Synthetic oligosaccharides: recent advances

Geert-Jan Boons

Oligosaccharides play essential roles in many biological processes and it has been recognized that these type of compounds may provide important leads for drug development. Recent advances in chemical and enzymatic synthesis of oligosaccharides make it possible to more reliably prepare a wide range of oligosaccharides that can be used for SAR studies. Synthetic procedures allow the preparation of designed glycomimetics with improved pharmacokinetics and better binding affinities.

Glycoconjugates are the most functionally and structurally diverse molecules in nature, and it is now well established that protein- and lipid-bound saccharides play essential roles in many molecular processes impacting on eukaryotic biology and disease[1,2], such as fertilization, embryogenesis, neuronal development, hormone activities, the proliferation of cells and the organization of cells into specific tissues. Marked changes in cell-surface carbohydrates occur with tumour progression, which appears to be closely associated with metastasis[3]. Furthermore, carbohydrates are capable of inducing a protective antibody response, and this immunological reaction is a major contributor to the survival of the organism during infection[4]. Oligosaccharides have also been found to control the development and defence mechanisms of plants[5].

Agents in current use

Several carbohydrate-based compounds with important pharmaceutical applications[6] are currently on the market, many of which can be classified as monosaccharides or simple disaccharides (Figure 1). For example, the monosaccharide streptozotocin is used to treat malignant insulinomas and Hodgkin's disease. Another simple sugar with a strong biological activity is the fructose sulphamate, topiramate, which is a new prototype of an antiepileptic drug and is now in late-phase clinical trials. 4-Guanidino-Neu5Ac2en, an analogue of neuraminic acid, is currently being developed as an anti-influenza A and B drug[7]. The design of this compound was based on the crystal structure of influenza virus neuraminidase (sialidase) and 5-*N*-acetylneuraminic acid. Lactulose was one of the first disaccharides to be used therapeutically, as an agent against chronic constipation and hepatic coma. It should also be noted that many pharmaceutically important compounds are glycosylated. For example, the avermectin antibiotics possess carbohydrate substituents that are crucial for their biological activity.

Increased appreciation of the roles of carbohydrates in biological processes and the advances made in the analysis and chemical synthesis of oligosaccharides have stimulated the development of more complex carbohydrates as potential therapeutics. For example, it was found recently that selectins, which are membrane-bound adhesion receptors expressed on endothelial cells near a site of inflammation, recognize the tetrasaccharide antigen sialyl Lewis X (SLe^x; Figure 2)[8]. This saccharide has been synthesized both chemically and enzymatically and is currently being developed as an acute anti-inflammatory drug.

Oligosaccharide analogues

It should be realized that several disadvantages are associated with the use of complex oligosaccharides (e.g. SLe^x) as therapeutic agents. In many instances, they display unfavourable pharmacokinetics, are often metabolically unstable and are poorly absorbed orally. In addition, many carbohydrates bind

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with low affinity (mmol or μmol range) to proteins, which complicates their use as drugs. These properties have stimulated the development of oligosaccharide analogues. Several oligosaccharides have been prepared in which one or more of the glycosidic oxygen atoms have been replaced by another atom (N, S, C). For example, a thio-SLe^x analogue has been synthesized, but it did not exhibit any biological activity. It is believed that the replacement of an exocyclic anomeric oxygen atom with sulphur may dramatically alter the conformational properties of a saccharide and hence will result in loss of biological activity. It is possible, however, to make dramatic changes in the saccharide structure and still maintain or enhance a required activity. For example, SAR studies have shown that the carboxylic acid of neuraminic acid and the C-2 and C-3 hydroxy groups of the fucoside are critical functionalities of SLe^x required for recognition (Figure 2). This information was used to design templates that present the required functional groups in their preferred orientation. α-D-Mannopyranosyloxybiphenyl-substituted carboxylic acid (TBC265) is such a compound and displays these functionalities in the required orientation. This compound has a greater *in vitro* potency then the parent SLe^x tetrasaccharide and an *in vivo* efficacy in small animal models of inflammation (Figure 2)[9]. Furthermore, compounds of this class have been shown to be orally bioavailable. It should be noted that the IC_{50} values of TBC265 for E-, P- and L-selectin inhibition are at the millimolar level. Other studies have shown that the biological affinity of saccharide ligands can be markedly enhanced by presenting them in clusters (multivalent saccharide ligands)[10].

The above developments will make it possible to design glycomimetics that may find application in areas where

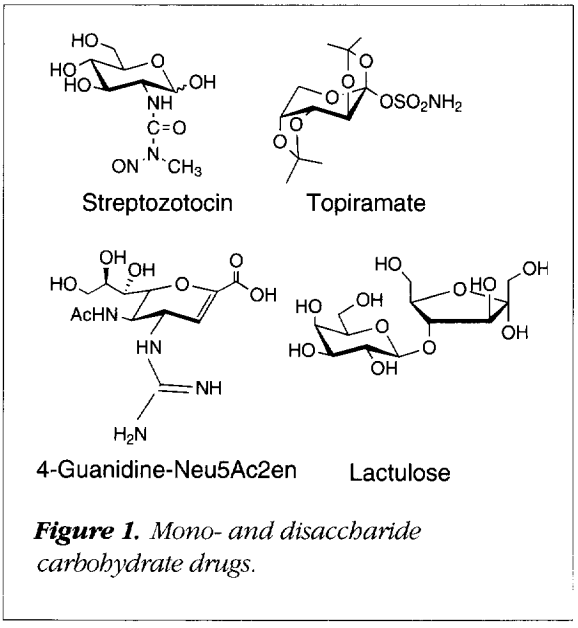


Figure 1. *Mono- and disaccharide carbohydrate drugs.*

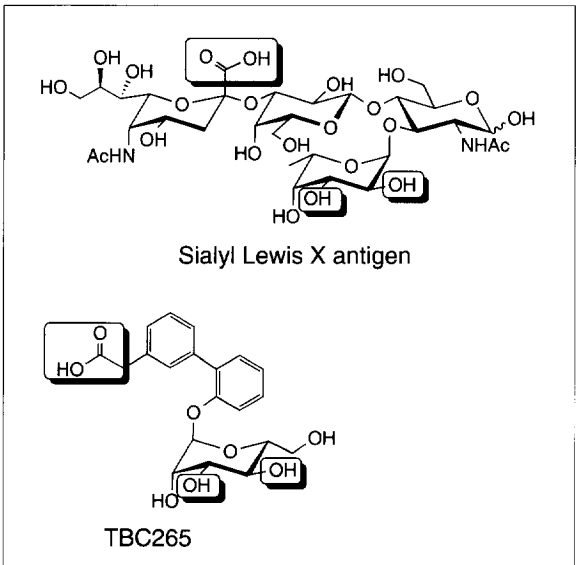


Figure 2. *Chemical structures of sialyl Lewis X and an analogue (TBC265) that has improved pharmacological properties.*

selectin-mediated mechanisms are thought to be important (e.g. reperfusion injury, psoriasis, septic shock, rheumatoid arthritis, asthma, cancer and inflammatory bowel disease).

The heparin sulphates are another class of saccharides with important biomedical applications. They are complex linear sulphated polysaccharides whose initial biosynthetic products are extensively modified by *N*- and *O*-sulphation and uronate epimerization. Heparin is widely used as an anticoagulant. It binds with high affinity to the plasma protein antithrombin III (AT III), thereby accelerating its inhibitory activity towards factor Xa and thrombin – two serine proteases involved in blood coagulation. The AT III-binding region of heparin consists of a unique pentasaccharide domain. A synthetic analogue of this domain has been developed that can accelerate AT III-mediated inhibition of factor Xa but not of thrombin (Figure 3)[11]. This pentasaccharide, which is produced completely synthetically (multi-kg scale), is now in late-phase clinical trials. There is also a growing body of literature indicating important neurobiological roles for heparin sulphate proteoglycans; examples include neuroepithelial growth and differentiation, neurite outgrowth, nerve regeneration, axonal guidance and branching, deposition of amyloid plaques in Alzheimer's disease and astrocyte proliferation[12]. It is to be expected that synthetic analogues of heparin may find application in the treatment of several neurodiseases.

Other pharmaceutical applications

Carbohydrates may also be used in other pharmaceutical applications. Saccharides that are recognized by cell-specific proteins may be used as drug delivery systems[13]. Also,

carbohydrate-based contraceptives may be developed which are targeted to lectins on the cell membrane of sperm cells[14]. Neoglycopeptides may find application as novel vaccines and may have many advantageous properties compared to traditional vaccines[15]. Derivatives of bacterial lipopolysaccharides with endotoxin antagonistic activity may provide drugs for the treatment of Gram-negative sepsis[16].

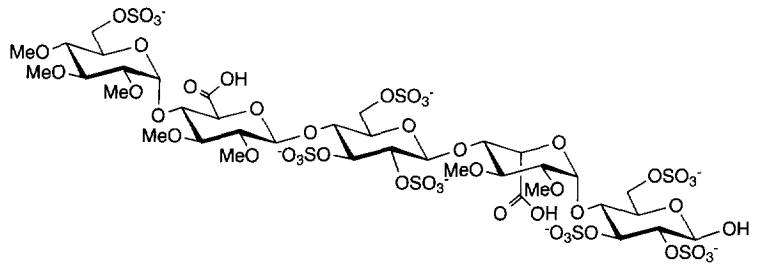


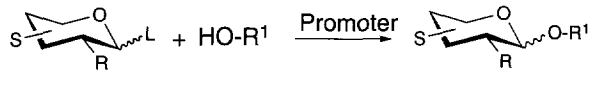
Figure 3. *Chemically modified heparin derivative.*

Enzymatic procedures have been developed to help overcome the problems associated with the chemical synthesis of oligosaccharides. However, the number of enzymes available for glycosidic bond synthesis is still very limited.

Preparation of complex oligosaccharides

It is clear that a wide range of well-defined oligosaccharides are required in order to study the SARs of the processes described above. Organic[17–22] and enzyme-mediated syntheses[23–25] provide an important means of obtaining such molecules.

The chemical synthesis of oligosaccharides is much more complicated than the synthesis of other biopolymers such as peptides and nucleic acids. The difficulties in the preparation of complex oligosaccharides result from the greater number of possibilities for the combination of monomeric units to form oligosaccharides. In addition, the glycosidic linkages have to be introduced stereospecifically (α/β selectivity). To date, there are no generally applicable methods or strategies for oligosaccharide synthesis and hence the preparation of these molecules is very time consuming. Nevertheless, contemporary carbohydrate chemistry makes it possible to execute complex, multistep synthetic sequences that give oligosaccharides consisting of as many as 20 monosaccharide units. The preparation of oligosaccharides of this size is only possible when each synthetic step in the assembly of the oligosaccharide is high yielding and, furthermore, the formation of each glycosidic linkage is highly stereoselective. Apart from this, the assembly of the monomeric units should be highly convergent.



Scheme 1. *Chemical interglycosidic bond formation. (See Box 1 for factors that influence the α/β ratio.)*

Chemical glycosidic bond synthesis

Chemical interglycosidic bond formation is generally achieved by condensing a fully protected glycosyl donor, which bears a leaving group (L) at its anomeric centre, with a suitably protected glycosyl acceptor (R^1OH) that often contains only one free hydroxyl group (Scheme 1). Traditionally, the most widely used glycosylation methods utilized anomeric halide derivatives of carbohydrates as glycosyl donors[17]. However, these compounds are often unstable and relatively extreme conditions are required for their preparation. The orthoester[26] and imidate[27] procedures were the first attempts to find alternatives to the glycosyl halide methodologies. Since then, many other leaving groups for the anomeric centre have been reported (see Figure 4)[21]. However, of these glycosyl donors, the anomeric fluorides, trichloroacetimidates and thioglycosides have been the most widely used. These compounds can be prepared under mild conditions, are sufficiently stable to be purified and stored for a considerable period of time, and undergo glycosylation under mild conditions. By selecting the appropriate reaction conditions, high yields and good α/β ratios can be obtained (see Box 1).

Box 1. Factors that influence the α/β ratio in glycosylations[a]

Substituent R: participating versus non-participating
Orientation substituent R: equatorial versus axial
Type of substituents (S) at donor and acceptor
Type of leaving group (L)
Type of promoter
Solvent
Temperature
Pressure

[a]See Scheme 1 for glycosylation reaction.

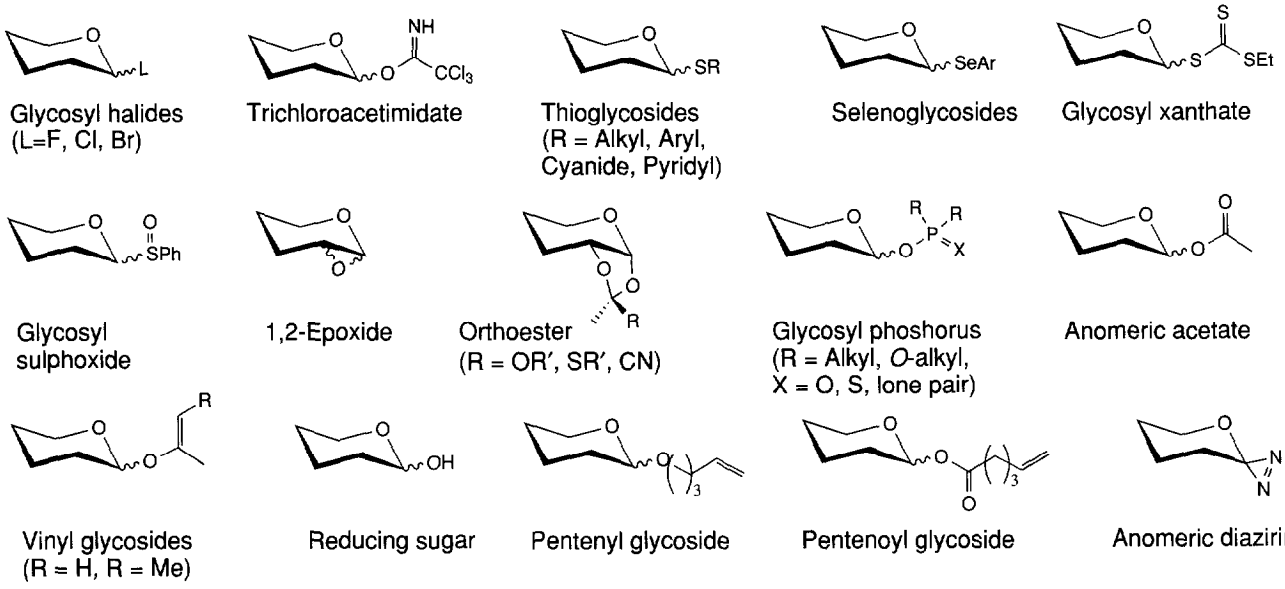


Figure 4. *Commonly used glycosyl donors.*

The anomeric linkages can be classified according to the relative and absolute configuration at C-1 and C-2 (Figure 5): the 1,2-*cis*- and 1,2-*trans*-2-D-*glycero* series (allo-, gluco-, gulo- and galactopyranosides) and the 1,2-*cis*- and 1,2-*trans*-2-L-*glycero* series (altro-, manno-, ido- and talo-galactopyranosides). In addition, some miscellaneous glycosidic linkages can be identified, including 2-deoxyglycosides and 3-deoxy-2-keto-ulo(pyranosylic) acids.

Anomeric control in chemical glycosylation

The stereoselective introduction of the glycosidic linkage is one of the most challenging aspects in chemical oligosaccharide synthesis. The nature of the protecting group at C-2 of the glycosyl donor is a major determinant of the anomeric selectivity. A protecting group at C-2 that can perform neighbouring group participation during glycosylation will give 1,2-*trans* glycosidic linkages (Scheme 2a). Thus, after removal of the leaving group (L), the acyl protecting group of **2** will perform neighbouring group participation to give a more stable dioxonium ion (**3**). Nucleophilic attack of the alcohol at the anomeric centre of intermediate **3** will result in the formation of a 1,2-*trans* glycoside (**4**). β-Linked products will be obtained with glucosyl-type donors, and mannosides will give α-glycosides. On the other hand, when a non-assisting functionality is present at C-2, the reaction conditions (e.g. solvent, temperature, promoter) will determine the anomeric selectivity. For example, the '*in situ* anomerization procedure' introduced by Lemieux and coworkers will result mainly in the formation of α-glycosides[28,29]. In this type of reaction, an activator catalyses the equilibration between the α- and β-halides (**5** and **6**, respectively) and the equilibrium is shifted strongly towards the α-bromide (**5**) because this compound is stabilized by the anomeric effect. However, the energy barrier for nucleophilic attack by an alcohol is lower for the β-halide (**6**). Therefore, glycosylation will take place from this intermediate and mainly α-glycosides (**7**) will be formed (Scheme 2b). High α-anomeric selectivities have been obtained with other anomeric leaving groups. For example, trimethylsilyltriflate-mediated couplings of perbenzylated

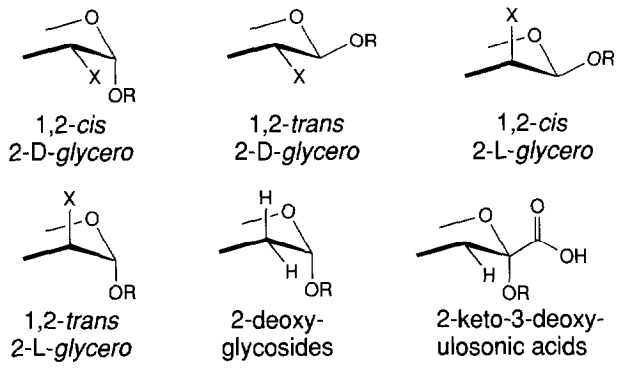
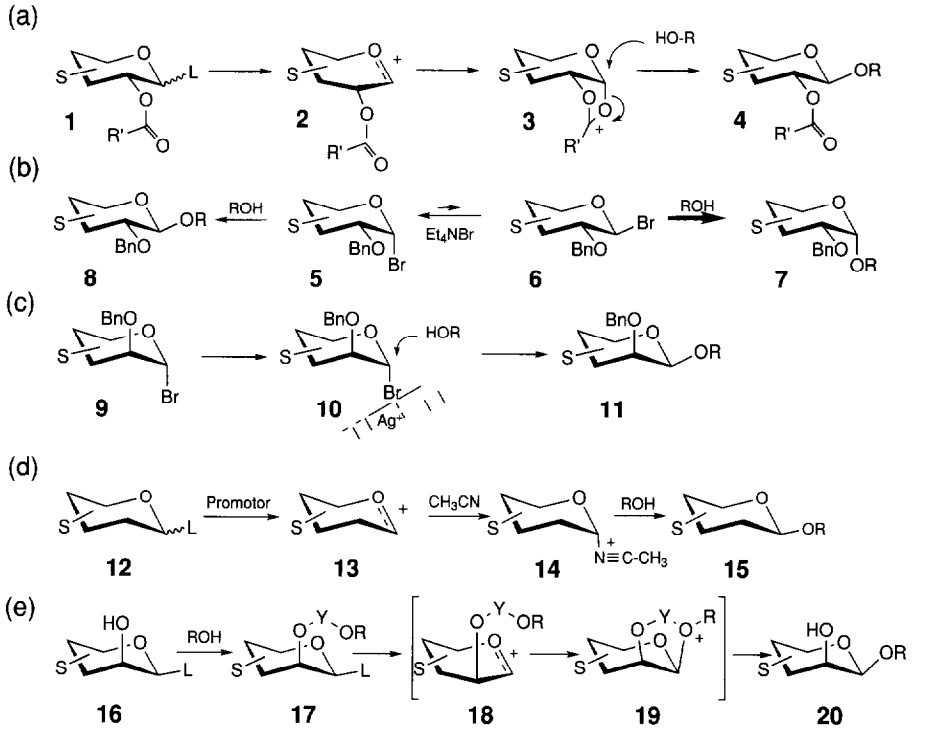


Figure 5. *Different types of glycosidic linkages.*



Scheme 2. *Methods for the stereoselective formation of glycosidic linkages. (a) Preparation of 1,2-*trans *glycosides by neighbouring group participation; (b) preparation of α-glycosides by* in situ *anomerization; (c) glycosylation by inversion of configuration; (d) preparation of β-glycosides by participation of the solvent acetonitrile; (e) synthesis of β-mannosides by intramolecular aglycone delivery.*

trichloroacetimidates at low temperature give, in many cases, excellent α-selectivities. It has also been reported that thioglycosides and glycosyl fluorides give high α-selectivities.

The *in situ* anomerization procedure requires a fast equilibrium between an α- and β-ion pair (**5**, **6**). However, some glycosylation procedures are based on preventing this preequilibration, and glycosylation will thus proceed via inversion of configuration. For example, glycosylation of α-halides (**9**) in the presence of an insoluble silver salt results mainly in β-glycoside formation (**11**) (Scheme 2c)[30–33]. In this case, anomerization of the halide is restricted because of lack of nucleophiles in the reaction mixture and the reaction will therefore proceed with inversion of configuration. Silver silicate and silver silicate–aluminate have often been used as the heterogeneous catalyst. These catalysts have proved to be valuable in the preparation of β-linked mannosides which can not be prepared by neighbouring group participation or *in situ* anomerization.

The stereochemistry of a glycosylation can also be controlled by a participating solvent. The most marked example is the use of acetonitrile, which in many cases leads to the formation of an equatorial glycosidic bond[34–38]. Several research groups have independently proposed that this reaction proceeds via an α-nitrilium ion (**14**) which is generated under S_N1 conditions (**12**→**13**). Nucleophilic substitution of the nitrilium ion by an alcohol will lead to β-glycosidic bond formation (**15**) (Scheme 2d). An important requirement for the reaction is the absence of a participating functionality at C-2. Unfortunately, this method gives low β-selectivities for mannosides.

Recently, β-mannosides have been prepared in a highly stereoselective manner by an intramolecular aglycone delivery approach[39–42]. In this case, the sugar alcohol (ROH) is first linked via an acetal or silicon tether (Y = CH_2 or $SiMe_2$, respectively) to the C-2 position of a mannosyl donor (**16**→**17**) and, subsequently, activation of the anomeric centre of this adduct (**17**) forces the aglycone to be delivered from the β-face of the glycosyl donor (Scheme 2e).

It is important to note that the approaches discussed above are in many cases unreliable and often result in modest α/β selectivities. For example, the constitution of the glycosyl donor and of the acceptor (e.g. type of saccharide, leaving group at the anomeric centre, protection and substitution pattern) has a major effect on the α/β selectivity. It should be realized that often many reaction conditions need to be examined to achieve acceptable results.

Convergent block synthesis

Oligosaccharides can be prepared by a linear glycosylation strategy or by block synthesis. In a linear glycosylation strategy, monomeric glycosyl donors are added to a growing saccharide chain. However, it is more efficient to use oligosaccharide building blocks as glycosyl donors and acceptors (convergent approach). Glycosyl bromides have been used in block synthesis, but the results were often disappointing, especially when labile bromides were used[17].

A variety of glycosyl donors are now available which can be prepared under mild conditions, are sufficiently stable to

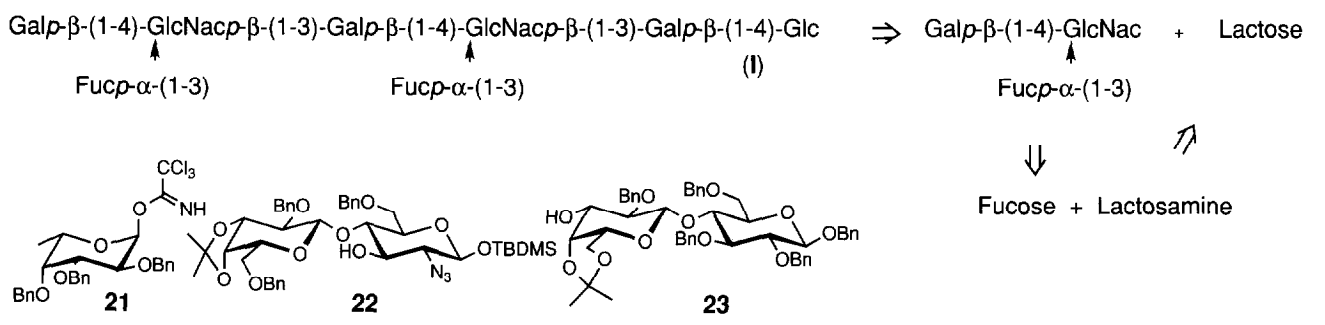


Figure 6. *Retrosynthetic analysis for the preparation of sialyl Lewis X (SLe^x).*

be purified and stored for a considerable period of time and which undergo glycosylation under mild conditions; by selecting the appropriate reaction conditions they give high yields and good α/β ratios. These features allow the preparation of oligosaccharides by efficient block syntheses.

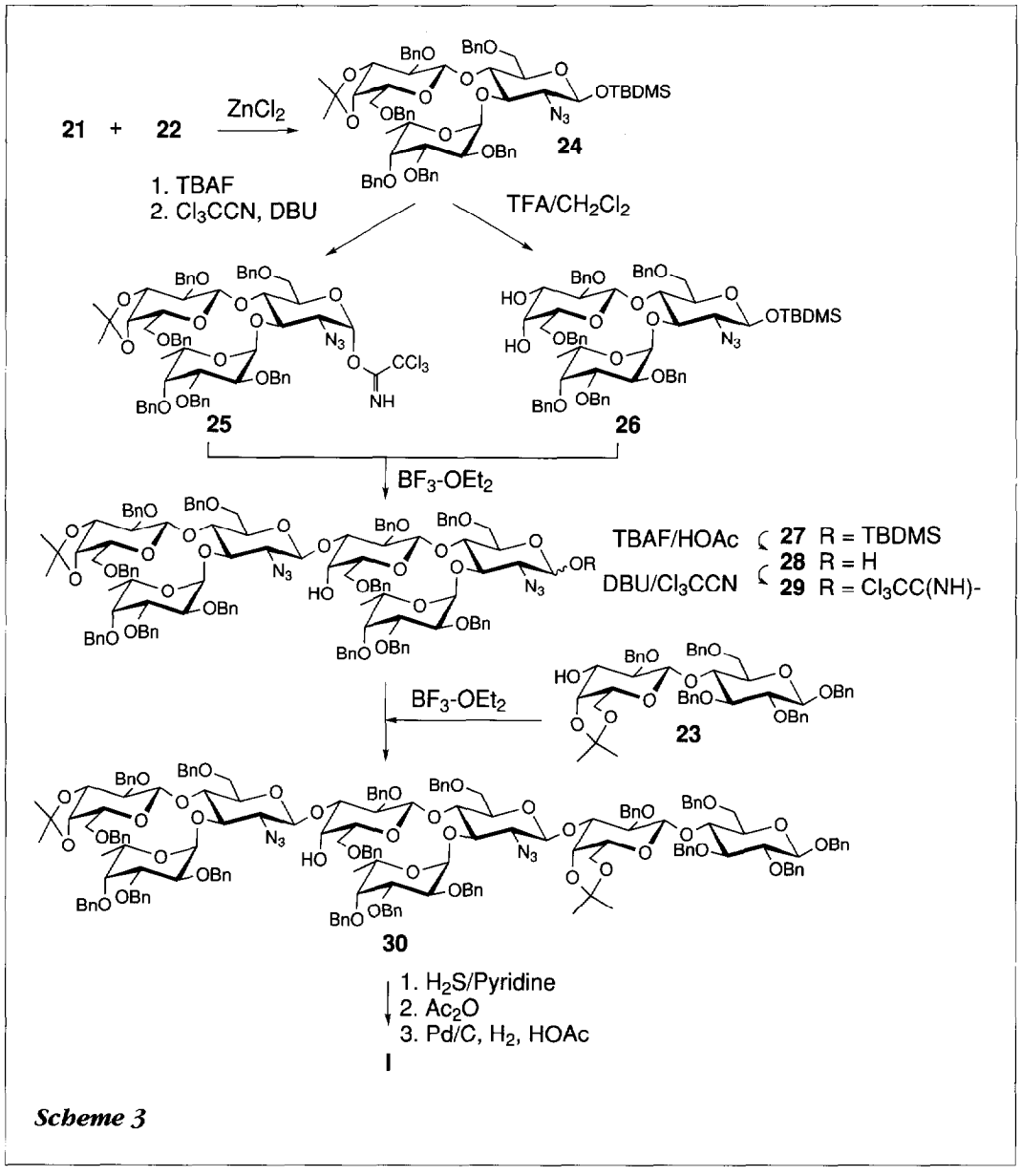
The favourable properties of the trichloroacetimidate methodology have been exploited in the block synthesis of the prominent tumour-associated dimeric antigen Lewis X (Le^x)[43,44]. The retrosynthetic strategy is depicted in Figure 6. In order to make efficient use of common building blocks, it was decided to disconnect the octasaccharide into two trimeric units and a lactoside residue. The trisaccharide was further disconnected into a fucose and a lactosamine moiety and the latter was readily available from lactose. Thus, the strategy was designed in such a manner that optimal use could be made of the disaccharide lactose, which is available at low cost. In such an approach, the number of glycosylation steps is considerably reduced. The key building blocks for the preparation of the target compound **I** were **21**, **22** and **23**.

The azidolactose building block **22** was prepared by azidonitration of lactal, followed by selective protection. The selectively protected lactoside **23** was readily available from lactose via a sophisticated protecting group interconversion strategy. α-Fucosylation of acceptor **22** with the very reactive fucosyl donor **21**, under 'inverted procedure' conditions[45], gave trisaccharide **24** in a 89% yield (Scheme 3). The trisaccharide (**24**) was converted into the required glycosyl donor (**25**) and acceptor (**26**). Coupling of glycosyl donor **25** with acceptor **26** in the presence of BF_3–Et_2O as catalyst gave the hexasaccharide **27** in a 78% yield. In the latter reaction, the higher acceptor reactivity of the equatorial 3-OH group with respect to the axial 4-OH was exploited. The synthesis of octasaccharide **30** required the repetition of the above strategy, i.e. conversion of the anomeric TBDMS group into a trichloroacetimidate functionality (**27**→**29**) and coupling of the trichloroacetimidate (**29**) with the lactoside unit (**23**) (64%). Finally, target molecule **I** was obtained by reduction of the azido group of **30**, followed by acetylation of the amino group and hydrogenation under acidic conditions.

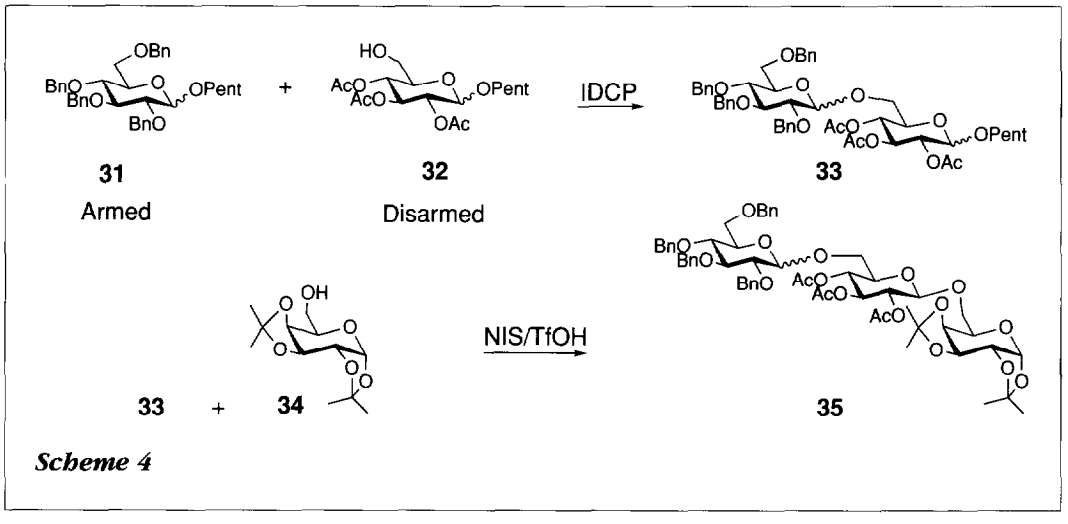
The described glycosylation strategy is highly convergent and makes optimal use of the common trisaccharide **24**. Furthermore, efficient use was made of the commercially available dimer lactose and, finally, the donor trichloroacetimidates could be prepared in high yield and behaved very well in the glycosylation reactions (high yields and high anomeric selectivities). The latter point requires some attention. It should be realized that some types of glycosidic linkages can be constructed rather easily, whereas others impose great difficulties. In planning a synthetic scheme, the disconnections should be chosen in such a way that the block assembly will not create problems. Furthermore, difficult glycosylations should be performed in an early stage of the synthesis.

Chemoselective glycosylations and one-pot multistep glycosylations

An important requirement of convergent oligosaccharide synthesis is ease of accessibility of oligosaccharide building blocks. Fraser-Reid and coworkers have introduced[19] a chemoselective glycosylation (armed–disarmed glycosylation strategy), which allows the preparation of this type of unit with a minimum of protecting group manipulations. They have shown that pentenyl glycosides having a protected C-2 ether can be coupled chemoselectively to C-2 benzoylated pentenyl glycosides. The chemoselectivity relies on the fact that an electron-withdrawing C-2 ester deactivates (disarms), and an electron-donating C-2 ether activates (arms), the anomeric centre. Thus, coupling of armed donor **31** with disarmed acceptor **32**, in the presence of the mild activator iodonium dicollidine perchlorate (IDCP), gave the dimer **33** as an anomeric mixture in a yield of 62% (Scheme 4). Next, the disarmed dimer **33** could



Scheme 3



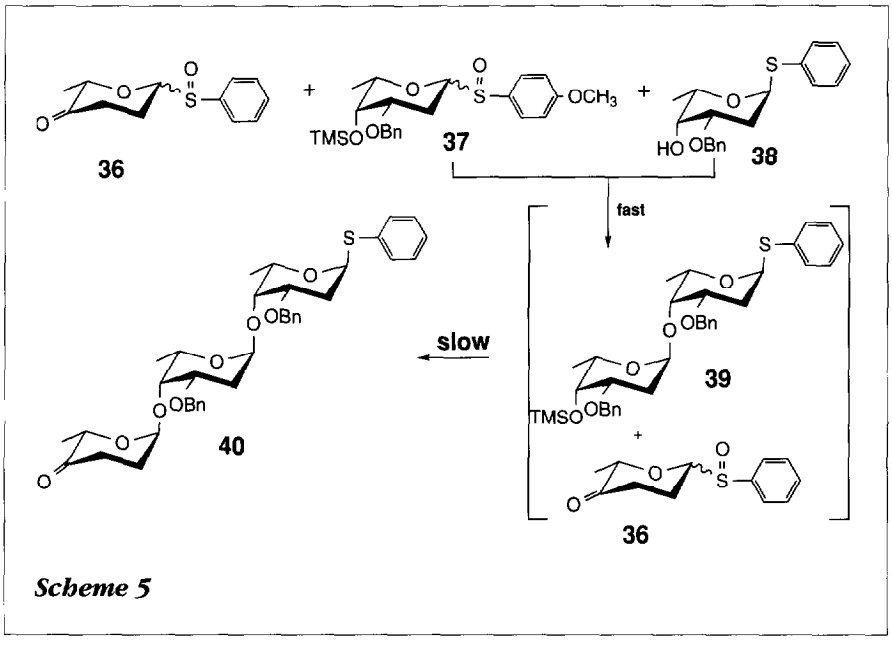
Scheme 4

be further glycosylated with acceptor **34**, using the more powerful activating system *N*-iodosuccinimide/catalytic triflic acid (NIS/TfOH) to yield the trisaccharide **35** (60%). Thus, this chemoselective glycosylation approach allows the preparation of a trisaccharide without a single protecting group manipulation between the glycosylations.

Chemoselective glycosylations have also been developed for thioglycosides[46–48] and glycals[49]. In the case of thioglycosides, a relatively wide range of glycosyl donors and acceptors with differential reactivities have been developed, allowing the preparation of larger oligosaccharide building blocks.

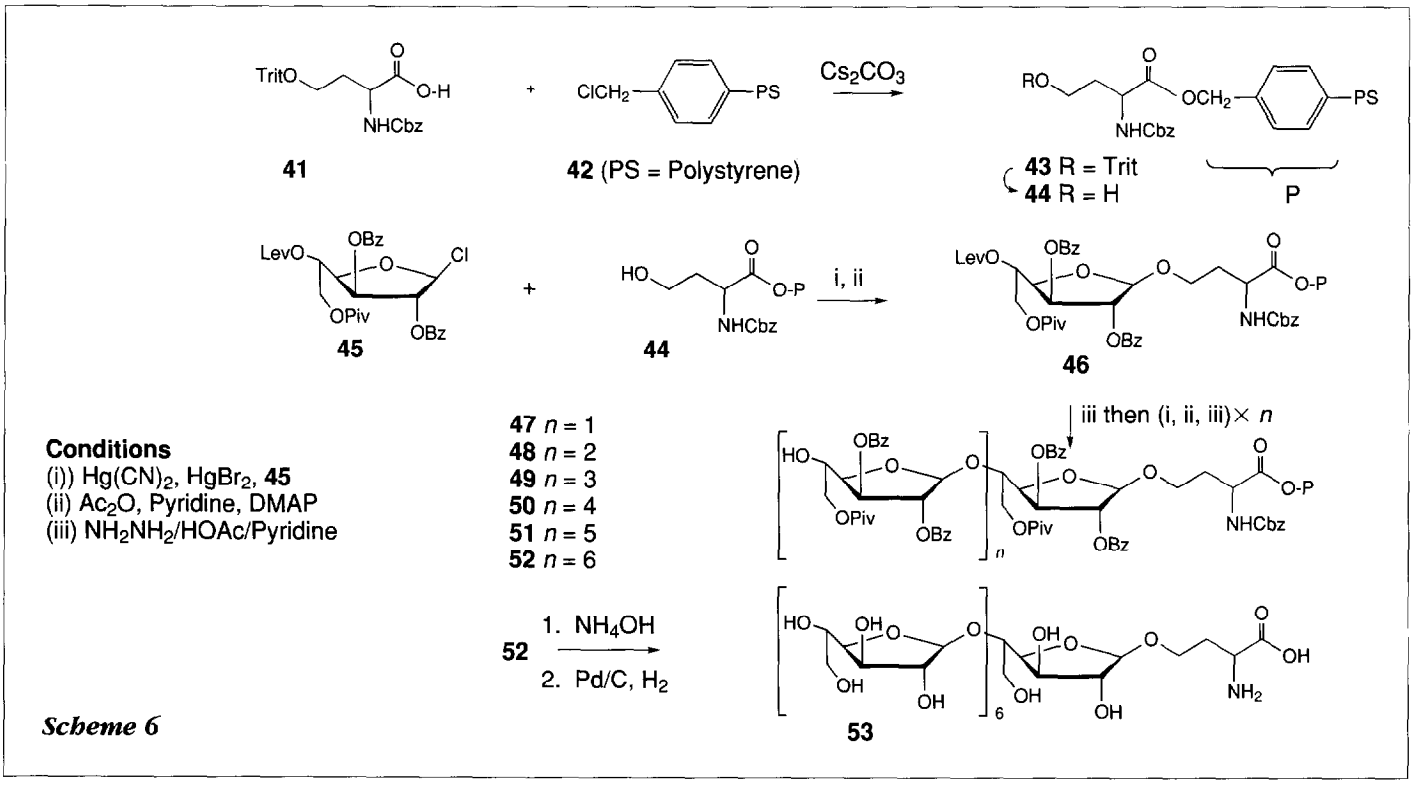
Recently, several methods have been reported to perform chemoselective glycosylations as a one-pot procedure. Kahne and coworkers[50] described a glycosylation method based on activation of anomeric sulphoxides with triflic anhydride (Tf_2O) or triflic acid (TfOH). Mechanistic studies revealed that the rate-limiting step in this reaction is triflation of the sulphoxide; therefore, the reactivity of the glycosyl donor could be influenced by the substituent in the para-position of the phenyl ring and the following reactivity order was established: OMe > H > NO_2. The reactivity difference between a *p*-methoxyphenyl sulphonyl donor and an

unsubstituted phenylsulphonyl glycosyl acceptor is large enough to permit selective activation. In addition, silyl ethers are good glycosyl acceptors when catalytic triflic acid is the activating agent, but react more slowly than a corresponding alcohol. These features opened the way for a one-pot synthesis of a trisaccharide (**40**) from a mixture of monosaccharides (**36**, **37** and **38**) (Scheme 5)[51]. Thus, treatment of this mixture with triflic acid resulted in the formation of trisaccharide **40** in a 25% yield. No other trisaccharides were isolated and the only other coupling product was dimer **39**. The products of the reaction indicate that the glycosylation takes place in a sequential manner. First, the most reactive *p*-methoxyphenylsulphenyl glycoside (**37**) is activated and reacts with alcohol **38** and not with the silyl ether of another molecule **37**. In the second stage of the reaction, the less reactive silyl ether of disaccharide **39** reacts with the less reactive sulphoxide **36** to give trisaccharide **40**. The phenylthio group of **40** can be oxidized to a sulphoxide which was used in a subsequent glycosylation. The trisaccharide obtained is part of the natural product ciclumycin 0, and, despite the relatively low yield of the coupling reactions, this methodology provides a very efficient route for this compound.



Scheme 5

Several variations of this concept have been reported[52–54]. For example, Ley and Priepke[52] prepared the trisaccharide unit that is derived from the common polysaccharide antigen of a group B streptococcus by a facile one-pot two-step synthesis. In this strategy, a glycosyl donor and acceptor were



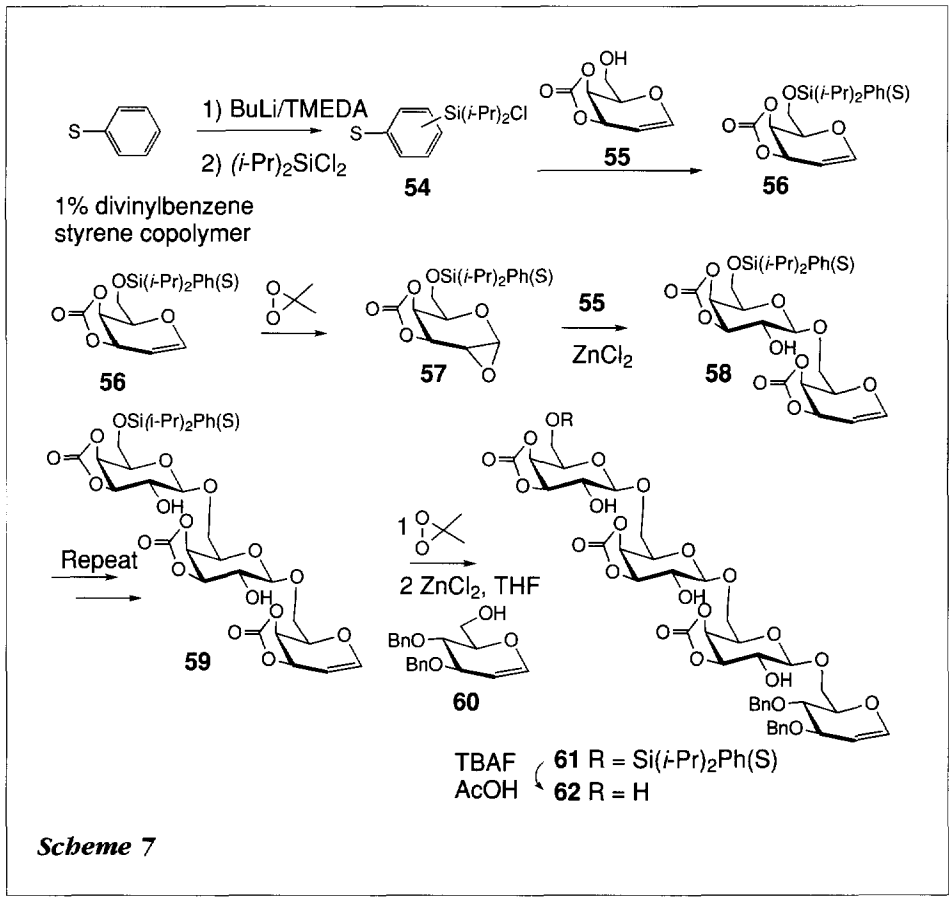
Scheme 6

coupled to give a disaccharide. Next, a second acceptor and more activator were added to the reaction mixture to prepare a trisaccharide. This type of glycosylation strategy allows the construction of several glycosidic linkages by a one-pot procedure and avoids time-consuming work-up and purification steps. It should be realized, however, that this type of reaction will only give satisfactory results when all the glycosylations are high yielding and highly diastereoselective. For example, by exploiting neighbouring group participation, it is relatively easy to form 1,2-*trans* glycosides selectively. Other types of glycosidic linkages may impose problems.

Solid-phase oligosaccharide synthesis

In the early 1970s, inspired by the success of solid-phase peptide and oligonucleotide syntheses, several research groups attempted to develop methods for solid-supported oligosaccharide synthesis[55–58]. However, since no powerful methods for glycosidic bond formation were available, the success of these methods was limited and only simple di- and trisaccharides could be obtained.

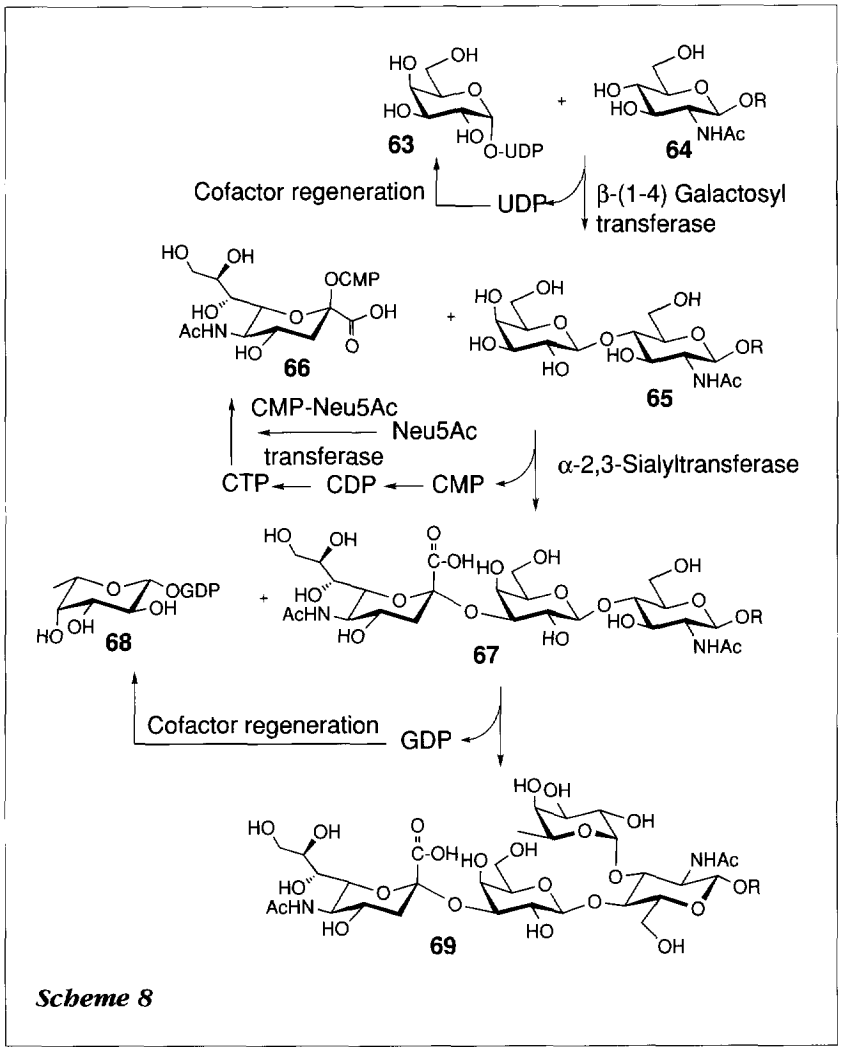
In 1987, Van Boom and coworkers reported[59] the solid supported synthesis of a D-galactofuranosyl heptamer. The synthetic approach that they followed is illustrated in Scheme 6. The selectively protected L-homoserine (**41**) was linked to the Merrifield polymer chloromethylpolystyrene (**42**) to give the derivatized polymer **43**. The loading capacity of the polymer was 0.5 mmol/g resin. Acid hydrolysis of the trityl group of **43** gave **44**, and coupling of the chloride (**45**) with the immobilized **44** under Koenigs–Knorr conditions afforded the polymer-linked homoserine glycoside **46**. It was observed that the coupling reaction had not gone to completion and, in order to limit the formation of shorter fragments, the unreacted hydroxyl groups were capped by treatment with acetic anhydride in the presence of pyridine and *N,N*-dimethylaminopyridine (DMAP). Elongation was performed as follows: the levulinoyl (Lev) group of **46** was removed by treatment with a hydrazine/pyridine/acetic acid mixture, the released alcohol was coupled with chloride **45** and the unreacted hydroxyl groups were capped by acetylation. After repeating this procedure five times (n = 6), the heptasaccharide **52** was released from the resin by basic hydrolysis. Under these conditions the benzoyl (Bz) and pivaloyl (Piv) protecting groups were also removed. Finally, cleavage of the benzyloxycarbonyl (Cbz) group by hydrogenolysis over Pd/C gave **53** in an overall yield of 23%.



Scheme 7

Kahne and coworkers[60] described the solid supported synthesis of oligosaccharides using anomeric sulphoxides as donors. In the procedure of Van Boom and Kahne, the anomeric centre of a saccharide is linked to the solid support and glycosyl donors are added to the growing chain. Recently, Danishefsky and coworkers[61,62] reported an inverse approach using the incoming sugars as glycosyl acceptor. The basic strategy involves attachment of a glycal to a polymer support, followed by epoxidation to provide a 1,2-anhydro derivative. This polymer-bound glycosyl donor is then treated with a solution of a protected glycal, acting as a glycosyl acceptor, to give a polymer-bound disaccharide. Reiteration of this reaction sequence provides larger oligosaccharides which ultimately are retrieved from the support (Scheme 7). The commercially available 1% divinyl benzene–styrene copolymer was employed and the glycal was attached to

this resin using a diisopropylsilyl ether linker. Such a linker is stable under the reaction conditions employed, but can be cleaved by fluoride ion treatment. In previous studies, a diphenyldichlorosilane linker was used, but it was found to be inferior to the diisopropylsilyl linker. Lithiation of the copolymer followed by quenching with diisopropyldichlorosilane provided **54**. The silylated polymer was reacted with a solution of galactal (**55**) in dichloromethane and Hünig's base to give the corresponding dialkylsilyl-linked polymer construct (**56**). The loading of the solid support was 0.9 mmol/g resin. The double bond of the polymer bound glycal (**56**) was activated by epoxidation with 3,3-dimethyldioxirane, and the epoxide **57** thus obtained was reacted with a tetrahydrofuran solution of **54** in the presence of $ZnCl_2$ to give the polymer-bound dimer **58**. The glycosidation procedure required a 6–10-fold excess of solution-based glycosyl acceptor and 2–3 equivalents of promoter, although in some reactions less acceptor and shorter reaction times have been used. It should also be noted that no glycosylation at the 2-position was observed. Twice repetition of this two-step procedure (epoxidation, glycosylation) provided a polymer-bound tetrasaccharide (**61**) which was released from the solid support by treatment with tetra-*n*-butylammonium fluoride (TBAF). The method allowed the preparation of tetrasaccharide **62** in a 74% overall yield.



Scheme 8

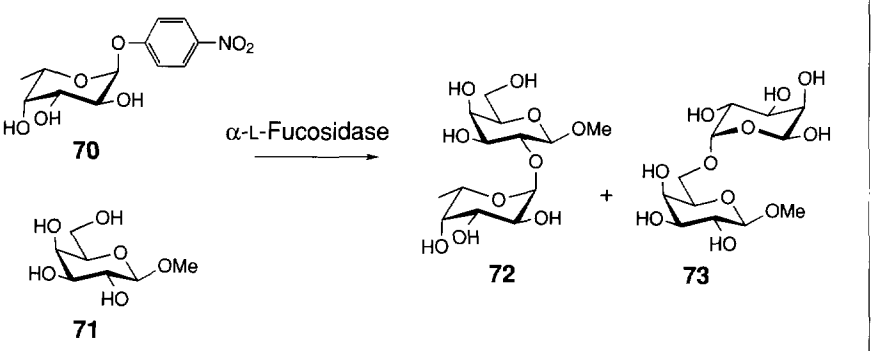
An advantageous aspect of this solid supported approach is that no capping step is required because any unreacted epoxide will hydrolyse in the washing procedure. On the other hand, in the case of a very difficult glycosylation step, most of the solid-support linked glycosyl donor may decompose, lowering the overall yield. In the procedures of Van Boom and Kahne, excess donor can be used to achieve acceptable yields in difficult glycosylation reactions.

The rates of reactions on a solid support are generally reduced compared to solution-based methods. Krepinsky and others addressed this problem by using a polymer-supported solution synthesis of oligosaccharides[63–65]. This strategy is based on the fact that a polyethylene-polymer-supported saccharide is soluble under conditions of glycosylation but insoluble during the work-up procedure.

Enzymatic and semisynthetic glycosylation strategies

The need for increasingly efficient methods of oligosaccharide synthesis has stimulated the development of enzymatic methods[23–25]. These methods bypass the need for protecting groups since the enzymes control both the regio- and the stereoselectivity of glycosylation. Two basic approaches for enzymatic oligosaccharide synthesis are available: the use of glycosyltransferases and the application of glycosylhydrolases.

Glycosyltransferases are essential enzymes for oligosaccharide biosyntheses and transfer a sugar residue from a sugar–nucleotide mono- or diphosphate to a maturing oligosaccharide chain. These enzymes are highly regio- and stereoselective and have been obtained by isolation or by cloning and overexpression. The group of Wong and Paulson have employed glycosyltransferases for the preparation of the SLe^x ligand (Scheme 8)[66]. They overexpressed galactosyl-, fucosyl- and



Scheme 9

neuramyltransferases, which were obtained in relatively large amounts. A new method for *in situ* nucleotide diphosphate regeneration was developed. This procedure avoids the use of very expensive cofactors and also prevents product inhibition of the enzymes. For example, condensation of galactosyl-UDP (**63**) with *N*-acetylglucosamine (**64**) gives stereo- and regioselective formation of disaccharide **65** and UDP. The UDP is regenerated by a sophisticated enzyme cascade to give galactosyl-UDP which can be used in a next reaction cycle. The disaccharide **65** is a substrate for the enzyme α-2,3-sialyltransferase, and coupling with CMP-Neu5Ac gave trisaccharide **67**. Finally, **67** was fucosylated by fucosyl-GDP (**68**) in the presence of a fucosyltransferase to give the target compound (**69**). This method allows the preparation of multigram quantities of the SLe^x tetrasaccharide.

The number of available glycosyltransferases is still very limited and these enzymes are highly substrate specific; thus the possibilities to prepare analogues are limited.

Glycosylhydrolases were traditionally used for the degradation of oligosaccharides. However, the reverse hydrolytic activity of glycosidases can be exploited in glycosidic bond formation. This method allows the preparation of several di- and trisaccharides. For example, reaction of *p*-nitrophenyl α-fucopyranoside (**70**) with methylgalactoside (**71**) in the presence of α-L-fucosidase gave a mixture of the disaccharides **72** and **73** (Scheme 9)[67]. The product ratio can be influenced by the reaction solvent. Glycosylhydrolases are much more readily available than glycosyltransferases but are in general less stereoselective and lower yielding.

Recently, a combined sequential use of a glycosidase together with a glycosyltransferase and cofactor regeneration was used for the preparation of sialyl T antigen[68].

In order to overcome problems associated with chemically and enzymatically based methods, combined approaches have been developed[69–72]. In such an approach, glycosidic linkages that are very difficult to introduce chemically are introduced enzymatically, and vice versa. The latter approach has proved to be extremely valuable for the introduction of neuramic acid units into an oligosaccharide.

Enzymatic oligosaccharide synthesis has also been performed on solid supports[73].

Conclusion and future perspectives

The development of improved glycosylation procedures has made it possible to plan the preparation of an oligosaccharide much more reliably, and tri- and tetrasaccharides can now be synthesized routinely. Convergent synthetic strategies have become available that allow the convenient assembly of complex oligosaccharides from properly protected building units involving a minimum number of synthetic steps.

Methods for solid-phase oligosaccharide synthesis have been reported. However, a major problem for the general application of solid-phase techniques is that glycosylation reactions often proceed with modest yield and poor anomeric selectivity. The latter point needs some attention.

Traditionally, chemists have focused on the preparation of single compounds. However, combinatorial chemistry has afforded the possibility of preparing mixtures of compounds that can be used for biological screening[74]. Thus, medicinal carbohydrate chemists should perhaps consider constructing small libraries of saccharides in which the compounds differ only in anomeric configuration (i.e. the oligosaccharides would then be prepared as mixtures of anomers). An important requirement for this type of synthesis would be the availability of saccharide building blocks that can readily be converted into donors and acceptors[75]. An attempt has been made to construct more complex libraries[76]; however, the preparation of large saccharide libraries will be impractical since the introduction of a glycosidic linkage is less well developed than amide and phosphodiester bond synthesis.

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