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Recent Developments in Polymer Supported Syntheses of Oligosaccharides and Glycopeptides

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1. INTRODUCTION

Carbohydrates are biologically important molecules and have received considerable interest in recent years.[1] This is a consequence of improvements in analytical methods which have allowed isolation and identification of even small quantities of these biomolecules. Thus, it has been possible to reach a far better understanding of their biological roles. For example, it is now well established that carbohydrates play key roles in both normal and pathological biological recognition processes[2] and, as such, they are implicated in chronic inflammatory, viral and bacterial infections[3] as well as in tumorigenesis and metastasis.[4] Considerable effort has

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recently been expended on determining how carbohydrates function as recognition signals as well as developing strategies to block undesirable interactions between cell surface carbohydrates and their protein targets.[5]

Since the preparation of biologically important oligosaccharides typically requires multistep transformations involving iterative protection-glycosidation-deprotection reactions with chromatographic purification of intermediates at each stage of the synthesis,[6] such preparations would greatly benefit from developments in polymer supported oligosaccharide synthetic strategies. Polymer supported syntheses offer many advantages over solution phase reactions including increased yields, due to the ability to add excess reagent to drive reactions to completion, and increased speed of synthesis, due to the elimination of purification processes. Major problems can be expected when such polymer supported approaches are applied to the syntheses of oligosaccharides, because the preparation of a specific carbohydrate requires the stereospecific formation of each new glycosidic bond in high yield. Such processes have been demonstrated to be very sensitive even to slight structural or electronic variations in the glycosyl donor or acceptor.[7]

However, if successful, such strategies would not only allow the rapid and efficient synthesis of target molecules but also the synthesis of polymer bound, fully deprotected saccharides for use directly and repeatedly in biological assays.[8] This is an invaluable tool for glycobiology.[9] It is for this reason that polymer supported oligosaccharide synthesis has received such attention from pioneering carbohydrate chemists. The polymer supported synthesis of oligosaccharides lags behind that of peptides and oligonucleotides,[10] but much progress has been made using an array of polymers, linkers and glycosidation strategies. Recent reviews on polymer bound reactions highlight the different types of polymers and linkers available[11] as well as the different methods for monitoring polymer supported reactions. However, little mention of reactions relevant to oligosaccharide assembly are given.

Two main strategies can be envisaged for solid-phase oligosaccharide synthesis entailing either attachment of the acceptor or of the donor to the solid support. In the former strategy, an acceptor is bound to the solid support, usually at the anomeric position, and a solution based donor and promoter are administered for the coupling step. In the second approach, glycosyl donors are bound to the solid support by a suitable hydroxyl group and then reacted with solution phase acceptors. These approaches are discussed in detail below.

2. TYPES OF POLYMERS AND LINKER SYSTEMS

Polymer Systems

There are many different polymers and linker systems that are used in solid phase oligosaccharide synthesis[11] and these will be highlighted during the course of this review. It will be seen that the Merrifield resin has been used extensively in the solid phase synthesis of oligosaccharides, which, no doubt, is a result of its success in the solid-phase synthesis of peptides.[12] However, such reactions require swelling of the polymer by the solvent for efficient reactions to occur. Furthermore, reactions using this resin are often solvent and temperature dependent, so that in certain cases control of the anomeric geometry and the regioselectivity of glycosidation reactions is somewhat limited. Controlled-pore glass (CPG) has been proposed as a good alternative solid support,[13] because no preswelling is required and it has proven utility in solid phase oligonucleotide synthesis. Recent developments have also included the grafting of polyoxyethylene onto polystyrene crosslinked resins to produce Tentagel and related resins.[14] Although these have a lower loading capacity than polystyrene based resins, their swelling properties in polar solvents are much improved.

Of perhaps greater utility for all polymer supported reactions are soluble polymer supports, which allow homogenous rather than heterogeneous reactions to occur.[15] Analysis of the polymer bound reactions then does not require cleavage of intermediates and products from the solid support. Initial attempts to create a soluble polymer support on which oligosaccharides could be constructed involved copolymerising one of four 6-*O*-vinylbenzoyl glucopyranose derivatives with styrene (Figure 1).[16] In this way polymers of varying saccharide content were obtained and the solubility properties were similar to polystyrene itself.

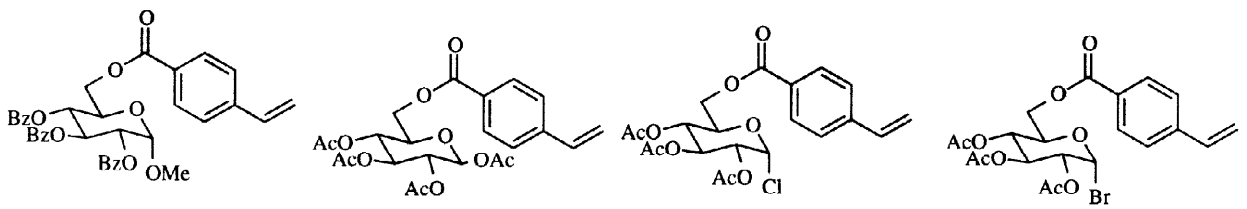


Figure 1 : Monosaccharides used for copolymerisation with styrene.

Alternative soluble homopolymers, which have been used in oligosaccharide synthesis, are given in Figure 2.

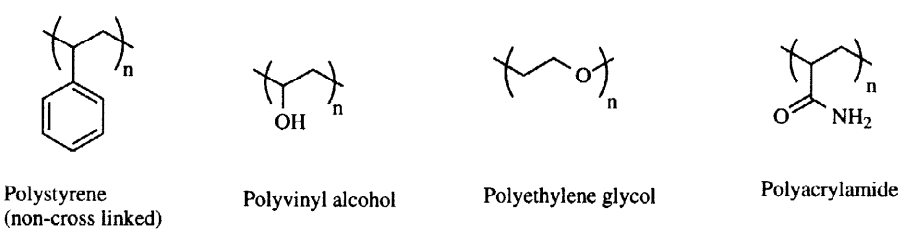


Figure 2 : Soluble homopolymers used in oligosaccharide synthesis.

Linker Systems

When selecting a linker for attaching a saccharide unit to a polymer, careful consideration must be given to the reaction conditions to which the linker will be exposed. Furthermore, it must be possible to release the newly constructed oligosaccharide from the linker and polymer at the end of the synthesis without any decomposition of the glycosidic bonds. A range of linkers have been developed that are labile under different conditions, and, again, specific points of interest will be highlighted within the case studies that follow. An acid labile linker, 5-(4-(9-fluorenylmethyloxycarbonyl)aminomethyl-3,5-dimethoxyphenoxy)valeric acid (PAL) (Figure 3), has been utilised for the solid-phase synthesis of *C*-terminal peptide amides including human gastrin-1,[17] and some base labile succinate linkers have been employed for linking a range of acceptors to Tentagel (*vide infra,* Schemes 3 and 4).[18] However, under certain circumstances, limitations associated with the succinoyl ester linkage have been identified.[19] For example, such linkages are prone to migration under acidic conditions, and cannot be incorporated at the anomeric centre of sugars, limiting their applicability to general glycosidation and protecting group manipulations.

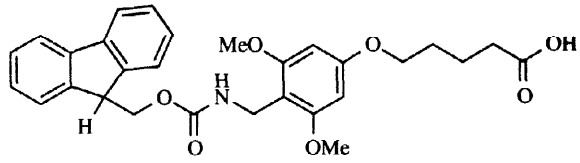
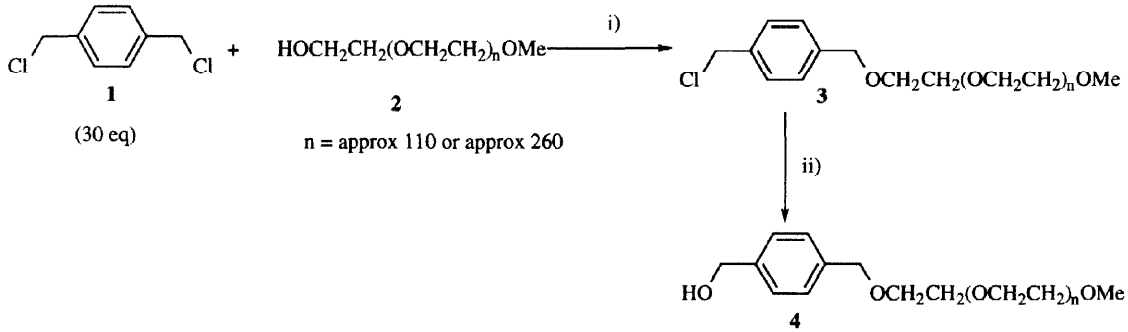


Figure 3 : PAL, an acid labile linker.

An alternative α,α'-dioxy-*p*-xylyl (DOX) linker allowed attachment of monosaccharides (including glucosamine derivatives) to polyethyleneglycol monomethylether (MPEG) by any hydroxyl group including that at the anomeric centre.[20] It is stable under most glycosidation conditions and can be easily removed at the end of a synthesis,by hydrogenolysis, to afford either the free hydroxyl group, or a *p*-tolylmethyl ether, which resembles a benzyl ether in its chemical properties. The polymer-support-linker system is readily prepared from the reaction of the monomethylether of MPEG **1** with excess α,α'-dichloro-*p*-xylene **2** following the Williamson ether synthesis (Scheme 1).[21] The material **3** thus produced can either be used directly for incorporation at non-anomeric hydroxyl groups, or can be hydrolysed to produce MPEG-DOX-OH **4**. This can then be reacted with trichloroacetimidate donors to introduce the linker to the anomeric position for further manipulations. Such an approach has successfully led to the preparation of D-mannopentaose, a structural moiety of cell surface D-mannans of pathogenic yeasts.[20]



i) NaH, NaI, THF, 96h; ii) 10% aq Na_2CO_3, 70°C, 16h

Scheme 1 : Synthesis of DOX linker.

Ideally, all linkers would be removable under neutral conditions and therefore much effort has been spent on such linkers. In particular, a whole range of photolabile linkers have been established and some examples of the more useful types available, including Rich's linker **6** are given in Figure 4 (R=sugar).[22]

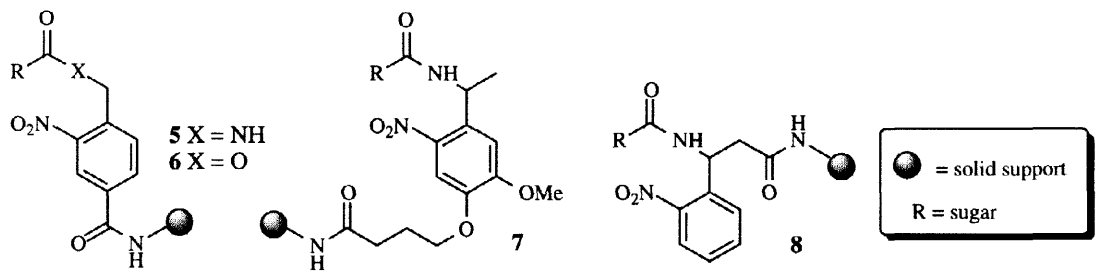
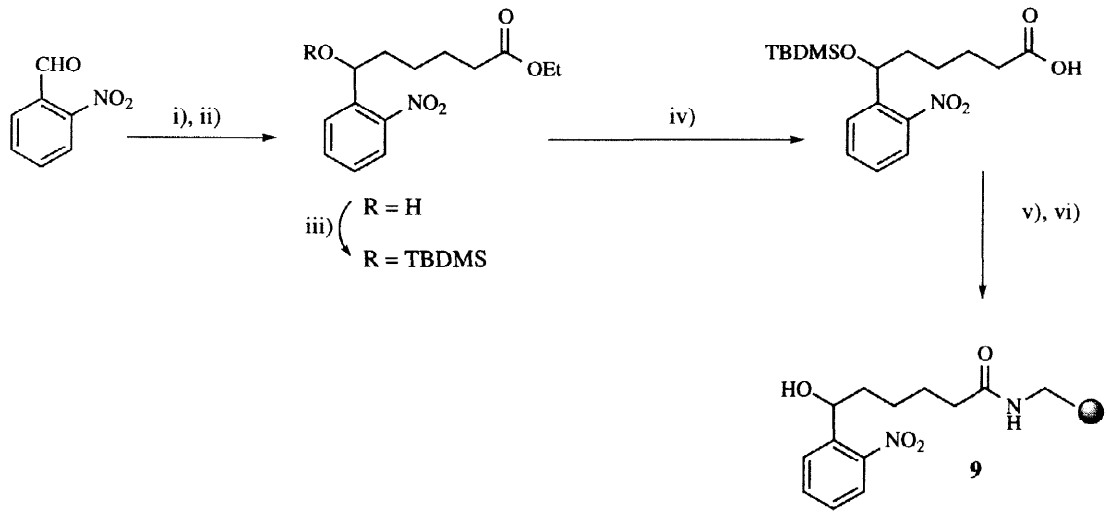


Figure 4 : Photolabile linkers.

The original photolabile linkers **5**[22a] and **6**[22b] (Rich's linker) suffer from slow cleavage kinetics. Hence, a further series of linkers **7**[22c] and **8**[22d] were prepared, and these afforded better yields of deprotected compounds upon photolysis. Such protecting groups have been utilised in the solid phase synthesis of a heptasaccharide phytoalexin elicitor.[23] A further derivative of Rich's linker **6** has been prepared by Fraser-Reid.[24] On the basis of the mechanism for photocleavage of *o*-nitrobenzyl derivatives[25] he reasoned that a secondary benzylic hydrogen should be more easily abstracted under photochemical conditions than a secondary proton. This afforded improved cleavage kinetics and deprotection yields. The synthesis of the linker **9** is outlined below (Scheme 2).



i) ZnI_2, naphthalene, Li, THF, 16h then ethyl 5-bromovalerate; ii) Zn, BF_3OEt_2, THF, 88%; iii) TBDMSCl, imidazole, DMF, 97%; iv) Na_2CO_3, MeOH : H_2O; v) DIC, DMAP, amine resin, NMP; vi) TBAF, THF

Scheme 2 : Synthesis of alternative photolabile linker.

A six carbon chain of linker **9** was chosen to remove the problems of elimination and lactonization. As the cleavage of perbenzyl galactose attached to the polymer *via* this linkage was found to be effective (89%) under mild reaction conditions, its utility for the solid-phase synthesis of a branched trimannan oligosaccharide was investigated (Scheme 7).

Alternative linkers which are cleaved under essentially neutral conditions include an α-chymotrypsin sensitive linker (Scheme 21)[26] and an allylic ester linker (HYCRAM™) (Scheme 23),[27] which is removed using a palladium(0)-catalyst.[28] The latter linker is particularly suitable for glycopeptides since it is compatible with reaction conditions necessary for *N*- and *O*-deprotection of amino acids. These systems are described in more detail throughout the report.

3. INTERMEDIATES COMMONLY EMPLOYED IN POLYMER SUPPORTED OLIGOSACCHARIDE SYNTHESIS

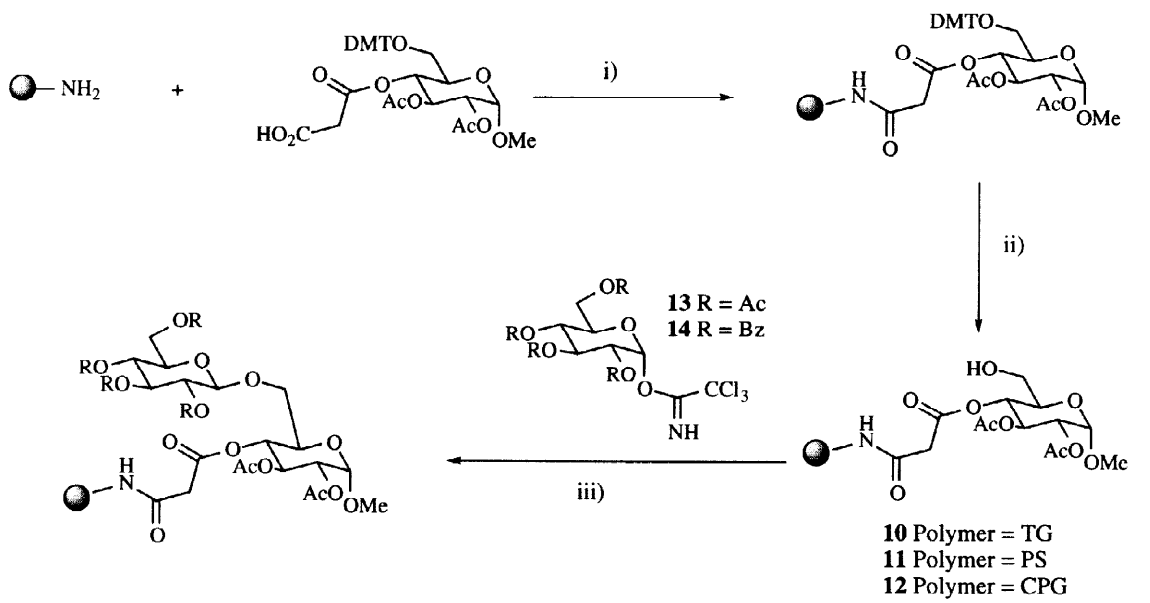
Many strategies reported for the polymer supported syntheses of oligosaccharides take advantage of recent successful developments in the solution phase syntheses of oligosaccharides. Hence, the more widely used anomeric groups, glycosidation conditions, and tactics for controlling the geometry of newly formed bonds

developed for solution phase reactions have been applied to polymer supported strategies. Trichloroacetimidates, *n*-pentenyl glycosides, anomeric sulfoxides and glycals are the most widely used intermediates in polymer supported oligosaccharide strategies and examples of specific syntheses employing these intermediates are discussed below.

Trichloroacetimidates

Trichloroacetimidates are key intermediates in many solution phase syntheses of oligosaccharides.[29] A number of promoters may be employed for the solution phase activation of trichloroacetimidates including $BF_3.OEt_2$[30] and derivatives of trifluoromethylsulfonic acid, such as silver triflate[31] and trimethylsilyl- or triethylsilyl triflate.[32] However, some acceptors are either unreactive or hindered and such promoters are ineffective. As polymer supported reactions often proceed slower than solution reactions, a more effective promoter was required for the reaction of MPEG bound acceptors with trichloroacetimidates. Dibutylboron triflate was found to be an efficient promoter[33] and excellent glycosidation yields of up to 85-95% were obtained.

An investigation to identify which solid-support afforded best glycosidation results with a small range of trichloroacetimidate promoters was undertaken by Adinolfi and co-workers.[18a] Acceptors **10**, **11** and **12**, bound to Tentagel (TG), polystyrene (PS) and controlled pore glass (CPG) respectively, were used in glycosidation reactions with trichloroacetimidate and phosphoramidite donors for the synthesis of saccharo-nucleotide conjugates.[19] α-D-Methylglucopyranoside was anchored to the solid support through a succinate linkage involving the C-4 OH of the monosaccharide. Coupling to peracetate or perbenzoate trichloroacetimidates **13** or **14** was then investigated using a range of Lewis Acid promoters (Scheme 3).



i) DCC / pyr; ii) a) Cap excess amine sites on polymer with Ac_2O, pyr; b) 10% Cl_2HCCO_2H / DCM; iii) See Table 1

Scheme 3 : Investigation of the effect of polymer and promoter on glycosidation results

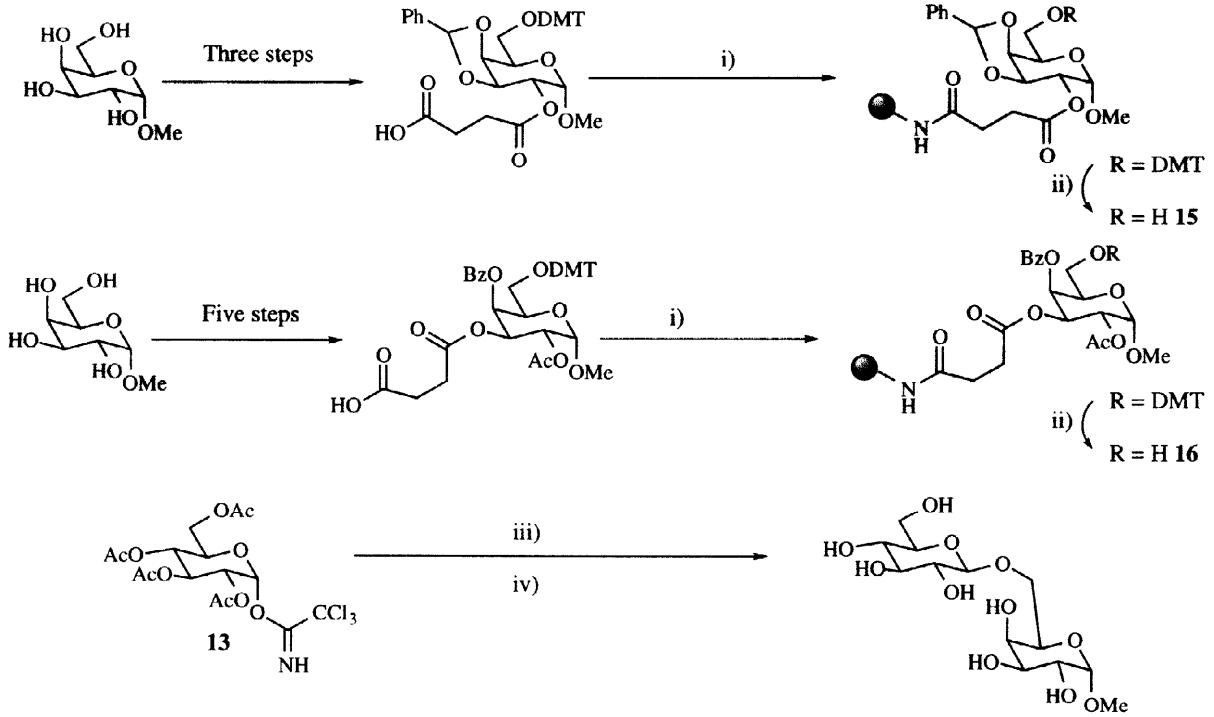
The results obtained with the different polymers, donors and promoters are presented in Table 1 below.

Support	Donor, R =	Promoter (eq)	Yield (%)
TG	Ac	TMSOTf (5)	45
TG	Ac	$BF_3.OEt_2$ (5)	60
PS	Ac	TMSOTf (5)	45
PS	Ac	$BF_3.OEt_2$ (5)	35
CPG	Ac	TMSOTf (1.2)	55
CPG	Ac	TMSOTf (2.5)	65
CPG	Ac	$BF_3.OEt_2$ (2.5)	60
TG	Bz	TMSOTf (5)	50
TG	Bz	$BF_3.OEt_2$ (5)	65
PS	Bz	TMSOTf (5)	90
CPG	Bz	TMSOTf (5)	95

Table 1 : Glycosidation results

CPG proved to be the best support producing typical glycosidation yields of 55 to 95%, and this strategy allowed access to glyconucleotides for antisense investigation.

Some interesting observations have been made on the compatibility of standard carbohydrate protecting groups with the more forcing reaction conditions required for polymer bound oligosaccharide assembly.[18b] A succinamide linker was again employed to attach Tentagel to either the C-2 or C-3 hydroxyl group of a glycosyl acceptor. A range of glycosidation reactions were investigated with the acceptors **15** and **16**, trichloroacetimidate **13** donor, and a number of activating conditions (Scheme 4). In contrast to solution-phase reactions,[34] it was found that the yields of glycosidation were greatly affected by the activating conditions. Furthermore, as is often encountered with solid-phase reactions, yields were improved by exposing the intermediates to the reaction media a number of times. These effects are highlighted in Table 2.



i) NH_2-Tentagel, DCC (3.5 equiv.), pyr, rt, 16h; ii) Cl_2CHCO_2H, DCM (2% w/w, repeat 3 times); iii) **15** or **16** and See Table 2; iv) NH_4OH, rt, 4h

Scheme 4 : Further investigation of promoter effect on glycosidation results.

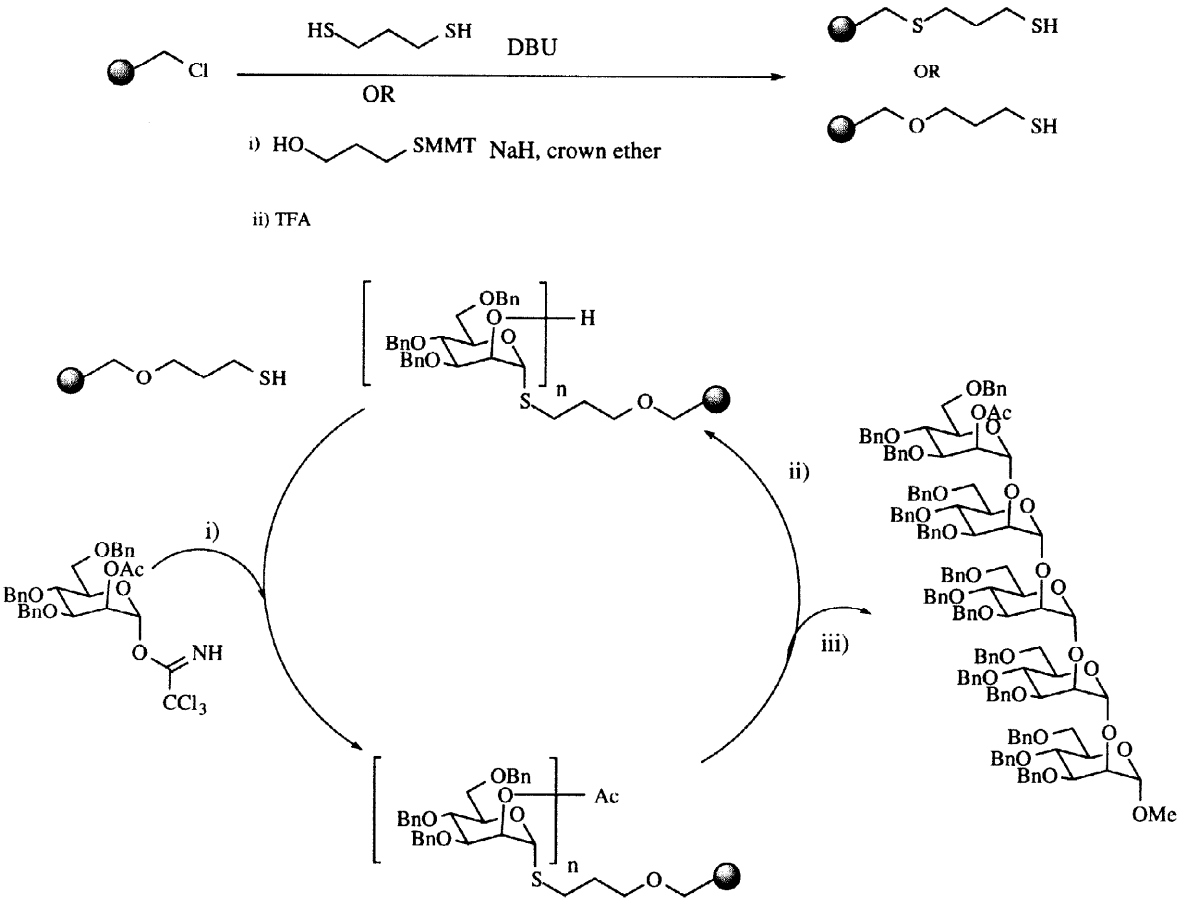
Acceptor	Promoter	Solvent	Yield (%)	Reaction time (h)
15	TfOH (0.1 equiv)	DCM : Cyclohexane 2:1	55	2
15	TMSOTf (0.1 equiv)	DCM : Cyclohexane 2:1	55	2
15	BF_3OEt_2 (0.1 equiv)	DCM : Cyclohexane 2:1	52	2
15	$LiClO_4$	DCM	3-5	48
16	TfOH (0.1 equiv)	DCM : Cyclohexane 2:1	50 (60)[a]	2
16	TMSOTf (0.1 equiv)	DCM : Cyclohexane 2:1	45 (55)[a]	2
16	BF_3OEt_2 (0.1 equiv)	DCM : Cyclohexane 2:1	70 (90)[a]	2
16	$LiClO_4$	DCM	3-5	48

[a] Numbers in brackets indicate yields afforded by performing the coupling reaction on the solid support twice.

Table 2 : Glycosidation results.

The dimethoxytrityl group (DMT) was found to be an effective temporary C-6 protecting group in this strategy. It was concluded that acceptor **16** was a more effective acceptor than **15**, because of the instability of the benzylidene protecting group in acceptor **15**. Interestingly, although $LiClO_4$ is a good activator of trichloroacetimidates in solution phase reactions, its utility in solid-phase coupling reactions is poor.

Trichloroacetimidate donors have also been used in conjunction with alkyl thiol acceptors linked to chloromethylated polystyrene / divinylbenzene copolymers. Using these polymer systems, pentasaccharides containing repeated Glu-α-(1,6) linkages[35] or Man-α-(1,2) linkages[36] have been synthesised*via* the iterative process outlined in Scheme 5. Conditions for glycosylation by monitoring the progress of the reaction by cleaving small amounts of the products from the polymer and analysing the supernatant thus produced by tlc, HPLC and MALDI-TOF techniques. The trichloroacetimidate donors have already been employed in a similar solution phase strategy.[37]

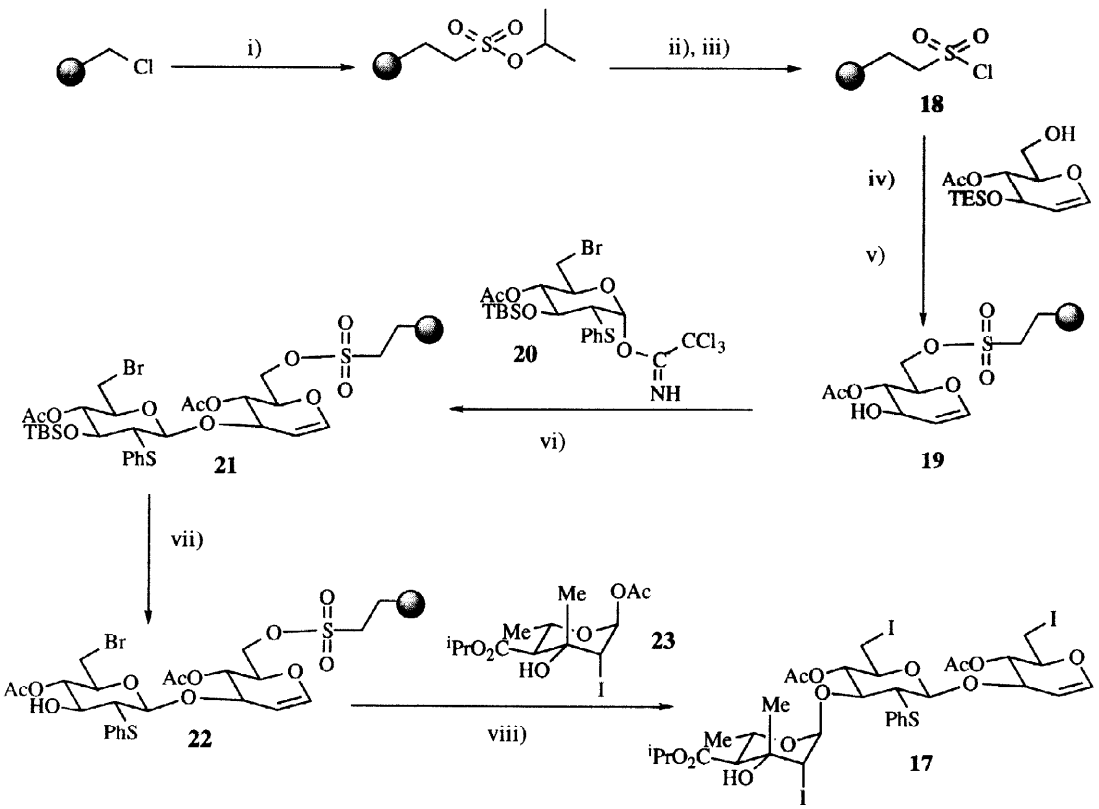


i) Trichloroacetimidate donor (3 eq.), TMSOTf (0.3 eq.), DCM; ii) DCM / 0.5 M NaOMe; iii) NBS (4 eq.), THF / MeOH, 9 : 1.

Scheme 5 : Iterative glycosidation procedure.

An acetate group is employed as a temporary orthogonal protecting for the C-2 hydroxyl group and neighbouring group participation results in selective formation of the required α-linkage.

A linker was carefully designed to allow attachment of a carbohydrate to a polymer support and to allow concomitant incorporation of additional functional groups upon removal of the linked saccharide from the polymer.[38] Thus, entry to 6-deoxy oligosaccharides **17**, which are integral components of antibiotic and cytostatic agents,[39] has been possible by using sulfonate esters to link the sugar residues to polymer supports. The required sulfonyl chloride resin **18** was prepared by the solution phase method of Widlanski[40] in an overall yield of 70% over three steps (Scheme 6).



i) $^{i}PrOSO_2CH_2Li$ (20 eq.), THF, -78°C to -25°C, 20h.; ii) NaI (30 eq.), acetone, 55°C, 20h; iii) SO_2Cl_2 (5 eq.), PPh_3, polymer-CH_2Cl_2, 0°C to 23°C, 4.5 h., 70% over 3 steps; iv) Et_3N, DCM, 23°C; v) HF-pyr, THF, 23°C; vi) TMSOTf, 4Å ms, DCM, 4h, -78°C, repeat; vii) a) Ac_2O, pyr; b) $Et_3N(HF)_3$; viii) a) TMSOTf, -78°C, 4h, repeat; b) Ac_2O, pyr.; c) NaI, 2-butanone, 67% overall yield based on glycal **19**

Scheme 6 : Synthesis of 6-deoxy oligosaccharides.

Glycosidations of the polymer bound acceptor **19** with trichloroacetimidate donor **20** proceeds well, with best yields being obtained upon repetition of the coupling reaction. Only a trace of the α-anomer of **21** is observed, which is in contrast to the analogous solution phase reaction,[41] (β : α anomers in the ratio of 4-5 : 1). Any unreacted acceptor **19** is capped by treatment with acetic anhydride and pyridine. The silyl ether of **21** is deprotected under standard conditions to afford acceptor **22** and further glycosidation is performed with acceptor **23**. The oligosaccharide is displaced from the resin by treatment with iodide and compound **17** is a precursor of olivomycin A.

n-Pentenyl glycosides

The use of *n*-pentenyl glycosides (NPG) in the solution phase syntheses of oligosaccharides is well documented.[42] When extending this approach to polymer supported strategies two options exist.[43] Firstly, the NPG donor can be anchored to a polymer support through a hydroxyl group, and subsequently coupled to an excess of acceptor in the solution phase. Alternatively, the acceptor can be bound to the polymer support and reacted with excess NPG donor in solution. Both approaches have been investigated using polystyrene and Tentagel as the polymer supports. The latter approach was found to afford best yields and many NPG donors were reacted with Polystyrene or Tentagel bound acceptors. As with solution phase techniques,[44] donors

possessing C-2 phthalimide and ester groups afforded 1,2-*trans*-glycosides, and the selectivity of donors with non-participating C-2 groups was influenced by the reaction solvent. Bulky *O*-6 substituents favoured formation of the α-anomers (Table 3).

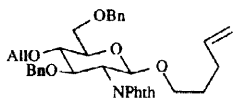
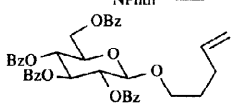
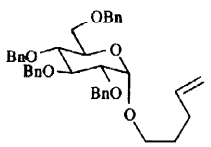
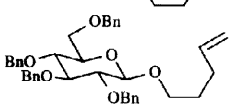
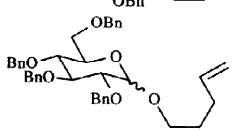
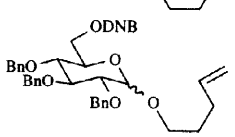
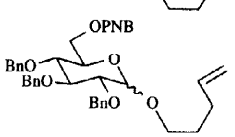
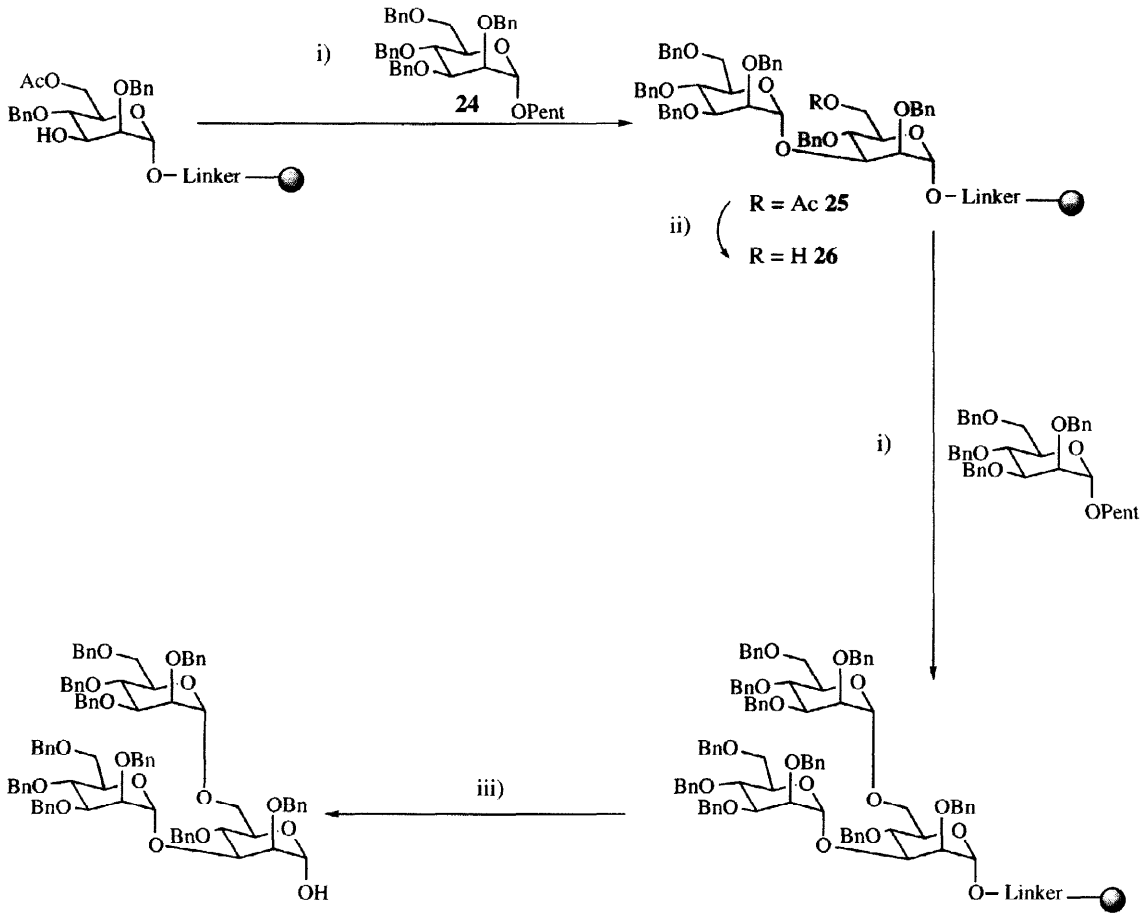
Donor	Solvent	α / β
	DCM	0 : 1
	DCM	0 : 1
	DCM	1 : 1
	DCM	1.1 : 1
	Et_2O : DCM 11 : 1	1.8 : 1
	DCM	9 : 1
	DCM	4 : 1

Table 3 : Effect of donor structure on anomeric ratio of products.

These anomeric ratios were measured using gel phase ^{13}C nmr techniques[45] and it was possible to improve this process further using polystyrene-grafted crowns as a solid support in conjunction with Rich's linker.[22a] This afforded both a polymer support that was more amenable to parallel synthesis, and a linker that facilitated monitoring.

A NPG donor **24** has also been used for the synthesis of a branched trimannan oligosaccharide using a derivative of Rich's linker (Scheme 7). A key point of this synthesis is a chemoselective deprotection of the acetate group of **25** to afford acceptor **26** whilst the saccharide is still bound to the resin.

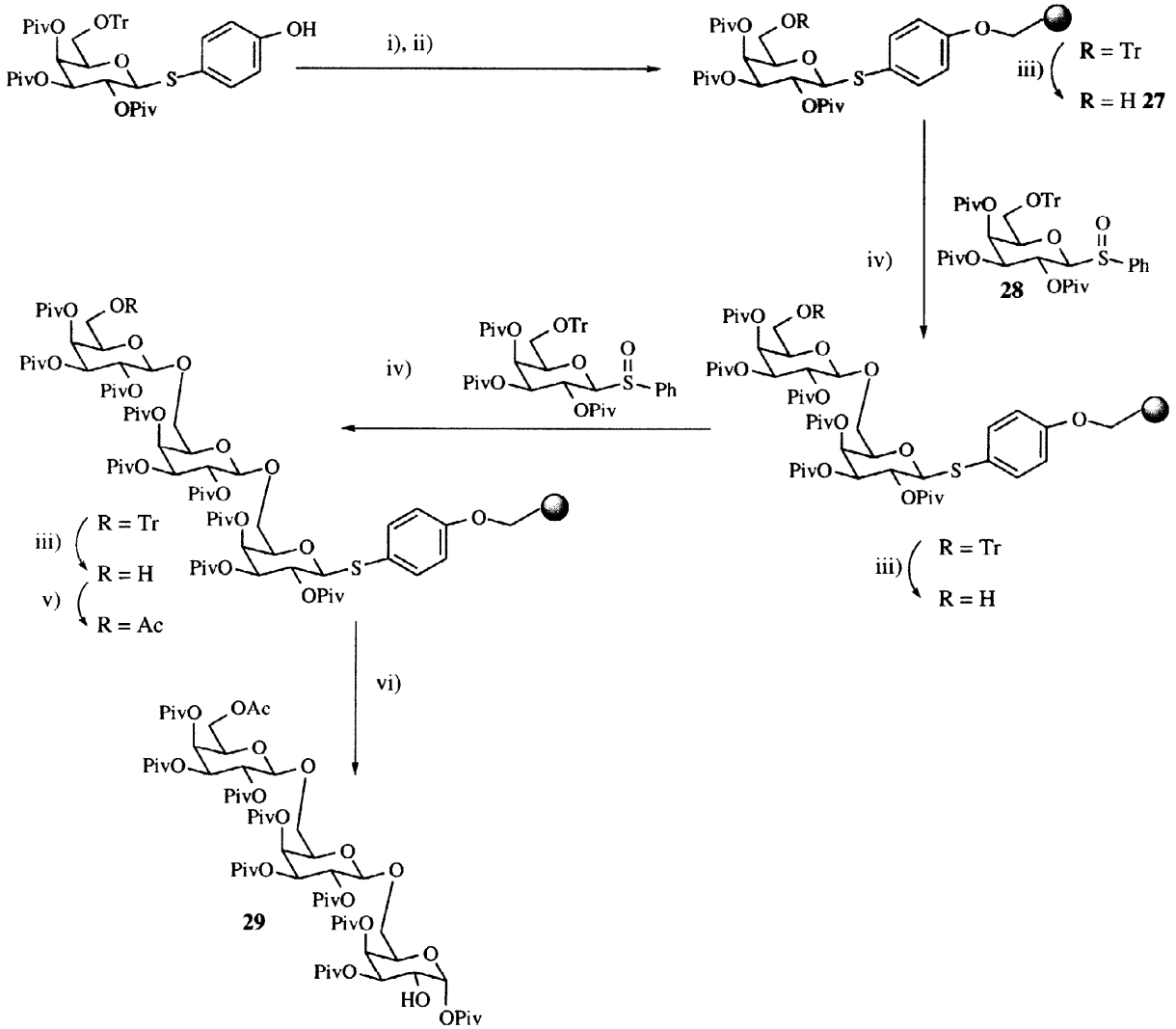


i) NIS, TESOTf, DCM; ii) NaOMe, MeOH, THF; iii) hv, THF, 15h; 42% overall yield

Scheme 7 : Synthesis of a branched trimannan oligosaccharide.

Anomeric sulfoxides

As mentioned before, many reactions which work efficiently in solution are ineffective with polymer supported substrates. Often, such inefficiencies are due to low reactivity of the glycosyl donors and acceptors at high dilution. Kahne has shown that anomeric sulfoxides, which are extremely reactive glycosyl donors even at low temperatures in solution phase reactions,[46] are particularly useful in polymer supported reactions because of the high levels of reactivity and excellent stereocontrol (Scheme 8).[47] Moreover, since both the glycosyl sulfoxide and the activating agent (Tf_2O) are relatively non-polar, favourable partitioning into the non-polar matrix of the insoluble solid support should occur. Kahne thus investigated the feasibility of attaching a monosaccharide to Merrifield resin *via* a *p*-hydroxythiophenyl linkage and reacting this acceptor **27** with a solution phase sulfoxide donor **28** (Scheme 8). The thiophenyl ether linkage is stable under typical reaction conditions employed for oligosaccharide assembly, but is easily hydrolysed with mercuric trifluoroacetate. An overall yield of 52% for the synthesis of oligosaccharide **29** was obtained. This procedure has been expanded to allow specific entry to either α- or β-linked oligosaccharides with secondary alcohols as polymer bound acceptors, using the *p*-hydroxythiophenyl glycoside of 2-azido-2-deoxy-4,6-*O*-benzylidene-D-glucose.[47] Such methodology may pave the way for synthesis of Le^x and Le^a analogues.



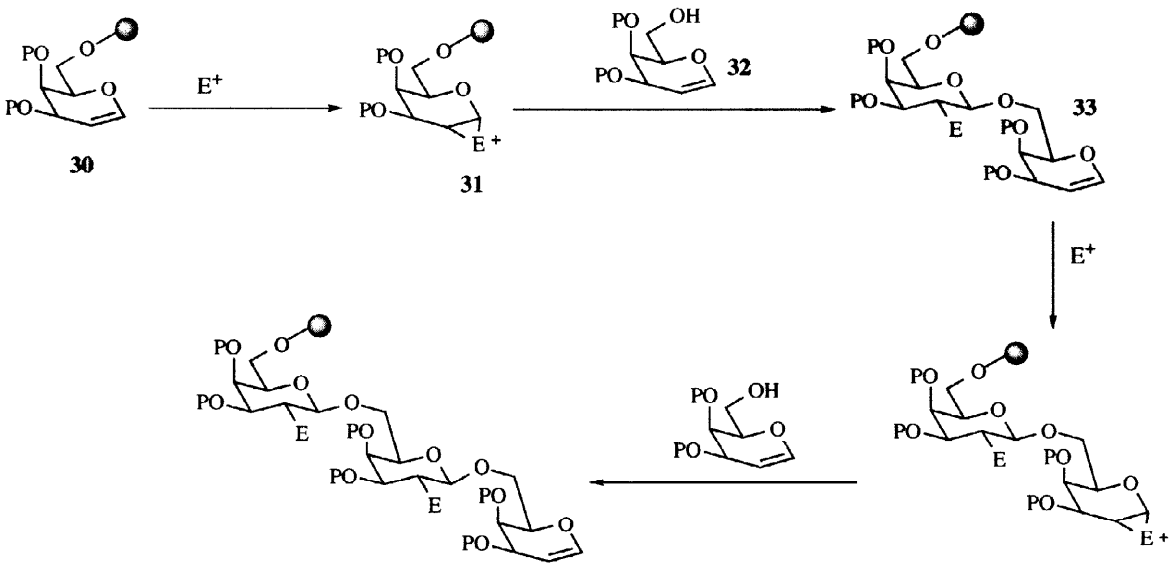
i) TMSCl, Et_3N, THF, 0 to 25°C, 50min.; ii) Polymer-CH_2-Cl, CsF, DMF, 60°C, 24 h.; iii) CF_3CO_2H, DCM; iv) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine, DCM, -78°C to -60°C; v) Ac_2O, pyr; vi) $Hg(OCOCF_3)_2$, DCM, H_2O, rt, 5h.

Scheme 8 : Glycosidation reaction employing anomeric sulfoxides as donors.

The value of glycosyl sulfoxides has also been exemplified in the synthesis of carbohydrate libraries (Scheme 29).[48]

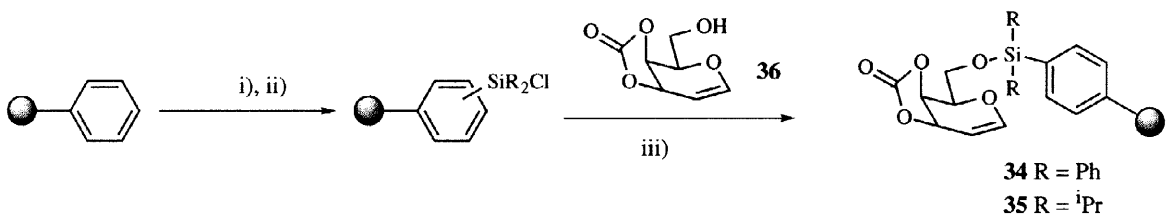
Glycals

Danishefsky has published extensively on the use of glycals for the solution phase synthesis of carbohydrates, and many biologically important oligosaccharides can now be synthesised using the glycal methodology.[49] Recent work has shown that glycal donors **30**, which are linked to a silyl based polymer support, for example *via* the most reactive C-6 hydroxyl group, can be activated with electrophiles. In an analogous fashion to the solution phase protocol,[50] the activated species **31** can be trapped by an acceptor **32** to afford a polymer bound oligosaccharide **33**. If a solution phase glycal acts as the acceptor the extended polymer bound glycal can be further exposed to an identical activation / trapping protocol such that an iterative process can be developed for the synthesis of extended oligosaccharides of both the linear and branched types (Scheme 8).



Scheme 8 : Application of polymer bound glycals

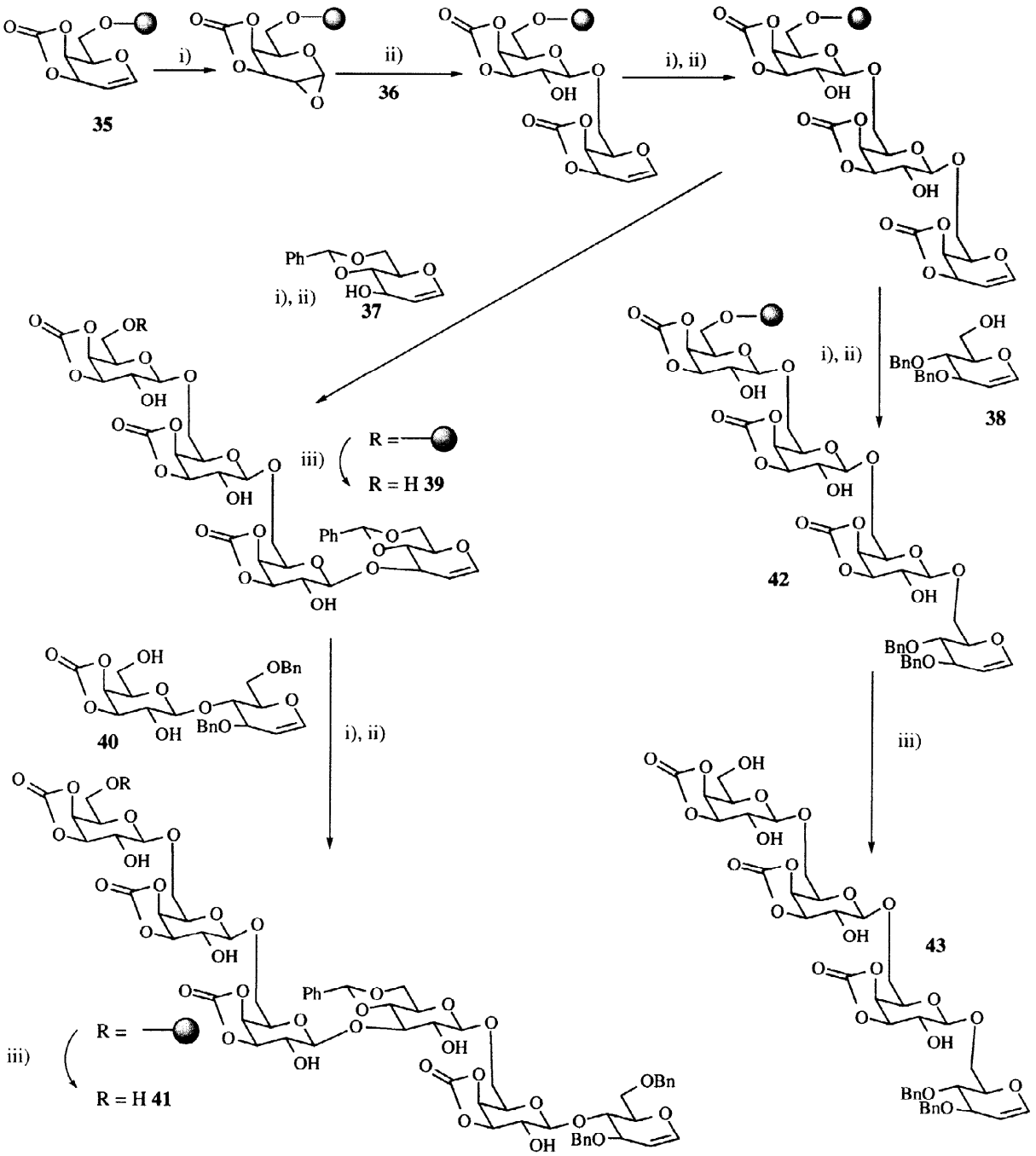
This glycal approach has proved particularly useful when a silicon based linker is employed to attach the glycal to a commercially available polystyrene support and dimethyldioxirane (DMDO) is used as the electrophilic species, allowing formation of epoxide intermediates. Initial attention focused upon a diphenylsilyl linker,[50] but more recent studies have found the diisopropylsilyl spacer to be superior.[51] The polymer-linker systems **34** and **35** are prepared by treating a 1 % divinylbenzenestyrene copolymer with butyl lithium, and quenching the anion thus formed with diphenyl- or diisopropyldichlorosilane. A glycal derivative, such as **36**, is added as a solution in dichloromethane, together with Hünig's base, to afford the dialkyl- or diarylsilyl linked glycal polymers **34** and **35** (Scheme 9). The diphenylsilyl linked polymer has a loading capacity in the order of 0.6 mmol of glycal / g of polymer, and the diisopropyl analogue displays loading of greater than 0.9 mmol of glycal / g of polymer.



i) BuLi, TMEDA, cyclohexane; ii) R_2SiCl_2, benzene; iii) $^iPr_2NEt$, DCM, DMAP

Scheme 9 : Synthesis of polymer bound glycal systems.

The polymer bound glycal system **35** has been employed for the synthesis of many biologically important oligosaccharides and is not limited to acceptors such as **36** and **38** with primary hydroxyl groups. It is equally effective with less reactive acceptors such as **37** with a secondary C-3 hydroxyl group, disaccharide acceptors such as **40**, and for the synthesis of branched chain compounds such as oligosaccharide **41**. The extent of this is highlighted in Scheme 10. Since any unreacted epoxide intermediate is destroyed by hydrolysis under the reaction conditions, no entities with deletions in the interior of the chain are detected.



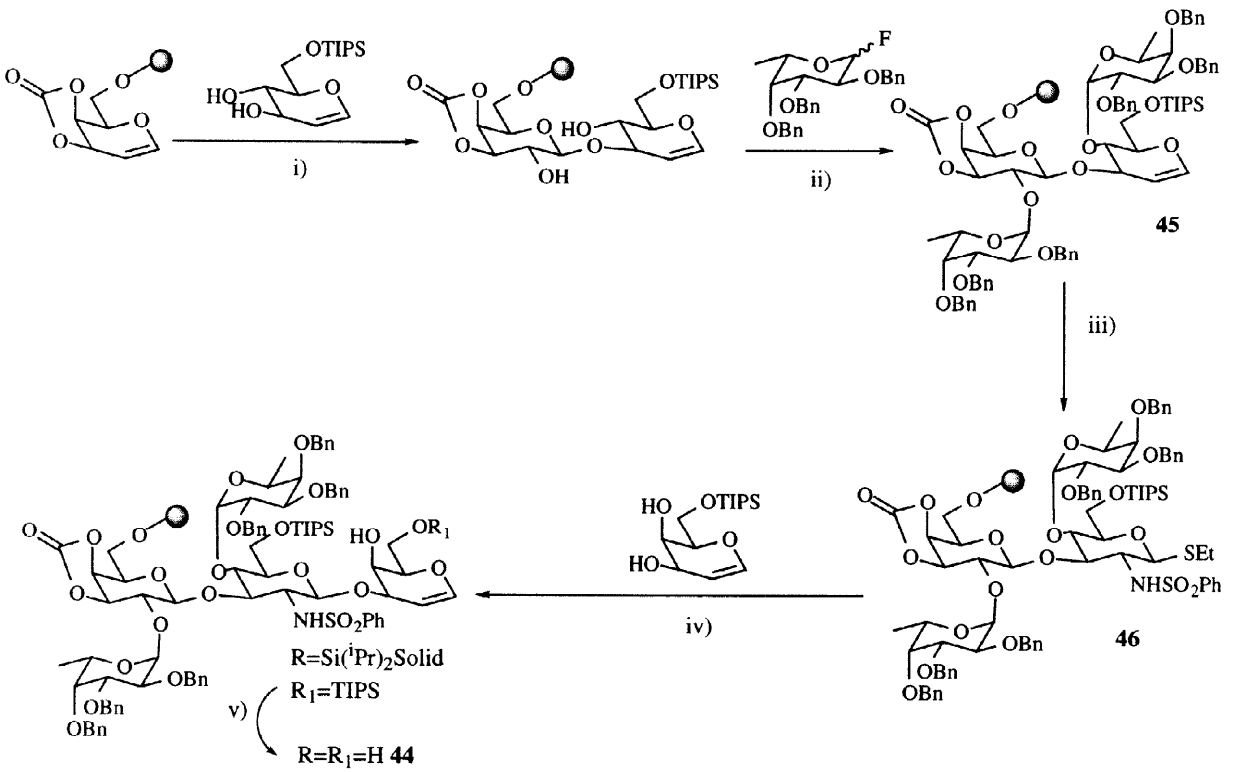
i) DMDO, DCM; ii) acceptor, $ZnCl_2$, THF; iii) TBAF, AcOH, THF

Scheme 10 : Further uses of glycals in polymer bound strategies.

Tetrasaccharide **43** was retrieved from the solid support, by treatment with buffered TBAF, in 32% overall yield from glycal **35**. This corresponds to an approximate yield of 70% per two step coupling sequence. Likewise, tetrasaccharide **39** and hexasaccharide **41** were formed in overall yields of 66% and 29% respectively from glycal **35**.

In recent years the syntheses of the A, B and H antigens as well as of the Lewis families have received considerable interest. As the solution phase protocols are generally laborious and time consuming, a need existed for rapid, automated alternatives. However, as is often encountered, a reaction that worked efficiently in the

solution phase was inefficient when applied to a polymer supported system and this initially limited the applicability of Danishefsky's glycal methodology for the synthesis of the Lewis^y glycal **44**. For example, a key sulfonamidoglycosylation strategy, effective in solution phase, was ineffective with polymer bound glycal **45**. Thus, initial attempts at synthesising **44** on a polymer support involved cleavage of **45** from the polymer and completion of the synthesis in the solution phase.[51] However, a refinement to the methodology,[52] which had already proved effective in the solution phase for reactions involving hindered or weakly reactive acceptors, was successfully applied to these polymer supported reactions. A two stage sulfonamidoglycosylation methodology, which involved conversion of polymer supported glycal **45** into an ethylsulfonyl 2-amidoglucosyl donor **46**, was successfully applied (Scheme 11).



i) a) DMDO, DCM; b) $ZnCl_2$, THF, 75%; ii) $Sn(OTf)_2$, DTBP, 4Å ms, THF / toluene (1 / 4), 65%; iii) a) $I(coll)_2ClO_4$, $PhSO_2NH_2$, DCM, 0°C; b) LHMDS, EtSH, DMF, -40 to 0°C, 64%; iv) MeOTf, DTBP, 4Å ms, DCM, 0 to 20°C, 8h, 71%; v) TBAF / AcOH, THF, 40°C, 18h.

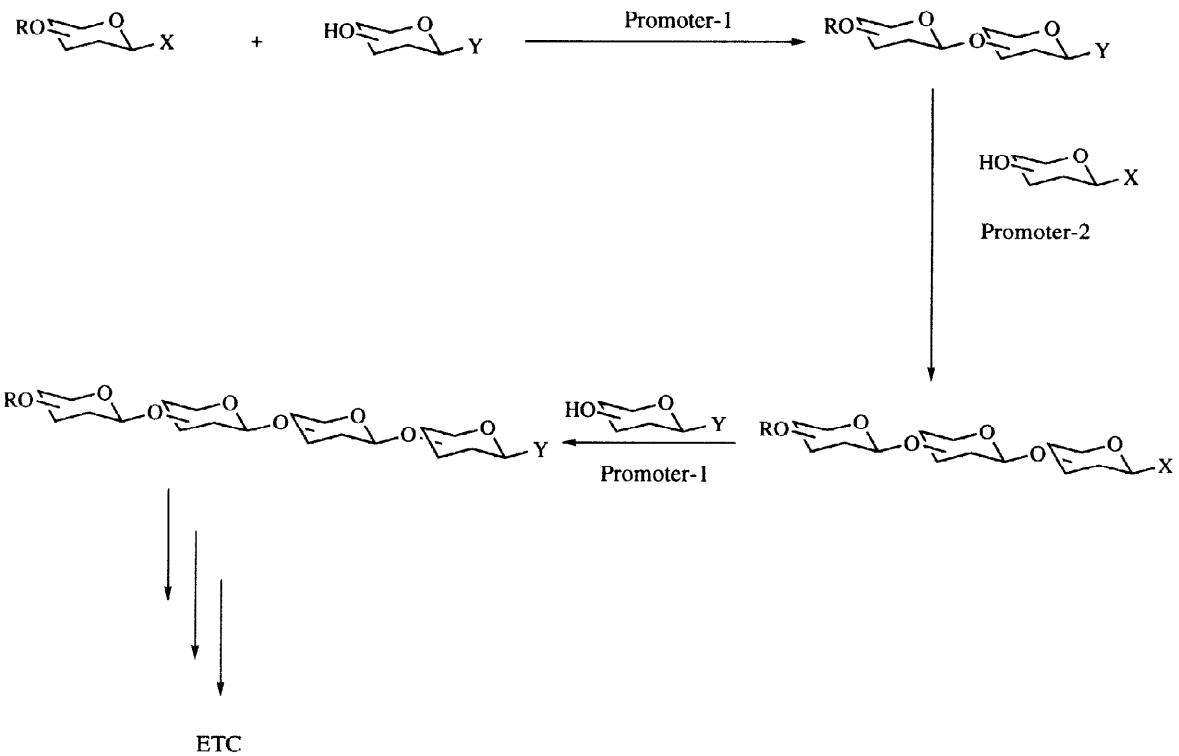
Scheme 11 : Further uses of glycals in polymer bound strategies

Danishefsky's glycal methodology has also made considerable impact in the polymer supported synthesis of glycopeptides (Scheme 27).[53]

4. TACTICS EMPLOYED IN POLYMER SUPPORTED OLIGOSACCHARIDE SYNTHESIS

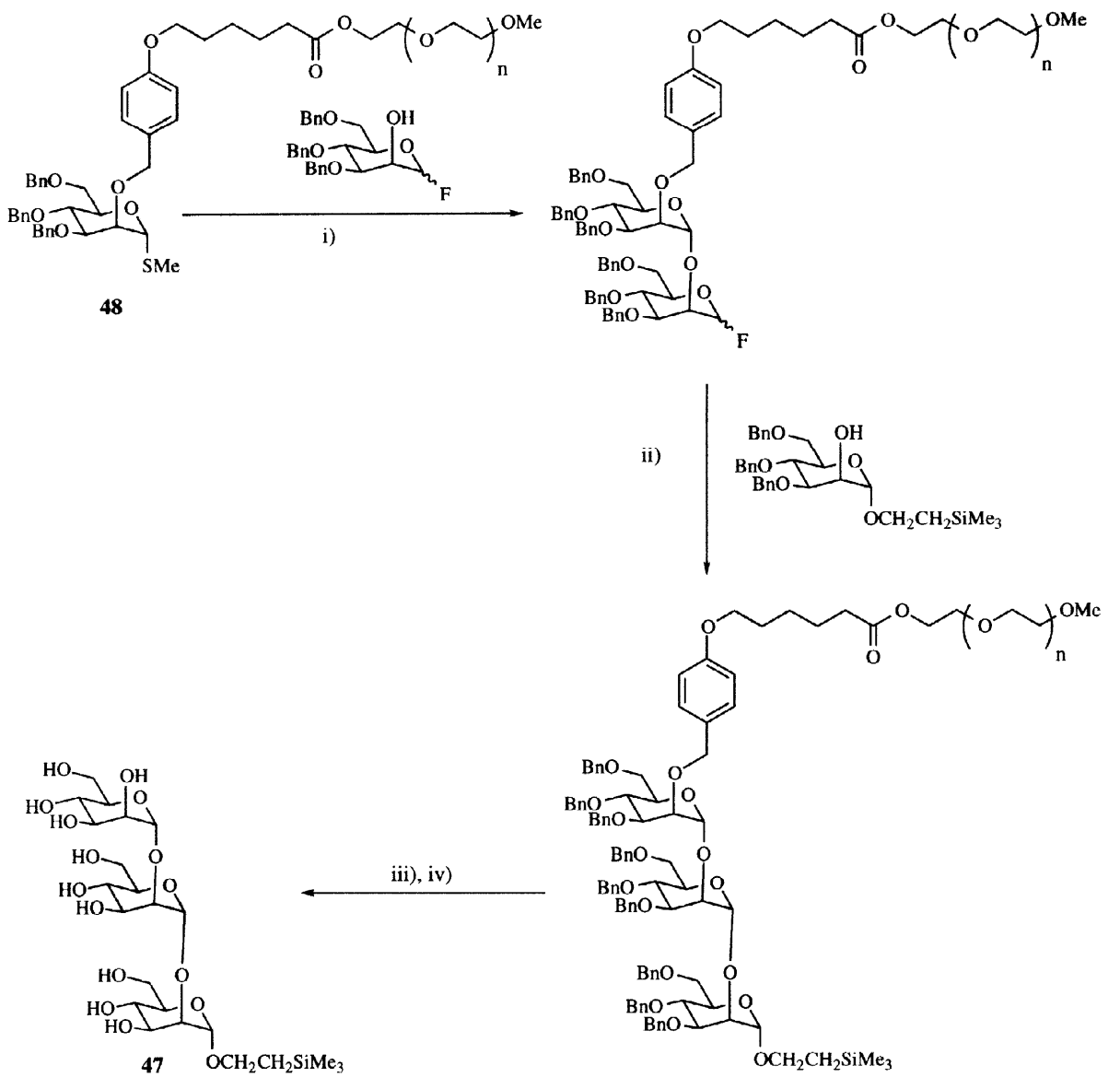
Orthogonal glycosidation

Oligosaccharide synthesis has been improved by the development of orthogonal glycosidation strategies and one-pot syntheses are consequently possible.[54] In such approaches, two donors displaying different anomeric groups (X and Y), which are activated by *different* promoters, are employed (Scheme 12).



Scheme 12 : Orthogonal glycosidation strategies.

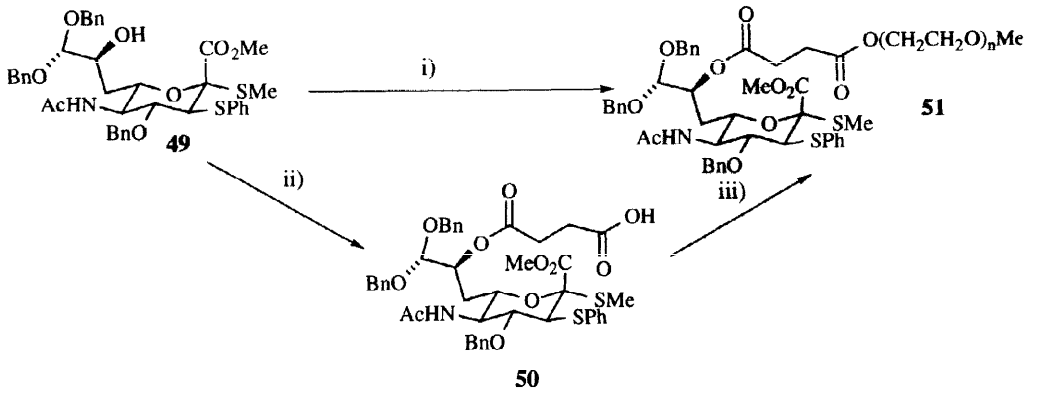
An iterative glycosylation protocol allows elongation of the glycan chain with alternate use of, for example, thioglycoside and glycosyl fluoride donors. This approach has been extended to polymer supported syntheses of, for example, an α-(1,2) linked mannotrioside **47**, in 40% overall yield from the polymer bound donor **48** (Scheme 13), and a tetrasaccharide corresponding to a partially protected form of the GPI anchor, in 42% from a polymer bound disaccharide.[55] In both cases PEG monomethyl ether (MPEG) was used as the polymer support since it provides a means of performing solution phase polymer supported synthesis, hence alleviating the problems of analysis and pseudo-high dilution often encountered with solid phase strategies.



i) $MeOSO_2CF_3$, MeSSMe, 4Å ms, DCM, rt, 89%; ii) Cp_2HfCl, $AgOSO_2CF_3$, 4Å ms, DCM, 0°C to rt, 99%; iii) NaOMe, MeOH; iv) H_2, $Pd(OH)_2$, MeOH-EtOAc, 40% overall yield

Scheme 13 : Orthogonal glycosidation strategies in polymer supported synthesis of a trimannan oligosaccharide.

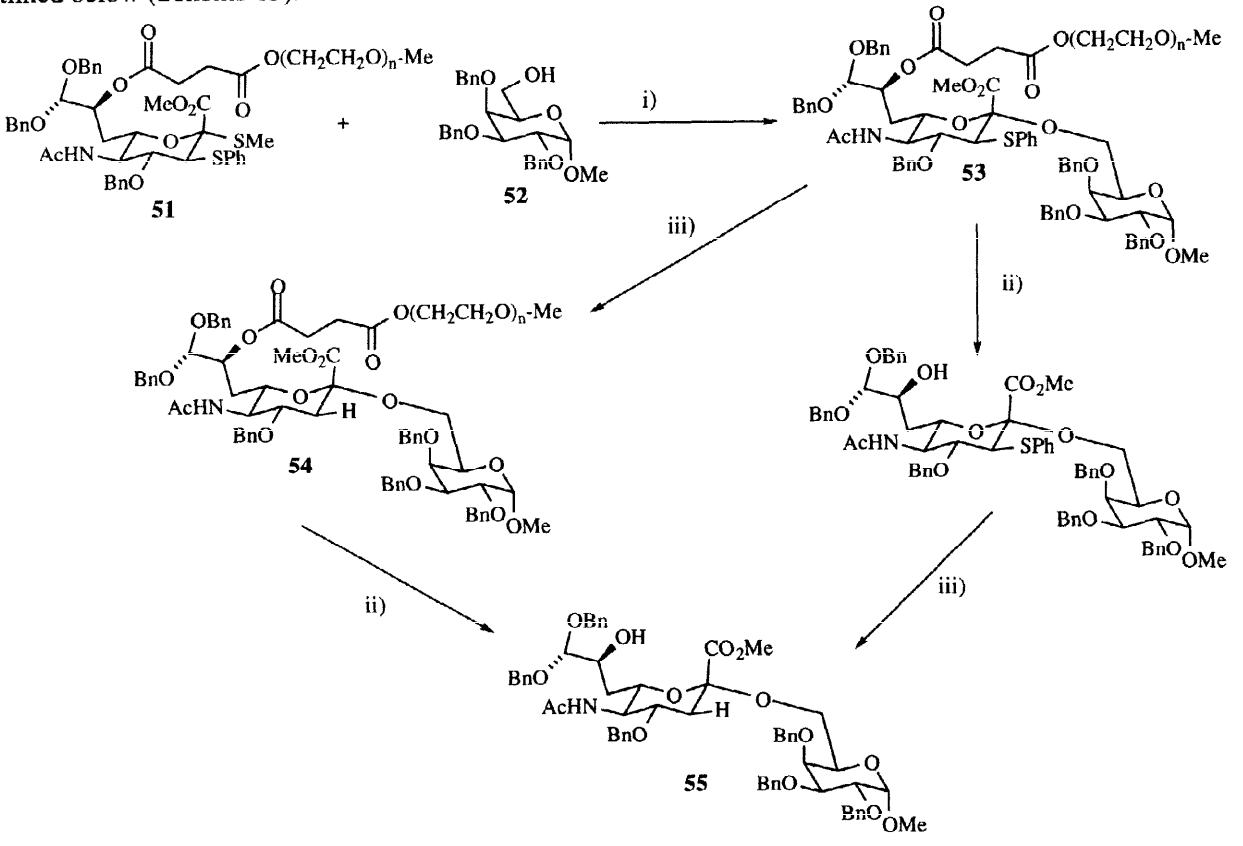
Recent results[56] have indicated that this strategy may also be potentially useful for the syntheses of oligosaccharides containing NeuAc at the non-reducing end. The initial attachment of a NeuAc thioglycoside to a polymer support allows subsequent glycosidation reactions to be performed with a range of solution phase acceptors. This can either be achieved *via* reaction of the donor **49** with succinylated PEG monomethyl ether (MPEGSu) or by a two step reaction involving formation of the 8-*O*-succinate **50**, which is subsequently coupled with MPEG. In both cases the coupling reaction is promoted by the action of 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) and polymer bound **51** of equal quality is obtained (Scheme 14).



i) MPEGSu, MSNT, *N*-methylimidazole, DCM, rt, 91h 50-55%; ii) succinic anhydride, pyr, DMAP, 75°C, 17h, 63%; iii) a) MPEG, MSNT, *N*-Methylimidazole, DCM, 28°C, 38h, 55-65%; b) Ac_2O, pyr, DCM, rt, 14h

Scheme 14 : Preparation of polymer bound NeuAc donor.

A chiral auxiliary was used in the initial polymer supported NeuAc glycosidation reaction,as a means of controlling the stereochemistry of the glycosidic bond.[57] Activation of the polymer-bound donor **51** was achieved using $MeSSMe_2.OTf$ (DMTST)[58] and the preliminary coupling results obtained with acceptor **52** are outlined below (Scheme 15).



i) DMTST, DCM, 4Å ms, -40 to 27°C, 22h; ii) 0.1M MeONa, MeOH, rt, 14h; iii) Ph_3SnH, AIBN, toluene, 80°C, 22h

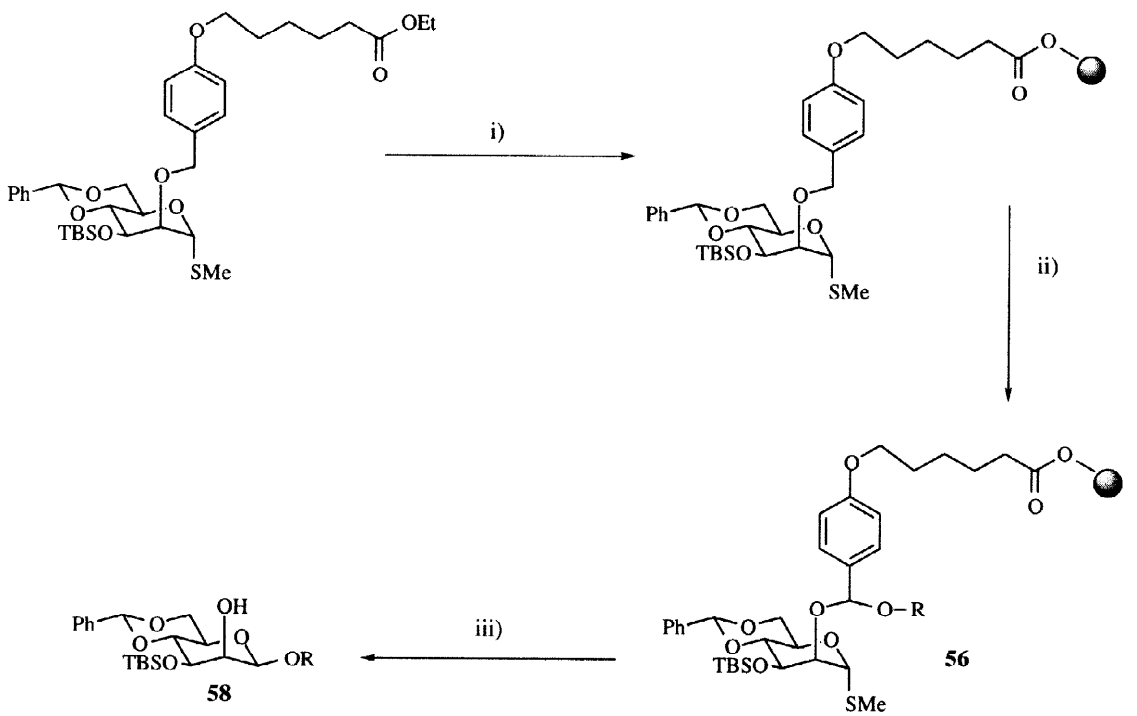
Scheme 15 : Glycosidation reaction of polymer bound NeuAc donor.

The thiophenyl group could be reductively cleaved either from **53** at the polymer-bound stage, to afford **54**, or after cleavage of the oligosaccharide from the polymer. The overall yield of synthesis of **55** was 65-70% with the α-anomer being the most favoured product. Indeed, less than 2% of the β-anomer was obtained. Less reactive acceptors also proved of use in this approach.

This recent report therefore indicates that polymer bound thiomethyl NeuAc is a suitable donor for glycosidation reactions, and hence may well afford oligosaccharides with NeuAc at their non-reducing end if incorporated within an orthogonal glycosylation strategy.

Intramolecular aglycon delivery

A recent report has highlighted an elegant method for the polymer supported synthesis of β-mannosidic linkages that utilises the linker for the stereoselective delivery of a carbohydrate acceptor.[59] Hence, entry to β-mannosidic linkages can be achieved following an extension of the powerful intramolecular aglycon delivery approach already developed for solution phase syntheses.[60] This approach is unusual in that, after completion of the reaction, the product itself is released into the non-polymeric phase, leaving the by-products bound to the polymer (Scheme 16). This is in contrast to most other solid phase strategies which leave impurities and by-products within the solution phase.



i) a) NaOH / ^{t}BuOH; b) PEG Monomethyl ether, EtO_2C-N=N-CO_2Et, Ph_3P, DCM-THF, 80%; ii) ROH **57**, DDQ, 4Å ms, DCM, rt, 3h; iii) MeOTf, DBMP, 4Å ms, $ClCH_2CH_2Cl$, See Table for conditions and yields.

Scheme 16 : Intramolecular aglycon delivery approach.

The MPEG bound mixed acetal **56** is precipitated from solution by addition of *tert*-butylmethyl ether (TBME) to allow its purification prior to initiation of the key glycosidation reaction using 2,6-di-*tert*-butyl-4-methylpyridine. A range of acceptors, ROH **57**, have been utilised in this approach, as highlighted in Table 4.

This approach is compatible with orthogonal glycosidation strategies, and, in all cases, affords only the required β-anomer of disaccharide **58**.

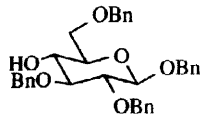
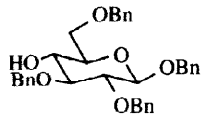
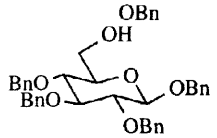
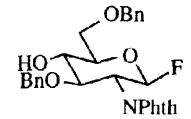
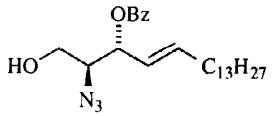
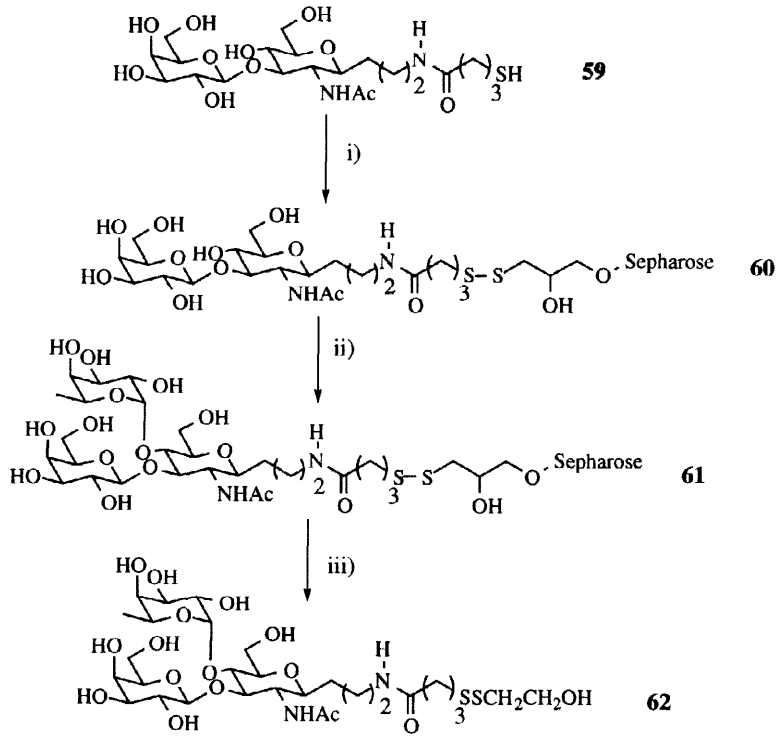
ROH, 57	Equivalents of ROH	T / time (°C / h)	Yield (%)
	2.6	40 / 21	50
	2.0	40 / 40	48
	1.9	20 / 8	43
	1.9	40 / 120	37
	2.3	40 / 22	54

Table 4 : Range of acceptors investigated.

5. POLYMER SUPPORTED OLIGOSACCHARIDE SYNTHESIS EMPLOYING ENZYMES

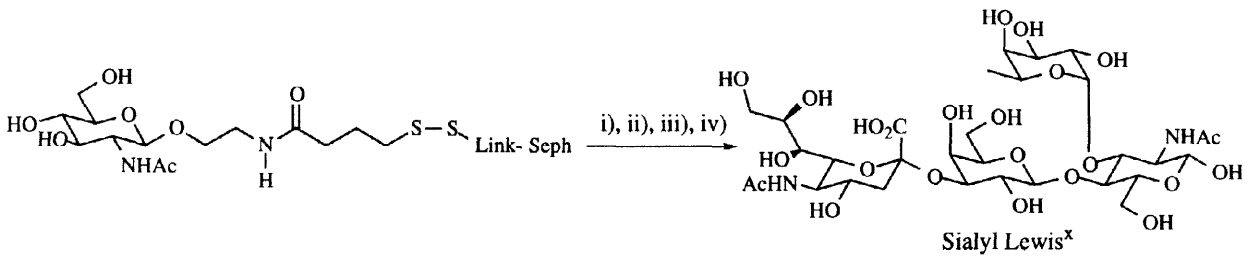
Many advantages offered by enzymes in the solution phase syntheses of oligosaccharides,[61] including their ability to initiate highly regio- and stereoselective reactions without employing protecting groups, have recently been successfully transferred to polymer supported strategies.[62] Water soluble glycopolymers have received increasing attention as high performance primers for the enzymatic synthesis of glycoconjugates, because of their high efficiency in glycosidation reactions. This can be attributed both to the polymeric sugar cluster effect[63] and to the simple procedures available for the purification of the products.[64] Recent investigations in these areas have allowed the syntheses of complex carbohydrates of biological importance, including Lewis^a and Sialyl Lewis^x.[65] For the former target, a disaccharide acceptor **59** is bound to Sepharose *via* a disulfide linkage to afford **60**, which is reacted with GDP-fucose, in the presence of fucosyltransferase, to afford the polymer bound Lewis^a trisaccharide **61**. This is released from the polymer by treatment with mercaptoethanol, affording the trisaccharide **62** in an overall yield of 68% (Scheme 17).



i) Activated thiopropyl-Sepharose; ii) Fucosyltransferase, GDP-Fucose; iii) 2-Mercaptoethanol, 68% overall yield.

Scheme 17 : Use of fucosyltransferase for the synthesis of Lewis[a].

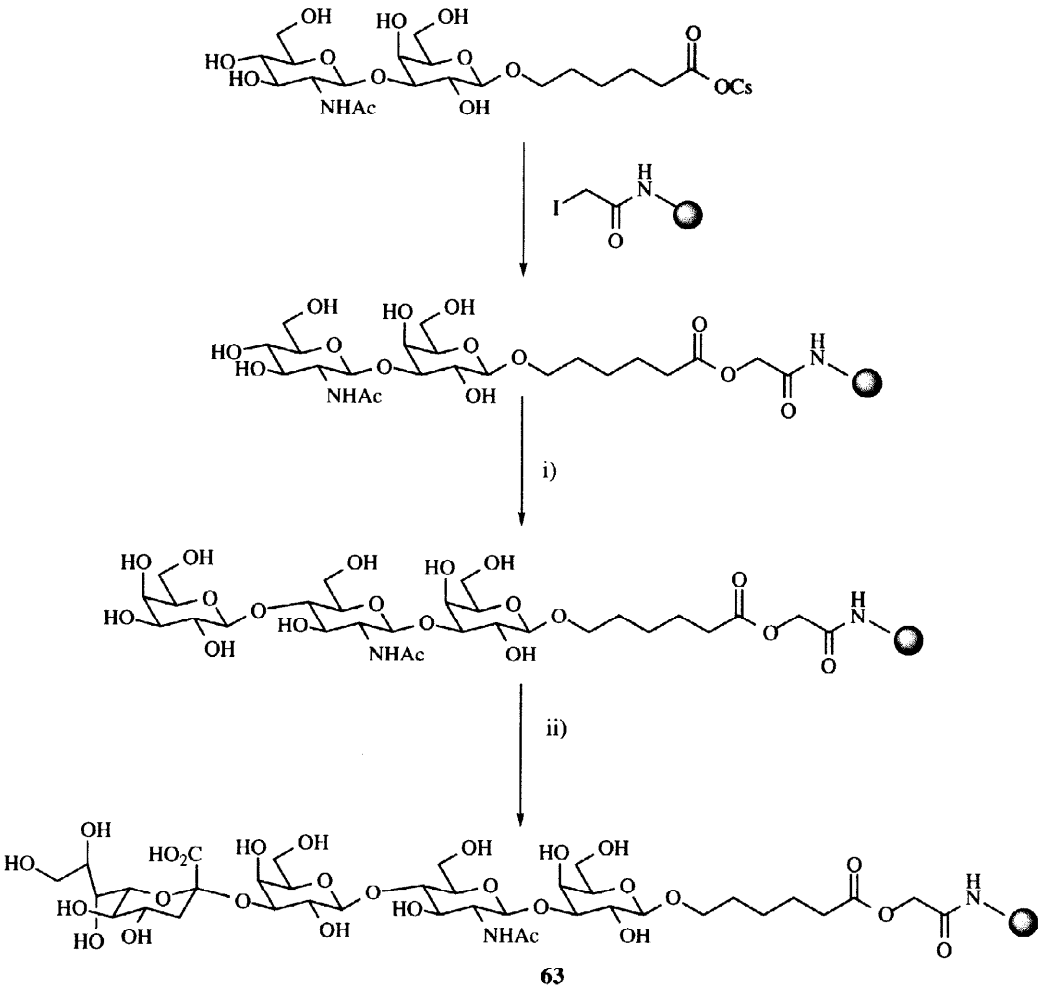
Further research revealed that the length of the linker had a profound effect on the yield of the reaction. Hence, when a linker of approximate length 15Å was employed, yields of only 70% were obtained for the enzyme mediated coupling reactions. Fucosyl transferase may ne of such a size (diameter of approximately 60 Å) that some sites on the gel are inaccessible, and therefore unreactive. However, when longer linkers of 47-71 atoms were incorporated (by the successive reaction of thiobutyrolactone and 2,2'-dithiopyridine) the yield of reactions with galactosyltransferase improved to 89-98%. This yield was deemed satisfactory, and this method was used in a polymer supported enzymatic synthesis of a complex oligosaccharide, Sialyl Lewis[x]. The strategy employed is highlighted in Scheme 18, which shows that the overall yield of the reaction was a remarkable 57%.



i) Galactosyl transferase, UDP-Gal; ii) Sialyl transferase, CMP-Neu5Ac; iii) fucosyl transferase, GDP-fucose; iv) a) DTT; b) P2Biogel, 57% overall

Scheme 18 : Enzymatic synthesis of Sialyl Lewis[x].

An alternative method for overcoming steric problems, which may hamper the ability of enzymes to act upon acceptors bound to solid supports, is to incorporate a monosaccharide residue between the linker and acceptor monosaccharide to serve as a hydrophobic spacer. Such an approach was utilised for the polymer supported synthesis of inhibitors designed to block the adhesin process mediated by E-selectin and *H. pylori*.[66] CPG was chosen as a suitable polymer support since it is amenable for use in both aqueous and organic solvents. A linker which could be cleaved under basic or neutral conditions was chosen rather than an acid-labile linker, deprotection conditions for which might initiate fragmentation of glycosidic bonds (Scheme 19).[17]



63

i) UDP-Gal, galactosyl transferase; ii) CMP-NeuAc, α-(2,3)-Sialyl transferase.

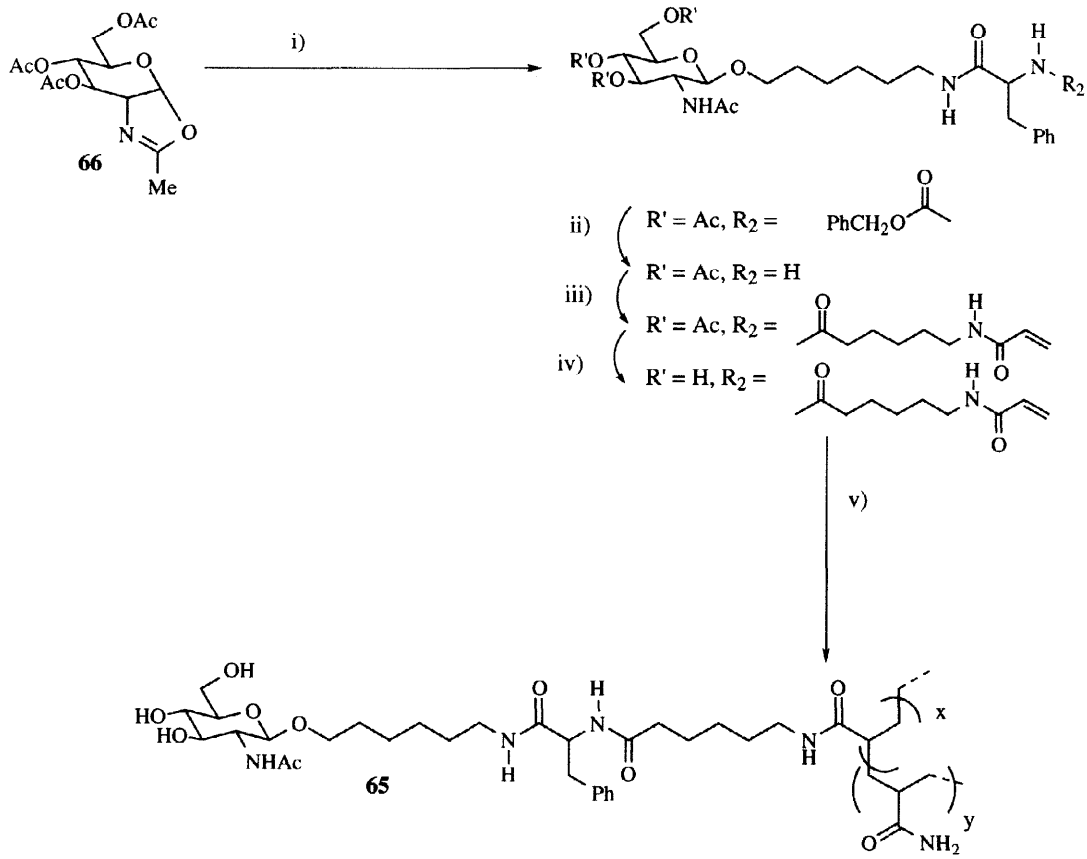
Scheme 19 : Synthesis of adhesin inhibitors.

The synthesis mirrors its counterpart in the solution phase.[67] Cleavage of the tetrasaccharide **63** from the polymer was achieved by treatment with hydrazine.

Many *soluble* polymer supported liquid-phase oligosaccharide syntheses have been performed using enzymes. For example, lactose derivatives have been obtained in average yields of 27% by the action of galactosyltransferase on saccharide units attached to polyvinylalcohol by photolabile nitrobenzyl linkages.[68] Linkage of saccharides to polyacrylamide poly(*N*-acryloxysuccinimide) copolymer by similar nitrobenzyl groups has also produced suitable substrates for galactosyl transferase, allowing synthesis of lactose derivatives and

subsequent incorporation of sphingosine to produce glycosphingolipids. Photolytic removal of the oligosaccharide from the polymer and purification afforded lactosyl sphingosine in 54% yield.[69]

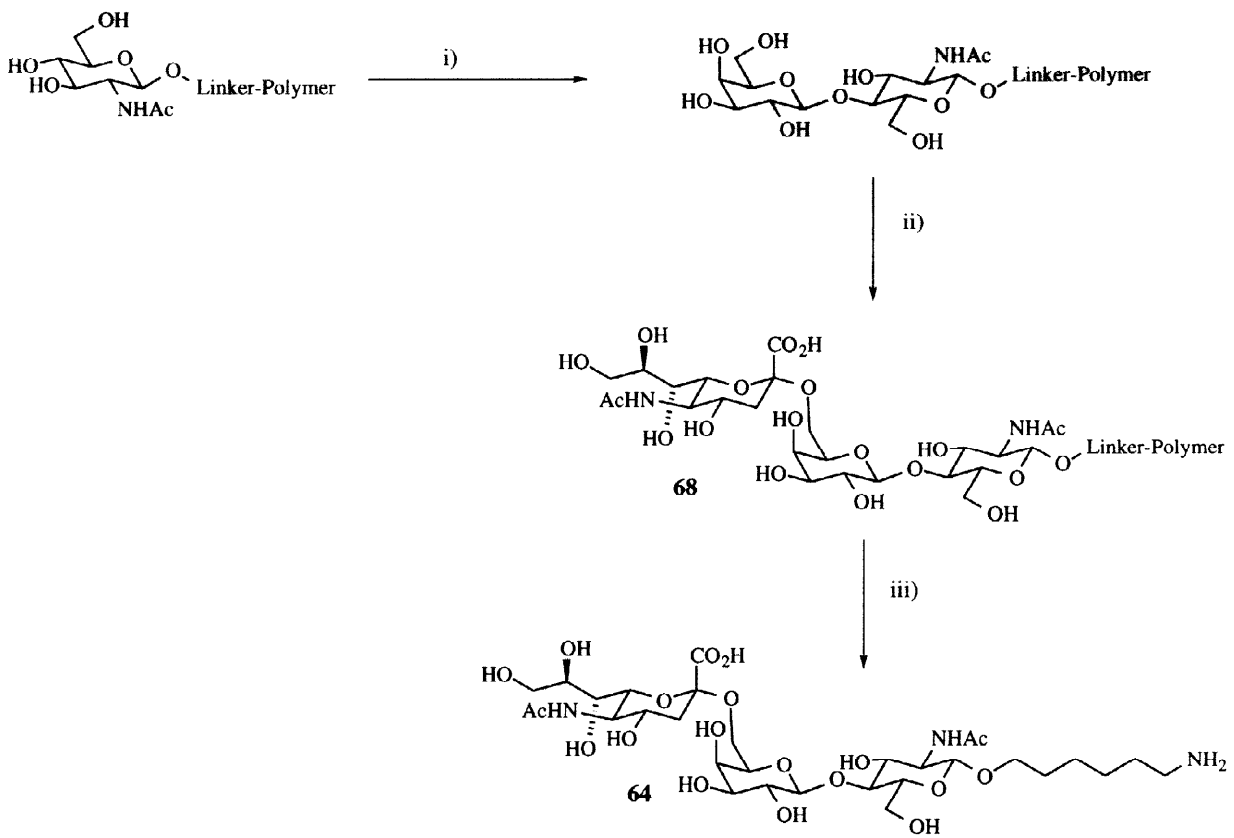
In an interesting extension ofthis approach, a novel support has been developed which allows attachment of GlcNAc, for subsequent enzymatic elaboration, to a polymer, through an α-chymotrypsin-sensitive spacer.[26] This has allowed the facile preparation of a 6-aminohexylglycoside of sialooligosaccharide **64** as outlined in Scheme 21 below. Synthesis of the required polymer linked GlcNAc residue **65** commences with the reaction of oxazoline **66** with the alcohol **67** (Scheme 20).



i) Z-Phe-NH-$(CH_2)_6$-OH **67** (2 eq), CSA, DCM, 70°C, 30min, 36%; ii) H_2, Pd / C, MeOH, 50°C, quant.; iii) HC=C(O)NH$(CH_2)_5CO_2H$ (1.1 eq), EtOH, PhH, 77%; iv) NaOMe, MeOH, THF, quant.; v) a) HC=C(O)NH_2 (4 eq), TMEDA (0.4 eq), APS (0.16 eq), DMSO-H_2O, 50°C; b) gel filtration on Sephadex G-25 with 0.05M CH_3COONH_4 (aq), 93%

Scheme 20 : Formation of polymer bound GlcNAc.

N-Deprotection of the incorporated phenyl alanine moeity and condensation of the amine thus formed with 6-acrylamidocaproic acid afforded a polymerizable GlcNAc derivative. De-*O*-acetylation and copolymerisation of the sugar monomer in the presence of ammonium persulfate (APS) and *N,N,N'N'*-tetramethylethylenediamine (TMEDA) as promoters then affords the primer polymer **65** displaying glucosamine units suitable for subsequent enzymatic elaboration. In this report, galactosyl and sialyl transferases were used to afford the polymer-bound trisaccharide **68**. This was readily removed from the polymer support using α-chymotrypsin (Scheme 21).



i) a) Gal T (1.0 unit), UDP-Gal (1.3 eq), α-lactalbumin, HEPES buffer (pH 6.0), 37°C, 24h; b) gel filtration on Sephadex G-25 with 0.05M CH_3COONH_4 (aq); ii) a) α (2-6)-SialylT (0.1 unit), CMP-Neu5Ac (1.5 eq), phosphatase alkaline (20 units), bovine serum albumin (2 mg), NaN_3 (14.38μM), $MnCl_2$ (1.58μM), sodium cacodylate buffer (pH 7.4), 37°C, 45h; b) gel filtration on Sephadex G-25 with 0.05M CH_3COONH_4 (aq); iii) a) α-chymtrypsin (1.0mg), Tris-HCl buffer (pH 7.8), 40°C, 24h; b) gel filtration on Sephadex G-25 with 0.05M CH_3COONH_4 (aq), 72% overall yield.

Scheme 21 : Enzymatic synthesis of a 6-aminohexylglycoside of sialooligosaccharide.

The range of enzymes available for use in enzymatic syntheses of oligosaccharides is constantly increasing. Hence, it is to be expected that the number of polymer supported strategies employing enzymes will also increase over the next few years.

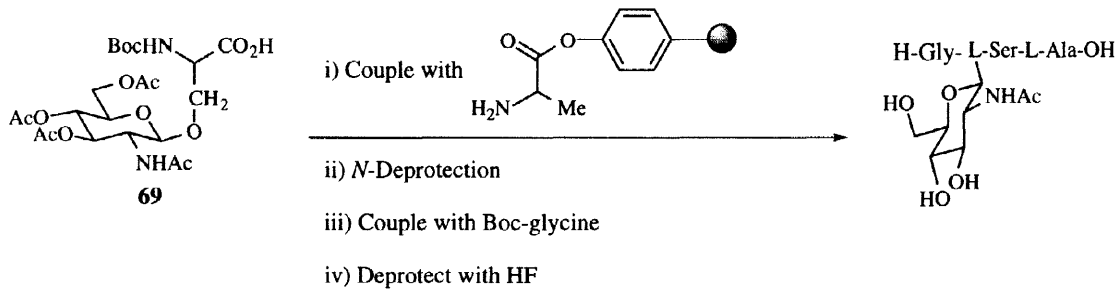
6. POLYMER SUPPORTED SYNTHESIS OF GLYCOPEPTIDES

Since the polymer supported synthesis of peptides is a well established process,[70] the majority of progress in the polymer supported synthesis of glycopeptides has involved the linkage of the glycopeptide to the polymer support *via* the peptide moiety rather than *via* the carbohydrate moiety. Many strategies utilise the phthalimide (Phth) group for temporary protection of the C-2 amino functionality of the oligosaccharide but problems are often encountered if the Phth group is removed whilst the oligosaccharide is still bound to the solid phase. An *N*-dithiasuccinoyl (Dts) group has been proposed as a useful alternative to the Phth group and this is removed readily and quantitatively under mild conditions by thiolysis or other reductive methods.[71]

Two classes of glycopeptides exist, namely the *O*-linked glycopeptides and the *N*-linked glycopeptides.

O-Linked Glycopeptides

The first reported method for the synthesis of *O*-linked glycopeptides[72] involved attachment of Merrifield resin to alanine which was subsequently coupled with an *N*-(*tert*-butyloxycarbonyl)-*O*-glycosyl serine derivative **69**. Subsequent removal of the *N*-Boc group on serine with TFA allowed formation of a new amine ready for subsequent iterative coupling to the required amino acids using the solid phase technique (Scheme 22).



Scheme 22 : General strategy for formation of *O*-linked glycopeptides.

The glycosylated somatostatin derivative **70** (Figure 5) was also synthesised in a similar strategy commencing with resin-linked cysteine.[73]

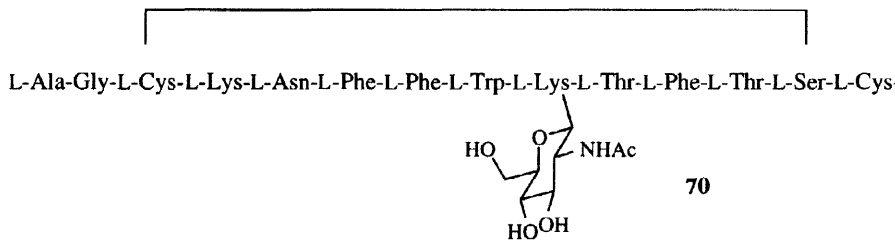
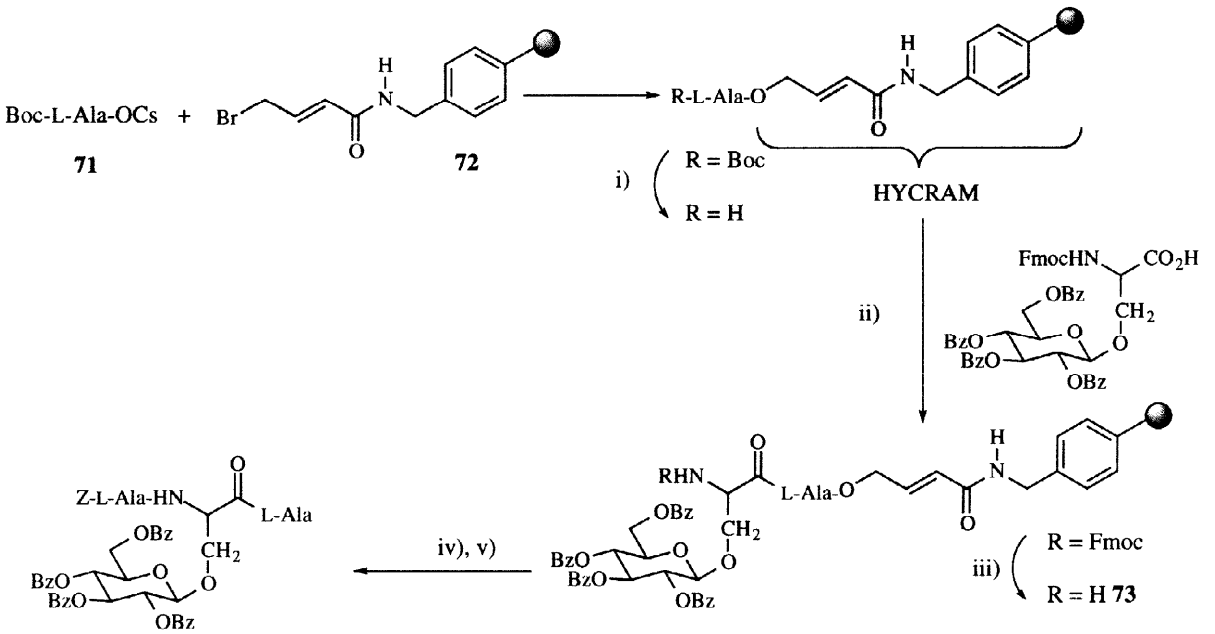


Figure 5 : Glycosylated somatostatin derivative.

A new HYCRAM™ linker compatible with the reaction conditions necessary to effect *N*- and *O*-deprotection of amino acids has been introduced for improved polymer supported syntheses of glycopeptides.[29] Amino acids are easily attached to the polymer by reaction of the caesium salt of the *N*-protected amino acid, for example **71**, with 2-(4-bromo-2-butenamido)methyl-polystyrene (HYCRAM™) 72 (Scheme 23).

The glycotripeptide **73** is readily removed from the resin using palladium(0)-catalysed allyl ester cleavage.[28] Under the essentially neutral conditions employed, the other protecting groups, as well as the *O*-glycosidic bond, remained intact. A similar approach has been used for the syntheses of a glycosylated HIV peptide T-derivative.[74]



i) TFA; ii)DCC, HOBt; iii) Morpholine; iv) Z-L-Ala-OH, DCC, HOBt; v) $[Ph_3P]_4Pd(0)$, morpholine.

Scheme 23 : Synthesis of a glycotripeptide using the HYCRAM linker.

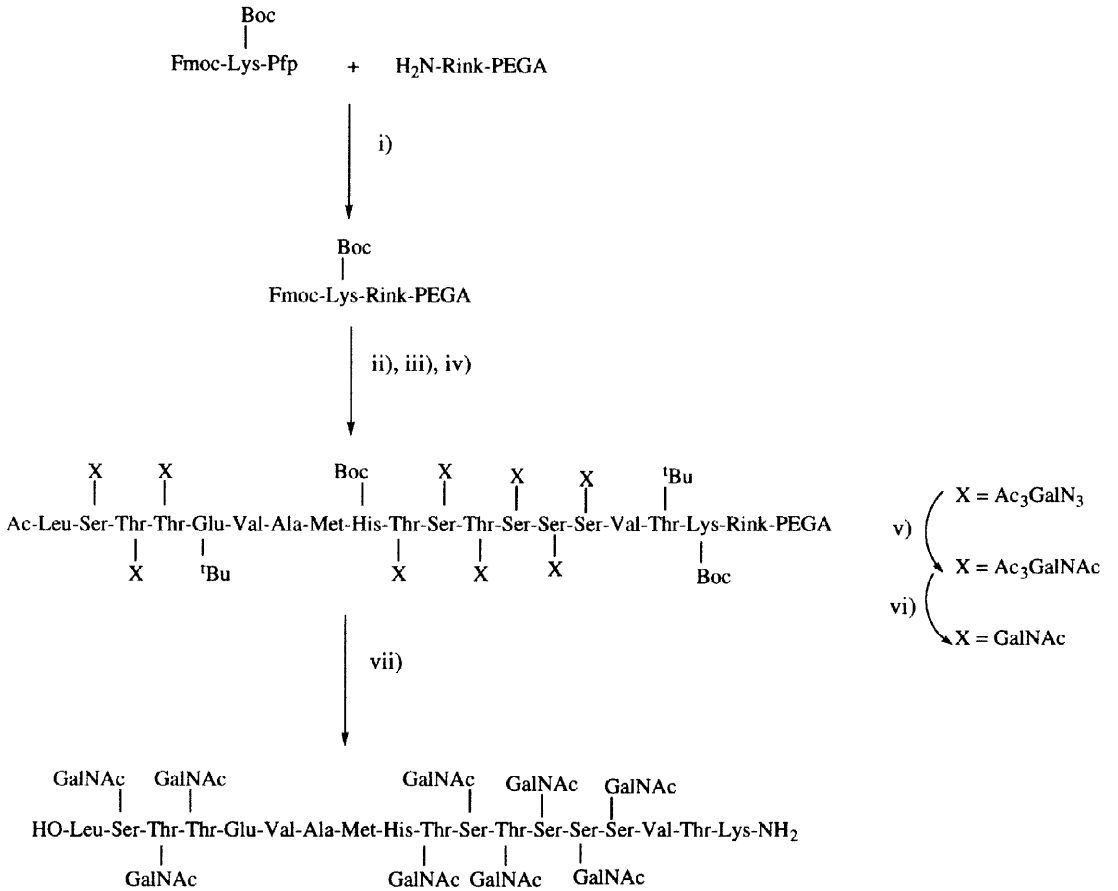
Alternative resins reported for peptide synthesis include one displaying acid-sensitive alkoxylbenzyl ester anchoring groups, and it is used in conjunction with Fmoc amino acids,[75] as well as a commercial SASRIN™ resin having a dialkoxybenzyl anchoring group.[76] In both cases, cleavage of the glycopeptide from the resin is achieved by treatment with TFA, although the latter requires milder conditions than the former. A series of dimeric through to octameric (1-5) amide linked sialooligomers have also been prepared on the Rink resin with Fmoc protected amino acids.[77]

O-Glycopeptides containing α-glycosidically linked 2-azido-2-deoxy-D-galactose residues at any desired site are easily prepared from the initial solution phase coupling of galactosyl chlorides with pentafluorophenyl esters of amino acids. The pentafluorophenyl activated esters are then used directly for incorporation of solid-phase polymers such as polydimethylacrylamide.[78] Subsequent elaboration of the peptide moiety then proceeds as above.

O-Glycopeptides bound to a solid phase support can be further glycosylated using standard conditions. The glycosyl acceptors are normally prepared using established methods in solid-phase glycopeptide synthesis and allow entry to branched or linear glycopeptides. In particular, a highly glycosylated glycopeptide mucin core structures located on cancer cells,[79] a glycosylated hexapeptide of human sialophorin located on leucocytes and platelets which plays a critical role in independent T-cell adhesion,[80] and a glycopeptide from human glycophorin A^N with T^n-antigenic structures[81] have been synthesised using this technique.

Orthogonal protecting groups are of optimum use in the above strategies to allow easy elongation of the peptide or oligosaccharide on the solid support. The effect of bulky saccharides on the efficiency of peptide synthesis has been investigated using PEGA resin (polyethyleneglycol poly-*N,N*-dimethyl acrylamide copolymer resin) as the polymer support (Scheme 24).[81] Interestingly, the non-glycosylated peptide required longer reaction times for amino acid coupling reactions than the glycosylated analogue. Azido protecting groups were

used at the C-2 position of galactose and nine of these were simultaneously converted to the required acetamido groups on the solid support.

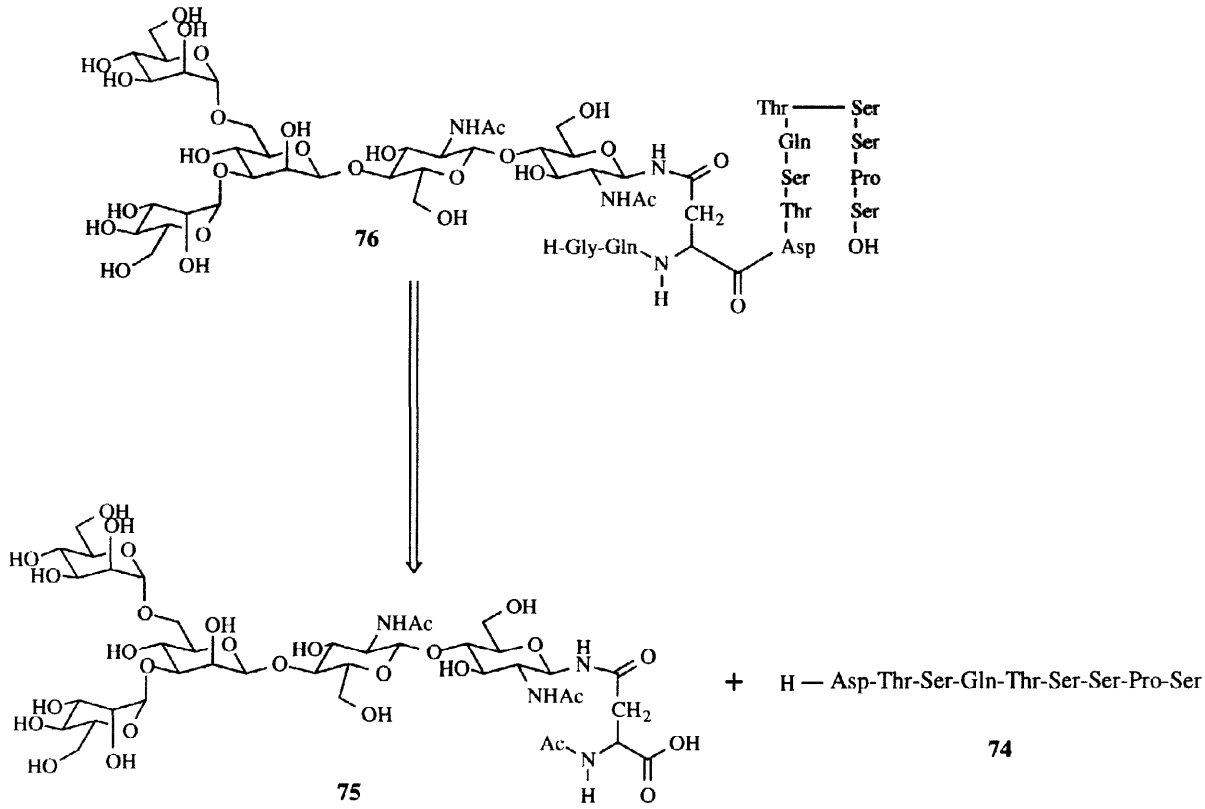


i) DhbtOH-DM; ii) 20% piperidine-DMF; iii) coupling with N^{α}-Fmoc Amino Acid-Pfp ester, N^{α}-Fmoc-Ser (Ac_3GalN_3)-Pfp ester or N^{α}-Fmoc-Thr (Ac_3GalN_3)-Pfp ester; iv) repeat steps ii) and iii); v) Thioacetic acid; vi) hydrazine-MeOH; vii) 19 : 1 TFA : H_2O

Scheme 24 : Effect of bulky saccharides on the efficiency of polymer bound peptide synthesis.

N-*Linked Glycopeptides*

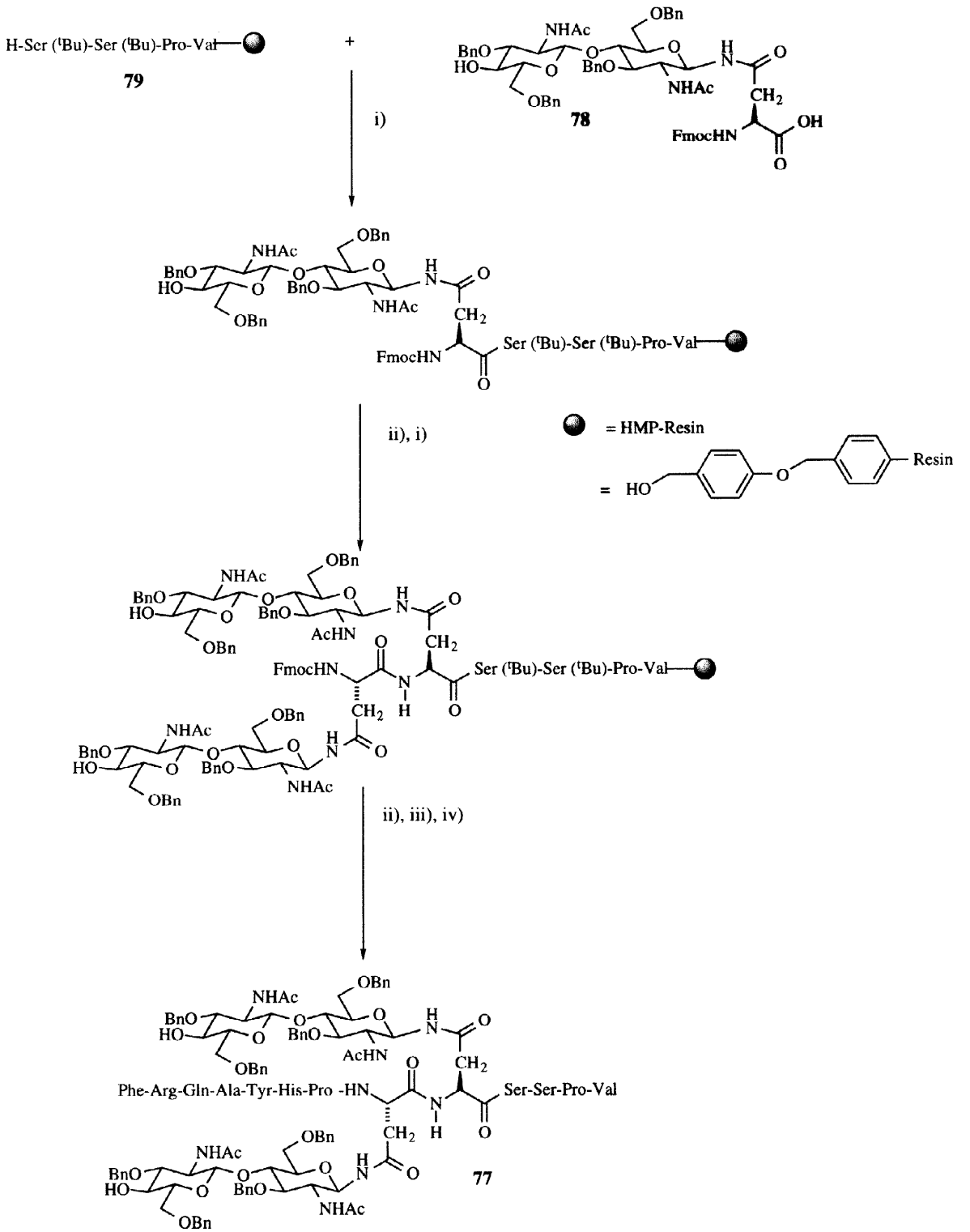
A range of *N*-linked glycopeptides have been synthesised on a polymer support, including the CD 52 glycopeptide.[82] This GPI-anchored glycoprotein is expressed on virtually all human lymphocytes and monoclonal antibodies raised against the antigen have proved useful both *in vivo* and *in vitro* for the prevention of bone marrow transplant rejection. The synthetic strategy involved the automated synthesis of the Asp^4-Ser^{12} peptide **74** on an HMP resin using the traditional Fmoc method. Manual coupling to the Asn pentasaccharide **75**, synthesised using solution techniques, was achieved using HOBT and DCC in 98% yield (Scheme 25). The glycopeptide was then reapplied to the automatic synthesiser and elongated to afford the target glycopeptide in 94%. Pleasingly, no steric problems were encountered and the glycopeptide was released by treatment with 94% TFA with 2.5% 1,2-dimercaptoethane. These conditions also removed the side chain amino acid protecting groups and the overall process resulted in an 81% yield of glycopeptide **76**. Less than 2% of product with degraded sugar moieties was detected .



Scheme 25 : Synthesis of CD 52 glycopeptide.

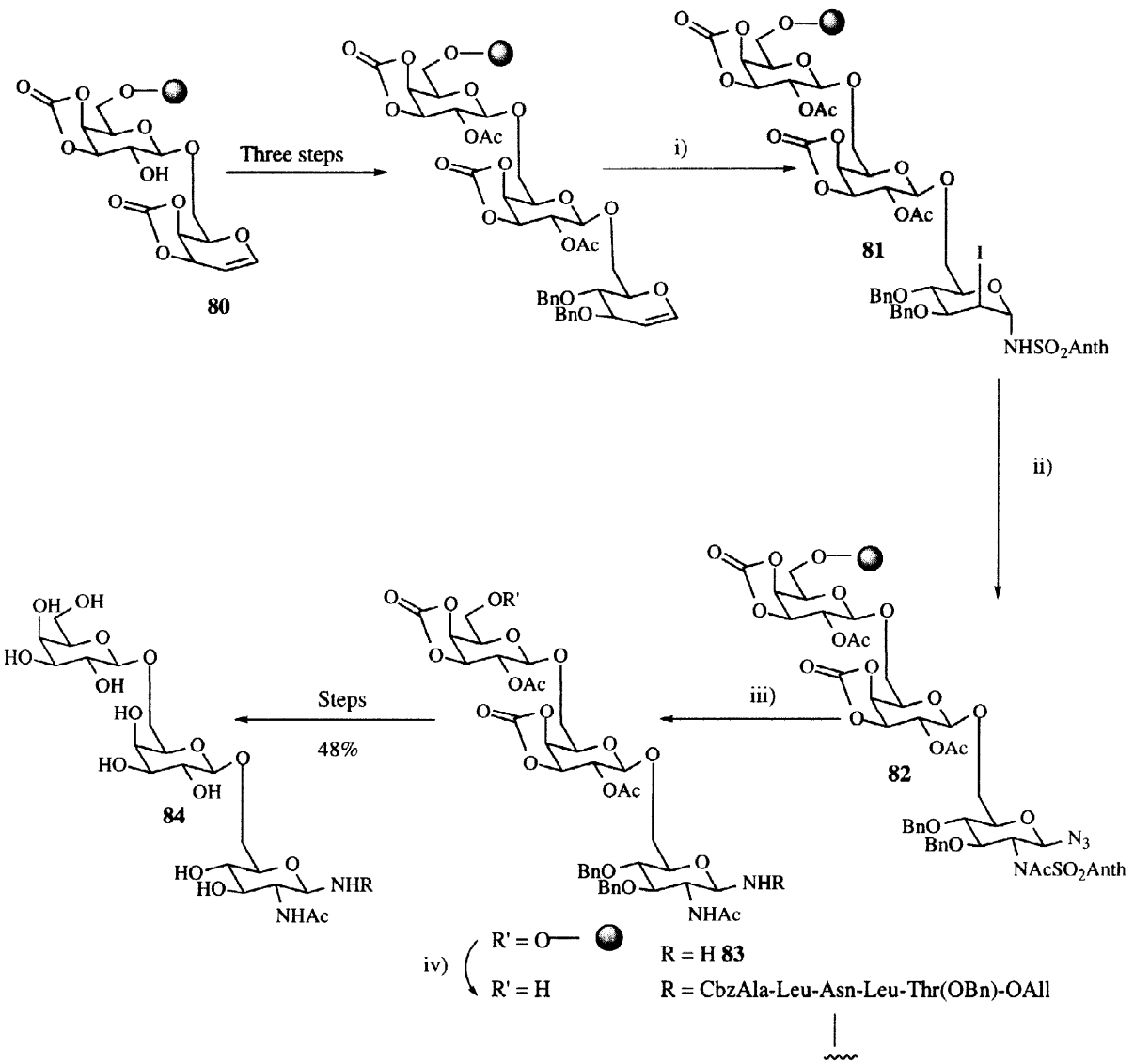
An *N*-linked glycosylated peptide fragment of the IL-8 receptor, containing two vicinal oligosaccharide chains **77**, has also been prepared as outlined in Scheme 26.[83] The disaccharide **78** was prepared using solution techniques.

When considering glycopeptide synthesis, the carbohydrate portion can either be prepared synthetically[56] or can be released in a suitable form from natural glycoproteins using routine hydrazinolysis techniques.[84] For example, a series of *N*-linked oligosaccharides have been released intact in their unreduced form from fetulin and ribonuclease B using hydrazinolysis. The respective glycosylamines are then formed and coupled to the side chain of activated aspartic acid derivatives, thus allowing their incorporation into, for example, a multiple-column peptide synthesis of glycopeptide T-cell epitope analogues. One strategy that involves the construction of the oligosaccharide on a polymer support was reported by Danishefsky, and utilises the glycal methodology previously described (*vide supra*).[53] Both linear and branched oligosaccharides can be constructed in this manner and are subsequently modified with anthracenesulfonamide in an azasulfonamidation sequence of reactions to afford, for example, polymer bound trisaccharide **81** (Scheme 27). Conversion of **81** to **82** and subsequent reduction affords the anomeric amine **83**, which is coupled to a peptide prepared using traditional solution or solid phase techniques. Removal from the polymer support with HF-pyridine provides the glycopeptide **84** in 37% overall yield. This constitutes an average yield of approximately 90% per step over the ten steps from the polymer bound glycal **80**.



i) HOBt, DCC; ii) 20% piperidine / NMP, 97% over 3 steps; iii) peptide coupling-repeat steps ii) and iii); iv) TFA, 1,2-dimercaptoethane, water, thioanisole, phenol, 40:1:2:2:3, rt, 1h, 19%

Scheme 26 : Synthesis of a glycosylated fragment of the IL-8 receptor.



i) 9-Anthracenesulfonamide, $I(coll)_2ClO_4$; ii) $^nBu_4NN_3$; iii) $HS(CH_2)_3SH$, $^iPr_2NEt$; iv) Peptide, HDQ

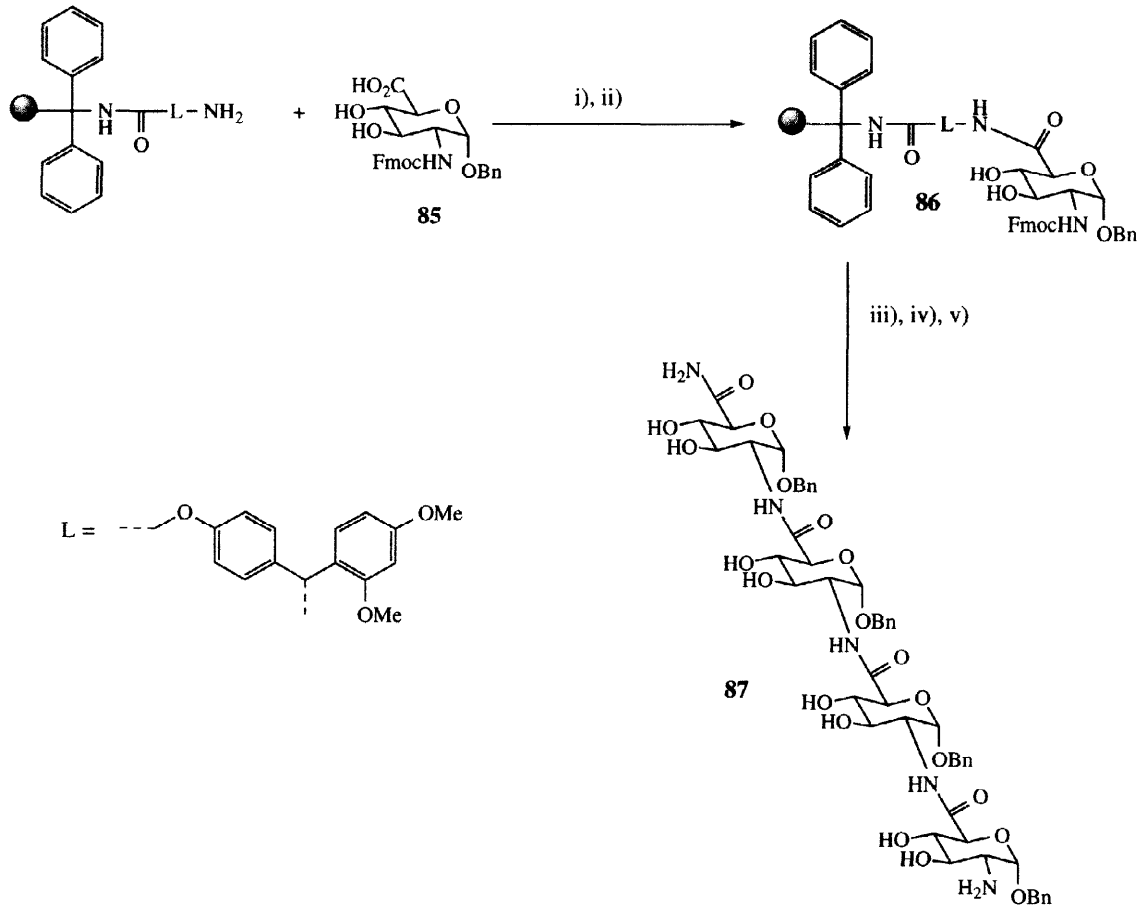
Scheme 27 : Polymer supported synthesis of saccharide moiety.

Furthermore, since the glycopeptide contains orthogonal protecting groups on the *C*- and *N*- termini of the peptide, the peptide chain itself can be extended with the glycopeptide bound to the polymer.

Glycomimetics

Carbohydrate amino acids have recently been used for the preparation, in solution, of oligosaccharide mimetics.[85] The assembly of carbohydrate amino acids has also been achieved using polymer supported peptide assembly methods. A benzhydrylamine polystyrene resin, functionalised with an Fmoc amide linker based on Rink's resin, has been employed.[86] An Fmoc-protected carbohydrate amino acid, glucosaminouronic acid **85**, was then attached to the resin and linker unit, using *in situ* activation of the carboxylic acid group with *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TATU) to afford **86**.[87] Interestingly, subsequent couplings were performed without prior protection of the C-3 and C-4 hydroxyl groups of the uronic

acid building blocks. The tetrasaccharide product **87** was released from the solid support under acidic conditions (Scheme 28).



i) Fmoc-amide linker - benzhydrylamine polystyrene, TATU, DIPEA, DMF, rt, 80min; ii) 20% Piperidine, DMF, rt, 14min; iii) **85**, TATU, DIPEA, rt, 45min per cycle; iv) 20% piperidine in DMF, rt, 14min; repeat steps iii) and iv) 3 times; v) 50% TFA, DCM, rt, 2 x 2h, 70%.

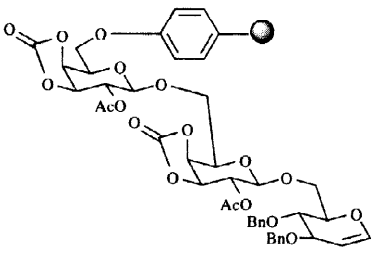
Scheme 28 : Polymer bound synthesis of glycomimetics.

As highlighted above, few methods exist for the synthesis of the oligosaccharide moiety of glycopeptides on polymer supports. Hence, it is to be expected that further advances in polymer supported syntheses of glycopeptides will probably focus upon the development of such procedures.

7. ANALYTICAL TECHNIQUES

A major limitation in polymer supported oligosaccharide synthesis is the difficulty in characterising reaction products or intermediates bound to the polymer. Normally, cleavage of the product from a small portion of the polymer is required and the resulting product is analysed using classical techniques. A non-destructive analytical method would offer many advantages and, in this context, the development of new nmr techniques is significant. Nmr analysis of compounds bound to solvent-swollen resins is possible, but the quality of the spectra, particularly the breadth of the peaks obtained, depends on the type of resin. Recently, a Magic Angle Spinning

technique using high resolution probes (HR-MAS) has been found to afford better ^{1}H and ^{13}C spectra of small resin bound compounds.[88] This approach has recently been extended to allow analysis of a trisaccharide glycal, bound to Merrifield resin and this is the largest molecule analysed by HR-MAS to date (Figure 6).[89]



Key ^{1}H nmr features: Glucal 1-H 6.4 ppm
Acetyl CH_3 approx. 2.10 ppm
Two $PhCH_2$ 4 doublets at 4.83, 4.69, 4.64, 4.55 ppm
^{i}Pr 0.80-1.20 ppm

Key ^{13}C nmr features: Two acetyl C=O at 169.3 and 169.1 ppm
Two carbonate C=O at 154.3 and 153.6 ppm
Two anomeric C and Glycal C-2 at 90-100 ppm

Figure 6 : NMR data for polymer bound glycal.

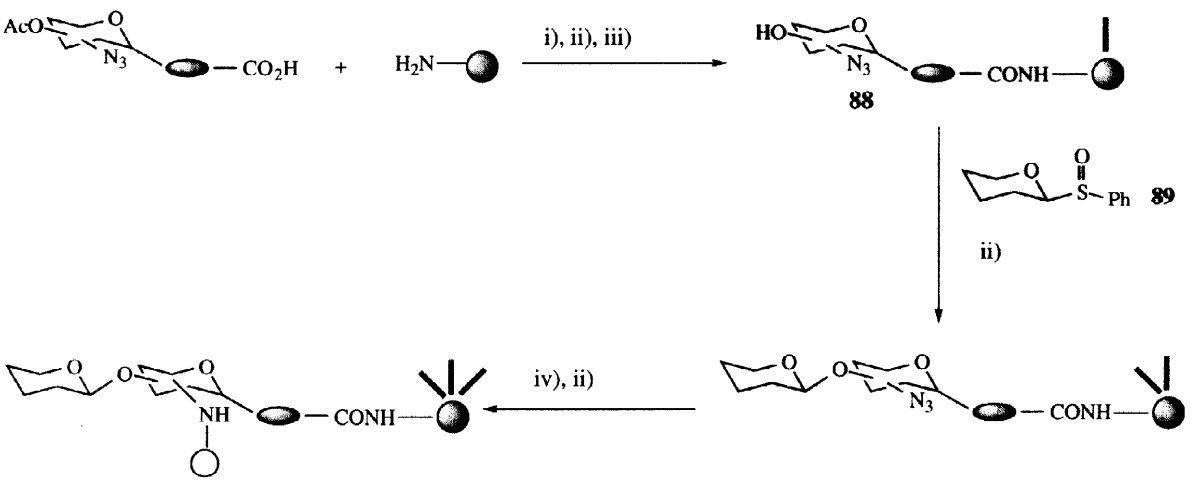
Detailed ^{1}H, ^{13}C and ^{1}H-^{13}C HMQC NMR data have been obtained for polymer bound intermediates and products, and this has enabled the rapid confirmation of a successful trisaccharide synthesis. Typical nmr experiments using this technique were complete within 2 hours. Any signals from the polystyrene backbone were reduced in the ^{1}H nmr spectrum using spin-echo techniques. Although the lines obtained in the spectra were still broader than in the resin-cleaved products, the specific peaks highlighted in Figure 6 allowed unequivocal confirmation of the proposed structure within a short period of time.

8. CARBOHYDRATE BASED COMBINATORIAL LIBRARIES[90]

The conformationally rigid and functionally rich carbohydrate system is unparalleled in its value as a molecular scaffold[91] and, as such, effective methods for creating libraries of carbohydrates would have a great impact on combinatorial chemistry drug-discovery programmes. Polymer-supported synthesis of combinatorial libraries can either be performed in the solid or solution phase. The latter eliminates some of the potential problems associated with insoluble polymers.

Solid phase polymer supported approaches

Whilst the synthesis of combinatorial libraries of peptides and oligonucleotides for structure-activity relationships is relatively common,[92] carbohydrate based approaches are relatively rare. A solid phase carbohydrate library has been prepared using Kahne's sulfoxide methodology.[47] The "split and mix" method has been employed to generate 1300 di- and trisaccharides attached to Tentagel solid support, through the reaction of 6 monomer acceptors bound to Tentagel **88** with 12 glycosyl sulfoxide donors **89**. Azido sugars were included among the selection of donors and acceptors, allowing the introduction of further structural diversity (Scheme 29). A single deprotection step then afforded the resulting library of di- and trisaccharides which were screened, whilst still bound to the polymer, against the *Bauhinia purpurea* carbohydrate binding protein. In this way, polyvalent presentation of oligosaccharides on the surface of the cells was mimicked by the presentation of multiple oligosaccharides on the surface of the polymer. Two ligands that bound more tightly than the natural carbohydrate were identified, and their structures were determined by Still's encoding protocol.[93]



i) Immobilization; ii) coding; iii) de-*O*-acetylation; iv) reduction then amine acylation with 20 acyl groups

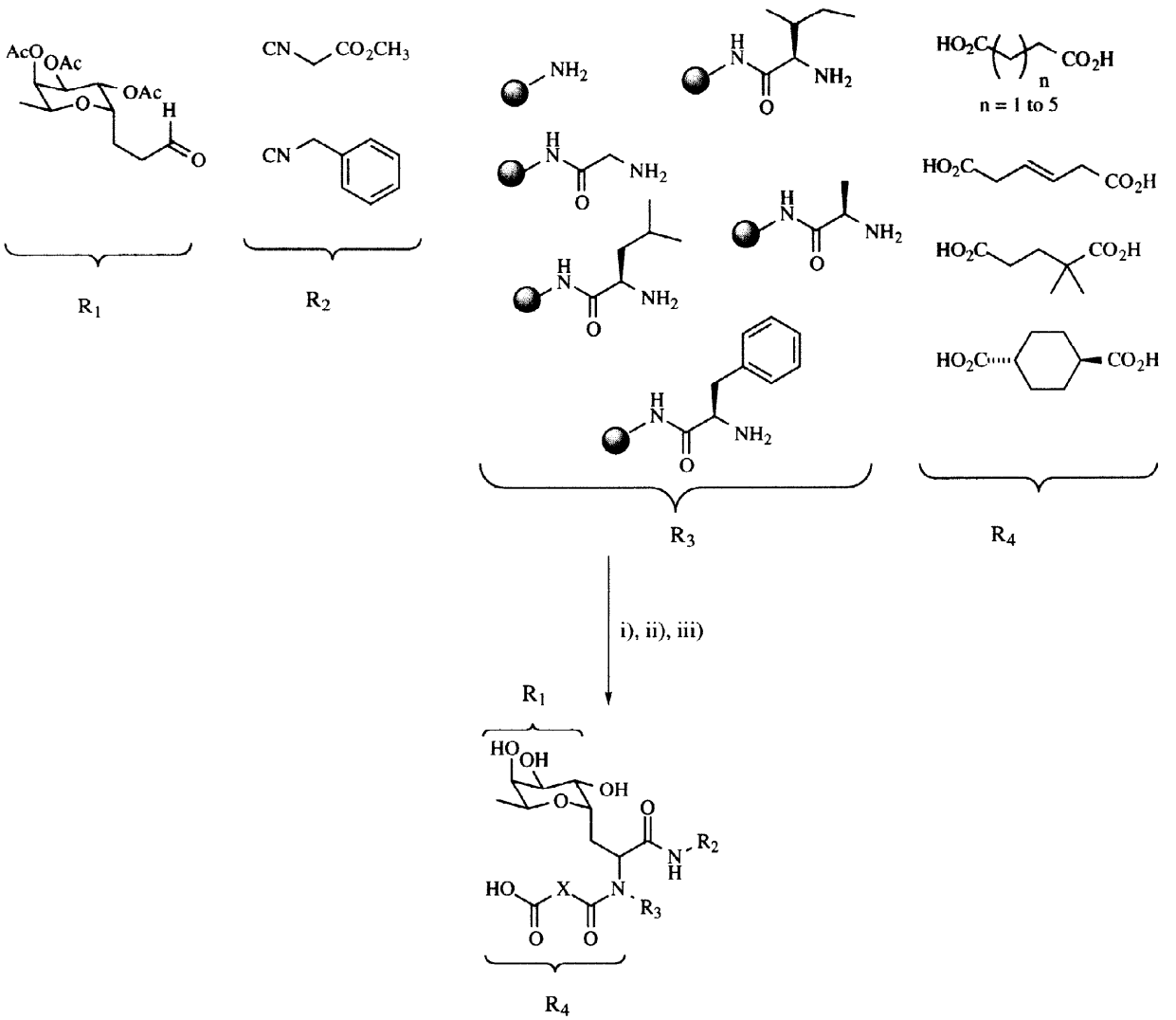
Scheme 29 : Generation of oligosaccharide library using sulfoxide donors.

More progress has been made in the synthesis of glycopeptide libraries. The peptide is normally attached to a solid support such as Tentagel using an acid labile linker such as 5-(4-aminoethyl-3,5-dimethoxyphenoxybenzyl)valeric acid (PAL) or Rink's linker. Libraries of compounds can then be generated either by coupling the amine group of amino sugars with the activated carboxylic acid moieties of the peptides, or by coupling the amine group of peptides with carboxylic acid groups of carbohydrate uronic acids. For example, a small glycopeptide library consisting of 23 glycopeptides has been generated by coupling pentafluorophenyl ester derivatives of 5 polystyrene linked peptides with 18 1-amino-1-deoxy saccharide derivatives under routine conditions.[94] Coupling yields of 50-80% were observed when uncharged oligosaccharides were employed, but yields of only 30-50% were obtained with charged analogues. In order to incorporate an on-bead encoding mechanism, a further strategy was developed which involved attachment of the 1-amino-1-deoxysaccharides to one of the active esters of disuccinimidyl suberate (DDS).[95] The glycoconjugates thus produced were immobilised onto Tentagel resin and used in the generation of combinatorial libraries for screening against digoxigenin-labelled Neu5Ac α-(2,3) Gal specific lectin, and fluorescently labelled lectins. The bead containing the bound ligand for each of these receptors was identified using an enzyme-linked assay and a precipitative stain or flow cytometry technique.

The alternative approach of coupling the amine group of peptides with the carboxylic acid moiety of uronic acids has been exemplified by Chan and co-workers for the construction of a library of approximately 350 peptidoglycan monomer analogues for screening as inhibitors of bacterial peptidoglycan biosynthesis.[96] Twelve mono- and disaccharide building blocks, each containing a muramic acid sugar derivative were coupled to the terminal amino group of a small library of 22 peptides linked to polystyrene through a chlorotrityl linker.

Standard organic reactions that have been optimised on solid supports have also been applied to the synthesis of carbohydrate derivatives for use in inhibition studies. For example, the Ugi four component condensation reaction, which utilises aldehydes, carboxylic acids, isocyanides and amines to produce α-acylamino amides, has been applied to the synthesis of a library of *C*-glycoside glycoconjugates.[97] *C*-1-Acetaldehyde-*C*-glycosides or *C*-1-acetic acid *C*-glycosides were employed as the aldehyde and / or acid components and Rink resin bearing an amine was used as the amine component in the generation of a library of

96 discrete compounds for investigation of the ability of sialyl Lewis^x glycomimetics to target cell adhesion receptors (Scheme 30).



i) MeOH / THF / CH_2Cl_2, 36h; ii) 1M NaOMe; iii) 20% TFA, CH_2Cl_2

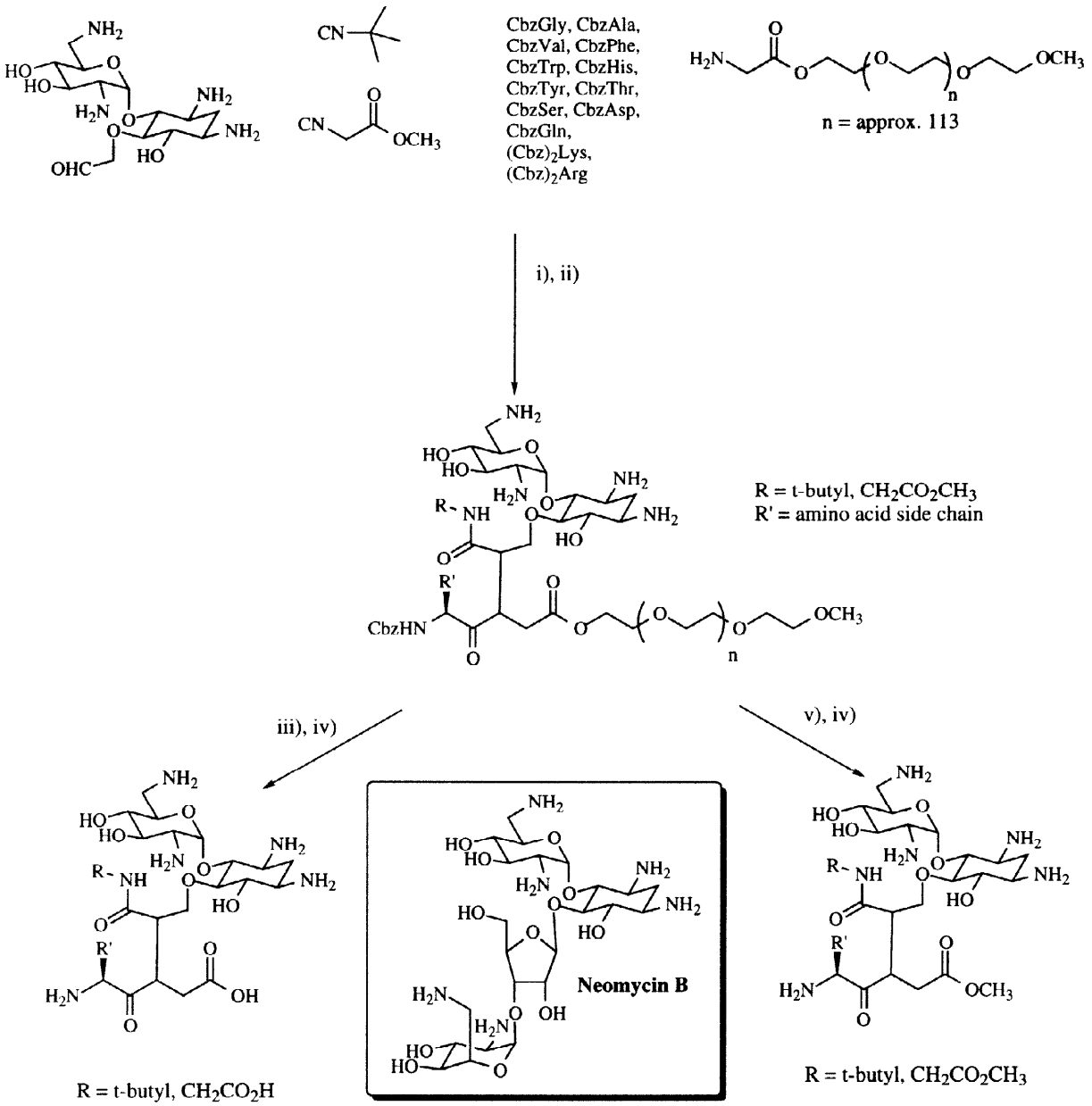
Scheme 30 : Ugi four component condensation for the combinatorial synthesis of sialyl Lewis^x glycomimetics.

Cleavage of the acetate groups was achieved under basic conditions and the products thus formed were readily cleaved from the polymer under acidic conditions.

Solution phase polymer supported approaches

The use of soluble polymers for the synthesis of mimetics of the aminoglycoside antibiotic neomycin B has recently been described using a Ugi-type multiple component condensation (MCC) strategy on a polyethylene glycol soluble polymer.[98] The three components required for this reaction, namely aldehydes, isocyanide derivatives and amines were derived from neamine derivatized at the C-5 hydroxyl group with an acetaldehyde unit, *tert*-butyl isocyanide and isocyanoacetic acid methyl ester, and 13 different *N*-protected amino acids. The library of compounds always displayed the same neamine unit with different appended peptides. The products of

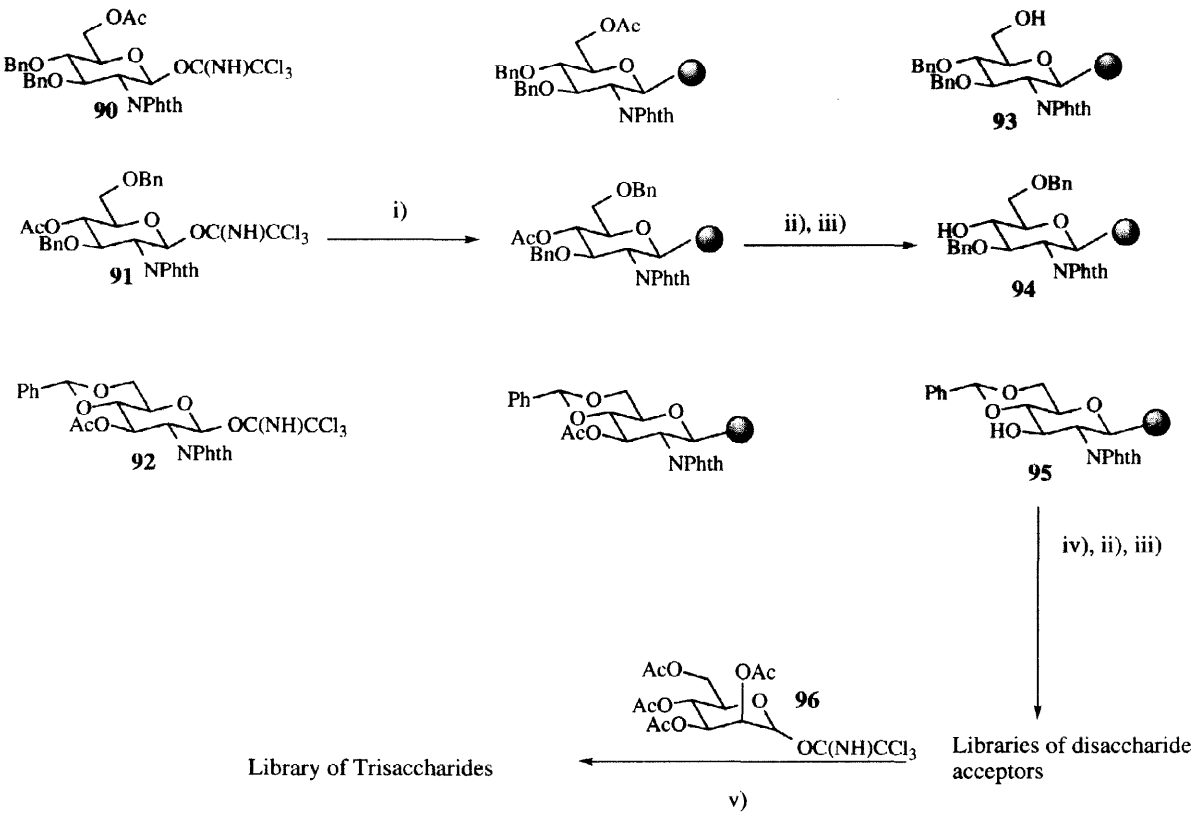
the MCCs were isolated by precipitation with ether, and subsequent treatment with base allowed concomitant deprotection of the acetate protecting groups on the neamine unit and removal of the compounds from the polymer. The Cbz protecting group was then removed under standard hydrogenolysis conditions (Scheme 31).



i) CF_3CH_2OH, rt, 5 days; ii) ether; iii) LiOH; iv) 10% Pd / C; v) NaOMe

Scheme 31 : Ugi-type MCC strategy for synthesis of Neomycin B analogues.

The pool and split method for forming libraries of oligosaccharides in solution has recently proved successful for the synthesis of α-Man-(1,4)-β-GlcNAc-(1,4)-GlcNAc and α-Man-(1,4)-β-GlcNAc-β-(1,N) GlcNAc[99] using polyethylene glycol as the soluble polymeric resin (Scheme 32).



i) MPEG, TESOTf, CH_2Cl_2; ii) pool; iii) MeOH / DBU; iv) glycosylate individually with **90**, **91** and **92** / TfOH; v) BF_3OEt_2

Scheme 32 : Pool and split method for forming libraries of oligosaccharides.

Three trichloroacetimidate donors **90**, **91** and **92**, which each have one temporary acetate protecting group are linked to methyl PEG *via* an α,α'-xylo linker. After de-*O*-acetylation and pooling, the polymer bound monosaccharides **93**, **94** and **95** are individually glycosidated with further quantities of **90**, **91** and **92** to afford libraries of disaccharides. Subsequent pooling and de-*O*-acetylation affords sub-libraries of acceptors, which are mannosylated with tetra-*O*-acetyl mannosyl trichloroacetimidate **96** to afford a library of trisaccharides. One limitation of this method, however, is the inability of PEG to be encoded with currently available coding tags.

All of the procedures outlined above have been reported in recent years, which is a reflection of the current interest in combinatorial chemistry. As screening processes continue to improve in efficiency, the value of combinatorial approaches to carbohydrate libraries of compounds using polymer supported strategies will increase.

9. FUTURE PERSPECTIVES

The considerable research effort spent on the development of polymer supported syntheses of oligosaccharides has brought many rewards. Indeed, methods are now available for the reliable syntheses of many specific classes of oligosaccharides on polymer supports. However, compared with polymer supported peptide and oligonucleotide syntheses, oligosaccharide strategies are still in their infancy with further developments in polymer supported syntheses of oligosaccharides relying upon progress in solution phase

strategies. Furthermore, many polymer supported reactions are only applicable on a small scale so that often access to only limited amounts of biologically important oligosaccharides can be obtained. The importance of synthesising only one isomer of a required target also emphasises the need to develop oligosaccharide assembly techniques, both in the solution and solid phase, which are both regio- and stereoselective.

Until analytical techniques for monitoring solid phase polymer supported syntheses of oligosaccharides improve, soluble polymer supported methodologies will remain popular. Researchers will continue to strive for novel polymers and linker systems which will be compatible with the protecting groups and glycosidation conditions routinely employed in the syntheses of oligosaccharides of biological importance. Hence, polymer supported oligosaccharide assembly can be identified as a contemporary research area of utmost importance.

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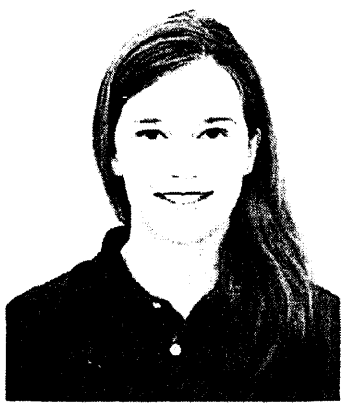

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