donor **26** (**Scheme 1.12**). The

activation of the anomeric centre leads to stereospecific intramolecular aglycon delivery *via* a five-membered transition state **28** to give the *cis*-linked glycoside **29**.



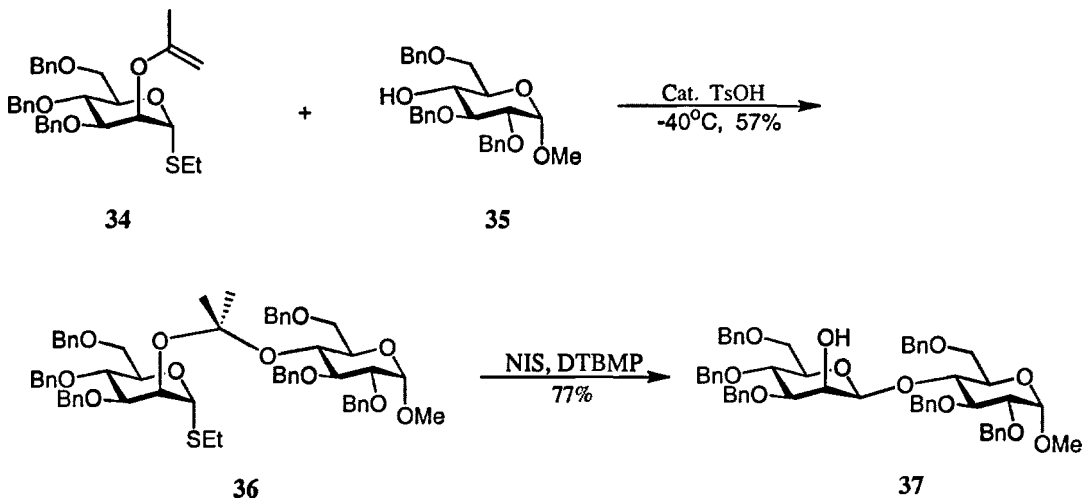
Scheme 1.12

A silicon tether, which was designed by Stork and co-workers, could be readily introduced under mild basic conditions (**Scheme 1.13**). The anomeric sulfoxide of compound **32** was activated by Tf_2O and the β-linked mannoside **33** was formed in an excellent yield with complete stereoselectivity.



Scheme 1.13

Hindsgaul *et al.* reported a dimethyl ketal tether for the same purpose (**Scheme 1.14**). After treatment of vinyl ether **34** with a stoichiometric amount of **35** in the presence of catalytic amount of TsOH, the resulting adduct **36** was reacted with NIS and DTBMP to produce β-mannoside **37** in a yield of 77% without formation of the α-anomer.



Scheme 1.14

A modification of the acetal strategy was reported by Ogawa and co-workers[69,70] They demonstrated that an intramolecular *p*-methoxybenzylidene acetal tether can be introduced by treating a mixture of a mannoside, bearing a *p*-methoxybenzyl group at C-2, and glycosyl acceptor with DDQ. Activation of the anomeric methylthio group with MeOTf leads to the formation of β-mannosides in good yields.

Apart from the synthesis of β-mannosides, the concept of intramolecular aglycon delivery is also applicable to the synthesis of 1,2-*cis* glucoside[71] and branched oligosaccharides.[72]

1.2 Solid phase oligosaccharide synthesis

Despite many recent advances, solution based synthesis of complex oligosaccharides still has 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 the techniques of solid phase synthesis to the preparation of oligosaccharides. In principle, large excess of reagents can be used to drive glycosylation reactions to completion and the excess of 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 can be employed on solid support.[73]

1.2.1 Linkers for Solid Phase Organic Synthesis[74]

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 cleavage should be efficient under conditions that do not damage the final product. One of the key challenges is to utilise cleavage reagents that are easily removed from the cleaved product. Many linkers don't meet all of these criteria.

1.2.2 Polymers

The most commonly used polymer backbone in solid phase synthesis is polystyrene, crosslinked with 1 or 2% divinylbenzene.[75] 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 (3-6 mL/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 crosslinking, up to *ca.* 5% provide resins more stable 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.[76] In contrast to the swellable resins, these supports show a permanent porosity, and no swelling is necessary. They are characterised by better mechanical and thermal stability, but their loading capacity is lower than polystyrene based supports. An example of this kind of support is controlled pore glass (CPG).[77]

It is important to note that resin parameters like crosslinking, swelling properties and bead size have a major effect on the outcome of a reaction that happens on the support, and they have to be considered.

Another type of support has been obtained by grafting polyethylenglycol (PEG) chains onto polystyrene crosslinked resin.[78] The resin thus obtained (Tentagel), even though presenting a lower loading than crosslinked polystyrene, has proved to be more effective than normal

polystyrene for automated peptide synthesis, owing to improved swelling and mechanical properties. This PEG grafted polymer swells in all the 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 chains behave as though they were in solution,[79] 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.[79]

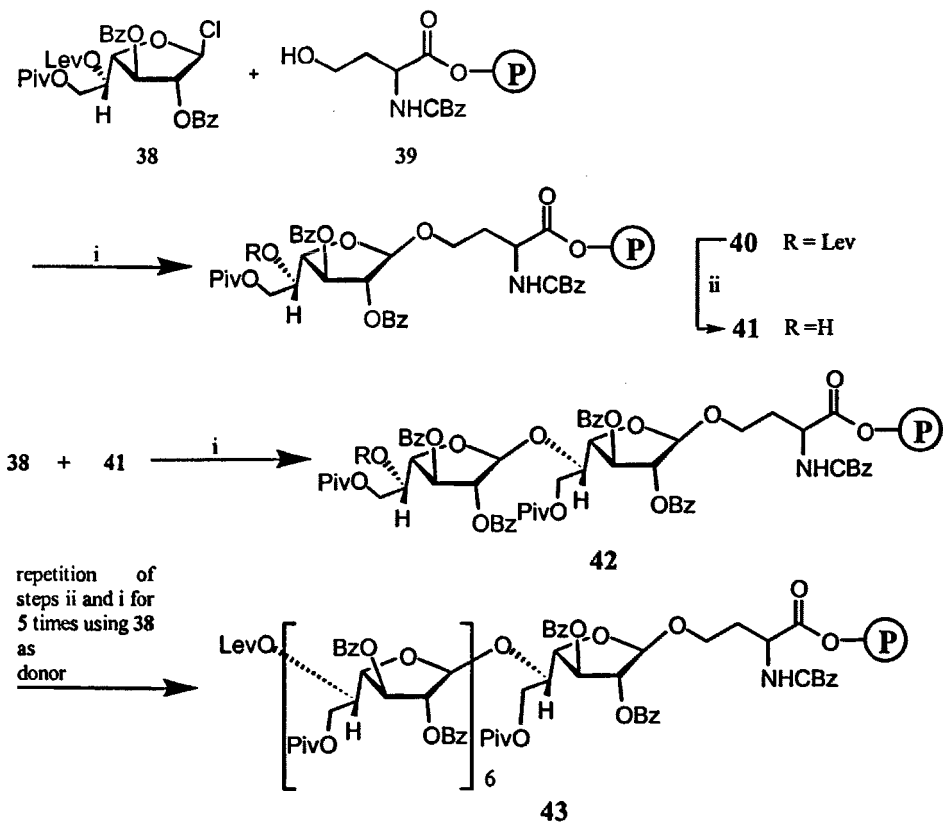
A major limitation of using solid support is the difficulty in characterizing the products while still on the resin. Analysis of the products can be performed by cleaving the product from a small portion of the polymer and analysis by TLC or MS. Recently, magic angle spinning (MAS) has been used for observation of small molecules bound to a resin.[80]

An alternative to the use of a solid support is the use of soluble polymeric supports.[81] In this approach, purification of the products is achieved by adding the polymer with a solvent, such as hydrocarbons, that induces precipitation of the macromolecular 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 functionalised PEG of molecular mass between 3000-20000 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.[81] Soluble polymeric supports allow to follow individual reaction using NMR and other techniques without the need to cleave a fraction of the product from the support itself.

1.2.3 Development of Solid phase Oligosaccharide Synthesis

The first attempts to synthesise oligosaccharides on solid support were conducted in the early seventies,[82,83] 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.

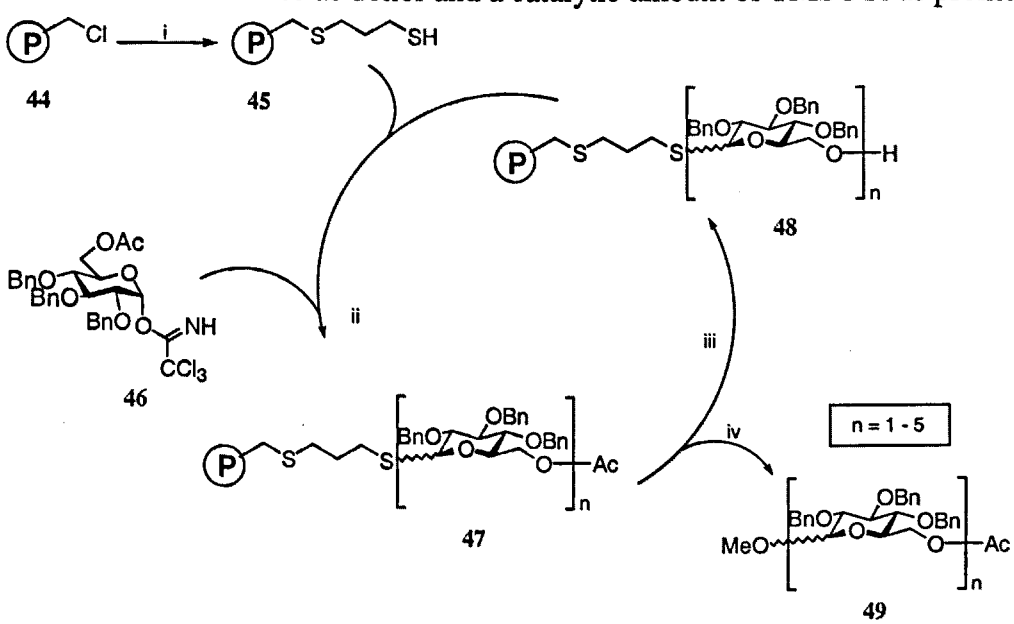


Reagents and conditions: i. $Hg(CN)_2$ / $HgBr_2$ ii. NH_2NH_2 / HOAc/ Pyridine

Scheme 1.15

In 1987, van Boom *et al.*[84] reported the synthesis of β-(1→5)-linked D-galactofuranosyl heptamer on solid support. The anomeric centre of the first sugar residue was linked to L-homoserine derivatised Merrifield polystyrene **39** and D-galactofuranosyl chloride **38** was employed as a glycosyl donor for chain elongation, using $Hg(CN)_2/HgBr_2$ as promoter (**Scheme 1.15**). 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 **43** was obtained after six repeated glycosylations in a yield of 23% .

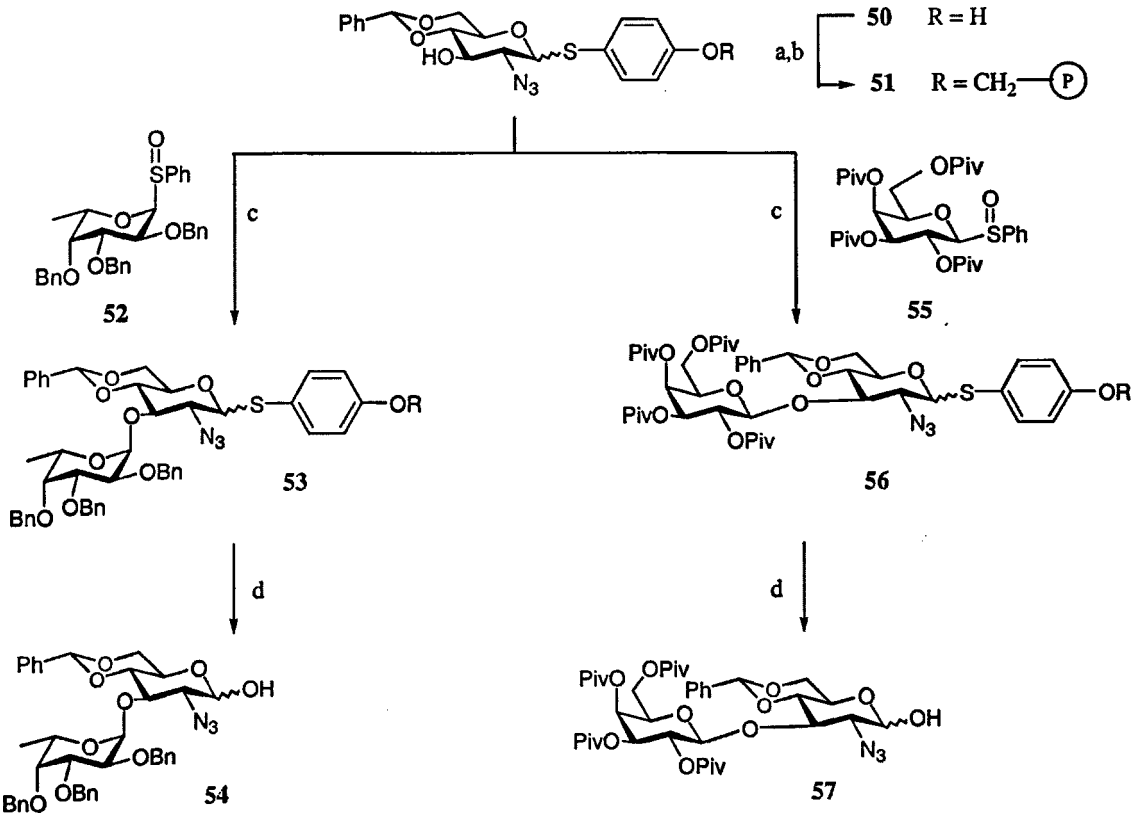
Recently, Schmidt *et al.*[77,85] described the synthesis of oligosaccharides on solid support using trichloroacetimidates as glycosyl donors. Glycosyl donor **46** was attached to the solid support through a thiol linker that can be cleaved by reaction with NBS (**Scheme 1.16**). Synthesis of linear pentasaccharide **49** (n=5) was achieved by cleaving the acetyl protecting group from compound **47** and glycosylating the so obtained acceptor **48** using trichloroacetimidate **46** as donor and a catalytic amount of TMSOTf as promoter.



Conditions: i). propanedithiol, DBU ii) TMSOTf, DCM, r.t. iii) DCM / 0.5 M NaOMein methanol iv) DMTSB, DIPEA, DCM / MeOH

Scheme 1.16

Kahne and co-workers[86] 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 dependent on the protecting groups of the donor itself. At low temperature, excellent stereochemical 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 **54** and **57** where obtained stereoselectively with overall yield of 67% and 64% respectively after cleavage from the Merrifield resin used as a solid support (**Scheme 1.17**).



Conditions: a. Cs_2CO_3, MeOH. b. P—CH_2Cl, N-methylpyrrolidinone, 55°C
c. Tf_2O, DTMPB, -60--30°C, DCM. d. $Hg(OCOCF_3)_2$, DCM, H_2O

Scheme 1.17

Solid phase methodology has also been employed in the combinatorial synthesis of carbohydrate libraries. Kahne and co-workers[87] reported the synthesis of a library of

approximately 1300 di- and trisaccharides using sulfoxide donors. The library was synthesised by a mix and split strategy starting from the monomers shown in **Figure 1.1.** Six different monomers were attached separately onto Tentagel resin beads. Next, twelve different glycosyl sulfoxide donors were coupled separately to mixtures of beads containing all six monomers. The beads were then combined, and the azido groups were reduced to amines. The beads were split into nineteen portions and eighteen were allowed to react with different acylating reagents. Finally, all beads were recombined and deprotected to give a polymer bound di- and trisaccharide target library.

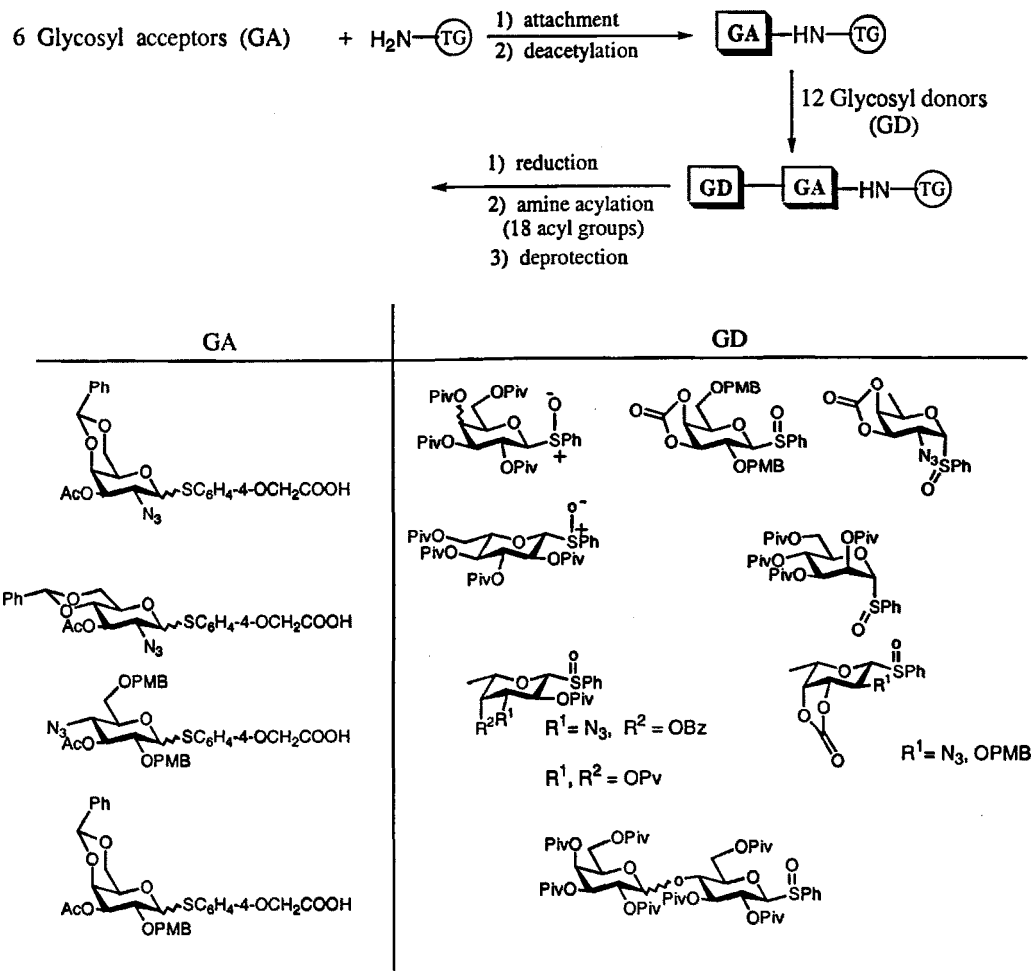
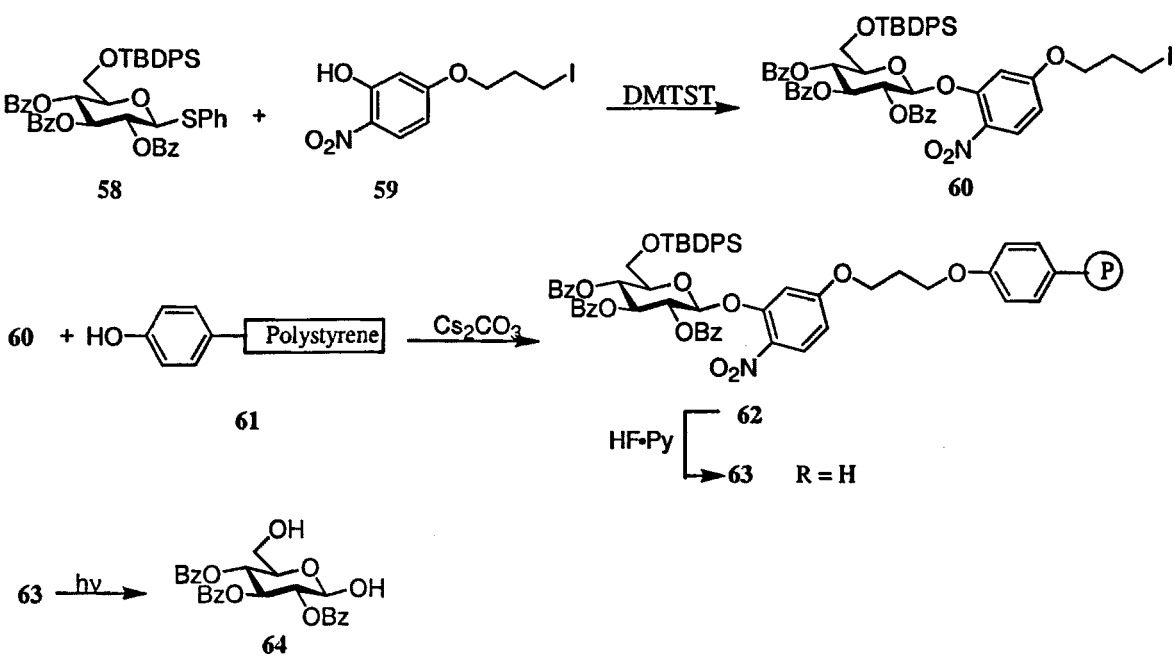


Figure 1.1

To facilitate identification of the products, the beads were encoded with chemical tags after each combinatorial step in order to record the reaction history of each bead, according to the

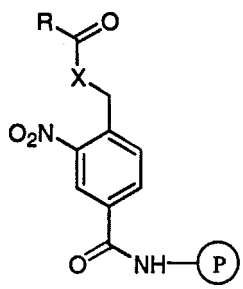
procedure developed by Still and co-workers.[88,89] In Still's procedure, beads-tagging molecules that encode both the step number and the chemical reagent used in that step are attached to the beads after each step of the synthesis. The tagging molecules are not sequentially connected. At the end of the synthesis, cleavage from a bead and capillary GC analysis allows separation and identification of the tags, and thus of the oligosaccharide bound to the bead.

A *o*-nitrobenzyl ether photolabile linker was used by Nicolaou and co-workers[90] for the synthesis of a heptasaccharide phytoalexin elicitor (HPE). This linker is stable to the reaction conditions used during the synthesis but can be easily cleaved by exposure to UV light. A monosaccharide was initially attached to the resin following the procedure described in **Scheme 1.18** and subsequently cleaved by irradiation in order to demonstrate the feasibility of this approach.

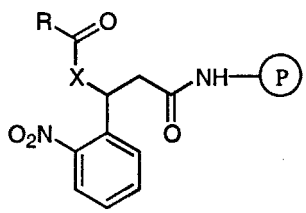


Scheme 1.18

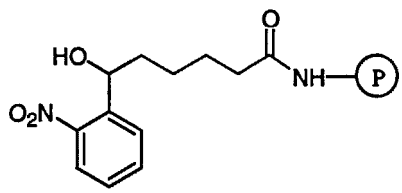
Recently a similar type of photocleavable linker, **67**, was developed by Fraser-Reid and co-workers[91] and was used in their synthesis of a branched trimannan. They anticipated that linker **66** should be cleaved faster then linker **65**, due to the secondary benzylic group present in **66** being abstracted more easily then the primary one present in linker **65** (**Figure 1.2**). Linker **67** was constructed by connecting it to the polymer through an alkyl chain, and by changing the amide or ester function used to bind the substrate with an hydroxyl functionality. The length of the chain in **67** was chosen in order to avoid elimination from an α/β unsaturated system as well as lactonisation of the alcohol into the carbonyl moiety during preparation of the linker. After these modification, linker **67** was found to be cleaved at a speed comparable to linker **66**. During the subsequent synthetic sequence pentenyl glycosides were used as glycosyl donors.



65a X = NH
65b X = O



66

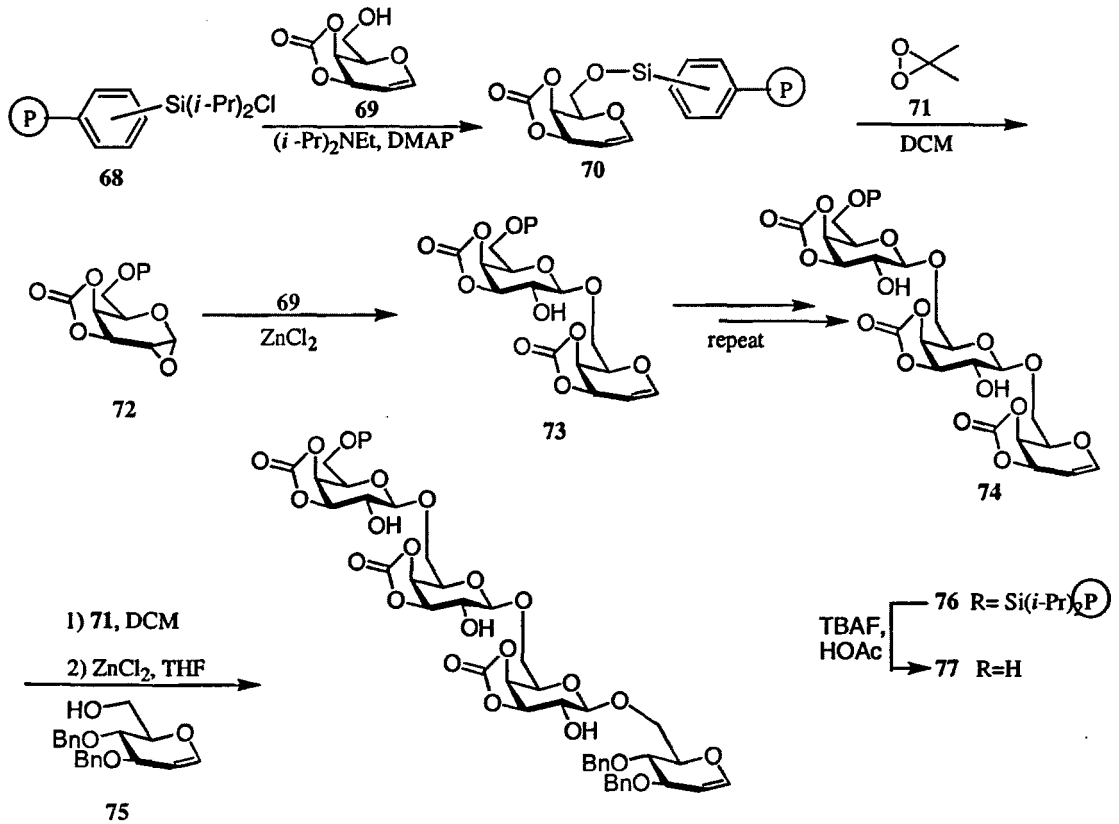


67

Figure 1.2

Two options are available when choosing the direction of elongation of the oligosaccharide chain: from the non-reducing end to the reducing end or from the reducing end to the non-reducing end. The latter approach has generally the distinct advantage of allowing to use excess of glycosyl donor in order to drive glycosylation reactions to completion. The procedures described above are an example of this type of approach. However, Danishefsky *et*

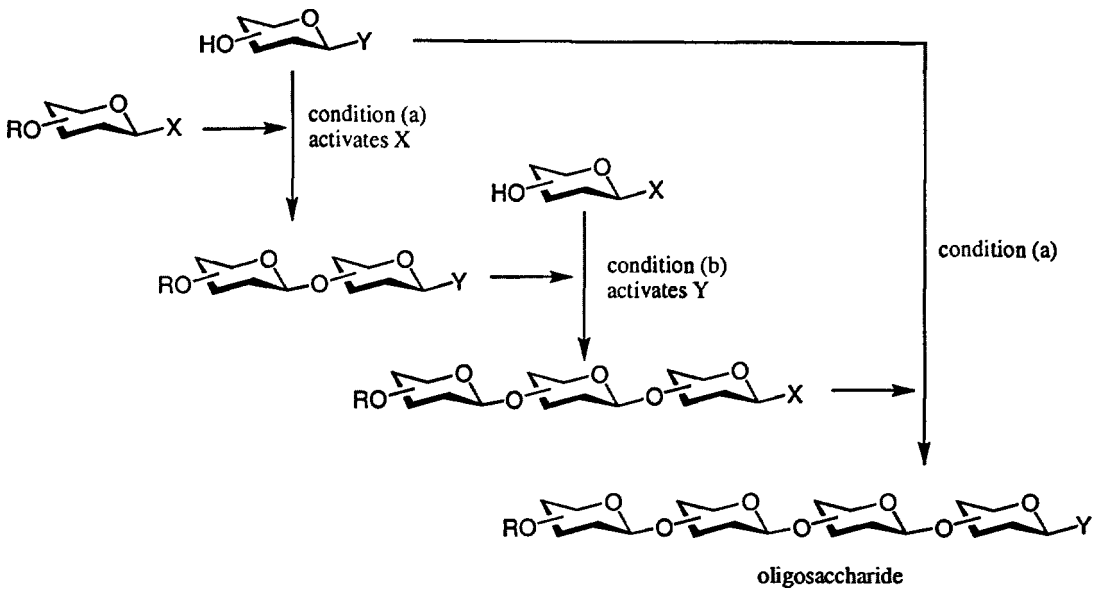
al.[92-94] reported a method for the assembly of oligosaccharides on solid support based on the opposite approach of chain elongation, *i.e.* from the non-reducing end to the reducing end.



Scheme 1.19

As illustrated in **Scheme 1.19**, a glycal was linked through a silyl ether linker to a divinyl benzene polystyrene copolymer. The polymer-bound glycal, upon activation by epoxidation, functioned as a glycosyl donor, giving the β-anomer only. Indeed, epoxide **72** reacted with large (6-10 fold) excess of glycal **69** in THF in the presence of $ZnCl_2$ to give polymer-bound disaccharide **73**. Repetition of this two step process of epoxidation with **71** followed by glycosylation with **69** afforded trisaccharide glycal **74**. The polymer bound **74** was then epoxidised and coupled with glycal **75**. Tetrasaccharide **77** was obtained in 74% overall yield after the treatment of **76** with TBAF. The advantage of this method arises from its self-policing nature. Unreacted epoxide after each glycosylation step was hydrolysed during work-

up and the resulting 1,2-diol may be subject to further degradation. Therefore, the failure to couple leads to chain termination and no capping step is required.

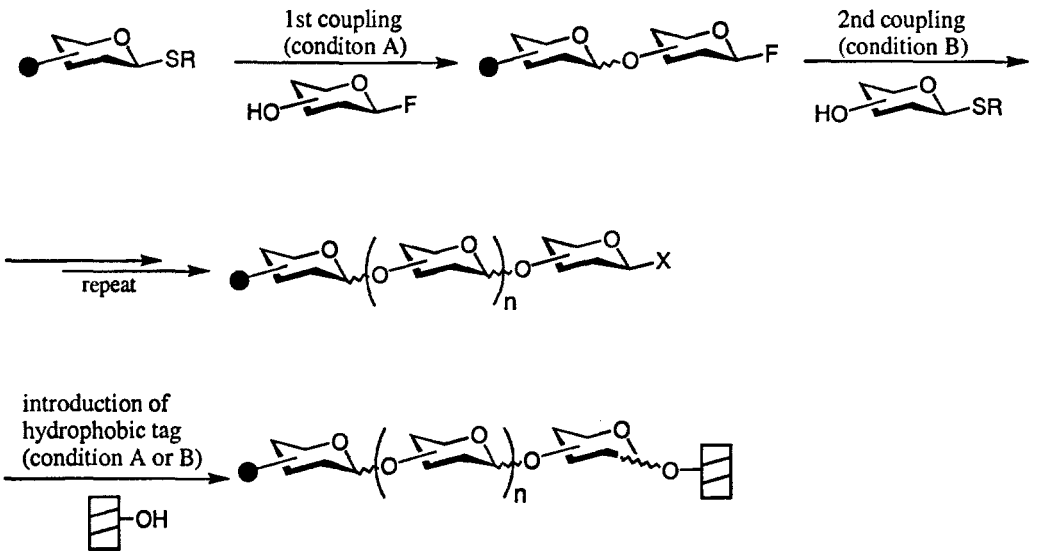


Scheme 1.20

Ogawa *et al.*[95] employed an orthogonal glycosylation strategy on solid support. In such a strategy, first developed in solution,[96] two sets of chemically distinct glycosyl donors and activation conditions are used, and the product of each glycosylation can act as a glycosyl donor in a subsequent reaction without performing any manipulations at the anomeric centre, consequently reducing the number of synthetic steps. In order to make this approach practical, the anomeric leaving group (X, Y) and the protecting groups (R) should be carefully selected. X should be unaffected under the conditions that activate Y and *vice versa*. For the purpose of this strategy, the phenylthio group was selected as X and fluoride as Y **(Scheme 1.20)**.

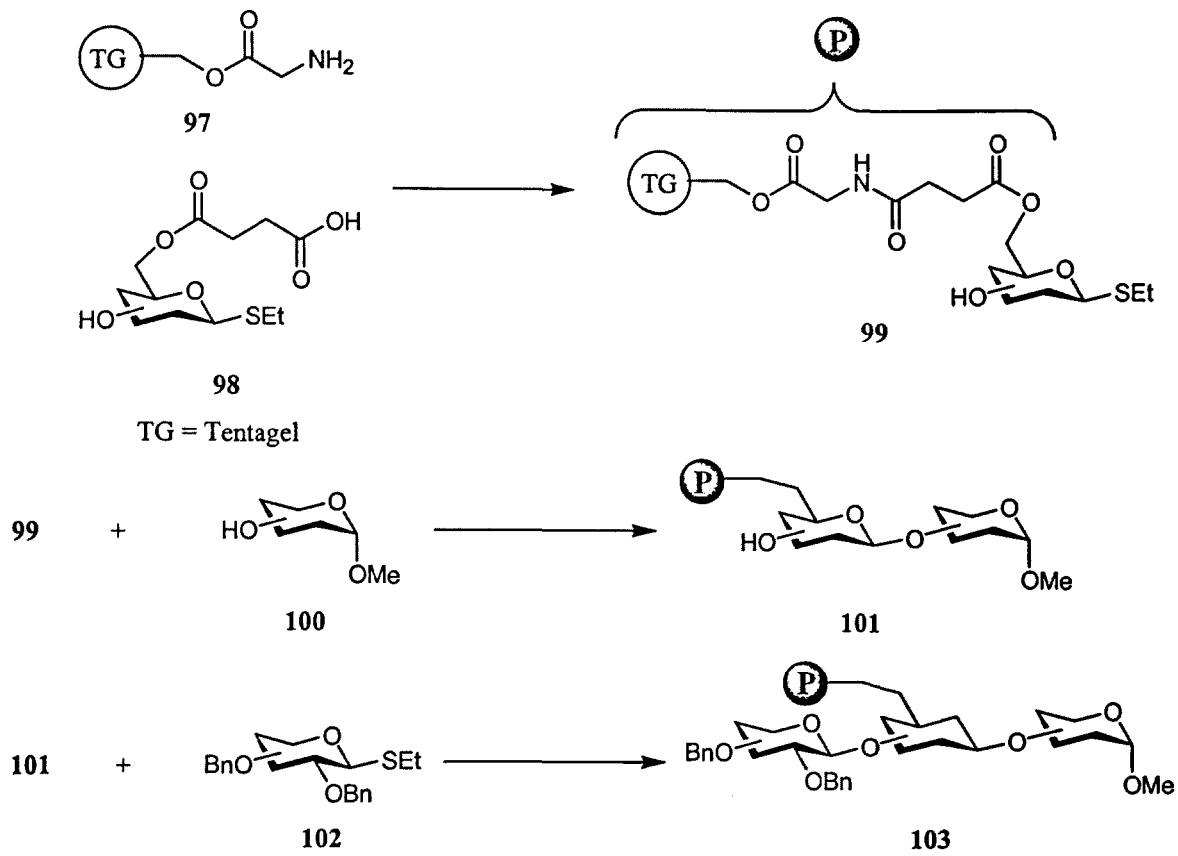
The fact that glycosyl fluoride or thioglycosides immobilised on a resin act as a glycosyl donor in chain elongation can potentially hamper the process. All by-products accumulate on the solid support together with the desired product, and this complicates the process of detachment of the product from the resin and its purification. Ogawa[95] addressed this problem

by introducing a hydrophobic tag (*e.g.* 2-trimethylsilylethyl group) at the reducing end of the target oligosaccharide chain. The tag allows facile separation of the target oligosaccharide from all the other polymer bound side-products. The essence of this method is depicted in **Scheme 1.21**. A trimannoside was prepared using this methodology.



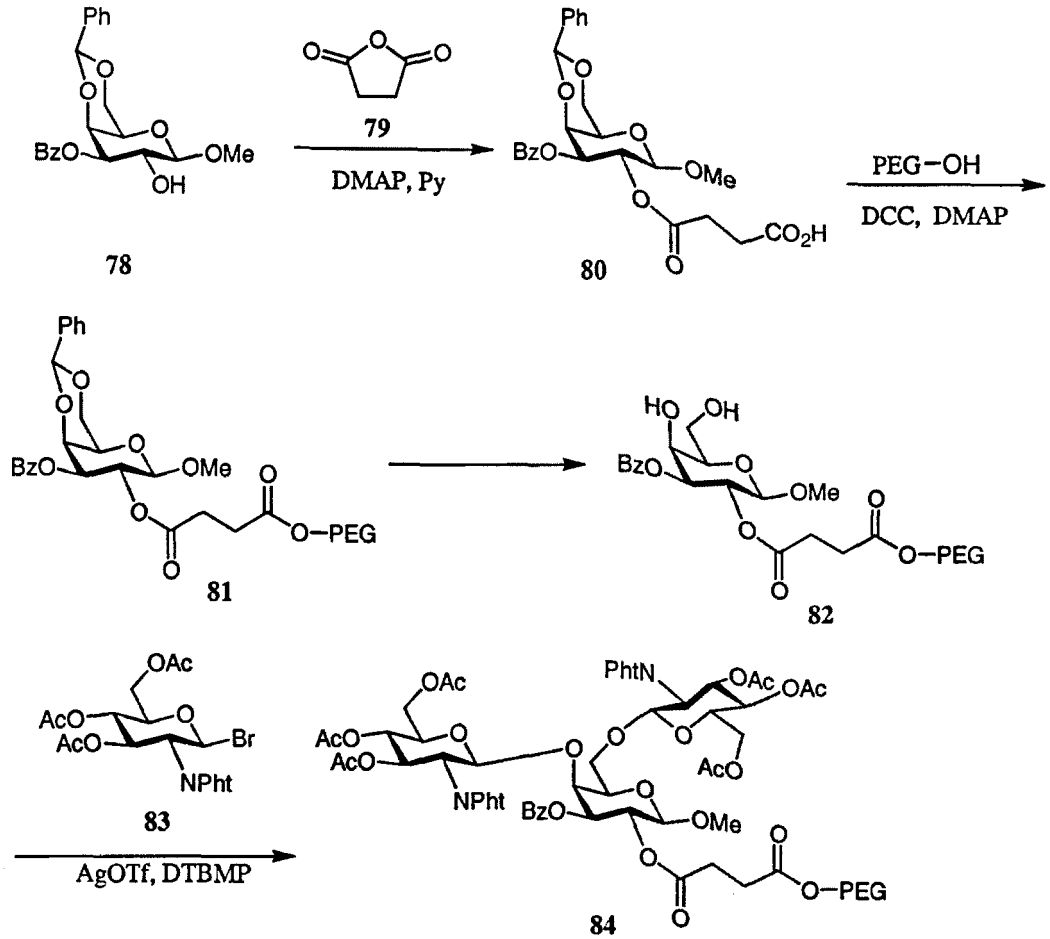
Scheme 1. 21

Recently Boons *et al.*[97] reported the synthesis of a combinatorial saccharide library on solid support whereby all glycosydic linkages were intentionally synthesised as mixtures of anomers. A novel two-directional approach was used in which every saccharide immobilised on solid support can act as a glycosyl donor as well as an acceptor. The essence of this procedure is described in **Scheme 1.22**. Glycosyl donor **98**, after being linked to the solid support through a succinoyl bridge, can act both as a glycosyl donor and a glycosyl acceptor.



Scheme 1.22

In general, the rate of a reaction on solid support is remarkably reduced when compared to the same reaction in solution phase. This is probably due to the fact that it is more difficult for the reagents to access the immobilised substrate. Krepinsky and co-workers[98] addressed this problem by utilizing polyethyleneglycol mono-ethyl ether (MPEG) as the supporting polymer. The MPEG-carbohydrate conjugate is soluble in the solvent used for the glycosylation reactions. After a reaction is completed, it can be precipitated by addition of hydrocarbons or dietyl ether. The resulting insoluble mixture can be filtered and washed, thus removing impurities and excess of reagents and allowing to recover the pure product after cleavage from the polymeric support. The solubility of the reactants allows to achieve reaction kinetics and anomeric control similar to those observed in solution chemistry. A succinoyl diester bridge was the first used as a linker to link a carbohydrate to MPEG. (**Scheme 1.23**)

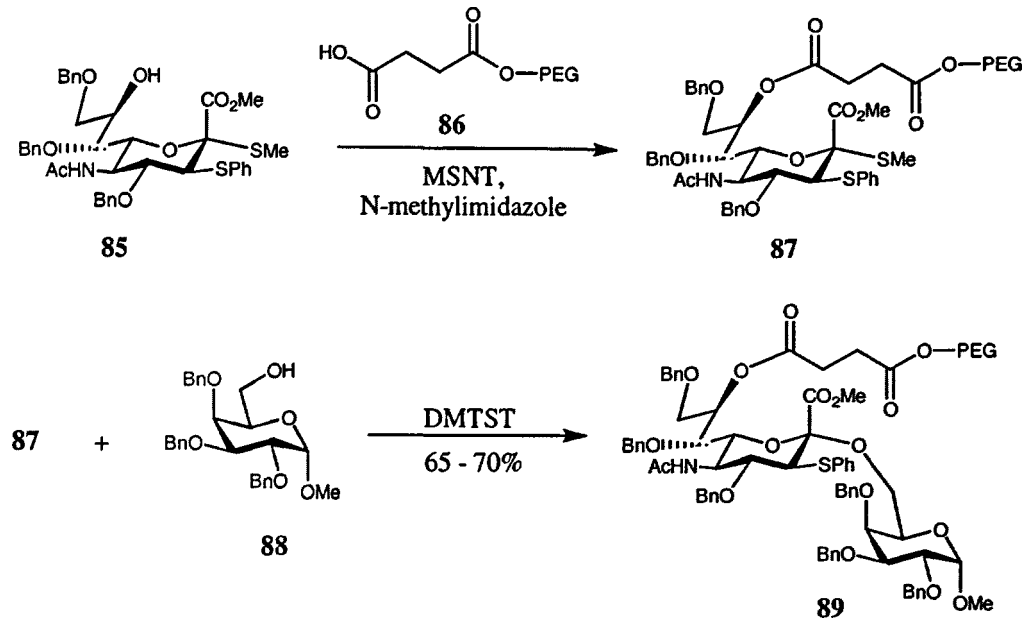


Scheme 1.23

The glycosylation of a MPEG-bound glycosyl acceptor can be driven to completion by repeated addition of a glycosylating agent. The progress of the glycosylation can be monitored by NMR spectroscopy. The saccharide is finallly detached from the MPEG-Su by basic hydrolysis.

In 1993, van Boom and co-workers[195] reported the synthesis of a heptasaccharide having phytoalexin elicitor activity by using MPEG-succinoyl methodology.

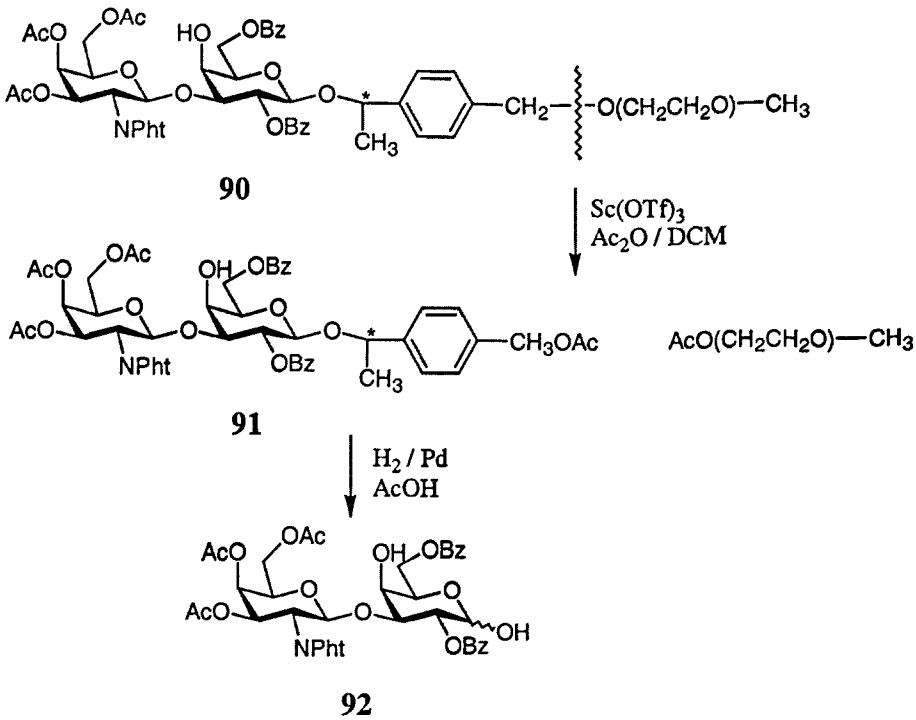
Ogawa *et al.*[196] immobilised a sialic acid thioglycosyl donor **85** using the same methodology. The polymer bound thioglycoside **87** was reacted with galactosyl acceptor **88** to give α-linked disaccharide **89** in good yield (**Scheme 1.24**).



MSNT = 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole

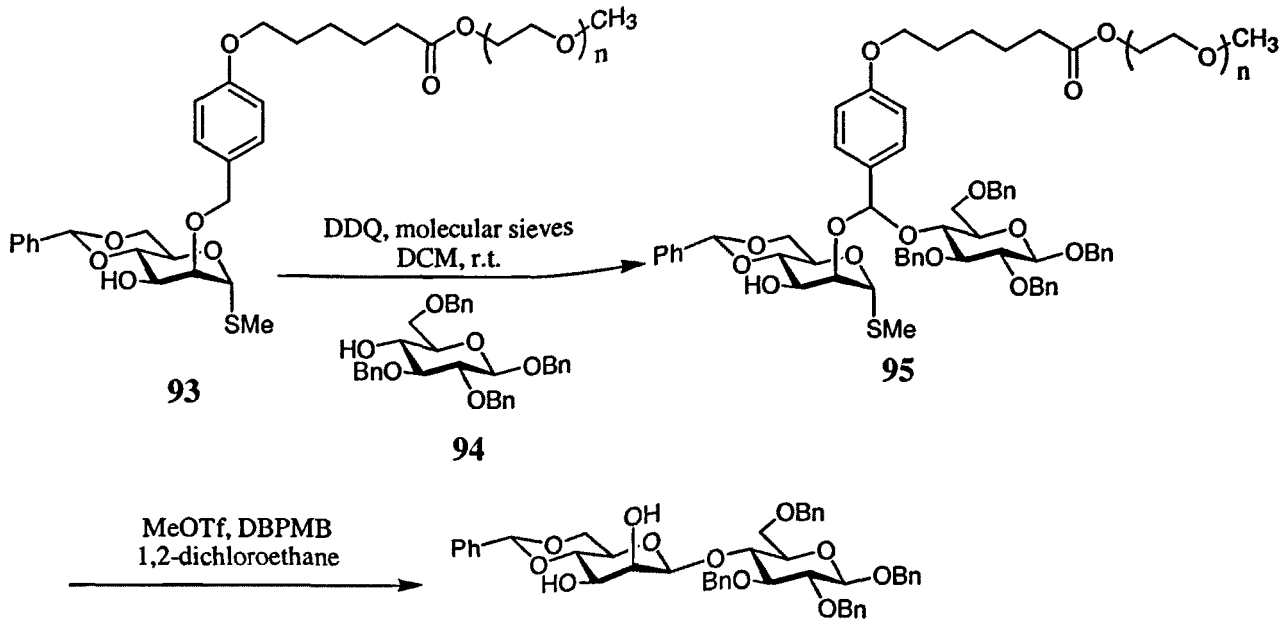
Scheme 1.24

Despite the success of this methodology, the succinoyl diester linker excludes the use of basic and strong acidic conditions, thus limiting its applicability. α,α'-Dioxyxylyl diether (-$CH_2C_6H_4CH_2O$-) was found to be an effective alternative.[99] It is stable under most reaction conditions and can be easily removed by hydrogenolysis, either completely to give free OH, or MPEG can be selectively removed to leave the hydroxyl protected with a *p*-tolylmethyl group. A pentasaccharide was prepared using the combination of this linker and MPEG. Recently, Whitfield and co-workers[100] reported a new protocol for the cleavage of oligosaccharide bound to MPEG through a derivative of the dioxyxyl linker. Polymer bound disaccharide **91** was cleaved from the polymer by treatment with hard Lewis acid $Sc(OTf)_3$ in the presence of Ac_2O (**Scheme 1.25**). The site of cleavage was surprisingly between the benzylic carbon of the dioxyxyl linker and the terminal oxygen of MPEG. Sc^{3+} is thought to form a complex with PEG thus determining the selectivity of this cleavage.



Scheme 1.25

Ito *et al.* [101] combined the use of PEG resin and of *p*-methoxybenzyl intramolecular aglycon delivery for the stereoselective synthesis of a β-mannoside on solid phase. In this approach, a thiomethyl donor and a glycosyl acceptor were tethered through a polymer-supported *p*-methoxybenzyl acetal bridge, as seen in compound **95** (**Scheme 1.26**). The thiomethyl donor was then activated using MeOTf as promoter, and product **96** was obtained in a yield of 50%. The most attractive feature of this approach is that the product is released in solution upon formation, while most of the byproducts are retained on the polymer.



Scheme 1.26

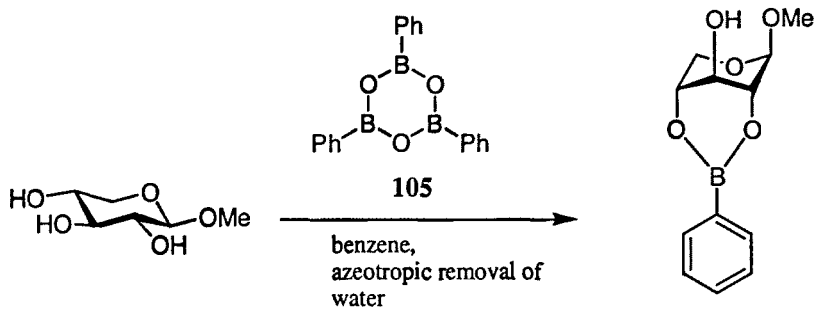
1.3 Applications of Boronic Acid in Carbohydrate Chemistry

The reaction between 1,2- or 1,3-diols and aryl- and alkylboronic acids to form boronate esters was first described by Kuivila and co-workers in 1954.[102] A saccharide carrying a suitable 1,2- or 1,3-diol can form a cyclic boronate. A wide range of boronates, mostly phenylboronate, have been synthesised. Most of the investigation regarding the preparation of boronates of carbohydrates was performed by Ferrier[103] during the sixties and the seventies.

1.3.1 Preparation of boronate esters of carbohydrates

Boronates of carbohydrates can be formed easily. A commonly used procedure to prepare boronates entails treating a diol, or carbohydrate, with an equimolecular amount of boronic acid in benzene or toluene. The water that is formed is azeotropically removed in a Dean-Stark distillation head.

For example, ethyl β-D-xylopyranoside **104** reacted with an equimolecular amount of triphenylboroxin **105**, that was formed by dehydration of boronic acid. After removal of the solvent by evaporation and crystallisation from dry petroleum ether, boronate **106** was obtained in a yield of 89%[104] (**Scheme 1.27**).



104 **106**

Scheme 1.27

If the carbohydrate is not sufficiently soluble in benzene or toluene, other solvents such as 1,4-dioxane or pyridine can be employed. Whereas methyl α-D-glucopyranoside reacts with phenylboronic acid in benzene, yielding the corresponding 4,6-*O*-boronate, the boronate ester of methyl α-D-mannoside must be prepared using 1,4-dioxane as solvent.[105] Another method applicable to benzene-insoluble carbohydrates involves the addition of the saccharide to a solution of boronic acid, or of the corresponding cyclic anhydride, in water, or preferably in methanol. Under these conditions, some boronate esters spontaneously precipitate. Pyridine has been used as a solvent in particular for the formation of boronates of nucleosides.[106,107] Also acetone[108-110] and DMF[111] have been used as solvent. In one case, the use of pyridine led to the isolation of the product as a pyridine complex.[112] Crystallisation of β-D-fructopyranose 2,3:4,5-*bis*(benzeneboronate) from light petroleum ether yielded material containing 1 mole of pyridine *per* mole of monosaccharide.

It is important to note that the purification of boronate esters of carbohydrates is in general performed only by crystallisation. It is not possible to purify these compounds by silica gel column chromatography. Also, it is not possible to perform TLC analysis on most boronates of carbohydrates as they will hydrolyse on silica gel. Distillation of boronates of carbohydrates has also been reported.[106]

Determination of the structure of boronates of carbohydrates and of polyols is in general not an easy task. Boronates of various carbohydrates have been prepared during the late sixties and early seventies, and their structure was determined by chemical methods: unesterified hydroxyl groups were substituted and, after cleavage of the boronate ester, the corresponding products were characterised and compared with known compounds. However, characterisation by chemical methods of some of these compounds might have been not sufficiently rigorous, in case the derivatisation of the boronate could have altered the structure of the boronate itself.[103] The use of ^{1}H NMR and ^{11}B NMR has improved the situation, but ambiguity is still possible, especially in those cases when the structure of the boronates can change while the boronate is in solution. Furthermore, preparation of the same boronate by different methods, *e.g.* using a different solvent, might result in different regioselectivity of the reaction.[103]

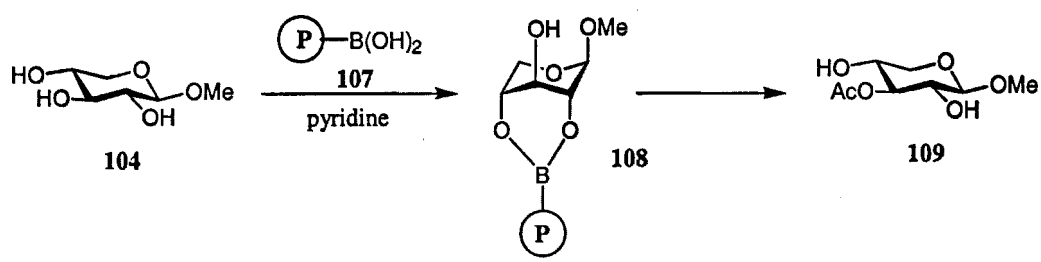
Boronates of glycosides and of related compounds can be characterised more easily then the corresponding derivatives of alditols and sugars, mostly because the monosaccharide does not have the possibility of changing the structure by converting from the furanose to the pyranose form and from α- to β-anomer.

1.3.2 Removal of boronate groups

Reported procedures for cleavage of boronate esters from carbohydrates or other diols include treatment with acetone/water (4/1, v/v), H_2O_2/EtOAc, acetic anhydride/pyridine and 1,3-propanediol/acetone.[113] The latter method removes the boronate by an exchange reaction. Another diol that has been used for this purpose is 2-methylpentane-2,5-diol in acetic acid.[114] Cleavage of boronate esters was also performed by eluting a solution of a boronate through a short pad of silica gel.[115]

1.3.3 Stability of boronate esters in chemical reactions

It is possible to acetylate free hydroxyl groups of boronates of carbohydrates. Acetyl chloride in pyridine has been used for the acetylation of boronates of glycosides,[104,105,116-118] alditols[119] and nucleosides.[120] Products were often purified by distillation prior to crystallisation. Benzoylation has also been performed. Products were purified by crystallisation, since the products are not sufficiently volatile to perform a distillation. Benzoyl chloride[121] and benzoic anhydride[104] have been used as acylating reagents. It was also possible to prepare chloroacetylated derivatives.[110]

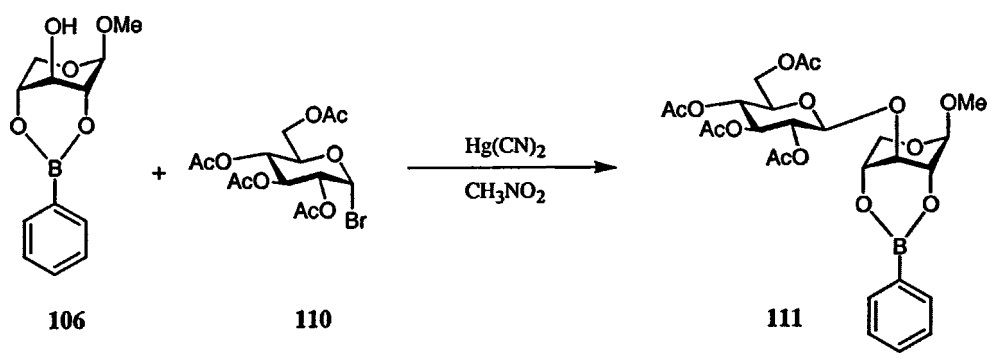


Scheme 1.28

Fréchet *et al.*[122] successfully acetylated and benzoylated a variety of glycosides bound on polystyrylboronic acid **107**. This polymer proved to be an efficient and very selective protecting group. In a one pot procedure, a suspension of a glycoside and the resin (a slight

excess) was heated in pyridine and water removed by azeotropic distillation. The solution phase was then removed and unbound monosaccharide recovered. The resin was then suspended in dry pyridine, and the acylating reagent added. After stirring overnight at room temperature, the solvent was removed and the resin washed with dry pyridine. Cleavage of the product from the solid support was achieved using several portions of acetone/water (4/1, v/v). This procedure is illustrated in **Scheme 1.28** for the preparation of methyl 3-*O*-acetyl-α-D-xylopyranoside **109** starting from methyl α-D-xylopyranoside **104** in a yield of 87% after cleavage from the polymer, and crystallisation from ethyl acetate/heptane. An attractive feature of this procedure is that it was possible to reuse the polymer and that no loss of activity was observed with repeated use.

Liao *et al.* have recently used polystyrylboronic acid as solid support to prepare in a similar one pot procedure 4,6-dimethylpyrimidin-2-yl derivatives of methyl α-D-glucopyranoside.[123] Other ester derivatives of boronates of glycosides, alditols and nucleosides have been prepared, such as N-phenylcarbamates,[104,116] phosphates and trimethylsilyl ethers.[124]



106 **110** **111**

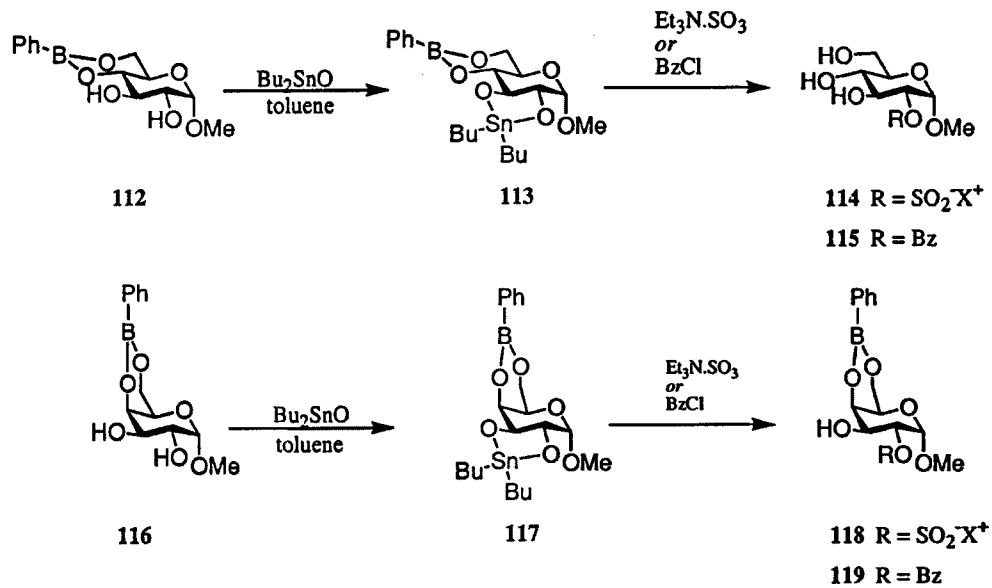
Scheme 1.29

The phenylboronate group has been shown to be stable under Koenigs-Knorr glycosylation conditions, and disaccharide **111** was synthesised by coupling donor **110** and benzyl β-D-

xylopyranoside 2,4-*O*-phenylboronate **106** using $Hg(CN)_2$ as a promoter with a yield of 29%[116] (**Scheme 1.29**).

The phenylboronate group has been found to be stable during acetic anhydride dimethylsulfoxide oxidation of hydroxyl groups[125,126] and to oxidation with PCC.[115]

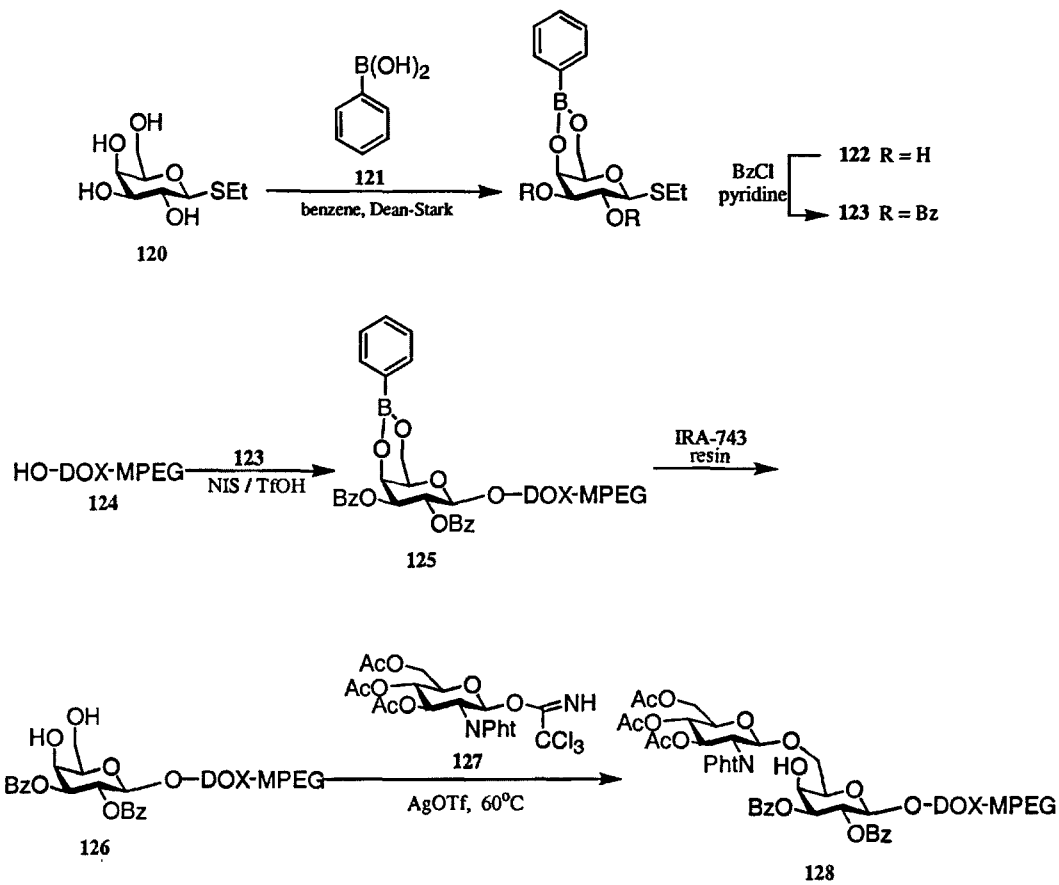
Phenylboronates are also stable to the conditions of stannylation and have been used for selective sulfonation to produce monosulfated monosaccharides by Vasella and co-workers.[127] The 4,6-*O*-phenylboronates **112** and **116** were treated with Bu_2SnO under reflux in toluene with azeotropic removal of water to obtain respectively the stannanedinyl compounds **113** and **117** respectively (**Scheme 1.30**). Sulfonation with $Et_3N{\cdot}SO_3$ or benzoylation provided the corresponding 2-sulfates **114** and **118** and 2-benzoates **115** and **119** respectively, after simultaneous removal of both the stannanediyl and boranediyl residues by flash silica gel chromatography, thus avoiding an additional deprotection step. In these procedure phenylboronic acid is acting as a temporary protecting group.



Scheme 1.30

Recently Whitfield and co-workers[128] also employed phenylboronic acid as a temporary protecting group. Thioglycoside donor **123** was prepared *via* benzoylation of boronate **122**

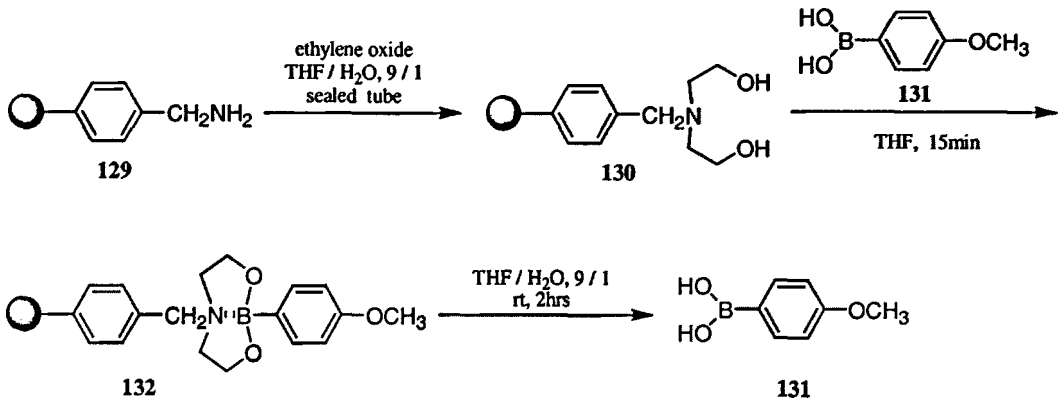
and then successfully used to glycosylate the soluble polymer MPEG-DOX-OH, using NIS/TfOH as a promoter (**Scheme 1.31**). The boronate ester was readily removed by shaking a dry acetonitrile solution of the MPEG polymer with the borate specific resin Amberlite IRA-743 and the resulting diols regioselectively glycosylated using a trichloroacetimidate as glycosyl donor and silver triflate as a promoter .



Scheme 1.31

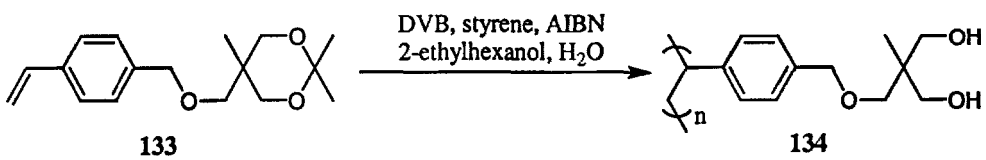
Hall *et al.* developed an efficient solid support to immobilise boronic acids.[129] This support, *N,N*-diethanolaminomethyl polystyrene (DEAM-PS) **130**, could be useful as scavenger, to remove unreacted boronic acids in reactions such as Suzuki couplings, or to facilitate the sometime troublesome purification of boronic acids. Resin **130** can couple almost quantitatively to equimolecular amounts of aryl-, alkenyl- and alkylboronic acid in dry THF

in a few minutes. The formation of stable resin bound adduct **132**, where nitrogen is coordinated to boron, explains the effectiveness of this linker. Using a glycerol polystyrene resin gave lower yields of loaded boronic acids (**Scheme 1.32**).



Scheme 1.32

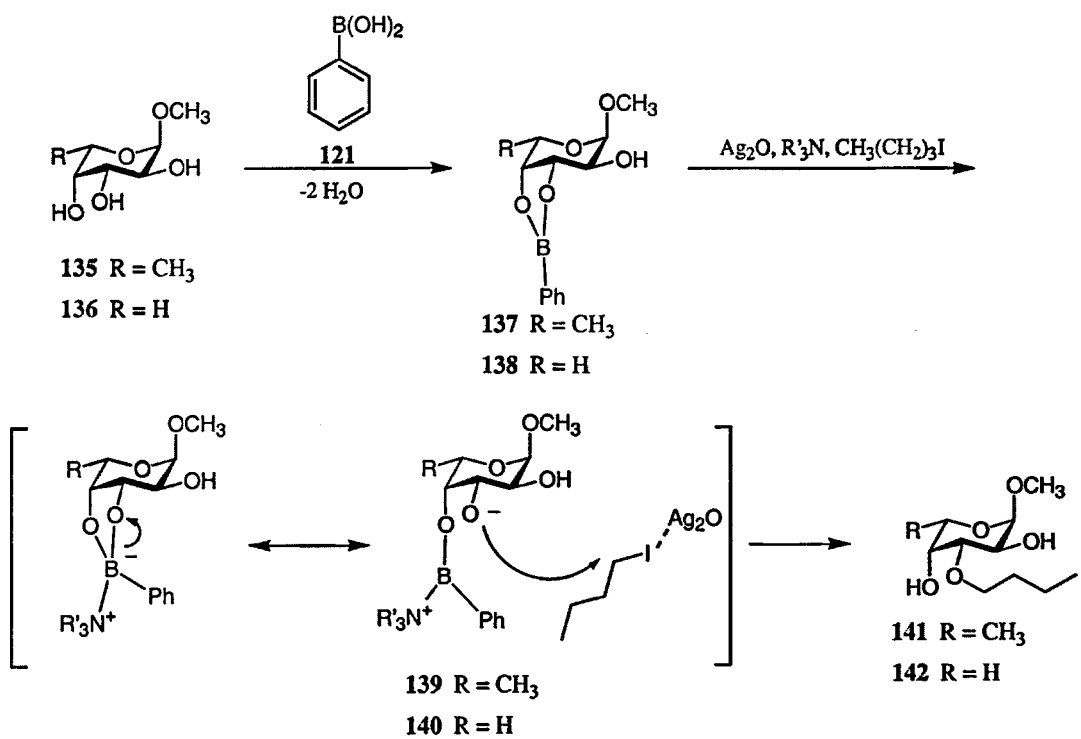
Another linker was developed soon after by Carboni *et al.*.[130] A macroporous polystyrene was prepared by polymerisation of **133** using 2-ethyl-hexanol as porogen. The obtained polymer **134** proved effective in reacting with boronic acids, and a protocol for cleaving and binding boronic acids was developed (**Scheme 1.33**).



Scheme 1.33

A potential alternative to the multistep protection-deprotection procedures commonly adopted for oligosaccharide synthesis, that essentially rely on selective deactivation of all but one hydroxyl group, would involve the complexation-induced activation of a particular hydroxyl group. This is illustrated by the successful application of Sn reagents in the selective alkylation,[131,132] acylation,[133,134] and sulfation of unprotected saccharides. Aoyama and co-workers[135] successfully used of arylboronic acid to regioselectively alkylate unprotected glycosides. Upon treatment with phenylboronic acid **121** in benzene, with azeotropic removal

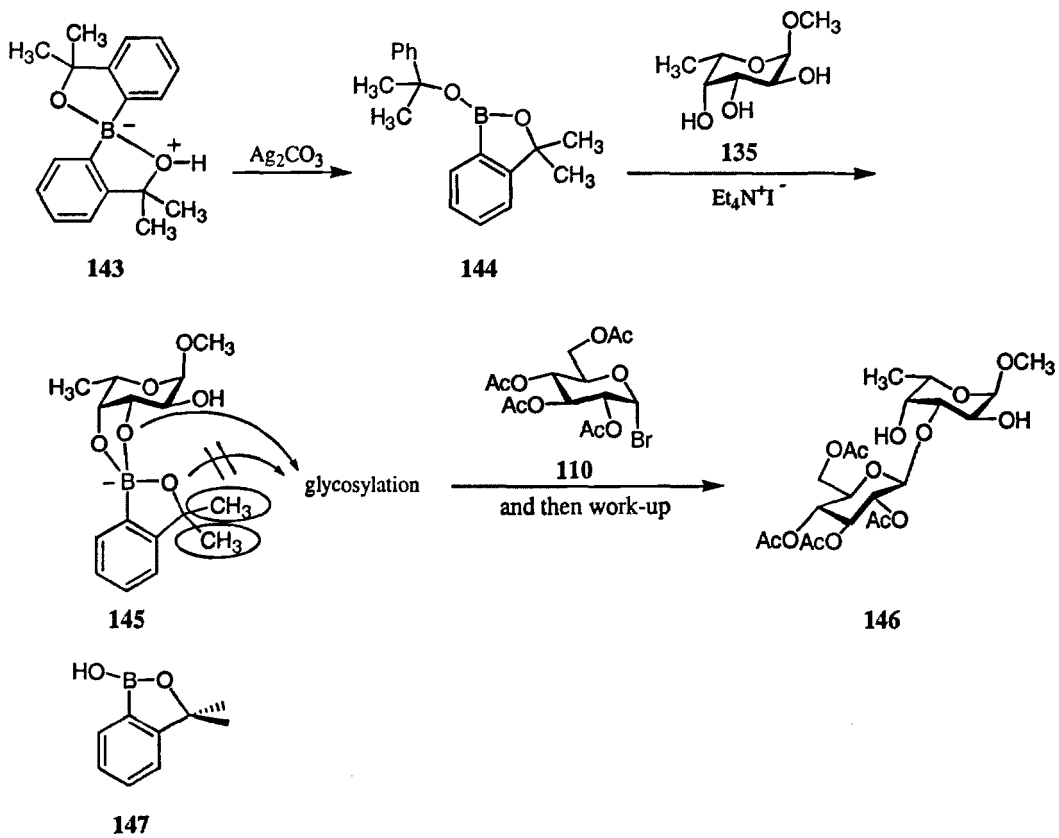
of water, methyl α-L-fucopyranoside **135** formed 3,4-*O*-boronate **137** exclusively. Treatment of **137** with Ag_2O (5eq.), triethylamine (1eq.) and iodobutane (1eq.) in benzene at reflux for 22h afforded 3-*O*-butylated product **141** in a yield of 50%. The yield was improved to 80% by addition of another one eqivalent of triethylamine and iodobutane in small portions over a period of 24h. Product **141** was purified by silica gel column chromatography of the reaction mixture after this was filtered through a short pad of silica gel. When the reactions was performed in identical conditions on glycoside **135**, no product was observed, thus clearly indicating that complexation-induced activation of the otherwise unreactive 3-OH group is in fact taking place.



Scheme 1.34

The proposed mechanism for this activation is showed in **Scheme 1.34**: an essential feature is the Ag^+- and amine-promoted activation respectively of the electrophilic center in iodobutane and of the nucleophilic centre in the boronate. The regioselectivity can be explained by the fact that the 3-OH is equatorial, while 4-OH is axial. Further, the 4-OH is hindered by the

methyl group. Upon alkylation of arabinoside **136**, which lacks the methyl group, a 4:1 mixture of the 3-*O*- and 4-*O*-alkylated regioisomers was obtained.



143 **144** **145** **146** **147**

Scheme 1.35

When phenylboronic acid was used in an attempt to selectively glycosylate the boronate of fucoside **135**, using glycosyl bromide **110** as a donor and Ag_2CO_3 and $Et_4N^+I^-$ as promoters, no product was obtained.[136] Aoyama and co-workers decided to employ modified arylboronic acid **147**, but while preparing it they instead obtained diarylborinic acid derivative **143** (**Scheme 1.35**). This turned out to be an excellent promoter of the desired glycosylation. In the presence of Ag_2CO_3, borinate **143** undergoes facile protonolysis of B–C bond to give the dimethylbenzyl derivative **144**. Upon treatment with fucoside **135** in THF in presence of $Et_4N^+I^-$ and molecular sieves 4Å, boronate **144** readily formed boronate **145**. Addition of

donor **110** to the mixture gave disaccharide **146** in good yield. Other glycosides were also selectively glycosylated using the same procedure.

1.3.4 Interaction between Carbohydrates and Aromatic Boronic Acids in water

Boronic acid and saccharides, and diols in general, establish an equilibrium in water between the bound and the unbound form. In a basic solution, boronates exist as tetragonal species, where boron is sp^3, and an HO^- is coordinated to boron, that carries a negative charge as shown in **Figure 1.3**.

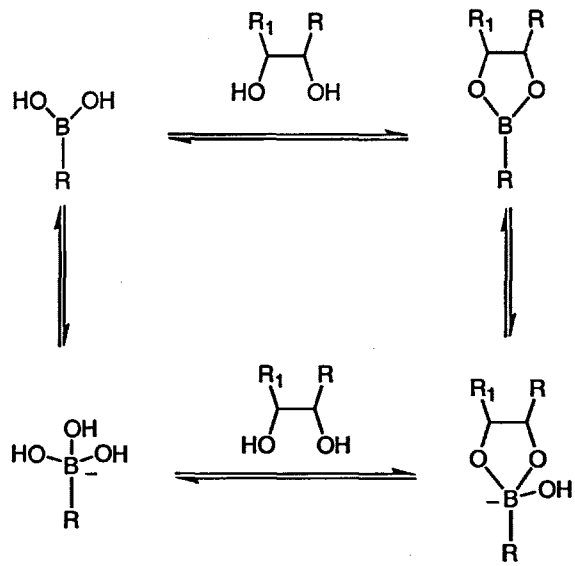


Figure 1.3

It is difficult to predict the regiochemistry of the reaction of a saccharide with boronic acids. Reaction with 1,2- and 1,3-diols, that form respectively 5- and 6-membered rings, is favourite. Furthermore, saccharides can exist in solution in the furanose or pyranose form and an equilibrium is established between the α- and the β-anomers. Analysis by ^1H or ^{13}C NMR may not lead to unequivocal conclusions about the structure, especially when more then one boronate is in equilibrium with the free saccharide. This explains why despite many efforts no

unequivocal conclusions about the structure of boronate esters of carbohydrates in water have been reached.

Coupling constants have been determined with different methods, including pH depression,[137] potentiometric titration,[138] calorimetry,[139] polarimetry,[140] circular dicroism,[140,141] fluorescence,[142,143] absorption[144] and voltametry.[145] A general finding is that among monosaccharides D-fructose forms the most stable complex, followed by D-galactose, D-mannose and D-glucose. Simple diols, such as ethylene glcycol, appeared to have much smaller formation constants than saccharides. Eggert and co-workers[146] have recently investigated the formation of the complex between D-fructose and *p*-tolylboronic acid under both neutral non-aqueous and alkaline aqueous conditions by combining the measurement of J_{CC} coupling constant with the standard information provided by ^{1}H and ^{13}C NMR spectroscopy. This equilibria was shown to have a much higher degree of complexity than was previously thought. Measurements were conducted at different molar ratios of saccharide and boronic acid. In alkaline aqueous solution, nine different complexes were observed. In DMSO, five different complexes were found. Assignement of all these structures was possible by measurement of their J_{CC} coupling constants.

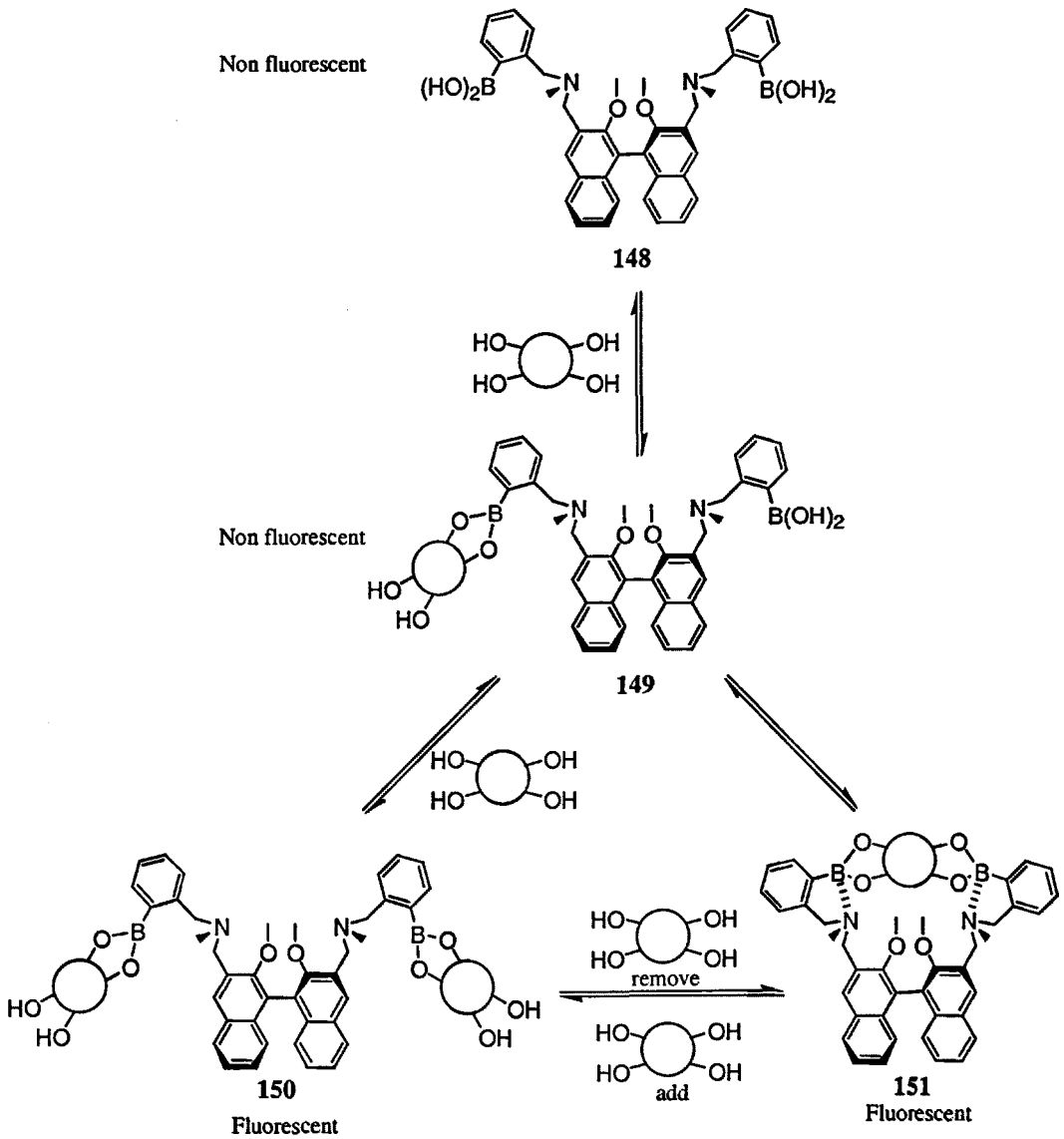
This observation concerning the complexity of these mixtures of complexes leads to the conclusion that earlier studies on binding constants, with their lack of knowledge of the stoichiometry and the proportions of the complexes formed, show no relationship between the physical properties and the complexes formed.

1.3.5 Boronic acids as sensors for the carbohydrates

There is a strong demand for the development of new, efficient, selective and cheap sensors for glucose and other saccharides. Many receptors that have been developed are based on hydrogen bonding interactions.[147] The efficiency of such interactions has been well demonstrated in non aqueous systems, but in aqueous media competitive hydrogen bonding by the solvent presents a serious limitation. Boronic acids readily form covalent bonds with saccharides in aqueous media and therefore they offer an attractive moiety to design synthetic receptors. Recently, aromatic boronic acid derivatives have been indeed employed for the construction of receptors for saccharides.[148] The presence of hydroxyl groups with only small differences in reactivity creates problems in designing saccharide receptors. On the other hand, the high number of hydroxyl groups is an advantage if these hydroxyl groups can be used in the recognition process. When two boronic acids are arranged in a suitable orientation in a molecule, a saccharide may be bound in a 1:1 complex through the molecule's head and tail boronic acid groups, as in compound **151 (Scheme 1.36)**.

The recognition events can be followed using a broad range of physical properties, such as fluorescence, UV-visible absorption, circular dichroism and electrochemistry. Shinkai and co-workers have explored this concept and have synthesised new sensor molecules now reported to be capable of distinction between various carbohydrates.[148] James *et al.*[143] reported the discrimination of D and L monosaccharides using a compound that acts as a sensor by virtue of its fluorescence response upon formation of a boronate ester with the guest species. Receptor **148** contains two boronic acid groups that can react with saccharides and incorporates a fluorescent naphtyl moiety. Esterification with each monosaccharide alters the intensity of the fluorescence, through suppression of photoinduced electron transfer from the

nitrogen to the naphtyl moiety. Different monosaccharides alter the intensity to a different degree, enabling them to be distinguished. In fact, upon reacting with D-glucose, receptor **148** becomes markedly more fluorescent than when a boronate ester with L-glucose is formed. Thus, the two enantiomers can be distinguished. Enantiomers of other monosaccharides were also recognised using sensor **148**.



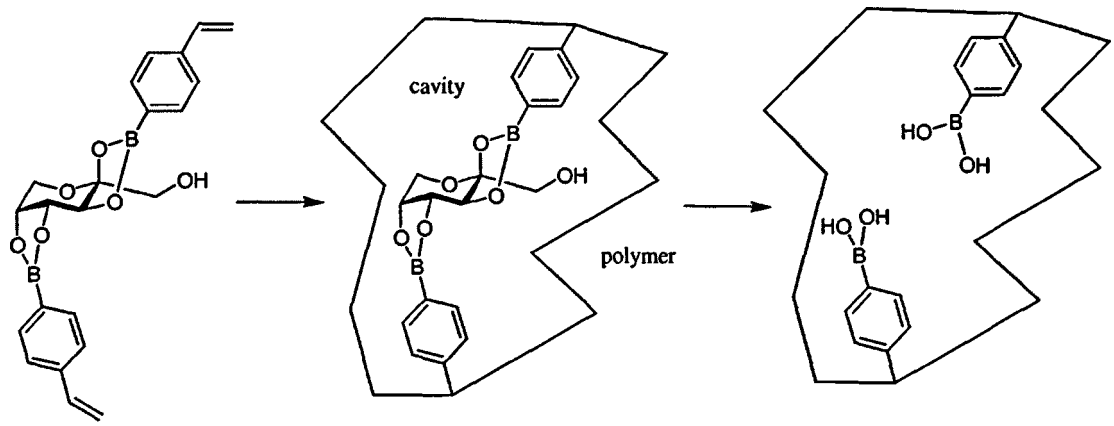
Scheme 1.36

These findings complement previous findings from James *et al.*[149] who had built and tested a boronic acid based receptor tuned toward binding D-glucose selectively.

Although much progress in the construction and design of boronic acid-based sensors has been made, future development will require a greater insight of the structural and electronic properties of boronic ester saccharide complexes.

1.3.7 Molecular imprinting

Boronic acids have also been used to prepare polymers imprinted with saccharides. Imprinting of organic polymers was first reported by Wülff *et al.*.[150] In this process, crosslinked polymers are formed around a molecule that acts as a template (**Scheme 1.37**). When the template is removed, an imprint containing reactive groups with binding capability remains behind in the polymer. Promising areas of development for molecular imprinting include chromatography, catalysis, biosensor technology and the production of artificial antibodies. Interactions used in the process of imprinting can be covalent or non covalent. Covalent interactions have the advantage that the groups involved in the binding are precisely fixed in space during the polymerisation, but they are suitable only if a high percentage of the templates can be removed after the polymerisation. In the case of non-covalent interactions, the removal of the template is straightforward. But for the construction of catalysts, the orientation of the binding and catalitically active groups in the cavity are of greater significance, and therefore covalent interactions would be overall advantageous. The boronic acid moiety proved to be very suitable for preparing imprinted polymer based on covalent binding. Polymerisation of β-D-fructopyranose 2,3:4,5-bis-*O*-(4-vinyl-phenylboronate) (**152**) in presence of a large amount of crosslinking agent gave a polymer that, upon removal of the template, was used to resolve a racemate of the template.[151]



152 153 154

Scheme 1.37

1.4 Concluding remarks

Most of the advances in oligosaccharide synthesis mentioned above were developed during the last decade. By employing these methodologies properly, it is possible to execute complex multi-step syntheses to prepare biologically active saccharide sequences. However, it is difficult to predict which methodology will be best suited for a given synthetic challenge. A powerful and general method that gives both an excellent chemical yield and stereoselectivity has not yet appeared. It is therefore necessary to develop new methodologies and to improve the existing ones. The first aim of this thesis is to employ aromatic boronic acids as protecting groups in solution and as linkers on solid phase and to explore the convenience of their use in oligosaccharide synthesis.

Currently, there is a considerable interest in utilizing the binding properties of boronic acid in building receptors for the recognition of carbohydrates. Future work on this receptors will focus on improving the binding selectivity of the substrate. In order to achieve this goal, a more detailed knowledge of the structure and properties of boronate esters of carbohydrates is

necessary. The second aim of this thesis is to investigate some aspects of the structure of boronate esters of carbohydrates.

Chapter II

Phenylboronic Acid as Protecting Group for Oligosaccharide Synthesis

2.1 Introduction

In the last two decades, the synthesis of oligosaccharides in solution has seen a dramatic improvement with the introduction of new effective and reliable glycosyl donors, such as thioglycosides, fluorides, trichloroacetimidates and pentenyl glycosides, and of a vast array of new protecting groups.[6,7] Nevertheless, almost all of these protecting groups have limitations, and there is a need to introduce new protecting groups with useful properties.

Aryl- and alkylboronic acids can be used as protecting group for carbohydrates and are compatible with reactions such as acylations and benzylations. In the sixties, one example was reported by Ferrier *et al.*[116] where phenylboronic acid was used as a protecting group in a glycosylation reaction (see **Scheme 1.29**). Since then, boronate esters have not been widely used as protecting groups for carbohydrates, mostly because boronate esters of carbohydrates cannot be purified by commonly used chromatographic procedures. Recently, boronic acids have received renewed attention with the development of sensors for carbohydrates and of systems for the selective transport of monosaccharides and nucloetides across membranes. During the course of this research, Whitfield *et al.*[128] reported the use of phenylboronic acid as a temporary protecting group for the synthesis of a disaccharide on a soluble MPEG-DOX-OH polymeric support (see **Scheme 1.31**).

We envisaged that boronate esters could find novel applications in oligosaccharide synthesis and in particular that boronic acid could be used as a linker for solid supported synthesis of oligosaccharides (see **Chapter III**). Therefore it was considered necessary to investigate the compatiblity of boronate esters with some of the newly developed synthetic methodologies

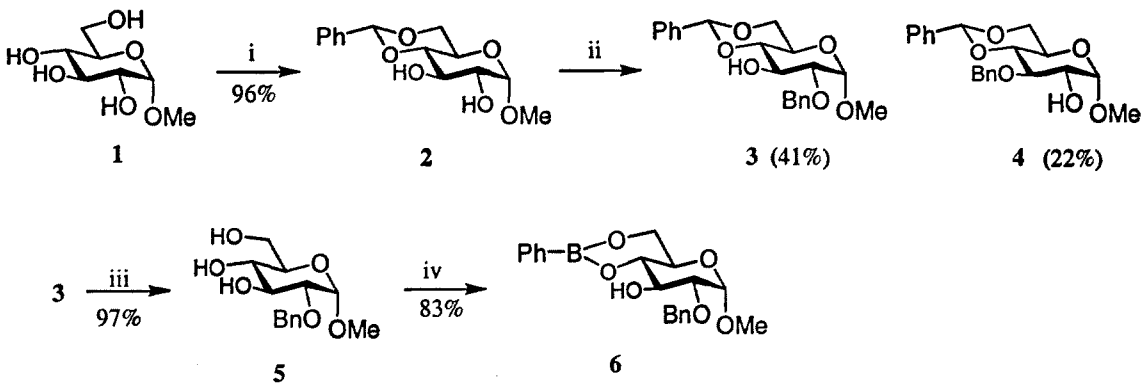
for the synthesis of oligosaccharides. Phenylboronic acid was used as a protecting group in glycosylation reactions using different glycosyl donors In first instance thioglycosides, fluorides and trichloroacetimidates were employed.

2.2 Results and discussion

In order to investigate the compatibility of phenylboronate ester as a protecting group in glycosylations with thioglycosides, fluorides and trichloroacetimidates, glycosyl acceptors **6** (**Scheme 2.1**) and **9** (**Scheme 2.2**) and glycosyl fluoride **15** (**Scheme 2.3**), thioglycoside **18** (**Scheme 2.4**) and trichloroacetimidate **21** (**Scheme 2.5**) were prepared. Subsequently, glycosyl fluoride **15** and thioglycoside **18** were reacted with glycosyl acceptor **6** and trichloroacetimidate **21** was reacted with glycosyl acceptor **9**.

Glycosyl acceptor **6** was prepared in four steps starting from methyl α-D-glucopyranoside (**1**) (**Scheme 2.1**). Reaction of **1** with benzaldehyde dimethyl acetal using camphorsulfonic acid as a catalyst gave methyl 4,6-*O*-benzylidene acetal **2** in a yield of 96%. Compound **2** was then benzylated using benzyl bromide and NaH in DCM in the presence of a phase transfer catalyst.[152] Under these conditions mono-benzylation was mainly achieved, and products **3**[152] and **4**[152] were obtained in a yield of 41% and 22% respectively. The benzylidene of **3** was then cleaved using acetic acid/water (4/1, v/v) at 60 °C and compound **5**[153] was obtained in 97% yield. In order to prepare the boronate ester, **5** and phenylboronic acid in toluene were heated under reflux with azeotropic removal of water in a Dean-Stark apparatus.[103] Boronate **6** precipitated upon cooling. Purification by silica gel column chromatography was not possible, because boronates of carbohydrates are unstable on silica gel. Attempts to crystallise **6** were not successful. Also, it proved impossible to obtain a mass spectrum of this compound

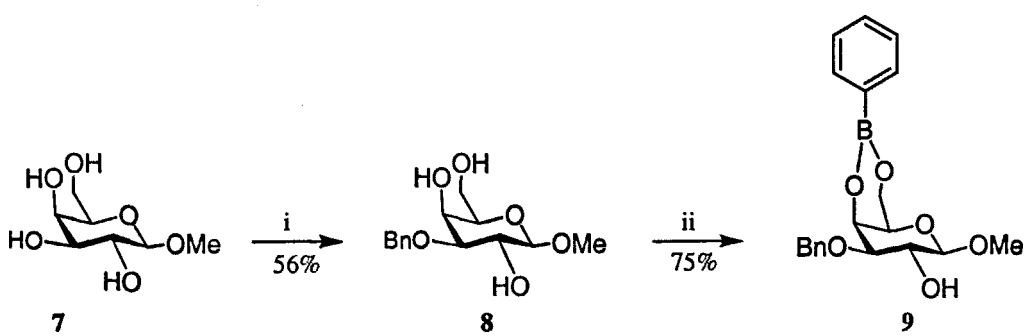
by MALDI or FAB techniques. Both ^{1}H and ^{13}C NMR spectra are in agreement with the expected product **6**, showing a single compound which is clearly different from **5**.



Reagents and conditions: i. MeCN, CSA, $PhCH(OMe)_2$ ii. BnBr, DCM, NaOH aq., $Bu_4N^+Br^-$, 40°C iii. $AcOH/H_2O$ (4/1), 60°C iv. $PhB(OH)_2$, toluene, Δ

Scheme 2.1

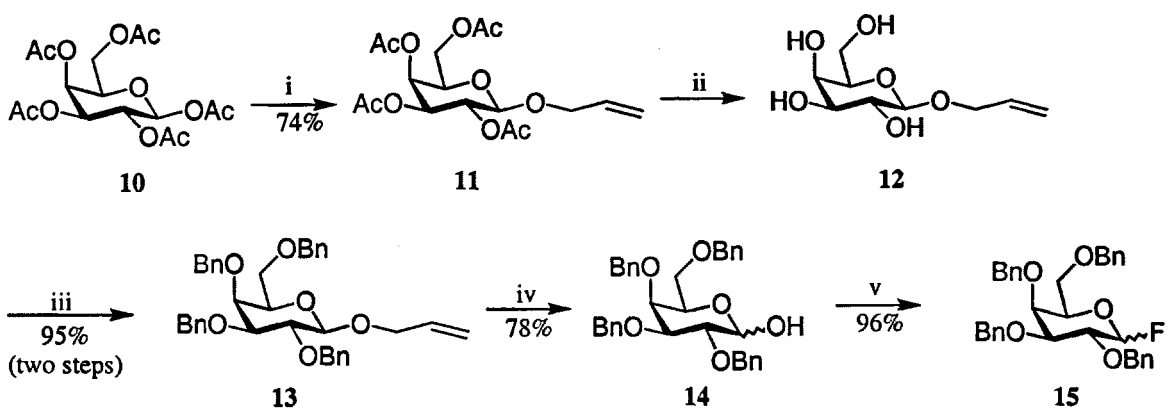
Glycosyl acceptor **9** was prepared in two steps starting from galactoside **7** **(Scheme 2.2).** Compound **7** was treated with dibutyltindimethoxide in DMF to give a stannylated intermediate that was selectively benzylated by addition of benzyl bromide and cesium fluoride. After aqueous work-up, crystallisation of the product gave compound **8**[154] in a yield of 56%. Compound **8** was treated with phenylboronic acid in benzene under reflux with azeotropic removal of water to give product **9** after crystallisation from benzene in a yield of 75%.



Reagents and conditions: i. BnBr, $Bu_2Sn(OMe)_2$, CsF ii. $PhB(OH)_2$, toluene, Δ

Scheme 2.2

Glycosyl fluoride **15**[24] was prepared starting from β-D-galactose pentaacetate (**10**) (**Scheme 2.3**). Thus, **10** was allylated at the anomeric position by treatment with $SnCl_4$ and allyl alcohol, affording **11**[155] in a yield of 74%. The allyl glycoside **11** was then deacetylated using NaOMe in methanol[156] to give **12**[157] which was used without further purification. Subsequent benzylation with benzyl bromide and NaH in DMF gave the fully protected compound **13**[158] in 95% yield. The allyl group of **13** was isomerised and cleaved by a one-pot two-step reaction using $PdCl_2$ as a catalyst in acetic acid/water,[159] to give **14**[160] in 78% yield. Fluorination with diethylaminosulfur trifluoride (DAST) in dry THF[25,26] at –30°C afforded **15** in a yield of 96% after purification by silica gel column chromatography.

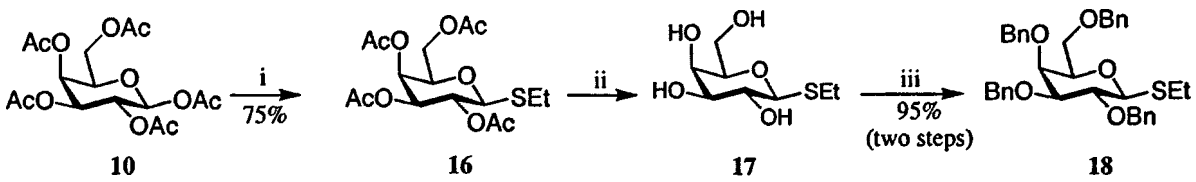


Reagents and conditions: i. $SnCl_4$, allyl alcohol, DCM, 0°C ii. MeONa, MeOH iii. BnBr, NaH, DMF, 0°C iv. $PdCl_2$, NaOAc, AcOH, H_2O, 70°C v. DAST, THF, -30°C

Scheme 2.3

Thioglycosyl donor **18** was prepared in three steps starting from galactose pentaacetate **10** (**Scheme 2.4**). Thus, treatment of **10** with ethanethiol and $ZrCl_4$ in DCM[161] gave the thioglycoside **16**[161] in a yield of 75%. Only the β-anomer was obtained, due to the neighbouring group participation of the acetyl group at C-2. Compound **16** was then deacetylated using NaOMe in MeOH and, without any further purification of compound

17,[162] benzylated using standard conditions. Compound **18** was obtained after purification by silica gel column chromatography in a yield of 95%.

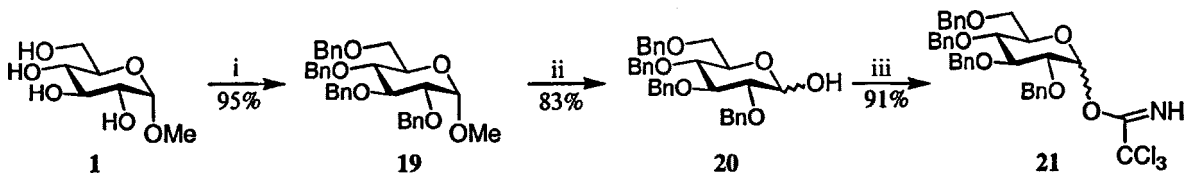


10 **16** **17** **18**

Reagents and conditions: i. $ZrCl_4$, EtsH, DCM, 0°C ii. MeONa, MeOH iii. BnBr, NaH, DMF, 0°C

Scheme 2.4

Trichloroacetimidate **21**[29] was prepared starting from methyl α-D-glucopyranoside (**1**) (**Scheme 2.5**). Thus, **1** was fully benzylated by treatment with BnBr and NaH in DMF to afford **19** in a yield of 95%. Hemiacetal **20** was obtained by refluxing **19** in acetic acid/water (4/1, v/v) with a catalytic amount of H_2SO_4. Crystallisation of the product from ethanol gave **20** in 83% yield. Compound **20** was treated with trichloroacetonitrile in the presence of a catalytic amount of NaH to give **21** as a mixture of anomers (α/β, 4/1) in 91% yield.[163]



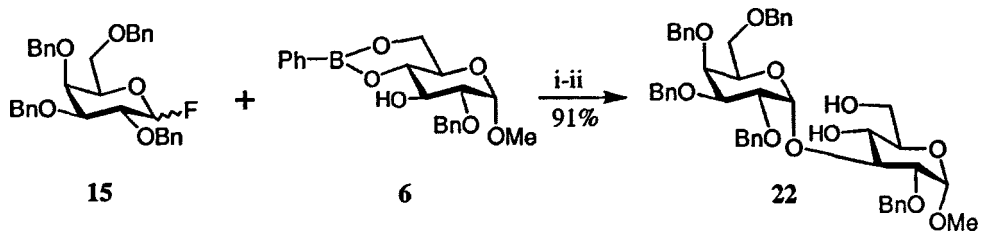
1 **19** **20** **21**

Reagents and conditions: i. BnBr, NaH, DMF, 0°C ii. AcOH / H_2O (4/1,v/v), H_2SO_4 cat., 100 °C iii. Cl_3CCN, NaH, DMF

Scheme 2.5

In first instance, attention was focused on the glycosylation of glycosyl fluoride **15** with glycosyl acceptor **6** (**Scheme 2.6**). Glycosyl fluorides offer the advantage of glycosylations at low temperature. Thus, coupling of glycosyl fluoride **15** with glycosyl acceptor **6** in the presence of lutidine and a suspension of $AgOTf/Cp_2ZrCl_2$[18] in DCM/ether (1/1, v/v) at –78 °C

gave **22** in a yield of 91%. The major side product that was formed in this reaction was trehalose.



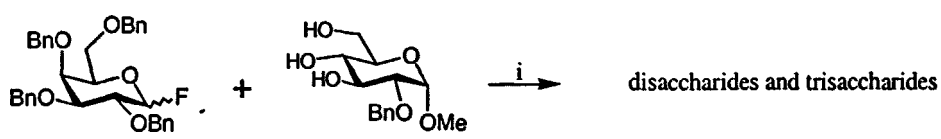
15 **6** **22**

Reagents and conditions: i. $AgOTf/Cp_2ZrCl_2$, 2,6- lutidine, DCM/Ether, 1/1, -78°C
ii. aq. $NaHCO_3$ (10%)

Scheme 2.6

It is important to note that the phenylboronate ester was cleaved either during the aqueous work-up or the chromatographic purification. This feature should be regarded as a possible advantage of arylboronic acids as protecting groups for oligosaccharide synthesis because it avoids a deprotection step after glycosylation.

Lutidine possibly has a dual role in this reaction. Nitrogen containing bases are known to stabilise boronate esters by coordination with tetrahedral boron.[164] In addition, the acid generated during the glycosylation reaction is neutralised by lutidine. The same reaction, but without the addition of lutidine, gave the product in a disappointing yield of 30%.



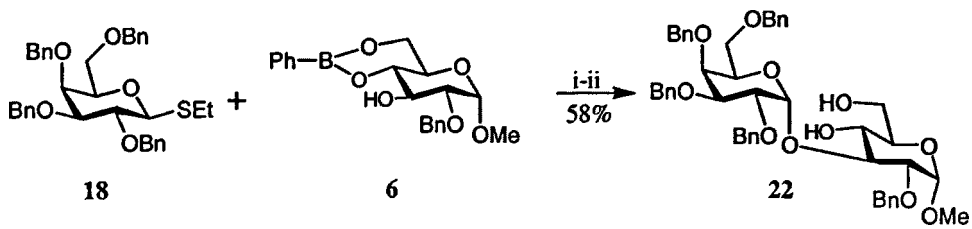
15 **5**

Reagents and conditions: i. $AgOTf/Cp_2ZrCl_2$, 2,6-lutidine, DCM/Ether, 1/1, -78°C

Scheme 2.7

As a control reaction, a glycosylation was performed using glycoside **5** as the glycosyl acceptor (**Scheme 2.7**). As expected, in the absence of phenylboronic acid as protecting group, an intractable mixture of products was obtained. MALDI-TOF spectrometry performed

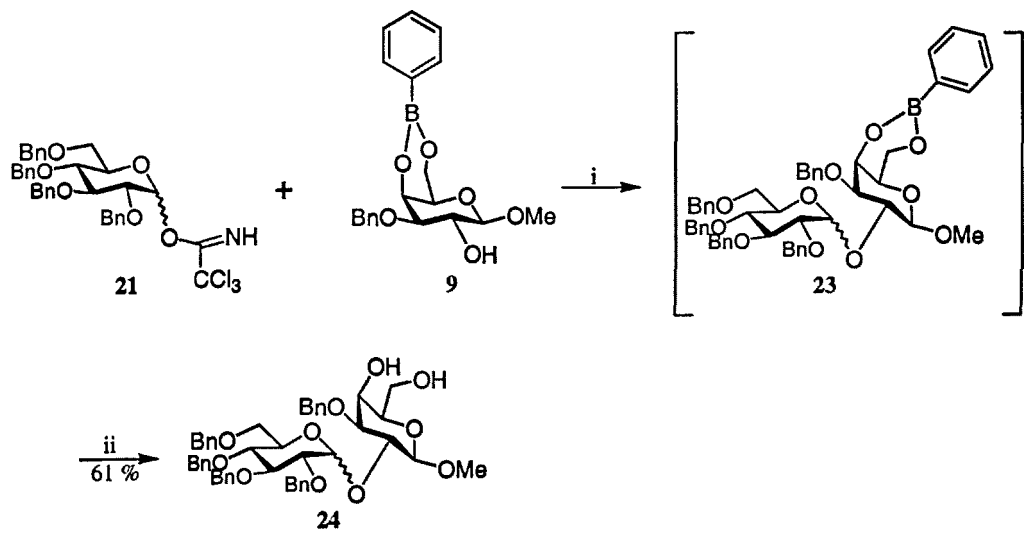
on the crude product revealed that trisaccharides had been formed as side products. Using **6** as glycosyl acceptor, no trisaccharides were observed. It can be concluded that phenylboronic acid is an appropriate protecting group for the C-4,6 diol of the glycosyl acceptor.



Reagents and conditions: i. IDCP, DCM/Ether, 1/1, 0°C ii. aq. $NaHCO_3$ (10%)

Scheme 2.8

Glycosylation of thioglycosyl donor **18** and acceptor **6** in DCM using IDCP[51] as promoter gave, after after aqueous work-up followed by purification by silica gel column chromatography, disaccharide **22** in a yield of 58% (**Scheme 2.8**). Analysis by MALDI-TOF of both the crude and the isolated products showed that no trisaccharides had been formed, confirming that the boronate ester is stable under the glycosylation conditions.



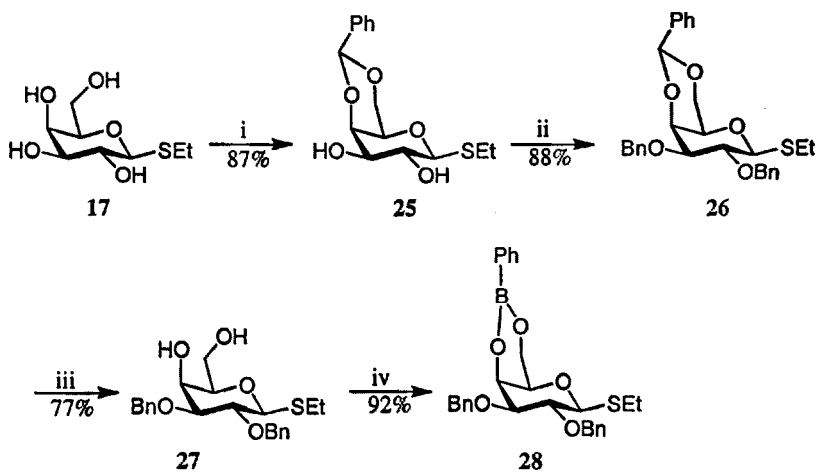
Reagents and conditions: i. TMSOTf, DCM, -60°C ii. aq. $NaHCO_3$

Scheme 2.9

Glycosyl acceptor **9** was reacted with trichloroacetimidate **21** in the presence of a catalytic amount of TMSOTf in DCM to give **24** in a yield of 61% (α/β, 1/1) (**Scheme 2.9**). It

appeared that the boronate ester functionality of intermediate **23** was stable on TLC. Even more surprisingly, aqueous work-up did not result in hydrolysis of the boronate ester. The boronate ester was cleaved during silica gel column chromatography.

Encouraged by these results, attention was turned to the use of phenylboronic acid as a protecting group for glycosyl donors. Thus, glycosyl donor **28**[128] was prepared in four steps starting from thioglycoside **17** (**Scheme 2.10**). The reaction of **17** with benzaldehyde dimethyl acetal using camphorsulfonic acid as a catalyst gave 4,6-*O*-benzylidene acetal **25**[165] in a yield of 87%. Compound **25** was benzylated using benzyl bromide and NaH in DMF to give product **26**[166] (88%). The benzylidene acetal was removed by treatment with acetic acid/water (4/1, v/v), to afford thioglycoside **27**[166] in 77% yield. Compound **27** and phenylboronic acid were refluxed in benzene with azeotropic removal of water. Upon cooling, boronate **28** precipitated as a white solid. Re-crystallisation from benzene gave **28** as a white crystalline solid (92%).

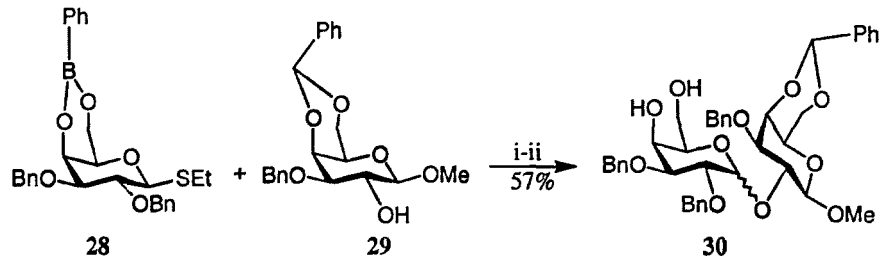


17 25 26 27 28

Reagents and conditions: i. MeCN, CSA, $PhCH(OMe)_2$ ii. BnBr, NaH, DMF iii. AcOH / H_2O (4/1, v/v) iv. $PhB(OH)_2$, benzene, Δ

Scheme 2.10

Glycosylation of glycosyl acceptor **29**[167] using thioglycoside **28** as a glycosyl donor and NIS and TMSOTf as promoter system[46,47,168] in DCM at 0°C gave disaccharide **30** (57%), after removal of the boronate protecting group (**Scheme 2.11**).



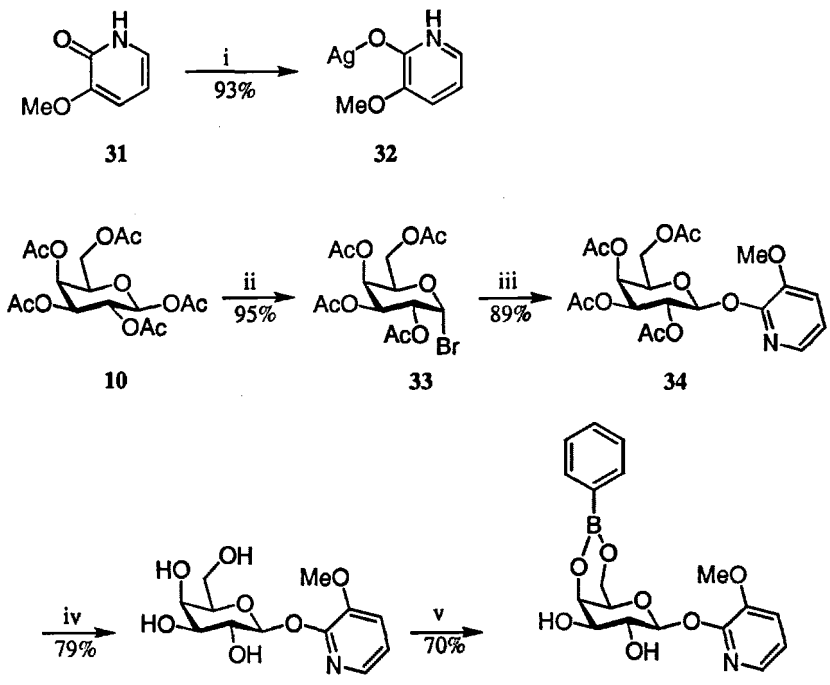
Reagents and conditions: i. NIS, TMSOTf, DCM, 0°C ii. aq. $NaHCO_3$ (10%)

Scheme 2.11

Next, it was decided to investigate the compatibility between phenylboronic acid and 3-methoxy-2-pyridyl glycosyl donors. These fully unprotected glycosyl donors were developed by Hanessian and co-workers.[169] They can be activated in nitromethane or DMF by a catalytic amount of TfOH or MeOTf. Unprotected glycosyl donors are of no practical use in oligosaccharide synthesis because a large excess of glycosyl acceptor is required in order to suppress self-condensation of the donor. Unprotected glycosyl donors could find an application in solid phase oligosaccharide synthesis. In this case, the unprotected glycosyl donor would be immobilised on a solid support and self-condensation of the donor would be suppressed. It was envisaged that a boronic acid derivatised polymer would be an ideal solid support for 3-methoxy-2-pyridyl glycosyl donors.

Thus, 3-methoxy-2-pyridyl β-D-galactopyranoside (**35**)[169] was prepared in three steps starting from β-D-galactose pentaacetate (**10**) (**Scheme 2.12**). Compound **10** was converted into the corresponding α-bromide by treatment with HBr/AcOH (33%, v/v) in DCM[170] (95%) and used without any further purification. A suspension of compound **33** and silver 3-methoxy-2-pyridoxide **32** in toluene under reflux afforded fully acetylated 3-methoxy-2-

pyridiyl galactoside **34** in 89%.[169] Compound **32** was previously prepared from 3-methoxy-2-(1*H*)-pyridone (**31**) by treatment with an equimolecular amount of sodium hydroxide in water and silver nitrate (**Scheme 2.12**).[169] Glycoside **35** was obtained by deacetylation of compound **34** by treatment with sodium methoxide in methanol/THF and purified by crystallisation from ethanol (79%). Boronate **36** was prepared by refluxing **35** and phenylboronic acid in benzene. Compound **36** precipitated as white crystals upon cooling and was obtained in a yield of 70% after recrystallisation from benzene.

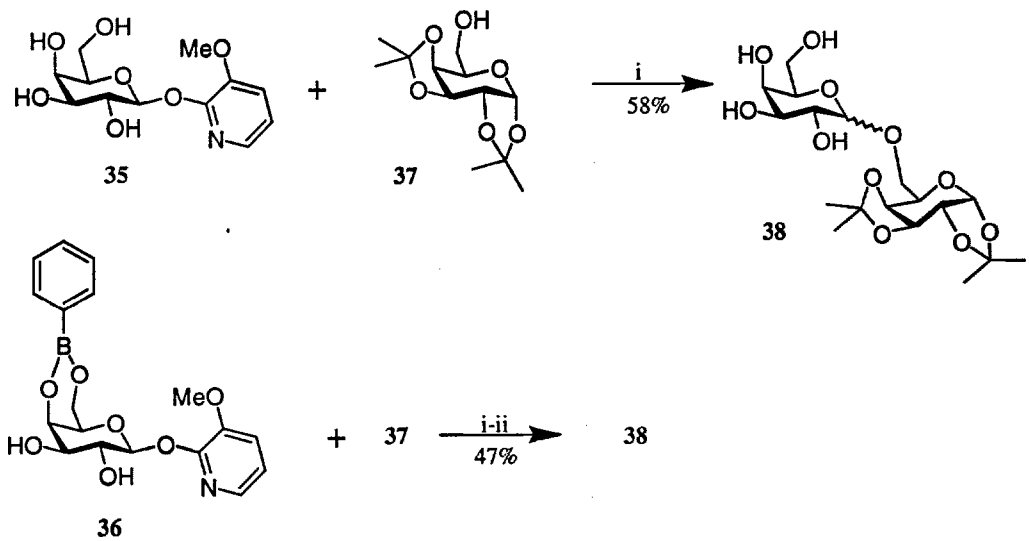


Reagents and conditions: i. $AgNO_3$, NaOH, H_2O ii. HBr-AcOH (33%), DCM iii. **32**, toluene, reflux iv. MeONa, MeOH/THF v. $PhB(OH)_3$, benzene, Δ

Scheme 2.12

Glycosylations were then performed using 3-methoxy-2-pyridyl glycosyl donors **35** and **36** (**Scheme 2.13**). 1,2:3,4-Di-*O*-isopropylidene acetal **37** was used as a glycosyl acceptor. Glycosylation of ten equivalents of **37** using fully unprotected donor **35** was conducted in nitromethane at room temperature using 0.2 eq. of MeOTf[169] (with respect to **35**) as promoter. Disaccharide **38**[171,172] was obtained in a yield of 58% (α/β, 1/1). Glycosylation of

37 (10 eq.) with boronate **36** in nitromethane using MeOTf as promoter, followed by purification by silica gel column chromatography, gave **38** in a yield of 47% (α/β, 1.5/1). A marked difference in the reactivity of the two donors was observed. Glycosyl donor **35** was consumed within 5 min of the addition of the promoter to the solution, glycosyl donor **36** was consumed in 2 hr. This could be explained by the fact that the phenylboronate group, in analogy with a benzylidene acetal, makes the conformational change of the pyranose ring required for the formation of the oxycarbonium ion energetically unfavourable.



Reagents and conditions: i. MeOTF, $MeNO_2$ ii. silica gel column chromatography

Scheme 2.13

2.3 Conclusions

Boronate esters perform as competent protecting groups in glycosylation reactions when using thioglycosides, trichloroacatimidates and glycosyl fluorides as glycosyl donors. The major limitation of their usage as protecting groups is that purification by standard chromatographic methods is not possible. This makes boronate esters only useful when a temporary protection is needed. On the other side, the successful outcome of the glycosylations where

phenlyboronic acid is employed as a protecting group suggests that phenylboronic acid may be a suitable linker for the solid phase synthesis of carbohydrates.

Chapter III
Polystyrylboronic Acid as Solid Support for Oligosaccharide Synthesis

3.1 Introduction

The success of solid phase syntheses of polypeptides and oligonucleotides has promoted investigations of oligosaccharide synthesis by polymer supported methods. Solid phase synthesis offers advantages over traditional solution phase approaches, notably that large excess of reagents can be used to drive a reaction to completion and that time-consuming work-up procedures and chromatographic purifications can be avoided. However, the preparation of well-defined oligosaccharides by a solid supported approach is complicated by several factors. Many glycosylations result in formation of mixtures of anomers and after several coupling steps, a complex mixture of products will be produced. In addition, the reactivities of glycosyl donors and acceptors are often significantly reduced after the immobilisation on a solid support. In order to overcome the many drawbacks and limitations associated with the current methodologies, there is a great need for the development of novel methodologies and of new polymers and linkers.

In this section, the investigation on the properties of polystyrylboronic acid as solid support for oligosaccharide synthesis is described. Polystyrylboronic acid presents several attractive features: the procedures for loading and unloading of the glycosides are simple and effective and the support is potentially reusable and has a high-loading capacity.

In order to prove the versatility of this support, a series of disaccharides was prepared on this solid support by employing different glycosyl donors. A trisaccharide was then synthesised by a procedure comprising the removal of a temporary protecting group from a disaccharide

attached to the solid support followed by glycosylation. A novel strategy was also developed where an anomeric mixture of disaccharides was formed on solid support, detached from the polymer and, after separation of the α- and β-anomers, reloaded onto the polymer to allow the continuation of the synthetic procedure. This sequence enables to overcome the problem of the lack of stereoselectivity often encountered in solid supported oligosaccharide synthesis, and may be attractive when it is very easy to load and unload the saccharide from the resin.

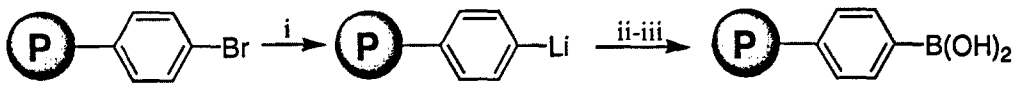
3.2 Results and Discussion

The most commonly used polymer backbones used for oligosaccharide synthesis on solid support are polystyrene and polyethyleneglycol derivatised polystyrene (Tentagel). While the high mobility and flexibility of the polyethyleneglycol moieties of Tentagel ensures high reactivities of the immobilised compounds and good swelling properties, this polymer has low loading and poor resistence to mechanical damage. Conversely, polystyrene derivatised solid supports have a very high loading capacity, though the rigidity of the backbone and the low polarity of the reactive space of the polymer decrease the reactivity of the polymer bound glycosyl donors and acceptors. It was decided to prepare and employ boronic acid derivatives of both polystyrene and Tentagel.

3.2.1 Polystyrylboronic acid

Polystyrylboronic acid (**41**) was prepared from commercial 4-bromopolystryrene (**39**, Novabiochem, loading: 1.97 mmol/g, crosslinked with 1% DVB) (**Scheme 3.1**). The polymer was first lithiated by treatment with 5 equivalents of *n*-BuLi in toluene at 65 °C.[173] After cooling to room temperature, the solvent was removed, and the lithiated resin was washed twice with dry toluene. The resin was swollen in dry THF and stirred in presence of excess of

trimethylborate for 18 h at room temperature, filtered, washed with THF and stirred in a mixture of dioxane/water/hydrochloric acid (4/2/1, v/v/v) at 60 °C. The solution was removed, and the resin washed extensively with dioxane/water (1/1, v/v), dioxane, acetone, and finally methanol. The polymer was filtered and dried under *vacuo* in presence of P_2O_5.



39 40 41

Reagents and conditions: i. *n*-BuLi, toluene, 65°C, 4.5 h ii. $B(OMe)_3$, THF, r.t. iii. dioxane, aq. HCl, 60°C

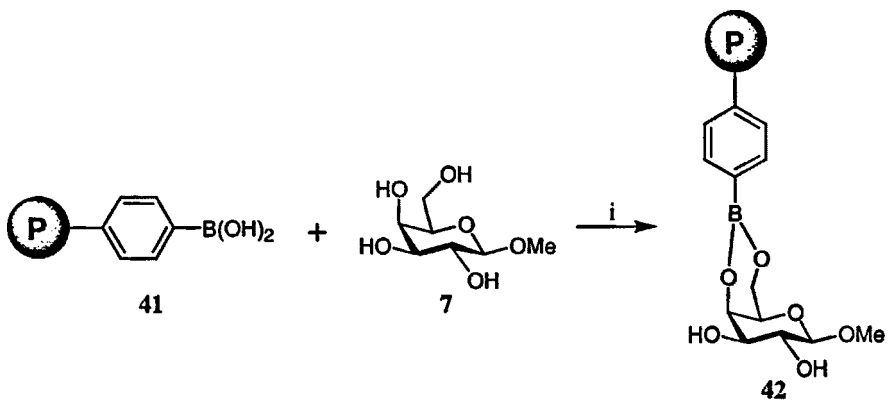
Scheme 3.1

Fréchet and coworkers[173] observed that the outcome of the lithiation reaction varies considerably depending on the type of polymer, the degree of bromination and the solvent used. Lithiation of 1% cross linked polystyrene in THF gave different results depending on the degree of functionalisation. In particular, highly substituted polymers, for example a polymer containing 3 mequiv/gram of bromine, required several successive treatments with *n*-BuLi, while less substituted ones, containing approximately 1-1.5 mequiv/g, required a single treatment. When benzene or toluene were used, however, a single treatment with n-BuLi was sufficient to complete lithiation of bromide from a 1% crosslinked polymer containing 3 mequiv/gram of bromide. This difference in reactivity cannot be explained by the different swelling properties in benzene, toluene or THF, since all three solvents have excellent swelling properties. It was instead proposed that in the more polar solvent, THF, ionic repulsions limit the accessibility of the reagent thus causing the reaction to stop once a fraction of the functional groups have reacted with *n*-BuLi.

Formation of boronate esters of carbohydrates is normally achieved by refluxing a glycoside in toluene or benzene with azeotropic removal of water. While polystyrylboronic has good

swelling properties in benzene and toluene, these solvents were found to be unsuitable to achieve loading, because of the poor solubility of unprotected glycosides. Thus, no loading was observed when a suspension of methyl β-D-galactopyranoside (7) and polystyrylboronic acid (41) was refluxed for 1 h in benzene. Interestingly, a similar reaction between glycoside 7 and phenylboronic acid does go to completion in benzene, and no undissolved glycoside is observed after a reaction time of 20 minutes. Pyridine proved to be an ideal solvent for loading glycosides on polystyrylboronic acid, most probably due to the good solubility of unprotected glycosides in this solvent.

Methyl β-D-galactopyranoside (7) was successfully loaded on the polymer by heating the polymer and the monosaccharide at 60 °C in pyridine for 1 h, and then at 80 °C under reduced pressure for another 1 h. In the case of polystyrylboronic acid, around 10 mL/g are necessary to swell the polymer fully. After cooling, the solvent was removed by filtration (**Scheme 3.2**). It was observed that it is very important to use a limited amount of solvent in order to keep the concentration of the substrate inside the volume of the polymer as high as possible.

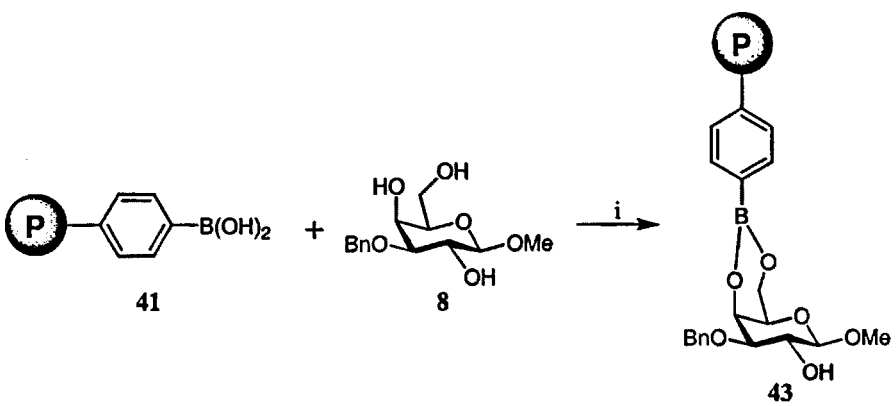


Reagents and conditions: i. pyridine, Δ

Scheme 3.2

After removal of the solvent, the resin was washed successively with pyridine, toluene, DMF and dichloromethane. It was found that the use of solvents containing moisture resulted in cleavage of the glycoside from the resin. This problem can be avoided by using a limited

amount of properly dried solvents when washing the polymer. Furthermore, use of protic solvents (*e.g.* methanol) to wash the resin resulted in cleavage of the glycoside from the resin. The loading of the polymer, intended as the quantity of boronic acid functionalities present on the polymer, was determined by the following procedure: an excess of glycoside **7** was reacted with the polymer, and the amount of glycoside recovered from the solution phase and from the washings the polymer at the end of the reaction was measured. Then glycoside **7** was cleaved from the polymer by refluxing a suspension of the polymer in acetone/water (4/1, v/v) at 80 °C for 30 minutes.[122] The hot suspension was then poured into a sintered glass filter and the resin washed several times with dichloromethane, methanol and acetone. All the washings were combined, the solvent was evaporated and the amount of glycoside **7** thus obtained was measured. The same procedure was performed by reacting an excess of glycoside **8** with the polymer following the same procedure employed to load **7** on the resin (**Scheme 3.3**). In both case it was found that ~75% of the bromide present in the starting material had been converted to boronic acid functionalities accessible for binding monosaccharides, assuming that all boronic acid functionalities are occupied by the glycoside.

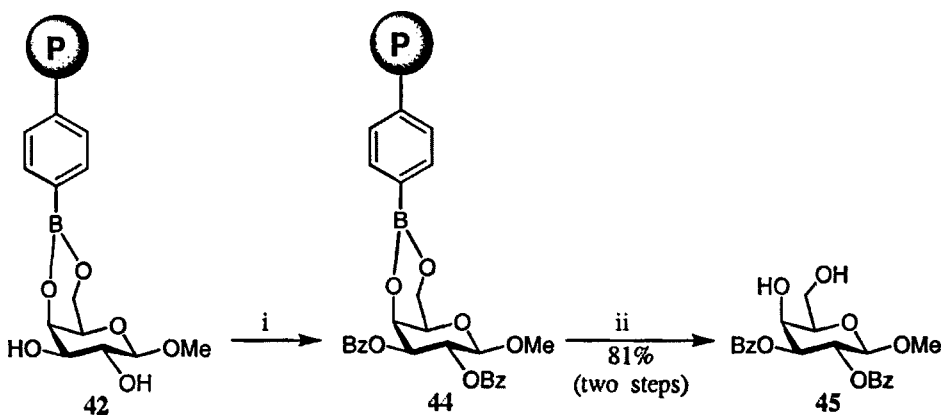


Reagents and conditions: i. pyridine, Δ

Scheme 3.3

Thus, the polystyrylboronic acid prepared following the procedure above described above has a loading of 1.6 mmol/g.

In order to confirm that glycoside **7** was effectively attached to the polymer, the polymer bound glycoside **42** was benzoylated following the procedure described by Fréchet *et al.*[122] Polymer bound glycoside **42** was reacted in pyridine with benzoyl chloride in the presence of a catalytic amount of DMAP (**Scheme 3.4**). After filtration of the resin, the solution phase was concentrated and analyzed by TLC and MALDI-TOF to determine if any 2,3-*O*-benzoylated glycoside was released into the solution phase during the washing and if any fully benzoylated glycoside was present in the solution phase due to release of glycoside into the solution phase during the reaction. The 2,3-*O*-benzoylated and the fully benzoylated glycosides were not detected. Cleavage of product **45**[174] from the solid support was achieved by refluxing a suspension of the polymer in acetone/water (4/1, v/v) at 80 °C for 30 minutes.[122] Silica gel column chromatography performed on the residue gave product **45** in a yield of 81%.

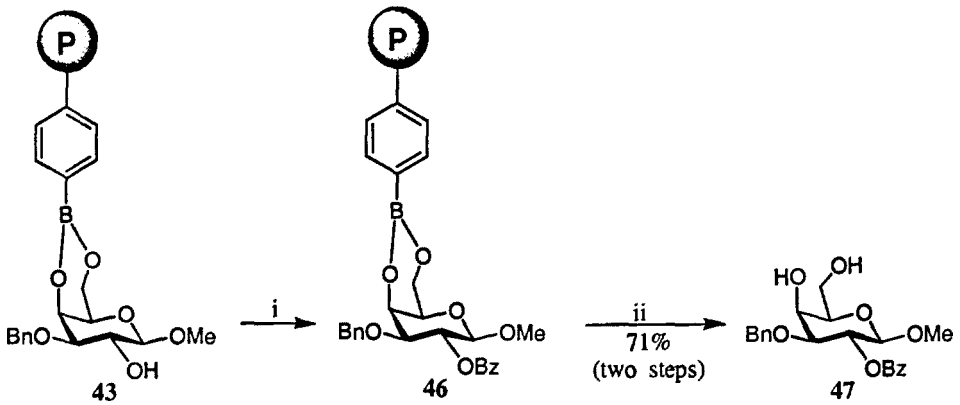


Reagents and conditions: i. BzCl, DMAP, 16 h, pyridine ii. Acetone / water (4/1, v/v), 80°C, 30min

Scheme 3.4

In order to verify that the loading of **8** on the resin had also been effective, polymer bound glycoside **43** was benzoylated using benzoyl chloride in pyridine following the procedure previously described (**Scheme 3.5**). Monobenzoylated glycoside **47** was obtained after silica

gel column chromatography in a yield of 71%. No unreacted starting material **8** was detected after cleavage of the product from the resin.

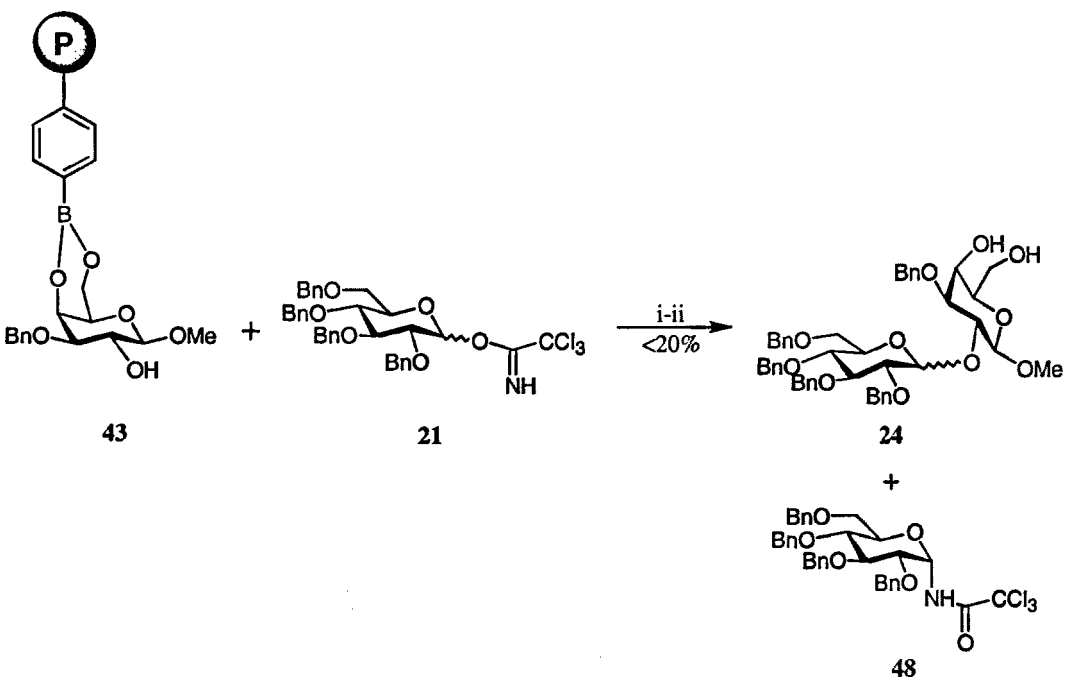


Reagents and conditions: i. BzCl, DMAP, pyridine, 16 h ii. acetone/water (4/1, v/v), 80°C, 30min

Scheme 3.5

It was then decided to use polymer bound glycoside **43** as a glycosyl acceptor, and to perform a series of glycosylations using different glycosyl donors: glycosyl fluorides, trichloroacetimidates and thioglycosides. Thus 0.5 equivalents of **8**, relative to the amount of boronic acid functionalities present on the polymer, were loaded on polymer **41**. It was thought that the excess of boronic acid functionalities would ensure that the loading went to completion, and it was anticipated that unreacted boronic acid functionalities would not interfere in subsequent glycosylations. Indeed, no glycosyl acceptor **8** was found in the filtrate and in washings at the end of the reaction. In the first instance, attempts to glycosylate polymer bound glycoside **43** were performed using 5 eq. of trichloroacetimidate **21** in DCM at 0 °C in the presence of a catalytic amount of TMSOTf (**Scheme 3.6**). In general, reactions performed on solid phase require a large amount of glycosyl donor in solution to drive the reactions to completion. After 2 h, all glycosyl donor **21** was consumed. TLC and MALDI-TOF analysis on the material cleaved from a small sample of beads (~1mg) showed presence of the expected product **24** (~20%) but also of a large quantity of unreacted starting material **8**. The reaction was repeated at –60 °C, but no improvement was observed. In both cases, a

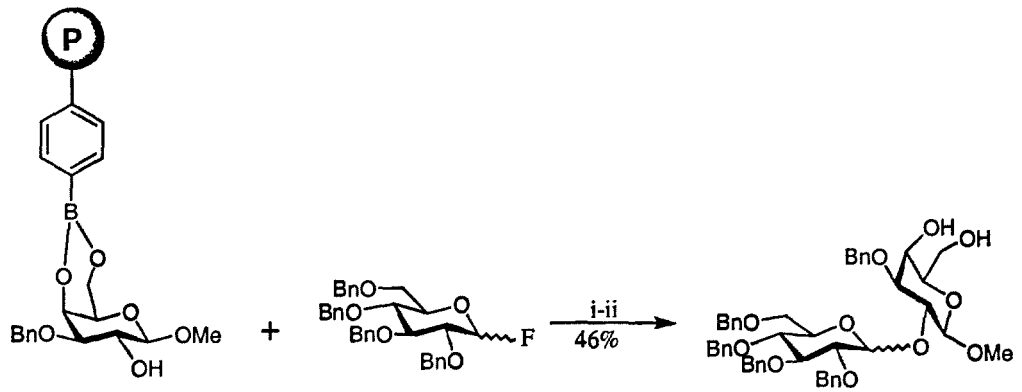
significant amount of the trichloroacetamide **48** resulting from the rearrangement of glycosyl donor **21** was formed as demonstrated by MALDI-TOF and ^{1}H NMR analysis. This rearrangement has been previously observed to occur in cases when the reactivity of the glycosyl acceptor is very low.[37]



Reagents and conditions: i. TMSOTf, DCM, 0°C ii. aceton /water (4/1, v/v), 80°C, 30min

Scheme 3.6

Next, glycosyl fluoride **49**[25,175] was employed as a glycosyl donor in the presence of the promoter system Cp_2ZrCl_2 and AgOTf[18](**Scheme 3.7**). After cleavage from the resin, product **24** was obtained in a yield of 46%, together with a large amount of unreacted glycoside **8**. This glycosylation presents the disadvantage that unsoluble silver salts, that cannot be separated from the resin by filtration, are formed during the reaction.

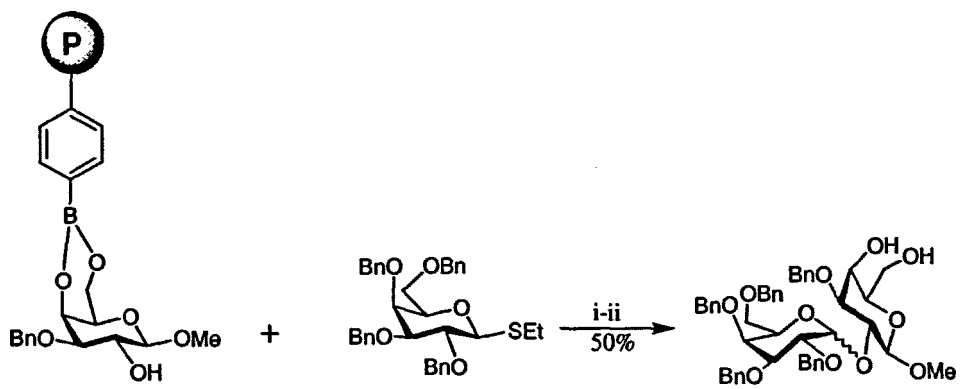


43 49 24

Reagents and conditions: i. Cp_2ZrCl_2, AgOTf, DCM, r.t. ii. acetone/water (4/1, v/v), 80°C, 30 min

Scheme 3.7

Finally, thioglycoside **18** was used as a glycoysl donor. A glycosylation was conducted at room temperature and TMSOTf and NIS were used as promoter system. The reaction was followed by TLC, and glycosyl donor **18** was consumed within 5 min (**Scheme 3.8**). The overall yield of disaccharide **50** was a disappointing 50% (α/β=1/1) and a large amount of glycosyl acceptor was found unreacted (50%).



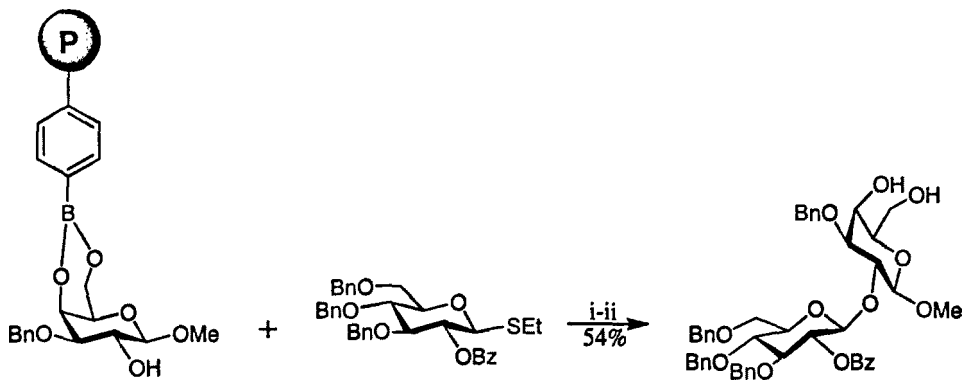
43 18 50

Reagents and conditions: i. NIS, TMSOTf, DCM, r.t. ii. acetone/water (4/1, v/v), 80°C, 30 min

Scheme 3.8

Next, thioglycoside **51**,[176] with a benzoyl protecting group at C-2, was used as a glycosyl donor. The presence of an acyl protecting group at C-2 favours the formation of the β-anomer only. The reaction of compound **51** with polymer supported glycoside **43** in the presence of NIS and TMSOTf as promoter system showed rapid consumption of thioglycosyl donor **51**

(**Scheme 3.9**). Compound **52** was obtained in a yield of 54% after cleavage from the resin, while unreacted glycosyl acceptor **8** was recovered (46%).



43 **51** **52**

Reagents and conditions: i. NIS, TMSOTf, DCM, r.t. ii. acetone/water (4/1, v/v), 80°C, 30 min

Scheme 3.9

When the reaction was repeated again with a further 5 eq. of glycosyl donor **51**, only a small increase in the yield was obtained (58%). The same reaction performed in the presence of freshly prepared DMTST[40] at 0 °C gave disaccharide **52** in a yield of 43%. Even though different yields were obtained with different glycosyl donors, it appeared that the best yields achieved were around 50%. Repetition of the reaction was not effective: part of the polymer bound glycosyl acceptor appeared to be unreactive, and was recovered as glycoside **8** at the end of the reaction. It became clear that this was an inherent property of the polymer. All the glycosylations up to this point were performed on the same batch of loaded polymer, which had been prepared by reacting **8** with an excess of polymer **41.**

It was decided to prepare a new batch of polymer loaded with glycoside **8.** This time, an excess of **8** (1.3 eq.) was reacted with polymer **41.** As expected, 0.3 eq of unreacted **8** was recovered in the solution phase and in the washings at the end of the loading procedure.

The next step consisted of repeating some of the previous glycosylations with this fully loaded polymer. Thus, a glycosylation was performed using glycosyl donor **51** and polymer

bound glycosyl acceptor **43** in the presence of NIS and TMSOTf as promoter system. The glycosyl donor was consumed in less than 10 minutes as indicated by TLC. After cleavage of the product from the resin, disaccharide **52** was isolated in 96% yield. TLC analysis conducted on the material cleaved from a small amount of beads (~ 1mg) showed that all polymer bound glycoside **8** had been consumed. The same glycosylation was repeated using three equivalents of glycosyl donor, with respect to the glycosyl acceptor, compared to five eqivalents used previously. In this case, disaccharide **52** was still obtained in a yield of 96%. When the reaction was performed using 1.5 equivalents of glycosyl donor, the yield decreased to 85% and unreacted glycosyl acceptor (15%) was recovered.

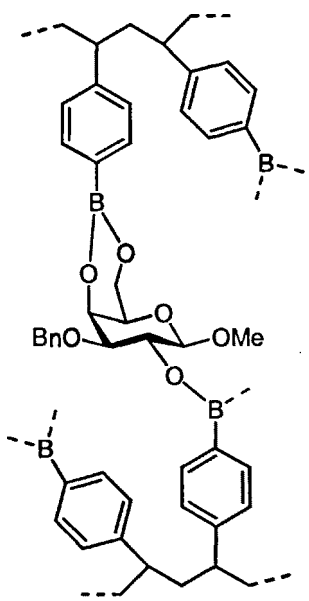
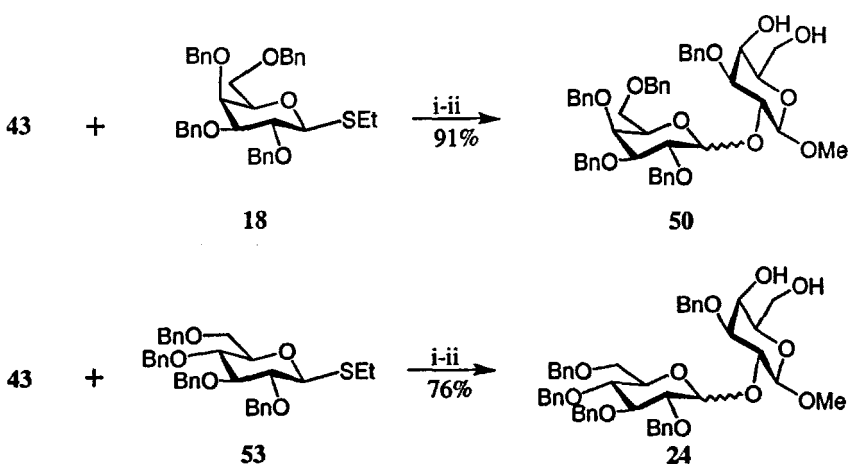


Figure 3.1

The marked difference in the outcome of glycosylations performed using fully loaded polymer compared to partially loaded polymer is possibly due to the fact that unreacted boronic acid functionalities present in the resin interfere in glycosylation reactions by reacting with the hydroxyl group at C-2 of the glycosyl acceptor, thus acting as a protecting group and preventing glycosylation (**Figure 3.1**).

Different promoters were used to activate glycosyl donor **51**. The use of DMTST and MeOTf[38,39] as promoter gave discaccharide **52** in a yield of respectively 25% and 28%. In both cases, most of the starting material **8** was recovered unreacted. It is evident that the combination of TMSOTf and NIS is the promoter system of choice when performing glycosylation using thioglycosides as glycosyl donors and polystyrylboronic acid bound glycosides as glycosyl acceptors.

Next, reaction of thioglycoside **18** with polymer **43** gave disaccharide **50** in 91% yield (α/β = 1.5/1), while glycosylation of **43** using fully benyzlated glucoside **53** gave disaccharide **24** in 76% yield (α/β = 1/1) (**Scheme 3.10**).



Reagents and conditions: i. NIS, TMSOTf, DCM, r.t.
ii. acetone/water (4/1, v/v), 80°C, 30 min

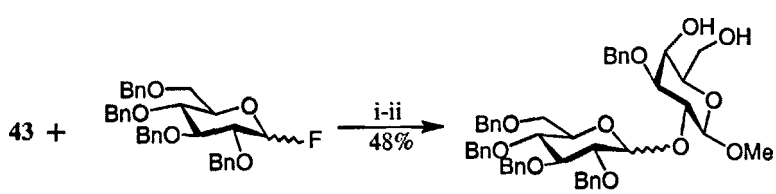
Scheme 3.10

It is noted that the more reactive fully benzylated thioglycoside donors are less effective to glycosylate the polymer bound acceptor. This could be explained by the fact that the rate of reactions on solid phase are controlled by diffusion of reagents into the reactive volume of the polymer. Therefore, a very reactive glycosyl donor may decompose before diffusing into the polymer. The very good performance of the less reactive glycosyl donor **51**, which carries a

benzoyl group at C-2, suggests that the reactivity of glycosyl donors employed in solid phase synthesis can be tuned in order to maximise the yields of glycosylations.

The glycosylations with fluorides and trichloroacetimidates as glycosyl donors were repeated. Glycosyl fluoride **49** was reacted with **43** using Cp_2ZrCl_2 and AgOTf as promoter system to give disaccharide **24** after cleavage from the resin in 48% yield (α/β = 2/1). A large amount of glycosyl acceptor (52%) was recovered unreacted. In an attempt to increase the yield, the glycosylation reaction was repeated with 5 eq. of glycosyl donor. Thus, after a first glycosylation TLC analysis of a small amount of crude product released from a few beads (~1mg) showed that glycosyl acceptor was still present. TLC analysis after a second glycosylation, showed no improvement in the ratio between product and unreacted glycosyl acceptor and an overall yield of 48% of disaccharide **24** was still obtained.

When trichloroacetimidate **21** was reacted with polymer bound **43**, disaccharide **24** was obtained in a yield of 56% (α/β = 2/1). It can be noted that when the glycosyl acceptor **8** was loaded on the polymer using an excess of polymer, a yield of less then 20% was obtained in the same glycosylation. An attempt to increase the yield of the reaction by repeating the glycosylation gave no improvement in the overall yield of disaccharide **24** (**Scheme 3.11**).



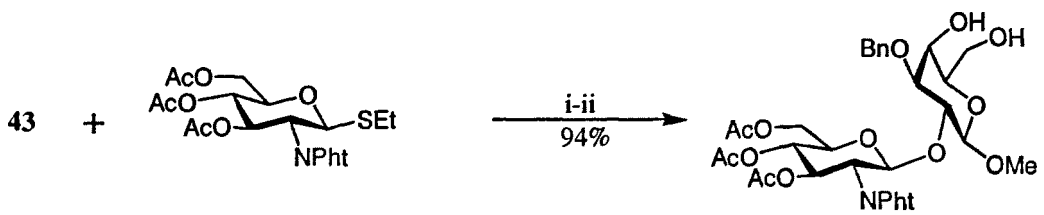
49 **24**

Reagents and conditions: i. Cp_2ZrCl_2, AgOTf, r.t. ii. acetone/water (4/1, v/v), 80°C, 30 min

Scheme 3.11

It was decided to employ a suitably protected aminosugar as a glycosyl donor. Reaction of five equivalents of aminosugar **54**[38] with glycosyl acceptor **43** gave disaccharide **55** in a yield

of 94%. Only the β-linked product was obtained, due to the participating nature of the C-2 phthaloyl protecting group (**Scheme 3.12**).



54 **55**

Reagents and conditions: i. TMSOTf, DCM, 0°C ii. acetone / water (4/1, v/v), 80°C, 30min

Scheme 3.12

Surprisingly, glycosylations using glycosyl donors **56**[177] and **57**[178] were not successful. A large amount of polymer bound starting material **8** was left unreacted and side products were detected (**Figure 3.2**).

56 **57**

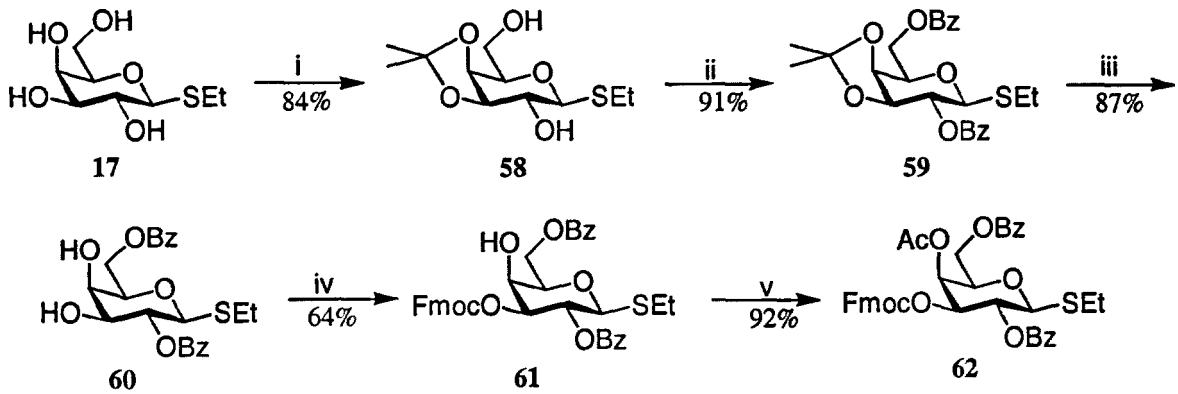
Figure 3.2

A further challenge posed the construction of oligosaccharides on solid support employing the boronic acid linker. Solid supported oligosaccharide synthesis requires in general the removal of a temporary protecting group after each glycosylation step in order to generate a free hydroxyl function that is then available for next glycosylation step. A requirement of this temporary protecting group is that it should be orthogonal with other protecting groups present and with the linker used to attach the oligosaccharide to the solid phase .

The boronic acid linker is stable to mild acidic and basic conditions. However, it can be cleaved by water, protic solvents and mixtures of water and organic solvents. A protecting group that can be cleaved under either basic or acidic conditions but without the use of water

or protic solvent is thus necessary to allow the elongation of disaccharides. With this requirements in mind, Fmoc was selected as a temporary protecting group. This protecting group has been extensively used as *N*-protecting group in the synthesis of oligopeptides, and has also been recently used as an *O*-protecting group in oligosaccharide synthesis.[90,179,180] It is stable to acidic conditions and can be promptly cleaved under mild basic conditions.

Scheme 3.13

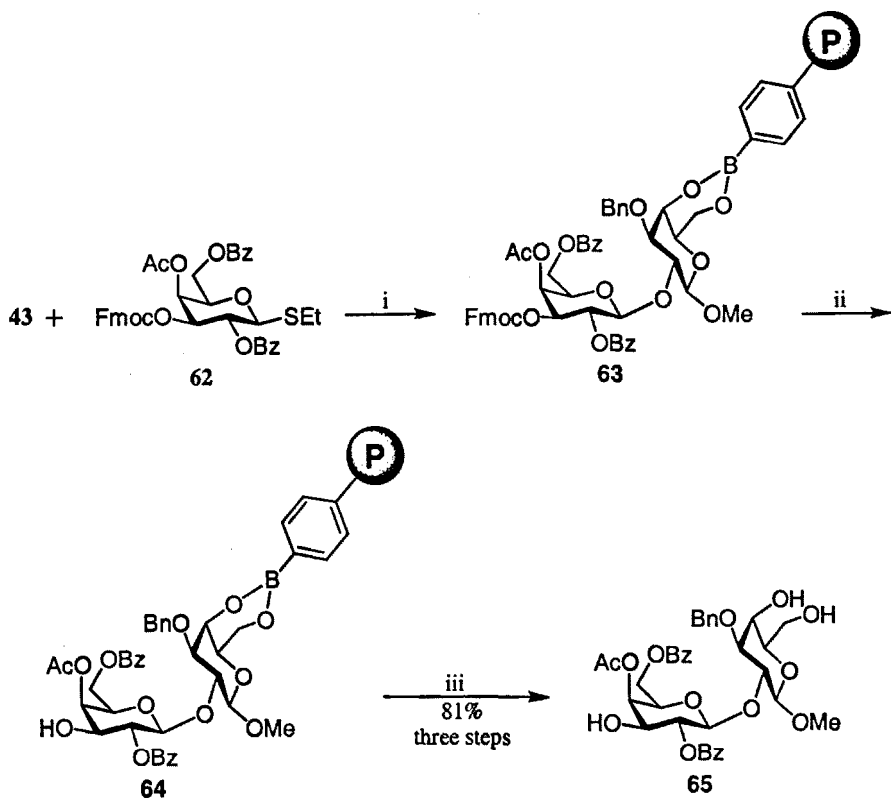


Reagents and conditions: i. 2,2-dimethoxy propane, toluenesulfonic acid ii. benzoyl chloride, DMAP, pyridine iii.AcO H/ H_2O, 60°C iv. FmocCl, pyridine v. acetic anhydride, pyridine

Glycosyl donor **62** was prepared in five steps starting from thioglycoside **17**. Compound **17** was reacted with 2,2-dimethoxypropane which served as reagent as well as solvent in the presence of a catalytic amount of *p*-toluensulfonic acid to give the 3,4-*O*-isopropylidene[181] substituted thioglycoside **58**[182] in 84% yield (**Scheme 3.13**). Benzoylation of **58** with benzoyl chloride in pyridine in presence of a catalytic amount of DMAP gave compound **59**[183] in a yield of 91%. Removal of the isopropylidene acetal was performed by heating **59** at 60 °C in acetic acid/water (4/1, v/v) to give **60**[183] in 87% yield. Treatment of compound **60** with FmocCl in pyridine gave selective reaction of the 3-OH to afford **61** in 64% yield. It should be noted that although Fmoc is sensitive to basic conditions, the Fmoc substituted compound **61** can actually be formed in pyridine, because cleavage of Fmoc in pyridine is a slow

reaction. Finally, acetylation of compound **61** in pyridine/acetic anhydride gave the desired glycosyl donor **62** in a yield of 92%.

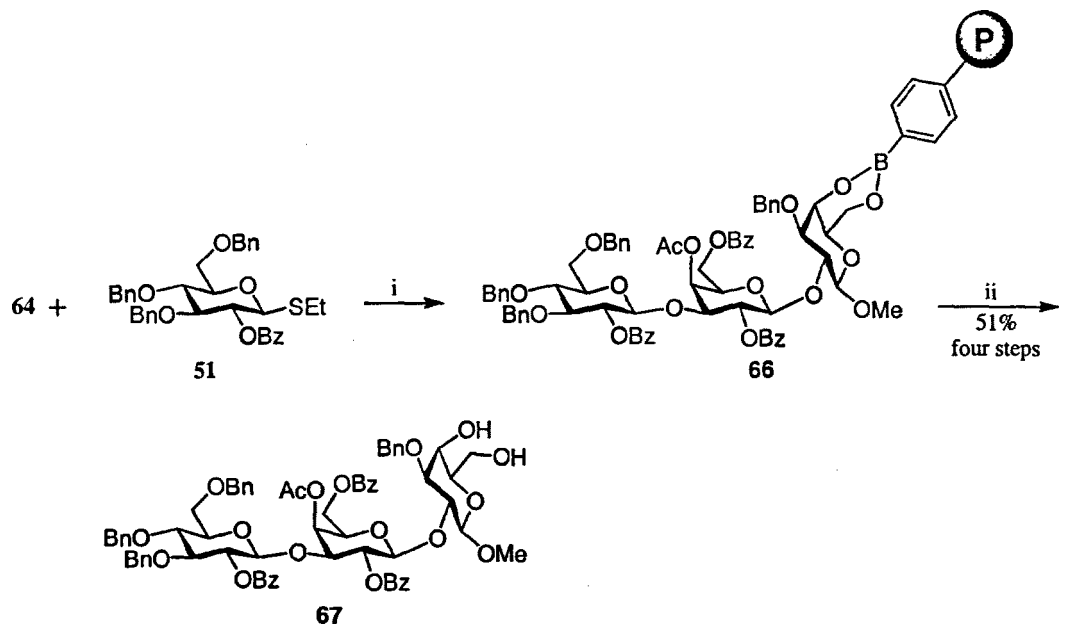
Glycosylation of polymer bound glycosyl acceptor **43** with glycosyl donor **62** in the presence of NIS and TMSOTf as promoter system in DCM gave polymer bound disaccharide **63** **(Scheme 3.14)**. TLC and MALDI-TOF analysis showed that all glycosyl acceptor was consumed and that only one product was formed. Cleavage of the Fmoc protecting group was performed by stirring the polymer in DCM/Et_3N (1/1, v/v) for 20 minutes at room temperature. After washing the resin, disaccharide **65** was cleaved from the resin by treatment with acetone/water (4/1, v/v) at 80 °C. Disaccharide **65** was obtained in a yield of 91% after silica gel column chromatography.



Reagents and conditions: i. TMSOTf, NIS, DCM, r.t. ii. Et_3N/DCM , r.t. iii. acetone/water (4/1, v/v), 80°C, 30 min

Scheme 3.14

In order to synthesise a trisaccharide, the sequence of glycosylation and removal of the temporary protecting group on the solid phase was repeated. Compound **64** was carefully dried and glycosylated at room temperature with ten equivalents of glycosyl donor **51**, in the presence of NIS and TMSOTf as promoter. TLC analysis showed that no polymer bound glycosyl acceptor **65** was left unreacted. After cleavage from the resin, trisaccharide **67** was obtained in a yield of 51% (**Scheme 3.15**).



Reagents and conditions: i. TMSOTf, NIS, DCM, r.t. ii. acetone/ water (4/1, v/v), 80°C, 30 min

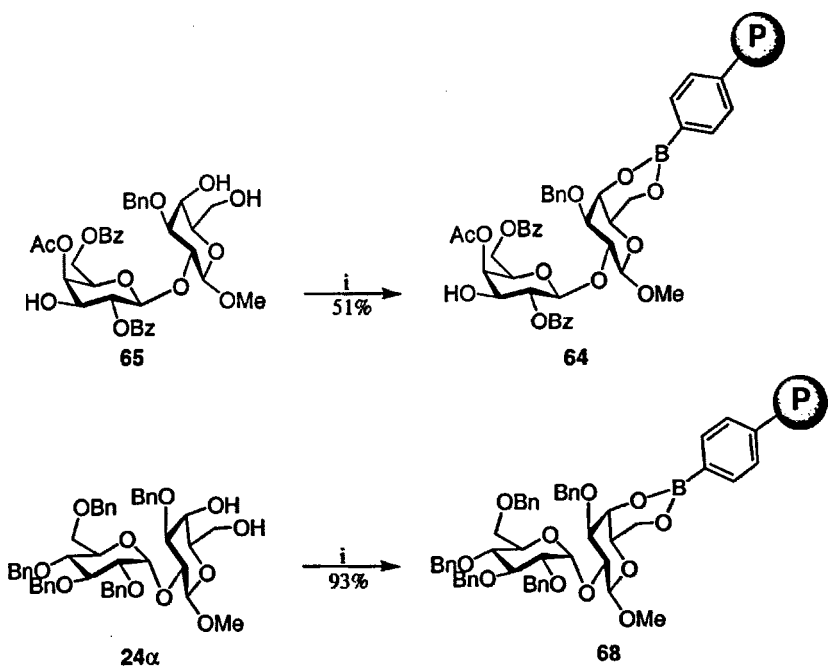
Scheme 3.15

The synthesis of trisaccharide **67** shows the versatility of the boronic acid linker for the solid supported synthesis of oligosaccharides.

One of the most attractive properties of polystyrylboronic acid as a solid support are the mild conditions employed for the loading: a suspension of the polymer and of the saccharide are heated at 80 °C in pyridine. Any saccharide having a suitable unprotected diol can in principle react with boronic acid. The experiments conducted during our research up to now have shown that there is no substantial difference between the reactivity of of phenylboronic acid and of its polymeric counterpart.

Another attractive feature is the ease of cleavage from the resin. No reagents are necessary, only water is required to cleave the boronate linkage. This means that after cleavage, the products are dissolved or suspended in water, or in a mixture of organic solvent and water, that can be easily removed by evaporation. These features are highly desirable for linkers employed in the solid phase synthesis of oligosaccharides.

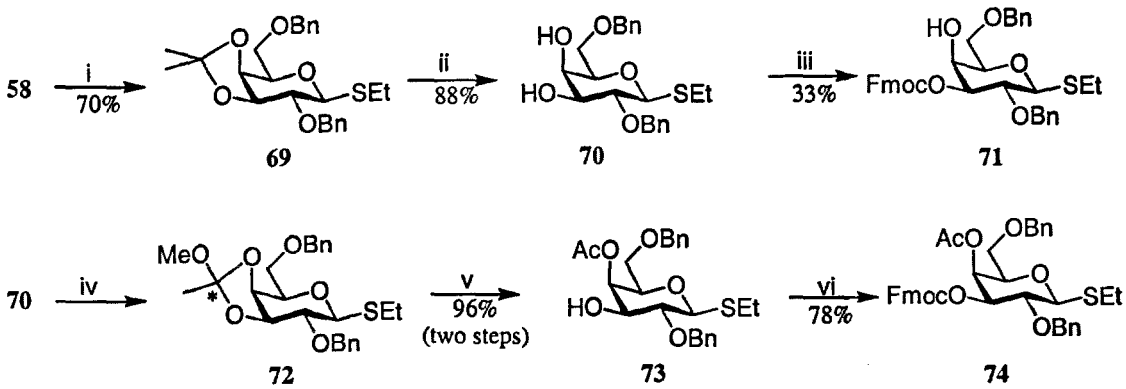
Next, disaccharides were attached to the solid support. Thus, two disaccharides, **65** and **24α** were loaded onto polymer **41**, according to a procedure identical to the one which was used to load monosaccharides. The yield of both reactions was determined by measuring the amount of unreacted disaccharide that was recovered after filtration of the resin followed by repetitive washings. In the case of **24α** the yield was 93%. This yield is very similar to the result obtained when loading monosaccharides such as methyl 3-*O*-benzyl-β-D-galactopyranoside **8**. In contrast, the yield for the loading of compound **65** was only 51% **(Scheme 3.16)**.



Reagents and conditions: i. 41, pyridine, 80°C, Δ

Scheme 3.16

It was envisaged that the possibility of loading a disaccharide on the solid support would allow us to develop a new synthetic strategy whereby an anomeric mixture of disaccharides could be formed on the solid support, detached from the polymer and after separation of the α- and β-anomers, reloaded onto the polymer in order to continue the synthetic sequence. This sequence would enable the problem of the lack of stereoselectivity often encountered in solid supported oligosaccharide synthesis to be addressed and may be attractive when it is very easy to load and unload the saccharide from the resin, as in the case of polystyrylboronic

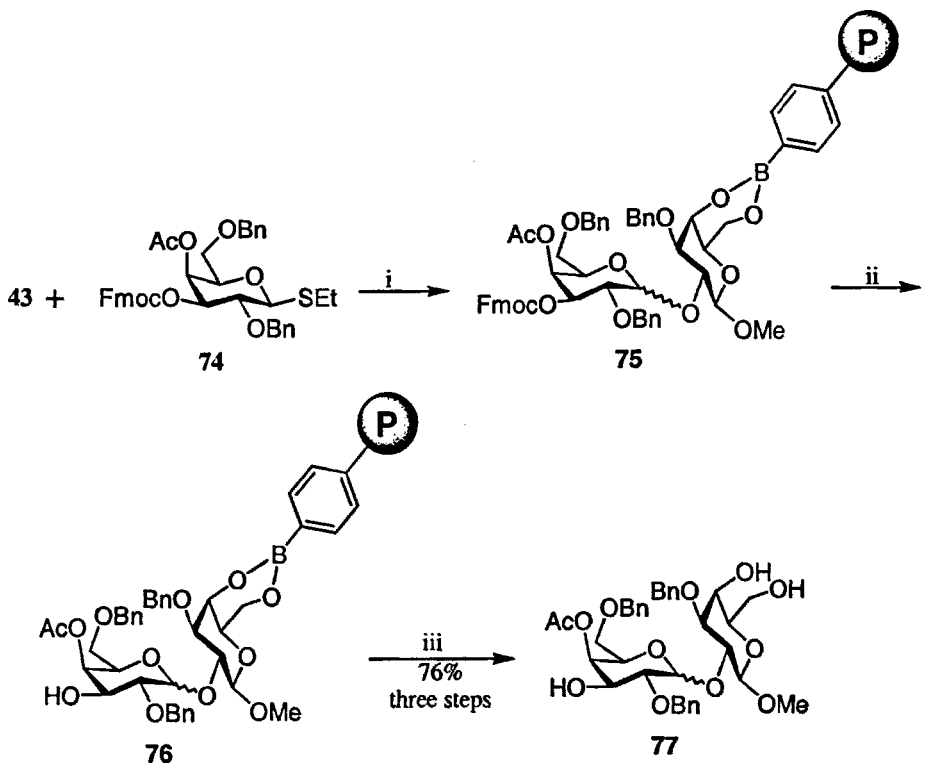


Reagents and conditions: i.BnBr, NaH, DMF ii. AcOH/H_2O, 60°C iii. FmocCl, pyridine iv. trimethyl orthoacetate, *p*- toluensulfonic acid, benzene v. AcOH/H_2O, r.t. vi. FmocCl, pyridine acid.

Scheme 3.17

Glycosyl donor **74** was synthesised in five steps starting from isopropylidene acetal **58**. Compound **58** was benzylated with benzyl bromide and NaH in DMF affording thioglycoside **69**[182] in 70% yield. Cleavage of the isopropylidene acetal was achieved in a yield of 88% by heating compound **69** in acetic acid/water (4/1, v/v) at 60 °C. Reaction of compound **70** with one equivalent of Fmoc chloride gave a mixture of products and the yield of desired compound **71** was a disappointing 33%. The corresponding benzoylated compound **60** (see **Scheme 3.15**) could be selectively derivatised with Fmoc at 3-OH, due to the difference in reactivity between the equatorial 3-OH and the axial 4-OH, but the difference in reactivity

between 3-OH and 4-OH in compound **70** is not large enough to allow a regioselective reaction. In fact, both hydroxyls are made electron rich and activated by the electron releasing benzyl groups. Instead, C-4 *O*-acetylated thioglycoside **73** was obtained following an alternative facile and high yielding procedure. First, treatment of compound **70** with trimethyl orthoacetate and a catalytic amount of *p*-toluensulfonic acid in benzene gave orthoester **72** quantitatively, as observed by TLC analysis. Acetolysis of **72** gave the desired acetylated compound **73** in 96% yield for the two steps.[184] Less then 20 minutes were necessary to complete this procedure. Reaction of compound **73** with FmocCl in pyridine afforded



Reagents and conditions: i. TMSOTf, NIS, DCM, r.t. ii. Et_3N/DCM , r.t.
iii. acetone/water (4/1, v/v), 80°C, 30 min

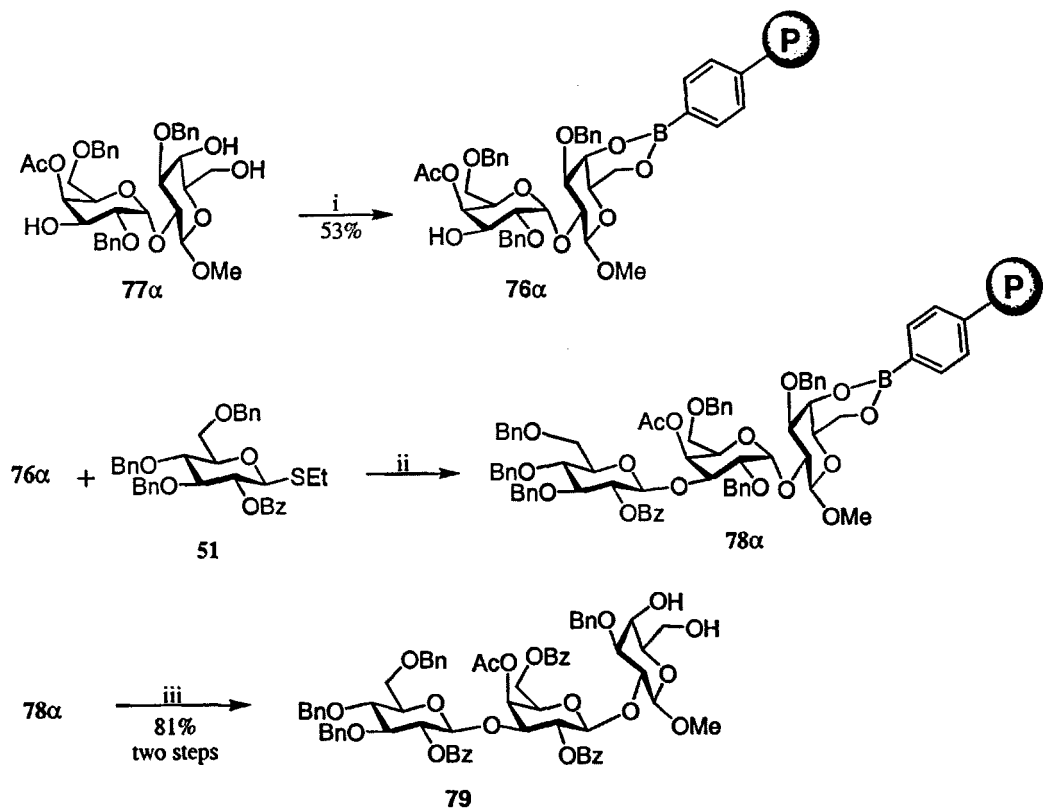
glycosyl donor **74** and the yield of this step was 78% (**Scheme 3.17**).

Scheme 3.18

Glycosylation of polymer bound glycosyl acceptor **43** with thioglycoside **74** in DCM using NIS and TMSOTf as promoter system was followed by cleavage of the Fmoc protecting

group. This was achieved by stirring the resin in trietylamine/DCM (1/1, v/v) for 20 minutes. Disaccharide **77** was cleaved from the resin by heating the polymer in acetone/water (4/1, v/v) for 30 minutes. The overall yield was 76% (α/β = 1.6/1) and the α- and the β-anomer were separated by preparative TLC (**Scheme 3.18**).

Disaccharide **77α** was then successfully reloaded on polystyrylboronic acid **41** in a yield of 69%, as determined by measuring the amount of disaccharide recovered in the solution phase after the loading procedure. Polymer bound disaccharide **78α** was then glycosylated using thioglycoside **51** as a glycosyl donor. After cleavage from the polymer, trisaccharide **79** was obtained in a yield of 81% (**Scheme 3.19**).

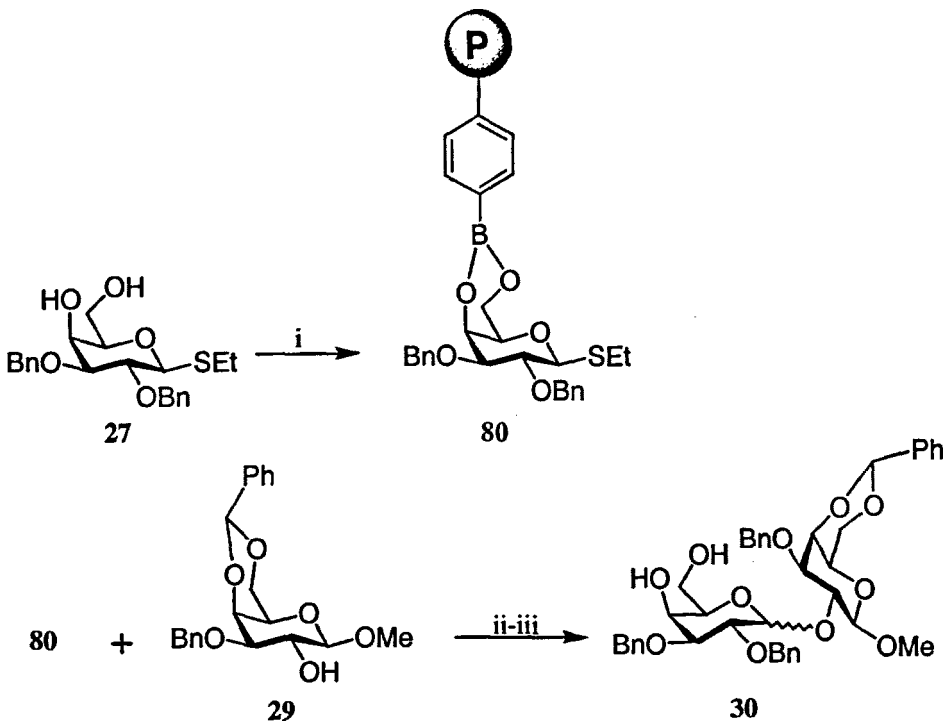


Reagents and conditions: i.pyridine, Δ ii. TMSOTf, NIS, DCM, r.t. iii. acetone/water (4/1, v/v), 80°C, 30 min

Scheme 3.19

It was then investigated whether it was possible to build an oligosaccharide chain on polystyrylboronic acid starting from the non-reducing end. Thus, thioglycoside **27**[166] was

bound to polymer **41** following the procedure previously described in yield of 98% (**Scheme 3.20**). Activation of the polymer bound glycosyl donor using NIS and TMSOTf in presence of 5 equivalents of glycosyl acceptor **29**, followed by cleavage from the resin gave, after purification by silica gel column chromatography, disaccharide **30** in a yield of 68%.



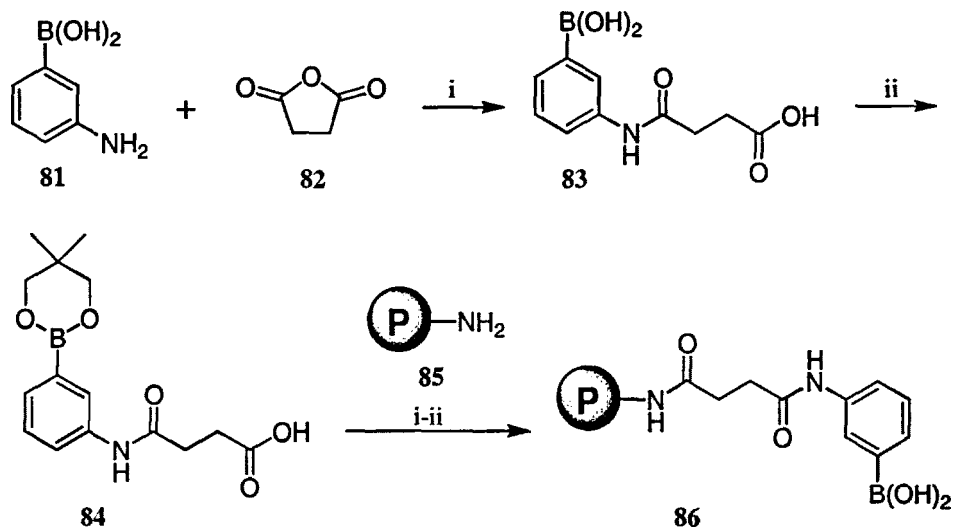
Reagents and conditions: i. **41**, pyridine, Δ ii.TMSOTf, NIS, DCM, r.t. iii. acetone/water (4/1, v/v), 80°C, 30 min

Scheme 3.20

3.2.2 Boronic acid derivatised Tentagel

In order to prove the versatility of the phenylboronic acid linker, it was decided to derivatise Tentagel polymer with boronic acid functionalities. Thus, carboxylic acid **83**[185] was prepared by reacting 3-amino-phenylboronic acid **81**[186] with succinic anhydride **82**. Purification of **83** was difficult. Silica gel column chromatography and attempts to crystallise the product were unsuccessful. Derivatisation of **83** with 2,2-dimethyl-1,3-propanediol gave compound **84** in a yield of 56% after crystallisation from benzene. Reaction of **84** with amino derivatised

Tentagel (Novasyn TG amino resin HL, Novabiochem, loading: 0.43 mmol/g) was performed using benzotriazole-1-yl-oxy-tris-pyrrolodino-phosphonium (PyBOP)[187] and DIPEA in DMF. The disappearance of free amino functionalities was verified by performing a Kaiser test on a small sample of beads (~1mg). The diol was cleaved using the same procedure used to cleave saccharides from the resin (**Scheme 3.21**).

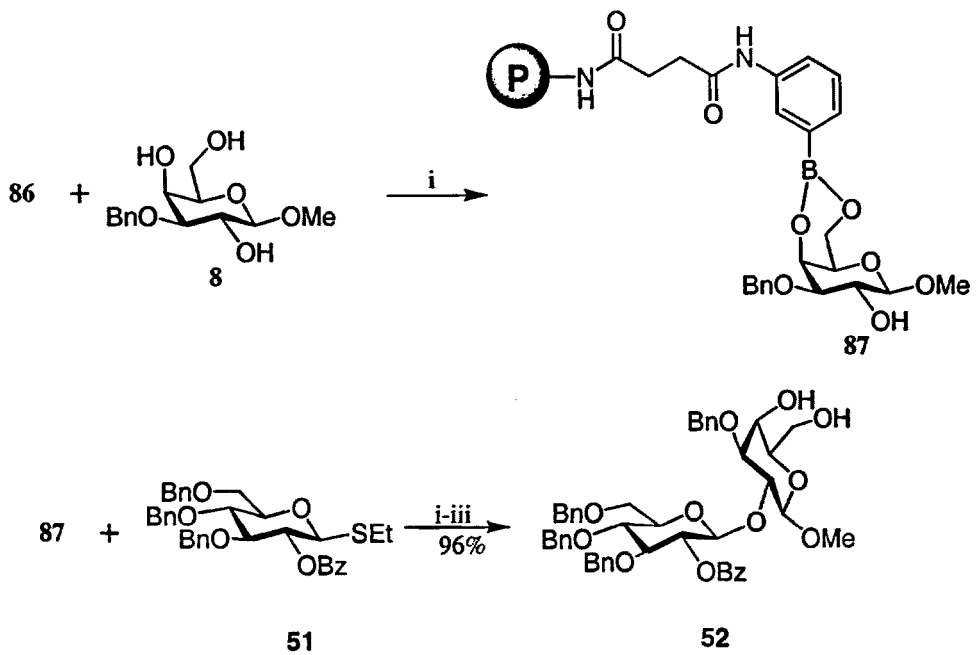


Reagents and conditions: i. pyridine, r.t. ii.1,3-propandiol, benzene, Δ
iii. PyBOP, DIPEA, DMF iv. acetone/water, 80°C

Scheme 3.21

The loading of this polymer was not determined and in first instance the number of boronic acid functionalities was considered equal to the number of amino functionalities originally present on the polymer, assuming that the amide coupling and the deprotection of the boronic acid had been quantitative. One equivalent of glycosyl acceptor **8** was reacted with boronic acid derivatised Tentagel **86**, following the procedure previously described for polystyrylboronic acid. The resin was washed with dichloromethane, DMF and again dichloromethane and dried with P_2O_5 *in vacuo*. Glycosylation with glycosyl donor **51** was performed in dichloromethane at room temperature, with NIS and TMSOTf as promoter

system. The suspension was shaken, because Tentagel can be mechanically damaged by mechanical stirring. The glycosyl donor was completely consumed within one hour, and TLC analysis showed that almost no unreacted glycosyl acceptor was present on the polymer. After cleavage of the saccharide from the resin and purification by silica gel column chromatography, disaccharide **52** was obtained in 45% yield over three steps (**Scheme 3.22**).



Reagents and conditions: i. pyridine, 80°C, Δ ii. NIS, TMSOTF, DCM
iii. acetone/waater, 60°C

Scheme 3.22

3.3 Conclusions

The boronic acid linker proved valuable for the synthesis of disaccharides on solid support. Glycosylations with a series of different glycosyl donors were performed, and thioglycosides proved to be the best glycosyl donors for glycosylations using polystyrylboronic acid bound glycosides as acceptors.

Loading of saccharides on the resin and cleavage from the resin are performed using very mild conditions, and disaccharides and trisaccharides could be prepared using this solid

support. It was found that unreacted boronic acid functionalities can determine the outcome of the glycosylation, and that it is important that all those functionalities are bound to a glycoside. Polystyrylboronic acid is potentially a reusable polymer, but investigation about the reusability for glycosylation reactions has not yet been performed.

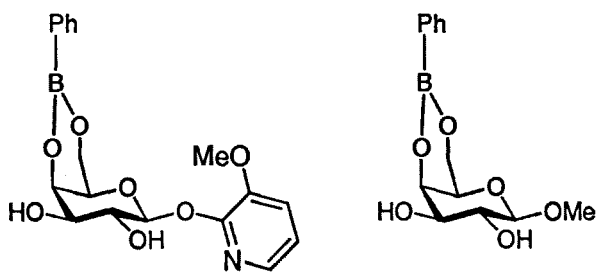
Oligosaccharide synthesis on solid support requires to perform glycosylations as well as removal of temporary protecting groups from the growing oligosaccharide chain. While glycosylations are performed in strictly anhydrous conditions, many deprotection steps require the presence of water or other protic solvent. The fact that the boronic acid linker is sensitive to water and protic solvents appears to limit the applicability of this linker. Fmoc was successfully used as a temporary protecting group that can be cleaved without affecting the linkage between polystyrylboronic acid and the substrate, but further investigations are required to determine which other temporary protecting groups are compatible with the boronic acid linker.

Chapter IV

Structural Motifs in Crystals of Boronate Esters of Carbohydrates

4.1 Introduction

Boronic acids have recently found an application as sensors for carbohydrates.[148] Information about the structure of boronate esters would be valuable to optimise the design of such saccharide sensors, but surprisingly very little structural information regarding boronate esters of saccharides and glycosides is available. In fact, in the Cambridge Structural Database (CSD) only one X-ray structure of a boronate ester of a carbohydrate can be found.[188] In this chapter the crystal structures of 3-mehtoxy-2-pyridyl β-D-galactopyranoside 4,6-*O*-phenylboronate (**36**) and of methyl β-D-galactopyranoside 4,6-*O*-phenylboronate (**88**) are described (**Figure 4.1**).



36 **88**

Figure 4.1

4.2 Results and discussion

Phenylboronate ester **36** was prepared by heating **35** and phenylboronic acid in benzene with azeotropic removal of water. It was noted that immediately after removing of the flask from the oil bath, crystals of **36** started to precipitate. After few minutes compound **36** was completely precipitated in the form of white fibre-like crystals. This observation prompted us

to conduct further investigation. Many other boronates of carbohydrates had been prepared during this research, but no one had shown to crystallise so quickly. Instead, crystallisation of some boronates proved difficult and sometime not possible at all. Further, it was possible to crystallise compound **36** from eighteen different solvents. Small needle-shaped crystals were obtained from dioxane, THF, ethanol and cyclohexanone. Fibre-like crystals, that are unsuitable for diffractometric analysis, were obtained from methanol, chloroform, propanol, butanol, diethylene glycol, *iso*-amyl alcohol, benzene, *iso*-butanol, dichloromethane, nitromethane, acetonitrile, acetone, ethyl acetate and 2-butanone.

It is well known that boronate esters can interact with amines to form tetrahedral complexes. This property is exploited to develop sensors for carbohydrates.[148] It was envisaged that an *intermolecular* interaction between the nitrogen of the pyridyl moiety and boron might be present in the structure of **36**, and in order to verify this hypothesis it was decided to obtain a crystal structure of boronate **36**.

Single crystals suitable for diffractometric analysis were obtained only from THF and from ethanol by slow evaporation from a saturated solution in the respective solvent. Attempts to obtain small crystals from benzene were not successful. In both cases, the solvent was found to be included in the crystal by ^{1}H NMR analysis in a ratio of 1:1. In the case of the crystals obtained from THF, also a molecule of water was included in the crystal in a ratio of 1:1, as later observed from the X-ray structure.

Accidentally, crystallisation of methyl β-D-galactopyranoside 4,6-phenylboronate **88** from ethanol also provided single crystals of the quality required for X-ray analysis. Also in this case molecules of solvents were included in the crystal in a 1:1 ratio.

X-ray structures of two different crystals of compound **36** were obtained. The first crystal is relative to compound **36·MeOH (= 36A)** and the second to the compound **36·THF·H_2O**

(=**36B**). Also a crystal structure for compound **88**, relative to the compound **88·EtOH** was obtained.

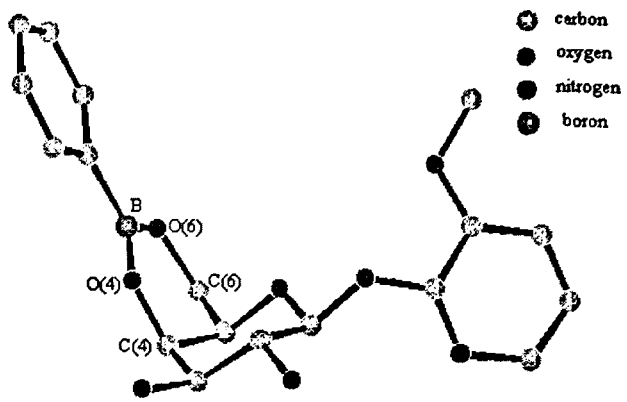


Figure 4.2

In the crystal structure of **36A**, the phenyl ring of **36** is co-planar with the BO_2 group and the methoxy group is co-planar with the pyridyl group. The length of the B---O and B---C bonds, and the value of the O---B---C angles are consistent with data reported in literature[188] (**Figure 4.2**).

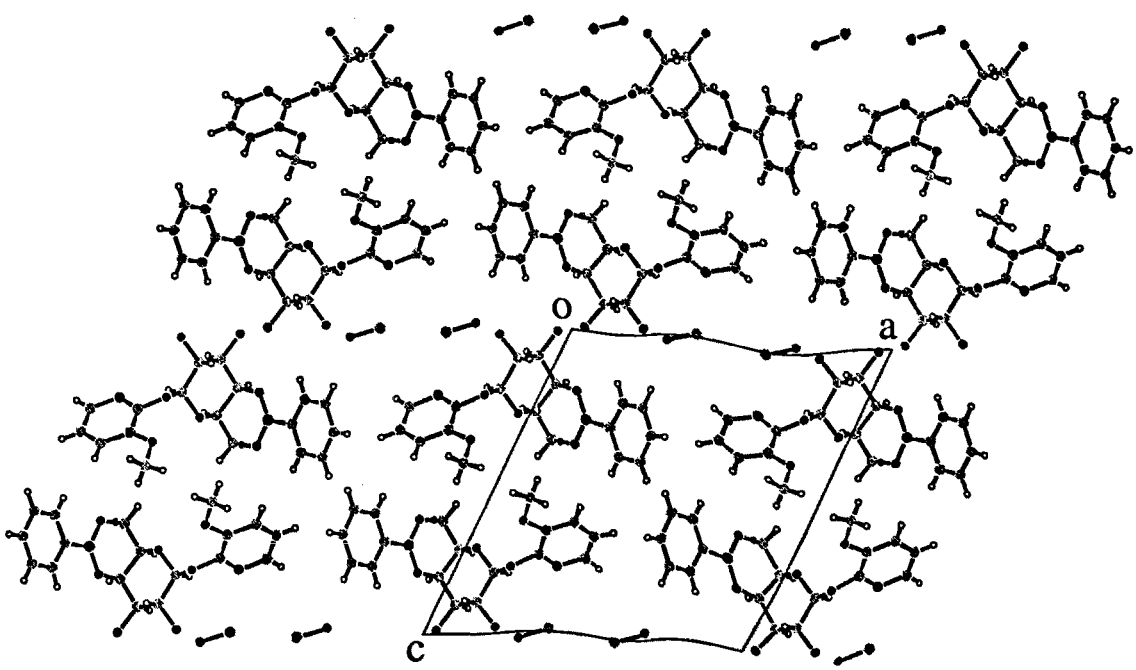


Figure 4.3

Molecules of **36** are related by translation along the **b**-axis (perpendicular to the plane of the picture in **Figure 4.3**), forming separate stacks of phenyl ring and pyridyl ring along this axis. In each stack, the distance between the centres of adjacent rings is *ca.* 5.1Å with an interplanar distance of *ca.* 4.0 Å. This excludes the possibility of π–π interaction. Along the **b**-axis, the structure contains channels occupied by methanol molecules.

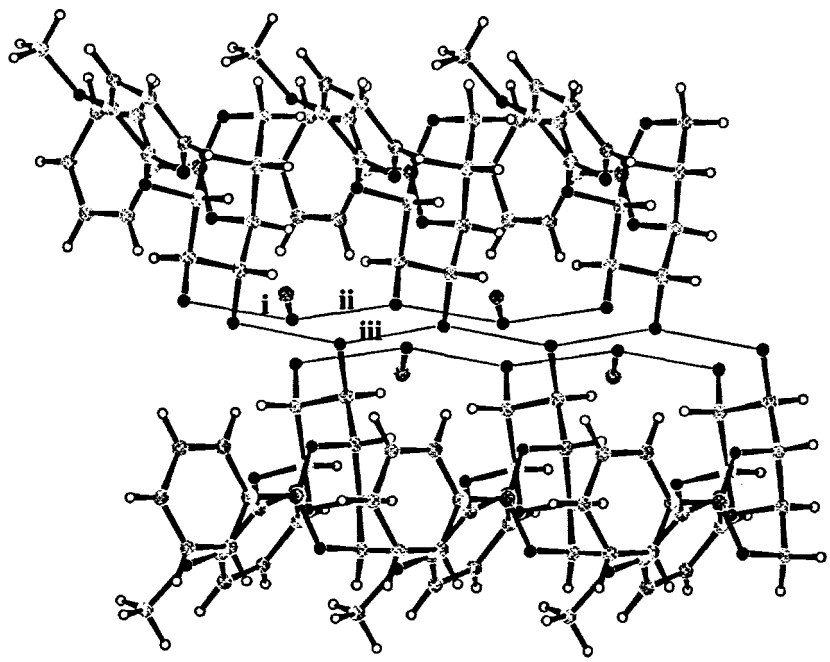


Figure 4.4

Molecules of **36** and of methanol are linked by hydrogen bonding, and there are also hydrogen bonding interactions between adjacent molecules of **36**. One hydroxyl group of **36** is involved in hydrogen bonds to two methanol molecules (O---O distances 2.88 Å and 2.73 Å, **i** and **ii** in **Figure 4.4**), whereas the other hydroxyl group of **36** is involved in a hydrogen bond to the hydroxyl group of a neighbouring molecule of **36** (O---O distance 2.84 Å, **iii** in **Figure 4.4**). These different types of hydrogen bonding form hydrogen bonding chains along the **b**-axis.[189] The side-by-side arrangement of the hydrogen bonded stacks can be visualised as a double layer of molecule **36** parallel to the **ab** plane (horizontal in **Figure 4.4**). The

methanol molecules are located in the interior of the double layer, which has hydrophilic character.On the other hand, the outer surfaces of the double layer are hydrophobic. Adjacent double layers interact with each other through van der Waals interactions (**Figure 4.5**).

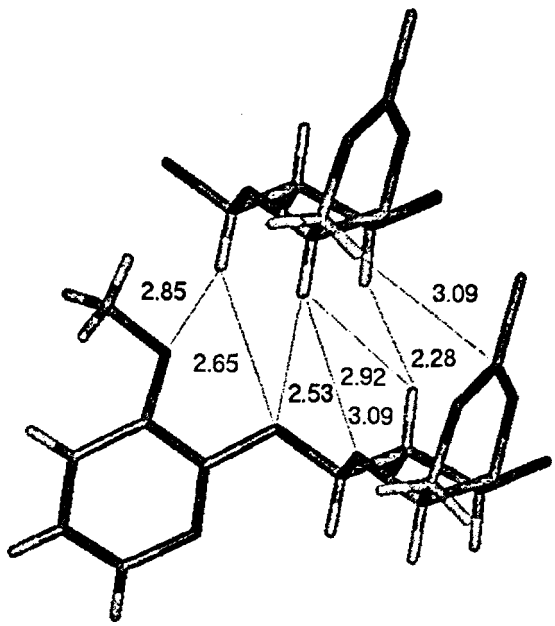


Figure 4.5

In the crystal structure of **36B**, the phenyl ring of **36** is coplanar with the BO_2 group and the methoxy group is co-planar with the pyridyl group (as also observed for **36A**). The main difference between the molecules of **36** in structures **36A** and **36B** concerns the conformation of the pyridyl ring. The torsion angle N---C---O---C (representing rotation of the C-(pyridyl)-O-(saccharide) bond is 48° in **36B** and 26° in **36A**. In the crystal structure of **36B**, the molecules of **36** are also stacked along the **b**-axis with similar stacking distances to those in the crystal structure of **36A** (**Figure 4.6**). In the crystal structure of **36B**, the channels formed by molecules of **36** are occupied both by disordered THF molecules and water molecules.

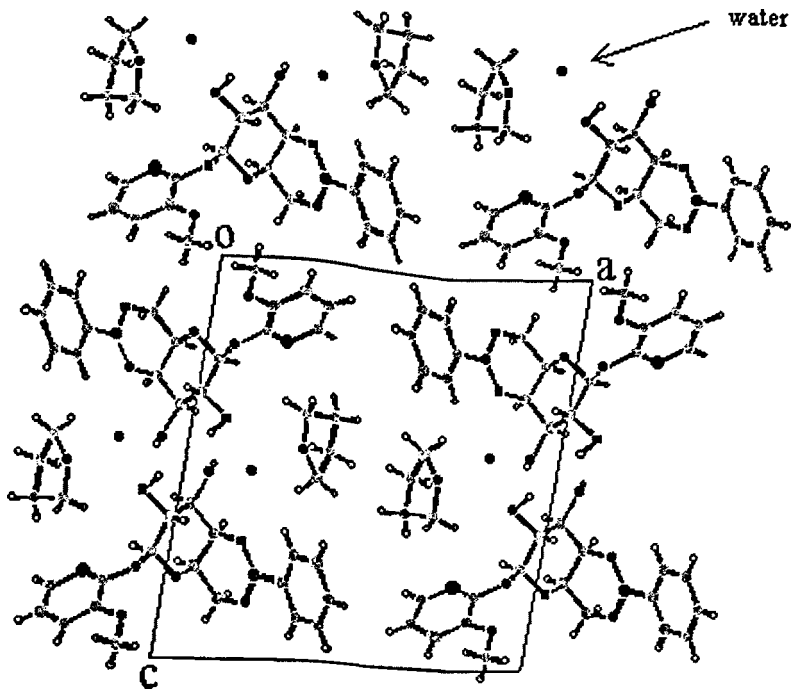
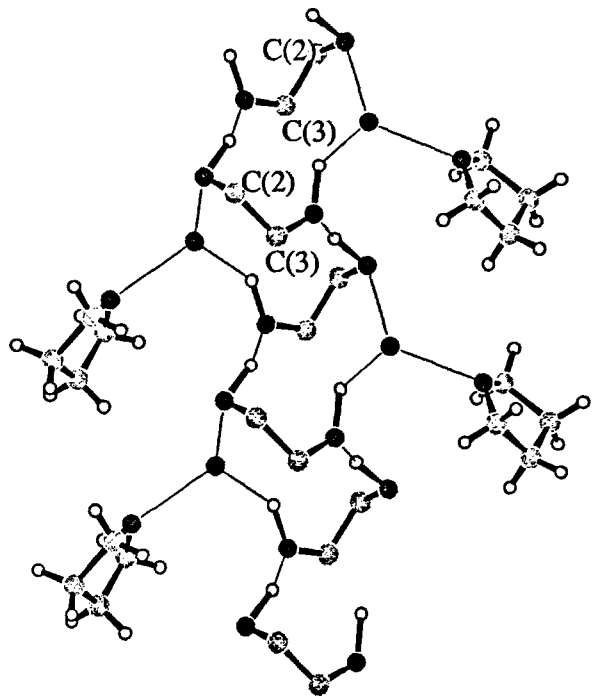


Figure 4.6

The structure contains the following types of hydrogen bonds: between **36** and water molecules (O---O distance 2.70 Å) and between water molecules and THF molecules (O---O distance ~2.6 Å). Each hydroxyl group of a given molecule of **36** interacts through hydrogen bonding with another molecule of **36** and with a water molecule. The structure of **36B** can be again described in terms of a double layer of molecules of **36** parallel to the **ab** plane (horizontal in **Figure 4.7**).



Only part of the structure is represented.

Figure 4.7

The THF molecules and the water molecules are located in the interior of the double layer, which has a hydrophylic character. Again, the outer surfaces of the double layer are hydrophobic. As for the crystal structure of **36A**, adjacent double layers interact with each other through van der Waals interactions.

Not surprisingly in view of the different solvent molecules involved, the hydrogen bonding patterns in the crystal structure of **36A** and **36B** are different and hence the geometry arrangements in the interior of the double layers in these structures are different. However, the arrangement of **36** on a given exterior surface of the double layer is essentially identical in structures **36A** and **36B**. Moreover, the interactions between the hydrophobic surfaces of adjacent double layers in the two structures are virtually identical, suggesting that these structures have a particularly favorable geometric arrangement of molecules of **36** at the interface between the double layers. The structure of **36** seems to cause steric complimentarity

between the hydrophobic faces of **36**, thus forming a robust structural motif common to **36A** and **36B** (**Figures 4.8**).

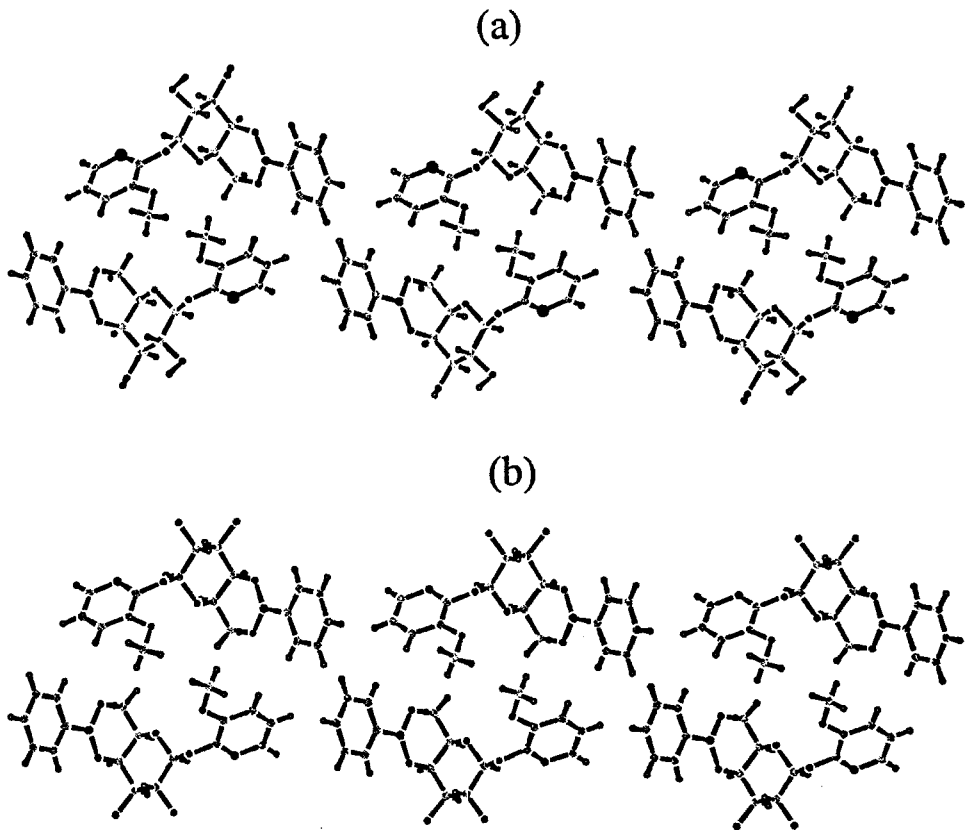


Figure 4.8

In **Figure 4.9** the double layers of compound **36** observed in structures **36A** and **36B** are superimposed.

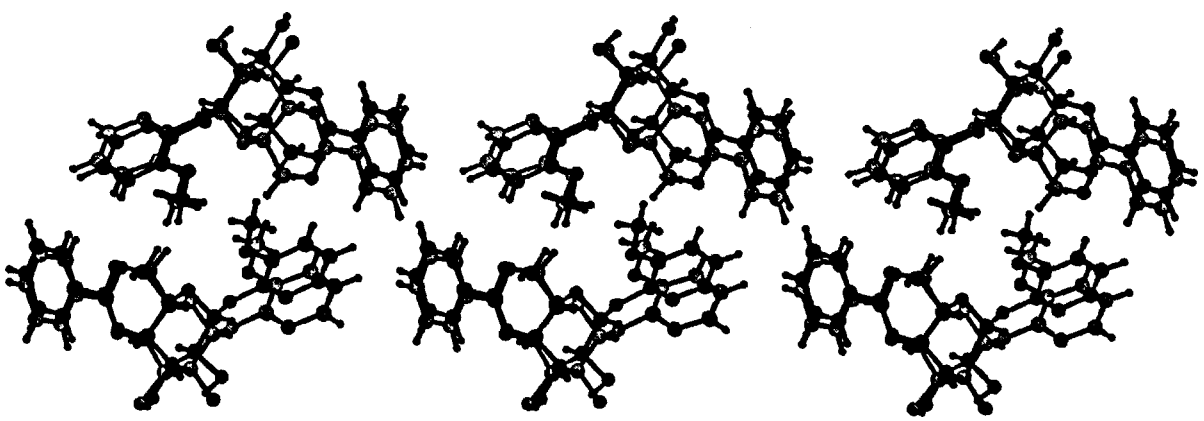


Figure 4.9

It is interesting to note that the pyridyl group is not involved in any kind of hydrogen bonding, and also that no interaction between B and N is observed.

The structure of **88·EtOH** shows many similarities with the ones of **36A** and **36B**. Again, the phenyl ring of **88** is coplanar with the BO_2 group (**Figure 4.10**).

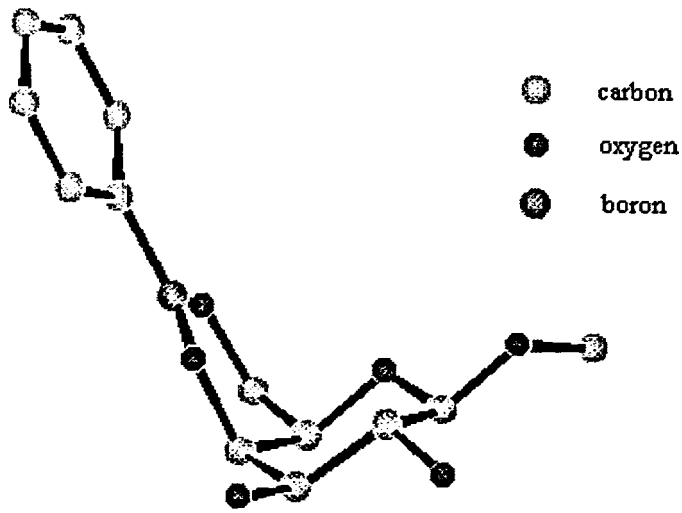


Figure 4.10

The molecules of **88** are stacked along the **b** axis with similar stacking distances to those observed in the other two structures. The channel formed along the **b**-axis by molecules of **88** is occupied by molecules of ethanol (**Figure 4.11**).

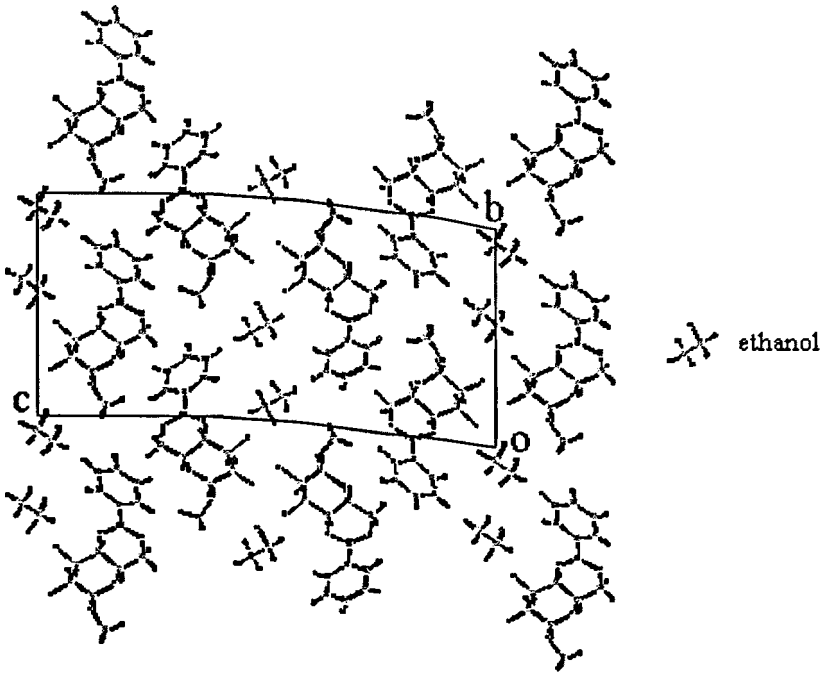


Figure 4.11

The structure contains two types of hydrogen bonding: between one hydroxyl and the ethanol (O----O distance 2.87 Å) and between the other hydroxyl and methanol (O----O distance 2.70 Å). In this case a chain of hydrogen bonds along the **b**-axis is not formed (**Figure 4.12**).

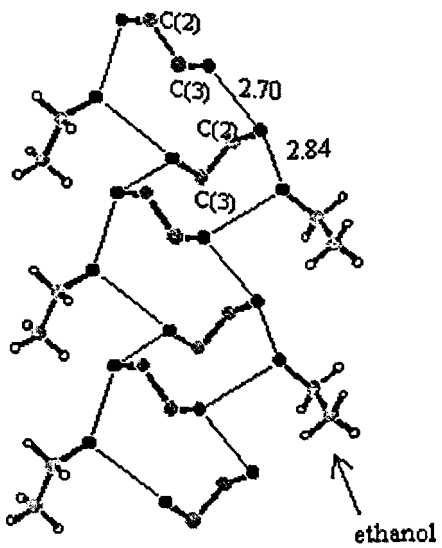


Figure 4.12

Also the structure of **88·EtOH** can described in terms of a double layer of **88** parallel to the **ab** plane. The molecules of ethanol are located in the interior of the double layer, which has

hydrophylic character. Again, the outer surfaces of the layer are hydrophobic, and adjacent double layers interact with each other through van der Waals interactions (**Figure 4.13**).

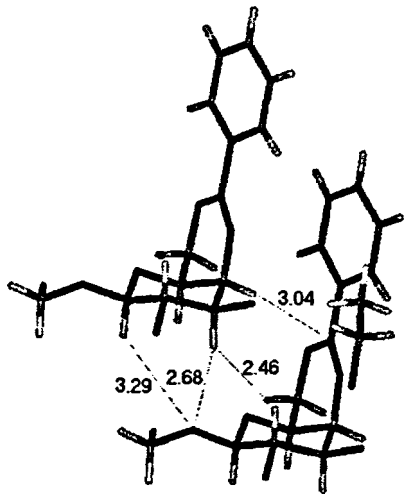


Figure 4.13

4.3 Conclusions

The X-ray structures of **36A**, **36B** and **88·EtOH** provide valuable structural information about 4,6-*O*-boronate esters of β-D-galactosides. Furthermore, in all the three structures, a robust structural motif consisting of double layers of the glycoside boronates held together by a network of hydrogen bonding is observed.

The observation of robust structural motifs in the crystalline state is of great interest for a branch of supramolecular chemistry known as *crystal engineering*.[190,191] It is in general very difficult to predict the structure of crystals of organic molecules. The structure depends on a subtle balance of intermolecular interactions which can be achieved for a given conformation in a particular packing arrangement. At the current level of knowledge, computational methods are not available to perform such a complex calculation. *Crystal engineering* is

interested with the prediction of the structure of crystals of organic molecules through the observation of a large number of structures and the identification of recurrent structural patterns that can subsequently be used as "supramolecular synthons" for the design of organic solids with defined structures and properties.

While most of the interest has been directed toward the identification and the use of patterns of strong intermolecular interactions such as multiple hydrogen bonding,[192] few examples of design of supramolecular synthons based on steric complimentarity or other weak interactions have been reported.[193]

Structures **36A**, **36B** and **88·EtOH** show a structural motif characterised by van der Waals interactions and steric complementarity. This motif is preserved in structures **36A** and **36B** despite the inclusion of different solvent in the structure, and could be considered a supramolecular synthon based on hydrophobic interactions and shape complimentarity.

The information provided by this and many other available crystal structures is of great importance in the development of new drugs. Although it is well accepted that the binding of a drug to a receptor is mediated by ion-ion interactions, hydrogen bonding, dipole-dipole interactions, lipophilicity and shape complimentarity, the relative contribution of each of these interactions is still poorly understood. It is nevertheless apparent that hydrophobicity is a major source of binding in drug-receptor interactions,[194] and that a detailed knowledge of hydrophobic interactions is necessary for the design of new drugs.

Chapter V

Experimental Section

5.1 General methods

Chemicals were purchased from Aldrich and Fluka and used without further purification. The resin for solid support synthesis was purchased from Novabiochem. Molecular sieves were activated at 350 °C for 3 h *in vacuo*. All solvents were distilled from the appropriate drying agents; dichloromethane, benzene and toluene were distilled from P_2O_5 and stored over 4 Å molecular sieves. Diethyl ether, THF, and 1,4-dioxane were distilled from CaH_2, redistilled from $LiAlH_4$ and stored over sodium wire. Pyridine and acetonitrile were distilled from CaH_2 and stored over 4 Å molecular sieves. DMF was stirred over CaH_2 for 16 h, then distilled under reduced pressure and stored over 4 Å molecular sieves. Methanol was distilled from sodium and stored over 4 Å molecular sieves. All reactions were performed under anhydrous conditions and monitored by TLC on Kiesegel 60 F_{254} (Merck). Detection was effected by examination under UV light (254 nm) and by charring with 10% sulphuric acid in methanol. Flash Column Chromatography was performed on silica gel (Merck, mesh 70-230). Size-exclusion column chromatography was performed on Sephadex LH-20 (Pharmacia Biotech AB, Uppsala Sweden) and dichloromethane/methanol (1/1, v/v) was used as eluent. Extracts were evaporated under reduced pressure at <40 °C (bath). All the ^1H NMR, ^{19}F NMR and ^{13}C NMR spectra were recorded on a Bruker Ac 300, Bruker AMX 400, Bruker DRX 500, Varian Mercury 300, Varian Inova 500 or Varian Inova 600 spectrometer. For ^1H and ^{13}C NMR spectra recorded in $CDCl_3$ and DMSO, chemical shifts (δ) are given in ppm relative to solvent peaks ($CDCl_3$: ^1H, δ=7.26; ^{13}C, δ=77.23; DMSO: ^1H, δ=2.52; ^{13}C, δ=39.51). Coupling constants are measured in Hertz (Hz). FAB mass spectra were recorded using a VG ZabSpec

spetcrometer with *m*-nitrobenzoyl alcohol as matrix. Negative ion matrix assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectra were recorded using a Kratos Kompact instrument using a *trans*-3-indoleacrylic acid matrix. Optical rotations were measured on a Jasco P-1020 polarimeter, and $[\alpha]_D$ are given in units of deg cm^3 g^{-1}.

All crsytal X-ray diffraction experiments where carried out on a Rigaku R-Axid II diffractometer equipped with an area detector and a rotating anode source. Graphite-monchromaated Kα radiation (Mo, λ=0.71069 Å for **36·MeOH** and Cu, λ=0.71069 Å for **36·THF·H_2O** and for **88·EtOH**) was used. All strutcture were solved and refined by standard methods.[197-198]

5.2 Synthetic section

Methyl 3-*O*-Benzyl-β-D-galactopyranoside (8). A solution of methyl β-D-galactopyranoside (7, 2.00 g, 10.3 mmol) and dibutyltin-dimethoxide (3.54 mL, 15.5 mmol) in dry DMF (10 mL) was stirred for 90 min at room temperature under reduced pressure. Benzyl bromide (1.35 mL, 11.3 mmol) and cesium fluoride (2.35 g, 15.5 mmol) were added, and stirring was continued for 16 h. TLC analysis (dichloromethane/methanol, 1/1, v/v) showed that the reaction was complete. The solvent was evaporated. The residue was dissolved in dichloromethane (20 mL) and washed with aqueous KF (2 x 10 mL), aqueous $NaHCO_3$ (2 x 10 mL) and aqueous NaCl (2 x 10 mL). The organic layer was collected, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and the residue was crystallised from petroleum ether (40-60 °C)/ethyl acetate to give **8** as a white crystalline solid (1.63 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.48-7.21 (m, 5H, Ar-H), 4.76 (s, 2H, CH_2Ph), 4.20 (d, 1H, H-1, $J_{1,2}$=7.9 Hz), 4.19 (d, 1H, H-4, $J_{3,4}$=3.5 Hz). 4.00 (dd, 1H, H-6a, $J_{5,6a}$=6.1 Hz, $J_{6a,6b}$=11.4 Hz), 3.85 (dd, 1H, H-6b, $J_{5,6b}$=4.8 Hz), 3.80 (dd, 1H, H-2, $J_{2,3}$=9.4 Hz), 3.58 (s,

3H, OCH_3), 3.54 (dd, 1H, H-5), 3.46 (dd, 1H, H-3). ^{13}C NMR (75 MHz, DMSO) δ: 127.80 (2x), 127.26 (2x), 126.86 (5*C*H, Ar), 104.29 (C-1), 81.19, 74.92, 69.41, 64.50 (C-2, C-3, C-4, C-5), 70.06, 60.24 (C-6, CH_2Ph), 55.77 (OCH_3). FAB-MS: *m/z* 285 $[M+H]^+$.

Methyl 3-*O*-Benzyl-β-D-galactopyranoside 4,6-*O*-phenylboronate (9). A suspension of methyl 3-*O*-benzyl-β-D-galactopyranoside (**8**, 220 mg, 0.77 mmol) and phenylboronic acid (94 mg, 0.77 mmol) in dry toluene was heated under reflux for 20 min using a Dean-Stark apparatus. After cooling a white precipitate formed which was filtered and recrystallised from benzene, to give **9** as a white crystalline solid (215 mg, 75% yield). ^{1}H NMR (300 MHz, DMSO) δ: 7.74 (d, 2H, Ar-H), 7.50-7.27 (m, 8H, Ar-H), 5.31 (d, 1H, 2-OH, J=6.6 Hz), 4.76 (AB q, 2H, OCH_2Ph, J_{AB}=11.9 Hz), 4.67 (d, 1H, H-4, $J_{3,4}$=2.6 Hz), 4.30 (d, 1H, H-6a, $J_{6a,6b}$=11.4 Hz), 4.26 (d, 1H, H-1, $J_{1,2}$=7.5 Hz), 4.11 (d, 1H, H-6b), 4.00 (s, 1H, H-5), 3.53 (dd, 1H, H-3, $J_{2,3}$=9,7 Hz), 3.50-3.42 (m, 1H, H-2), 3.39 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.74 (Cq, Ar), 133.26 (2x), 130.58, 127.93 (2x), 127.41 (2x), 127.26 (2x), 127.07 (10*C*H, Ar), 103.76 (C-1), 79.37, 68.78, 67.45, 67.33 (C-2, C-3, C-4, C-5), 70.14 (*C*H_2Ph), 64.38 (C-6), 56.04 (O*CH*$_3$). FAB-MS: *m/z* 369 $[M-H]^+$. m.p. 214.5-215.6 °C. $[\alpha]_D^{25}$ +69.6° (*c* 1.25, CH_2Cl_2).

Ethyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (16). To a stirred solution of β-D-galactose pentaacetate (**10**, 5 g, 12.8 mmol) in dichloromethane (150 mL) at 0 °C was added ethanethiol (0.95 mL, 12.8 mmol) and $ZrCl_4$ (2.8 g, 12 mmol) and the reslulting suspension was stirred for 1 h. An additional quantity of ethanethiol (0.19 mL, 2.6 mmol) was added and the mixture was stirred at 0 °C for a further 1 h. TLC analysis (ethyl acetate/petroleum ether (60-80 °C), 1/1, v/v) indicated that all the starting material had been consumed. The reaction

mixture was diluted with dichloromethane (100 mL) and filtered. The filtrate was washed successively with ice-cold water (2 x 50 mL), aqueous $NaHCO_3$ (15%, v/w, 2 x 50 mL) and water (2 x 50 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was crystallised from ethanol to give **16** as a colorless crystalline solid (4.29 g, 11.0 mmol, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.44 (d,1H, H-4, $J_{3,4}$=2.9 Hz), 5.25 (t, 1H, H-2, $J_{2,3}$= $J_{1,2}$= 9.9 Hz), 5.05 (dd, 1H, H-3, $J_{2,3}$=9.9 Hz, $J_{3,4}$=3.31 Hz), 4.50 (d, 1H, H-1, $J_{1,2}$=9.9 Hz), 4.18 (dd, 1H, H-6a, $J_{5,6a}$=6.3 Hz, $J_{6a,6b}$=11.0 Hz), 4.10 (dd, 1H, H-6b, $J_{5,6b}$=6.3 Hz), 3.94 (t, 1H, H-5), 2.84-2,64 (m, 2H, SCH_2), 2.17, 2.09, 2.05, 2.00 (4s, each 3H, CH_3CO), 1.28 (t, 3H, SCH_2CH_3, J=7.3 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.59, 170.44, 170.29, 169.79 (4CO), 84.24 (C-1), 74.53, 72.08, 67.44, 67.36 (C-2, C-3, C-4, C-5) 61.65 (C-6), 24.56 (SCH_2CH_3), 21.01, 20.86 (2x), 20.78 (4C, CH_3CO), 15.04 (SCH_2CH_3). FAB-MS: *m/z* 393 $[M+H]^+$.

Ethyl 1-Thio-β-D-galactopyranoside (17). A solution of NaOMe in methanol (1%, 5 mL) was added (pH of solution: 11-12) to a solution of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**16**, 4.00 g, 10.2 mmol) in methanol/DCM (30 mL, 5/1, v/v). The solution was stirred at room temperature for 10 min. The reaction mixture was neutralised by addition of DOWEX 50 (H^+) resin and filtered. The filtrate was concentrated *in vacuo*. Product **17** was obtained as a white solid (2.28 g, 99%) and used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.86 (d, 1H, OH, J=5.3 Hz), 4.72 (d, 1H, OH, J=5.3 Hz), 4.52 (t, 1H, 6-O*H*, $J_{6a,OH}$=$J_{6b,OH}$=5.3 Hz), 4.34 (d, 1H, OH, J=4.4 Hz), 4.20 (d, 1H, H-1, $J_{1,2}$=8.8 Hz), 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, 2H, SCH_2), 1.18 (t, 3H, SCH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 85.29 (C-1), 79.04, 74.64,

69.69, 68.35 (C-2, C-3, C-4, C-5), 60.54 (C-6), 22.98 (SCH_2CH_3), 15.13 (SCH_2CH_3). FAB-MS: *m/z* 225 $[M+H]^+$.

Methyl 2-*O*-Benzyl-β-D-glucopyranoside 4,6-*O*-phenylboronate (6). A suspension of methyl 2-*O*-benzyl-β-D-glucopyranoside (**5**, 5.00 g, 17.6 mmol) and phenylboronic acid (3.14 g, 17.6 mmol) in dry toluene was heated under reflux for 20 min with azeotropical removal of water. After cooling a solid precipitated from the solution. This was filtrated to give **6** as a brown solid (5.41 g, 83% yield). ^{1}H NMR (300 MHz, DMSO) δ: 7.78-7.51 (m, 10*CH*, Ar), 5.56 (d, 1H, 3-OH, *J*=4.0 Hz), 4.85 (d, 1H, H-1, $J_{1,2}$=3.3 Hz), 4.68 (AB q, 2H, OCH_2Ph, J_{AB}=12.2 Hz), 4.18 (dd, 1H, *J*=4.0 Hz, *J*=9.6 Hz), 3.93 (t, 1H, *J*=10.0 Hz), 3.83-3.72 (m, 3H), 3.42-3.35 (m, 1H), 3.32 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.82 (Cq, Ar), 133.93 (2x), 133.93, 128.38 (2x), 128.22 (2x), 127.71, 127.63, 127.49 (10*CH*, Ar), 103.76 (C-1), 79.37, 68.78, 67.45, 67.33 (C-2, C-3, C-4, C-5), 70.14 (CH_2Ph), 64.38 (C-6), 56.04 (OCH_3).

Ethyl 2,3,4,6-Tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (18). A solution of ethyl 1-thio-β-D-galactopyranoside (**17**, 2.24 g, 10.0 mmol) in DMF (30 mL) was added dropwise to a suspension of NaH (60% dispersion, 2.4 g, 60.0 mmol) in DMF (50 mL) at 0 °C and the mixture was stirred for 30 min at 0 °C. Benzyl bromide (5.7 mL, 48.0 mmol) was added dropwise and the mixture was stirred at room temperature for 3 h. The excess of NaH was quenched by addition of MeOH (2 mL). The resulting mixture was poured into ice-cold water (100 mL) and extracted with diethyl ether (5 x 50 mL). The ether layers were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was crystallised from hexane and the product was washed with cold petroleum ether (60-80 °C) to give **18** as a crystalline solid (5.08 g, 8.7

mmol, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.38-7.08 (m, 20H, Ar-H), 4.83 (AB q, 2H, OC*H*$_2$Ph, J_{AB}=10.3 Hz), 4.78 (AB q, 2H, OC*H*$_2$Ph, J_{AB}=11.6 Hz), 4.73 (s, 2H, OC*H*$_2$Ph), 4.43 (AB q, 2H, OC*H*$_2$Ph, J_{AB}=11.8 Hz), 4.42 (t, 1H, H-2, $J_{1,2}$=$J_{2,3}$=9.9 Hz), 3.96 (d, 1H, H-4, $J_{3,4}$=2.5 Hz), 3.83 (t, 1H, *J*=9.4 Hz), 3.63-3.53 (m, 4H), 2.83-2.63 (m, 2H, SC*H*$_2$), 1.30 (t, 3H, SCH$_2$C*H*$_3$, *J*=7.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.78, 138.41, 138.33, 137.89 (4Cq, Ar), 128.50-127.53 (20*C*H, Ar), 85.35 (C-1), 84.13 (C-2), 78.47, 77.22, 73.57 (C-3, C-4, C-5), 75.83, 74.47, 73.60, 72.75, 68.84 (C-6, 4*C*H$_2$Ph), 24.85 (S*C*H$_2$CH$_3$), 15.13 (SCH$_2$*C*H$_3$). FAB-MS: *m/z* 585 $[M+H]^+$.

Methyl 2-*O*-Benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-α-D-galactopyranoside (22). A suspension of methyl 2-*O*-benzyl-α-D-glucopyranoside 4,6-*O*-phenylboronate (**6**, 50 mg, 0.13 mmol), 2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl fluoride (**15**, 143 mg, 0.26 mmol), one equivalent of lutidine (14 mg, 0.13 mmol) and molecular sieves (4Å, 150 mg) in dichloromethane/ether (2 mL, 1/1, v/v) was stirred for 1 h at room temperature. The mixture was cooled to -78 °C. A solution of AgOTf (70 mg, 0.26 mmol), Cp_2ZrCl_2 (40 mg, 0.13 mmol) and molecular sieves (4 Å, 150 mg) in dichloromethane/ether (2 mL, 1/1, v/v) was stirred at room temperature for 1 h, cooled to -78 °C and added dropwise to the above mentioned mixture. The resulting suspension was stirred for 1 h. The mixture was filtered through Celite®, diluted with dichloromethane (10 mL) and washed with aqueous $NaHCO_3$ (15%, v/w, 2 x 5 mL) and aqueous NaCl sat. (2 x 5 mL). The organic layers were collected, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether (60-80 °C)/ethyl acetate, 1/1, v/v), to afford the product **22** as a white solid (100 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.52-7.19 (m, 25H, Ar-H), 5.10 (d, 1H, H-1', $J_{1',2'}$=4.0 Hz), 4.89

(d, 1H, J=12.0 Hz, CH_2Bn), 4.76-4.74 (m, 5H, CH_2Bn), 4.73 (d, 1H, J=12.8 Hz, CH_2Bn), 4.57 (d, 1H, J=12.0 Hz, CH_2Bn), 4.44 (d, 1H, J=12.6 Hz, CH_2Bn), 4.41 (d, 1H, H-1, $J_{1,2}$=4.0 Hz), 4,32 (d, 1H, J=12.6 Hz, CH_2Bn), 4.19 (ddd, 1H, H-5, $J_{4,5}$=1.0 Hz, $J_{5,6b}$=6.0 Hz, $J_{5,6b}$=8.2 Hz), 4.12 (dd, 1H, H-2', $J_{2',3'}$=9.4 Hz), 4.08 (dd, 1H, H-4', $J_{3',4'}$=3.5 Hz, $J_{4',5'}$=1.6 Hz), 4.03 (dd, 1H, H-3'), 3.85-3.71 (m, 3H, H-3, H-5', H-6'b), 3.63-3.49 (m, 3H, H-4, H-6a, H-5'), 3.41 (dd, 1H, H-6b, $J_{6a,6b}$=9.6 Hz), 3.32 (dd, 1H, $J_{2,3}$=9.5 Hz), 3.39 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.77-127.36 (30*C*H, Ar), 101.45, 98.66 (C-1, C-1'), 84.47, 79.63, 78.92, 76.90, 74.61, 71.85, 70.43, 70.24 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 74.93, 74.68, 73.75, 73.57, 72.68, 68.39, 63.08 (C-6, C-6', 5CH_2), 55.39 (OCH_3). FAB-MS: *m/z* 829 $[M+Na]^+$.

Methyl 3-*O*-Benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-β-D-galactopyranoside (24). The polymer bound glycosyl acceptor (**43**, 30 mg, 31 μmol) was placed in a round-bottomed flask, just covered with dichloromethane (~1 mL) and allowed to swell for 15 min. Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**53**, 108 mg, 0.18 mmol) and 4Å molecular sieves (200 mg, beads) were added and the resulting suspension was stirred at room temperature for 15 min. NIS (40 mg, 0.18 mmol) and TMSOTf (3.2μL, 0.018 mmol) were added, and the mixture was stirred at room temperature for 10 min, until TLC analysis (petroleum ether (60-80 °C)/ethyl acetate, 8/2, v/v) showed complete consumption of **53**. The reaction was quenched by adding triethylamine (0.1 mL). The mixture was filtered and the resin was washed successively with dichloromethane (2 x 2 mL), DMF (2 x 2 mL) and again dichloromethane (2 x 2 mL). The molecular sieves were removed by decanting and the resin was filtered and dried in vacuo for 12 h. The resin was refluxed in acetone/water (4/1, v/v, 5 mL) for 30 min. The polymer was filtered and washed with dichloromethane (20 mL) and methanol (20 mL). The filtrates were combined and concentrated. The residue was

purified by silica gel column chromatography (petroleum ether (60-80 °C)/ethyl acetate, 8/2, v/v) to give **24** as a colorless syrup (19 mg, 76%). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.37-7.10 (m, 50H, Ar-H), 5.55 (d, 1H, H-1'α, $J_{1'\alpha,\,2'\alpha}$=3.5 Hz), 4.99 (d, 1H, C*H*$_2$Bn, *J*=11.0 Hz), 4.93 (d, 1H, C*H*$_2$Bn, *J*=11.0 Hz), 4.90 (d, 1H, H-1'β, $J_{1,2}$=7.9 Hz), 4.85–4.78 (m, 8H, C*H*$_2$Bn), 4.69 (d, 1H, C*H*$_2$Bn, *J*=11.8 Hz), 4.65-4.48 (m, 6H, C*H*$_2$Bn), 4.46 (d, 1H, H-1α, $J_{1,2}$=7.9 Hz), 4.44 (d, 1H, C*H*$_2$Bn, *J*=12.0 Hz), 4.39 (d, 1H, H-1β, *J*=7.4 Hz), 4.27 (d, 1H, C*H*$_2$Bn, *J*=12.5 Hz), 4.19 (d, 1H, H-3'α), 4.12 (d, 1H, H-4α, *J*=3.3 Hz), 4.42-3.93 (m, 6H), 3.90 (dd, 1H, *J*=9.8 Hz, *J*=10.0 Hz), 3.87 (dd, 1H, *J*=11.4 Hz, *J*=5.6 Hz), 3.82 (dd, 1H, *J*=9.9 Hz, *J*=4.1 Hz), 3.70 (d, 1H, H-4β, *J*=3.4 Hz), 3.67 (t, 1H, *J*= 10.1 Hz), 3.65-3.46 (m, 5H), 3.53 (s, 3H, *OCH*$_3$α), 3.51 (s, 3H, *OCH*$_3$β, *3*.41-3.39 (m, 1H), 3.33 (dd, 1H, *J*=2.5 Hz, *J*=10.4 Hz), 3.27 (dd, 1H, *J*=1.2 Hz, *J*=10.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.96-136.75 (10Cq, Ar), 128.80-127.46 (25*C*H, Ar-α/β), 104.82 (C-1α), 102.86 (2x) (C-1β, C-1' β), 96.10 (C-1'α), 85.18, 83.01, 82.30 (2x), 79.74, 78.02 (3x), 75.81 (3x), 75.08 (3x), 74.20, 73.81 (2x), 73.55 (2x), 73.00, 72.64 (2x), 72.45, 72.22, 70.23, 69.06, 68.34, 66.96, 66.47, 62.78 (C-2α, C-3α, C-4α, C-5α, C-6α, C-2'α, C-3'α, C-4'α, C-5'α, C-6'α, C-2β, C-3 β, C-4 β, C-5 β, C-6 β, C-2'β, C-3'β, C-4'β, C-5'β, C-6'β, 5*C*H$_2$Ph-α/β), 57.41, 56.89 (O*C*H$_3$, O*C*H$_3$'). FAB-MS: *m/z* 807 $[M+H]^+$ (Found: $[M+H]^+$, 807.3797. $C_{48}H_{55}O_{11}$ requires *m/z*, 807.3744). *Anal.* Calcd. for $C_{48}H_{54}O_{11}$: C 71.44, H 6.75; found: C 71.82, H 6.85 .

Methyl 3-*O*-Benzyl-2-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-4,6-benzylidine-β-D-galactopyranoside (30). Polystyrylboronic acid (**41**, 70 mg) was placed in a round-bottomed flask. Enough pyridine was added to swell the polymer. After 15 min ethyl 2,3-di-O-benzyl-1-thio-β-D-galactopyranoside (**27**, 59 mg, 0.146 mmol) was added to the suspension, and this

was heated at 60 °C for 1 h, and then heated at 80 °C for 1 h under reduced pressure. The reaction was followed by TLC (dichloromethane/methanol, 95/5, v/v). After cooling, the resin was filtered, washed with dry pyridine (2 x 2 mL), dry toluene (2 x 2 mL) and co-evaporated from dry toluene (2 x 2 mL). The filtrate and the washings were concentrated under reduced pressure. Unreacted **27** (13 mg, 23%) was recovered. The loaded polymer was dried in vacuo for 48 h over P_2O_5. The dried polymer was placed in a round-bottomed flask, and enough dichloromethane (4 mL) was added to cover and swell the polymer. Molecular sieves (4Å, 300 mg, beads) and glycosyl acceptor (**29**, 210 mg, 0.56 mmol) were added to the suspension and this was stirred for 15 min. NIS (25 mg, 0.11 mmol) and TMSOTf (2 µL, 11 µmol) were added to the suspension, and stirring was continued at room temperature for 1 h. The reaction was quenched with triethylamine (0.1 mL). The resin was filtered and washed with dichloromethane (2 x 2 mL), DMF (2 x 2 mL) and dichloromethane again (2 x 2 mL). The product was cleaved from the resin by refluxing in acetone/water (4/1, 5 mL) for 30 min. The resin was filtered, washed with dichloromethane (2 x 20 mL) and methanol (2 x 20 mL). The filtrate and the washings were combined and evaporated under reduced pressure. The residue was further purified by silica gel column chromatography (dichloromethane/methanol, 95/5) to give **30** as a white solid (47 mg, 60%, based on recovered glycosyl donor). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.58-7.20 (m, 20H, Ar-H), 5.58 (d, 1H, H-4', $J_{3',4'}$=3.4 Hz), 5.49 (s, 1H, PhC*H*), 4.83-4.62 (m, 5H), 4.53-4.26 (m, 3H), 4.26-4.25 (m, 1H), 4.17-4.05 (m, 3H), 3.92-3.75 (m, 3H), 3.75-3.62 (m, 1H), 3.61-3.53 (s, 3H, OCH_3), 3.45-3.35 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.71, 138.65, 138.53, 137.24 (4Cq, Ar), 128.73-126.50 (20*C*H, Ar), 104.88 (C-1'), 101.06 (Ph*C*H), 96.90 (C-1), 75.95, 75.55 (2x), 74.88 (2x), 74.10, 73.29, 72.85, 72.07, 69.45, 66.60, 66.36 (2x), 62.46 (C-2, C-3, C-4, C-5, C-6, C-2', C-3', C-4', C-5', C-6', 3CH_2Ph), 56.97 (OCH_3). FAB-MS: *m/z* 715 $[M+H]^+$ (Found: $[M+Na]^+$, 715.3141.

$C_{41}H_{47}O_{11}$ requires *m/z*, 715.3118). *Anal.* Calcd. for $C_{41}H_{46}O_{11}$: C 68.89, H 6.49; found: C 69.21, H 6.79 . $[\alpha]_D^{25}$ +29.8° (*c* 0.40 CH_2Cl_2).

(3-Methoxy-2-pyridyl) 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (34). A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**33**, 6.17 g, 15.0 mmol) and silver 3-methoxy-2-pyridoxide (**32**, 5.55 g, 15.0 mmol) in dry toluene (70 mL) was refluxed for 1 h. The mixture was filtered over Celite® and washed with dichloromethane (100 mL). The filtrate and the washings were combined and concentrated under reduced pressure. Purification by silica gel column chromatography (petroleum ether (40-60 °C)/ethyl acetate/dichloromethane, 1/1/1, v/v/v) gave **34** as a white solid (6.08 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.72 (d, 1H, Ar-H), 7.12 (d, 1H, Ar-H), 6.95 (dd, 1H, Ar-H), 6.22 (d, 1H, H-1, $J_{1,2}$=8,4 Hz), 5.58 (dd, 1H, H-2, $J_{2,3}$=10.1 Hz), 5.47 (d, 1H, H-4, $J_{3,4}$=3,5 Hz), 5.18 (dd, 1H, H-3) 4.22-4.10 (m, 3H, H-5, H-6a, H-6b), 3.85 (s, 3H, O*CH*$_3$), 2,16 (s, 3H, *CH*$_3$CO), 2.01 (s, 6H, CH3CO), 1.96 (s, 3H, CH3CO). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.34, 170.29, 170.12, 169.37 (4CO), 151.71, 144.42 (2Cq, Ar), 136.94, 119.62, 119.13 (3*C*H, Ar), 94.25 (C-1), 71.45 (2x), 68.80, 67.30 (C-2, C-3, C-4, C-5), 61.40 (C-6), 56.30 (O*C*H$_3$), 20.95 (3x), 20.89 (4C, *C*H$_3$CO). FAB-MS: *m/z* 456 $[M+H]^+$.

(3-Methoxy-2-pyridyl) β-D-Galactopyranoside (35). A solution of (3-methoxy-2-pyridyl) 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**34**, 6.0 g, 13.1 mmol) and NaOMe/methanol solution (25%, w/v, 0.15 mL) in 35 mL of methanol/THF (6/1, v/v) was stirred at room temperature for 1 h. The mixture was cautiously neutralised with DOWEX 50 (H^+) ion-exchange resin and filtered. The filtrate was concentrated under reduced pressure. The residue was recrystallised from ethanol, affording **35** as white crystals (2.08 g, 55%). ^{1}H NMR (300

MHz, $CDCl_3$) δ: 7.70 (d, 1H, Ar-H), 7.35 (d, 1H, Ar-H), 7.01 (t, 1H, Ar-H), 5.88 (d, 1H, H-1, $J_{1,2}$=7.9 Hz), 5.06 (d, 1H, OH, *J*=5.7 Hz), 4.84 (d, 1H, OH, *J*=5.7 Hz), 4.59-4.53 (m, 1H), 4.50 (d, 1H), 3.80 (s, 3H, O*CH*$_3$), 3.77-3.69 (m, 1H), 3.68-3.57 (m, 1H), 3.56-3.37 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 144.49, 136.28 (2Cq, Ar), 128.18, 111.07, 110.27 (3*C*H, Ar), 88.40 (C-1), 67.52, 65.58, 60.70, 52.74, 46.85 (C-2, C-3, C-4, C-5, C-6). FAB-MS: *m/z* 288 $[M+H]^+$.

(3-Methoxy-2-pyridyl) β-D-Galactopyranoside 4,6-*O*-phenylboronate (36). Phenylboronic acid (0.21 g, 1.74 mmol) was added to a suspension of (3-methoxy-2-pyridyl) β-D-galactopyranoside (**35**, 0.50 g, 1.74 mmol) in dry benzene (30 mL). The reaction mixture was refluxed for 20 min under Dean-Stark conditions. Upon cooling a white precipitate formed which was filtered and recrystallised from ethanol to give **36** as white crystals (0.45 g, 70%). ^{1}H NMR (300 MHz, DMSO) δ: 7.81 (d, 2H, Ar-H), 7.70 (d, 1H, Ar-H), 7.47 (t, 1H, Ar-H), 7.42-7.31 (m, 3H), 7.02 (dd, 1H), 6.05 (d, 1H, H-1, $J_{1,2}$=7.9 Hz), 5.24 (d, 1H, 2-*OH*, *J*=5.3 Hz), 5.19 (d, 1H, 3-*OH*, *J*=6.1 Hz), 4.38 (d, 1H, H-4, $J_{3,4}$=2.2 Hz), 4.29 (dd, 1H, H-6a, $J_{5,6a}$=1.8 Hz, $J_{6a,6b}$=11.9 Hz), 4.16 (s, 1H, H-5), 3.98 (d, 1H, H-6b), 3.74 (s, 3H, O*CH*$_3$), 3.68 (dd, 1H, H-3, $J_{2,3}$=2.6 Hz), 3.62 (dd, 1H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 151.80, 143.69 (2Cq, Ar), 136.27, 133.83 (2x), 130.88, 127.62 (2x), 119.14, 118.57 (8*C*H, Ar), 95.20 (C-1), 72.45, 71.00, 69.25, 68.24 (C-2, C-3, C-4, C-5), 64.29 (C-6), 55.26 (O*CH*$_3$). FAB-MS: *m/z* 396 $[M+Na]^+$. *Anal.* Calcd. for $C_{18}H_{20}O_7NB$: C 57.93, H 5.40, N 3.75; found: C 57.61, H 5.76, N 4.40 . m.p. 156.4-156.7 °C. $[\alpha]_D^{25}$ –55.9° (*c* 0.43 CH_2Cl_2).

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(D-galactopyranosyl)-α-D-glucopyranoside (38). A solution of **36** (130 mg, 0.35 mmol) and 1,2:3,4-di-*O*-isopropylidene-α-D-glucopyranoside

(**37**, 0.906 g, 3.5 mmol) in dry nitromethane (5.0 mL) was stirred at room temperature. A solution of MeOTf in nitromethane (1M, 70µl, 70 µmol) was added. After 4 h the reaction was quenched by addition of pyridine (0.1 mL). Evaporation of the solvent and purification by silica gel column chromatography (dichloromethane/methanol, 9/1, v/v) gave 69 mg of disaccharide **38** as a colorless syrup (47%, α/β = 1.5/1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.55 (d, 1H, H-1β, $J_{1,2}$=4.8 Hz), 5.52 (d, 1H, H-1α, $J_{1,2}$=4.9 Hz), 4.92 (d, 1H, H-1'α, $J_{1,2}$=3.1 Hz), 4.53-4.51 (m, 2H), 4.36-4.30 (m, 3H), 4.28-4.19 (m, 2H), 4.12-3.52 (m, 14H), 3.51-3.50 (m, 1H), 3.25-3.10 (m, 2H), 3.08 (t, 1H, *J*=3.6 Hz), 1.55 (s, 6H, $(CH_3)_2CH$), 1.45 (s, 6H, $(CH_3)_2CH$), 1.32 (s, 12H, $(CH_3)_2CH$). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 109.61, 109.59, 108.93, 108.07 (4C, $C(CH_3)_2$), 104.13, 99.43, 96.39 (2x) (C-1α, C-1'α, C-1β, C-1'β), 74.73, 73.63, 71.47, 70.90, 70.75, 70.34, 70.11, 69.35, 68.91, 68.01, 67.64, 66.89, 62.18, 61.48, 53.73 (C-2α, C-3α, C-4α, C-5α, C-6α, C-2'α, C-3'α, C-4'α, C-5'α, C-6'α, C-2β, C-3 β, C-4 β, C-5 β, C-6 β, C-2'β, C-3'β, C-4'β, C-5'β, C-6'β), 26.19 (2x), 26.13 (2x), 25.07, 24.66 (2x), 24.52 (2 $C(CH_3)_2$-α/β). FAB-MS: *m/z* 445 $[M+Na]^+$.

Polystyrylboronic acid (41). A suspension of 4-bromopolystyrene (**39**, 1.00 g, purchased from Novabiochem) and n-BuLi (6.0 mL, 1.6M in hexane, 9.6 mmol) in toluene (10 mL) was stirred at 65 °C for 4 h 30 min. The solution became cloudy. After cooling, the polymer was filtered under argon, and the solvent was removed. THF (10 mL) and trimethyl-borate (2.2 mL, 19.3 mmol) were added to the suspension, and this was stirred at room temperature overnight. The resin was then filtered. The polymer was suspended in a dioxane/water/HCl solution (22.5 mL, 4/4/1, v/v/v) and stirred at room temperature for 2 h. The resin was filtered, washed with dioxane/water (100 mL, 3/1, v/v), dioxane (100 mL), acetone (100 mL) and methanol (100 mL) and dried *in vacuo* for 48 h to give 0.94 g of resin **41**.

Loading of methyl 3-*O*-benzyl-β-D-galactopyranoside (8) on polystyrylboronic acid. Polystyrylboronic acid **41** (400 mg) was placed in a round bottomed flask with enough pyridine to cover and swell the polymer (~5 mL). Methyl 3-O-benzyl-β-D-galactopyranoside (**8**, 240 mg, 0.84 mmol) was added and the suspension was stirred for 1 h at 60 °C, and then for 1 h at 80 °C under reduced pressure. The solvent was evaporated. The polymer was co-evaporated from dry pyridine (2 x 3 mL), dry toluene (3 x 3 mL), and the residue was successively washed with dry pyridine (2 x 2 mL), dry DMF (2 x 2 mL) dry toluene (2 x 2 mL) and dry dichloromethane (2 x 2 mL) and dried *in vacuo* over P_2O_5 for 48 h. The filtrate and the washings were collected and evaporated. Unreacted starting material (66 mg, 23%) was recovered.

Methyl 3-*O*-Benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-β-D-galactopyranoside (50). The polymer bound glycosyl acceptor (**43**, 20 mg, 18 μmol) was placed in a round-bottomed flask just covered with dichloromethane (~1 mL) and allowed to swell for 15 min. Ethyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-galactopyranoside (**18**, 51 mg, 80 μmol) and 4Å molecular sieves (250 mg, beads) were added, and the suspension was stirred at room temperature for 15 min. NIS (20 mg, 0.08 mmol) and TMSOTf (1.6μL, 9 μmol) were added and the mixture was stirred at room temperature for 10 min until all the glycosyl donor had been consumed, as indicated by TLC analysis (petroleum ether (60-80 °C)/ethyl acetate, 8/2, v/v). The reaction was quenched by addition of triethylamine (0.1 mL). The mixture was filtered and the resin was washed successively with dichloromethane (2 x 2 mL), DMF (2 x 2 mL) and again dichloromethane (2 x 2 mL). Dichloromethane (2 mL) was added and molecular sieves were removed by decanting, The resin was filtered and dried in vacuo for 12

h. Then, the resin was refluxed in acetone/water (4/1, v/v, 5 mL) for 30 min. The polymer was filtered and washed with dichloromethane (20 mL) and methanol (20 mL). The filtrates were combined and the solvent evaporated. A colorless syrup was obtained. Purification by silica gel column chromatography (petroleum ether (60-80 °C)/ethyl acetate, 8/2, v/v) yielded **50** as a colorless syrup (13 mg, 91%, α/β = 1/1). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40-7.18 (m, 50H, Ar-H), 5.54 (d, 1H, H-1'α, $J_{1'\alpha,2'\alpha}$=4.0 Hz), 4.95 (dd, 1H, *J*=1.3 Hz, *J*=10.9 Hz), 4.92 (d, 1H, *J*=11.4 Hz), 4.83-4.70 (m, 7H), 4.62 (d, 1H, *J*=12.0 Hz), 4.58-4.50 (m, 5H), 4.42 (s, 2H), 4.41 (d, 1H, H-1α, $J_{1,2}$=7.3 Hz), 4.38-3.52 (m, 4H), 4.28 (d, 1H, *J*=11.9 Hz), 4.08 (dd, 1H, H-2'α, $J_{2'\alpha,3'\alpha}$=9.3 Hz), 3.98-3.88 (m, 10H), 3.85-3.77 (m, 3H), 3.62 (t, 1H, *J*=6.9 Hz), 3.60 (dd, 1H, *J*=3.5Hz, *J*=9.7 Hz), 3.59-3.53 (m, 2H), 3.49 (s, 3H, $OCH_3\alpha$), 3.46 (s, 3H, $OCH_3\beta$), 3.44-3.43 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 139.21-137.45 (10Cq, Ar), 18.70-127.36 (*25CH*, Ar-α/β), 104.85, 103.39, 103.19, 96.59 (C-1$_\alpha$, C-1$_\beta$, C-1$_\alpha$', C-1$_\beta$'), 82.75, 81.37, 80.37, 79.83, 79.07, 76.47, 75.21, 74.90, 74.82, 74.11, 73.96, 73.85, 73.67, 73.46, 73.32, 73.18, 73.06, 72.60, 72.38, 72.10, 69.24, 68.97, 68.87, 67.10, 66.80, 62.75, (C-2α, C-3α, C-4α, C-5α, C-6α, C-2'α, C-3'α, C-4'α, C-5'α, C-6'α, C-2β, C-3 β, C-4 β, C-5 β, C-6 β, C-2'β, C-3'β, C-4'β, C-5'β, C-6'β), 56.84 ($OCH_3\alpha$), 56.73 ($OCH_3\beta$). FAB-MS: *m/z* 807 $[M+H]^+$ (Found: $[M+H]^+$, 807.3726. $C_{48}H_{55}O_{11}$ requires *m/z*, 807.3744). *Anal.* Calcd. for $C_{48}H_{54}O_{11}$: C 71.44, H 6.75; found: C 71.57, H 6.86.

Methyl 3-*O*-Benzyl-2-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-galactopyranoside (52). The polymer bound glycosyl acceptor (**43**, 30 mg, 31 μmol) was placed in a round-bottomed flask just covered with dichloromethane (~1 mL) and allowed to swell for 15 min. Ethyl 2-*O*-benzoyl-3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**51**, 66

mg, 0.11 mmol) and 4Å molecular sieves (200 mg, beads) were added and the suspension was stirred at room temperature for 15 min. NIS (25 mg, 0.11 mmol) and TMSOTf (2μL, 10 μmol) were added and the mixture was stirred at room temperature for 1 h, until all the glycosyl donor had been consumed, as indicated by TLC analysis (petroleum ether (60-80 °C)/ethyl acetate, 8/2, v/v). The reaction was quenched by addition of triethylamine (0.1 mL). The resin was filtered, and washed successively with dichloromethane (2 x 2 mL), DMF (2 x 2 mL) and again dichloromethane (2 x 2 mL). Molecular sieves were removed by decanting. The resin was filtered, washed and dried in *vacuo* for 12 h. The resin was refluxed in acetone/water (4/1, v/v, 5 mL) for 30 min. The polymer was filtered and washed with dichloromethane (20 mL) and methanol (20 mL). The filtrates were combined and concentrated. Purification of the residue by silica gel column chromatography (petroleum ether (60-80 °C)/ethyl acetate, 8/2, v/v) yielded **52** as a colorless syrup (26 mg, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.97-7.91 (m, 2H, Ar-H), 7.56-7.48 (m 2H, Ar-H), 7.40-7.08 (m, 21H, Ar-H), 5.36 (t, 1H, H-2', $J_{1',2'}$=$J_{2',3'}$=7.5 Hz), 4.99 (d, 1H, H-1'), 4.83 (d, 1H, J=11.0 Hz), 4.78-4.54 (m, 5H), 4.38 (AB q, 2H, OCH_2Ph, J_{AB}=12.1 Hz), 4.30 (d, 1H, H-1, $J_{1,2}$=7.4 Hz), 3.92-3.76 (m, 6H), 3.72 (dd, 1H, J=4.5 Hz, J=11.5 Hz), 3.66 (d, 1H, H-4, $J_{3,4}$=3.3 Hz), 3.62-3.54 (m, 1H), 3.49 (s, 3H, OCH_3), 3.38-3.30 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.25 (Ph*C*O), 138.30, 138.10, 137.88, 137.81, 130.26 (5Cq, Ar), 133.02-127.67 (25*C*H, Ar), 103.48, 101.38 (C-1, C-1'), 83.18, 80.33, 78.21, 78.03, 75.57, 74.62, 73.78, 67.47 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 75.20 (2x), 73.83, 72.59, 68.92, 62.75 (C-6, C-6', 4*C*H_2Ph), 57.62 (O*C*H_3). FAB-MS: *m/z* 843 $[M+Na]^+$ (Found: $[M+Na]^+$, 843.3376. $C_{48}H_{52}O_{12}Na$ requires *m/z*, 843.3356). *Anal.* Calcd. for $C_{48}H_{52}O_{12}$: C 70.23, H 6.38; found: C 70.58, H 6.72 . m.p. 92.9-93.4 °C. $[\alpha]_D^{25}$ +11.2° (*c* 0.31, CH_2Cl_2).

Methyl 3-*O*-Benzyl-2-*O*-(2-deoxy-2-phtalimido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-β-D-galactopyranoside (55). The polymer bound glycosyl acceptor (**43**, 17 mg, 18 μmol) was placed in a round-bottomed flask just covered with dichloromethane and allowed to swell for 15 min. Ethyl 2-deoxy-2-phtalimido-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (**54**, 42 mg, 87 μmol) and 4 Å molecular sieves (250 mg, beads) were added and the suspension was stirred at room temperature for 15 min. NIS (20 mg, 89 μmol) and TMSOTf (1.6μL, 9μmol) were added and the mixture was stirred at room temperature for 30 min until all the glycosyl donor had been consumed, as indicated by TLC analysis (petroleum ether (60-80 °C)/ethyl acetate, 1/1, v/v). The reaction was quenched by adding triethylamine (0.1 mL). The mixture was filtered and the resin was washed successively with dichloromethane (2 x 2 mL), DMF (2 x 2 mL) and again dichloromethane (2 x 2 mL). Dichloromethane was added and the molecular sieves were removed by decanting. The resin was filtered and dried in vacuo for 12 h. The resin was refluxed in acetone/water (4/1, v/v, 5 mL) for 30 min. The polymer was filtered and washed with dichloromethane (20 mL) and methanol (20 mL). The filtrates were combined and the solvent evaporated to give a colorless syrup. Purification by silica gel column chromatography (dichloromethane/methanol, 99/1, v/v) yielded **55** as a colorless syrup (12 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.70-7.52 (m, 4H, Ar-H), 7.35-7.13 (m, 3H, Ar-H), 7.00-6.93 (m, 2H, Ar-H), 5.74-5.62 (m, 2H), 4.40-4.18 (m, 5H), 4.07 (d, 1H, *J*=12.6 Hz), 3.92-3.78 (m, 2H), 3.70-3.56 (m, 3H), 3.54 (s, 3H, OCH_3), 2.10, 2.03, 2.02 (3s, 9H, CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.75, 170.14, 169.36 ($3CH_3CO$), 167.87 (2x) (2*C*O, NPht), 137.48, 131.59 (2Cq, Ar), 134.22 (2x), 128.69 (2x), 128.01 (2x), 127.4 (2x), 123.55 (9*C*H, Ar), 103.61, 99.11 (C-1, C-1'), 79.47, 79.19, 73.71, 72.28, 71.25, 69.18, 67.07, 57.32, 55.42 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', OCH_3), 72.08, 62.72, 62.48 (C-6, C-6', CH_2Ph), 21.12, 20.97, 20.76 (3C, CH_3CO). FAB-MS: *m/z* 724 $[M+Na]^+$. *Anal.* Calcd. for

$C_{34}H_{39}NO_{15}$: C 58.20, H 5.60, N 2.00; found: C 58.07, H 5.79, N 1.95 . $[\alpha]_D^{25}$ +13.1° (*c* 0.26 CH_2Cl_2).

Ethyl 3,4-*O*-Isopropylidene-1-thio-β-D-galactopyranoside (58). To a mixture of ethyl 1-thio-β-D-galactopyranoside **17** (3 g, 13.4 mmol) in 2,2-dimethoxypropane (60 mL) was added toluensulfonic acid (50 mg) and the mixture was stirred at room temperature. After 12 h, TLC (ethyl acetate) indicated that all the starting material was consumed. The solution was neutralised with Et_3N (0.2 mL) and concentrated under reduced pressure. The residue was crystallised from ether to give compound **58** as colorless crystals, while the mother liquor was concentrated and the residue purified on silica gel column (ethyl acetate) (2.9 g, 11.3 mmol, total yield 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.26 (d, 1H, H-1, $J_{1,2}$=10.2 Hz), 4.22 (dd, 1H, *J*=5.5 Hz, *J*=1.6 Hz) 4.09 (t, 1H, *J*=6.9 Hz, $J_{1,}$=10.2 Hz), 3.98 (dd, 1H, *J*=7.1 Hz, *J*=11.0 Hz), 3.92-3.85 (m, 1H), 3.81-3.76 (m, 1H), 3.56 (dd, 1H, *J*=7.1 Hz, *J*=10.2 Hz), 2.81-2.68 (m, 2H, SCH_2CH_3),1.53 (s, 3H, $(CH_3)_2C$), 1.38 (s, 3H, $(CH_3)_2C$), 1.32 (t, 3H, SCH_2CH_3) ^{13}C NMR (75 MHz, $CDCl_3$) δ: 110.55 (Cq, $(CH_3)_2C$), 85.75 (C-1), 79.32, 77.32, 74.18, 72.32 (C-2, C-3, C-4, C-5), 62.81 (C-6), 28.45, 26.63 ($(CH_3)_2C$), 24.84 (SCH_2CH_3), 15.70 (SCH_2CH_3). FAB-MS: *m/z* 287.01 $[M+Na]^+$.

Ethyl 2,6-Di-*O*-benzoyl-1-thio-β-D-galactopyranoside (60). Ethyl 2,6-di-O-benzoyl-4,6-O-isopropylidene-1-thio-β-D-galactopyranoside (**59**, 500 mg, 1.06 mmol) was dissolved in acetic acid/water (80%, 12.5 mL) and the solution stirred at 60 °C for 12 h. The solution was concentrated under reduced pressure. The residue was dissolved in dichloromethane (30 mL) and washed with water (2 x 20 mL), aqueous $NaHCO_3$ (2 x 20 mL), aqueous NaCl (2 x 20 mL) and dried ($MgSO_4$). The organic layer was filtered and concentrated *in vacuo*.

Crystallisation of the residue from dichloromethane/hexane gave **60** as a white crystalline solid (400 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.15 (m, 4H, Ar-H), 7.65-7.57 (m, 2H, Ar-H), 7.52-7.40 (m, 4H, Ar-H), 5.30, (t, 1H, H-2, $J_{1,2}$=$J_{2,3}$=10.1 Hz), 4.72 (dd, 1H, H-6a, $J_{5,6a}$=6.6 Hz, $J_{6a,6b}$=11.4 Hz), 4.63 (d, 1H, H-1), 4.56 (dd, 1H, H-6b, $J_{5,6b}$=6.6 Hz), 4.09 (d, 1H, H-4, $J_{3,4}$=2.6 Hz), 3.92 (t, 1H, H-5, $J_{5,6a}$=$J_{5,6b}$=6.6 Hz), 3.91-3.80 (m, 1H, H-3), 2.75 (m, 2H, S*C*H_2CH$_3$), 1.26 (t, 3H, SCH$_2$*C*H_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.02, 166.71 (2*C*O), 129.66, 129.60 (2Cq, Ar), 133.54, 133.49, 130.15 (2x), 129.87 (2x), 128.59 (2x), 128.54 (2x) (10*C*H, Ar), 83.54 (C-1), 76.30, 73.80, 72.59, 69.10 (C-2, C-3, C-4, C-5), 69.27 (C-6), 24.45 (S*C*H$_2$CH$_3$), 15.35 (SCH$_2$*C*H$_3$). FAB-MS: *m/z* 455 $[M+Na]^+$.

Ethyl 2,6-Di-*O*-benzoyl-3-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-galactopyranoside (61). To a cooled (0 °C) solution of ethyl 2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside (**60**, 200 mg, 0.46 mmol) in pyridine (2 mL) was added 9-fluorenylmethylchloroformate (131 mg, 0.51 mmol). The reaction was followed by TLC (petroleum ether (60-80 °C)/ethyl acetate, 4/1, v/v) until the starting material **60** was completely consumed (~1 h). Methanol (0.2 mL) was added to quench the reaction. Solvent was evaporated under reduced pressure and the residue was co-evpaorated from dry toluene (3 x 5 mL). The residue was dissolved in dichloromethane (10 mL) and washed with water (2 x 5 mL) and saturated aqueous NaCl (2 x 5 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether (60-80 °C)/ethyl acetate, 4/1, v/v). to give **61** as a white solid (194 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.10-8.00 (m, 2H, Ar-H), 7.72-7.67 (m, 2H, Ar-H), 7.62-7.18 (m, 13H, Ar-H), 7.14-7.07 (m, 1H, Ar-H), 5.70 (t, 1H, H-2, $J_{1,2}$=$J_{2,3}$=9.9 Hz), 5.09 (dd, 1H, H-3, $J_{3,4}$=3.0 Hz), 4.69 (dd, 1H, H-6a, $J_{5,6a}$=8.2 Hz, $J_{6a,6b}$=11.3 Hz), 4.66 (d, 1H, H-1), 4.57 (dd, 1H, H-6b,

$J_{5,6b}$=6.6 Hz), 4.36-4.22 (m, 3H), 4.17-3.95 (m, 2H), 2.87-2.27 (m, 2H, SCH_2CH_3), 1.25 (t, 3H, SCH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.49, 165.27, 154.35 (3*C*O), 143.16, 142.98, 141.30, 141.20, 129.66, 129.48 (6Cq, Ar), 135.47, 133.41, 130.04, 129.88, 128.59, 128.51, 127.97, 127.93, 127.27, 127.20, 125.20, 125.10, 120.09 (18*C*H, Ar), 84.09 (C-1), 78.28, 76.21, 68.25, 67.57 (C-2, C-3, C-4, C-5), 70.59 (CH_2-Fmoc), 63.05 (C-6), 46.74 (*C*H-Fmoc), 24.46 (SCH_2CH_3), 13.21 (SCH_2CH_3). FAB-MS: *m/z* 677 $[M+Na]^+$ (Found: $[M+Na]^+$, 677.1830. $C_{37}H_{34}O_9Na$ requires *m/z*, 677.1821). *Anal.* Calcd. for $C_{37}H_{76}O_9S$: C 67.88, H 5.23; found: C 67.97, H 5.52 . m.p. 70.6-71.4 °C. $[\alpha]_D^{25}$ +26.8° (*c* 1.13, CH_2Cl_2).

Ethyl 4-*O*-Acetyl-2,6-di-*O*-benzoyl-3-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-galactopyranoside (62). Ethyl 2,6-di-*O*-benzoyl-3-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-galactopyranoside (**61**, 100 mg, 0.15 mmol) was added to a solution of pyridine/acetic anhydride (3 mL, 2/1, v/v). The solution was stirred at room temperature until TLC (petroleum ether (60-80 °C)/ethyl acetate, 4/1, v/v) showed that all the starting material had disappeared (~1 h). The reaction was quenched by addition of methanol (0.2 mL). The solvent was removed under reduced pressure and the residue co-concentrated from dry toluene (3 x 5 mL). The residue was dissolved in dichloromethane (10 mL) and washed with water (2 x 5 mL) and saturated aqueous NaCl (2 x 5 mL). The organic phase was collected, dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel column chromatography (petroleum ether (60-80 °C)/ethyl acetate, 4/1, v/v) to yield **62** as a white solid (98 mg, 92%). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.08-8.00 (m, 4H, Ar-H), 7.73-7.67 (m, 2H, Ar-H), 7.61-7.42 (m, 2H, Ar-H), 7.50-7.39 (m, 5H, Ar-H), 7.38-7.28 (m, 3H, Ar-H), 7.32 (t, 1H, Ar-H), 7.20 (t, 1H, Ar-H), 5.76 (d, 1H, H-4, $J_{3,4}$=2.8 Hz), 5.63 (t, 1H, H-2, $J_{1,2}$=$J_{2,3}$=9.9 Hz), 4.57 (dd, 1H, H-1), 4.57 (dd, 1H, H-6a, $J_{5,6a}$=6.6 Hz, $J_{6a,6b}$=11.3 Hz), 4.41-

4.29 (m, 2H), 4.27-4.10 (m, 3H), 2.84-2.71 (m, 2H, SCH_2), 2.26 (s, 3H, CH_3CO), 1.27 (t, 3H, SCH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.23, 166.08, 165.26, 154.22 (4*C*O), 143.43-120.04 (18C, Ar), 84.48 (C-1), 76.17, 74.85, 68.25, 67.53(C-2, C-3, C-4, C-5), 70.74, 62.72 (CH_2-Fmoc, C-6), 46.68 (*C*H-Fmoc), 24.93 (SCH_2CH_3), 15.23 (SCH_2CH_3). FAB-MS: *m/z* 696 $[M]^+$. *Anal.* Calcd. for $C_{39}H_{36}O_{10}S$: C 67.23, H 5.21; found: C 67.19, H 5.30 . m.p. 82.0-82.9 °C. $[\alpha]_D^{25}$ +15.6° (*c* 0.20, CH_2Cl_2).

Methyl 3-*O*-Benzyl-2-*O*-(2,6-di-*O*-benzoyl-4-*O*-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside (65). The polymer bound glycosyl acceptor (**43**, 17 mg, 18 μmol) was placed in a round-bottomed flask just covered with dichloromethane and allowed to swell for 15 min. Ethyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-glucopyranoside (**62**, 55 mg, 79 μmol) and 4Å molecular sieves (300 mg, beads) were added, and the suspension was stirred at room temperature for 15 min. NIS (20 mg, 89 μmol) and TMSOTf (3.2μL, 18 μmol) were added, and the mixture was stirred at room temperature for 1 h, until all the glycosyl donor had been consumed, as indicated by TLC analysis (petroleum ether (60-80 °C)/ethyl acetate, 8/2, v/v). The mixture was filtered, and the resin was washed successively with dichloromethane (2 x 2 mL), DMF (2 x 2 mL) and again dichloromethane (2 x 2 mL). Dichloromethane was added and molecular sieves were removed by decanting. The resin was filtered and dried in vacuo for 12 h. The polymer was placed in a round-bottomed flask and dichloromethane/trietylamine (1.6 mL, 1/1, v/v) was added. The suspension was stirred for 30 min at room temperature. Cleavage of Fmoc was observed through TLC analysis (ethyl acetate/petroleum ether (60-80 °C), 2/1, v/v). The resin was filtered and washed with dichloromethane (2 x 2 mL). TLC analysis conducted on a small number of beads (0.5 mg) showed complete consumption of the starting material. Cleavage of

the disaccharide from the resin was achieved by refluxing in acetone/water (4/1, v/v, 5 mL) for 30 min. The polymer was filtered and washed with dichloromethane (20 mL) and methanol (20 mL). The filtrates were combined and the solvent evaporated. A colorless syrup was obtained. Purification by silica gel column chromatography (petroleum ether (60-80 °C)/ethyl acetate, 8/2, v/v) yielded **65** as a colorless syrup (11 mg, 91%). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.09-7.95 (m, 4H, Ar-H), 7.59–7.50 (m, 2H, Ar-H), 7.43-7.40 (m, 2H, Ar-H), 7.40-7.35 (m, 2H, Ar-H), 7.12-7.09 (m, 3H, Ar-H), 7.0-7.00 (m, 2H, Ar-H), 5.57 (d, 1H, H-4', $J_{3',4'}$=2.5 Hz), 5.31 (dd, 1H, H-2', $J_{1',2'}$=8.2 Hz, $J_{2',3'}$=10.0 Hz), 5.08 (d, 1H, H-1'), 4.55 (dd, 1H, H-6a', $J_{5',6a'}$=6.6 Hz, $J_{6a',6b'}$=11.3 Hz), 4.38 (dd, 1H, H-6b', $J_{5',6b'}$=6.6 Hz), 4.37 (AB q, 2H, OCH_2Ph, J_{AB}=12.2 Hz), 4.28 (d, 1H, H-1, $J_{1,2}$=7.8 Hz), 4.09 (t, 1H, H-5'), 4.00 (dd, 1H, H-3'), 3.87 (t, 1H, H-2, $J_{2,3}$=7.8 Hz), 3.85 (dd, 1H, H-6a, $J_{5,6a}$=6.6 Hz, $J_{6a,6b}$=11.6 Hz), 3.69 (dd, 1H, H-6b, $J_{5,6b}$=6.6 Hz), 3.61 (d, 1H, H-4, $J_{3,4}$=3.1 Hz), 3.47 (s, 3H, OCH_3), 3.38 (dd, 1H, H-3), 3.33 (t, 1H, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.88, 167.02, 166.15 (3CO), 137.65, 129.69, 129.60 (3Cq, Ar), 133.46 (2x), 130.04 (2x), 129.84 (2x), 128.59, 128.46 (4x), 128.04 (2x), 127.79 (2x) (15*C*H, Ar), 103.86 (C-1), 101.67 (C-1'), 79.62 (C-3), 79.30 (C-2), 75.01 (C-2'), 73.86 (C-5), 73.01 (*C*H_2Ph), 72.12 (C-3'), 71.59 (C-5'), 70.07 (C-4'), 67.73 (C-4), 62.66 (C-6), 62.44 (C-6'), 57.41 (O*C*H_3), 21.17 (*C*H_3CO). FAB-MS: *m/z* 719 $[M+Na]^+$. *Anal.* Calcd. for $C_{36}H_{40}O_{14}$: C 62.06, H 5.79; found: C 62.21, H 5.99 . $[\alpha]_D^{25}$ –4.9° (*c* 0.46, CH_2Cl_2).

Methyl 3-*O*-Benzyl-2-*O*-(4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-(2-benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-β-D-galactopyranosyl)-β-D-galactopyranoside (67).

The polymer bound glycosyl acceptor (**43**, 20 mg, 18 μmol) was placed in a round-bottomed flask just covered with dichloromethane and allowed to swell for 15 min. Ethyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-3-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-glucopyranoside (**62**, 55 mg,

79 μmol) and 4Å molecular sieves (300 mg, beads) were added, and the suspension was stirred at room temperature for 15 min. NIS (18 mg, 78 μmol) and TMSOTf (1.42μL, 8 μmol) were added, and the mixture was stirred at room temperature for 1 h until all the glycosyl donor had been consumed, as indicated by TLC analysis (petroleum ether (60-80 °C)/ethyl acetate, 8/2, v/v). The mixture was filtered, and the resin was washed successively with dichloromethane (2 x 2 mL), DMF (2 x 2 mL) and again dichloromethane (2 x 2 mL). After removal of the molecular sieves, achieved by decanting, the resin was filtered and dried *in vacuo* for 12 h. The polymer was suspended in dichloromethane/trietylamine (1.2 mL, 1/1, v/v) and stirred for 20 min at room temperature. Cleavage of Fmoc was observed through TLC analysis (ethyl acetate/petroleum ether (60-80 °C), 2/1, v/v). The resin was filtered and washed with dichloromethane (2 x 2 mL). TLC analysis conducted on a small number of beads (~0.5 mg) showed that no starting material was present. The polymer was dried *in vacuo* over P_2O_5 for 48 h. After drying, the resin was placed in a round-bottomed flask and just covered with dichloromethane (1 mL). Ethyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**51**, 108 mg, 180 μmol) and molecular sieves (4Å, beads) were added to the suspension, and the resulting mixture was stirred at room temperature for 15 min. NIS (20 mg, 89 μmol) and TMSOTf (1.6 μL, 9 μmol) were added. All glycosyl donor was consumed in less then 20 min, as determined by TLC analysis (petroleum ether (40-60 °C)/ethyl acetate, 1/1, v/v). The reaction was quenched by addition of triethylamine (0.1 mL). The resin was then filtered and washed with dry dichloromethane (2 x 2 mL), dry DMF (2 x 2 mL) and dry dichloromethane (2 x 2 mL). Molecular sieves were removed by decanting, and the resin was dried *in vacuo* for 12 h. The trisaccharide was detached from the resin by refluxing the resin in acetone/water (4/1, v/v, 5 mL) for 30 min. The polymer was filtered and washed with dichloromethane (20 mL) and methanol (20 mL). The filtrates were combined and the solvent

evaporated. A colorless syrup was obtained. Purification by silica gel column chromatography (petroleum ether (60-80 °C)/ethyl acetate, 8/2, v/v) yielded trisaccharide **67** as a colorless syrup (9 mg, 50%). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.09 –7.99 (m, 2H, Ar-H), 7.72-7.59 (m, 4H, Ar-H), 7.57-6.96 (m, 29H, Ar-H), 5.64 (d, 1H, H-4', $J_{3',4'}$=3.5 Hz), 5.46 (dd, 1H, H-2', $J_{1',2'}$=7.9 Hz, $J_{2',3'}$=9.2 Hz), 5.14 (t, 1H, H-2', $J_{1',2'}$=$J_{2',3'}$=8.8 Hz), 4.93 (d, 1H, H-1'), 4.70 (d, 1H, *J*=10.9 Hz), 4.60 (d, 1H, H-1'', $J_{1'',2''}$=7.5 Hz), 4.58-4.43 (m, 5H), 4.37-4.31 (m, 2H), 4.18 (d, 1H, H-1, $J_{1,2}$=7.5 Hz), 4.14 (d, 1H, *J*=12.8 Hz), 4.02 (dd, 1H, H-3', $J_{2',3'}$=9.6 Hz, $J_{3',4'}$=3.1 Hz), 4.00-3.96 (m, 1H), 3.79 (m, 2H), 3.70-3.62 (m, 3H), 3.62-3.56 (m, 2H), 3.52-3.46 (m 2H), 3.41 –3.37 (m, 1H), 3.37 (s, 3H, OCH_3), 3.23 –2.95 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.32, 166.21, 164.73, 164.41 (4CO), 138.40-127.59 (42*C*H, Ar), 103.70 (C-1), 101.75 (C-1'), 101.24 (C-1''), 82.87, 79.61, 79.09, 77.93, 76.63, 75.50, 75.18, 74.96, 73.85, 73.68, 73.04, 72.51, 71.90 (2x), 69.95, 69.36, 67.78, 63.03, 62.62 (C-2, C-3, C-4, C-5, C-6, C-2,' C-3', C-4', C-5', C-6', C-2'', C-3'', C-4'', C-5'', C-6'', $4CH_2Ph$), 57.27 (OCH_3), 21.11 (CH_3CO). FAB-MS: *m/z* 1255 $[M+Na]^+$. *Anal.* Calcd. for $C_{70}H_{72}O_{20}$: C 68.17, H 5.88; found: C 67.81, H 5.27 . $[\alpha]_D^{25}$ +12.7° (*c* 0.16, CH_2Cl_2).

Ethyl 2,6-Di-*O*-benzyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (69).

Ethyl 3,4-O-isopropylidene-1-thio-β-D-galactopyranoside (**58**, 784 mg, 2.96 mmol) was dissolved in DMF (10 mL) and added dropwise to a suspension of NaH (60% dispersion, 480 mg, 12.0 mmol) in DMF (15 mL) at 0 °C and the mixture was stirred for 30 min at 0 °C. To this mixture was added dropwise benzyl bromide (0.84 mL, 7.10 mmol) and the mixture was stirred at room temperature. After 3 h, the excess of NaH was quenched by addition of MeOH (2 mL). The resulting mixture was poured into ice-cold water (50 mL) and extracted with diethyl ether (5 x 50 mL). The organic layers were combined, dried ($MgSO_4$), filtered and

concentrated *in vacuo*. The residue was crystallised from hexane/ethyl acetate, to give **69** as a white solid (900 mg, 2.02 mmol, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.44-7.39 (m, 2H, Ar-H), 7.38-7.22 (m, 8H, Ar-H), 4.76 (AB q, 2H, OC*H*$_2$Ph, J_{AB}=11.4 Hz), 4.60 (AB q, 2H, OC*H*$_2$Ph, J_{AB}=12.3 Hz), 4.43 (d, 1H, H-1, $J_{1,2}$=9.7 Hz), 4.26-4.20 (m, 2H, H-3, H-4), 3.91 (t, 1H, H-5, $J_{5,6a}$=$J_{5,6b}$=6.2 Hz), 3.80-3.70 (m, 2H, H-6a, H-6b), 2.45 (dd, 1H, H-2, $J_{2,3}$=3.1 Hz), 2.73 (m, 2H, SC*H*$_2$CH$_3$), 1.43, 1.35 (2s, 12H, 2C(C*H*$_3$)$_2$), 1.31 (t, 3H, SCH$_2$C*H*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.33, 137.96 (2Cq, Ar), 128.48 (2x), 128.44 (2x), 128.36 (2x), 127.83, 127.74 (3x) (10*C*H, Ar), 110.11 (*C*(CH$_3$)$_2$), 83.96 (C-1), 79.78, 79.33, 75.92, 74.19 (C-2, C-3, C-4, C-5), 73.80, 73.69, 69.91 (C-6, 2*C*H$_2$Ph), 28.17, 26.6 (C(*C*H$_3$)$_2$), 25.03 (S*C*H$_2$CH$_3$), 15.32 (SCH$_2$*C*H$_3$). FAB-MS: *m/z* 443 [M–H]$^+$.

Ethyl 2,6-Di-*O*-benzyl-1-thio-β-D-galactopyranoside (70). A solution of ethyl 2.6-di-*O*-benzyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (**69**, 2.36 g, 5.0 mmol) in aqueous acetic acid (80%, 50 mL) was stirred at 50 °C. After 5 h, TLC (ethyl acetate/petroleum ether (60-80 °C), 1/1, v/v) indicated the completion of this reaction and the solution was concentrated under reduced pressure. Silica gel column chromatography of the residue gave **70** as a white solid (1.90 g, 4.4 mmol, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.44-7.23 (m, 10H, Ar-H), 4.70 (AB q, 2H, OC*H*$_2$Ph, J_{AB}=11.0 Hz), 4.57 (s, 2H, OC*H*$_2$Ph), 4.42 (d, 1H, H-1, $J_{1,2}$=9.2 Hz), 4.01 (d, 1H, H-4, $J_{3,4}$=3.1), 3.81-3.65 (m, 2H), 3.64-3.57 (m, 2H), 3.52 (t, 1H, H-2, $J_{2,3}$=9.2 Hz), 2.77 (m, 2H, SC*H*$_2$CH$_3$), 1.33 (t, 3H, SCH$_2$C*H*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.17, 137.86 (2Cq, Ar), 128.62 (2x), 128.54 (2x), 128.39 (2x), 128.09, 127.90, 127.82 (2x) (10*C*H, Ar), 85.16 (C-1), 79.14, 77.14, 75.08, 69.69 (C-2, C-3, C-4, C-5), 75.54, 73.87, 69.74 (C-6, 2*C*H$_2$Ph), 25.22 (S*C*H$_2$CH$_3$), 15.58 (SCH$_2$*C*H$_3$). FAB-MS: *m/z* 427 [M+Na]$^+$.

Ethyl 2,6-Di-*O*-benzyl-3-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-galactopyranoside (71). 9-Fluorenylmethylchloroformate (402 mg, 1.55 mmol) was added to a cooled (0 °C) and stirred solution of ethyl 2,6-di-*O*-benzyl-1-thio-β-D-galactopyranoside (**70**, 629 mg, 1.55 mmol) in pyridine (6 mL) previously cooled to 0 °C. Stirring at 0 °C was continued for 1 h until all of the starting material 70 had been consumed as indicated by TLC analysis. Methanol (0.2 mL) was added to quench the reaction. The solvent was removed under reduced pressure. and the residual syrup co-concentrated from dry (3 x 5 mL). The residue was dissolved in dichloromethane (10 mL), and washed with water (2 x 5 mL) and saturated aqueous NaCl (2 x 5 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residual syrup was purified by silica gel column chromatography (petroleum ether (60-80 °C)/ethyl acetate, 4/1, v/v) to give **71** as a colorless syrup (320 mg, 32%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.77 (d, 2H, Ar-H), 7.61 (d, 2H, Ar-H), 7.50-7.20 (m 1H, Ar-H), 4.78 (AB q, 2H, OC*H*$_2$Ph, J_{AB}=10.5 Hz), 4.77 (dd, 1H, H-3, $J_{2,3}$=9.7 Hz, $J_{3,4}$=2.6 Hz), 4.58 (AB q, 2H, OC*H*$_2$Ph, J_{AB}=11.8 Hz), 4.48 (d, 1H, H-1, $J_{1,2}$=9.7 Hz), 4.45-4.33 (m, 2H, C*H*$_2$-Fmoc), 4.26 (d, 1H, H-4, $J_{3,4}$=2.6 Hz), 4.27-4.19 (m, 1H, C*H*-Fmoc), 3.85 (t, 1H, H-2), 3.60-3.45 (m, 2H, H-6a, H-6b), 3.6 (t, 1H, H-5, $J_{5,6a}$=$J_{5,6b}$=4.8 Hz), 2.90-2.70 (m, 2H, SC*H*$_2$CH$_3$), 1.33 (t, 3H, SCH$_2$C*H*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 154.50 (*C*O), 143.41, 143.27, 141.36 (2x), 137.87, 137.64 (6Cq, Ar), 128.59 (2x), 128.38 (2x), 128.18 (2x), 127.99 (3x), 127.86 (3x), 127.29 (2x), 125.29, 125.17, 120.17 (2x) (18*C*H, Ar), 85.44 (C-1), 80.87, 76.63, 76.18, 75.82, 73.97, 70.31, 69.78, 68.39 (C-2, C-3, C-4, C-5, C-6, 2*C*H$_2$Ph, *C*H$_2$Fmoc), 46.96 (1C, *C*H-Fmoc), 25.17 (S*C*H$_2$CH$_3$), 15.41 (SCH$_2$*C*H$_3$). FAB-MS: *m/z* 627 $[M+H]^+$. *Anal.* Calcd. for $C_{37}H_{38}O_7S$: C 70.90, H 6.11; found: C 70.93, H 5.96 . $[\alpha]_D^{25}$ +28.6° (*c* 1.00, CH_2Cl_2).

Ethyl 4-*O*-Acetyl-2,6-di-*O*-benzyl-1-thio-β-D-galactopyranoside (73). Ethyl 2,6-di-*O*-benzyl-1-thio-β-D-galactopyranoside (**70**, 0.75 g, 1.85 mmol) was added to a mixture of triethylorthoacetate (3 mL), dry benzene (3 mL) and p-toluenesulfonic acid monohydrate (4 mg, 0.02 mmol). The solution was stirred for 30 min at room temperature. Triethylamine (0.8 mL) was added and the solution was poured into ice-cold water (20 mL). Extraction with diethyl ether (3 x 20 mL) and evaporation of the solvent provided 72 as a colorless syrup which contained triethylorthoacetate. The syrup was dissolved in 80% aqueous acetic acid (6 mL) and the solution was kept at room temperature for 10 min. TLC (ethyl acetate/petroleum ether (60-80 °C), 2/5, v/v) indicated the completion of the reaction. The solution was then concentrated *in vacuo* and **73** was obtained as white crystals (820 mg, 1.83 mmol, 99% overall yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.45-7.25 (m, 10H, Ar-H), 5.39 (d, 1H, H-4, $J_{3,4}$=3.1 Hz), 4.76 (AB q, 2H, OCH_2Ph, J_{AB}=11.0 Hz), 4.49 (AB q, 2H, OCH_2Ph, J_{AB}=11.9 Hz), 4.48 (d, 1H, H-1, $J_{1,2}$=9.2 Hz), 3.80 (dd, 1H, H-3, $J_{2,3}$=9.2 Hz), 3.75 (t, 1H, H-5, $J_{5,6a}$=$J_{5,6b}$=6.1 Hz), 3.57 (dd, 1H, H-6a, $J_{6a,6b}$=9.7 Hz), 3.50 (dd, 1H, H-6b), 3.49 (t, 1H, H-2), 2.90-2.70 (m, 2H, SC$H_2$$CH_3$), 2.07 (s, 3H, C$H_3$CO), 1.34 (t, 3H, SCH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.17 (1C, CH_3CO), 138.03, 137.79 (2Cq, Ar), 128.60 (2x), 128.51 (2x), 128.37 (2x), 128.12, 127.97 (2x), 127.89 (10CH, Ar), 85.38 (C-1), 79.11, 76.20, 74.00, 70.42 (C-2, C-3, C-4, C-5), 75.72, 73.80, 68.41 (C-6, 2CH_2Ph), 25.53 (SCH_2CH_3), 21.13 (CH_3CO), 15.35 (SCH_2CH_3). FAB-MS: *m/z* 475 $[M+H]^+$.

Ethyl 2,6-Di-*O*-benzyl-3-*O*-(9-fluorenylmethoxycarbonyl)-4-*O*-acetyl-1-thio-β-D-galactopyranoside (74). To a stirred solution of ethyl 4-*O*-acetyl-2,6-di-*O*-benzyl-1-thio-β-D-

galactopyranoside (**73**, 200 mg, 0.55 mmol) in pyridine (3 mL) at 0 °C, 9-fluorenylmethylchloroformate (Fmoc-Cl, 155 mg, 0.60 mmol) was added. After 15 min the reaction was quenched with methanol (0.2 mL). Solvent was removed under reduced pressure, and the residue co-concentrated from dry toluene (3 x 5 mL). The residual syrup was then dissolved in dichloromethane (10 mL) and washed with water (2 x 5 mL) and saturated aqueous NaCl (2 x 5 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether (60-80 °C)/ethyl acetate, 4/1, v/v). to give **74** as a white solid (222 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.77 (d, 2H, Ar-H), 7.62 (d, 1H, Ar-H), 7.59 (d, 1H, Ar-H), 7.44-7.38 (m, 2H, Ar-H), 7.37-7.23 (m, 12 h, Ar-H), 5.63 (d, 1H, H-4, $J_{3,4}$=3.3 Hz), 4.88 (d, 1H, H-3, $J_{2,3}$=9.9 Hz), 4.79 (AB q, 2H, OCH_2Ph, J_{AB}=10.6 Hz), 4.56 (d, 1H, H-1, $J_{1,2}$=9.9 Hz), 4.52 (q, 1H, C*H*-Fmoc, *J*=3.7 Hz), 4.49 (AB q, 2H, OCH_2Ph, J_{AB}=11.7 Hz), 4.35-4.27 (m, 2H, CH_2-Fmoc), 3.85 (t, 1H, H-5, $J_{5,6a}$=$J_{5,6b}$=6.2 Hz), 3.71 (t, 1H, H-2), 3.57 (dd, 1H, H-6a, $J_{6a,6b}$=9.5 Hz), 2.79 (m, 2H, SCH_2CH$_3$), 2.09 (s, 3H, CH_3CO), 1.34 (t, 3H, SCH$_2$CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.19, 154.19 (2CO), 143.82, 143.19, 141.40, 141.33, 137.75, 137.71 (6Cq, Ar), 128.54 (2x), 128.42 (2x), 128.21 (2x), 128.03 (2x), 128.93 (4x), 127.24 (2x), 125.40, 125.22, 120.12 (2x) (18*C*H, Ar), 85.57 (C-1), 78.75, 76.40, 75.84, 68.12 (C-2, C-3, C-4, C-5), 75.89, 73.78, 70.48, 68.10 (C-6, 2*C*H$_2$Ph, *C*H$_2$Fmoc), 46.94 (*C*H-Fmoc), 25.59 (S*C*H$_2$CH$_3$), 21.03 (*C*H$_3$CO), 15.37 (SCH$_2$*C*H$_3$). FAB-MS: *m/z* 669 [M+H]$^+$ (Found: [M+H]$^+$, 669.2495. $C_{39}H_{41}O_8S$ requires *m/z*, 669.2522). *Anal.* Calcd. for $C_{39}H_{40}O_8S$: C 70.04, H 6.03; found: C 70.01, H 6.16. m.p. 103.2-103.8 °C. $[\alpha]_D^{25}$ +2.5° (*c* 1.30, CH_2Cl_2).

Methyl 3-*O*-Benzyl-2-*O*-(4-*O*-acetyl-2,6-Di-*O*-benzyl-D-galactopyranosyl)-β-D-galactopyranoside (77). A suspension of polymer bound glycosyl acceptor (**43**, 33 mg, 35

μmol), glycosyl donor (**74**, 120 mg, 0.180 mmol) and molecular sieves (4Å, 300 mg) in dichloromethane (2.0 mL) was stirred for 15 min at room temperature. NIS (20 mg, 0.180 mmol) and TMSOTf (1.6 μL, 20 μmol) were added to the suspension. After stirring for 15 min, the resin was filtered and washed with dichloromethane (2 x 2 mL), DMF (2 x 2 mL) and dichloromethane (2 x 2 mL), molecular sieves were removed by decantating, and the resin was dried *in vacuo* for 24 h. The resin was stirred in dichloromethane/trietylamine (2.5 mL, 4/1, v/v) for 1 h at room temperature, filtered, washed with dichloromethane (4 x 2 mL) and dried *in vacuo* for 12 h. The α/β mixture of disaccharides was cleaved from the resin by refluxing in acetone/water (5.0 mL, 4/1, v/v) for 30 min. The resin was filtered and washed with dichloromethane (2 x 20 mL) and methanol (2 x 20 mL). The filtrates and the washings were combined and concentrated *in vacuo*. The residue was further purified by preparative TLC (petroleum ether/ethyl acetate, 9/1, v/v) to give the α-anomer **77α** (8 mg) and the β-anomer **77β** (8 mg), both as a syrup (68%, α/β = 1/1). NMR data for α- anomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 15H, Ar-H), 5.63 (d, 1H, H-4', $J_{3',4'}$=3.5 Hz), 5.32 (d, 1H, H-1', $J_{1',2'}$=2.1 Hz), 4.80 (d, 1H, *J*=11.9 Hz), 4.67-4.52 (m, 3H), 4.48 (t, 1H, *J*=6.1 Hz), 4.43-4.32 (m, 2H), 4.30-4.25 (m, 1H), 4.12-4.05 (m, 1H), 4.02-3.78 (m, 4H), 3.70 (dd, 1H, *J*=3.5 Hz, *J*=10.1 Hz), 3.60 (dd, 1H, *J*=3.1 Hz, *J*=9.7 Hz), 3.55-3.40 (m 1H), 3.51 (s, 3H, OCH_3), 3.33 (dd, 1H, *J*=5.7 Hz, *J*=9.7 Hz), 3.27 (dd, 1H, *J*=6.2 Hz, *J*=9.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.96 (CO), 138.20, 137.89, 137.03 (3Cq, Ar), 128.86 (2x), 128.65 (2x), 128.55, 128.47 (2x), 128.40 (2x), 128.09, 127.88 (2x), 127.78 (2x), 127.64 (15CH, Ar), 104.77, 95.78 (C-1, C-1'), 79.90, 76.22, 72.97, 70.81, 68.16, 67.86, 66.71 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 73.46, 72.23, 71.94, 68.59, 62.77 (C-6, C-6', 3CH_2Ph), 56.90 (OCH_3), 21.15 (CH_3CO). FAB-MS: *m/z* 691 $[M+Na]^+$ (Found: $[M+Na]^+$, 691.2700. $C_{36}H_{44}O_{12}Na$ requires *m/z*, 691.2730). *Anal.* Calcd. for $C_{36}H_{44}O_{12}$: C 64.66, H 6.63; found: C

64.83, H 7.01 . $[\alpha]_D^{25}$ 26.5° (*c* 0.19, CH_2Cl_2). NMR data for β-anomer: ^1H NMR (300 MHz, $CDCl_3$) δ: 7.57 (m, 15H, Ar-H), 5.58 (d, 1H, H-4', $J_{3',4'}$=5.1 Hz), 4.88 (d, 1H, *J*=7.9 Hz), 4.82 (AB q, 2H, OCH_2Ph, J_{AB}=11.9 Hz), 4.63-4.44 (m, 4H), 4.43-4.39 (m, 4H), 4.00-3.92 (m, 3H), 3.86-3.71 (m, 3H), 3.63-3.44 (m, 5H), 3.49 (s, 3H, OCH_3), 2.08 (s, 3H, CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.98 (CO), 138.64, 137.55, 136.48 (3Cq, Ar), 128.69 (2x), 128.54 (4x), 128.24 (3x), 128.07 (2x), 127.99 (2x), 127.93, 127.84 (15CH, Ar), 103.09 (C-1, C-1'), 81.48, 80.21, 77.07, 74.01, 72.60, 69.93, 66.92 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 75.05, 73.84, 72.52, 68.39, 62.83 (C-6, C-6', $3CH_2Ph$), 56.49 (OCH_3), 21.19 (CH_3CO). FAB-MS: *m/z* 691 $[M+Na]^+$ (Found: $[M+Na]^+$, 691.2751. $C_{36}H_{44}O_{12}Na$ requires *m/z*, 691.2730). *Anal.* Calcd. for $C_{36}H_{44}O_{12}$: C 64.66, H 6.63; found: C 64.31, H 6.82. $[\alpha]_D^{25}$ +1.9° (*c* 0.50, CH_2Cl_2).

Methyl 3-*O*-Benzyl-2-*O*-(4-*O*-acetyl-2,6-di-*O*-benzyl-3-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-α-D-galactopyranosyl)-β-D-galactopyranoside (79).

Polystyrylboronic acid (**41**, 20 mg) was placed in a round-bottomed flask and covered with enough pyridine (~0.6 mL) to swell the polymer completely. Disaccharide **77α** (30 mg, 45 μmol) was added, and the suspension was heated for 1 h at 60 °C, and then at 80 °C under reduced pressure for 1 h. The resin was filtered and washed with dry pyridine (2 x 2 mL) and dry toluene (2 x 2 mL). The filtrate was evaporated, and unreacted starting material (14 mg, 47%) was recovered. The resin was co-evaporated from dry toluene (2 x 1.0 mL), and then dried *in vacuo* over P_2O_5 for 24 h. The loaded resin **76α** was placed in a round-bottomed flask and dichloromethane (2.0 mL) was added. The suspension was stirred for 10 min to allow the resin to swell. Ethyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**51**, 134 mg, 0.22 mmol) and molecular sieves (300 mg, Å, beads) were added to the suspension, and this

was stirred for 15 min. NIS (50 mg, 0.22 mmol) and TMSOTf (4.0μL, 20 μmol) were added to the suspension. After 15 min, the reaction was quenched by the addition of triethylamine (0.1 mL). The resin was filtered and washed with dichloromethane (2 x 2 mL), DMF (2 x 2 mL) and dichloromethane (2 x 2 mL). The product was released from the resin by stirring the polymer in acetone/water (5 mL, 4/1, v/v) for 30 min. The polymer was filtered and washed with dichloromethane (2 x 20 mL) and methanol (2 x 20 mL). All the filtrates and the washings were collected and concentrated under reduced pressure. Purification of the residue by silica gel column chromatography (petroleum ether (40-60 °C)/ethyl acetate, 8/2, v/v) gave **79** as a colorless syrup (24 mg, 83% for three steps, based on recovered acceptor). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.95–7.92 (m, 2H, Ar-H), 7.52-7.48(m, 1H, Ar-H), 7.40-7.15 (m, 30H, Ar-H), 7.08-7.04 (m, 2H, Ar-H), 5.43 (d, 1H, H-1', $J_{1',2'}$=3.5 Hz), 5.41 (d, 1H, H-4', $J_{3',4'}$=3.5 Hz), 5.31 (t, 1H, H-2'', $J_{1'',2''}=J_{2'',3''}$=8.4 Hz), 5.02 (d, 1H, H-1''), 4.74 (AB q, 2H, OCH_2Ph, J_{AB}=11.4 Hz), 4.71 (AB q, 2H, OCH_2Ph, J_{AB}=10.5 Hz), 4.56 (d, 1H, *J*=12.3 Hz), 4.56-4.32 (m, 7H), 4.30-4.21 (m, 2H), 3.98-3.92 (m, 2H), 3.89 (t, 1H, *J*=9.7 Hz), 3.86-3.68 (m, 6H), 3.67-3.60 (m, 2H), 3.56 (dd, 1H, *J*=3.4 Hz, *J*=9.7 Hz), 3.54-3.42 (m, 2H), 3.40 (s, 3H, OCH_3), 3.25 (dd, 1H, *J*=6.1 Hz, *J*=10.1 Hz), 3.37 (dd, 1H, *J*=5.3 Hz, *J*=10.5 Hz), 2.02 (CH_3CO). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.33, 165.00 (2CO), 139.12, 138.48 (2x), 138.09 (2x), 137.22 (2x) (7*C*H, Ar), 133.13-127.17 (35*C*H, Ar), 104.59 (C-1), 100.95 (C-1''), 96.16 (C-1'), 83.05, 80.14, 78.25, 76.50, 75.39, 74.40, 74.15, 73.15, 72.08, 71.44, 67.88, 66.76 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2'', C-3'', C-4'', C-5''), 75.34, 75.07, 73.60, 73.31, 72.79, 72.39, 69.37 (2x), 62.80 (C-6, C-6', C-6'', 6CH_2Ph), 56.70 (OCH_3), 30.05, 21.11 (2CH_3CO). FAB-MS: *m/z* 1227 $[M+Na]^+$. *Anal.* Calcd. for $C_{70}H_{76}O_{18}$: C 69.75, H 6.36; found: C 69.38, H 6.21. $[\alpha]_D^{25}$ +63.2° (*c* 0.29, CH_2Cl_2).

Methyl β-D-Galactopyranoside 4,6-*O*-phenylboronate (88). Phenylboronic acid (0.628 g, 5.15 mmol) was added to a suspension of methyl β-D-galactopyranoside (**7**, 1.00 g, 5.15 mmol) in dry toluene (30 mL). The reaction mixture was refluxed under Dean-Stark conditions for 20 min. Upon cooling a white precipitate was formed which was filtered and recrystallised from tolueneto give **88** as a white crystalline solid (1.28 g, 89%). ^{1}H NMR (300 MHz, DMSO) δ: 7.63 (d, 2H, Ar-H), 7.37 (t, 1H, Ar-H), 7.26 (t, 2H, Ar-H), 5.20 (d,1H, 2-OH, J=4.7 Hz), 5.08 (d, 1H, 3-OH, J=6.4 Hz), 4.32 (d, 1H, H-4, $J_{3,4}$=2.6 Hz), 4.32-4.22 (m, 1H, H-6a), 4.20 (d, 1H, H-1, $J_{1,2}$=7.9 Hz), 4.07 (d, 1H, H-6b, $J_{6a,6b}$=12.3 Hz), 3.97 (s, 1H, H-5), 3.51 (ddd, 1H, H-3, $J_{2,3}$=9.1 Hz), 3.36 (s, 3H, OCH_3), 3.28 (ddd, 1H, H-2). ^{13}C NMR (75 MHz, DMSO) δ: 133.51 (2x), 130.51, 127.27 (2x) (5*C*H, Ar), 103.99 (C-1), 72.10, 70.82, 69.76, 67.60 (C-2, C-3, C-4, C-5), 64.32 (C-6), 56.00 (O*C*H$_3$). FAB-MS: *m/z* 303 [M+Na]$^+$.

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