REVIEW

Recent developments in oligosaccharide synthesis

Benjamin G. Davis

Department of Chemistry, University of Durham, Science Laboratories, South Road, Durham, UK DH1 3LE

Received (in Cambridge, UK) 24th March 2000
Published on the Web 27th June 2000

Covering: the literature from October 1995 until December 1999.

1 Introduction

This review aims to update developments in the synthesis of oligosaccharides from 1996 to the end of the last century. This is a period during which the focus of glycosylation chemistry has changed. For almost 150 years the importance of sugars has driven methods for creating bonds between them. That this chemistry has been one of elegance and ingenuity and has attracted the brightest and the best minds to strive for methods attests to the richness of carbohydrate chemistry. Yet, in spite of this, the clear fact remains: we have singularly failed to develop a general solution—glycosylation chemistry is still not routine, predictable or generally accessible. This is perhaps a fact more keenly felt by those outside the carbohydrate community than within it: often these are the people who require just a method—just a simple reply to "How do I make this?".

For the majority of the last 150 years we have been cataloguing new methods—a vast array of specialised synthetic knowledge for glycoside formation. We have available now many tens of methods but nearly all share a common link with the chemistry of Fischer and of Koenigs and Knorr that was used at the turn of the 19th century. True, efficiencies have greatly improved and many elements of control and selectivity (each typically peculiar to a given system) are now available. Yet the urgent requirement for more general methods that will give us the power to do more than scratch the surface of the discoveries and suggestions that glycobiology offers us is now shifting many goals of glycosylation chemistry away from simple variations on the Koenigs–Knorr theme. Instead, new themes themselves are sought. Two examples covered in some detail in this review are tethered glycosylation (or intramolecular aglycon delivery) and polymer supported glycosylation. Both are in their infancy, being essentially topics of concentrated interest for only ten years. Yet both offer the potential for a revolution in glycosylation chemistry in seeking to alter our fundamental approaches. Furthermore, enzyme catalyzed chemistry—often just an addition in passing to many accounts—is considered here as an integral part of glycosylation chemistry. Indeed, as many of the examples below will illustrate it is often careful combined use of the best that traditional and enzymatic methods have to offer that are the most expeditious.

Fundamental and classical aspects of glycosylation chemistry have been covered previously both in several seminal reviews[1–5] and in excellent texts[6–9] and it is not the intention of this review either to go over this ground or to catalogue all examples of glycosylations. Instead, the aim is to continue in format and context where Boons' excellent review of 1996 left off.[2] Certain areas that may be considered glycosylation in a broader context, such as glycoconjugate synthesis,[10] C-glycoside formation,[11] cyclic oligosaccharide synthesis[12] or furanoside synthesis are covered elsewhere and are excluded here in favour of a more concentrated examination of the synthesis of the O to pyranosyl bond. Synthetic strategy for oligosaccharide formation[3] and methods for the construction of sugar–amino acid links[13,14] have also been ably reviewed previously and are not considered explicitly in this review other than to illustrate the use or achievements of novel glycosylation methodology.

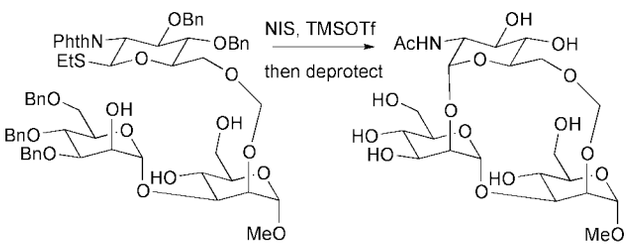
DOI: 10.1039/a809774g

2 Glycosyl donors

2.1 Thio/selenoglycosides

Thioglycosides and the closely related in behaviour selenoglycosides are amongst the most widely used choices of glycosyl donors. This popularity is partly due to their ready synthesis and partly to their easy conversion into sulfoxides, thereby offering an alternative glycosyl donor source. Their use has recently been reviewed[15] and several modifications have appeared.

Boons and co-workers have described the synthesis of a conformationally restricted trisaccharide **2** (Scheme 1) as a lectin binding ligand.[16,17] The macrocyclization of **1** was achieved in 34% yield using a thioglycoside activated by NIS–TMSOTf, whereas the corresponding trichloroacetimidate gave only 10%. In both cases the methylene acetal was untouched. An attempt to cyclize using the formation of this acetal as a final step failed. The use of an O-2 acetate thiomannosyl donor allowed the formation of the initial Manα(1,3)Man† link in **1**.



Scheme 1

Galactosyl and abiquosyl (a 3,6-dideoxy sugar) thioglycosides were the donors of choice in the syntheses of a similarly conformationally restricted trisaccharide lectin ligand.[19] α-Rhamnosylation of O-3 in diacetoneglucose using a thioglycoside donor allowed the synthesis of Verbascoside, a bioactive phenylethyl glycoside.[20]

I_2 has been shown to be a cheaper, milder, and more convenient reagent for the activation of armed thioglycosides than most standard methods and is compatible with a range of protecting groups.[21] *N*-Phenylselenophthalimide (N-PSP) or iodosobenzene with $Mg(ClO_4)_2$ have also been described as new reagents for thioglycoside activation.[22] The conditions are sufficiently mild to allow the use of 6-*O*-trityl groups on the donor, which as a result increases α-selectivity, presumably by sterically shielding the β face. Iodosobenzene can also be used in conjunction with other Lewis acids as an activator for thioglycosides, whilst TMSOTf and $Sn(OTf)_2$ promote β-selectivity by non-participatory donors, $SnCl_4$ and $AgClO_4$ as the Lewis acids promoted α glycosidation.[23]

Activation of disaccharide thioglycosyl donors with the radical cation reagent tris(4-bromophenyl)ammoniumyl hexachloroantimonate can lead to activation and cleavage of the intersaccharide glycosidic linkage rather than the reducing-end thioglycosidic linkage.[24] Amino acid esters and some saccharides have been α-fucosylated using a methyl iodide activated perbenzyl 2-pyridylthio donor.[25] 2-Methylbut-2-ene has been used as an alternative acid scavenger in NIS–AgOTf activations of thioglycosides.[26] Furthermore an *N*,*N*-dibenzylthiogalactosaminide also acts as a useful glycosyl donor forming β-galactosides with high stereoselectivity.[27] This selectivity was attributed to the formation of an intermediate α-aziridinium species. The benzyl groups were easily removed through hydrogenolysis.

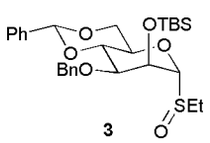
A thioglycoside carrying a hindered *O*-benzylhydroxyamino group in the α-face position at C-3 of a 3,3-disubstituted-2-deoxy glycoside has been used for stereoselective formation of β-glycosides which were then elaborated to complete the first synthesis of the 3-nitro-sugar containing Cororubicin trisaccharide.[28]

The mechanism of thioglycoside activation has only been poorly studied, and a useful study of the anomerization of thioglycosides has been conducted.[29] Whilst thioglycosides with small aglycons equilibrate between α and β anomers in the presence of catalytic iodonium dicollidine perchlorate (IDCP), thioglycosides with larger aglycons do not. Through labelling studies this anomerization was shown to be an intermolecular process. Furthermore, the nature of the aglycon affects stereoselectivity, with a higher α selectivity (3.5 : 1) being obtained from both α- and β-*tert*-butyl thioglycosides than from α- and β-ethyl thioglycosides (2 : 1). This identical product distribution from both anomers in both cases supports intimate ion paired intermediates.

In a fundamentally new approach to oligosaccharide formation, open chain *O*,*S*-acetals can be formed from the AgOTf–2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) activated reaction between open chain thioacetal chlorides and a glycosyl acceptor and then cyclized using NIS–TfOH to give furanoside[30] or septanoside[31] containing oligosaccharides depending on the protection regime selected.

2.2 Sulfoxides

The sulfoxide method also continues to be one of the most popular due to its ability to glycosylate even relatively non-nucleophilic molecules. Crich and Sun have observed a remarkable reversal in the stereoselectivity of mannosylation by **3** which is dependent on the order of addition alone.[32] This has allowed the efficient formation of a number of β-mannosides under these "inverse addition" conditions[33] and appears to represent one of the most generally efficient solutions to the "β-mannose problem". Recently, using this method a linear trisaccharide from *Hyriopsis schlegelii* glycosphingolipid was synthesized through β-mannosylation of the O-4 of glucose using an O-2 alkylated sulfoxide donor and β-xylosylation under Ag^+ catalyzed Koenigs–Knorr conditions.[34,35]



Kahne and Yan have used Lewis-a (Le^a), Lewis-b (Le^b) and Lewis-x (Le^x) determinants as model target systems that may be synthesized under standard reaction conditions with the excellent aim of making glycosylation chemistry accessible to the non-expert.[36] Using sulfoxide donors, a generalized set of conditions (2 equivalents of sulfoxide, 1 equivalent of Tf_2O, DCM, −78 °C) allowed the ready synthesis of these trisaccharides. Although the reliability and generality of sulfoxides is well demonstrated by this work, it is somewhat of an overstatement to say that this work is now accessible to all—especially given the protecting group manipulations that are additionally required.

Boeckman has described the use of a highly reactive *para*-methoxybenzylated phenyl sulfoxide donor with high β-selectivity that may be of use for the glycosylation of hindered alcohols where other functional groups prevent the use of benzyl or pivaloyl.[37]

The combined use of $BF_3{\cdot}Et_2O$ and DTBMP allowed the glycosylation of the highly sensitive vancomycin aglycon.[38] Since β stereoselectivity in the glucosylation of the very hindered phenol that is the tyrosine residue 4 side chain was required, a participatory strategy was adopted. To prevent orthoester formation, C-2 pivalates are typically employed but their removal required conditions incompatible with vanco-

† IUPAC symbols for monosaccharide residues and short form are followed in this review.[18]

mycin. Therefore an azidobutyryl group was used which may be removed by the reduction of azide to amine. Unfortunately, orthoester formation is a problem in this less hindered acyl group. Kahne and co-workers reasoned that lack of orthoester formation in comparable glycosylation systems that instead use $BF_3{\cdot}Et_2O$ catalysis is due to rearrangement to glycoside products of any orthoester formed. However, reactions of sulfoxides in the presence of $BF_3{\cdot}Et_2O$ but without DTBMP fail. Worries that $BF_3{\cdot}Et_2O$ and DTBMP would form adducts were dispelled both by previous reports of DTBMP's preference for protic acids and the successful resulting reactions using both together. This valuable system may also allow general suppression of orthoester formation in other glycosylation systems. Use of $BF_3{\cdot}Et_2O$ also allows formation of the second α vancosamine linkage without dehydration to nitrile of the D-asparagine amide in vancomycin. Interestingly, in both these glycosylations, no protection of the D-asparagine is required despite the previously reported ability of sulfoxides to glycosylate amides. Moreover no dehydration in these earlier amide glycosylations had been seen. This powerful glycosylation methodology has also allowed the establishment of the mechanism of action of carbohydrate-altered vancomycin derivatives.[39] Whilst vancomycin inhibits transpeptidation in peptidoglycan biosynthesis by binding D-Ala-D-Ala, analogues which show activity against vancomycin resistant strains of bacteria actually inhibit the transglycosylation stage. Further variations in the carbohydrate portion should allow these antibiotic specificities to be optimized. In an only slightly later and independent disclosure Nicolaou and co-workers used a trichloroacetimidate glucosyl donor bearing a C-2 allyloxycarbonyl group for anchimeric assistance, and a vancosaminyl fluoride donor.[40] High α selectivity in the latter glycosylation was attributed to participation by an O-4 acetate.

A cheap and simple H_2O_2–Ac_2O–SiO_2 in DCM system has been described for both small and large scale preparation of glycosyl sulfoxides from corresponding thioglycosides.[41]

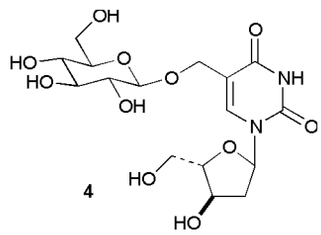
2.3 Glycals

A thorough and useful review of the glycal methodology has been published.[42] A one-pot variation on the glycal method that does not require oxidation prior to activation has been described.[43] Reaction of protected glucals with Ph_2SO and Tf_2O in the presence of $ZnCl_2$ and an acceptor leads to β-glucosides. The use of $Ph_2S^{18}O$ led to almost complete label incorporation and suggests the formation of a 1,2-anhydrosugar intermediate by transfer of oxygen from the sulfoxide; this was further supported by isolation of the anhydrosugar. A stereoselective assembly of an arabinogalactotetrasaccharide used stepwise elongation of the sugar chain from the reducing end with 1,2-anhydroglycoside and $ZnCl_2$ activation building blocks (or "reversed glycal" approach) and was completed by the use of arabinofuranosyl thioglycoside mediated glycosylation of the reducing end residue OH-2.[44] Glycal methodology has also been used to synthesize hydroxylysine β-glycopeptides found in collagen.

The use of a fluorous benzyl protected glucal allowed α-2-deoxyglycoside synthesis in benzotrifluoride (BTF) and subsequent fluorous three-phase extraction, as a simplified form of purification, thereby extending the powerful fluorous-phase technique to oligosaccharide synthesis.[45]

2.4 Trichloroacetimidates

Schmidt and co-workers have used trichloroacetimidates of a participatory galactosyl and a non-participatory fucosyl donor to construct C_2-symmetric tetrasaccharide Lewis carbohydrate determinant mimics based on a Galβ,βGal trehalose core.[46] Participatory glucosyl trichloroacetimidates have been used in the synthesis of the modified nucleoside **4** from the variant surface glycoprotein gene in *Trypanosoma brucei*, which



4

was used as its phosphoramidite in an automated DNA synthesiser.[47]

Lipase enzymes can be used for catalyzing the selective deacylation of OH-1 in the preparation of 1-hydroxy precursors to trichloroacetimidates.[48] Commercially available dibutylboron triflate (DBBT) has been demonstrated as an effective alternative activator of trichloroacetimidates to $BF_3{\cdot}Et_2O$ or trialkylsilyl triflates that allowed glycosylation of both monosaccharide acceptors and an MPEG polymer support.[49] As a hard Lewis acid DBBT failed to activate thioglycosides.

A so-called inverse-Schmidt procedure in which catalytic TMSOTf and acceptor are premixed before trichloroacetimidate addition gave high yields in the synthesis of a nonasaccharide.[50] An ingeniously designed 6-*O*-acetyl-2-*O*-benzoyl-3,4-*O*-dibenzyl building block allowed selective deprotection and therefore access to O-6 only or O-6 and O-2 as required. The participatory O-2 Bz also ensured stereocontrol.

2.5 Pent-*n*-enyl glycosides

Molecular modelling gave modified activation energies for pent-*n*-enyl glycoside hydrolysis that are in excellent agreement with the experimentally observed trends for a series of conformationally restricted pentenyl glycosides bearing benzylidene and dispiroketal protecting groups.[51] The more the restriction during the flattening of the pyran ring to form the glycosyl cation, the slower the hydrolysis. On the basis of these calculations, the negative effects of dispiroketal are attributed to poor solvation, rather than conformational restriction.

In related methodology Kunz and Leuck have suggested the use of (*S*)-pent-4-enyl thioglycosides as glycosyl donors that may be activated by NIS–TfOH, although it is unclear whether these donors are iodinated at the anomeric S atom or at the C=C bond followed by thiolane formation akin to *O*-pentenyl glycosides.[52]

2.6 Halides

The use of 2,3,4-tri-*O*-acetyl-6-*O*-pivaloylgalactopyranosyl halides as novel glycosyl donors in the synthesis of Lewis saccharides allowed participatory β-galactosylation followed by later selective deacetylation using hydrazine hydrate in EtOH, whilst leaving the 6-*O*-pivaloyl group intact. The bulk of this *O*-6 protection allowed selective *O*-3 over *O*-4 sulfation and sialylation.[53] An I_2–DDQ activator system has proved a highly effective alternative to traditional heavy metal salts in the formation of simple glycosides from glycosyl halides.[54]

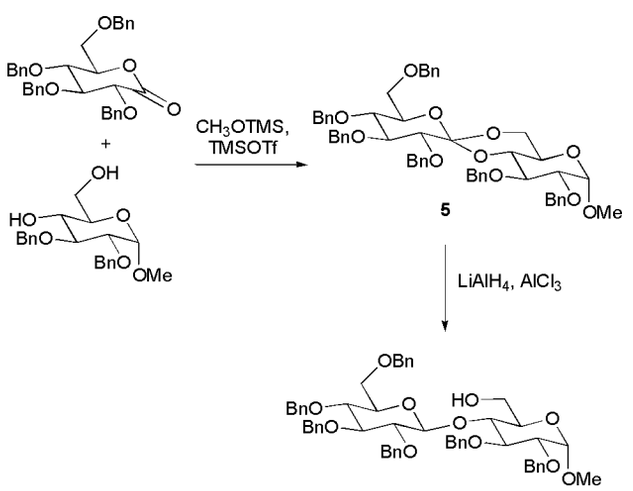
The preparation and use of glycosyl fluorides has been thoroughly reviewed.[55] The tricky β-glucosylation of phenols and carboxylic acids can be achieved efficiently by peracetylated glucosyl fluoride using a combination of a Lewis acid, such as $BF_3{\cdot}Et_2O$ and a hindered base, such as DTBMP.[56] However this reaction is not applicable to the glucosylation of simple alcohols indicating perhaps the requirement for an anion as a nucleophile. Various solid acids such as Nafion-H have been used to activate tetrabenzyl glycosylfluorides. SO_4–ZrO_2 proved most efficient giving good yields of α-mannosides; interestingly the stereoselectivity was reversed upon addition of 5 Å molecular sieves, allowing β-mannoside formation with moderate selectivity.[57] A selective armed–disarmed approach has been

applied to the activation of glycosyl fluorides using Cp_2HfCl_2–AgOTf.[58] Activation of glycosyl fluorides can be achieved in good to excellent yields using a trityl salt.[59] Interestingly, within this study the use of *tert*-BuCN, BTF and drierite proved superior in reactivity and selectivity to more conventional solvents, such as DCM, and drying agents such as molecular sieve. The pore size of the molecular sieve also had a dramatic effect on yield.

Glycosyl iodides are infrequently used as glycosyl donors and have largely to date been generated *in situ*. Gervay and Hadd have described a simple synthesis from glycosyl acetates which allows their isolation.[60] Although their use with certain C, N and O nucleophiles including phenoxides and carboxylates, excitingly indicated a high degree of S_N2-type character, disappointing competition by either elimination or *in situ* anomerization with sugar alkoxides or simple alcohols, respectively, gave largely glycal or α-glycosides as products and therefore appears to limit their potential use in oligosaccharide synthesis. Trimethylsilylation followed by reaction with TMSI allowed formation of persilylated L-fucosyl iodide, a donor which gave moderate to excellent yields of α-fucosides without activation.[61]

2.7 Orthoesters and acetates

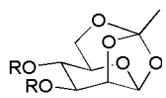
The introduction and regioselective cleavage of the 4,6-benzylidene acetal in glucose to give O-6 or O-4 benzylglucosides has long been used as a protecting group manipulation trick in carbohydrate chemistry. An ingenious extension of this idea has been used for the regio- and stereo-selective formation of glycosyl(1,4)glucosides.[62,63] Thus, gluco-, galacto- and mannopyranosylidene acetals of O-6 and O-4 of glucose such as **5** (Scheme 2) were regio- and stereo-selectively cleaved using $LiAlH_4$–$AlCl_3$ to give β(1,4) products in excellent yields.[62] Glycosyl(1,4)galactosides were also synthesised through the formation of ketals with O-3 and O-4 of a galactoside acceptor and reductive cleavage with $LiAlH_4$–$AlCl_3$ to give exclusively β-linked products of glucose, galactose and notably mannose.[63] The orthoester, or sugar acetal, precursors can be readily prepared from the corresponding sugar lactones using $TMSOCH_3$ and TMSOTf.[64]



Scheme 2

1,2,6-Orthoesters of mannose **6** act as glycosyl donors when activated by $BF_3{\cdot}Et_2O$ allowing differentiation of OH-6 (left free) and OH-2 (becomes OAc) after α-mannosylation.[65] They may be prepared from the corresponding unprotected 1,2-orthoester using pyridinium triflate or imidazolium chloride as catalysts.

In a remarkably simple protocol, α-alkyl glycosides and disaccharides can be prepared from peracetates of galactose and glucose as glycosyl donors using $FeCl_3$ in DCM as an activator.[66] These high α-selectivities go against the expected β-selectivity in what are typical examples of participatory systems and presumably arise through the equilibration of initial β products to more stable α ones.



R = Bn or Ac

6

Crich and co-workers have examined orthoester formation and reaction in xylosyl donor systems and this work supports previous mechanisms.[67] Through clear use of ^{13}C NMR, the bridged dioxoalenium cation was observed. In the presence of non-nucleophilic base DTBMP, the cation was intercepted by alcohol acceptor to give acid sensitive orthoesters whereas in the absence of base glycoside was formed, perhaps by acid catalyzed rearrangement of orthoester intermediate.

2.8 Vinyl glycosides

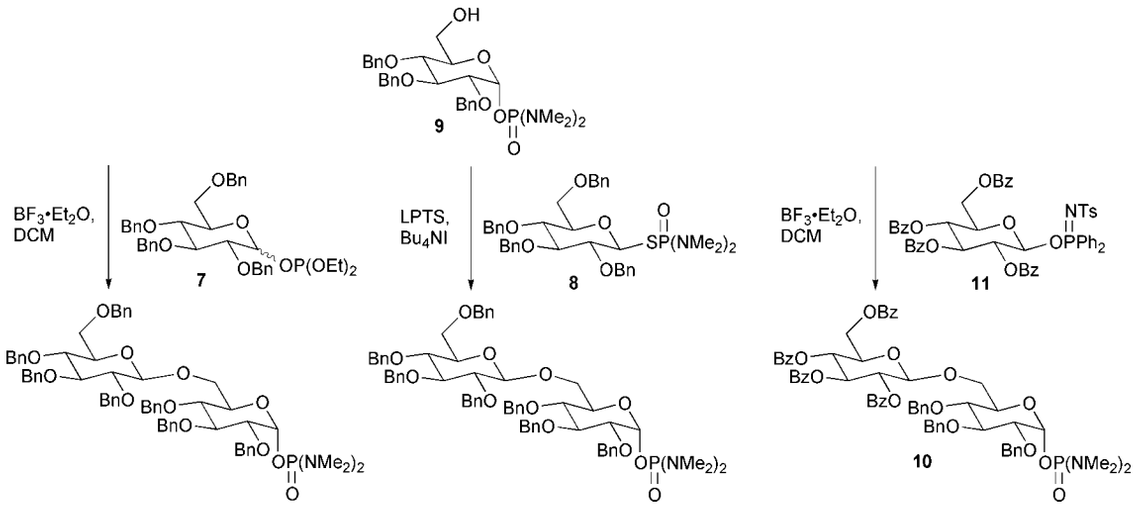
Boons and co-workers have now reported a refinement to their isomerization procedure for the conversion of allyl glycosides to vinyl glycosides, which may be activated using Lewis acids.[68] Better yields are obtained when the Wilkinson's catalyst normally used is pre-treated with BuLi. This isomerization procedure allowed the use of inactive allyl glycosides (so-called latent) as acceptors which can be synthesized from glycosyl halides and but-3-en-2-ol and converted into active vinyl glycosides that when activated by TMSOTf act as donors.[69] This strategy led to the synthesis of a number of disaccharides through glycosylation with moderate stereoselectivity using non-participatory donors. Vinyl glycoside donors of α,β-unsaturated esters and ketones may be synthesised by Bu_3P catalyzed Michael addition to the corresponding alkynic esters or ketones and activated by 9–12 equivalents of TMSOTf at −40 to −50 °C.[70] Vinyl glycosides have also proved useful in the synthesis of glycosyl phosphates.[71]

Mechanistic studies on isopropenyl α- and β-glucopyranosides have shown that the sole mechanism of their hydrolysis is *via* enol ether cleavage following an irreversible rate limiting C-protonation step that occurs four times faster for the α anomer.[72] The lack of a glycosidic cleavage pathway may bring into question their utility as glycosyl donors under certain conditions. Isoprenyl glycosides can be readily prepared from corresponding glycosyl halides or acetates using bis-(acetonyl)mercury or Petasis' reagent, respectively.[73] Electrophilic addition reactions were favoured by non-polar solvents such as DCM. Interestingly, *tert*-butyl alcohol favoured transglycosylation, and this is the first example of such an effect by protic solvents perhaps as a result of specific cationic intermediate solvation. Their selective activation by TMSOTf over pentenyl or thioglycosides opens up one-pot opportunities.

2.9 Anomeric phosphorus containing compounds

Given that Nature's choice of glycosyl donor, glycosyl nucleotide diphosphates, contains an O–P bond at the anomeric centre it is somewhat surprising that such phosphorus containing compounds have been relatively rarely used in chemical systems. However, the utility of a number of such systems has recently been demonstrated.

Hashimoto's glycosyl diethyl phosphites, such as **7** (Scheme 3), have proved a particularly versatile donor type in that the stereoselectivity of glycosylation may be well controlled through conditions alone. For example, activation of **7** with $BF_3{\cdot}Et_2O$ gives some of the highest β-glucoside selectivities seen for non-participatory donors,[74] whereas activation of **7**



Scheme 3

with 2,6-di-*tert*-butylpyridinium iodide in the presence of Bu_4NI allowed mild *in situ* anomerization *via* the glycosyl iodide and very high (90–95%) α-glucoside selectivity.[75] Furthermore, the latter conditions are mild enough to be compatible with acid sensitive donors and acceptors.

Such glycosylphosphites may also be converted into phosphorimidate glycosyl donors using phenyl azide in a Staudinger reaction.[76] These may be activated under a wide variety of conditions: Lewis acids (TMSOTf, $BF_3{\cdot}Et_2O$, $ZnCl_2$, $LiClO_4$), alkylating agents (MeI) or salts (lutidinium tosylate (LPTS)). The choice of activator for these non-participatory donors also affected their stereoselectivity in glycosylation. For example, galactosyl and glucosyl donors gave predominantly α using LPTS–Bu_4NI but mainly β using TMSOTf.

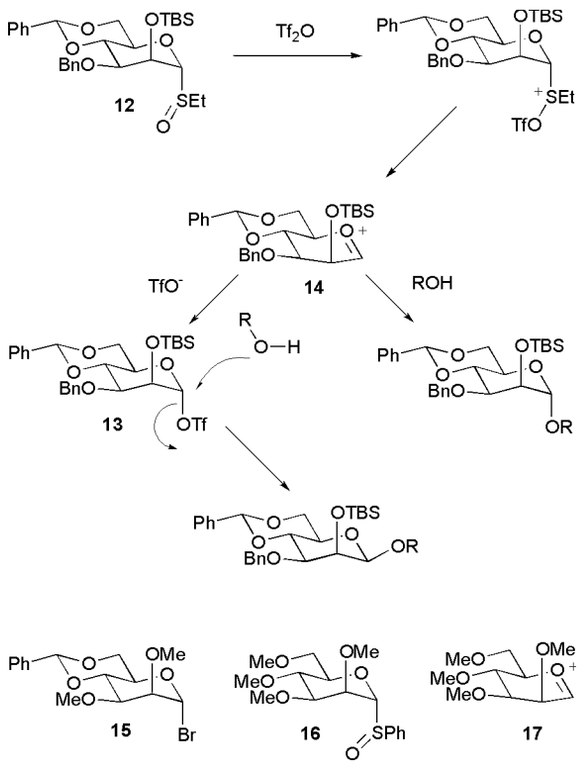
The relative merits as glycosyl donors of tetrabenzylglycosyl dimethyl, diethyl and dibenzyl phosphites, when activated with neutral concentrated perchlorate solutions, have also been investigated.[77] Although, dimethyl and dibenzyl phosphites give only moderate yields, diethyl phosphites gave some good yields of glycosides especially with $Ba(ClO_4)_2$. Stereoselectivities in these systems were only slight.

Glucosyl phosphates, which often show only a moderate activity as glycosyl donors, can be activated under neutral conditions using concentrated solutions of lithium perchlorate in DCM in the presence of lithium iodide to form glycosyl iodides which then react without further activation.[78] For non-participatory donors, higher α-selectivities through the use of lithium iodide were obtained. This was attributed to β-iodide formation followed by α attack by the acceptor, possibly by an *in situ* anomerization type process. Singh has adapted the use of diphenylphosphinyl as a leaving group from peptide coupling chemistry and shown that both non-participatory glycosyl diphenylphosphinate and propane-1,3-diyl phosphate donors give largely β-*O*-glycosides in TMSOTf catalyzed glycosidations.[79] The method also gave good yields of an L-fucoside and an umbilliferone D-galactoside.[80] Interestingly Hanessian and co-workers have reported that glycosyl phosphates may be synthesised directly from *unprotected* glycosyl 3-methoxypyridine donors using phosphoric acid.[81] Also, glycosyl phosphates have been synthesized in a flexible strategy from vinyl glycosides.[71]

The use of phosphorus based leaving groups has allowed the exploitation of their various chemoselectivities for armed–disarmed approaches.[82] In a useful comparative study, protecting group based armed (ether protected) and disarmed (acyl protected) strategies worked well for glycosyl phosphoramidates activated by TMSOTf. Such a strategy was used successfully to develop two alternative routes to the globoceramide Gb_3.[83] Leaving group tuning in a latent-active type approach also proved successful in such systems. More reactive phosphorodiamidimidothioates, such as **8** (Scheme 3), or glycosylphosphites can be used to glycosylate an armed phosphoramidate, such as **9**, using LPTS or $BF_3{\cdot}Et_2O$ as an activator, respectively. Furthermore, the orthogonality of phosphinimidates to phosphoramidates for $BF_3{\cdot}Et_2O$ allowed the synthesis of **10** despite the acceptor **9** being armed by benzyl protection and the donor **11** being disarmed by acyl protection. Tribenzylmono-*O*-2-pivaloyl protected glucosyl phosphorodithioates, in contrast to acetyl protected counterparts,[84] may be activated using MeOTf to give moderate yields of β-glucosides.[85]

2.10 Anomeric sulfonates

Crich and co-workers have conducted a long-needed thorough mechanistic investigation into the intermediacy of glycosyl triflates in so-called "inverse addition" glycosyl sulfoxide and related reactions.[86] The resulting scheme (Scheme 4) explains well the selectivity dependency on mixing order and the high β-selectivity observed. Prior activation of **12** by Tf_2O allows formation of α-triflate **13** (detected by ^1H, ^{13}C, ^{19}F NMR) which then reacts in an S_N2-like manner. However, in the presence of a nucleophile that is more powerful than TfO^-, the intermediate oxonium ion **14** reacts to give the stereoelectronically preferred α-mannosides. Interestingly, AgOTf activation of a mannosyl bromide **15** gave similar α-triflate intermediates. Similarly, thioglycosides may be activated and converted into glycosyl triflates using PhSOTf,[87,88] including the first examples of the selective β-mannosylation of tertiary alcohols *e.g.* adamantol. Furthermore, ^{13}C NMR chemical shift values of ~105 ppm for C-1 indicate that the intermediates detected are true glycosyl triflates and not intimate oxonium–triflate ion pairs (the corresponding glycosyl cation's chemical shift would be expected to be >200 ppm). The formation of a greater proportion of α-mannosides for the more conformationally flexible **16** (Scheme 4), was explained by the greater stability of the corresponding oxonium ion **17** which reacts to give α-mannoside. The benzylidene protected oxonium ion **14** is too high in energy, due to the strain introduced into the fused system on going to a sofa conformation, to enter the reaction manifold and so all glycosylation proceeds *via* S_N2-reaction of the α-triflate. Interestingly, this proposal is supported by the work of Fraser-Reid and co-workers on the rates of hydrolysis of corresponding pentenyl glycosides.[51] In this context, it is noteworthy that Ogawa has mentioned that his intramolecular aglycon delivery (IAD) approach also requires a cyclic 4,6-acetal for optimal β-mannosylations.[89] Consistent with the S_N2-like model, decreasing the size of the O-2 substituent TBDMS > Bn > TMS increases the β-selectivity due to reduced hindrance of the β-face.[88] A remarkable reversal of stereoselectivity is seen



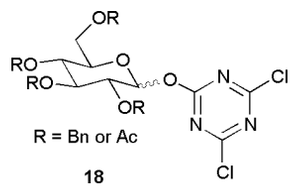
Scheme 4

in the corresponding D-gluco series as these produce α-glucosides in excellent yields (apart from methanol as an acceptor which gave high β-selectivities).[90] This reversal has been explained by an *in situ* anomerization of the α-triflate to the more reactive β-triflate. It is proposed that enhanced anomeric effect in the corresponding mannose series, which gave such high β-selectivity, prevents sufficiently rapid equilibration (either to β-triflate or glycosyl cation) thereby precluding Curtin–Hammett type kinetics required for such anomerization.

It seems likely that a related, efficient one-pot procedure for the direct activation of 1-hydroxy glucosyl donors using diphenyl sulfoxide and Tf_2O also proceeds *via* such triflate intermediates following the collapse of a glycosyl oxosulfonium intermediate.[91] This method also allows C, S, N and O glucosylation in good yield.

2.11 Other donor types

Schmidt has selectively alkylated OH-1, the most acidic hydroxy group, with heterocyclic chlorides and fluorides to form compounds such as **18**, which under mild acid catalysis will form pyridinones in a manner akin to trichloroacetimidates forming amides.[92] Thus TMSOTf in ether activation of non-participatory β-imidates gave, as for trichloroacetimidates, good yields of α products and the corresponding participatory tetraacetyl compounds gave β, though in a lower yield as compared with trichloroacetimidates. The ease of formation and potential for leaving group tuning in these systems hold great promise.



Similarly, as Scheme 5 shows, other electron withdrawing group-substituted 2-pyridyl glycosides can function both as glycosidase catalyzed glycosyl donors (see Section 8.1) or as glycosyl donors activated by TMSOTf.[93]

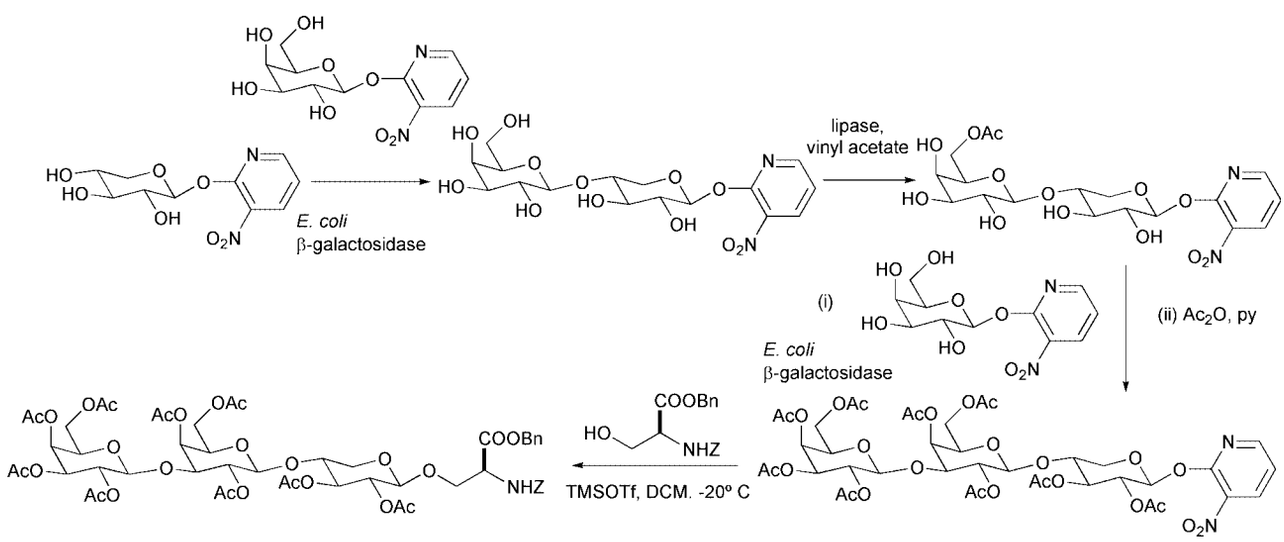
Solid acids have been investigated for the activation of tetrabenzyl 1-hydroxysugars as donors.[94] In contrast to others tested, heteropolyacid $H_4SiW_{12}O_{40}$, which also serves to dehydrate the reaction mixture, gave excellent yields of glycosides with good to high α-stereoselectivities.

Electrolysis of aryl telluroglucosides allows their mild oxidative activation and hence O-glycosidation.[95] Moderate β-stereoselectivities for non-participatory donors were obtained. The efficiency was highly dependent on the oxidative potential of the donor, which itself was dependent on the nature of the aglycon and protecting groups. This in turn allowed the demonstration of an armed–disarmed type approach in which a more easily oxidized benzyl-protected donor was exclusively activated in the presence of a benzoyl protected donor. This approach could also be extended given the relatively easy and mild activation of telluroglycosides under these conditions as compared with other chalcogenoglycosides (see Section 3.5 for an example of a one electron oxidation of selenoglycosides approach). Failure to observe a reversible electron transfer wave even at high scan rates indicated the short life time of the proposed radical cation intermediate.

3 Strategies for stereoselective formation of anomeric bond

3.1 Choice according to stereochemistry and functionality at C-1 and C-2

As previous discussions have emphasised,[2,3,6] the key step in



Scheme 5

deciding a strategy for oligosaccharide synthesis is often dependent on the stereochemistry of the glycosidic linkage. To this end, division according to *cis* and *trans* at C-1 and 2 of the unit to be transferred usefully unifies strategies for apparently different linkages. For example, α-mannosides and β-glucosides although seemingly very different to the uninitiated are in fact both *trans* at C-1 relative to C-2 and therefore readily formed through anchimeric assistance from so-called participatory C-2 groups such as esters. Similarly, *cis* configurations such as α-gluco or β-manno require non-participatory groups at C-2. Of these, the β-mannoside link is the most challenging as it cannot even be synthesised by taking advantage of the anomeric effect, an option that is available to α-glucoside formation. β-Mannoside formations are therefore often used as true tests of novel glycosylation methodology. The recent increased access to β-mannosylation strategies is the subject of a dedicated review[96] and has led to the synthesis of a number of natural products containing the β-mannoside linkage. One such is caloporoside **35** (see later), a phospholipase inhibitor, which both Crich and Barba[97] (a partial synthesis) and Furstner and Konetzki[98] (a total synthesis) have targeted using their respective direct and indirect methods. Finally, it should be noted that although in this review a distinction has been made between traditional C-2 acylated participatory donors (Section 3.2) and those that are not C-2 acylated (non-participatory, Section 3.3), there are several recent studies described in this latter section that have highlighted the possibility of more remote participation either from C-4 or C-6 acylated compounds.

3.2 Anchimeric assistance or neighbouring group participation (NGP)

The high propensity of 2,6-di-*O*-acyl-3,4-*O*-isopropylidene-D-galactopyranosyl donors to transfer acyl groups to acceptors has been explored using density function theory (DFT) calculations.[99] The results suggest that bridged dioxolonium ions interacting with the alcohol acceptor *via* a long C–O bond to the orthoester carbon are the most stable intermediates. They also suggest that acyl transfer products are kinetic whereas β-glycosides are thermodynamic.

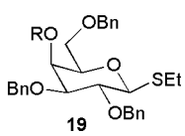
This problem of transfer of acyl groups from O-2 acylated glycosyl donors to the OH of the putative acceptor has also been investigated for polymer supported (PEG-based) oligosaccharide syntheses.[26] O-2 Pivaloyl groups do, as previously demonstrated, partially suppress acyl transfer but interestingly the additional use of more sterically hindered nucleophiles as acceptors completely suppresses it; it is suggested that the transition state for acyl transfer is more sterically crowded compared with that for glycosylation and therefore more sensitive to additional bulk.

Kahne and co-workers[100] have described the use of a hindered 2,2-dimethyl-β-ketoacetate protecting group at C-2 to allow β-control without orthoester formation. Two participatory glycosylations using Koenigs–Knorr conditions with a D-fucosyl donor followed by a glycosyl trichloroacetimidate allowed the formation of the Tricolorin A macrolide disaccharide Glcβ(1,2)D-Fuc.[101] The macrolide "belt" was then closed by olefin metathesis. An alternative synthesis also used double sequential participation but with a trichloroacetimidate fucosyl donor followed by macrolactonization. Lowary and Subramaniam have used neighbouring group participation in an O-2 acetate thiomannoside to construct a linear Man*p*α(1,2)-Man*p*α(1,2)Man*p*α(1,6)Ara*f* tetrasaccharide.[102]

3.3 Non-participatory glycosylations

In the absence of C-2 NGP, stereoselectivity is typically less determined and a catch all category of non-participatory reactions covers many aspects of glycosylation mechanism. Formation of a glycosyl cation (favoured by polar solvents) almost by definition means that absolute control of the stereochemistry of glycosidic bond formation is lost. Stereoelectronics dictate that such cations will tend to form α products for galactosyl and mannosyl donors and α : β mixtures for glucosyl donors. However, their interception either by counterions (leading either to covalent intermediates or intimate ion pairs) or certain solvent molecules (*e.g.* CH_3CN) may dramatically affect stereoselectivity. Furthermore, although S_N1 glycosyl cation formation by analogy with acetal hydrolysis is a commonly assumed intermediate pathway, stereocontrol may in fact be a consequence of a high degree of S_N2-like character with concomitant inversion of configuration—either of the donors directly or of some of the above intercepted-cation intermediates.

The α-anomeric selectivity of glycosidations of non-participatory tetrabenzyl thioglucosides is improved by using 1 : 1 toluene–dioxane as the reaction solvent and IDCP as an activator.[103] The α-directional effect of dioxane may be due to participation of its oxygen atoms with a sugar oxonium intermediate. These results have recently been expanded in a study of α-galactosylation.[104] A valuable examination of reactions of donor type **19** in 1,4-dioxane–toluene provided convincing evidence for a 1,4-NGP mechanism, a mechanism previously suggested by Miljkovic and co-workers.[105] In exploring α-stereoselectivities potential steric (comparing R = OMe and OBn) and electron withdrawing (R = $COCF_3$, CH_2CF_3) factors were eliminated. These groups, none of which can provide anchimeric assistance, gave similarly low ~3 : 1 α : β-galactosyl ratios. However, using a variety of potential participatory benzoyl derivatives (R = Bz, pNO_2Bz, pMeOBz) ratios of 17 : 1, 14 : 1 and 32 : 1 respectively using NIS–TMSOTf activation were obtained. Final optimisation was achieved by using IDCP activation which gave exclusively α products. The R = Bz donor also allowed the synthesis of a number of disaccharides with high α-selectivity.



19

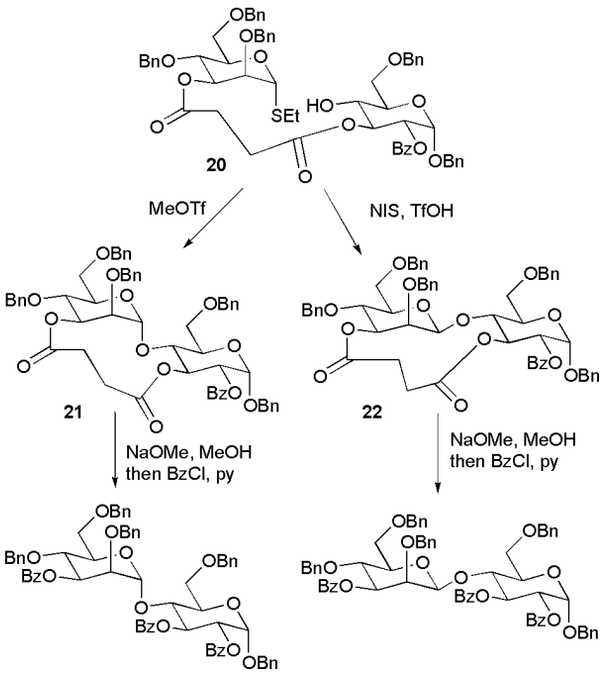
Interestingly, dependency of non-participatory glycosyl phosphite donor reactivities and stereoselectivities upon anomeric stereochemistry of the donors and upon whether the C-6 position is ether-protected, ester-protected or deoxygentaed have also led to the suggestion of a C-6 acyl participation mechanism akin to the above C-4 acylated donors and to more traditional participatory C-2 acylated donors.[106]

Alternatively bulk at C-6, such as trityl ether also appears to increase α-stereoselectivity for non-participatory glucosylations.[107]

3.4 Intramolecular aglycon delivery (IAD)

The idea of tethering the glycosyl donor and acceptor together is highly appealing as it raises ideas and concepts of reactants prearranged in space akin to activated enzyme–substrate complexes.

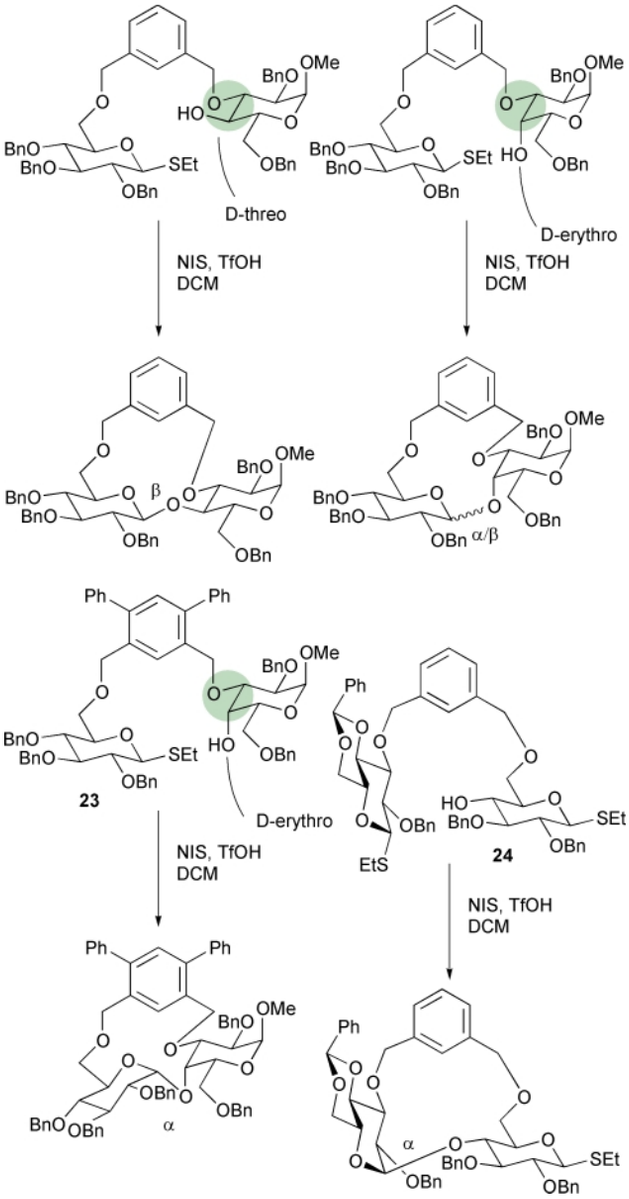
Such tethers may be temporary, lasting for the course of the reaction only, and in such cases doubt has been raised as to their intra- or inter-molecular nature.[108] This doubt is of course eliminated with tethers that are preformed and cleaved subsequent to glycosidation. Ziegler and Lemanski have described the use of a permanent tether for highly selective mannosylations.[109] Choice of tether position proved crucial. In contrast to succinoyl tethers at positions 2 or 6 of the mannosyl donor, which gave only moderate selectivity, tethering to position 3 to construct **20** (Scheme 6) gave very high stereoselectivities. In this method, the activation conditions dramatically affected stereoselectivity; whilst MeOTf gave exclusively α product **21**, the use of NIS–TfOH gave exclusively β product **22**, a truly remarkable reversal! Interestingly, selective β(1,4) mannosyl-



Scheme 6

ations by tethering to O-6 of the mannosyl donor, which had previously been of only moderate selectivity with the succinoyl tether, are transformed to highly selective ones when the tether is shortened by one methylene unit. Notably, although this latter method is successful for galactoside and glucoside acceptors it loses its selectivity for mannoside acceptors. Ziegler and Lemanski have also shown the operation of a double asymmetric induction by investigating the matching and mismatching of four O-6 to O-6 succinoyl tethered pairs of D- or L-glucose with D- or L-mannose.[110] The alteration of the tether point of attachment from O-6 on the donor to O-2 dramatically altered the ~1 : 1 α : β selectivity of the L-Man donor–D-Glc acceptor pair to 100% α due to NGP from C-2, thereby demonstrating the importance of the correct link point. Interestingly, the corresponding L-rhamnose–D-Glc pair showed a ~1 : 4 α : β preference and a degree of asymmetric induction in preference to NGP in this case. Succinoyl tethering of O-2 to O-3, glucosyl to glucosamine or glucoside gave α-stereoselective glycosylation despite an O-2 acyl group. A parallel O-2 to O-6 system gave α : β mixtures.[111]

Schmidt and co-workers have described exciting and comprehensive results through the use of a rigid *m*-xylylenyl diether linked system which allows good yields of Glcβ(1,*x*)Glc (for $x = 3,4$ or 6).[112] By reducing the tethering problem to the consideration of a relative configuration of the diol system of the acceptor—one OH of the diol is tethered *via* the tether linker, the other is the nucleophile for glycosylation—they have established that in their 14-membered ring systems 1,2-diol D- or L-*threo*- and L-*erythro*-systems give β-stereoselectivity with an O-6 tethered donor, whilst D-*erythro* gave an α : β mixture (Scheme 7). For the latter D-erythro system modifications to introduce 4,6-substituents to the xylylene spacer reduced the conformational space available in system **23** and led exclusively to α anomer formation. This type of clear analysis of the relative stereochemistry of the donor and acceptor has also led to valuable alternatives in the donor attachment. Thus, whilst O-6 attachment of a D-glucosyl donor leads to the presentation of the β-face to a D-*threo* acceptor diol configuration, attachment at O-3 of the same donor presents the α-face by formally inverting the relative stereochemistry of donor to acceptor (in the same way that left handed drinkers will drink from the opposite side of the cup to right-handed drinkers). This analysis was confirmed using an O-3 donor to O-6 acceptor



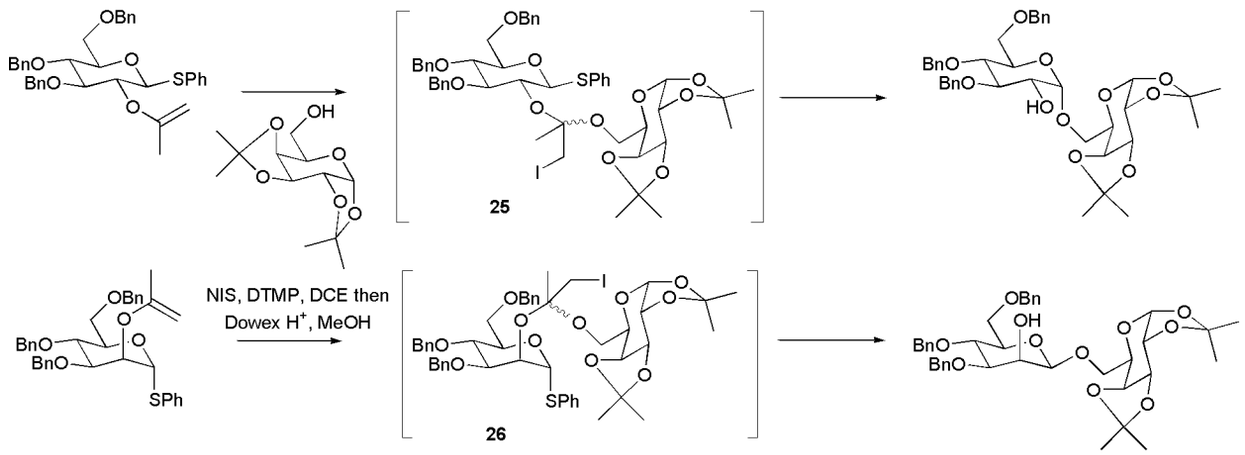
Scheme 7

diglucoside system **24**. A xylylene bridged, Glcβ(1,3)Glc linked product of these reactions was shown to adopt the rarely seen "*anti*" glycosidic linkage conformation.[113]

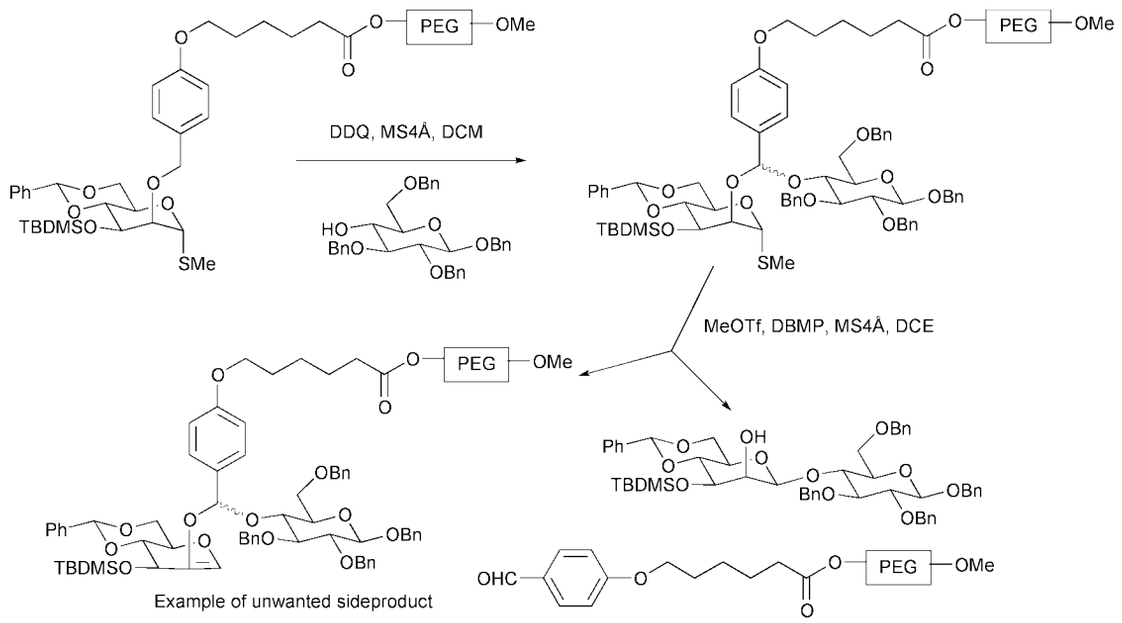
Stork provided one the very first examples of tethered O-glycosylation using a silyl ether tether between O-2 of a mannosyl sulfoxide donor and an acceptor. This work has now been extended to the glycosylation of O-2,3 and 6 of suitably protected tribenzyl glucosides to give good to excellent yields of disaccharides with high β-stereoselectivity.[114] Free OH-4 acceptors gave poor yields due to competing debenzylation and glycosylation of O-6.

The enol ether tethering system of Hindsgaul has recently been extended to an exciting one-pot system for the synthesis of α-gluco and β-manno disaccharides in good yield and with high stereoselectivity.[115] NIS can be used both to form the tethered mixed ketal **25** or **26** (Scheme 8) and then the subsequent activation of the thioglycoside donor. The intramolecularity of this method was indicated by excellent selectivities and confirmed by competition experiments with methanol as an acceptor.

Ogawa and Ito have ingeniously extended their O-2 *p*-methoxybenzyl tethering method to polymer support.[116] This so-called "gatekeeper" method uses a PEG methyl ether (MW 5000) as a soluble polymer support and its beauty is that only



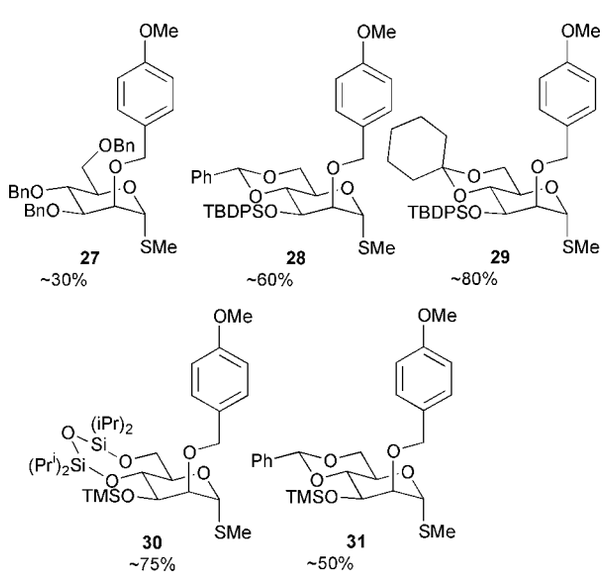
Scheme 8



Scheme 9

the desired product is released into the non-polymer phase. After precipitation of the PEG and filtration only product remains in solution (Scheme 9). In effect, the polymer only lets products pass—"if your glycoside bond's not formed, you're not coming in". They also note an analogy with glycosyltransferases, namely acceptor and donor bind separately to a polymer in an intentionally constrained manner resulting in stereoselectivity (and regioselectivity in the case of enzymes). In the solution phase Ogawa system, more rigid donors such as **29** improve yields up to ~80% over donors such as **27** or **28**.[89] The efficiency of **30**, which fails to operate in **31**, is attributed to the larger O-4,6 ring size. On the basis of nOe assignments and analysis, the stereochemistries at the acetal carbon in Ogawa's tethered intermediates have also been assigned.[117] Formation of the acetal from the 2-*O*-*p*-methoxybenzyl group proceeds with a high degree of diastereofacial selectivity with attack on the *Re* face of the oxonium intermediate to give (*R*) isomers. It was demonstrated that the intramolecular delivery proceeds with near equal efficiency for both (*R*) and (*S*) diastereoisomers.

A limited systematic study of tether types attached to the same positions in acceptor and donor has been conducted.[118] α-Selectivity is seen for diester linkers (glutaroyl, succinoyl and phthaloyl) but β-selectivity for silyl linkers in O-6 to O-6, glucose to glucose tethered system. The α-selectivity is highest for the so-called phthaloyl "molecular clamp", which is reminiscent of earlier work by Valverde and co-workers.[119] In addition a β-directing solvent effect, well known in acetonitrile



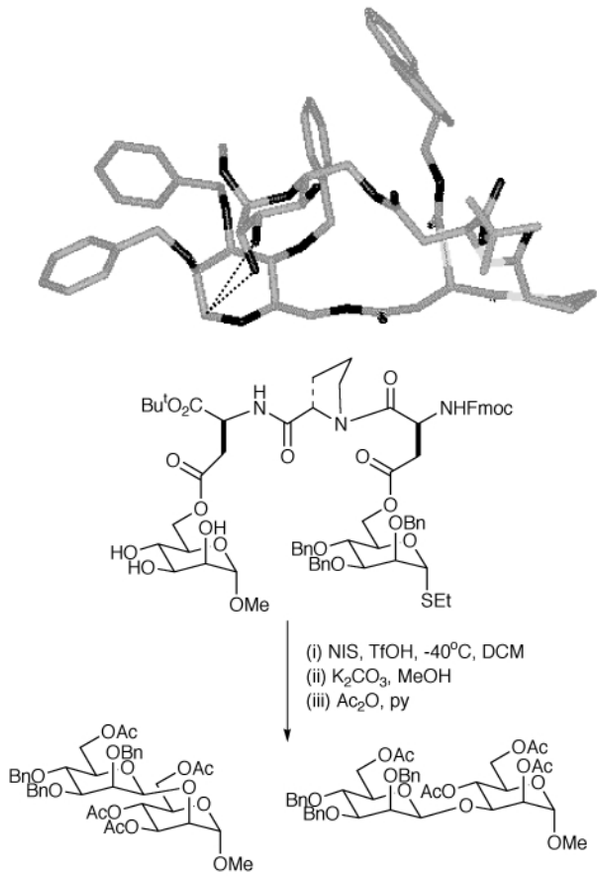
via the formation of α-nitrilium glycoside ion, appeared to be in operation in ether also.

Such tethered glycosylations have rather intensively focussed on similar linkages: silyl ethers, mixed ketals and simple flexible (alkyl *e.g.* sucinnoyl) or rigid (aryl) diesters. This is somewhat surprising. The intention of many of these IAD methods is to

alter glycosylation selectivity (regio- and stereo-) through the presence of the tether, yet only one use of a group of Nature's most readily available source of secondary structure—amino acids—has been reported.[120] This peptide-templated strategy exploits not only the structural benefits of peptide sequences but also the well-tried methods for their assembly. A number of mannose to mannose, O-6 to O-6 systems were constructed using di- and tri-peptide tethers in which the sugar–amino acid links were both Asp side chain esters. Upon activation, both increased regio- and stereo-selectivity were observed for these tethered systems over the corresponding untethered. The sequence Asp-Pro-Asp proved particularly successful—a finding that was rationalised through molecular modelling as being due to a well-defined turn in the tether that has the effect of orientating the OH-2 and OH-3 groups of the acceptor over the β-face of the donor (Scheme 10). Given the variety of natural and non-natural amino acids that are available to serve as stereodefined building blocks in this tethering system and their ready assembly, the great potential of this method is clear.

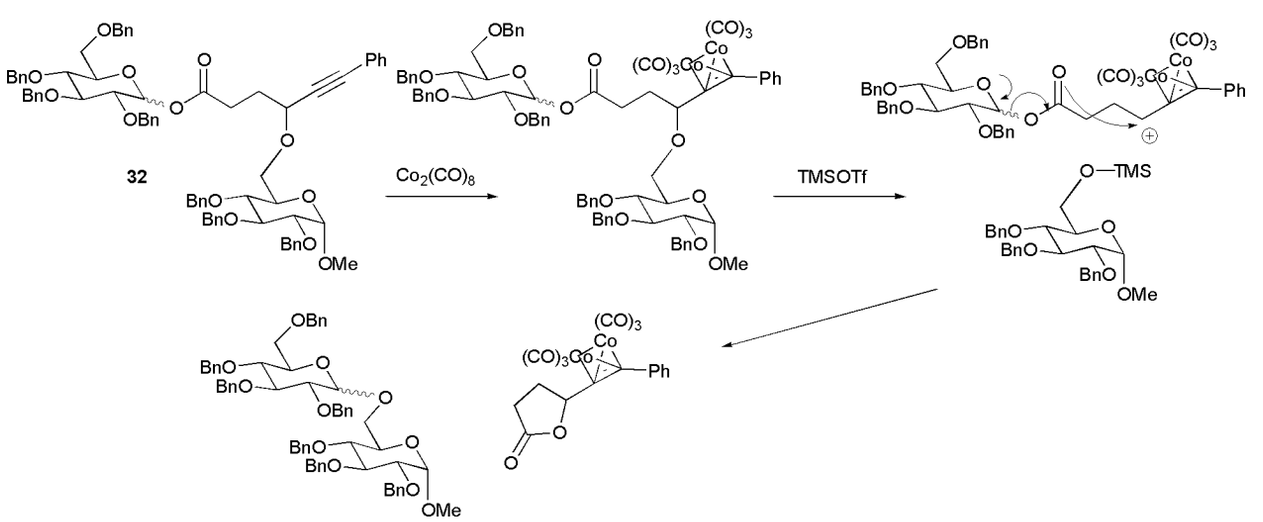
A novel tethered glycosylation based on the reaction of an alkyne–$Co_2(CO)_6$ complex has been described.[121] Following the construction of an alkyne containing tethered system, such as **32** (Scheme 11), conversion into complex and activation with TMSOTf led to what was described as "internal delivery" of the glycosyl acceptor and glycosylation in moderate yield. Interestingly, although the stereoselectivities and O-2 group–solvent participation that were observed seemed to suggest an intermolecular pathway, crossover experiments revealed only products consistent with an intramolecular mechanism.

The synthesis of a tethered H-type 2 oligosaccharide used the same tether that was intended for conformational restriction in the final product to also facilitate an intramolecular aglycon delivery approach. This allowed control of the stereoselectivity of the galactose–glucosamine link that was formed.[122]

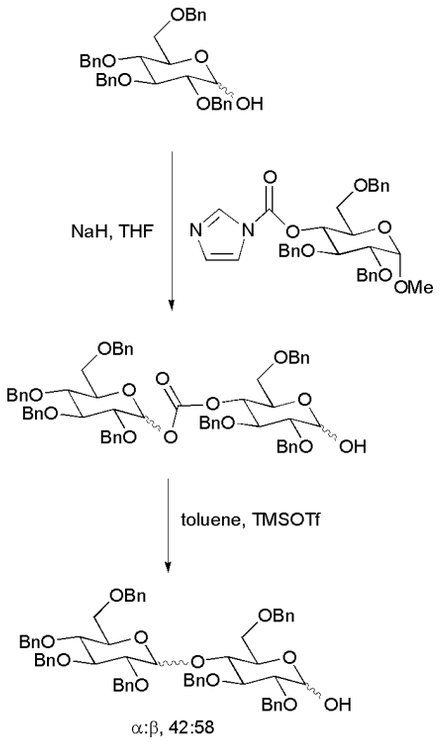


Scheme 10

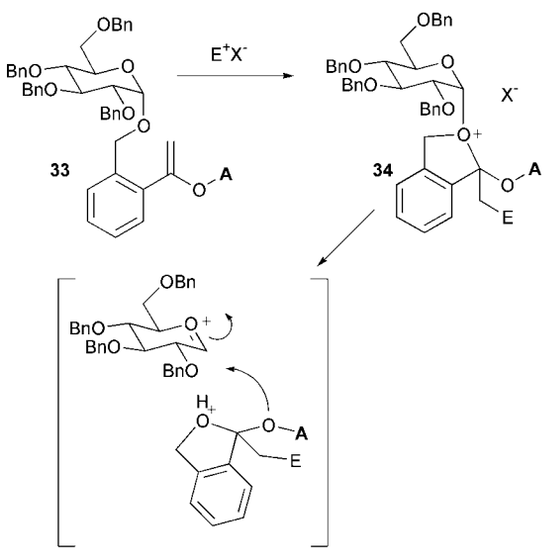
Few strategies have employed tethering *via* the donor leaving group (by necessity a temporary tether). A two step glycosylation procedure that involves the linking of two sugars by a mixed carbonate tethering from O-1 of a donor to the prospective glycosylation site of the acceptor, followed by silyl triflate activated decarboxylation, with concomitant glycosylation has been reported (Scheme 12).[123] Mixed glucosyl and galactosyl carbonates were constructed from acceptor-*p*-nitrophenyl or -imidazoyl carbonates. Subsequent results have shown that in these reactions only the β-carbonate reacts and that K_2CO_3 catalyzed carbonate formation using *N*-hydroxysuccinimidyl esters provides these in good yield.[124] Unsurprisingly, activation of these systems when the glycosyl donor bears a participatory O-2 group gave good yields of *trans*-1,2-glycosides. The low stereoselectivities obtained for the corresponding non-participatory systems have prompted Schmidt and Scheffler to investigate the nature of this glycosylation,[108] which was first described as intramolecular.[123] Competition experiments involving the activation of equimolar amounts of two different such carbonate tethered acceptor–donor systems led to complete scrambling thereby showing at least the partial if not total inter- rather than intra-molecular nature of these reactions. Recently, Schmidt and Scheffler have themselves provided another example of leaving group tethered glycosylation using the *cis* enol ether systems of type **33** (Scheme 13, where **A** is the acceptor).[125] Upon activation by an electrophile it was intended that the oxonium intermediate **34** would deliver acceptor **A** in close proximity to the ensuing glycosyl cation. If such a 1,3 shift were to occur with face selectivity, say in a solvent cage, then stereoselectivity might be expected. Alternatively, separation of leaving group and glycosyl cation in solution would lead to loss of selectivity. Thus the mechanistic possibilities for this tethered



Scheme 11



Scheme 12

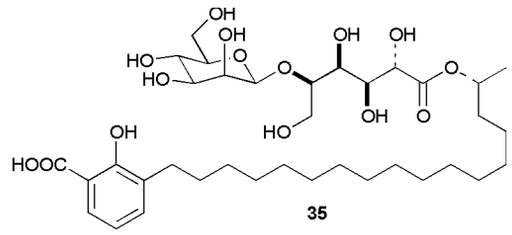


Scheme 13

reaction type might tread the boundary between inter- and intra-molecular by creating conditions for internal glycosylation *via* solvent cage with a formally intermolecular mechanism. Although stereoselectivities and subsequent scrambling in crossover experiments showed that PhSeOTf activation of these systems proceeded *via* an intermolecular mechanism, this concept still raises the interesting possibility of proximity controlled intermolecular glycosylations possibly, as suggested, within solvent cages.

3.5 Non-glycosyl cation based approaches

Furstner and Konetzki have described an indirect β-mannoside formation process which involves the formation of β-glucosides using a trichloroacetimidate donor with a participatory C-2 acetate, followed by inversion of configuration at O-2.[126] The latter step was achieved by high yielding ultrasound promoted S_N2 displacement of an O-2 triflate by AcO^- in a number of disaccharide systems. Furstner and Konetzki have used this method in an elegant total synthesis of caloporoside **35**, both segments being derived from glucose, which uses a Koenigs–Knorr glycosylation to construct the glycosidic link prior to C-2 inversion.[98]



35

A reversed mode glycosylation approach using 1,2 β-mannosyl or rhamnosyl stannylene acetals formed directly from their parent sugars as nucleophiles has been described as a solution to the β-mannoside problem.[127] Thus, displacement of primary and secondary triflates led to the formation of β(1,6) and β(1,4) linkages. Whilst more electron rich β-rhamnosyl stannylenes gave good yields, β-mannosyl stannylenes required O-3 protection to prevent migration of the stannylene acetal to O-3 with concomitant O-3 to O-6 or O-4 ether formation. A synthesis of the 1,1′-linked FG ring system of everninomycin utilized this method to stereoselectively form the required tricky Manβ(1,1′)αLyx linkage by reaction of the appropriate *manno*-stannane (ring F) with a *lyxo*-trichloroacetimidiate (ring G).[128] The generality of this method was shown for a number of glycosyl trichloroacetimidates. Interestingly, glycosyl fluorides gave good yields of branched (1,1′)(2,1″)-trisaccharides.

Alkylation of the alkoxides formed using NaH in DCM of 1-hydroxy sugars with allyl or benzyl bromide gives good yields of β- and α-glycosides in the absence and presence of Bu_4NI, respectively.[129] This stereoselectivity is attributed to enhanced reaction rate of Bu_4N^+ alkoxides as compared with Na^+ counterparts. This increased rate precludes Curtin–Hammett-type kinetics. Schmidt and Das have extended Lemieux's use of 1,4-additions by sugar alkoxides to 2-nitro-D-glycals for galactoside formation.[130] Stereoselectivity is base dependent: strong bases giving α-galactosides, weak amine bases giving β. Their use allowed the formation of Gal(1,6) disaccharides in exclusively α (KHMDS) or 2:3 β:α (DBU) stereoselectivities.

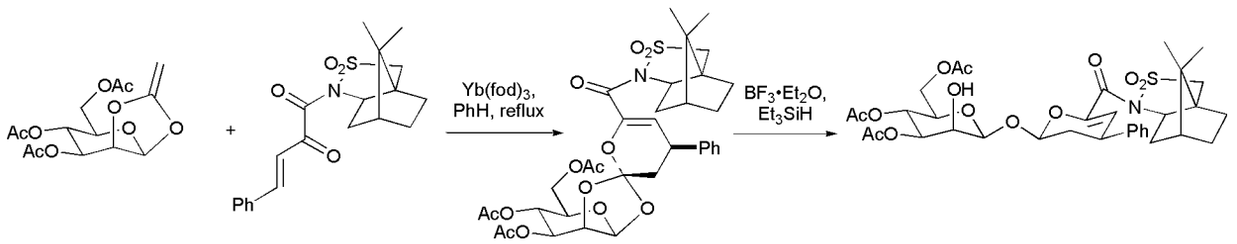
The oxidation potential and therefore the electron transfer from selenoglycosides is easier than that of their oxygen containing counterparts. Photoinduced one electron transfer of permethylated selenoglycosides using aromatic sensitizers allowed the formation of a radical cation, which collapsed to a glycosyl cation that allowed the first photochemical disaccharide formation.[131] The high β-selectivity is consistent with solvent participation by the acetonitrile used.

The use of the inverse electron demand hetero-Diels–Alder reactions of gluco and manno ketene acetal shown in Scheme 14, raises the possibility of using such methods for stereoselective trehalose synthesis.[132]

4 Chemoselectivity

4.1 Armed/disarmed

Whether the mechanism of a given glycosylation reaction possesses partial or complete S_N1 type character, the rate limiting step typically involves the development of positive charge in its transition state. As a consequence, the electronic effects of the substituents (both ring and anomeric) of a given glycosyl donor can markedly affect its reactivity. Thus, electron donating substituents such as found with ether protected donors tend to stabilize the rate-limiting transition state of glycosylation. Reactivity is therefore increased and such donors are termed "armed". Conversely, electron withdrawing substituents, such as esters, give rise to "disarmed" donors.



Scheme 14

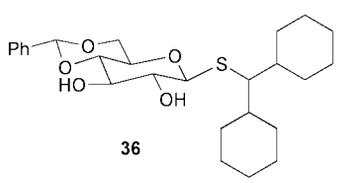
Such ideas of armed/disarmed have been expanded by additional concepts of active/latent (switching donor reactivity on or off *via* alteration of leaving group substituents), side-tracked (reversibly deactivating a donor before converting it back into its active form), one-pot (the use of layers of reactivity available through tuning to allow a controlled cascade of glycosylations with different donors one after another) or orthogonal (as for protecting groups—the use of donors that are activated under mutually exclusive conditions).

4.2 Active/latent: the effects of leaving group

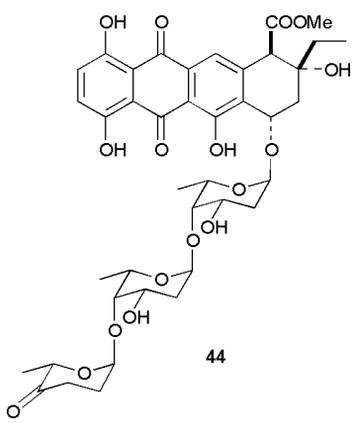
The nature of the leaving group of donors controls reactivity. In this way a reactive donor thioglycoside can react with a less reactive acceptor thioglycoside, which can itself act as a donor under more powerful activating conditions. Boons and co-workers have made a study of dicyclohexylmethyl thioglycosides as donors.[133] The bulk of the anomeric leaving group deactivates perbenzylated dicyclohexylmethyl thioglycoside to a so-called semi-disarmed level between perbenzyl ethyl thioglycoside and peracetyl ethyl thioglycoside. Furthermore, significant differences in reactivity between α and β dicyclohexylmethyl thioglycosides also allowed chemoselective activation. Application of this approach with the sterically less active dicyclohexylmethyl thioglycoside **36** as an acceptor and then donor allowed construction of a phytoalexin elicitor β-hexaglucoside.[134]

Fraser-Reid and Allen have prepared tetrasaccharide **43** from the protein to glycan linkage region of proteoglycans.[135] The key common intermediate benzylidene **37** (Scheme 15) was prepared *via* the corresponding pentenyl orthoester. The acylated counterparts react only to form orthoesters, yet benzoate **39** possesses sufficient reactivity whilst retaining a C-2 directing group for β-galactoside formation. Thus orthogonal glycosylation of **37** with trichloroacetimidate **38** gave the β-glucuronate-galactose disaccharide donor **39**. **41** was also used as a donor with the so-called "side-tracked" dibromoxyloside **40**. Thus, **40** was prepared from a pentenyl glycoside through bromination to act as an acceptor to give disaccharide **42** and then returned to a donor pentenyl glycoside through iodide induced elimination after the construction of tetrasaccharide **43**. The resultant donor **43** allowed formation of the glycopeptide bond.

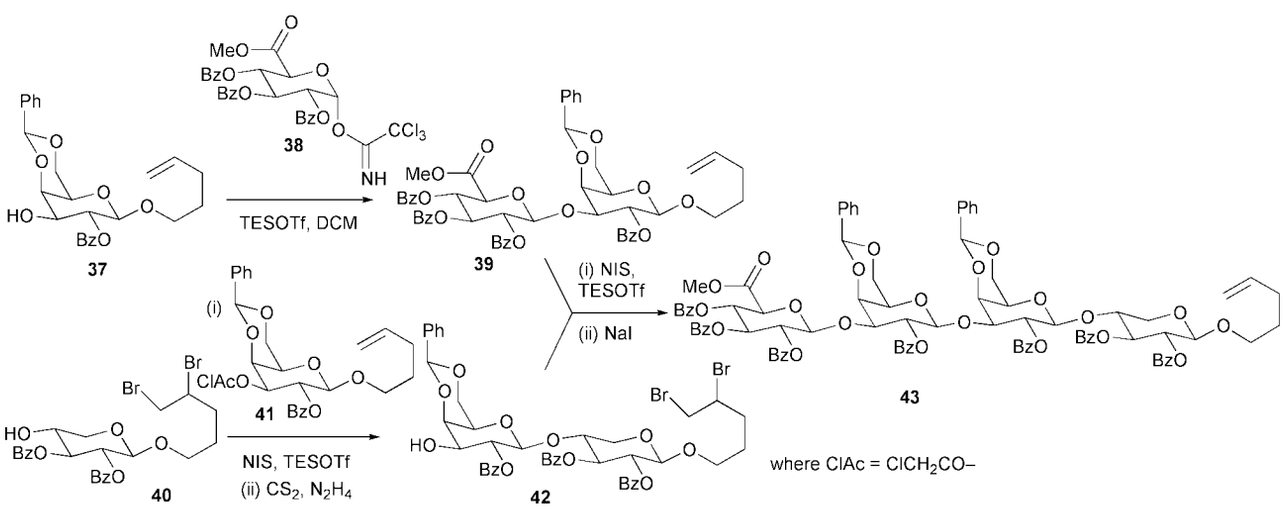
In a good example of using mechanistic understanding to improve efficiency, Kahne and co-workers have completed[136] a synthesis of ciclamycin 0 **44** that they started in 1993 but originally abandoned due to low yielding glycosylation of the aglycon with benzylated trisaccharide donor (16%); furthermore attempted deprotection led to cleavage of the aglycon–sugar linker, which is also benzylic. Another trisaccharide donor with DDQ-cleavable *p*-methoxybenzyl protection was therefore prepared. During the preparation, glycosylation of a thiophenyl glycoside acceptor led to oligomerization. It was



36



44



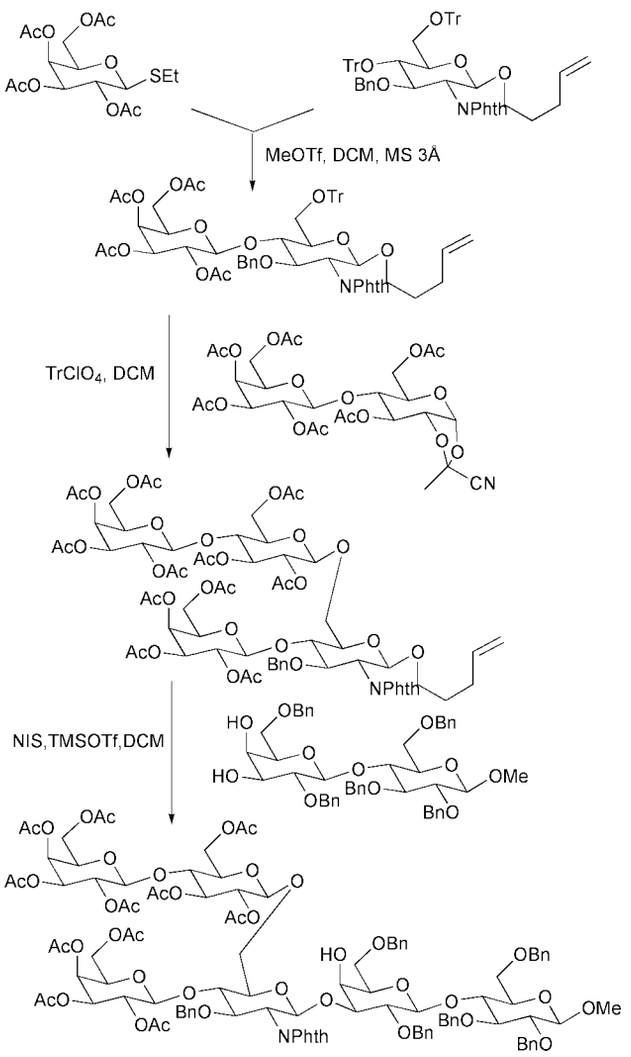
Scheme 15

reasoned that the PhSOTf that is released upon sulfoxide activation was in turn activating the thiophenyl group. This problem was solved using an alkene scavenger and a less nucleophilic, and therefore less easily activated, anomeric thiol substituent (2,5-dichlorothiophenol) in the acceptor. These optimisations to the construction of the trisaccharide donor allowed efficient final glycosylation and synthesis of ciclamycin 0 **44** in an overall 17% yield.

Roy and co-workers have used a *p*-nitrophenyl (pNP) phthalimide-protected *N*-acetylglucosaminide in a further example of a latent/active strategy.[137] After thioglycoside galactosylation and fucosylation, the latent pNP group was reduced and acetylated thereby allowing the active pNHAc phenylthiotrisaccharide that is formed to act as a donor in the synthesis of Lewis-x pentasaccharide.

4.3 Orthogonal glycosylations

Boons and Demchenko have described a highly convergent hexasaccharide synthesis which makes use of the orthogonal activation of thioglycoside, cyanoethylidenes and pentenyl glycosides with MeOTf, $TrClO_4$ and NIS–TMSOTf respectively (Scheme 16).[138] This is an exemplary synthesis that requires no further protecting group manipulations other than those required initially.



Scheme 16

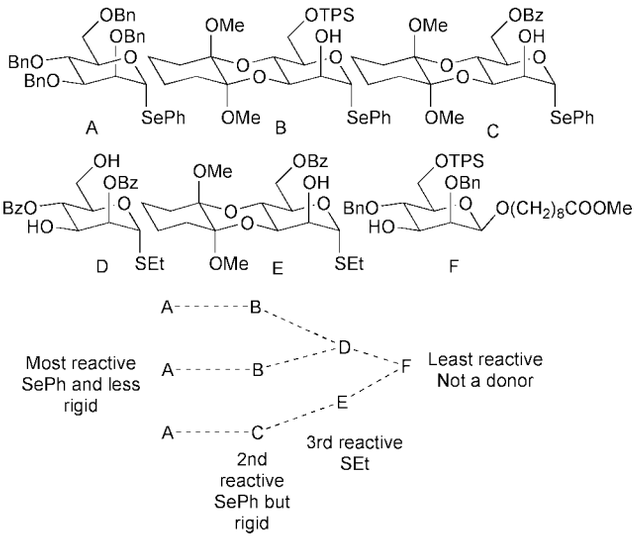
Sequential activation of orthogonal trichloroacetimidate (with TMSOTf) and thioglycosides (with subsequent addition of NIS) allowed rhamnosylations and glucosylations to create saponinglycosides.[139]

4.4 Reactivity tuning and one-pot reactions

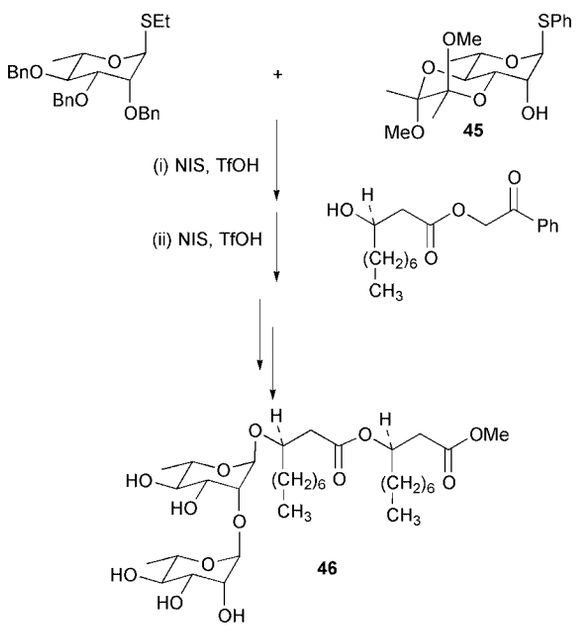
Wong and co-workers have published a comprehensive evaluation of the relative reactivities, through competition experiments followed by HPLC, of 50 thioglycoside donors as part of a newly initiated programme of one-pot oligosaccharide synthesis by reactivity tuning.[140] The data obtained have also allowed the development of a Macintosh programme, OptiMer, that will pick from the 50 suitable donors, on the basis of sufficient reactivity differences, those that will allow one-pot synthesis. To keep the goals of this project manageable, certain factors were held constant or limited in the study: activator (NIS, TfOH), leaving group (*p*-methylbenzenethiol), six sugar frameworks (glucose, galactose, mannose, fucose, *N*-acetylglucosamine, *N*-acetylgalactosamine) and 11 protecting group types. Some interesting and surprising correlations emerged. The increased reactivity of donors with a C-2 NHTroc as compared with phthaloylNH was noted. Stereochemistry gives rise to a reactivity order: fucosyl > galactosyl > glucosyl > mannosyl, attributed to an electron donating methyl group and O-4 lone pair participation in fucosyl alone and fucosyl *and* galactosyl, respectively. The glycosylation of a position was observed to have a slight deactivating effect in all cases; less so for glycosylation by ether protected sugars (1 to 2-fold) than for acylated (~10-fold), unless at O-2 where even glycosylation by perbenzylated sugars decreased activity ~5-fold: perhaps due to steric factors. In galactose, protection position has an influence in the order 4 > 3 > 2 > 6. Furthermore at C-2 in galactose, reactivity decreases with substituent BnO > BzO > PhthNH > ClAcO > N_3. The well known activating effects of ether over acyl were also observed but quantitatively varied with not only position but, once this variable was eliminated, with the nature of the other protecting groups present. This lack of absolute predictability led the authors to conclude that there was no simple numerical reactivity model to be found. However, from the data it appears that some form of polarization relationship might be in effect; the more electron withdrawing groups on the rest of the molecule, the less effect changing from ether to acyl would have and *vice versa*. Interestingly, an inverse correlation between the NMR chemical shift of the anomeric proton and normalised reactivity value was observed for the same core sugar structures, ranging from, for example, reactivity 17 000 (4.48 ppm) to 5.7 (4.98 ppm). Experiments to determine the self consistency of the method, which was largely based on comparison with 4 benchmark donors, showed an internal error of only ~10%. This valuable study lays down the groundwork for many more one-pot syntheses as the number of potential donors and corresponding reactivity relationships grows.

Ley and co-workers have published a further example of one-pot glycosylation that uses a single set of activation conditions (NIS–TfOH) and the four levels of donor reactivity outlined in Scheme 17.[141] Although low yields in certain coupling steps meant that a total one-pot approach was not feasible, this nonasaccharide synthesis remarkably utilized only five reaction protocols and points strongly to the advantages of reactivity tuned one-pot strategies. Recently, Ley and co-workers have also provided a systematic evaluation of reactivity tuning through protecting groups by comparing NMR-monitored glycosylations using benzoyl-, benzyl- and cyclohexane-1,2-diacetal-protected rhamnosyl and mannosyl donors.[142] Van Boom and co-workers have utilized Ley's one pot methodology to synthesize rhamnolipid **46** (Scheme 18) from butane-2,3-diacetal protected thioglycoside **45**.[143]

Boons and co-workers have described a convergent strategy that relied heavily on chemoselectivity and requires no protecting group manipulations other than introduction to the monosaccharide building blocks and final removal (Scheme 19).[144] Key points to note are (i) the IDCP activated glycosylation of **47** which relies on the greater reactivity both of 6-deoxy sugars and thioethyl glycosides; (ii) high nucleophilicity of the primary



Scheme 17



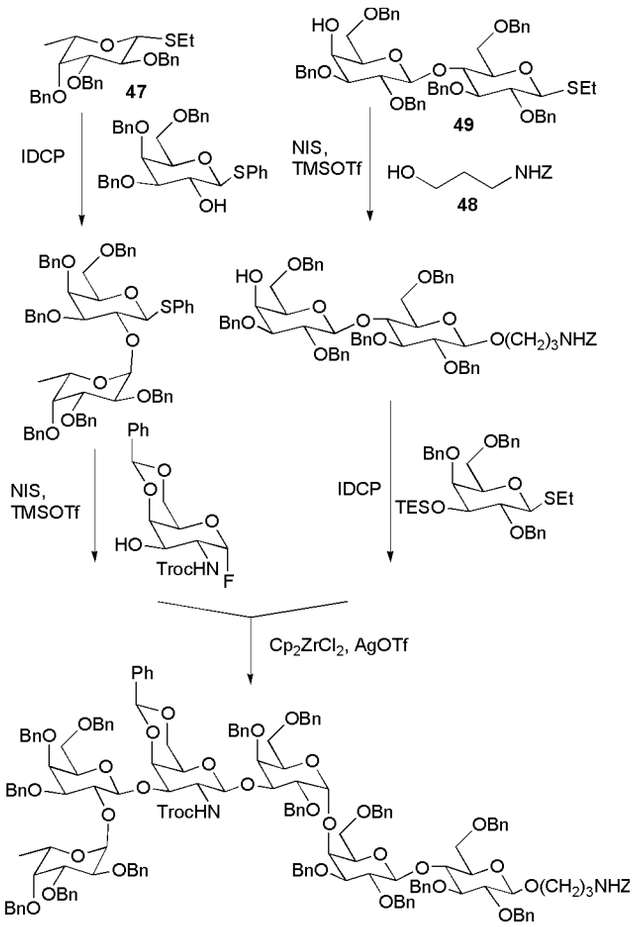
Scheme 18

hydroxy in **48** over the free axial hydroxy in **49** which prevents self condensation; (iii) apparent enhancement of nucleophilicity of a hydroxy group by a TES group (an unsilylated acceptor was much slower).

5 Regioselectivity

5.1 Protecting groups

The use of N-protection in 2-deoxy-2-amino sugar chemistry has been reviewed.[145] Pent-4-enoyl[146–149] C-2 amides and tetrachlorophthalimido[147–151] protected pentenyl glycosides have been described as C-2 protected *N*-acetylglucosamine donors. Tetrachlorophthalimides (TCPs) are particularly useful participatory C-2 N-protecting groups that are removed by 1,2-diaminoethane under more mild conditions than phthalimides.[150] They also show a useful level of compatibility with chloroacetyl, acetyl and benzoyl removal. 2,5-Dimethylpyrrole, which may be cleaved using hydroxylamine hydrochloride, has been used as a nitrogen protecting group in trichloroacetimidate donors.[152] The use of dimethylmaleoyl as an amino protecting group has been described and used in trichloroacetimidate donors.[153] It is introduced as its anhydride and cleaved using aq. NaOH then HCl (pH = 5) followed by acetylation to give NHAc. Hindsgaul and Qian have demonstrated



Scheme 19

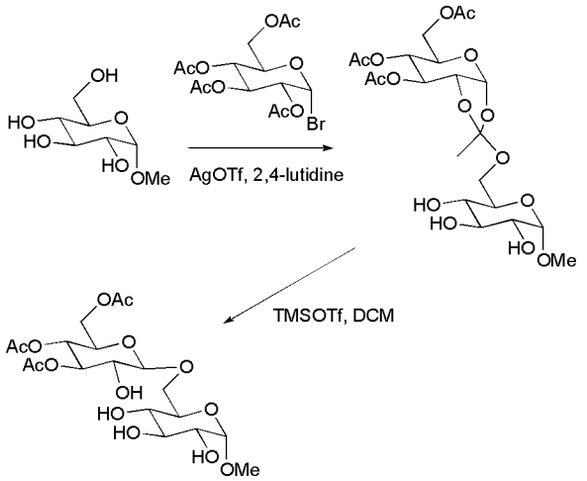
the use of the *p*-nitrobenzyloxycarbonyl (PNZ) group as a good participating substitute for 2-amino-β-glucoside formation.[154] It can be removed by hydrogenolysis or selectively in the presence of benzyl groups using sodium dithionite. The novel 2-naphthylmethyl protecting group can be selectively removed in the presence of benzyl ethers through careful hydrogenolysis.[155] *p*-Chlorobenzyl groups have been used to stabilize the α-fucoside linkage in Lewis-x trisaccharides under acidic conditions.[156]

Tritylated thioglycosides can be used as glycosyl donors as well as acceptors.[107] Stable under standard NIS–cat. TfOH conditions to act as donors, they are able to act as acceptors when activated by NIS–stoichiometric TMSOTf. This flexibility of reactivity opens up further opportunities for chemoselectivity using such glycosylations. Interestingly, the bulk of the O-6 trityl also increases α-stereoselectivity for non-participatory glucosylations.

5.2 Exploiting reactivity tuning and inherent reactivities

An elegant example that reports the exploitation of the differing nucleophilicity of hydroxy groups to allow a chemo- and regio-selective convergent strategy has been described.[157] This exploited the typical orders of nucleophilicity: primary hydroxy > equatorial secondary hydroxy > axial secondary hydroxy and hydroxy in ether protected sugars > hydroxy in ester protected sugars to allow, for example, the regioselective glycosylation of OH-3 of galactose over OH-4 and the use of thioglycoside donors with free hydroxy groups.

A new variation on the orthoester glycosylation method has been described[158] (Scheme 20). Regioselective formation of sugar–sugar orthoesters with OH-6 of the acceptor over other OH groups and with OH-3 over OH-4 in glucose when O-6 is protected is observed. Subsequent TMSOTf catalysed rearrangement led to highly regio- and stereo-selective (through



Scheme 20

C-2 participation) glycosylation. This method also provides a usefully stereoselective route to β,β-trehaloses.[159] Regioselective orthoester formation and subsequent rearrangement also allowed a selective O-6 glycosylation in the synthesis of the phytoalexin elicitor hexasaccharide, where Koenigs–Knorr glycosylation failed to distinguish O-4 from O-6.[160]

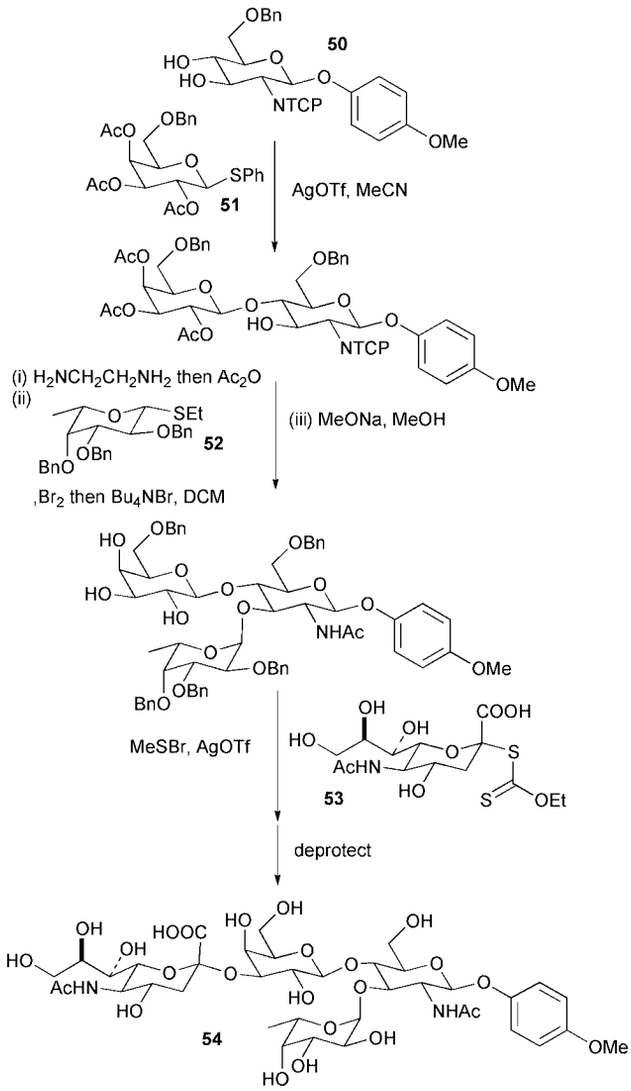
A one-pot strategy for branched oligosaccharide synthesis has been described.[161] Sequential activation of a mixture of a glycosyl bromide and then a thioglycoside in the presence of AgOTf and then NIS, TfOH, respectively, allowed sequential glycosylation of dihydroxy acceptors. The utility of this methodology was demonstrated by the one-pot preparation of 2,6-, 3,6-, 4,6- and 3,4-branched β-triglucosides by regioselective glycosylation of the primary OH-6 over OH-2, 3 or 4 and OH-3 over OH-4 in glucose. Problems of solubility of the methyl glycopyranoside acceptors in this method were overcome by the use of 1,2-ethylidene protection.[162] This method has also allowed the formation of a hexasaccharide phytoalexin inhibitor based on the synthesis of a 3,6-branched β-triglucoside motif which was formed through the regioselective formation of a 3,6-diorthoester.[162]

An important example of OH activation is illustrated by the use of the intermediate trityl ether monosaccharide acceptor in Scheme 16. This allowed selective galactosylation of the secondary O-4 over primary O-6. Notably a parallel reaction with OH-4 free fails.[138]

Two highly regioselective glycosylations have allowed a very efficient synthesis of sialyl Lewis-x (sLe^x).[163] Galactosylation of partially protected GlcNAc **50** (Scheme 21) proceeded with excellent stereoselectivity (due to neighbouring group participation) and regioselectivity (O-4 only). Subsequent fucosylation and then a regio- and stereo-selective sialylation gave sLe^x derivative **54** in a remarkable 29% yield from protected donors and acceptors **50**, **51**, **52** and **53**. Notably, attempts at selectively fucosylating **50** gave only good regioselectivity (still for O-4 and therefore not useful in the synthesis of sLe^x) or stereoselectivity —not both. This may indicate the existence of a mismatched pair as compared with the more selective use of D-galactose, which has an enantiomeric configuration to L-fucose.

5.3 Intramolecular aglycon delivery (IAD)

Effects of tethering position on regioselectivity line have been investigated for phthaloyl linked systems; whilst O-6 to O-2, donor to acceptor linking in mannosyl to glucoside-system **55**, gave O-3 glycosylation, the corresponding O-6 to O-6 linked systems gave O-4 glycosylation.[119] Similar regioselective effects were also seen in a glucosyl to glucoside system. Furthermore in a similarly tethered O-6 glycosyl, O-2 donor system, temperature dependent stereoselectivity (5 : 1 β : α at −78 °C; 1 : 1 at rt) was observed.



Scheme 21

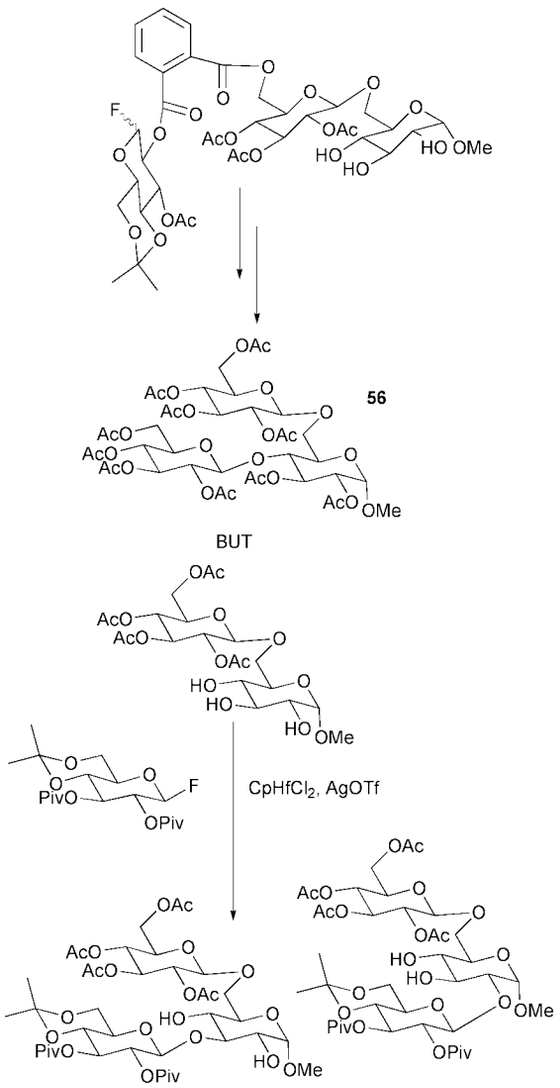
A rigid phthalate tether was also used in a regio- and stereoselective glycosylation strategy (christened "remote glycosylation") to form the β(1,4) link in branched triglucoside **56** (Scheme 22).[164] A parallel untethered glycosylation led only to O-2 or O-3 glycosylation.

5.4 One-pot methods

The regioselective orthoester strategy described in Section 5.2 has been extended to the synthesis of a number of 1,2-*trans* (1,3), (1,6) and 3,6-branched oligosaccharides from a 1,2-*O*-ethylidenated mannose acceptor.[165] The TMSOTf catalysed rearrangement, as well as appearing highly stereoselective due to dioxolonium formation, also maintains the regioselectivity of the orthoester formation, perhaps due to proximity effects.

6 Higher sugars

Dondoni and co-workers have described the use of thiazolylketose acetates such as **57** (Scheme 23), which may be activated using TMSOTf, to give ketosyl and ulonosyl glycosides,[166]



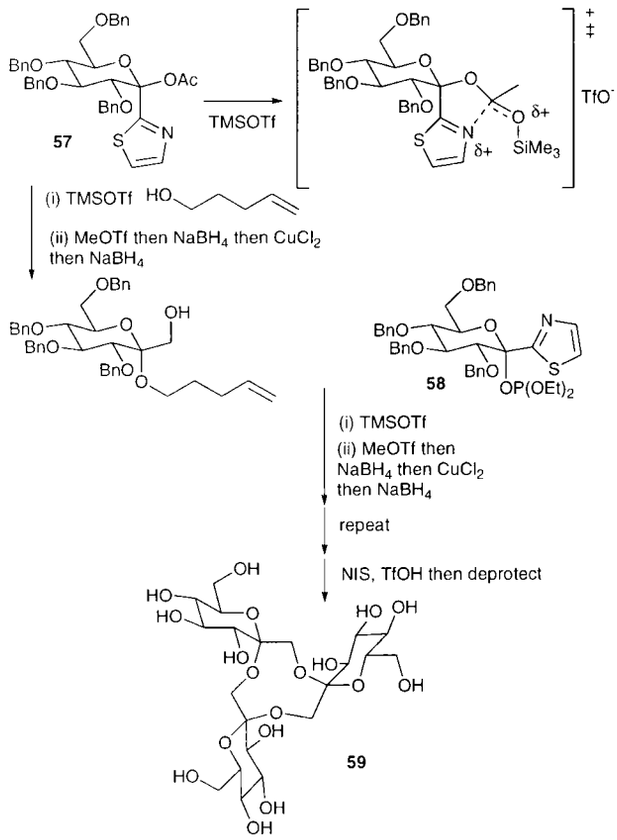
Scheme 22

the 1-C thiazolyl mannose and galactose compounds giving exclusively α products whilst the glucose gave approximately 1 : 1 α : β. The high reactivity of the acetate has been attributed to anchimeric assistance by the thiazolyl group following Lewis acid activation of the acetate. In a spectacular example of this method, **57** and the corresponding phosphite **58** were used to make the cyclic ketotrisaccharide **59**. This work has recently been reviewed.[167]

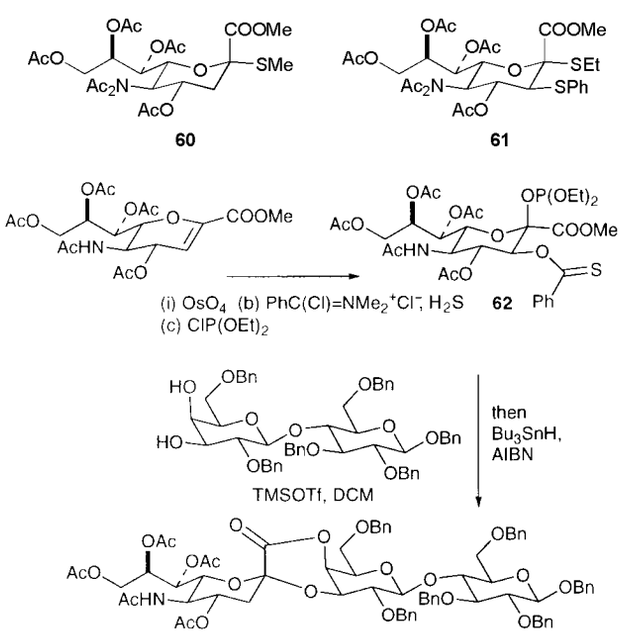
Glycal formation through elimination of the anomeric leaving group plagues the use of sialyl donors. Furthermore, alkylative activators may alkylate the C-5 NHAc group.[168] Remarkably diacetylation of the NHAc of *N*-acetylneuraminic acid donor to give **60** greatly increases its reactivity in the sialylation of galactosides[168] and the very poor nucleophile, the OH-8 of *N*-acetylneuraminic acid itself.[169] Sialyl donor **61** bearing 2 acetyl groups *and* a C-3 SPh also shares this enhanced reactivity.[170] Furthermore the SPh substituent reduces elimination by raising the pK_a of the H-3 proton and through episulfonium formation allows α-stereoselectivity.

A thorough investigation has revealed phosphite **62** as an excellent sialyl donor, which is easily prepared from sialic acid and as a result of a C-2 thionoester gives very high α-selectivities (Scheme 24).[171]

PhSOTf activated sialylation using xanthate donors gave high α-selectivities in the synthesis of a 1C-^{13}C labelled GM ganglioside[172] and in pentasaccharide ganglioside LM1 due to solvent participation by acetonitrile.[173] An *N*-glycolylneuraminic acid thioglycoside was used to synthesize a sea cucumber disaccharide ganglioside analogue from a protected



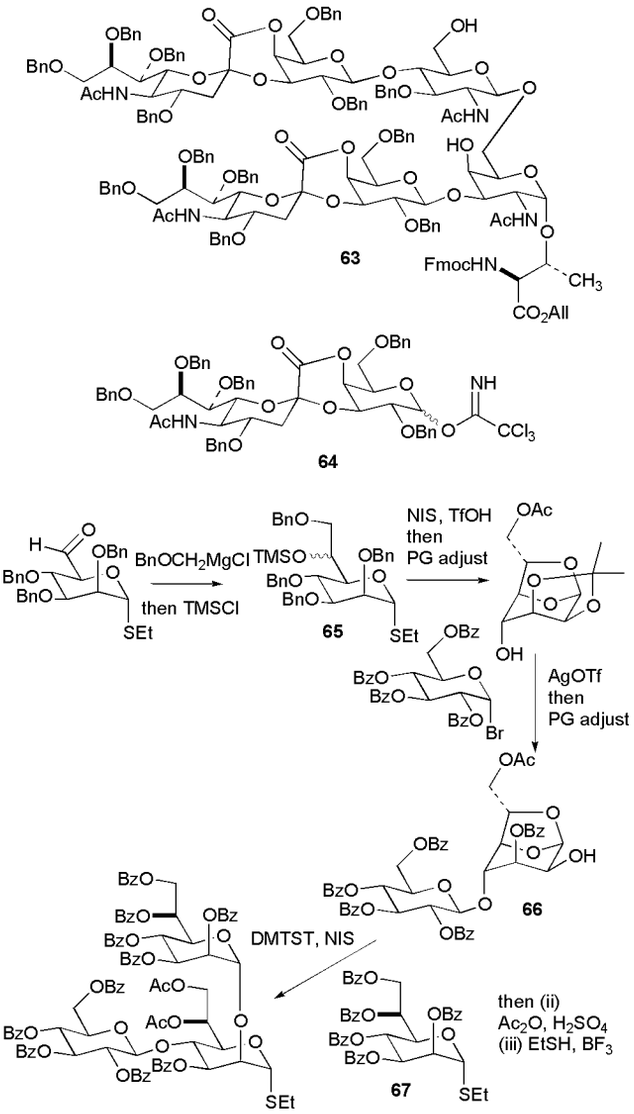
Scheme 23



Scheme 24

starfish cerebroside with α-selectivity.[174] In a *tour de force* of protecting group and glycosylation chemistry, the hexasaccharide glycopeptide **63** has been convergently constructed using an initial thioglycoside sialyl donor and trichloroacetimidate glycosides for block assembly *via* the key divergent lactone intermediate **64**.[175]

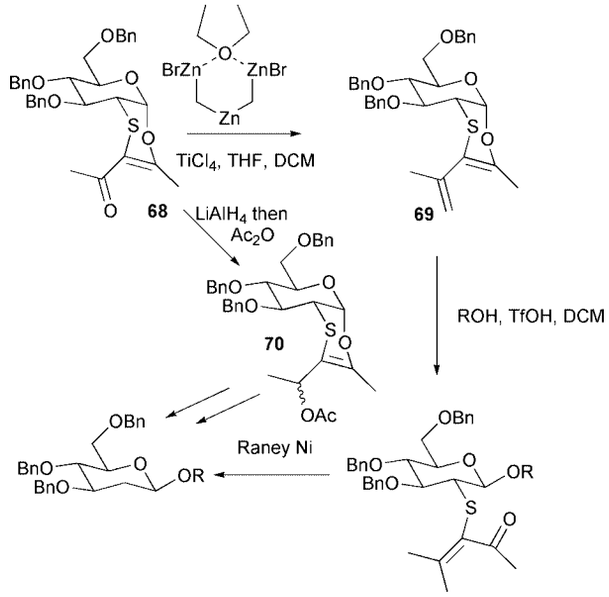
The use of 1,6-anhydrooligosaccharide **66** (Scheme 25) as an acceptor was central to the successful synthesis of a branched tetrasaccharide Kdo-containing core structure.[176] Kdo thioglycoside donor **67** glycosylated **66** in 70% yield, whereas no coupling was observed with the equivalent monocyclic acceptor. The 1,6-anhydro link was itself formed using NIS activation of thioglycoside **65**.



Scheme 25

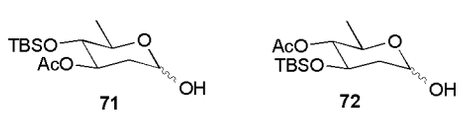
7 2-Deoxy sugars

A problem exists for 2-deoxyglycosides, namely the lack of assistance from C-2 substituents makes the preparation of pure anomers difficult. This usually means that the C-2 substituent is retained in order to aid stereocontrol and standard methods require the removal of the participatory or directing C-2 substituent after stereocontrol has been achieved. Franck and Marzabadi have presented a partial solution by extending the use of vinylglycosides as donors.[177] The cycloadduct **68** (Scheme 26), itself a slow donor, is converted to the more active **69** using the Nysted reagent. Activation using TfOH in DCM gives good yields of β-glycosides which are then readily desulfurized with Raney nickel. In a virtually identical approach, the acetate **70**, available from **68** by $LiAlH_4$ reduction and acetylation, has also been used.[178] MeOTf activation in $MeNO_2$ gives after 30 minutes β-glycosides but prolonged activation leads to equilibration, as indicated by colour changes, to the corresponding α anomers, presumably *via* a glycosyl cation intermediate. Collaborative work between these 2 groups on α-glucosystems such as **69** and the corresponding β-manno system suggests that contrary to previous suggestions in more rigid systems, that when considering vinyloxy leaving groups in flexible systems, β-*gluco* are more reactve than α-*manno*.[179] The beneficial use of Bu_4NOTf as an additive has led the authors to suggest that S_N2 reaction of anomeric triflates intermediates, as proposed by Crich (see Section 2.10), is the source of stereoselectivity.[180] The same cycloaddition methodology also allows



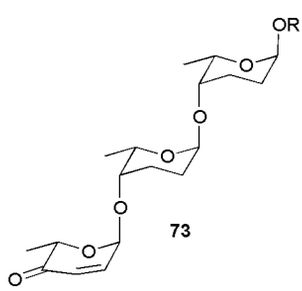
Scheme 26

the stereoselective synthesis of various aryl 2-deoxyglycosides such as α-tyrosine glucoside.[181] This type of heterocycloaddition was also used in the formation of a 2-deoxy steroid glycoside[138] and has led to the useful preparation of several glycosidic linkage motifs from antitumour agent aureolic acid.[136] High α-selectivities are observed in montmorillonite K-10 catalyzed glycosylations using **71**, but a dramatic reversal under the same conditions to give β-oliviosides using **72** was attributed to a C-4 participatory effect of the acetate in **72**.[182] The use of 2-deoxyglycosyl *N*,*N*-diisopropylphosphoramidites, prepared from the treatment of the corresponding pyranoses with ethoxy bis(diisopropylamino)phosphite in the presence of diisopropylammonium tetrazolide, as glycosyl donors allows the construction of 2-deoxyglycosidic linkages in good yield and with very high α-selectivities.[183] The use of removable thionoesters as C-2 participatory groups previously described for sialyl donors has been extended to stereoselective α- and β-2-deoxyglycoside syntheses from *manno* and *gluco*-trichloroacetimidates.[184]



BBr_3 and BCl_3 have been used as activators that allow formation of simple alkyl 2-deoxyglycosides from glycals, presumably through the formation of HBr and HCl respectively, in a manner that, depending on the glycal or acceptor, is superior to the use of CSA or TsOH.[185] High α-stereoselectivities are a result of equilibration under these reaction conditions.

2,3,6-Tridexoygenated trisaccharide **73**, a component of the aquayamycin antibiotic PI-080, has been prepared in an impressive, reiterative glycosylation then tungsten-mediated cyclization strategy that utilizes alkynol building blocks and is compatible with the terminal aculoside in **73**.[186]



The synthesis of **73** was also used to illustrate that when activated by Me_3OBF_4 at −78 °C, glycosyl tetrazoles of 2,3,6-trideoxysugars give adequate yields of α-glycosides.[187] These tetrazole donors may be formed by phosphoramidite activation of the corresponding 1-hydroxy sugars.

Kahne and co-workers have used their sulfoxide methodology[100] to reconstruct the glycosidic bond between vancosamine (a 3-amino-2-deoxy sugar) and the D-glucosyl residue in vancomycin. Only the required axial glycoside was isolated.

8 Enzymatic methods

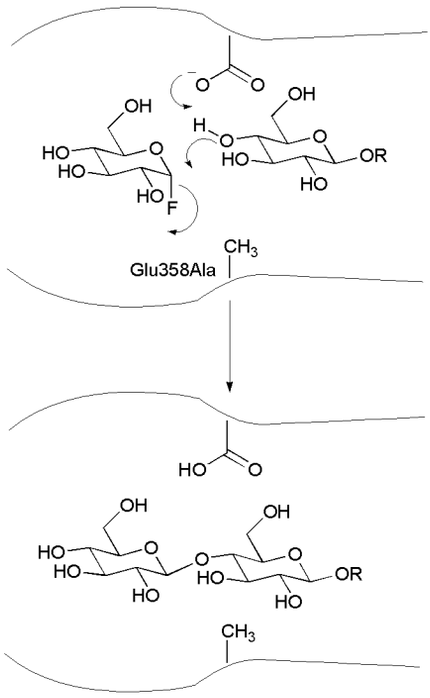
Enzymatic methods are now widely accepted as being as much a part of oligosaccharide synthesis as more traditional chemical methods. Their suitability will of course depend on the circumstances and target oligosaccharides and/or linkages. Given the often long-winded protection regimes required for most chemical glycosylations, the use of enyzme-catalyzed one step systems is clearly attractive. Thus, sialyltransferases or transialidases typically provide a method for sialylation superior to any chemical techniques. However, there will also be occasions when the complexity of enzyme systems, low yields, stringent specificities or the lack of an available catalyst will favour chemical systems. For example, until relatively recently examples of enzyme catalyzed β-mannosylations have been limited in number and/or effectiveness. Of course, these potential limitations of enzymes will diminish as usage continues to rapidly expand. Many of the exciting recent developments in this field have been covered in three excellent reviews,[188–190] and aspects of the use of glycosyltransferases and glycosidases applied to the synthesis of sLe^x have also been discussed.[191]

8.1 Glycosidases

An exemplary screening study using various pNP glycosides as donors and carbohydrates as acceptors, shows the sort of thoroughness that is required for establishing the true synthetic utility of glycosidases.[192] In this excellent work approximately 60 crude preparations were screened for 7 activity types and once identified, regioselectivities were determined.

In a highly elegant strategy Withers and co-workers have obtained very high yields for glycosylations using glycosyl fluoride donors with glycosidase mutants devoid of the nucleophilic active-site carboxylate.[193] These so-called glycosynthases allow oligosaccharide synthesis but are unable to hydrolyse O-glycosidic linkages. Thus, the Glu358Ala mutant of the glucosidase from *Agrobacterium sp*., in which the $–CH_2CH_2COO^-$ of glutamate is chopped back to just $–CH_3$, was created. This leaves an intact active site shape and general base catalyst but no natural activity. This mutant was then used with activated glucosyl fluoride donors and various acceptors to give high to excellent (66–92%) yields of oligosaccharides without concomitant hydrolysis (Scheme 27). In fact, the reaction is so efficient that the major problem of this method is oligomerization to tri-, tetra- and higher saccharides. The potential of this method was further illustrated by the use of the 2-deoxy-2-fluoroglucosides as acceptors that would have irreversibly inhibited a wildtype enzyme.

β-Galactosidases from *Bacillus circulans*[194,195] or *Bacillus singularis*[196] allow the synthesis of Galβ(1,4) disaccharides. *Bacillus circulans* galactosidase also shows this β(1,4) regioselectivity in the glycosidation of a wide range of acceptors[197] including thioglycosides.[194] This selectivity increases with the number of saccharide residues in the acceptor, as demonstrated by the synthesis of the trisaccharide Galβ(1,4)GlcNAcβ(1,6)-GalNAc in 48% yield.[197] Interestingly, enhanced β(1,3) regioselectivity is seen for this enzyme with β-1-*N*-acetamido-D-glucopyranose, a simple model for N-linked glycopeptides, as an acceptor, a result attributed to enhanced potential for hydrogen bonding by the acceptor aglycon.[198] This same study also thoroughly investigated the relative activities of



Scheme 27

B. circulans β-galactosidase with various organic cosolvents: acetonitrile was by far the most detrimental and 30% acetone v/v was finally chosen as optimal. Non-covalent coating of β-galactosidases with a polyhydroxy headgroup lipid allowed the use of these enzymes in non-polar organic solvents such as diisopropyl ether.[199] With simple alcohol acceptors the specificities of these systems appear to reflect those of the native unmodified enzymes in aqueous systems and conversions as high as 62% were reported, although oligosaccharide synthesis was not mentioned. The same lipid-coated galactosidase also showed superior activities in supercritical carbon dioxide ($scCO_2$) relative to diisopropyl ether and over non-coated enzyme in $scCO_2$.[200]

Regioselective β(1,4)-galactosylation of GlcNAcα(1,2)Man-αSPh using a galactosidase from *Bifidobacterium bifidium* proved a key step in the first synthesis of the serine tetrapeptide from α-dystroglycan, which contains a rare Man-Ser linkage.[201] Chemical glycosylation of the serine side chain through NIS, TfOH activation of a protected form of the thioglycoside that is the product of galactosylation followed by sialyltransferase mediated sialylation gave the final product in just 9 steps.

Screening of recombinant thermophilic glycosidases from the Diversa Corporation allowed the identification of high yielding β(1,4) galactosidases that formed *N*-acetyllactosamine from *N*-acetylglucosamine in 61% in 30 minutes using a pNP galactoside donor.[202] Ion exchange chromatography and the use of the thermophilic β-galactosidase Gly001-09 (operating temperature 85–90 °C) also allowed the synthesis of lactosamine using lactose and glucosamine as starting materials on a gram scale with good 1,4 regioselectivity.[203,204] Notably both the normally good 1,4 selectivity of *B. circulans* galactosidase, which gave 10% 1,4 and 6% 1,6, and the activity of bovine β(1,4)-galactosyltransferase, the latter due to its stringent substrate specificities, failed in this system.

It should be noted that the 1,4 selectivities shown by these β-galactosidases complements the 1,6 selectivity of *Escherichia coli* β-galactosidase and the 1,3 selectivity of bovine testes β-galactosidase. For example, the Galβ(1,3)GalNAcαSer TF determinant has been synthesized using a β-galactosidase from bovine testes.[205,206] Due to its broad donor specificity the β-glycosidase from the thermophilic microorganism *Thermus thermophilus* allows Galβ(1,3) autocondensation and Glcβ(1,3)

transglycosylation.[207] Reduced hydrolysis product and increased rates of conversion have been reported in transglycosylations to simple alcohols catalyzed by other thermophilic β-galactosidases in microwave irradiated, dry media.[208]

The importance of the so-called α-Gal epitopes to immunoregulatory therapies has prompted a number of enzymatic syntheses of Galα(1,3)Gal containing oligosaccharides. Crout and co-workers have described a practical (and simpler alternative to galactosyltransferases, see below) α-galactosidase approach which gives yields of α-Gal epitope saccharides of 42-48% with exclusive α(1,3) selectivity using the readily available α-galactosidase from *Penicillium multicolor*.[209] This has improved a previous sequential use of the β-galactosidase (*Bacillus circulans*) and an α-galactosidase (*Aspergillus oryzae*) to give a linear α-Gal epitope trisaccharide; until the use of the *P. multicolor* enzyme, lack of regioselectivity in the α-galactosylation had necessitated separation from the α(1,6) side product.[210] The α-galactosidase from *A. niger* gave only α(1,6) products whereas those of *Coffea arabica* and *A. oryzae* gave mixtures of Galα(1,6) and Galα(1,3) products.[211] A second galactosidase from *A. oryzae* interestingly gave only an α(1,6) *branched* trisaccharide product from lactose. Furthermore increasing the size of the anomeric substituent in the thioglycoside acceptor gave better yields.

The first use of a readily available β-mannosidase using *p*-nitrophenyl β-mannoside as a donor provides a vital enzyme-catalyzed alternative to the variety of chemical methods for this difficult to form linkage.[212] The enzyme was isolated from the crude extract of hexosaminidase available from *A. oryzae* and gave 26% yield of the β-*manno*-trisaccharide Manβ(1,4)GlcNAcβ(1,4)GlcNAc.

The β-*N*-acetylhexosaminidase from *Aspergillus oryzae* shows a β(1,4) selectivity for D-*gluco* substrates but a β(1,6) selectivity for D-*galacto* substrates.[197] Unusually, the same hexosaminidase catalyzes the transfer of *N*-acetylglucosamine from pNP-β-GlcNAc to the OH-1 of mannose to give as the major product a trehalose-type derivative and is the first example of a galactosidase catalyzed glycosylation of an anomeric hydroxy.[213]

The often broad acceptor specificity of glycosidases, whilst providing an advantage in terms of general utility, may lead to low regioselectivities. To overcome this problem, protease catalyzed regioselective acylation of O-6′ of lactose allowed selective α-galactosylation of O-3′ using the α-galactosidase from *Talaromyces flavus*, by blocking the competing O-6′ glycosylation site.[214] Simple glycosides may be synthesised by reverse hydrolysis from the corresponding parent sugars using an appropriate glycosidase in a reaction solution containing a high (up to 90%) proportion of the acceptor alcohol.[215] It was found that unlike the use of lipases, where almost all water can be excluded, at least 5% is required for the optimal activity of glycosidases. By drawing an analogy with acyltransferase biocatalysis, where the use of, for example vinyl acetate, is common due to the formation of non-nucleophilic and therefore non-competing acetaldehyde, vinyl galactosides have been suggested as donors for β-galactosidase catalysed glycosylations.[216] Although at room temperature standard NP donors gave better yields, at −7 °C yields of up to 80% were reported for vinyl glycosides. This reversal is attributed to a reduction in hydrolytic activity and a concomitantly lower reduction in transglycosylation activity. The use of nitropyridyl (3- and 5-) leaving groups offers the advantages of higher solubility over concentrated pNP glycoside donors (Scheme 5).[217] Saturated β-galactosyl, glucosyl and *N*-acetylglucosaminyl donor concentrations of 600, 300 and 50 mM as compared with 100, 100, and 5mM for pNP glycosides gave higher yields than pNP controls in the hands of the authors. Furthermore, increased reactivity (see the chemical activation of this same donor type in Section 2.11) allowed shorter reaction times.

8.2 Glycosyltransferases

Nine diantennary glycodelin oligosaccharides ranging from hepta- to undeca-saccharides have been synthesised using a combination of conventional chemical glycosylation and glycosyltransferase catalyzed elaborations.[218] Participatory selenomannosides allowed the synthesis of a branched Manα-(1,6)[Manα(1,3)]Man core. However, steric mismatches in glycosylation of the core with Lewis-x thioglycoside donors gave poor yields either under NIS–AgOTf activation or *via* the bromide at lower temperature. This problem was circumvented by using participatory lactosaminyl donors. Unfortunately steric hindrance led to reduced β-selectivities—a problem that was partly solved by reducing the reactivity of the donor through acyl rather than ether protection (thereby reducing the reactivity of the oxonium intermediate, which can give α products, relative to the dioxolonium). Choice of strategy for final elaborations depended on the linkage required. Fucα(1,3)GlcNAc and all sialyl links were introduced using fucosyltransferases and an α(2,3)sialyl transferase. Remaining Fucα(1,2)Gal links were achieved chemically. This general method demonstrates both the strengths (excellent strategic planning) and weaknesses (unpredictable steric mismatches and over specificity of glycosyltransferases) of current approaches and so illustrates well the challenges for oligosaccharide synthesis that remain.

Ingenious modifications of enzymatic systems often circumvent problems of inhibition by products. For example, the problem of inhibition by nucleotide diphosphate leaving group of glycosyltransferases has been solved by *in situ* regeneration of glycosyl nucleotide diphosphates (also an economic benefit as these are expensive and difficult to prepare). A new three enzyme system using sucrose synthase is driven in reverse to give glucosyl UDP, followed by epimerization with epimerase to give galactosyl UDP, which is the substrate for the third and key synthetic enzyme, galactosyltransferase.[219]

A thiol spacer arm-linked *N*-acetylglucosamine was loaded onto a thiopyridyl sepharose matrix and β(1,4)galactosyltransferase galactosylations revealed optimal efficiencies for the longest linker length (78 atoms).[220] Sequential β(1,4)Gal-T, Sial-T and Fuc-T reactions allowed the synthesis of SLe^x in 57% overall yield after cleavage from the support with DTT. *N*-Acetylglucosaminyltransferase and galactosyltransferase have also been extended to the use of sepharose-acceptor conjugates linked by a squarate linker.[221] Bovine β(1,4)galactosyltransferase has been used to galactosylate OH-4 of C-glucosides bearing hydroxymethyl and protected aminomethyl substituents at the pseudoanomeric centre.[222] Notably, the use of *meso* compounds opens up opportunities for enzymatic desymmetrization.

With the aim of preparing potential α-Gal epitope containing structures, the Wang group has used a recombinant bovine α(1,3)galactosyltransferase.[223] To ensure solubility of this naturally membrane-bound protein a truncated domain was designed. When the corresponding gene sequence was cloned into a readily available pET vector and then expressed in *E. coli*, this gave high specific activities well above those from extracting such enzymes from natural sources. This allowed ready access to α(1,3)Gal bond formation with a range of C-1 and C-2 modified lactose and lactosamine acceptors. A novel galactose epimerase α(1,3)galactosyltransferase fusion protein has also been described which utilizes the cheaper substrate UDP-Glc.[204]

A truncated yeast β(1,4)-mannosyltransferase has been expressed in *E. coli*.[224] Unlike, the membrane bound native form, this novel form is devoid of a hydrophobic membrane-anchor region and is therefore soluble. Furthermore, His-tagging (addition of a multihistidine sequence to a terminus of the protein) allowed its easy purification with a nickel affinity column. This also allowed immobilization and thereby stabil-

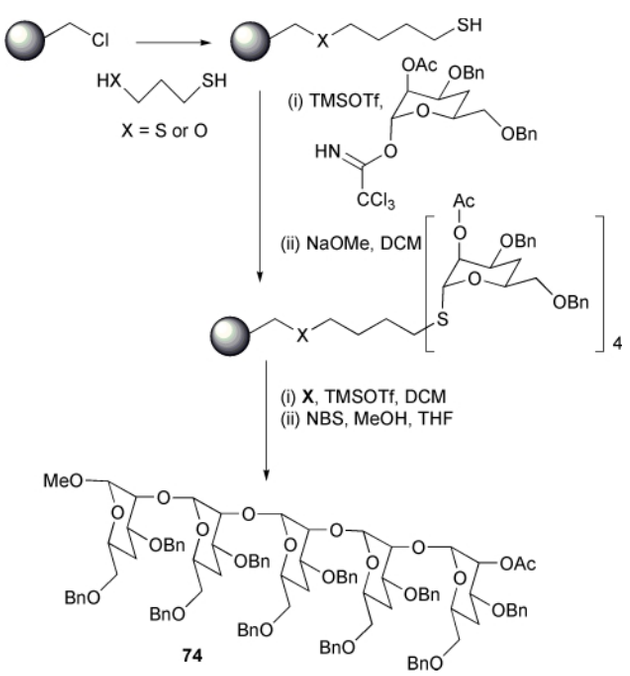
ization. This enzyme catalyzed the transfer of a β-mannosyl unit to OH-4′ of natural and mimetic chitobiosyl phospholipid acceptors from donor GDP-mannose. A Rhaα(1,3)Galβ-chromophore acceptor has been β(1,4)-mannosylated using a recombinant mannosyltransferase from *Salmonella* thereby expanding the substrate specificity from the native acceptor which is very specific and difficult to synthesise.[225]

9 Polymer supported methods and library syntheses

Two very useful and informative reviews have recently appeared in the area of polymer-supported oligosaccharide syntheses[226,227] and the following section should be viewed as an illustrative supplement to these works.

One of the very earliest examples of the use of trichloroacetimidates on polymer supports (see also Schmidt's thioglycoside linked system) utilized a glucoside acceptor attached to a succinoyl linker at O-2 or O-3. Glycosylation with peracetylglycosyl trichloroacetimidate proceeded in moderate yield.[228] Clearly, solid phase synthesis requires repetitive glycosylations and deprotection reactions in excellent yields. Schmidt and Rademann have designed a linker system that relies on a thioglycosyl linkage.[229,230] Merrifield's resin was functionalized to form either an O or S ether leaving a free thiol (a monomethoxytrityl intermediate allowed loading determination) that was glycosylated with a trichloroacetimidate donor (Scheme 28). Iterative glycosylations and deprotections led to the rapid and high yielding participatory formation of α(1,2)Man pentasaccharide **74**. The use of a fucosyl donor[230] and a glucosyl donor[229] (although the latter gave products as a mixture of anomers) has also been demonstrated. Analytical cleavage using Ag(I) salts or dimethyl(methylthio)sulfonium triflate (DMTST) allowed reaction monitoring by mass spectrometry, TLC or HPLC, whilst preparative cleavage was most successful using NBS. Swelling considerations necessitated the use of DCM in Zemplén deprotections and of dioxane instead of ether. Schmidt and co-workers have extended their method to mannosylation on mercaptopropyl-functionalized controlled-pore glass (CPG) since it does not require swelling.[231] Loading was assessed using Ellman's reagent. The use of an easily cleaved participatory phenylacetoxy C-2 allowed the synthesis of a Manα(1,2)Manα(1,2)Man trisaccharide.

A trichloroacetimidate was also used in initial solid phase approaches of the Ogawa group.[232] They created two linker



Scheme 28

types based on a *p*-alkoxybenzyl anomeric substituent on a lactoside acceptor. Glycosylation in three cycles using a lactosamine trichloroacetimidate donor followed by selective removal of a levulinoyl protecting group using hydrazinium acetate allowed the synthesis of a polylactosamine octasaccharide. Ogawa and Ito have also ingeniously extended their O-2 paramethoxybenzyl tethering method to polymer supports (Scheme 9).[116] They have also described an orthogonal glycosylation strategy using alternating thioglycoside and glycosyl fluoride donors on a soluble PEG support.[233] The complication of using a strategy that works from non-reducing-end to reducing-end, is the release of by-products from incomplete glycosylation into the final product mix upon cleavage. In this case, these were reduced by using a hydrophobic tag on the final reducing-end glycoside.

Nicolaou and co-workers have demonstrated the synthesis of a heptasaccharide phytoalexin elicitor[234] and a corresponding β-gluco dodecasaccharide[235] on Merrifield resin using linear sugar-by-sugar and reiterative block type construction strategies, respectively, starting from oligosaccharides tethered at the reducing-end anomeric centre. The use of orthogonally protected thioglycosides, activated by DMTST, allowed the construction of a trisaccharide block which was cleaved from the resin to give a trisaccharide thioglycoside. Iterative reaction of this with resin-bound tri-, hexa- and then nonasaccharide yielded the final product which was cleaved through irradiation with UV light due to the presence of a photolabile linker. β-Stereoselectivity in this dodecaglucoside was ensured by the use of participating O-2 benzoyl protection throughout.

Both participatory and non-participatory galactosyl, fucosyl, glucosyl and digalactosyl sulfoxide donors were used with resin bound 2-azido-2-deoxy galactoside and glucoside acceptors to construct a library of ~1300 carbohydrates on Tentagel.[236] Reduction of the azido group in the intermediate products allowed reaction with a number of acylating agents. From this library, a potent lectin ligand was identified.

Solid phase pentenyl glycoside systems have also been described.[237] Linking the acceptor to the resin (Merrifield or Tentagel) proved more efficient than linking OH-6 of the donor. Test glucosylations of an anomerically tethered OH-6 free glucoside gave β-stereoselectivity with participatory donors and showed that OH-6 bulk on the donor influenced stereoselectivity. Loading and reaction course were monitored by release of nitrobenzoates, which were used as protecting groups, and by gel phase NMR. The final system adopted for synthesis utilized polystyrene grafted crowns with a photocleavable linker and benzoylated and chloroacetylated donors which were successful despite their "disarmed" nature. In this way a linear Galβ(1,2)Manα(1,6)Glc trisaccharide was synthesized by exploiting O-2 participatory groups for stereocontrol.

Danishefsky and co-workers have overcome initial problems of efficiency in β(1,4)Glc formation using their solid phase diisopropylsilyl tethered glycal methodology by converting the 1,2-anhydrosugar to an SEt thioglucosyl donor bearing an O-2 pivaloyl for neighbouring group participation.[238] Such solid phase glycal assembly strategies can be monitored using magic-angle spinning (MAS) NMR to follow the appearance of the deshielded δ ~6 ppm glycal H-1 proton signal.[239] This on-resin technique was used to demonstrate an interesting enhanced β-glycosylation stereoselectivity on the solid phase as compared with solution. A very clear account of the use of glycal methodology on the solid phase has been published.[240] Solvent effects on PEG- and Merrifield-supported glycosylation have been studied.[241] α-Anomer favouring effects of ether–DCM or toluene and β-anomer favouring effects of acetonitrile, through solvent participation, were observed for non-participatory tetrabenzyl thioglucoside and fluoride donors. These mirror those typically found in solution. A diethylsilyl ether linker

system bound to O-6 of the glycosyl donor confirms previous results that hindered bases such as DTBMP allow the acid sensitive link to survive under glycosylation conditions.[242] Using this system, thioglycosides were most effective over fluorides and trichloroacetimidates, whilst sulfoxides failed to activate at all. A sulfonyl chloride resin prepared from Merrifield resin allowed the ready synthesis of precursors of 6- and 2-deoxy oligosaccharides.[243] The acceptor glycal was linked at O-6 before glycosylation and then cleaved using NaI to give a 6-iodo-oligosaccharide. Disaccharide synthesis on a soluble hyperbranched polymer that allows high loading levels was achieved for mannosylations using a thioglycoside donor and an acceptor linked to the polymer *via* a photocleavable anomeric linker.[244] A novel *p*-aminobenzyl type linker, that can be oxidatively cleaved by DDQ, has been used to link O-2 of a glucoside acceptor to Argopore resin, prior to the construction of a linear gluco(1,6) trisaccharide with non-participatory thioglycoside and trichloroacetimidate donors.[245] A novel aldehyde resin allows immobilisation of carbohydrates *via* acetal formation.[246] Schmidt and Knerr have used a linker that allows a ring closing olefin metathesis mediated cleavage to form 1-*O*-allyl glycosides.[247] Wong and co-workers have described the first examples of a non-destructive monitoring method for oligosaccharide synthesis and of the chemical synthesis of the tetrasaccharide motif of sLe^x on solid phase.[248] The presence of ^{13}C-labelled acetate groups in preliminary saccharidic building blocks and a ^{13}C-labelled methyl ester in the final sialic acid glycosyl donor was monitored using gated decoupling NMR in the presence of a relaxation agent. Comparison of these signals with that from the ^{13}C-labelled glycine linker as an internal standard allowed accurate quantitative monitoring of reaction courses for glycosylations, deacetylations and hydrolysis.

A two-directional solid phase approach has been described in which tethering of O-6 *via* a glycinylsuccinyl linker to Tentagel of a 2,3-di-*O*-benzyl thioglycoside allowed extension in both the reducing-end direction, by using the bound sugar as a donor, or in the non-reducing-end direction, by using the OH-4 of the bound sugar as an acceptor.[249] This approach was used in the split-and-mix construction of a small library of trisaccharides.

Low molecular weight PEG (MW550) has also been proposed as a support, although it does not offer any of the conventional advantages of PEG, *i.e.* it cannot be precipitated from solution.[250] However, column chromatographic purification is simplified as the conjugates remain on the base line in EtOAc but flow in DCM–MeOH mixtures. Difficulties in removing cleavage by-products may eclipse the potential advantages of this technique.

As for typical organic syntheses, the need for compound libraries has driven high throughput methods and consequently these are often intimately connected with solid phase approaches. A number of excellent reviews have already dealt with aspects of carbohydrate-containing library construction[251,252] and several illustrative examples of the potential power to synthesize large numbers of oligosaccharides have already been described in this section. Other examples include that of Boons and co-workers who used vinyl/allyl glycoside systems in a combinatorial approach that employed a solution phase split-and-mix (in fact a deprotect, split, glycosylate and mix) type strategy to prepare small library of 20 trisaccharides as mixture of α,β anomers.[253] The use of C-2 trifluoroacetamido sulfoxide donors has allowed the construction on Rink amide resin of an isocyanate and amide library based on an aminodeoxy disaccharide core motif.[254] A recent excellent review has highlighted many of the challenges of combinatorial oligosaccharide synthesis that remain (notwithstanding those still facing all types of oligosaccharide synthesis of reactivity, regioselectivity and stereoselectivity), such as investigating the glycosylation of unprotected acceptors.[255]

10 Conclusion and future directions

It is often the case that when confronted by some of the creative, efficient and effective methods that have been described in this review and those before it, the unfamiliar observer can be given the impression that oligosaccharide chemistry is done, finished or easy. However, in essence all the major goals of oligosaccharide chemistry have still to be achieved and the potential for impressive discoveries in this highly creative area is very high. Some glycosylation chemists argue that "there must be a simple answer"—a single general method that will selectively create all glycosidic linkages needed. Others point out that perhaps by definition the complexity and vast number of potential permutations of oligosaccharide assembly mean that rather than aiming for a unified method we should look for approaches that allow flexibility combined with a ready means for optimization. In either case, the goals remain and will remain until a generally available automated oligosaccharide synthesizer sits on the benches of glycoscientists.

11 References

1 H. Paulsen, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 155.
2 G. J. Boons, *Contemp. Org. Synth.*, 1996, **3**, 173.
3 G. J. Boons, *Tetrahedron*, 1996, **52**, 1095.
4 K. Toshima and K. Tatsuta, *Chem. Rev.*, 1993, **93**, 1503.
5 D. M. Whitfield and S. P. Douglas, *Glycoconjugate J.*, 1996, **13**, 5.
6 P. M. Collins and R. J. Ferrier, *Monosaccharides*, Wiley, Chichester, 1995.
7 S. H. Khan and R. A. O'Neill, in *Modern Methods in Carbohydrate Synthesis*, Harwood Academic, Amsterdam, 1996.
8 S. Hanessian, in *Preparative Carbohydrate Chemistry*, Marcel Dekker, New York, 1998.
9 G. J. Boons, in *Carbohydrate Chemistry*, Blackie Academic, London, 1998.
10 B. G. Davis, *J. Chem. Soc., Perkin Trans. 1*, 1999, 3215.
11 D. E. Levy and C. Tang, *The Chemistry of C-glycosides*, Elsevier Science, Amsterdam, 1995.
12 G. Gattuso, S. A. Nepogodiev and J. F. Stoddart, *Chem. Rev.*, 1998, **98**, 1919.
13 C. M. Taylor, *Tetrahedron*, 1998, **54**, 11317.
14 G. Arsequell and G. Valencia, *Tetrahedron: Asymmetry*, 1999, **10**, 3045.
15 P. J. Garegg, *Adv. Carbohydr. Chem. Biochem.*, 1997, **52**, 179.
16 N. Navarre, A. H. van Oijen and G. J. Boons, *Tetrahedron Lett.*, 1997, **38**, 2023.
17 N. Navarre, N. Amiot, A. van Oijen, A. Imberty, A. Poveda, J. Jimenez-Barbero, A. Cooper, M. A. Nutley and G. J. Boons, *Chem. Eur. J.*, 1999, **5**, 2281.
18 A. D. McNaught, *Pure Appl. Chem.*, 1996, **68**, 1919.
19 R. Alibes and D. R. Bundle, *J. Org. Chem.*, 1998, **63**, 6288.
20 H. I. Duynstee, M. C. de Koning, H. Ovaa, G. A. van der Marel and J. H. van Boom, *Eur. J. Org. Chem.*, 1999, 2623.
21 K. P. R. Kartha, M. Aloui and R. A. Field, *Tetrahedron Lett.*, 1996, **37**, 5175.
22 K. Fukase, Y. Nakai, T. Kanoh and S. Kusumoto, *Synlett*, 1998, 84.
23 K. Fukase, I. Kinoshita, T. Kanoh, Y. Nakai, A. Hasuoka and S. Kusumoto, *Tetrahedron*, 1996, **52**, 3897.
24 W. Zou and W. A. Szarek, *Chem. Commun.*, 1996, 1195.
25 H. B. Mereyala, S. R. Gurrala and R. R. Gadikota, *J. Chem. Soc., Perkin Trans. 1*, 1997, 3179.
26 T. Nukada, A. Berces and D. M. Whitfield, *J. Org. Chem.*, 1999, **64**, 9030.
27 H. L. Jiao and O. Hindsgaul, *Angew. Chem., Int. Ed.*, 1999, **38**, 346.
28 L. Noecker, F. Duarte, S. A. Bolton, W. G. McMahon, M. T. Diaz and R. M. Giuliano, *J. Org. Chem.*, 1999, **64**, 6275.
29 G. J. Boons and T. Stauch, *Synlett*, 1996, 906.
30 J. C. McAuliffe and O. Hindsgaul, *J. Org. Chem.*, 1997, **62**, 1234.
31 J. C. McAuliffe and O. Hindsgaul, *Synlett*, 1998, 307.
32 D. Crich and S. X. Sun, *J. Org. Chem.*, 1996, **61**, 4506.
33 D. Crich and S. X. Sun, *J. Org. Chem.*, 1997, **62**, 1198.
34 D. Crich and Z. M. Dai, *Tetrahedron Lett.*, 1998, **39**, 1681.
35 D. Crich and Z. M. Dai, *Tetrahedron*, 1999, **55**, 1569.
36 L. Yan and D. Kahne, *J. Am. Chem. Soc.*, 1996, **118**, 9239.
37 R. K. Boeckman and Y. G. Liu, *J. Org. Chem.*, 1996, **61**, 7984.
38 C. Thompson, M. Ge and D. Kahne, *J. Am. Chem. Soc.*, 1999, **121**, 1237.

39 M. Ge, Z. Chen, H. R. Onishi, J. Kohler, L. L. Silver, R. Kerns, S. Fukuzawa, C. Thompson and D. Kahne, *Science*, 1999, **284**, 507.
40 K. C. Nicolaou, H. J. Mitchell, N. F. Jain, N. Winssinger, R. Hughes and T. Bando, *Angew. Chem.*, *Int. Ed.*, 1999, **38**, 240.
41 R. Kakarla, R. G. Dulina, N. T. Hatzenbuhler, Y. W. Hui and M. J. Sofia, *J. Org. Chem.*, 1996, **61**, 8347.
42 S. J. Danishefsky and M. T. Bilodeau, *Angew. Chem.*, *Int. Ed. Engl.*, 1996, **35**, 1380.
43 V. Di Bussolo, Y. J. Kim and D. Y. Gin, *J. Am. Chem. Soc.*, 1998, **120**, 13515.
44 C. M. Timmers, S. C. M. Wigchert, M. A. Leeuwenburgh, G. A. van der Marel and J. H. van Boom, *Eur. J. Org. Chem.*, 1998, 91.
45 D. P. Curran, R. Ferritto and Y. Hua, *Tetrahedron Lett.*, 1998, **39**, 4937.
46 A. Geyer, S. Reinhardt, G. Bendas, U. Rothe and R. R. Schmidt, *J. Am. Chem. Soc.*, 1997, **119**, 11707.
47 M. de Kort, E. Ebrahimi, E. R. Wijsman, G. A. van der Marel and J. H. van Boom, *Eur. J. Org. Chem.*, 1999, 2337.
48 K. F. Hsiao, S. T. Chen, K. T. Wang and S. H. Wu, *Synlett*, 1996, 966.
49 Z. G. Wang, S. P. Douglas and J. J. Krepinsky, *Tetrahedron Lett.*, 1996, **37**, 6985.
50 J. Ning and F. Z. Kong, *Tetrahedron Lett.*, 1999, **40**, 1357.
51 R. Rodebaugh, J. S. Debenham, B. Fraser-Reid and J. P. Snyder, *J. Org. Chem.*, 1999, **64**, 1758.
52 M. Leuck and H. Kunz, *Carbohydr. Res.*, 1998, **312**, 33.
53 R. K. Jain, R. Vig, R. D. Locke, A. Mohammad and K. L. Matta, *Chem. Commun.*, 1996, 65.
54 K. P. R. Kartha, M. Aloui and R. A. Field, *Tetrahedron Lett.*, 1996, **37**, 8807.
55 M. Shimizu, H. Togo and M. Yokoyama, *Synthesis*, 1998, 799.
56 K. Oyama and T. Kondo, *Synlett*, 1999, 1627.
57 K. Toshima, K. Kasumi and S. Matsumura, *Synlett*, 1999, 813.
58 M. I. Barrena, R. Echarri and S. Castillon, *Synlett*, 1996, 675.
59 K. Takeuchi and T. Mukaiyama, *Chem. Lett.*, 1998, 555.
60 J. Gervay and M. J. Hadd, *J. Org. Chem.*, 1997, **62**, 6961.
61 T. Uchiyama and O. Hindsgaul, *Synlett*, 1996, 499.
62 T. Iimori, H. Ohtake and S. Ikegami, *Tetrahedron Lett.*, 1997, **38**, 3415.
63 H. Ohtake, T. Iimori and S. Ikegami, *Synlett*, 1998, 1420.
64 H. Ohtake, T. Iimori and S. Ikegami, *Tetrahedron Lett.*, 1997, **38**, 3413.
65 S. Hiranuma, O. Kanie and C. H. Wong, *Tetrahedron Lett.*, 1999, **40**, 6423.
66 S. K. Chatterjee and P. Nuhn, *Chem. Commun.*, 1998, 1729.
67 D. Crich, Z. M. Dai and S. Gastaldi, *J. Org. Chem.*, 1999, **64**, 5224.
68 G. J. Boons, A. Burton and S. Isles, *Chem. Commun.*, 1996, 141.
69 G. J. Boons and S. Isles, *J. Org. Chem.*, 1996, **61**, 4262.
70 Y. Osa, K. Takeda, T. Sato, E. Kaji, Y. Mizuno and H. Takayanagi, *Tetrahedron Lett.*, 1999, **40**, 1531.
71 G. J. Boons, A. Burton and P. Wyatt, *Synlett*, 1996, 310.
72 H. K. Chenault and L. F. Chafin, *J. Org. Chem.*, 1998, **63**, 833.
73 H. K. Chenault, A. Castro, L. F. Chafin and J. Yang, *J. Org. Chem.*, 1996, **61**, 5024.
74 S. Hashimoto, K. Umeo, A. Sano, N. Watanabe, M. Nakajima and S. Ikegami, *Tetrahedron Lett.*, 1995, **36**, 2251.
75 H. Tanaka, H. Sakamoto, A. Sano, S. Nakamura, M. Nakajima and S. Hashimoto, *Chem. Commun.*, 1999, 1259.
76 S. F. Pan, H. Li, F. Hong, B. Yu and K. Zhao, *Tetrahedron Lett.*, 1997, **38**, 6139.
77 H. Schene and H. Waldmann, *Eur. J. Org. Chem.*, 1998, 1227.
78 U. Schmid and H. Waldmann, *Tetrahedron Lett.*, 1996, **37**, 3837.
79 V. Hariprasad, G. Singh and I. Tranoy, *Chem. Commun.*, 1998, 2129.
80 H. Vankayalapati and G. Singh, *Tetrahedron Lett.*, 1999, **40**, 3925.
81 S. Hanessian, P. P. Lu and H. Ishida, *J. Am. Chem. Soc.*, 1998, **120**, 13296.
82 S. Hashimoto, H. Sakamoto, T. Honda and S. Ikegami, *Tetrahedron Lett.*, 1997, **38**, 5181.
83 S. Hashimoto, H. Sakamoto, T. Honda, H. Abe, S. Nakamura and S. Ikegami, *Tetrahedron Lett.*, 1997, **38**, 8969.
84 W. Kudelska and M. Michalska, *Synthesis*, 1995, 1539.
85 O. J. Plante and P. H. Seeberger, *J. Org. Chem.*, 1998, **63**, 9150.
86 D. Crich and S. X. Sun, *J. Am. Chem. Soc.*, 1997, **119**, 11217.
87 D. Crich and S. X. Sun, *J. Am. Chem. Soc.*, 1998, **120**, 435.
88 D. Crich and S. X. Sun, *Tetrahedron*, 1998, **54**, 8321.
89 Y. Ito, Y. Ohnishi, T. Ogawa and Y. Nakahara, *Synlett*, 1998, 1102.
90 D. Crich and W. L. Cai, *J. Org. Chem.*, 1999, **64**, 4926.
91 B. A. Garcia, J. L. Poole and D. Y. Gin, *J. Am. Chem. Soc.*, 1997, **119**, 7597.
92 U. Huchel, C. Schmidt and R. R. Schmidt, *Eur. J. Org. Chem.*, 1998, 1353.
93 T. Yasukochi, K. Fukase and S. Kusumoto, *Tetrahedron Lett.*, 1999, **40**, 6591.
94 K. Toshima, H. Nagai and S. Matsumura, *Synlett*, 1999, 1420.
95 S. Yamago, K. Kokubo and J. Yoshida, *Chem. Lett.*, 1997, 111.
96 J. J. Gridley and H. M. I. Osborn, *J. Chem. Soc.*, *Perkin Trans. 1*, 2000, 1471.
97 D. Crich and G. R. Barba, *Tetrahedron Lett.*, 1998, **39**, 9339.
98 A. Furstner and I. Konetzki, *J. Org. Chem.*, 1998, **63**, 3072.
99 T. Nukada, A. Berces, M. Z. Zgierski and D. M. Whitfield, *J. Am. Chem. Soc.*, 1998, **120**, 13291.
100 M. Ge, C. Thompson and D. Kahne, *J. Am. Chem. Soc.*, 1998, **120**, 11014.
101 A. Furstner and T. Muller, *J. Org. Chem.*, 1998, **63**, 424.
102 V. Subramaniam and T. L. Lowary, *Tetrahedron*, 1999, **55**, 5965.
103 A. Demchenko, T. Stauch and G. J. Boons, *Synlett*, 1997, 818.
104 A. V. Demchenko, E. Rousson and G. J. Boons, *Tetrahedron Lett.*, 1999, **40**, 6523.
105 M. Miljkovic, D. Yeagley, P. Deslongchamps and Y. L. Dory, *J. Org. Chem.*, 1997, **62**, 7597.
106 C. C. Lin, M. Shimazaki, M. P. Heck, S. Aoki, R. Wang, T. Kimura, H. Ritzen, S. Takayama, S. H. Wu, G. WeitzSchmidt and C. H. Wong, *J. Am. Chem. Soc.*, 1996, **118**, 6826.
107 G. J. Boons, S. Bowers and D. M. Coe, *Tetrahedron Lett.*, 1997, **38**, 3773.
108 G. Scheffler and R. R. Schmidt, *Tetrahedron Lett.*, 1997, **38**, 2943.
109 T. Ziegler and G. Lemanski, *Angew. Chem.*, *Int. Ed.*, 1998, **37**, 3129.
110 T. Ziegler and G. Lemanski, *Eur. J. Org. Chem.*, 1998, 163.
111 T. Ziegler, A. Ritter and J. Hurttlen, *Tetrahedron Lett.*, 1997, **38**, 3715.
112 M. Muller, U. Huchel, A. Geyer and R. R. Schmidt, *J. Org. Chem.*, 1999, **64**, 6190.
113 A. Geyer, M. Muller and R. R. Schmidt, *J. Am. Chem. Soc.*, 1999, **121**, 6312.
114 G. Stork and J. J. LaClair, *J. Am. Chem. Soc.*, 1996, **118**, 247.
115 S. C. Ennis, A. J. Fairbanks, R. J. Tennant-Eyles and H. S. Yeates, *Synlett*, 1999, 1387.
116 Y. Ito and T. Ogawa, *J. Am. Chem. Soc.*, 1997, **119**, 5562.
117 M. Lergenmuller, T. Nukada, K. Kuramochi, A. Dan, T. Ogawa and Y. Ito, *Eur. J. Org. Chem.*, 1999, 1367.
118 M. Wakao, K. Fukase and S. Kusumoto, *Synlett*, 1999, 1911.
119 S. Valverde, A. M. Gomez, J. C. Lopez and B. Herradon, *Tetrahedron Lett.*, 1996, **37**, 1105.
120 R. J. Tennant-Eyles, B. G. Davis and A. J. Fairbanks, *Chem. Commun.*, 1999, 1037.
121 C. Mukai, T. Itoh and M. Hanaoka, *Tetrahedron Lett.*, 1997, **38**, 4595.
122 S. A. Wacowich-Sgarbi and D. R. Bundle, *J. Org. Chem.*, 1999, **64**, 9080.
123 T. Iimori, T. Shibazaki and S. Ikegami, *Tetrahedron Lett.*, 1996, **37**, 2267.
124 I. Azumaya, T. Niwa, M. Kotani, T. Iimori and S. Ikegami, *Tetrahedron Lett.*, 1999, **40**, 4683.
125 G. Scheffler and R. R. Schmidt, *J. Org. Chem.*, 1999, **64**, 1319.
126 A. Furstner and I. Konetzki, *Tetrahedron Lett.*, 1998, **39**, 5721.
127 G. Hodosi and P. Kovac, *J. Am. Chem. Soc.*, 1997, **119**, 2335.
128 K. C. Nicolaou, F. L. vanDelft, S. R. Conley, H. J. Mitchell, Z. Jin and R. M. Rodriguez, *J. Am. Chem. Soc.*, 1997, **119**, 9057.
129 A. Lubineau, S. Escher, J. Alais and D. Bonnaffe, *Tetrahedron Lett.*, 1997, **38**, 4087.
130 J. Das and R. R. Schmidt, *Eur. J. Org. Chem.*, 1998, 1609.
131 T. Furuta, K. Takeuchi and M. Iwamura, *Chem. Commun.*, 1996, 157.
132 S. C. Johnson, C. Crasto and S. M. Hecht, *Chem. Commun.*, 1998, 1019.
133 R. Geurtsen, D. S. Holmes and G. J. Boons, *J. Org. Chem.*, 1997, **62**, 8145.
134 R. Geurtsen, F. Cote, M. G. Hahn and G. J. Boons, *J. Org. Chem.*, 1999, **64**, 7828.
135 J. G. Allen and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1999, **121**, 468.
136 J. Gildersleeve, A. Smith, K. Sakurai, S. Raghavan and D. Kahne, *J. Am. Chem. Soc.*, 1999, **121**, 6176.
137 S. D. Cao, Z. H. Gan and R. Roy, *Carbohydr. Res.*, 1999, **318**, 75.
138 A. Demchenko and G. J. Boons, *Tetrahedron Lett.*, 1997, **38**, 1629.
139 B. Yu, H. Yu, Y. Z. Hui and X. W. Han, *Tetrahedron Lett.*, 1999, **40**, 8591.
140 Z. Y. Zhang, I. R. Ollmann, X. S. Ye, R. Wischnat, T. Baasov and C. H. Wong, *J. Am. Chem. Soc.*, 1999, **121**, 734.
141 P. Grice, S. V. Ley, J. Pietruszka, H. M. I. Osborn, H. W. M. Priepke and S. L. Warriner, *Chem. Eur. J.*, 1997, **3**, 431.
142 N. L. Douglas, S. V. Ley, U. Lucking and S. L. Warriner, *J. Chem. Soc.*, *Perkin Trans. 1*, 1998, 51.

143 H. I. Duynstee, M. J. van Vliet, G. A. van der Marel and J. H. van Boom, *Eur. J. Org. Chem.*, 1998, 303.
144 T. Zhu and G. J. Boons, *Angew. Chem.*, *Int. Ed.*, 1999, **38**, 3495.
145 J. Debenham, R. Rodebaugh and B. Fraser-Reid, *Liebigs Ann./ Recl.*, 1997, 791.
146 R. Madsen, C. Roberts and B. Fraser-Reid, *J. Org. Chem.*, 1995, **60**, 7920.
147 S. L. Griffiths, R. Madsen and B. Fraser-Reid, *J. Org. Chem.*, 1997, **62**, 3654.
148 J. S. Debenham, R. Rodebaugh and B. Fraser-Reid, *J. Org. Chem.*, 1997, **62**, 4591.
149 L. Olsson, Z. J. Jia and B. Fraser-Reid, *J. Org. Chem.*, 1998, **63**, 3790.
150 J. S. Debenham and B. Fraser-Reid, *J. Org. Chem.*, 1996, **61**, 432.
151 J. S. Debenham, S. D. Debenham and B. Fraser-Reid, *Bioorg. Med. Chem.*, 1996, **4**, 1909.
152 S. G. Bowers, D. M. Coe and G. J. Boons, *J. Org. Chem.*, 1998, **63**, 4570.
153 M. R. E. Aly, J. C. Castro-Palomino, E. S. I. Ibrahim, E. S. H. El-Ashry and R. R. Schmidt, *Eur. J. Org. Chem.*, 1998, 2305.
154 X. P. Qian and O. Hindsgaul, *Chem. Commun.*, 1997, 1059.
155 M. J. Gaunt, J. Q. Yu and J. B. Spencer, *J. Org. Chem.*, 1998, **63**, 4172.
156 N. L. Pohl and L. L. Kiessling, *Tetrahedron Lett.*, 1997, **38**, 6985.
157 G. J. Boons and T. Zhu, *Synlett*, 1997, 809.
158 W. Wang and F. Kong, *J. Org. Chem.*, 1998, **63**, 5744.
159 W. Wang and F. Z. Kong, *Tetrahedron Lett.*, 1999, **40**, 1361.
160 W. Wang and F. Z. Kong, *Tetrahedron Lett.*, 1998, **39**, 1937.
161 H. Yamada, T. Kato and T. Takahashi, *Tetrahedron Lett.*, 1999, **40**, 4581.
162 W. Wang and F. Z. Kong, *J. Org. Chem.*, 1999, **64**, 5091.
163 U. Ellervik and G. Magnusson, *J. Org. Chem.*, 1998, **63**, 9314.
164 H. Yamada, K. Imamura and T. Takahashi, *Tetrahedron Lett.*, 1997, **38**, 391.
165 W. Wang and F. Z. Kong, *Angew. Chem.*, *Int. Ed.*, 1999, **38**, 1247.
166 A. Dondoni, A. Marra, I. Rojo and M. C. Scherrmann, *Tetrahedron*, 1996, **52**, 3057.
167 A. Dondoni and A. Marra, *Chem. Commun.*, 1999, 2133.
168 A. V. Demchenko and G. J. Boons, *Tetrahedron Lett.*, 1998, **39**, 3065.
169 A. V. Demchenko and G. J. Boons, *Chem. Eur. J.*, 1999, **5**, 1278.
170 N. Hossain and G. Magnusson, *Tetrahedron Lett.*, 1999, **40**, 2217.
171 J. C. Castro-Palomino, Y. E. Tsvetkov and R. R. Schmidt, *J. Am. Chem. Soc.*, 1998, **120**, 5434.
172 L. F. Tietze and D. Gretzke, *Eur. J. Org. Chem.*, 1998, 1895.
173 L. F. Tietze, C. O. Janssen and J. A. Gewert, *Eur. J. Org. Chem.*, 1998, 1887.
174 R. Higuchi, T. Mori, T. Sugata, K. Yamada and T. Miyamoto, *Eur. J. Org. Chem.*, 1999, 145.
175 L. Singh, Y. Nakahara and Y. Ito, *Tetrahedron Lett.*, 1999, **40**, 3769.
176 C. Bernlind and S. Oscarson, *J. Org. Chem.*, 1998, **63**, 7780.
177 C. H. Marzabadi and R. W. Franck, *Chem. Commun.*, 1996, 2651.
178 G. Capozzi, F. Mannocci, S. Menichetti, C. Nativi and S. Paoletti, *Chem. Commun.*, 1997, 2291.
179 A. Dios, C. Nativi, G. Capozzi and R. W. Franck, *Eur. J. Org. Chem.*, 1999, 1869.
180 R. W. Franck and C. H. Marzabadi, *J. Org. Chem.*, 1998, **63**, 2197.
181 G. Capozzi, C. Falciani, S. Menichetti, C. Nativi and B. Raffaelli, *Chem. Eur. J.*, 1999, **5**, 1748.
182 T. Jyojima, N. Miyamoto, Y. Ogawa, S. Matsumura and K. Toshima, *Tetrahedron Lett.*, 1999, **40**, 5023.
183 H. Li, M. J. Chen and K. Zhao, *Tetrahedron Lett.*, 1997, **38**, 6143.
184 J. C. Castro-Palomino and R. R. Schmidt, *Synlett*, 1998, 501.
185 K. Toshima, H. Nagai, Y. Ushiki and S. Matsumura, *Synlett*, 1998, 1007.
186 F. E. McDonald and H. Y. H. Zhu, *J. Am. Chem. Soc.*, 1998, **120**, 4246.
187 A. Sobti, K. J. Kim and G. A. Sulikowski, *J. Org. Chem.*, 1996, **61**, 6.
188 C. H. Wong, R. L. Halcomb, Y. Ichikawa and T. Kajimoto, *Angew. Chem.*, *Int. Ed. Engl.*, 1995, **34**, 521.
189 V. Kren and J. Thiem, *Chem. Soc. Rev.*, 1997, **26**, 463.
190 D. H. G. Crout and G. Vic, *Curr. Opin. Chem. Biol.*, 1998, **2**, 98.
191 Y. Ichikawa, *Trends Glycosci. Glycotechnol.*, 1997, **9**, S47.
192 M. Scigelova, S. Singh and D. H. G. Crout, *J. Mol. Catal. B: Enzym.*, 1999, **6**, 483.
193 L. F. Mackenzie, Q. P. Wang, R. A. J. Warren and S. G. Withers, *J. Am. Chem. Soc.*, 1998, **120**, 5583.
194 G. Vic, J. J. Hastings, O. W. Howarth and D. H. Crout, *Tetrahedron: Asymmetry*, 1996, **7**, 709.
195 E. Farkas and J. Thiem, *Eur. J. Org. Chem.*, 1999, 3073.
196 K. G. I. Nilsson, *Tetrahedron Lett.*, 1997, **38**, 133.
197 S. Singh, M. Scigelova, G. Vic and D. H. G. Crout, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 1921.
198 K. Priya and D. Loganathan, *Tetrahedron*, 1999, **55**, 1119.
199 Y. Okahata and T. Mori, *J. Mol. Catal. B: Enzym.*, 1998, **5**, 119.
200 T. Mori and Y. Okahata, *Chem. Commun.*, 1998, 2215.
201 I. Matsuo, M. Isomura and K. Ajisaka, *Tetrahedron Lett.*, 1999, **40**, 5047.
202 J. Li and P. G. Wang, *Tetrahedron Lett.*, 1997, **38**, 7967.
203 J. W. Fang, W. H. Xie, J. Li and P. G. Wang, *Tetrahedron Lett.*, 1998, **39**, 919.
204 J. W. Fang, X. Chen, W. Zhang, J. Q. Wang, P. R. Andreana and P. G. Wang, *J. Org. Chem.*, 1999, **64**, 4089.
205 U. Gambert and J. Thiem, *Eur. J. Org. Chem.*, 1999, 107.
206 U. Gambert and J. Thiem, *Carbohydr. Res.*, 1997, **299**, 85.
207 V. Chiffoleau-Giraud, P. Spangenberg, M. Dion and C. Rabiller, *Eur. J. Org. Chem.*, 1999, 757.
208 M. Gelo-Pujic, E. Guibe-Jampel, A. Loupy and A. Trincone, *J. Chem. Soc.*, *Perkin Trans. 1*, 1997, 1001.
209 S. Singh, M. Scigelova and D. H. G. Crout, *Chem. Commun.*, 1999, 2065.
210 G. Vic, C. H. Tran, M. Scigelova and D. H. G. Crout, *Chem. Commun.*, 1997, 169.
211 G. Vic, M. Scigelova, J. J. Hastings, O. W. Howarth and D. H. G. Crout, *Chem. Commun.*, 1996, 1473.
212 S. Singh, M. Scigelova and D. H. G. Crout, *Chem. Commun.*, 1996, 993.
213 V. Kren, E. Rajnochova, Z. Hunkova, J. Dvorakova and P. Sedmera, *Tetrahedron Lett.*, 1998, **39**, 9777.
214 L. Weignerova, P. Sedmera, Z. Hunkova, P. Halada, V. Kren, M. Casali and S. Riva, *Tetrahedron Lett.*, 1999, **40**, 9297.
215 G. Vic, J. J. Hastings and D. H. G. Crout, *Tetrahedron: Asymmetry*, 1996, **7**, 1973.
216 V. ChiffoleauGiraud, P. Spangenberg and C. Rabiller, *Tetrahedron: Asymmetry*, 1997, **8**, 2017.
217 T. Yasukochi, C. Inaba, K. Fukase and S. Kusumoto, *Tetrahedron Lett.*, 1999, **40**, 6585.
218 D. Depre, A. Duffels, L. G. Green, R. Lenz, S. V. Ley and C. H. Wong, *Chem. Eur. J.*, 1999, **5**, 3326.
219 A. Zervosen and L. Elling, *J. Am. Chem. Soc.*, 1996, **118**, 1836.
220 O. Blixt and T. Norberg, *J. Org. Chem.*, 1998, **63**, 2705.
221 O. Blixt and T. Norberg, *Carbohydr. Res.*, 1999, **319**, 80.
222 L. Panza, P. L. Chiappini, G. Russo, D. Monti and S. Riva, *J. Chem. Soc.*, *Perkin Trans. 1*, 1997, 1255.
223 J. W. Fang, J. Li, X. Chen, Y. N. Zhang, J. Q. Wang, Z. M. Guo, W. Zhang, L. B. Yu, K. Brew and P. G. Wang, *J. Am. Chem. Soc.*, 1998, **120**, 6635.
224 G. M. Watt, L. Revers, M. C. Webberley, I. B. H. Wilson and S. L. Flitsch, *Angew. Chem.*, *Int. Ed. Engl.*, 1997, **36**, 2354.
225 Y. X. Zhao and J. S. Thorson, *Carbohydr. Res.*, 1999, **319**, 184.
226 Y. Ito and S. Manabe, *Curr. Opin. Chem. Biol.*, 1998, **2**, 701.
227 H. M. I. Osborn and T. H. Khan, *Tetrahedron*, 1999, **55**, 1807.
228 M. Adinolfi, G. Barone, L. De Napoli, L. Guariniello, A. Iadonisi and G. Piccialli, *Tetrahedron Lett.*, 1999, **40**, 2607.
229 J. Rademann and R. R. Schmidt, *Tetrahedron Lett.*, 1996, **37**, 3989.
230 J. Rademann and R. R. Schmidt, *J. Org. Chem.*, 1997, **62**, 3650.
231 A. Heckel, E. Mross, K. H. Jung, J. Rademann and R. R. Schmidt, *Synlett*, 1998, 171.
232 H. Shimizu, Y. Ito, O. Kanie and T. Ogawa, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2841.
233 Y. Ito, O. Kanie and T. Ogawa, *Angew. Chem.*, *Int. Ed. Engl.*, 1996, **35**, 2510.
234 K. C. Nicolaou, N. Winssinger, J. Pastor and F. DeRoose, *J. Am. Chem. Soc.*, 1997, **119**, 449.
235 K. C. Nicolaou, N. Watanabe, J. Li, J. Pastor and N. Winssinger, *Angew. Chem.*, *Int. Ed.*, 1998, **37**, 1559.
236 R. Liang, L. Yan, J. Loebach, M. Ge, Y. Uozumi, K. Sekanina, N. Horan, J. Gildersleeve, C. Thompson, A. Smith, K. Biswas, W. C. Still and D. Kahne, *Science*, 1996, **274**, 1520.
237 R. Rodebaugh, S. Joshi, B. Fraser-Reid and H. M. Geysen, *J. Org. Chem.*, 1997, **62**, 5660.
238 C. S. Zheng, P. H. Seeberger and S. J. Danishefsky, *J. Org. Chem.*, 1998, **63**, 1126.
239 P. H. Seeberger, X. Beebe, G. D. Sukenick, S. Pochapsky and S. J. Danishefsky, *Angew. Chem.*, *Int. Ed. Engl.*, 1997, **36**, 491.
240 P. H. Seeberger and S. J. Danishefsky, *Acc. Chem. Res.*, 1998, **31**, 685.
241 S. Manabe, Y. Ito and T. Ogawa, *Synlett*, 1998, 628.
242 T. Doi, M. Sugiki, H. Yamada, T. Takahashi and J. A. Porco, *Tetrahedron Lett.*, 1999, **40**, 2141.
243 J. A. Hunt and W. R. Roush, *J. Am. Chem. Soc.*, 1996, **118**, 9998.

244 A. B. Kantchev and J. R. Parquette, *Tetrahedron Lett.*, 1999, **40**, 8049.
245 K. Fukase, Y. Nakai, K. Egusa, J. A. Porco and S. Kusumoto, *Synlett*, 1999, 1074.
246 S. Hanessian and F. Xie, *Tetrahedron Lett.*, 1998, **39**, 733.
247 L. Knerr and R. R. Schmidt, *Synlett*, 1999, 1802.
248 T. Kanemitsu, O. Kanie and C. H. Wong, *Angew. Chem.*, *Int. Ed.*, 1998, **37**, 3415.
249 T. Zhu and G. J. Boons, *Angew. Chem.*, *Int. Ed.*, 1998, **37**, 1898.
250 L. Jiang, R. C. Hartley and T. H. Chan, *Chem. Commun.*, 1996, 2193.
251 M. J. Sofia, *Drug Discovery Today*, 1996, **1**, 27.
252 M. J. Sofia, *Mol. Diversity*, 1998, **3**, 75.
253 G. J. Boons, B. Heskamp and F. Hout, *Angew. Chem.*, *Int. Ed. Engl.*, 1996, **35**, 2845.
254 D. J. Silva, H. M. Wang, N. M. Allanson, R. K. Jain and M. J. Sofia, *J. Org. Chem.*, 1999, **64**, 5926.
255 F. Schweizer and O. Hindsgaul, *Curr. Opin. Chem. Biol.*, 1999, **3**, 291.