1
Synthetic Methodologies

Chikako Saotome and Osamu Kanie

1.1
Introduction

Research directed toward revealing the functions of oligosaccharides is currently the subject of great attention, and so the synthesis of oligosaccharides as probes for functional investigation is being widely investigated. After decades of efforts since the first synthesis of the disaccharide sucrose [1], it has now become possible to synthesize a variety of oligosaccharides. For the successful synthesis of oligosaccharides, both chemical reactions and tactics are important concerns. This chapter focuses on the strategic aspect of oligosaccharide synthesis.

1.2
Tactical Analysis for Overall Synthetic Efficiency

For the efficient synthesis of oligosaccharides, both stepwise and convergent methods have to be employed (Fig. 1.1). The former format can be further divided into two subclasses: one in which synthesis starts from the reducing end (A), which has classically been used, and another in which synthesis starts from the non-reducing end (C). Format A has traditionally been used in oligosaccharide synthesis as it was difficult to transform anomeric protecting groups into the leaving groups required for the C format. The recent development of new anomeric protecting groups and some substituent groups that can be directly used as the leaving groups, however, have enabled the alternative format (C) to be used. The concept of stepwise synthesis is especially important for the construction of relatively small oligomers, but the convergent format (B) has to be employed for the synthesis of larger saccharides. This can be easily understood by simple tactical analysis in the cases of the synthesis of large oligosaccharides or oligosaccharides possessing repeating units in their structures.

Carbohydrate-based Drug Discovery. Edited by Chi-Huey Wong

ISBN: 3-527-30632-3

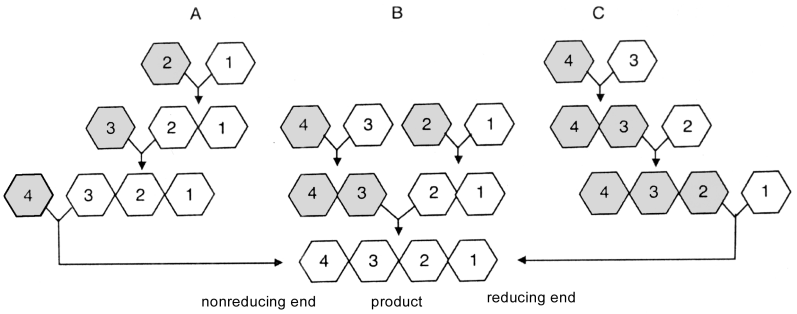
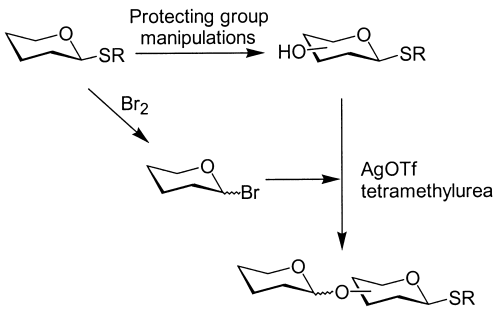


Fig. 1.1 Two stepwise methods and a convergent method in the synthesis of oligosaccharides. A: One of the stepwise methods, in which synthesis starts from the reducing end. B: The convergent method is especially advantageous for the synthesis of oligosaccharide with repeating structure. C: The other stepwise method, in which synthesis starts from the non-reducing end. Open hexagons: acceptors or protected forms. Gray hexagons: donors.

1.3 Methodological Improvements

One of the most important improvements in oligosaccharide synthesis is the discovery of the use of "stable" leaving groups that can function as protecting groups until exposed to certain activation conditions. This type of "potential leaving group" at the anomeric position is an ideal candidate intermediate in flexible synthetic strategies for oligosaccharides [2] (Scheme 1.1). The chemoselective glycosylation strategy that has emerged is based on tactical analysis aiming at efficient oligosaccharide synthesis [3–6, 7–23] (see Section 1.3.3).

The advancement of oligosaccharide synthesis is largely based on the development of good anomeric leaving groups and methods to control stereochemistry [24–26]. Regardless of the method used to control the stereochemistry of a given newly formed glycosidic linkage, one of the key factors is the reactivities and the stabilities of the leaving groups and the conditions used to activate one over another selectively.

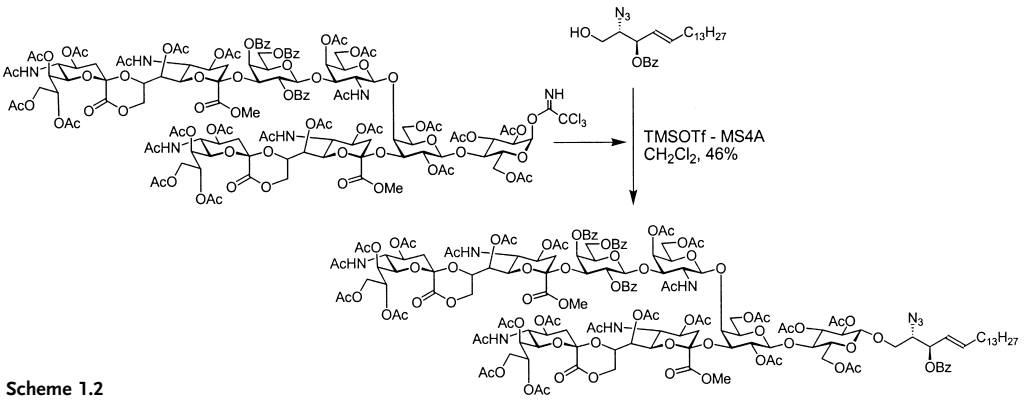


Scheme 1.1

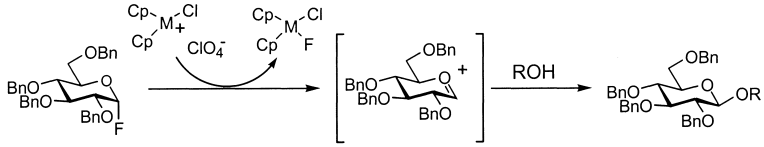
1.3.1 Chemistry

The first species to be recognized as a form of protected carbohydrate synthetic unit were alkyl- or phenylthio glycosides, the use of which allows anomeric centers to be readily converted into halides [3–5, 27] (Scheme 1.1). This so-called two-stage activation (see Section 1.3.3) is possible thanks to the stabilities of these compounds towards the acidic conditions generally used for glycosylation reactions and protecting group manipulations. In addition, they can be activated directly, which allows extremely flexible synthetic schemes for oligosaccharide synthesis, including the "armed and disarmed" concept [28], orthogonal strategy [29], the "active and latent" concept [30], and one-pot glycosylation [6, 21]. Thioglycosides can also be converted into more reactive sulfoxides, which have been shown to be useful both in solution and in solid-phase reactions [6, 31, 32].

One of the most powerful and popular anomeric leaving groups is the trichloroacetimidate group, which has been used for the synthesis of oligosaccharides in solution [33, 34]. One special characteristic of this group is its applicability for transferring large oligosaccharides onto aglycon moieties, such as in the case of azido sphingosine, a commonly used ceramide precursor, to afford a ganglioside precursor [35–37] (Scheme 1.2). Glycosyl trichloroacetimidates have also been shown to react in highly polar solvents such as DMF, which has allowed the glycosylation of unprotected glycosyl acceptors in a random manner [38–40]. Activation of the imidate donor can be achieved by use of $BF_3 \cdot OEt_2$, trimethylsilyl triflate (TMSOTf), triethylsilyl triflate (TESOTf), or silver triflate (AgOTf) [41]. TESOTf was introduced to avoid by-product formation (glycosyl fluoride in the case of $BF_3 \cdot OEt_2$ [42], or TMS ethers of the acceptor in the case of TMSOTf). Recently, the use of dibutylboron triflate (DBBOTf) to address both problems has been reported [43]. Trichloroacetimidate is also used in polymer-supported oligosaccharide synthesis and has been shown to be compatible with a variety of supports, including PEG [43, 44] (see Section 1.4.1), Merrifield-type resin [45–48], and CPG [49] (see Section 1.4.3).



Scheme 1.2



Scheme 1.3

Mukaiyama, in 1981, used $SnCl_2/AgClO_4$ as an activation system for glycosyl fluorides in ether to form glycosidic linkages [50]. Generally, however, it was the case that glycosyl fluorides were too stable to act as glycosylating agents in complex oligosaccharide syntheses. The situation changed after Suzuki's discovery of mild conditions with the use of $Cp_2MCl_2/AgClO_4$, where M is Hf or Zr [51, 52] (Scheme 1.3). Under these conditions, glycosyl fluorides can usually be activated at lower temperatures.

Glycals act as 1,2-protected sugars and are used as glycosylating agents. Traditionally, glycals were used to synthesize glycosides of 2-deoxy sugars by Fisher glycosylation or through 2-halo intermediates. Glycals were also used to produce ordinary glycosides via epoxides as the active agents. The advantage of glycals is their flexibility in the synthetic scheme, as has been shown in Danishefsky's research [53, 54] [see Section 1.4.3.5]. 2-Deoxy halosugars can be transformed into 2-amino-2-deoxy sugars by substitution reactions, which makes the glycals more useful strategically.

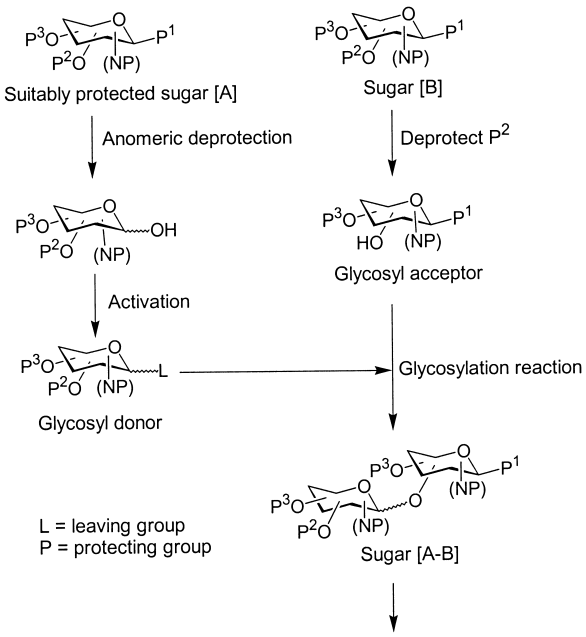
Other leaving groups (*n*-pentenyl, phosphite, phenylselenyl, etc.) have also been used for successful oligosaccharide synthesis [25, 26, 55]. In recent methods of synthesis of complex oligosaccharides, selective activation of a certain leaving group among others has allowed highly efficient syntheses [2, 56].

1.3.2
Protecting Group Manipulations

In oligosaccharide synthesis, particular sets of protecting groups have to be used, due to the multifunctional nature of carbohydrates (Scheme 1.4). The incorporation of protecting groups on functional groups needing to be protected and the order of deprotection have to be considered before the synthesis.

A general term "selectivity" has been used to describe these complex protecting group manipulations, but in 1977 a concept of chemical distinctiveness was introduced. The idea of orthogonal protection was defined by Baranay and Merrifield as "a set of completely independent classes of protection groups, such that each class can be removed in any order and in the presence of all other classes" [57]. Orthogonal protecting group manipulations are widely accepted, not only in peptide chemistry, but also in other fields including carbohydrate chemistry. The concept is summarized in Fig. 1.2. When individual hydroxy groups (two to five OHs) are protected with A, B, C, and D, respectively, and individual protecting groups can be removed in any order under certain conditions, the protecting groups can

Scheme 1.4



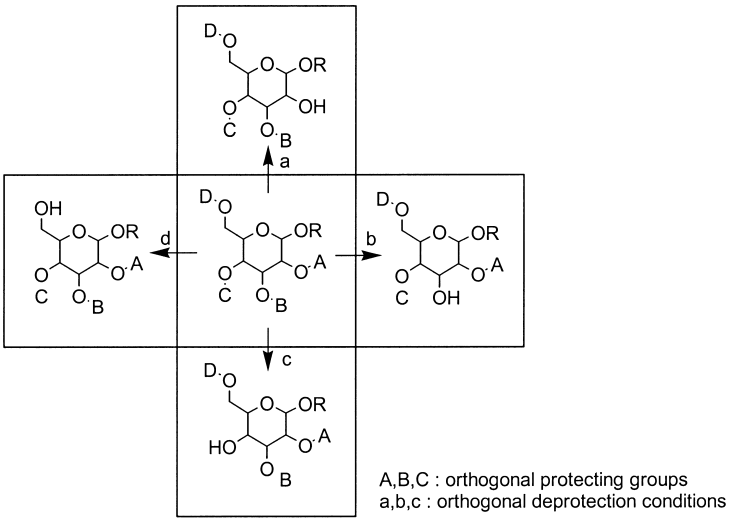


Fig. 1.2 Orthogonal protecting group manipulations. Protecting groups A–D can be removed in any order, eliminating tedious protecting group manipulations during complex oligosaccharide syntheses.

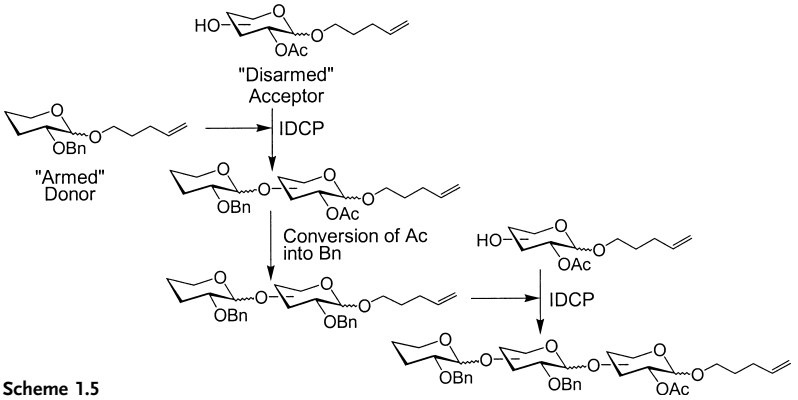
be said to be in an orthogonal relationship. The use of the concept is described well in Wong's work. As a representative set of orthogonal hydroxy protecting groups in carbohydrate chemistry, one combination of protecting groups and corresponding orthogonal deprotection conditions to have been used successfully [58] is A: chloroacetyl (a: $NaHCO_3/MeOH/H_2O$), B: methoxybenzyl (b: TFA/CH_2Cl_2), C: levulinyl (c: $NH_2NH_2/AcOH/THF/MeOH$), and D: TBDPS (d: HF/Pyr/AcOH/THF). Other sets are being investigated [59].

1.3.3 Modulation of the Reactivity of Glycosyl Donors

The reactivity of glycosyl donors can be controlled either through protecting groups or by anomeric leaving groups. Through the use of a set of molecules with suitable reactivities, oligosaccharides can be synthesized in the minimum possible number of operations.

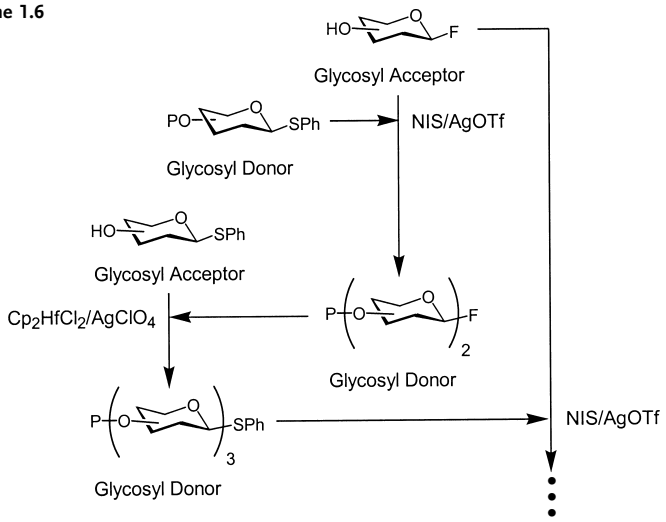
The "armed and disarmed" concept, which employs a single potential leaving group (the *n*-pentenyloxy group) at the anomeric positions both of the donor and of the acceptor was developed from the observation that the reactivities of glycosyl donors are affected by the protecting groups (i.e., ether or ester) [28] (Scheme 1.5). The utility of this methodology is obvious, since small fragments of oligosaccharides can be systematically synthesized in short steps, in which a "disarmed" unit can be transformed into an "armed" unit by exchanging the protecting groups. Alternatively, the coupling product can be directly used as a donor if exposed to slightly stronger activation conditions. The armed and disarmed concept has also proven to be applicable to glycals [8], thioglycosides [60], selenyl glycosides [61], and glycosyl phosphoroamidates [62]. Furthermore, it has also been shown that the reactivities of these potential glycosyl donors can be controlled by selection of protecting groups at positions other than *O*-2 [61, 63–65].

A strategically related but conceptionally independent method, orthogonal glycosylation, has been developed. The key feature of the orthogonal coupling concept

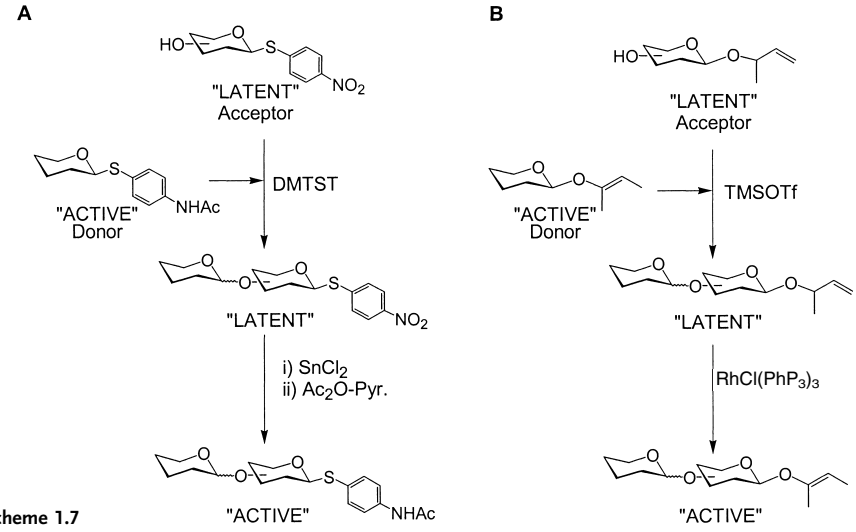


Scheme 1.5

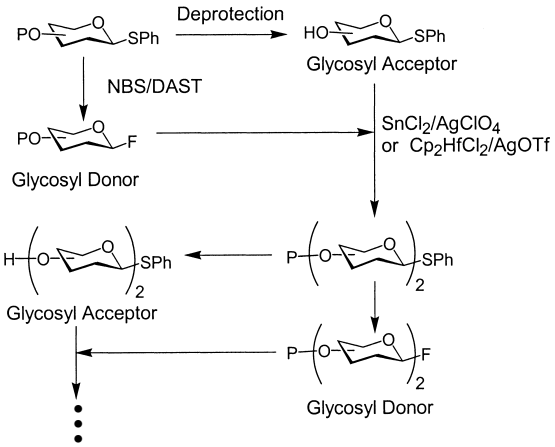
Scheme 1.6



is the combined use of two chemically distinct glycosylation reactions [29, 66, 67] (Scheme 1.6). A set of potential leaving groups and activation conditions for each group – phenylthio group and fluoride, and NIS/AgOTf and $Cp_2HfCl_2/AgClO_4$ – were used. Since the reactions of the set are mutually distinct, there is no need for reactivity control, so this methodology is conceptionally different from reactivity modulation methods. In addition, it has also been shown that the strategy can be applied to a polymer-supported oligosaccharide synthesis [67]. Another set of potential leaving groups with orthogonal reactivities has also been investigated [68].



Scheme 1.7



Scheme 1.8

Another tactic in oligosaccharide synthesis is the so called "active and latent" method. This method may be regarded as an extension of the traditional method without cleavage of the group but its transformation into an active species. One of the technique's successes is in the use of an allyloxy group as the protecting group at anomeric centers. It is later converted into a vinyl ether, which is readily activated in the glycosylation reaction [30, 69] (Scheme 1.7). However, this method is more likely related to the two-stage method discussed below.

The phenylthio group has commonly been used as a precursor of glycosyl donors such as glycosyl fluorides. The glycosyl fluorides can be activated chemoselectively without affecting the parent thioglycoside [3–5] (Scheme 1.8). In this way, extremely efficient syntheses of oligosaccharides possessing repeating sequences have been achieved in a convergent manner.

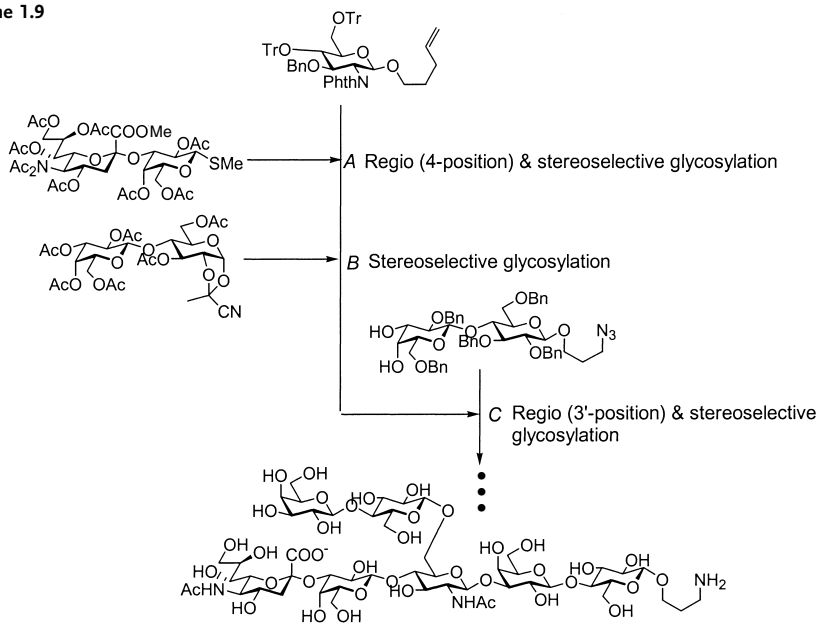
1.3.4
Block Synthesis

The importance of the convergent method (Fig. 1.1) is obvious in the synthesis of larger oligosaccharides (see also Section 1.2). This section covers several examples of oligosaccharide synthesis with special emphasis on the tactics. Because of the structural heterogeneity of the oligosaccharides involved, block synthesis is more suitable term than convergent synthesis to describe the synthesis.

The first example is the synthesis of a heptasaccharide reported by Boons et al., based on profound knowledge of carbohydrate chemistry [70] (Scheme 1.9).

The target heptasaccharide was first retrosynthetically taken into four blocks as shown in Scheme 1.9, the key feature of the synthesis being a reduced number of chemical steps after having four synthetic units. Sequential glycosylation reactions involving a 4,6-di-*O*-tritylated *n*-pentenyl glycoside derivative of glucosamine as a key unit were carried out with a methylthio glycoside, a cyanoethylidene, and an *n*-pentenyl glycoside as glycosyl donors. Another tactical ploy employed in this in-

Scheme 1.9



vestigation is the use of least protected acceptors. It is obvious that the use of least protected acceptors in a synthetic strategy involving multifunctional components as in carbohydrate synthesis is advantageous because one can eliminate time-consuming protective group manipulations [71]. In addition, the coupling reaction is free from any influence of nearby bulky protecting groups.

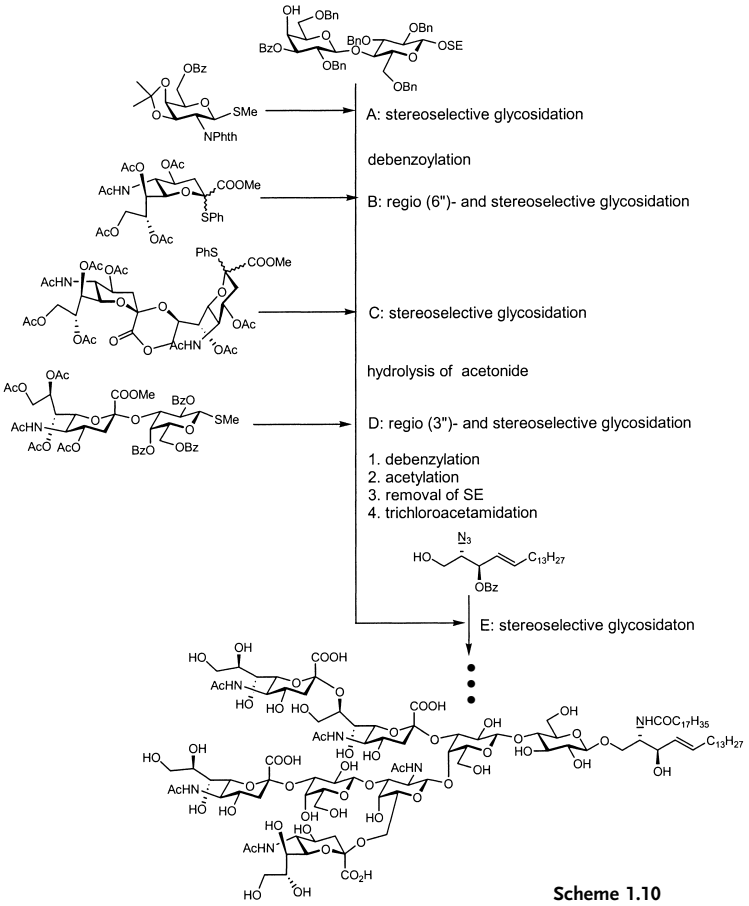
The issue, however, is the regiospecificity. One of the regiospecific glycosylations was carried out in the synthesis of sialyl galactose donor. A *N*-diacetylated methylthio glycoside of sialic acid and 4,6-benzylidene TMS ethyl galactoside were used as the donor and acceptor. The advantage of the diacetylated sialyl donor is the enhanced reactivity of thioglycoside due to the long range electronic effect [72]. It was reported that a higher yield than with *N*-acetyl derivative was obtained in a shorter reaction time, the stereochemistry being controlled through solvent effects.

The coupling product was further transformed into a thioglycoside. The second glycosylation was between the sialyl galactose donor and the 4,6-di-*O*-tritylated monosaccharide. The large steric hindrance of the 4-*O*-trityl group gave rise to polarization of the C–O bond of the secondary trityl ether, which enhanced reactivity and enabled regioselective glycosylation at the 4-*O* position. The neighboring participating effect of the 2-*O*-acetyl group permitted *β*-stereoselective glycosylation. The coupling product bearing a 6-*O*-trityl group was directly used as an acceptor for the next glycosylation reaction with cyanoethylidene lactosyl donor. Furthermore, since the anomeric position of the GlcN derivative was protected as an *n*-pentenyl glycoside, the formed pentasaccharide could again be directly used as a

donor to couple with another lactose derivative. In this glycosylation reaction, regioselectivity toward the equatorial 3-*O* position was achieved. After removal of all protecting groups, the introduced amino functionality at the reducing terminal was used to incorporate the saccharide onto polyacrylamide for biological assays.

When the target is a series of oligosaccharides, a more systematic and unified strategy is required. Common building blocks have to be carefully designed and used in the synthesis of multiple target saccharides. Excellent examples of this kind of research can be found in a course of synthetic work carried out by Hasegawa and Kiso [73] and by Schmidt [74].

As a representative systematic oligosaccharide synthesis, we focus on a synthesis of a ganglioside known as GQ1b*α* [75–77] (Scheme 1.10). In this synthesis, the synthetic plan is carefully designed on the basis of the frequency of the existence of a certain unit in oligosaccharide, and also on its natural abundance generally, which affects on availability of a unit. The lactose unit, which is always found as



Scheme 1.10

the reducing terminal of mammalian glycolipids, is therefore used. A commercially available α-2-8-linked dimer of sialic acid was utilized, eliminating difficult problems in constructing an α-2-8 sialyl sialic acid linkage. Sialyl α-2-3 Gal was selected as a donor unit for the reason that it is commonly found in a variety of gangliosides. Indeed, the disaccharide was used as the donor in the synthesis of GM1b, GD1a, GD1α, GT1aα etc. A stepwise method was applied for the introduction of the sialyl 2-6 GalNAc sequence, since it is a special case for the so-called α-series gangliosides. The 2-(trimethylsilyl)-ethyl group was used as a persistent protecting group for the anomeric position of the lactose unit. The stability of the group, together with the mild and selective conditions needed for its removal, enabled multiple glycosylation reactions and other protecting manipulations to be performed. Thioglycosides were used as the glycosyl donors throughout synthesis, except for the coupling of the octasaccharide unit and azidosphingosine. The trichloroacetimidate approach was used for this particular glycosylation, as it has been shown to be very successful for construction of this type of glycosidic linkage.

1.4 Accessibility

When a biologist wants to investigate the functions of oligosaccharides, one of the most important issues will be the accessibility of particular oligosaccharides. The strategic considerations described above are thus very important. To this end, automation in the synthesis of oligosaccharides strongly deserves consideration, as in the cases of functional investigations of oligonucleotides and oligopeptides. One evident approach for automation is based on solid-phase synthesis, through which tedious workup and chromatographic purification after every reaction are eliminated. This, however, can only be achieved once a reliable synthetic method – especially for the glycosylation reaction – has been developed, because there is only one chance for the purification. For this reason, strategic analysis with regard to the overall reaction yield is also required. PEG-based polymer-supported chemistry and also the recently developed fluorous-phase chemistry may be alternatives [78]. One-pot reactions can be considered to be advantageous if smaller numbers of coupling reactions are in mind, although in this case a different approach has to be taken to access larger oligosaccharides [6, 21]. Convergent synthesis is very useful in this instance.

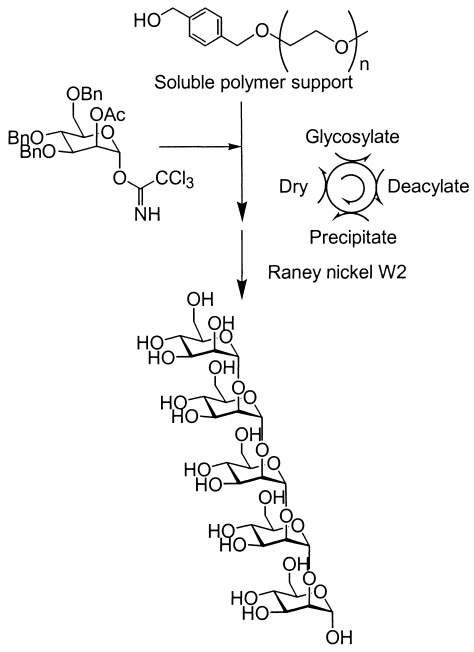
1.4.1 Solution-based Chemistry

Polyethyleneglycol monomethyl ether (MPEG) of molecular weight of approximately 5000 has been used as a support in oligosaccharide synthesis [43, 44, 67, 79–81]. A unique characteristic of this soluble polymer is that it can be precipitated by addition of *tert*-butyl methyl ether, facilitating isolation of polymer-bound

substances from the glycosylating agents and reagents used in the coupling reaction. In addition, since MPEG is soluble in various solvents used in solution-phase oligosaccharide synthesis, these reactions are solution-phase reactions, and so reaction conditions used for solution-phase oligosaccharide synthesis can be employed (Scheme 1.11). Alternatively, relatively short-chain MPEG can be used to facilitate rapid chromatographic isolation [82, 83]. Another advantage of the use of MPEG is that reaction progress can be monitored either by NMR or by mass spectrometric methods [83, 84] without cleavage from the support.

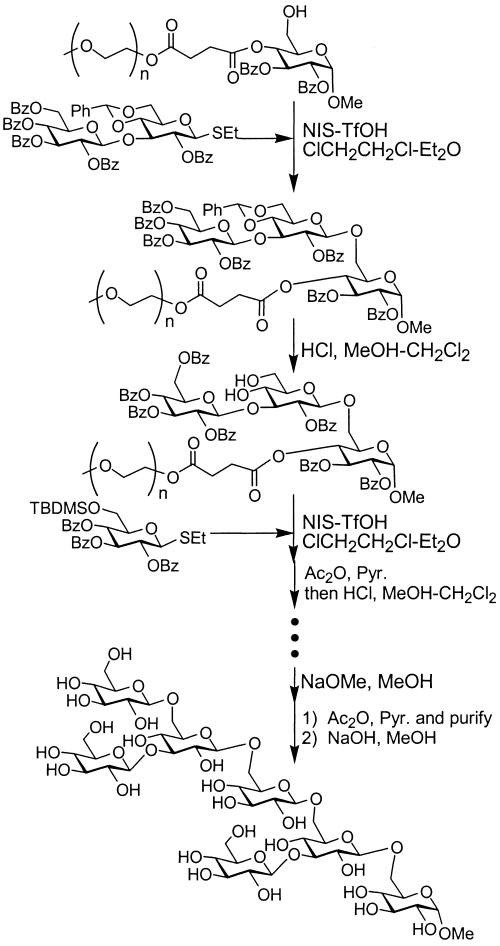
The recent target molecule in oligosaccharide synthesis is a heptasaccharide phytoalexin elicitor [80, 85]. A successful approach to the synthesis by use of the MPEG approach was reported in 1993 [79]. The MPEG was attached at the 4-OH group of a glucose unit through an ester linkage [80] (Scheme 1.12). On the basis of retrosynthetic analysis, three synthetic blocks were prepared. All glycosyl donors were synthesized as thioglycosides; protecting groups used were the minimum. After four coupling and deprotection reaction cycles, the heptasaccharide was synthesized in 18% overall yield. The synthetic scheme is very simple, which in turn indicates the strength of the method. In another case, an amino functionality was also used as an anchoring point [86].

Use of the orthogonal strategy [29, 66] described in Section 1.3.3 has also been reported [67] (Scheme 1.13). The synthetic plan also takes advantage of introduced hydrophobicity at the end of polymer supported synthesis, which facilitates the isolation of desired products, in addition to the advantage of the self-correction ef-



Scheme 1.11

Scheme 1.12

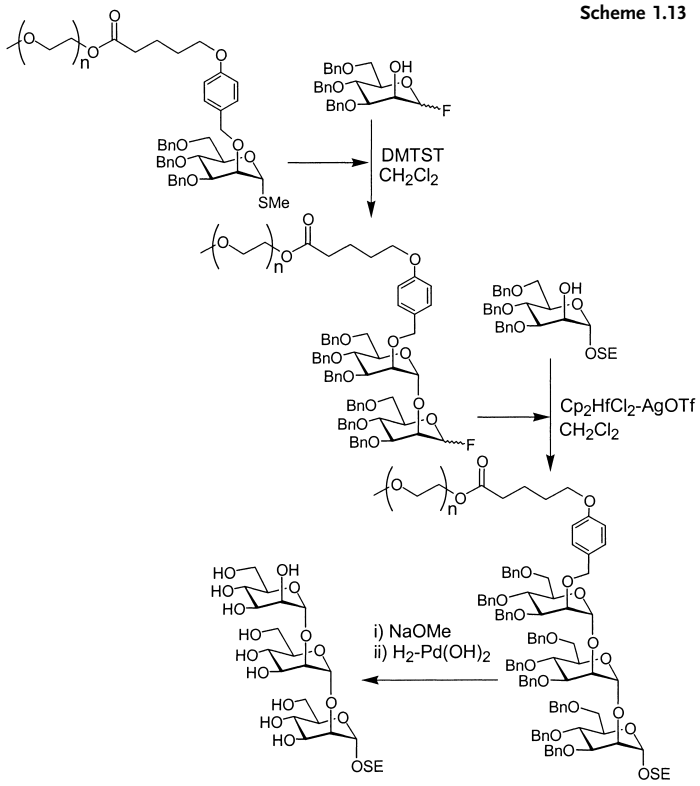


fect described in Section 1.4.3.5. In addition, thanks to the use of orthogonal sets of potential donors such as thioglycoside and glycosyl fluoride with leaving groups already installed, there is no need for activation on the support. It was later shown that the method can be used in combination with intramolecular aglycon delivery system [87–90] for the construction of β-mannopyranosides [91].

1.4.2
One-Pot Glycosylation

One of the important applications of methods based on anomeric reactivity modulation is the one-pot glycosylation method. If the reactivities of leaving groups at the anomeric centers of glycosyl units are differently controlled, a series of glycosylation reactions can be performed either all-in-one (A) [6] or sequentially (B) [20,

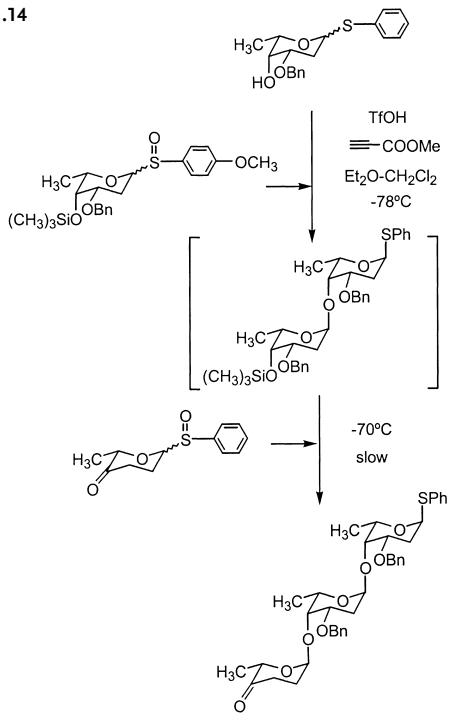
Scheme 1.13

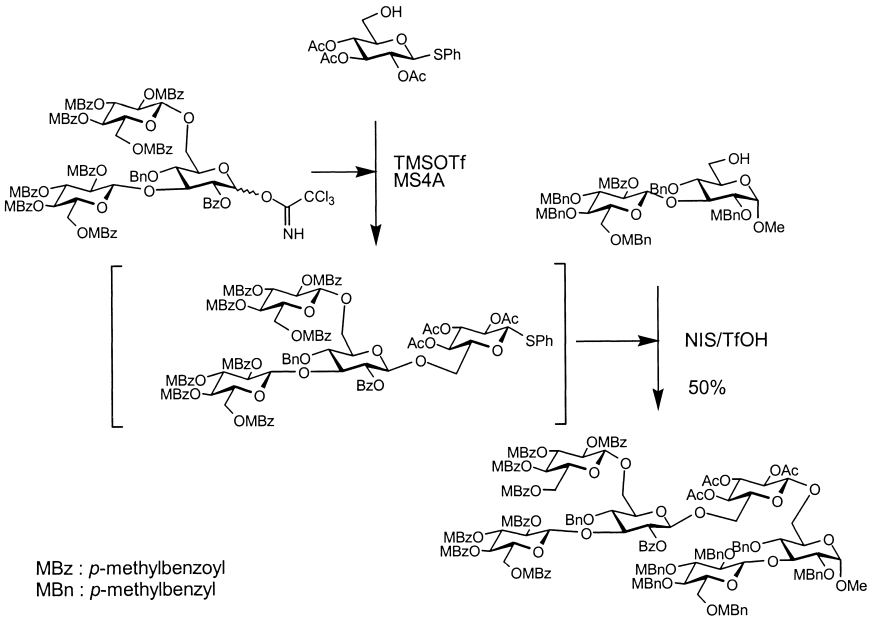


21, 92–101]. Despite its limitations arising from the identification and acquisition of glycosylating units with differently modulated reactivities, the method allows multiple coupling reactions to be performed in one-pot fashion, with obvious advantages over standard methods. Because this method eliminates the need for workup and purification steps during the operations, it should be regarded as equally as important as solid-phase synthesis. Careful purification must be performed after the reaction since there is no way to prevent the formation of deletion compounds. In an example of Format A, a phenylsulfoxide and a methoxyphenylsulfoxide were used as leaving groups. The reactivities of the acceptor molecules were also controlled by silylation of one of the hydroxy groups in the system [6] (Scheme 1.14).

One-pot sequential glycosylation (B) typically uses a series of leaving groups, requiring either that they can be activated under the same conditions or that a promoter used for the first coupling does not affect the other potential glycosyl donors [20, 21] (Scheme 1.15) (see also Section 1.4.6).

Scheme 1.14





Scheme 1.15

1.4.3 Solid-Phase Chemistry

The advantage of solid-phase reactions is the quick and simple workup process. Because only the growing molecule is attached on the support, other reagents used can be washed away by simple filtration. Higher reaction yields can generally be achieved by use of excess amounts of reagents. Furthermore, because of the simplicity of the process, it can be automated, allowing non-specialists to synthesize oligosaccharides.

There are basically two methods employed for solid-phase oligosaccharide synthesis. They differ in the direction of chain elongation: one starts from the reducing sugar (A) and the other is the opposite (B) (Fig. 1.3). Approach A is generally advantageous when both the polymer-supported glycosyl acceptor and the glycosyl donor are reactive enough to ensure completion of all glycosylations. The application of Approach B, on the other hand, is less straightforward, due to the following considerations. Firstly, every glycosylation inevitably gives rise to side reaction(s) (elimination, hydrolysis, etc.) together with the formation of the desired *O*-glycoside. These products arise largely from the glycosyl donor, so all products are accumulated on the support. In addition, transformation of the reducing-end anomeric position into a particular leaving group is required after each glycosylation. Nevertheless, it is necessary to make the choice of which method is to be used throughout the synthesis, because the synthetic schemes are completely reversed and so the choice of leaving group and protecting groups, including the linker, are different. Polymer-supported oligosaccharide syntheses appearing to date have been categorized in this context (Tab. 1.1).

1.4.3.1 Fundamentals of Solid-Phase Oligosaccharide Synthesis

To facilitate solid-phase oligosaccharide synthesis, several issues have to be addressed. The support may have a major influence on the reactions because of the demanding steric bulk close to the reaction site as well as physical properties. The choice of a suitable linker and protecting groups are important factors for the synthesis of multifunctional molecules such as oligosaccharides. Decisions regarding requirements for the reducing anomeric position after cleavage from the resin (i.e., hydroxy free or with a linker for further conjugation etc.) have to be made before the synthesis.

1.4.3.2 The Support

Polystyrene divinylbenzene cross-linked (PS) resins are mostly used, not only in carbohydrate chemistry but also in the synthesis of peptides and other small organic molecules. The main reason for this is their chemical stability toward a variety of chemical reaction conditions. However, there is room for improvement in areas such as swelling properties, which sometimes restrict the synthetic plan. Because of the multifunctionality of carbohydrates, it is to be expected that, for the

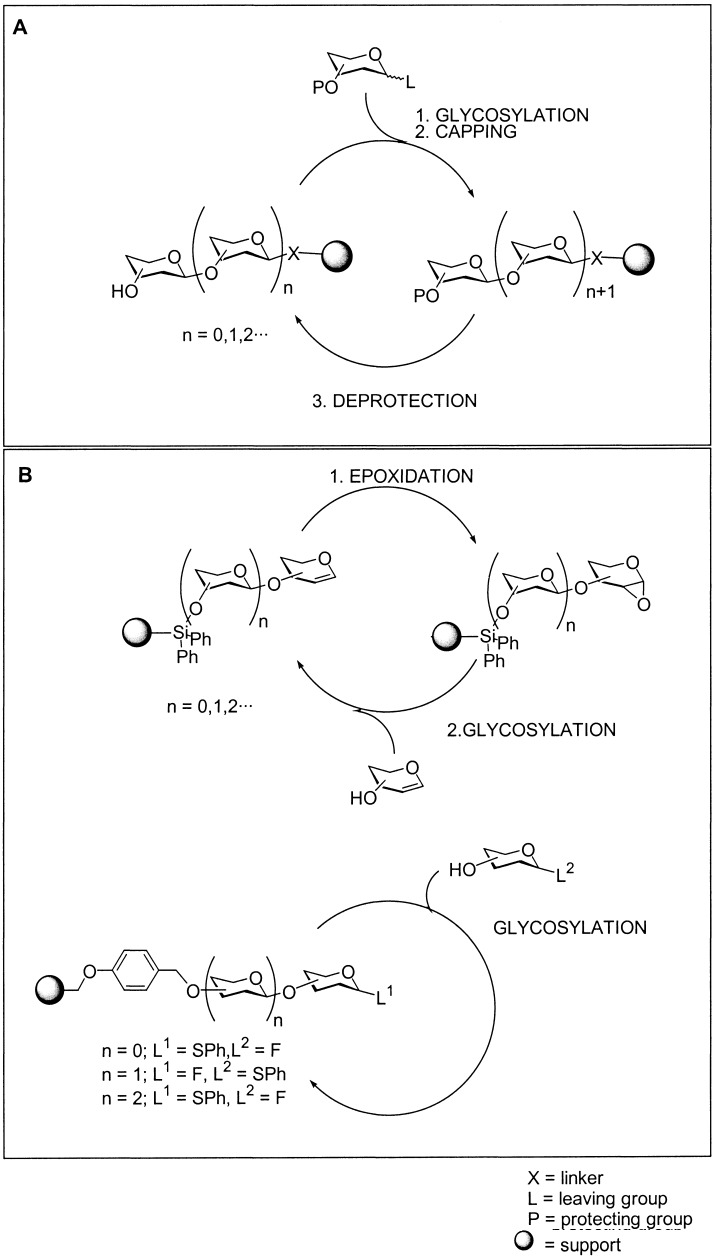
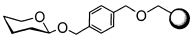
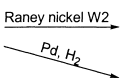
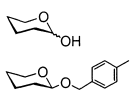
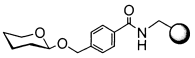
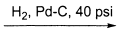
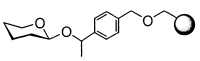
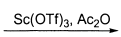
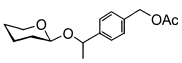
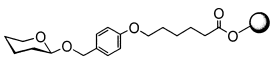
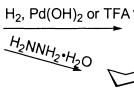
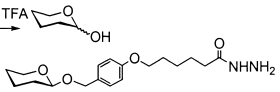
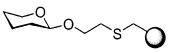
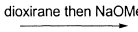
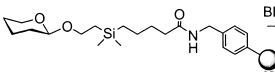
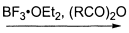
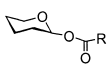
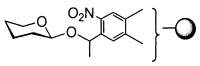
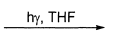
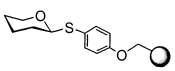
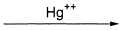
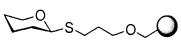
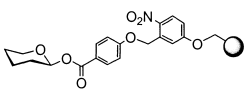
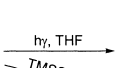
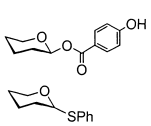
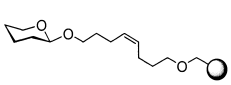
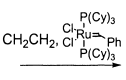
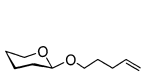
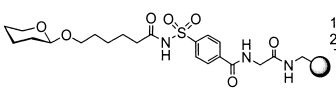
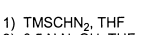
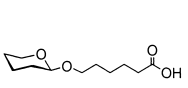
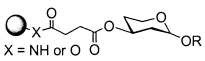
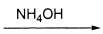
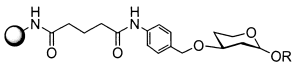
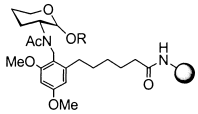
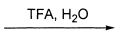
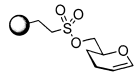
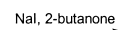
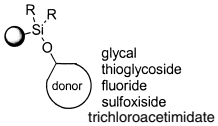
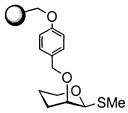


Fig. 1.3 Schematic representation of solid-phase oligosaccharide synthesis. A: A reducing sugar is attached on the support. The method typically operates in three steps for a cycle. B: A non-reducing end sugar is attached on the support, two examples being shown. One involves activation and glycosylation steps as a cycle and the other uses an orthogonal set of leaving groups, enabling a single step per cycle.

Tab. 1.1 Structures of linkers and the conditions of cleavage.

Category	*Structure of linkers*	*Condition of cleavage*	*Product*	*Support*	*Ref.*
A				MPEG	44
A				MPEG	139
A				MPEG PEG-PS	81
A, C				MPEG PS	140
A				MPEG	139
A				PS	141
A				PS	85, 104, 128
A				PEG-Ps PS	31, 32
A				PS CPG	46, 49
A, B				PS	103
B				PS	48
C				PEG-PS	134

Tab. 1.1 (cont.)

Category	*Structure of linkers*	*Condition of cleavage*	*Product*	*Support*	*Ref.*
D				MPEG PEG-PS	79, 80, 116, 142
D				PS	127
D				PS	86
D				PS	143
E				PS MPEG	67, 105– 113

synthesis of an oligosaccharide, many reactions will have to be performed on the support. The suitability of the resin under various reaction conditions therefore has to be taken into account [102].

Merrifield-type polystyrene (PS) resins have most often been used in oligosaccharide synthesis [45–48, 85, 103–113]. Their chemical stability and compatibility in organic synthesis are the reasons for this choice. The commonly used PS resin is cross-linked with divinyl benzene ($\sim$2%) with a relatively large "active" reaction surface (up to 3 mmol g^{-1}), depending on the type of functional group and linker. Solvent-dependent swelling properties and the steric bulk of the polymer may affect reactivity and stereochemical outcome.

In order to address problems with PS resins, polyethyleneglycol (PEG) has been incorporated into PS resins, allowing reactions to proceed under quasi-solution conditions. In addition, PEG is compatible with reactions that require polar solvents. PEG-PS resins can also directly be used in biological assays, which is important for high-throughput screening [32].

To improve the polymer further, PEG-based polyether-type resins have been developed, eliminating the swelling problem seen in PS-based supports [114, 115]. Large pore sizes facilitate enzymatic reactions, receptor-ligand binding studies, and so on, thus enabling biological assay after the completion of synthesis. Furthermore, some PEG cross-linked polymers do not contain any UV-absorbing components, and so can be used effectively in photometric assays.

Although controlled pore glass (CPG) has been used extensively in oligonucleotide synthesis, it has seldom been used in the field of oligosaccharide synthesis [49, 102, 116]. It has recently been shown, though, that CPG can serve as a solid support in oligosaccharide synthesis, glycosyl trichloroacetimidate and TMSOTf being successfully used as the glycosyl donor and activator, respectively.

1.4.3.3 **Linkers to the Support**

A variety of linkers have been used to connect protected carbohydrate units to the support. Some of the linkers used are categorized by the position of the functional group on the sugar unit (i.e., anomeric position and others; Tab. 1.1), since a linker at the anomeric position may have to be cleaved when the completely deprotected oligosaccharide is the target (A), while in other cases the aglycon part of the linker may be converted into a potential leaving group or other protecting group to be used in the synthesis to follow (B). A spacer may be left and can be used to connect the formed oligosaccharide with other materials (C). A linker at other hydroxy functions can be regarded as one of the protecting groups (D, E).

1.4.3.4 **Protecting Groups used in Solid-Phase Oligosaccharide Synthesis**

The basic combination of protecting groups consists of one for the anomeric position, one for the hydroxy group involved in chain-elongation, and "persistent" protecting groups for the others. Selective sequential reactions of these protecting groups are the minimum requirement not only in solid-phase oligosaccharide synthesis but also in solution-phase synthesis. When branching structures have to be constructed on the support, an orthogonal protection scheme has to be employed (see Section 1.3.2).

As long as this requirement is fulfilled, any kind of protecting groups can be used if deprotection is planned after cleavage from the support. If, however, deprotection is completed while the oligosaccharide is still attached to the support, the beads can be used in screening assays directly [31, 117]. For this approach, acid- and base-sensitive protecting groups are frequently used. In addition, substituted benzyl groups have been introduced not only to provide more flexibility in the synthetic scheme but also to compensate the problem of inability of removing the benzyl-protecting group frequently used in solution-phase synthesis [118–123]. Removal of benzyl-protecting groups cannot be achieved by catalytic hydrogenolysis, probably due to steric problems. To address this, it was shown recently that palladium nanoparticles can be used for the catalytic hydrogenolysis of solid-supported compounds [124].

1.4.3.5 **Solid-Phase Oligosaccharide Synthesis**

There is a choice in the positions of hydroxy groups that can be used to anchor a synthetic unit onto a polymer support. The majority of researchers have selected the anomeric position to be connected to the support (Fig. 1.3 A). Attachment of

the first sugar through the anomeric position is much more straightforward, since attachment and protection of the anomeric position are achieved at once. Substituted benzyl-, ethyl-, pentenyl-, and ester-type linkers have been used to attach reducing sugars to the support (Tab. 1.1 A–C). However, linking of sugars through the other hydroxy groups offers a number of advantages. One can install temporary anomeric protecting groups to facilitate transformation of the oligosaccharide bound to the support into a glycosyl donor [79, 80, 86, 116, 125, 126]. More options in the synthetic scheme gives flexibility in the synthesis of oligosaccharides, glycoconjugates, and libraries. When, however, the donor is attached to the support (Fig. 1.3B), several improvements have to be employed for this method to be useful or advantageous.

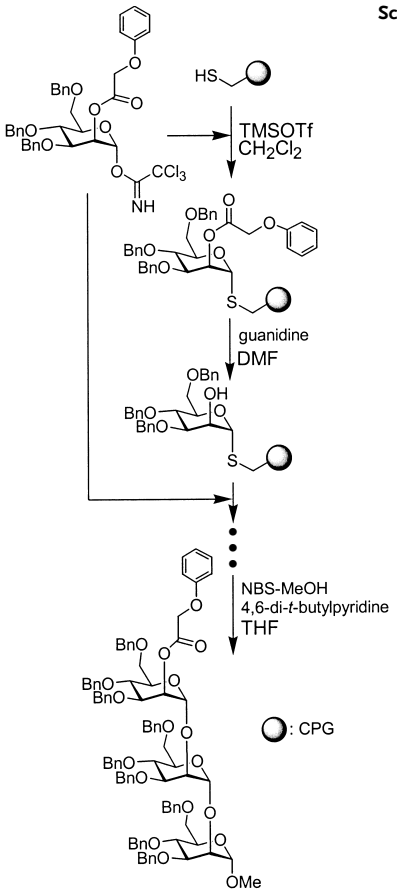
Synthesis Starting from the Reducing Terminus

Glycosyl trichloroacetimidates have been shown to be powerful glycosyl donors in solid-phase oligosaccharide synthesis [45–48, 127]. These donors appear to be unaffected by the polymer support used in the synthesis, so PS resins [45–48], PEG [43, 44], and CPG [49] have all been used as supports. Scheme 1.16 illustrates one approach, in which it was shown that this leaving group could be used on CPG. A very straightforward synthesis of an α-(1 → 2)-linked trimannoside has been reported. In order to push the reaction to consume unreacted acceptor, the glycosylation reaction was carried out twice per cycle, and over 95% yield was achieved for each coupling step. The reducing terminus was coupled to the support through a thioglycosidic linkage, which was cleaved the last stage. The phenoxyacetyl group was used as a temporary protecting group and removed by treatment with guanidine in the presence of benzyl groups as the persistent protecting group.

Glycosylation reactions with a carbohydrate monomer already attached to a peptide sequence constructed on a PEGA resin have been investigated [127] (Scheme 1.17). A solid-phase-bound glycosylated octapeptide, the sequence of which is a part of mucin MUC 2 protein, was used as an acceptor and coupled with a glycosyl trichloroacetimidate. Di- and trisaccharide portions were constructed through the use of mono- and disaccharide donors. In addition, the removal of the benzylidene group in one of the products followed by further glycosylation of the diol was achieved in stereo- and regiospecific manner. The glycosyl acetimidates were activated by TMSOTf throughout the synthesis, but interestingly it was reported that only freshly distilled reagent was effective, which is unlike the observation in solution-phase chemistry. Some influence of the support on the stereochemical outcome was also reported.

A solid-phase synthesis of one of the most complex oligosaccharide structures is depicted in Scheme 1.18 [103]. In this scheme, two cleavage sites were introduced in an extremely flexible and powerful approach. The linker consists of a nitrobenzyl group and an ester function. The reducing ester linkage was cleaved upon activation by a Lewis acid in the presence of a thiol to provide an oligosaccharide glycosyl donor for the convergent synthesis, and the other part of the linker is a

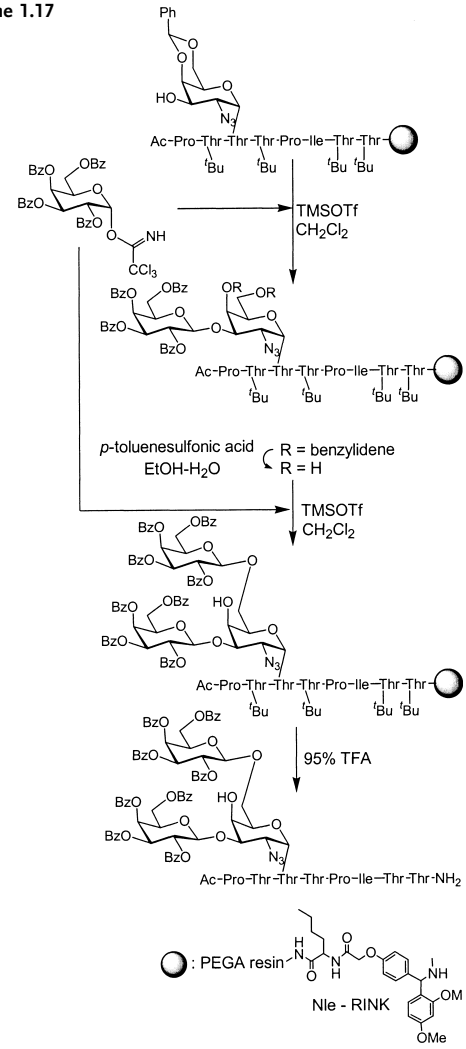
Scheme 1.16



photolabile group [85, 104, 128], so the constructed oligosaccharide can be released at the end of the chain-elongation without affecting other protecting groups. This helps in determining of the structure of the constructed oligosaccharide, since anomerically pure compounds are released. Suitably protected thioglycosides were utilized as glycosylation agents throughout the synthesis. After iterative coupling and deprotection reactions, the trisaccharide was released as a thioglycoside, which was used in the following convergent synthesis (see Fig. 1.1 B). Thus, a dodecasaccharide was synthesized on PS resin.

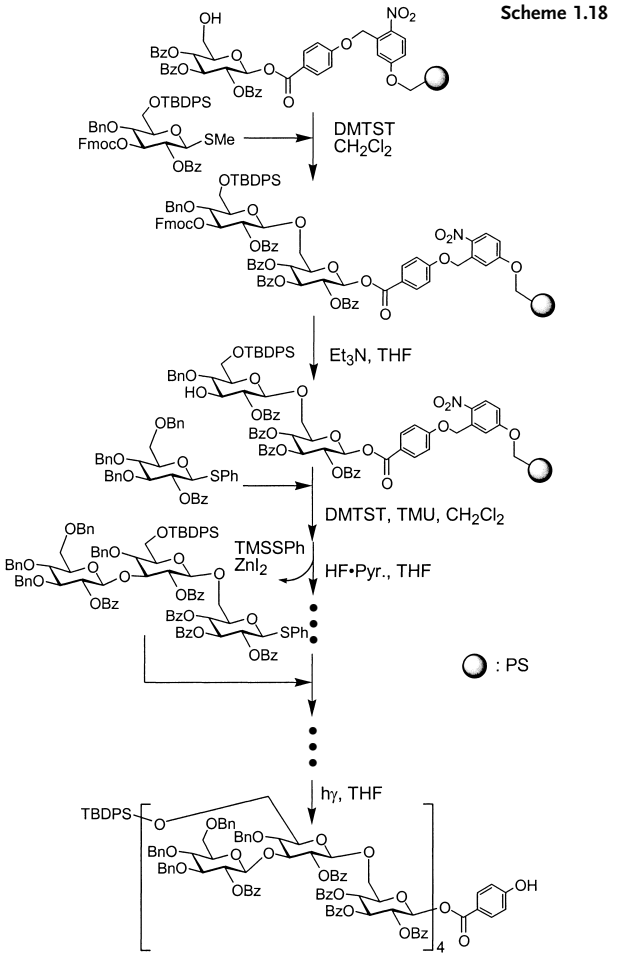
Glycosyl sulfoxides have also been used in the solid-phase synthesis of oligosaccharides (Scheme 1.19). A β-(1 → 6)-galacto-trioside was synthesized [32]. A combination of an acid-labile trityl group as a temporary protecting group, pivaloyl groups as persistent protecting groups and also as auxiliaries for β-selectivity, and a phenylsulfonyl group were used for the synthesis on PS resin. Anchoring was achieved through a thioglycoside, which was cleaved by the action of $Hg(OCOCF_3)_2$ at the end of the synthesis.

Scheme 1.17



One challenge in oligosaccharide synthesis is the sialylation reaction, and this has been addressed through solid-phase synthesis. The general strategy is the use of benzyl groups as the persistent protecting group, the acetyl group as a short-term protecting group, and thioglycosides as general glycosyl donors. Sialyl LeX tetrasaccharide was successfully synthesized, together with all possible anomers. The tetrasaccharides have carboxylic acid functionalities at the reducing terminus, and these can be used to form conjugates with various materials. In addition, the reaction process was monitored nondestructively by a gated decoupling ^{13}C NMR technique (Scheme 1.20; see Section 1.4.3.6).

Scheme 1.18

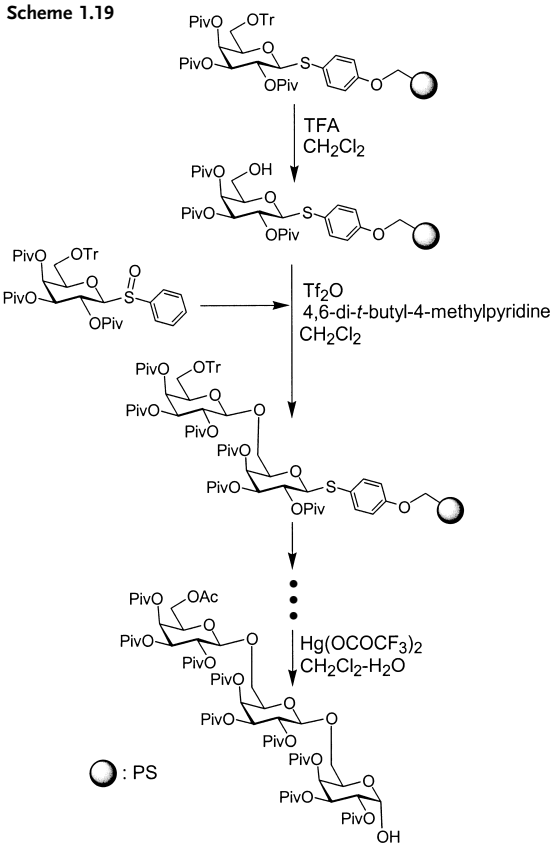


Pentenyl glycosides have also been used successfully as glycosyl donors in combination with PS resin as the support. The synthesis of a branched trimannoside was achieved through a chemoselective deprotection scheme [104]. An overall yield of 42% was achieved, an average yield of 87% per step.

Synthesis Starting from the Non-Reducing Terminus

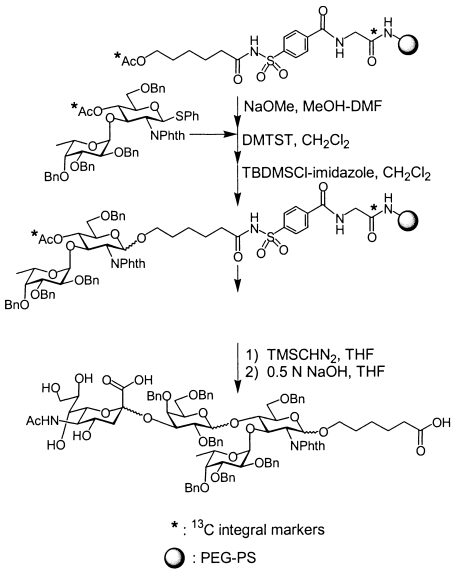
The assembly of oligosaccharides through the use of glycals as precursors of glycosyl donors has been investigated intensively [112, 129]. A characteristic of this method is the reversed synthetic direction (Fig. 1.1C), the glycosyl donor being attached to the support (Fig. 1.3 B). As stated in the literature, a major advantage of the reversed method is the self-suppressing effect of formation of sequence deletion compounds. When the activation step to obtain a highly active epoxy or

Scheme 1.19



equivalent donor is complete, the donor undergoes a glycosylation reaction, producing only the coupling product and the hydrolyzed donor. Since the formed by-product can be neither a substrate for the next activation reaction nor an acceptor for the next coupling, because of steric factors, these by-products do not affect further reactions. Therefore, regardless of the coupling yields, no capping step is required. Scheme 1.21 illustrates the glycal method [105, 112], by which a homotetrasaccharide was synthesized. The first carbohydrate was attached to a PS resin by silylation, and the double bond was oxidized with 2,2-dimethyldioxirane. The 1,2-epoxide produced was coupled with the primary OH group of a galactal acceptor to afford a β-galactosyl-linked disaccharide. Iterative reactions gave the tetrasaccharide in 32% overall yield. The β-stereocontrol of glycosylation with *galacto*-glycals notwithstanding, difficulty was reported in the case of *gluco*-type species, resulting in α-glycoside formation. To overcome this problem, the epoxide was converted into a thioglycoside with subsequent acylation, which acts as auxiliary to enhance β-stereoselectivity [110].

Scheme 1.20



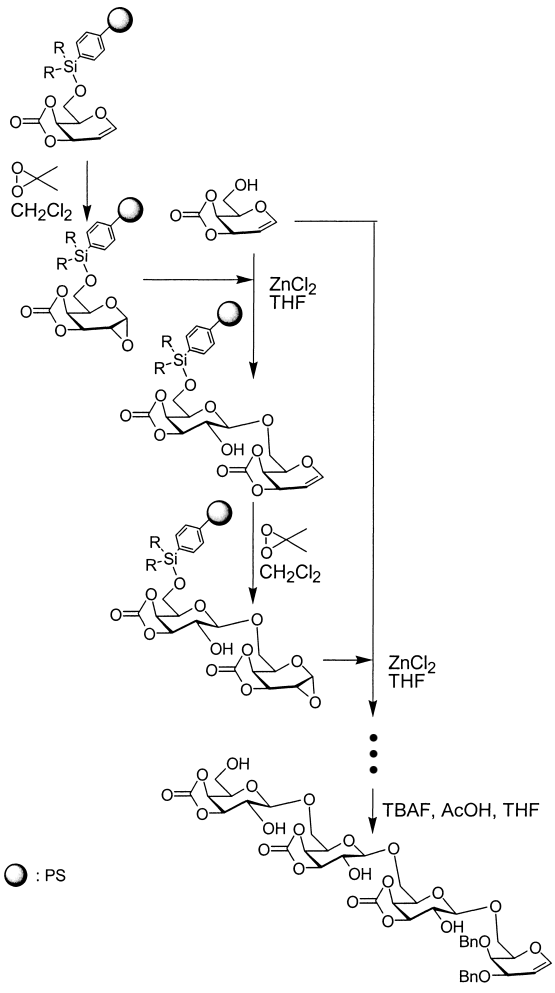
A route to 2-amino sugars was addressed by transformation of the glycal into a 2-phenylsulfonamido-thioglycoside by rearrangement-displacement of 2-iodo-1-phenylsulfonamide [111]. A structure found in the reducing terminus of *N*-linked oligosaccharide was also synthesized, the oligopeptide part being incorporated on the support at the final stage of the coupling process. This is one of the advantages of the strategy, since diversity other than carbohydrate in nature can be added after completion of the oligosaccharide synthesis [108].

1.4.3.6 **Monitoring of Reaction Progress**

Monitoring of reaction progress in solid-phase synthesis is very important, especially for the optimization of reaction conditions, because cleavage of the product from a support to analyze the reaction by TLC negates the advantage of solid-phase synthesis. Gravimetric analysis has classically been used, but resin breakdown and difficulties associated with incomplete "dryness" for analysis prevents quantitative measurements. For qualitative analysis, IR [45], gel-phase ^{13}C NMR [130], and MALDI TOF MS [49] have been used.

Methods based on nuclear magnetic resonance (NMR) are nondestructive and reliable techniques widely used in oligosaccharide synthesis. ^{1}H NMR spectroscopy is one of the most informative analytical methods, frequently used in organic chemistry. However, the spectra typically obtained for solid-bound compounds are broadened and it is difficult to obtain quantitative or even qualitative information, due to the short relaxation time of the macromolecule. An exception is found, however, in the case of the soluble polymer MPEG (see Section 1.4.1). Reaction progress in this case can be monitored easily by standard experimental techniques

Scheme 1.21



[67, 78]. Monitoring of the reaction progress of MPEG-based synthesis can also be performed by use of MALDI TOF MS [83, 131] (Fig. 1.4). Alternatively, high-resolution magic angle spinning NMR (HR-MAS) has also been used [106], and it has been shown that TOCSY is useful for obtaining coupling constants [132, 133] (Fig. 1.5).

To analyze the molecular structure of a product attached on the support, HR-MAS is probably the only method. Although the "high resolution" required for the coupling constants was not achieved by ^{1}H NMR, a trisaccharide bound to PS resin was analyzed on the basis of the chemical shifts of the anomeric protons and carbons after ^{1}H, ^{13}C, and HMQC experiments.

A conceptionally important and useful monitoring approach making use of ^{13}C NMR to monitor reaction progress focusing at a ^{13}C-enriched carbon center has

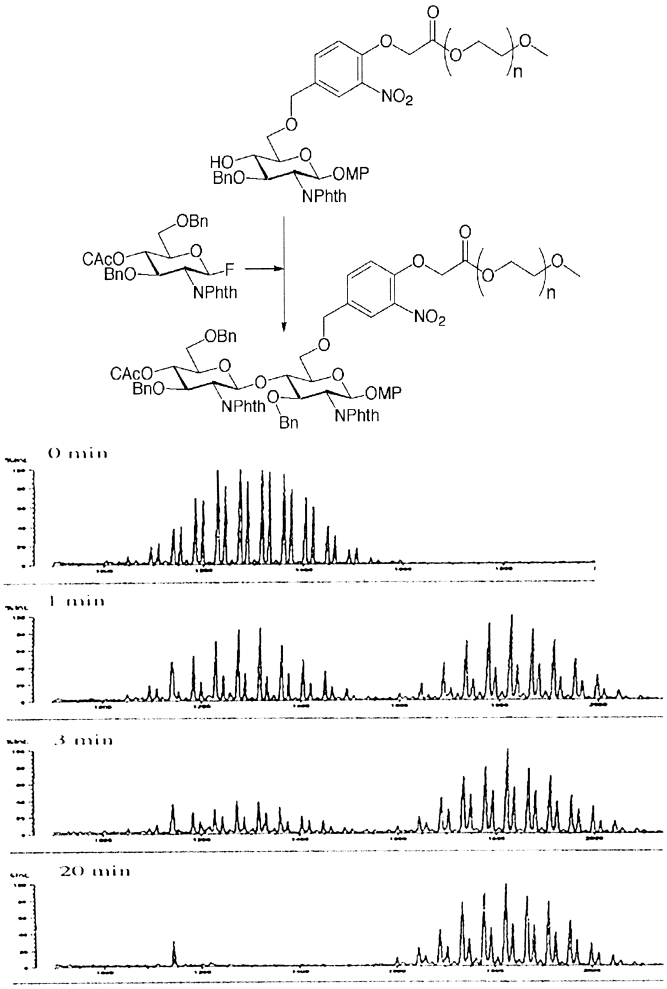


Fig. 1.4 Monitoring of MPEG-based oligosaccharide synthesis. MALDI-TOF mass spectrometry is used to monitor reaction progress in MPEG-supported oligosaccharide synthesis.

been reported [135] (Fig. 1.6; see Sections 1.4.3.5 and 1.4.4). In order to allow quantitative analysis of the solid-phase reaction by the so-called gel-phase ^{13}C NMR [130], the gated decoupling technique was used [136, 137]. ^{13}C-enriched temporary protecting groups and an internal ^{13}C marker have also been used, as well as a relaxation agent to obtain a short T_1 value. The method is particularly useful when only a small quantity of material has been synthesized and quantitative information is required. Through the use of gated decoupling techniques, the reaction progress can be monitored quantitatively without cleavage of the molecule from the resin. Since the yields are always given relative to an internal integral

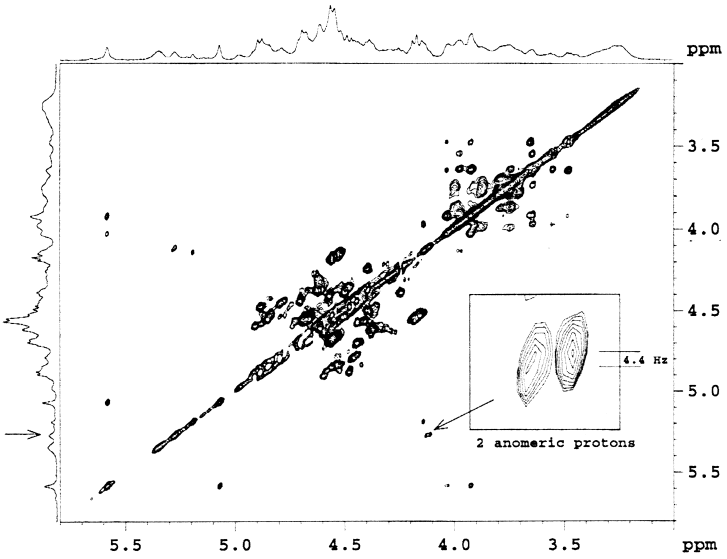


Fig. 1.5 Monitoring of solid-phase synthesis with HR-MAS. A TOCSY spectrum obtained by HR-MAS even gives anomeric coupling constants while the oligosaccharide is attached on the Merrifield resin.

marker, the chemical yields are determined regardless of the isolated yield, which is important for optimization of solid-phase reactions.

A more practical method, comparable to a Kaiser test in peptide synthesis, has been investigated [83]. In this method, a chloroacetyl group was used as a short-term protecting group for a hydroxy function for the next coupling reaction. Sequential treatment at the chloroacetyl stage with 4-(4-nitrobenzyl)pyridine and piperidine formed a zwitterion possessing a red color. Reaction progress could thus be monitored colorimetrically.

1.4.4
Automation

The emerging area of automation of oligosaccharide synthesis should contribute greatly not only to glycobiology but also to cell biology in general [59, 92, 93]. Access to structurally defined complex oligosaccharides has been very laborious, contrary to the needs for biological investigations. The final stage of a chemical synthetic method is the development of an automated system, and this has been addressed recently.

Seeberger used a modified peptide synthesizer equipped with a temperature-controlled reactor [135] (see Sections 1.4.3.5 and 1.4.4). The method was demonstrated in the cases of trichloroacetimidate and phosphate as the leaving groups; the trichloroacetimidate method is depicted in Fig. 1.7, which shows the sequential process program. In this way, non-specialists may soon be able obtain particu-

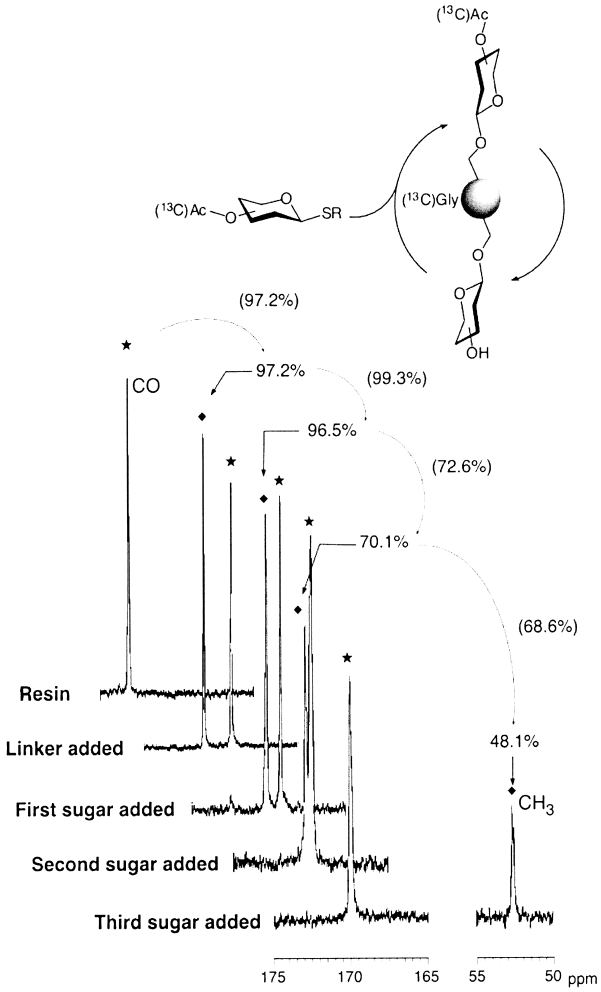
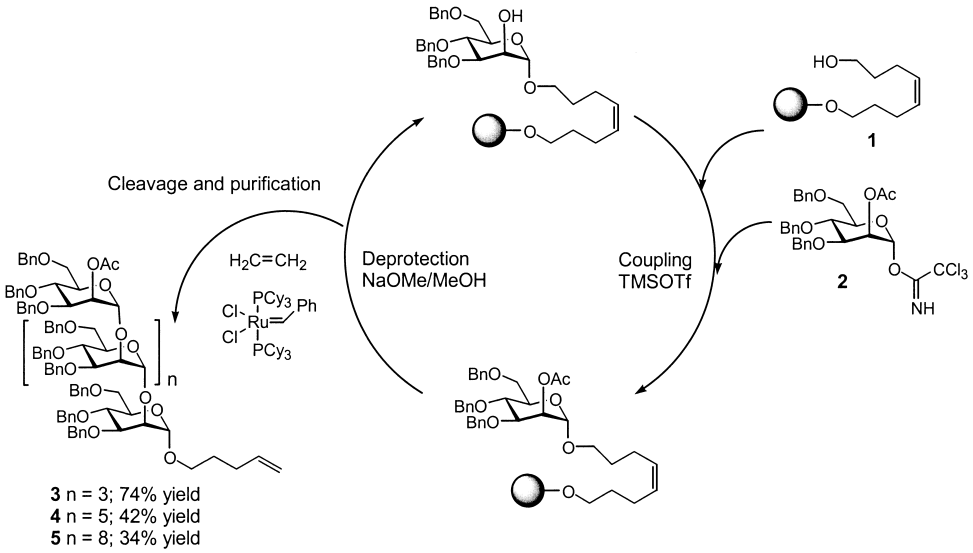


Fig. 1.6 Monitoring of solid-phase synthesis by ^{13}C NMR. Inverse gated decoupling ^{13}C NMR spectra with conventional NMR and use of a ^{13}C-enriched integral marker provide non-destructive monitoring of resin-bound (TentaGel) oligosaccharide synthesis.

lar oligosaccharides, although one issue pertinent for this type of system is the carbohydrate synthetic units. A variety of units have to be prepared one by one in a laboratory, and this matter still has to be resolved.

A different approach for automation by sequential one-pot glycosylation has also been developed (Fig. 1.8; see Section 1.4.2). A key issue in this approach is the use of a program to determine the glycosyl units needed in the synthesis of oligosaccharide. The basis of the method lies in analysis of the relative reactivities of carefully chosen suitably protected synthetic blocks in association with the armed and dis-



Step	Function	Reagent	Time (min)
1	Couple	10 equiv, donor and 0.5 equiv. TMSOTf	30
2	Wash	CH_2Cl_2	6
3	Couple	10 equiv, donor and 0.5 equiv. TMSOTf	30
4	Wash	CH_2Cl_2	6
5	Wash	1:9 MeOH:CH_2Cl_2	6
6	Deprotection	2×10 equiv. NaOMe (1:9 MeOH:CH_2Cl_2)	60
7	Wash	1:9 MeOH:CH_2Cl_2	4
8	Wash	0.2 M AcOH-THF	4
9	Wash	THF	4
10	Wash	CH_2Cl_2	6

Fig. 1.7 Automation of oligosaccharide synthesis based on solid-phase operations. Solid-phase oligosaccharide synthesis was automated for the first time with trichloroacetimidate and phosphate being successfully used as leaving groups.

armed concept [92, 93] (see Section 3.3). The choice of building blocks is stored in a database, from which researchers can select "suitable combinations" of glycosylating agents to be used in the one-pot sequential glycosylation. Expansion of the database is crucial for the success of this method, since estimation of the anomeric reactivities of differently protected carbohydrates is difficult. Another related problem can be seen when alkylthio and arylthio groups are used as glycosyl donor and acceptor. In some cases the former is preferentially activated, while in other cases the reactivity is reversed [137]. However, as long as a single substituent group at the anomeric position is used, the "programmable" oligosaccharide synthesis is considered effective since it has been shown that the relative reactivity number correlates with the chemical shift of the anomeric proton in the ^{1}H NMR [93].

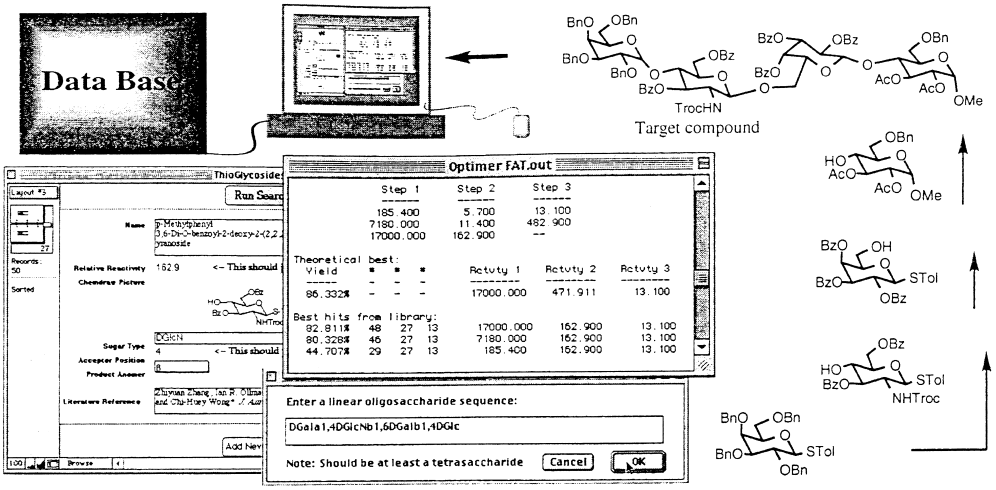


Fig. 1.8 A computer-assisted approach to the sequential one-pot synthesis of oligosaccharides. A database containing relative reactivities of synthetic units assists chemists in synthesizing target oligosaccharides.

1.5 Concluding Remarks

A variety of probes are needed for biological investigation of oligosaccharide functions. Methodological investigation is necessary for this purpose. Organic synthesis, enzymatic synthesis, isolation from natural sources, and/or combinations of each method can be utilized. This review summarizes the status of the organic synthesis of oligosaccharides, focusing on tactical aspects. The method best to be relied upon is an often debated matter, but it is important to obtain oligosaccharides by taking advantages of individual methods. One of the advantages of the synthetic method is that it is possible to access non-natural structures. Combinatorial oligosaccharide synthesis represents challenging but very important research in connection with approaches addressing infectious diseases. One has to take account of every aspect of current methods in oligosaccharide synthesis and to develop synthetic and engineering methods further in order for oligosaccharide probes to be available to all researchers.

1.6 References

1 Lemieux, R. U. *J. Am. Chem. Soc.* **1953**, *75*, 4118.
2 Boons, G.-J.; Huber, G. *Tetrahedron* **1996**, *52*, 1095–1121.
3 Koto, S.; Uchida, T.; Zen, S. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 2520–2523.
4 Nicolaou, K. C.; Dolle, R. E.; Papahatjis, D. P.; Randall, J. L. *J. Am. Chem. Soc.* **1984**, *106*, 4189–4192.
5 Lönn, H. *Carbohydr. Res.* **1985**, *139*, 105–113.
6 Raghavan, S.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 1580–1581.
7 Paulsen, H.; Tietz, H. *Carbohydr. Res.* **1985**, *144*, 205–229.
8 Friesen, R. W.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6656–6660.
9 Trumtel, M.; Veyrières, A.; Sinaÿ, P. *Tetrahedron Lett.* **1989**, *30*, 2529–2532.
10 Veeneman, G. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 275–278.
11 Veeneman, G. H.; van Leeuwen, S. H.; Zuurmond, H.; van Boom, J. H. *J. Carbohydr. Chem.* **1990**, *9*, 783–796.
12 Mori, M.; Ito, Y.; Uzawa, J.; Ogawa, T. *Tetrahedron Lett.* **1990**, *31*, 3191–3194.
13 Mehta, S.; Pinto, B. M. *Tetrahedron Lett.* **1991**, *32*, 4435–4438.
14 Marra, A.; Gauffeny, F.; Sinaÿ, P. *Tetrahedron* **1991**, *47*, 5149–5160.
15 Zegelaar-Jaarsveld, K.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1992**, *48*, 10133–10148.
16 Jain, R. K.; Matta, K. L. *Carbohydr. Res.* **1992**, *226*, 91–100.
17 Marra, A.; Esnault, J.; Veyrières, A.; Sinaÿ, P. *J. Am. Chem. Soc.* **1992**, *114*, 6354–6360.
18 Mehta, S.; Pinto, B. M. *J. Org. Chem.* **1993**, *58*, 3269–3276.
19 Sliedregt, L. A. J. M.; Zegelaar-Jaarsveld, K.; van der Marel, G. A.; van Boom, J. H. *Synlett* **1993**, 335–337.
20 Yamada, H.; Harada, T.; Miyazaki, H.; Takahashi, T. *Tetrahedron Lett.* **1994**, *35*, 3979–3982.
21 Yamada, H.; Harada, T.; Takahashi, T. *J. Am. Chem. Soc.* **1994**, *116*, 7919–7920.
22 Chenault, H. K.; Castro, A. *Tetrahedron Lett.* **1994**, *35*, 9145–9148.
23 Geurtsen, R.; Côté, F.; Hahn, M. G.; Boons, G.-J. *J. Org. Chem.* **1999**, *64*, 7828–7835.
24 Paulsen, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155–173.
25 Toshima, K.; Tatsuta, H. *Chem. Rev.* **1993**, *93*, 1503–1531.
26 Shimizu, M.; Togo, H.; Yokoyama, M. *Synthesis* **1998**, 799–822.
27 Garegg, P. J. *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 179–205.
28 Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583–5584.
29 Kanie, O.; Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1994**, *116*, 12,073–12,074.
30 Roy, R.; Andersson, F. O.; Letellier, M. *Tetrahedron Lett.* **1992**, *33*, 6053–6056.
31 Liang, R.; Yan, L.; Loebach, J.; Ge, M.; Uozumi, Y.; Sekanina, K.; Horan, N.; Gildersleeve, J.; Thompson, C.; Smith, A.; Biswas, K.; Still, W. C.; Kahne, D. *Science* **1996**, *274*, 1520–1522.
32 Yan, L.; Taylor, C. M.; Goodnow, Jr. R.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 6953–6954.
33 Schmidt, R. R.; Michel, J. *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 731–732.
34 Schmidt, R. R. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212–235.
35 Lassaletta, J. M.; Carlsson, K.; Garegg, P. J.; Schmidt, R. R. *J. Org. Chem.* **1996**, *61*, 6873–6880.
36 Ishida, H. K.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1994**, *260*, c1–c6.
37 Matsuzaki, Y.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1992**, *33*, 6343–6346.
38 Kanie, O.; Barresi, F.; Ding, Y.; Labbe, J.; Otter, A.; Forsberg, L. S.; Ernst, B.; Hindsgaul, O. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2720–2722.
39 Ding, Y.; Kanie, O.; Labbe, J.; Palcic, M. M.; Ernst, B.; Hindsgaul, O. in *Glycoimmunology*, Alavi, A.; Axford, J. S. Eds, Plenum Press, New York, **1995**, pp 261–269.
40 Ding, Y.; Labbe, J.; Kanie, O.; Hindsgaul, O. *Bioorg. Med. Chem.* **1996**, *4*, 683–692.

41 Douglas, S.P.; Whitfield, D.M.; Krepinsky, J.J. *J. Carbohydr. Chem.* **1993**, *12*, 131–136.

42 Nakahara, Y.; Nakahara, Y.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1998**, *309*, 287–296.

43 Wang, Z.-G.; Douglas, S.P.; Krepinsky, J.J. *Tetrahedron Lett.* **1996**, *37*, 6985–6988.

44 Douglas, S.P.; Whitfield, D.M.; Krepinsky, J.J. *J. Am. Chem. Soc.* **1995**, *117*, 2116–2117.

45 Rademann, J.; Schmidt, R.R. *Tetrahedron Lett.* **1996**, *37*, 3989–3990.

46 Redemann, J.; Geyer, A.; Schmidt, R.R. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1241–1245.

47 Shimizu, H.; Ito, Y.; Kanie, O.; Ogawa, T. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2841–2846.

48 Andrade, R.B.; Plante, O.J.; Melean, L.G.; Seeberger, P.H. *Organic Lett.* **1999**, *1*, 1811–1814.

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

50 Mukaiyama, T.; Murai, Y.; Shoda, S.; *Chem. Lett.*, **1981**, 431–432.

51 Suzuki, K.; Maeta, H.; Matsumoto, T.; late Tsuchihashi, G. *Tetrahedron Lett.*, **1988**, *29*, 3571–3574.

52 Suzuki, K.; Maeta, H.; Matsumoto, T. *Tetrahedron Lett.* **1989**, *30*, 4853–4856.

53 Danishefsky, S.J.; Bilodeau, M.T. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1380–1419.

54 Danishefsky, S.J.; Allen, J.R. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 836–863.

55 Fraser-Reid, B.; Udodong, U.E.; Wu, Z.; Ottosson, H.; Merritt, J.R.; Rao, C.S.; Roberts, C.; Madsen, R. *Synlett* **1992**, 927–942.

56 Schelhaas, M.; Waldmann, H. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2056–2083.

57 Barany, G.; Merrifield, R.B., *J. Am. Chem. Soc.* **1977**, *116*, 7363–7365.

58 Wong, C.-H.; Ye, X.-S.; Zhang, Z. *J. Am. Chem. Soc.* **1998**, *120*, 7137–7138.

59 Zhu, T.; Boons, G.J. *Tetrahedron: Asymmetry* **2000**, *11*, 199–205.

60 Veeneman, G.H.; van Leeuwen, S.H.; van Boom, J.H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334.

61 Grice, P.; Ley, S.V.; Pietruszka, J.; Priepke, H.W.M.; Walther, E.P.E. *Synlett* **1995**, 781–784.

62 Hashimoto, S.; Sakamoto, H.; Honda, T.; Abe, H.; Nakamura, S.; Ikegami, S. *Tetrahedron Lett.* **1997**, *38*, 8969–8972.

63 Wilson, B.G.; Fraser-Reid, B. *J. Org. Chem.* **1995**, *60*, 317–320.

64 Douglas, N.L.; Ley, S.V.; Lücking, U.; Warriner, S.L. *J. Chem. Soc., Perkin Trans. 1* **1998**, 51–66.

65 Cheung, M.-K.; Douglas, N.L.; Hinzen, B.; Ley, S.V.; Pannecoucke, X. *Synlett* **1997**, 257–260.

66 Kanie, O.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1996**, *37*, 4551–4554.

67 Ito, Y.; Kanie, O.; Ogawa, T. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2510–2512.

68 Chang, G.X.; Lowary, T.L. *Org. Lett.* **2000**, *2*, 1505–1508.

69 Boons, G.-J.; Isles, S. *Tetrahedron Lett.* **1994**, *35*, 3593–3596.

70 Demchenko, A.V.; Boons, G.-J. *J. Org. Chem.* **2001**, *66*, 2547–2554.

71 Hindsgaul, O.; Kanie, O. *Curr. Opin. Struct. Biol.* **1992**, *2*, 674–681.

72 Demchenko, A.V.; Boons, G.-J. *Chem. Europ. J.* **1999**, *5*, 1278–1283.

73 Ishida, H. *Carbohydrates in Chemistry and Biology*, Ernst, B.; Hart, G.W.; Sinaÿ, P.; Eds, Vol. 1, Chapt. 12, pp 305–317, **2000**.

74 Schmidt, R.R.; Jung, K.-H. *Carbohydrates in Chemistry and Biology*, Ernst, B.; Hart, G.W.; Sinaÿ, P. Eds, Vol. 1, Chap. 2, pp 5–59, **2000**.

75 Hotta, K.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1995**, *14*, 491–506.

76 Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1990**, *200*, 269–285.

77 Ishida, H.; Kiso, M. *J. Synth. Org. Chem. Jpn.* **2000**, *58*, 1108–1113.

78 Miura, T.; Hirose, Y.; Ohmae, M.; Inazu, T. *Org. Lett.* **2001**, *3*, 3947–3950.

79 Douglas, S.P.; Whitfield, D.M.; Krepinsky, J.J. *J. Am. Chem. Soc.* **1991**, *113*, 5095–5097.

80 Verduyn, R.; van der Klein, P.A.M.; Douwes, M.; van der Marel, G.A.; van Boom, J.H. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 464–466.

81 Mehta, S.; Whitfield, D. *Tetrahedron Lett.* **1998**, *39*, 5907–5910.

82 Jiang, L.; Hartly, C.; Chan, T.-H. *Chem. Commun.* **1996**, 2193.

83 Ando, H.; Manabe, S.; Nakahara, Y.; Ito, Y. *J. Am. Chem. Soc.* **2001**, *123*, 3848–3849.

84 Thürmer, R.; Meisenbach, M.; Echner, H.; Weiler, A.; Al-Qawasmeh, R.A.; Voelter, W.; Korff, U.; Schmitt-Sody, W. *Rapid Commun. Mass Spectrometry* **1998**, *12*, 398–402.

85 Nicolaou, K.C.; Winssinger, N.; Pastor, J.; DeRoose, F. *J. Am. Chem. Soc.* **1997**, *119*, 449–450.

86 Tolborg, J.F.; Jensen, K.J. *Chem. Commun.* **2000**, 147–148.

87 Barresi, F.; Hindsgaul, O. *J. Am. Chem. Soc.* **1991**, *113*, 9376–9377.

88 Stork, G.; Kim, G. *J. Am. Chem. Soc.* **1992**, *114*, 1087–1088.

89 Bols, M. *Tetrahedron*, **1993** *49*, 10049–10060.

90 Ito, Y.; Ogawa, T. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1765–1767.

91 Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1997**, *119*, 5562–5566.

92 Zhang, Z.; Ollmann, I.R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734–753.

93 Ye, X.-S.; Wong, C.-H. *J. Org. Chem.* **2000**, *65*, 2410–2431.

94 Manabe, S.; Ito, Y.; Ogawa, T. *Molecules Online* **1998**, *2*, 40–45.

95 Ley, S.V.; Priepke, H.W.M. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2292–2294.

96 Tsukida, T.; Yoshida, M.; Kurokawa, K.; Nakai, Y.; Achiha, T.; Kiyoi, T.; Kondo, H. *J. Org. Chem.* **1997**, *62*, 6876–6881.

97 Yamada, H.; Kato, T.; Takahashi, T. *Tetrahedron Lett.* **1999**, *40*, 4581–4584.

98 Yu, B.; Yu, H.; Hui, Y.; Han, X. *Tetrahedron Lett.* **1999**, *40*, 8591–8594.

99 Takeuchi, K.; Tamura, T.; Mukaiyama, T. *Chem. Lett.* **2000**, 122–123.

100 Takeuchi, K.; Tamura, T.; Mukaiyama, T. *Chem. Lett.* **2000**, 124–125.

101 Yoshida, M.; Kiyoi, T.; Tsukida, T.; Kondo, H. *J. Carbohydr. Chem.* **1998**, *17*, 673–682.

102 Adinolfi, M.; Barone, G.; Napoli, L.D.; Iadonisi, A.; Piccialli, G. *Tetrahedron Lett.* **1998**, *39*, 1953–1956.

103 Nicolaou, K.C.; Watanabe, N.; Li, J.; Pastor, J.; Winssinger, N. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1559–1561.

104 Rodebaugh, R.; Fraser-Reid, B.; Geysen, H.M. *Tetrahedron Lett.* **1997**, *38*, 7653–7656.

105 Danishefsky, S.J.; McClure, K.F.; Randolph, J.T.; Ruggeri, R.B. *Science* **1993**, *260*, 1307–1309.

106 Seeberger, P.H.; Beebe, X.; Sukenick, G.D.; Pochapsky, S.; Danishefsky, S.J. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 491–493.

107 Randolph, J.T.; Danishefsky, S.J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1470–1473.

108 Roberge, J.Y.; Beebe, X.; Danishefsky, S.J. *Science* **1995**, *269*, 202–204.

109 Randolph, J.T.; McClure, K.F.; Danishefsky, S.J. *J. Am. Chem. Soc.* **1995**, *117*, 5712–5719.

110 Zheng, C.; Seeberger, P.H.; Danishefsky, S.J. *J. Org. Chem.*, **1998**, *63*, 1126–1130.

111 Zheng, C.; Seeberger, P.H.; Danishefsky, S.J. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 786–787.

112 Danishefsky, S.J.; Bilodeau, M.T. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1380–1419.

113 Doi, T.; Sugiki, M.; Yamada, H.; Takahashi, T. *Tetrahedron Lett.* **1999**, *40*, 2141–2144.

114 Renil, M.; Meldal, M. *Tetrahedron Lett.* **1996**, *37*, 6185–6188.

115 Rademann, J.; Grøtli, M.; Meldal, M.; Bock, K. *J. Am. Chem. Soc.* **1999**, *121*, 5459–5466.

116 Adinolfi, M.; Barone, G.; De Napoli, L.; Iadonisi, A.; Piccialli, G. *Tetrahedron Lett.* **1996**, *37*, 5007–5010.

117 Liang, R.; Loebach, J.; Horan, N.; Ge, M.; Thompson, C.; Yan, L.; Kahne, D. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 10554–10559.

118 Yan, L.; Kahne, D. *Synlett* **1995**, 523–524.

119 Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 885–888.

120 Jobron, L.; Hindsgaul, O. *J. Am. Chem. Soc.* **1999**, *121*, 5835–5836.

121 Bouzide, A.; Sauvé, G. *Synlett* **1997**, 1153–1154.

122 Fukase, K.; Hashida, M.; Kusumoto, S. *Tetrahedron Lett.* **1991**, *32*, 3557–3558.

123 Fukase, K.; Yoshimura, T.; Hashida, M.; Kusumoto, S. *Tetrahedron Lett.* **1991**, *32*, 4019–4022.

124 Kanie, O.; Grotenberg, G.; Wong, C.-H. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 4545–4547.

125 Zhu, T.; Boons, G.-J. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1898–1900.

126 Fukase, K.; Nakai, Y.; Egusa, K.; Porco, J.A. Jr.; Kusumoto, S. *Synlett* **1999**, 1074–1078.

127 Paulsen, H.; Schleyer, A.; Mathieux, N.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 281–294.

128 Kantchev, A.B.; Parquette, J.R. *Tetrahedron Lett.* **1999**, *40*, 8049–8053.

129 Danishefsky, S.J.; Allen, J.R. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 836–863.

130 Rodebaugh, R.; Joshi, S.; Fraser-Reid, B.; Geysen, H.M. *J. Org. Chem.* **1997**, *62*, 5660–5661.

131 Thürmer, R.; Meisenbach, M.; Echner, H.; Weiler, A.; Al-Qawasmeh, R.A.; Voelter, W.; Korff, U.; Schmitt-Sody, W. *Rapid Commun. in Mass Spectrometry* **1988**, *12*, 398–402.

132 Plante, O.J.; Palmacci, E.R.; Seeberger, P.H. *Science* **2001**, *291*, 1523–1527.

133 Haase, W.-C.; Seeberger, P.H.; Pochapsky, S.S. *Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries*, Seeberger, P.H. Ed, Chapt. 8, pp 165–174.

134 Choudhury, A.K.; Mukherjee, I.; Mukhopadhyay, B.; Roy, N. *J. Carbohydr. Chem.* **1999**, *18*, 361–367.

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

136 Kanemitsu, T.; Kanie, O.; Wong, C.-H. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 3415–3418.

137 Kanemitsu, T.; Wong, C.-H.; Kanie, O. *J. Am. Chem. Soc.* **2002**, *124*, 3591–3599.

138 Jiang, L.; Hartley, R.C.; Chan, T.-H. *Chem. Commun.* **1996**, 2193–2194.

139 Manabe, S.; Ito, Y.; Ogawa, T. *Synlett* **1998**, 628–630.

140 Weigelt, D.; Magnusson, G. *Tetrahedron Lett.* **1998**, *39*, 2839–2842.

141 Kononov, L.O.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1997**, *38*, 1599–1602.

142 Hunt, J.A.; Roush, W.R. *J. Am. Chem. Soc.* **1996**, *118*, 9998–9999.