Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries

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PREFACE

Glycobiology has provided many compelling results that place oligosaccharides and glycoconjugates in the center of a host of signal transduction processes at the molecular and cellular levels. It was found that oligosaccharides in the form of glycoconjugates mediate a variety of events, including inflammation, immunological response, metastasis, and fertilization. Cell surface carbohydrates act as biological markers of various tumors and as binding sites for other substances, including pathogens.

A major impediment to the rapidly growing field of molecular glycobiology is the lack of pure, structurally defined complex carbohydrates and glycoconjugates. Although these molecules are often found only in low concentrations in nature, the identification and isolation of complex carbohydrates from natural sources is greatly complicated by their microheterogeneity. Detailed biophysical and biochemical studies of carbohydrates require sufficient quantities of defined oligosaccharides. The procurement of synthetic material presents a formidable challenge to the synthetic chemist. While the need for chemically defined oligosaccharides has steadily increased, the synthesis of these complex molecules remains time consuming and is carried out by a few specialized laboratories.

Many innovative methods in carbohydrate chemistry have been developed and are covered in several very recent (at the time of writing) books on this subject. Although the synthesis of oligopeptides and oligonucleotides has benefited greatly from the feasibility of conducting their assembly on polymer supports, solid support oligosaccharide synthesis has, after some reports in the 1970s, been deemed too difficult for a long time. More recent developments in solution-phase carbohydrate synthesis methodology, combined with a more general appreciation of the advantages of solid support synthesis, have led to renewed interest in this field. The advent of combinatorial chemistry has energized investigations into methods applicable to the generation of diverse libraries of oligosaccharides and glycoconjugates.

This book covers all of the most recent (at the time of writing) developments in the field of solid support oligosaccharide synthesis. Included are chapters discussing different synthetic strategies, glycosylation protocols, the use of solid supports versus soluble polymeric supports and “on-resin” analytical methods. Special topics such as the formation of β-glycosidic linkages on solid support are also discussed.

Combinatorial chemistry has provided new ways for the pharmaceutical industry and for academic researchers to address specific problems in a time- and resource-efficient manner. Given the involvement of specific oligosaccharide structures in signal transduction processes, combinatorial carbohydrate libraries are
expected to provide a wealth of information and to lead to a detailed understanding of
the structures involved in these processes. Because of the complexity of the task, only
few approaches have been reported. Different approaches are summarized in the last
part of this volume and keep the reader abreast of the latest developments in the field.

Finally, solid-phase glycopeptide synthesis is highlighted in two chapters
describing exciting new developments in this area.

This book covers the state-of-the-art developments in the field until the beginning
of the year 2000. At this time it becomes clear that the tremendous progress has set
the stage for the conception of an automated oligosaccharide synthesizer. The
ingenuity and hard work of many synthetic chemists will eventually lead to a situation
already familiar within the peptide and oligonucleotide arenas; defined oligo-
saccharides and glycoconjugates will become readily available for biochemical and
biophysical studies.

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1 Solid-Phase Carbohydrate Synthesis: The Early Work

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1.1 INTRODUCTION

The first steps toward solid-phase oligosaccharide synthesis date back to the early 1970s. Intriguing features associated with the solid-phase paradigm that prompted researchers to explore oligosaccharide synthesis on solid support included maximized yields by use of excess reagents, ease of purification, and synthesis speed. By 1970 solid-phase peptide synthesis, the concept of which had just been extended to the synthesis of depsipeptides, had already been automated. Given the immense impact of automated solid-phase oligopeptide and later oligonucleotide synthesis on the development of the biochemistry and biology of these molecules, the enthusiasm for developing related methodology for the synthesis of oligosaccharides is quite understandable.

The level of complexity associated with the synthesis of oligosaccharides on a polymer support is much greater than that associated with the other two classes of repeating biooligomers. While oligopeptides and oligonucleotides consist of merely linear chains, oligosaccharides, bearing up to four sites of potential elongation, are often branched, requiring flexible protecting group strategies for the effective differentiation of an array of similar functionality (hydroxyls and amines). The formation of a new stereogenic center in every glycosylation step further complicates oligosaccharide synthesis. Additionally, traditional acid-sensitive linker systems used for peptide synthesis are often incompatible with the Brönsted or Lewis acidic glycosylation conditions. Thus, a series of problems have to be considered in the planning process: (1) selection of an overall synthetic strategy and development of methods for attachment of the carbohydrate to the polymeric support through the “reducing” or the “nonreducing” end, (2) choice of a solid support material, (3) selection of a linker (“support-bound protecting group”) that is stable during the synthesis but can be easily cleaved when desired, (4) a highly flexible protecting group strategy, (5) stereospecific and high-yielding coupling reactions, and (6) “on resin” methods to monitor chemical transformations.
Because of the lack of availability of several of these required methodologies, the initial attempts described below were ultimately not continued. However, they explored most of the fundamental issues that provide the basis for solid-phase oligosaccharide synthesis practiced today. In this chapter we focus on pioneering work carried out in the 1970s.

1.2 SOLID-PHASE STRATEGIES

Fréchet and Schuerch were the first to report on the synthesis of di- and trisaccharides on a solid support in 1971. Glucosyl bromide 2 was attached to allyl alcohol functionalized Merrifield resin 1 by simple alcoholysis, preparing the first resin-bound monomer 3. The reaction was carried out in benzene or tetrachloromethane with excess donor over 2–4 days in the presence of 2,6-dimethylpyridine, providing 3 in yields up to 96%, as determined by weight gain of the resin. These conditions, resulting in a rather slow reaction, were chosen to minimize side reactions often associated with activation by metal ions. The use of p-nitrobenzoate as temporary protecting group at C6 aimed at achieving high \( \alpha \)-selectivity in the coupling reactions, as was established by solution studies. After removal of the \( p \)-nitrobenzoyl group, the coupling was reiterated twice yielding resin-bound trisaccharide 5 in near-quantitative stepwise yield. The yield was determined by weight gain of the resin and on the basis of the free hydroxyls of the latest attached sugar monomer. Cleavage from the resin was accomplished by ozonolysis followed by reduction of the ozonide with dimethyl sulfide in varying yields between 51% and 91% to furnish 2-hydroxyethyl glycoside 6. As no suitable analytical method was available at the time, a high degree of \( \alpha \)-glycosidic linkages in the product was assumed on comparison of the optical rotation of model compounds obtained by solution syntheses (Scheme 1.1A). Attempts to achieve \( \beta \)-selectivity in the solid-phase glycosylation by changing the electronic properties of the C6-protecting group were not successful. While long reaction times and the failure to selectively synthesize \( \beta \)-linked glycosides severely limited the generality of this approach, \( \alpha \)-linked 1,6-oligomers were prepared reasonably well.

Zehavi and coworkers introduced the original concept of a photolabile linkage of the first carbohydrate monomer to the polymeric phase (Scheme 1.1B). Applying essentially the same coupling conditions as Fréchet, disaccharide 8 was obtained in approximately 90% yield per coupling step. Unfortunately, photolytic release of the disaccharide from the resin did not proceed as well on a preparative scale as in previous solution-phase model studies, and debenzylated reducing isomaltose 9 was obtained in only 12.5%, based on resin-bound monomer. High \( \alpha \)-selectivity in these glycosylation reactions was demonstrated by digestion experiments using \( \alpha \)- and \( \beta \)-glycosidases.

Anderson and coworkers introduced a thioglycosidic linkage to the solid support in 1976 to afford the free reducing oligosaccharide after release from the support (Scheme 1.2A). Using a set of protecting groups similar to those mentioned above,
1.2 SOLID-PHASE STRATEGIES

resin-bound monomer 12 was obtained either by coupling of a stoichiometric amount of thiosugar 11 to chloromethylated polystyrene, or by glycosylation of the corresponding thiol functionalized resin with an excess of glucosyl donor in comparable yields (about 80%). The free C6 hydroxyl group was glycosylated with excess glucosyl donor 13 under repeated alcoholysis conditions to furnish support-bound disaccharide 14 in 75% yield. Refluxing disaccharide 14 in benzene in the presence of methyl iodide and benzyl alcohol as acceptor, furnished free disaccharide 15 as the major component in a mixture of products. Gas-liquid chromatography (GLC) analysis of the disaccharide fractions revealed an α/β-diastereomeric ratio of 11.5–19:1, confirming the findings by Schuerch and Zehavi on similar systems.9

In addition to differently functionalized polystyrene (Merrifield’s resin), controlled-pore glass, as a nonswelling inorganic polymeric support was already evaluated for solid-phase oligosaccharide synthesis in its pioneering days. Schuerch reported the attempted glycosylation of a zirconia-coated glass surface carrying unsaturated alcohol acceptor sites, but only poor glycosylation yields (<20%) could be achieved.15 A second attempt by Anderson et al. was based on porous glass beads (pore size 2500 Å) functionalized with bromobenzyl attachment sites.14 The first thiosugar monomer 11 was quantitatively coupled to the support and subsequently glycosylated up to the trisaccharide employing excess donor 13 in repeated alcoholysis reactions. However, coupling yields were low even after prolonged reaction times. HPLC analysis of the cleaved trisaccharide 19 showed essentially the same α/β ratios as for reactions carried out on polystyrene support (Scheme 1.2B).

In addition to these selective α-(1→6) glucosylations, several β-selective glycosylation reactions have been studied on the solid support making use of participating ester groups. Gagnaire and coworkers described two approaches to solid-phase oligosaccharide synthesis linking the first carbohydrate monomer to the polymeric support via an ester bond. Glucosamine acceptor 21 was immobilized by esterification with acid chloride functionalized “popcorn” polystyrene at the C6-hydroxyl. Benzoylation of the remaining free C4-hydroxyl and selective removal of the benzoyl propionate protecting group furnished acceptor 22. Repeated glycosylation with excess glucosamine chloride donor 23 employing Helferich conditions furnished β-linked disaccharide 24 in 85% yield.17 This was the first time that a sterically more hindered secondary acceptor was glycosylated on a polymer matrix. Cleavage with sodium methoxide in methanol/dioxane and subsequent reacetylation rendered free disaccharide 25 in 51%, yield based on polymer-bound monomer 22 (Scheme 1.3). A β-(1→6)-linked glucosamine dimer had previously been prepared on a “popcorn” polystyrene by the same group in a similar fashion.18 A major drawback of this approach on “popcorn” polystyrene was considerable loss of material at several stages during the syntheses due to partial solubilization of the matrix.

Gentiotetraose, an all β-(1→6)-linked tetramer of glucose was selectively prepared by Gagnaire and coworkers (Scheme 1.4).19 6-O-Trichloroacetylated glucosyl bromide 27 was attached to succinoylated 2% crosslinked polystyrene. After selective
Scheme 1.1 Early solid-phase approaches to α-(1→6)-linked oligosaccharides.
**Scheme 1.2** Solid-phase oligosaccharide synthesis employing thioglycosidic linkages to different solid supports.

**Scheme 1.3** Solid-phase synthesis of β-(1→3)-linked glucosamine dimers employing ester linkages to the support.
Scheme 1.4  Solid-phase synthesis of β-(1→6)-linked gentiotetraose employing ester groups for permanent protection, for temporary protection, and as linkage to the support.
removal of the trichloroacetyl group with ammonia in dioxane subsequent glycosylation with disaccharide donor 29, deprotection and glycosylation with glucosyl bromide 31 employing Helferich conditions afforded resin-bound gentiotetraose 32. Cleavage from the support with hydrazinium acetate furnished the crude tetramer 33 in almost 70% yield containing traces of di- and trisaccharides.

Changing the 2-O-acetyl group in 31 to a nonparticipating benzyl group resulted in preferential α-glycosylation yielding 76% of disaccharide (α/β ratio 4.4:1). The same support and donor were employed in the diastereospecific synthesis of β-(1→3)-linked glucose dimer laminarabiose in good yield on 2% crosslinked polystyrene. It should be noted that the stereochemical outcome of these solid-phase syntheses was virtually identical to that in solution phase. An interesting feature of these syntheses is the exclusive use of ester groups for both permanent and temporary protection of hydroxyl groups and attachment to the support. The esters were differentiated by their lability to treatment with base.

Fréchet proposed a resin-bound cyclic boronic acid ester as an unconventional mode of attaching the first sugar monomer to the solid phase (Scheme 1.5). This linkage was very selectively formed with cis-1,2 and cis-1,3-diols under mild azeotropic conditions, leaving one hydroxyl for further chain elongation. Simple hydrolysis of the cyclic esters afforded the free sugars. Unfortunately, couplings using monosaccharide 31 as glycosyl acceptor proceeded sluggishly and in poor yields, thus rendering this approach unattractive.

**Scheme 1.5** Disaccharide synthesis on a solid support using a cyclic boronic acid ester linker.

All the described solid-phase glycosylation protocols required long reaction times to proceed in reasonable yields because of the slower reaction kinetics on support than in solution. Furthermore, since no analytical means were available to monitor the progress of the reaction on the bead, development of optimal reaction conditions was difficult. The approach described by Guthrie and coworkers in the early 1970s for
Scheme 1.6  The first approaches to oligosaccharide synthesis employing soluble polymer supports.
polymer-supported oligosaccharide synthesis was unique in many respects. The polymer support was created by copolymerization of styrene with a sugar monomer suitably functionalized with a polymerizable O-protecting group. This linear polymer allowed for glycosylation reactions in homogeneous solution, thus avoiding some of the principal shortcomings of any solid-phase approach; on the other hand, the support could be readily precipitated for purification to take advantage of the solid-phase paradigm. This was the only approach utilizing a glycosyl donor that was generated on the support and was reacted with excess acceptor in solution. Carbohydrate monomers 38 and 39 were copolymerized with styrene to yield soluble polystyrene polymers 40 and 41, containing approximately 0.15 and 0.06 mol% of sugar monomer, respectively. Disaccharide formation was effected via glycosyl bromide 43 and orthoester 47 following the Kochetkov orthoester glycosylation method (Scheme 1.6). Treatment of the resin with potassium acetate in refluxing DMF (dimethylformamide) yielded gentiobiose octaacetate 51 in 42% yield, based on the support-bound monomer 41. When a benzoyl linkage to the support 40 was used instead of the arylsulfonyl linkage present in 41, cleavage by methanolysis furnished 23% of free disaccharide, based on available support-bound disaccharide 48.

This approach had several drawbacks. Most side reactions in glycosylation reactions occur within the glycosyl donor moiety, thus terminating the growth of the corresponding chain under this donor-bound paradigm. Moreover, repeated glycosylations could not be used to improve coupling yields. In addition, the strongly acidic conditions used for glycosyl bromide formation could affect acid-labile glycosidic bonds in the oligosaccharide chain. Another disadvantage of the soluble-polymer-supported synthesis was a substantial loss of material during the precipitation and filtration steps following each reaction on the support.


The pioneering work in solid-phase oligosaccharide chemistry provided the foundation for the rapid progress that several groups have made in the area as described in the following chapters. These early approaches explored some of the important fundamental issues, including different strategies (donor- vs. acceptor-bound synthesis), various solid supports (soluble and insoluble), and linker systems. Unfortunately, at that time “this approach [was] not competitive with the more classical solution chemistry methods, due mainly to the lack of suitable glycosidation reactions” (Fréchet), that would meet the demands and conditions of solid-phase synthesis. The absence of suitable on-bead analytical tools for effective reaction monitoring made reaction development particularly difficult. Soluble polymers circumvented in theory some problems associated with solid supported synthesis such as reaction kinetics and reaction monitoring. The considerable loss of material during the workup steps also compromised the advantages of the solid-phase paradigm, since it was less effective and more laborious than syntheses on solid support. Eventually, the field came to a complete standstill for a 20-year period, and with only one exception mentioned below, no further progress was reported. Major
advances in solution-phase oligosaccharide synthesis with regard to donor reactivity, glycosylation selectivity, protecting group diversity, and analytical techniques were necessary before solid-phase oligosaccharide synthesis (SOS) set the stage for the developments described in the following chapters.

During the 1980s and early 1990s some solid-phase and soluble-polymer-supported syntheses of bacterial capsular oligosaccharides were reported by chemical formation of phosphodiester bonds. Van Boom et al. reported the only notable advance based on chemical glycosylations on solid support, expanding the initial attempts to the solid-phase synthesis of a large antigenic oligosaccharide that exhibited properties of a synthetic vaccine (Scheme 1.7). Linear α-(1→5)-linked galactofuranosyl homopolymers of varying length, found to be immunologically active in Aspergillus and Penicillium species, were chosen as targets for a repetitive oligosaccharide synthesis on Merrifield resin. These synthetic structures were the basis for studies correlating oligosaccharide length and immunogenicity. Merrifield’s resin was functionalized with L-homoserine, resulting in a 0.5 mmol/g loading of acceptor resin. Coupling with a twofold excess of galactofuranosyl chloride employing Helferich conditions furnished resin-bound monosaccharide stereoselectively in 90% yield as determined after cleavage from the resin. In order to facilitate the purification of the final products, acetylation of any free hydroxyl groups was chosen as a capping step after each glycosylation reaction. Chain elongation was achieved by selective removal of the C5-levulinoyl protecting group in resin-bound monomer with hydrazine, pyridine, and acetic acid and subsequent β-stereospecific glycosylation with donor. The deprotection, glycosylation, and capping steps were reiterated up to the heptamer stage. Base hydrolysis released heptamer in 23% overall yield, corresponding to 89% average yield over 13 coupling and deprotection steps. Complete deprotection afforded the oligomers up to the heptamer in bioconjugatable form. These semisynthetic constructs were used for biological tests in rabbits demonstrating an increase of immunogenicity with increasing oligosaccharide chain length.

While chemical polymer-supported oligosaccharide synthesis with the abovementioned exception came to a halt during the 1980s, interest in enzymatic methods for oligosaccharide and glycopeptide synthesis on both insoluble and soluble polymeric supports continued since the first disclosure by Zehavi in 1983. The intriguing features of this approach include α/β-specificity and regioselectivity, which reduces the need for elaborate protecting group manipulations in many cases. These methods have been reviewed elsewhere and exceed the scope of this chapter.

High selectivity and substrate specificity of glycosyl transferases make them valuable catalysts for special linkages in polymer-supported synthesis. There is, however, still a rather limited set of enzymes available to date, and the need to synthesize a variety of natural and non-natural oligosaccharides prevails. Particularly with regard to combinatorial approaches, chemical solid-phase oligosaccharide synthesis promises to meet the demands most effectively.

With the development of novel, powerful, and selective glycosylating agents, exemplified by the introduction of glycosyl trichloroacetimidates to
Scheme 1.7  Solid-phase synthesis of an immunogenic heptasaccharide by van Boom et al.\textsuperscript{29}
polymer-supported oligosaccharide synthesis by Krepinsky in 1991, interest in chemical solid-phase oligosaccharide synthesis was revived. The following chapters give an overview of the achievements in the field, based on significant advances in glycosylation methodology, polymer supports, linker systems, “on bead” analytical tools, and protecting group development. Today, solid-phase oligosaccharide synthesis is competitive not only with classical solution-phase methods but also with many of the major problems solved, as automation has come within reach. These achievements promise significant impact on the glycosciences.

REFERENCES


REFERENCES


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2 The Glycal Assembly Method on Solid Supports: Synthesis of Oligosaccharides and Glycoconjugates

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2.1 INTRODUCTION

The three major classes of biopolymers found in eukaryotic systems are nucleic acids, proteins, and polysaccharides. The latter class is the most complex with respect to structural and stereochemical diversity. Polysaccharides indeed possess a massive "information" content. Furthermore, polysaccharides are commonly found in nature covalently attached (conjugated) to other biomolecules such as proteins, isoprenoids, fatty acids, and lipids.¹

Polysaccharides are involved in a number of significant biological functions, beyond merely acting as structural elements and serving as sources of energy.² For example, they play key roles in such processes as pathogen binding, inflammation, metastasis, and fertilization.³ To study such processes, there has been an increasing need to gain access to usable quantities of these materials in pure form.

Oligosaccharides and glycoconjugates in living cells often exist as closely related mixtures. Their isolation from natural sources in homogeneous form is therefore very difficult, involving tedious purification and difficult characterization. This sequence of steps tends to result in low yields. This difficult situation presents chemical synthesis with a major opportunity to positively affect progress in the biochemical understanding of the processes described above.⁴

The application of solid-phase synthesis and automation has revolutionized much of the chemical and biochemical research related to peptides and nucleic acids.⁵ Thus, it is likely that successful methods to synthesize oligosaccharides and glycoconjugates...
on solid supports could bring with them a similar impact in carbohydrate related research.

The development of methods for the synthesis of oligosaccharides on a polymer support requires the simultaneous solution to a myriad of problems. The high "information" content of these structures means that their synthesis involves a level of complexity that dwarfs the one associated with peptides and oligonucleotides. One must tackle the usual considerations concerning the nature of the support material, selection of a suitable linker, and monitoring of reaction progress, possibly by "on resin" techniques. Choices must also be made as to whether the carbohydrate attachment should occur via its "reducing" or "nonreducing" end. In a way this decision is not unlike that involved in undertaking to grow a peptide chain via its carboxy or amino terminus. However, unlike the solid-phase synthesis of the other classes of biopolymers, in oligosaccharide synthesis there is a more challenging requirement for the construction to differentiate among numerous and similar functionalities (hydroxyl or amino). An efficient and highly flexible protecting group strategy must be adopted. Finally, and perhaps most importantly, each glycosidic bond to be fashioned constitutes a new locus of stereogenicity. Therefore, high-yielding and stereospecific coupling reactions are necessary and must be amenable to being conducted with one component anchored to an insoluble matrix.

As this book will attest, remarkable progress has been achieved in the assemblage of oligosaccharides and glycoconjugates on solid support. In this chapter we report on our laboratory's advances, which have led to the efficient assembly of relatively complex and biologically relevant structures, including the Lewis^b blood group and globo-H polysaccharides. The synthesis of these compounds is also described.

2.2 WHY GLYCAL ASSEMBLY? STRATEGIC CONSIDERATIONS

As was alluded to above, two possible approaches immediately present themselves for the synthesis of oligosaccharides on solid support. These involve the decision as to the mode of attachment of the first carbohydrate to the matrix.

In one scenario, the first carbohydrate is anchored via its "reducing" end (see Scheme 2.1, case 1). Here the support-bound carbohydrate will function as an acceptor in the coupling step to a solution-based donor D. As the next cycle is contemplated, a unique acceptor hydroxyl must be exposed in the now elongated, resin-bound carbohydrate construct.

This strategy requires that the donor (D) of the previous step be furnished with a uniquely removable blocking group at the site of the next proposed elongation. Clearly, under the glycosylation conditions, the solution-based donor D cannot possess simultaneously a free hydroxyl and the intact glycosyl donating function. This need to expose a unique hydroxyl group in the polymer-bound construct will likely necessitate awkward functional group adjustments in the preparation of D.

Connection of the glycosyl acceptor to the solid support (case 1) allows for the use of excess donor D (usually more fragile than A), a feature that can be used to advantage to drive reactions to completion. Nevertheless, this strategy requires a capping step to prevent the formation of deletion sequences, which would complicate
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Final purification. Moreover, and perhaps most importantly for the broader context of glycoconjugate synthesis, the completed oligosaccharide construct would likely require retrieval from the solid matrix before conjugation to the peptide or lipid, unless this portion were already present as part of the linker to the solid support.

In the other scenario, which we have found more novel, for reasons that will be explained shortly, the oligomer undergoing elongation is mounted to the solid support somewhere in the nonreducing region. In this case, the reducing end (i.e., glycosyl donor portion) of the molecule is available for coupling to a solution-based acceptor A (Scheme 2.1, case 2).

The use of A has two requirements. The first is that the precise acceptor site on A be properly identified. The second is that the reducing end of A be functionalized so that new donor capacity can be installed at the anomeric carbon of the elongated construct, in anticipation of the next coupling event. Not unlike the possible situation of case 1, a serious question of functional group compatibility must be anticipated during glycosylation in case 2. During the coupling step, acceptor A cannot possess a fully equipped, next-stage anomeric donor function at the same time as it carries a free hydroxyl group.

The reason for favoring the second scenario described above has to do with our development of the “glycal assembly” method, which appeared to offer several advantages for the solid-phase synthesis of oligosaccharides via this strategy (case 2, Scheme 2.1). Scheme 2.2, with the expression 1→2→4, captures the essence of the method and reveals the potential attractiveness of this approach. In this scheme, the nature of E+ and of the oniumlike species 2 has been left unspecified, as well as any indication of stereochemistry. Nevertheless, it is apparent that the glycal terminus of 1 could be converted to a donor function as represented in a general way with 2. For

Scheme 2.1 Strategies for building an oligosaccharide chain: glycosyl acceptor linked via its reducing end (case 1) and donor linked via a nonreducing end (case 2).