Solid-Phase Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries

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I. Introduction

The transfer of information is a fundamental process of life and central to all cellular systems. From a biological perspective, information needs to be transmitted intracellularly and intercellularly and passed on from generation to generation. The three major biopolymers, proteins, nucleic acids, and glycoconjugates, are mainly responsible for information transfer. While the biological importance of proteins and nucleic acids has been appreciated for a long time, oligosaccharides in the form of glycoconjugates are less well understood and have only more recently generated interest. Glycolipids and glycoproteins[1] play a major role in inflammation, immune response, metastasis, fertilization, and many other biomedically important processes.[2,3] Specific carbohydrate structures have been identified as markers for certain types of tumors while others are binding sites for bacterial and viral pathogens.[4]

A major impediment to the rapidly growing field of molecular glycobiology is the lack of pure, structurally defined complex carbohydrates and glycocon-

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Wilm-Christian Haase, born 1969, received his chemistry Diplom from the Rheinische Friedrich-Wilhelms-Universität Bonn, Germany, in 1996, where he joined the group of Professor Dr. K. H. Dötz. During his dissertation work he explored the synthesis and application of Fischer-type glycosylidene complexes as novel organometallic reagents for the assembly of *C*-glycosidic linkages. As a DAAD/NATO-postdoctoral fellow, he then joined the group of Professor Dr. P. H. Seeberger at the Massachusetts Institute of Technology, Cambridge, MA, in 1999, where he focused on the development of methods for the combinatorial solid-phase synthesis of human milk oligosaccharides. His research interests include developing novel transition-metal-catalyzed as well as solid-supported methods for the synthesis of *C*-saccharides.

jugates. Besides the fact that 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.[5] While the need for chemically defined oligosaccharides has steadily increased in recent years, the synthesis of these complex molecules remains time-consuming and is carried out by a few specialized laboratories. Oligonucleotides[6] and oligopeptides,[7] on the other hand, are now routinely prepared on automated synthesizers, providing pure substances in a rapid and efficient manner. The effect of an automated oligosaccharide synthesizer on the field of glycobiology may be readily envisioned when considering the impact of automated solid-phase peptide and oligonucleotide synthesis on the biochemistry of these molecules. Solid-phase synthesis lends itself particularly well to automation and will be the focus of this review.

This retrospective begins with a brief outline of the central issues of carbohydrate chemistry. After reviewing the early work in the field from 1970 to 1991, different synthetic strategies will be discussed. The linkers used to connect the first monosaccharide to the polymeric support will be covered as will be special protecting groups that were developed for use on the solid support. Efforts to apply different glycosylating agents to the assembly of oligosaccharides on a polymer matrix will be reviewed, followed by a description of on-resin analytical methods. Next, a brief summary of special procedures for the assembly of unusual or difficult structures will be followed by an extensive review of a range of complex oligosaccharides prepared on polymer support. Finally, the efforts of different groups directed at the preparation of carbohydrates will be covered. The focus of all discussions will be on chemical methods, while enzymatic approaches will be mentioned where appropriate. The review will conclude with a comparison of the currently available methods and the prospects for the development of an automated solid-phase oligosaccharide synthesizer.

A. Challenges of Carbohydrate Chemistry

Organic chemists have been intrigued by the synthesis of complex oligosaccharides for over 100 years. The assembly of these natural products presents two crucial challenges. A multitude of functional groups (amino and hydroxyl) of similar reactivity on each monomer emphasize the need for effective differentiation to allow for access to branched structures. Furthermore, a new stereogenic center is created each time a glycosidic linkage is formed and complicates matters far beyond the synthetic situation encountered with peptides and nucleic acids. The need to purify the reaction products by chromatography after each step makes oligosaccharide synthesis a laborious, time-consuming, and expensive task.

Over the years a host of protective groups for the masking of amino and hydroxyl groups has been introduced by taking advantage of the reactivities of the respective protective groups. Even more importantly, a variety of anomeric groups that allow for the high-yielding, selective, and reliable formation of many glycosidic linkages have been developed. While much progress has been made, some linkages still remain difficult to install. In particular, the synthesis of large, branched oligosaccharides presents multiple

difficulties. Steric and electronic changes in either of the coupling partners can make each new glycosidic bond to be created a challenge.

B. Advantages of Polymer-Supported Synthesis

Judged by the immense impact of automated solid-phase peptide and oligonucleotide synthesis on the biochemistry of these molecules, an automated oligosaccharide synthesizer is expected to provide a fundamental impulse on the field of glycobiology. Solid-phase synthesis allows for removal of excess reagents used to drive the reaction to completion by simply washing the resin. Purification of the reaction products at the end of the synthesis minimizes the number of chromatographic steps required. The solid-phase synthesis paradigm lends itself particularly well to automation of the synthetic process.

C. Central Aspects of Solid-Phase Oligosaccharide Synthesis

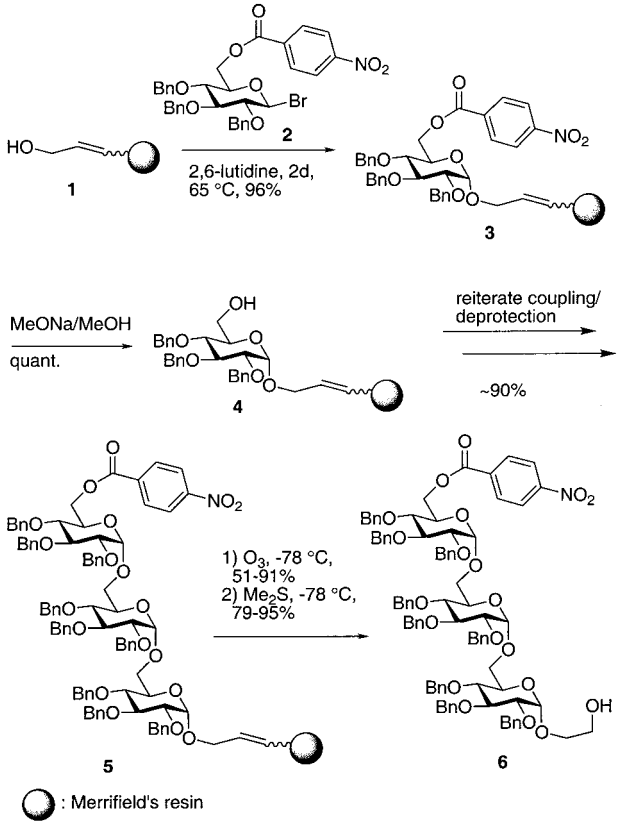
Several key issues have to be considered when contemplating the development of polymer-supported synthesis of oligosaccharides: (a) the design of an overall synthetic strategy with either the 'reducing' or the 'nonreducing' end of the growing carbohydrate chain attached to the support; (b) selection of a polymer and linker which has to be inert to all reaction conditions during the synthesis but is cleaved smoothly and effectively when desired; (c) a protecting-group strategy consistent with the complexity of the desired oligosaccharide; (d) stereospecific and high-yielding glycosylation reactions; (e) 'on-bead' analytical tools that facilitate reaction monitoring and enable a rational development of efficient protocols.

Different aspects of solid-phase oligosaccharide synthesis have been reviewed previously.[8] Here, we present a comprehensive summary of chemical (rather than enzymatic) carbohydrate synthesis on polymeric support and the preparation of combinatorial carbohydrate libraries.

II. Early Work

Inspired by the success of Merrifield's solid-phase peptide synthesis[9] that had just been applied to the synthesis of depsipeptides,[10] pioneering studies toward solid-phase oligosaccharide synthesis were carried out in the early 1970s.[11] Fréchet and Schuerch were the first to report on the synthesis of di- and trisaccharides on a solid support.[12] The first monosaccharide was connected via the anomeric position to allyl alcohol functionalized Merrifield resin **1** by reaction of glucosyl bromide **2**. Yields of up to 96% (as determined by weight gain) were obtained by reaction with excess glycosyl bromide over 2−4 days.[13] After removal of the temporary 4-nitro benzoyl protecting group,[14] two further couplings produced resin-bound trisaccharide **5** in near quantitative yield as judged by gravimetric analysis of the polymer. Cleavage from the resin was accomplished by ozonolysis followed by reduction of the ozonide with dimethyl sulfide to furnish 2-hydroxyethyl glycoside **6** (51−91% yield). A high degree of α-glycosidic linkages in the product was assumed based on the comparison of the optical rotation with model structures from solution-phase synthesis. These indirect methods had to suffice since no unambiguous analytical method was available at the time (Scheme 1).

Scheme 1

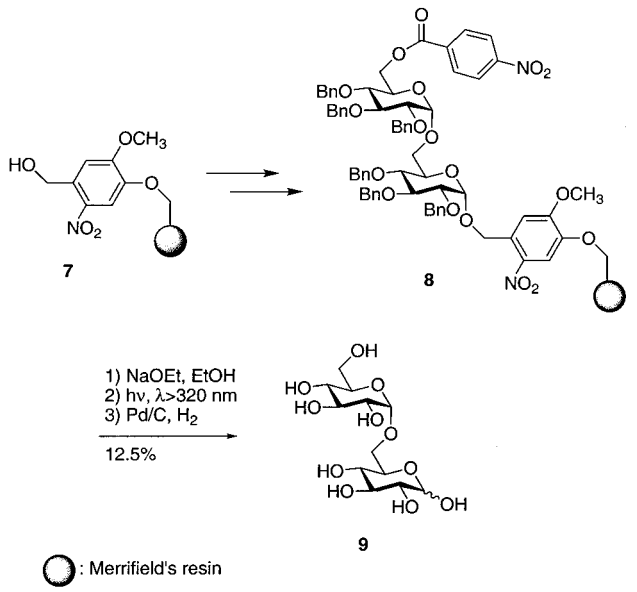


Attempts to achieve β-selectivity of the solid-phase glycosylation by altering the electronic properties of the C6 protecting group failed. This pioneering approach explored linkers, temporary protecting groups, and glycosylating agents. While it was quite successful in the preparation of α-linked 1→6-oligomers, drawbacks included long reaction times and the failure to selectively synthesize β-linked glycosides.

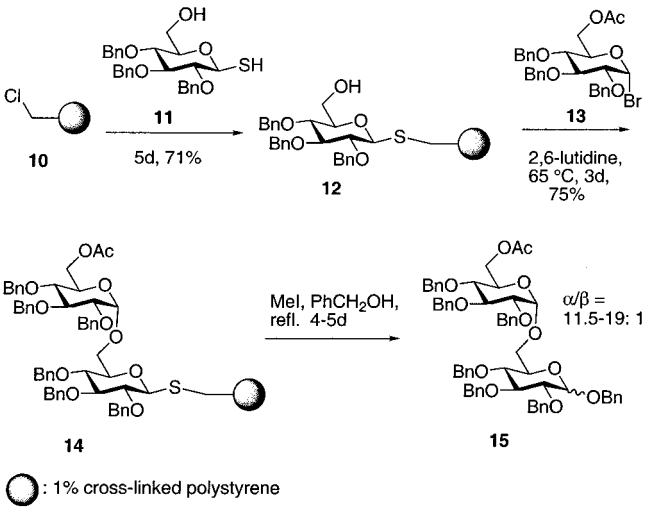
Zehavi et al. were the first to anchor a monosaccharide via a photolabile linkage to the polymer (Scheme 2).[15] Although model studies in solution had been promising,[16] photolytic release of disaccharide **8** from the resin did not meet expectations. Using glycosyl bromide donors, **8** was formed in approximately 90% yield per coupling step, but when cleaved and debenzylated on a preparative scale, isomaltose **9** was obtained in only 12.5% yield. Digestion experiments employing α- and β-glycosidases demonstrated high α-selectivity of the glycosylation reaction.

A thioglycosidic linkage[17] to the solid support that would release the reducing end of the oligosaccharide in form of the lactol was described by Anderson (Scheme 3).[18] Resin-bound monomer **12** was obtained either by coupling of thiosugar **11** to chloromethylated polystyrene or alternatively by glycosidation of thiol-functionalized resin with an excess of glucosyl donor. The C6 hydroxyl group was reacted with

Scheme 2



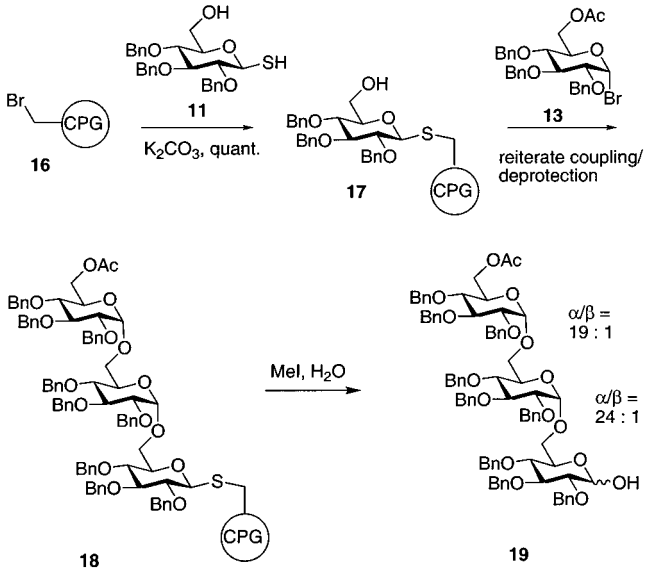
Scheme 3



excess glucosyl donor **13** to furnish support-bound disaccharide **14** in 75% yield. Cleavage from the polymer matrix was effected by refluxing **14** in the presence of methyl iodide and benzyl alcohol to prepare a mixture of products containing disaccharide **15** as major component. GLC analysis of the disaccharide fractions revealed the formation of anomeric mixtures during all couplings.

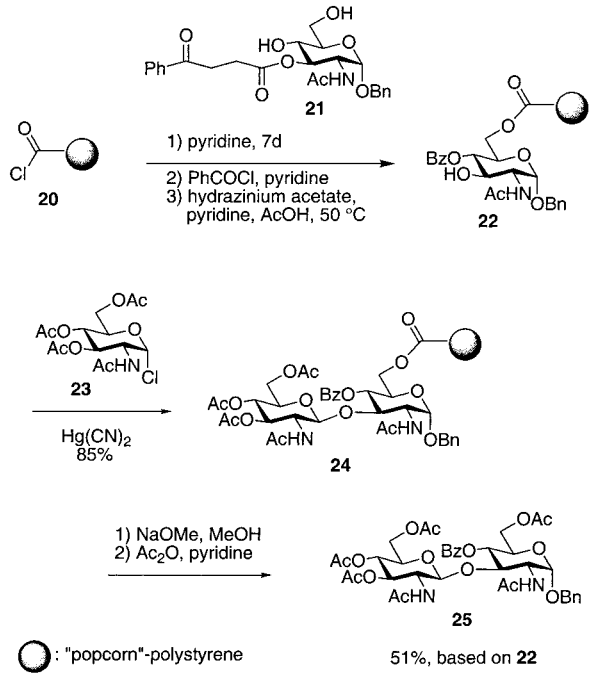
In addition to studies utilizing functionalized polystyrene (Merrifield's resin), controlled pore glass (CPG) was applied to solid-phase oligosaccharide synthesis as a nonswelling inorganic support. Schuerch reported the attempted glycosylation of a zirconia-coated glass-surface carrying unsaturated alcohol acceptor sites, but only poor glycosylation yields (<20%) were achieved.[19] A second attempt relied on the coupling of thiosugar monomer **11** to the bromobenzyl functionalized surface of porous glass beads.[20] Although the initial coupling to the support proceeded with almost quantitative yield, the subsequent couplings employing donor **13** suffered from low conversion even after prolonged reaction times. HPLC analysis of the cleaved trisaccharide **19** demonstrated that a mixture of α- and β-glycosidic linkages had been obtained (Scheme 4).

Scheme 4



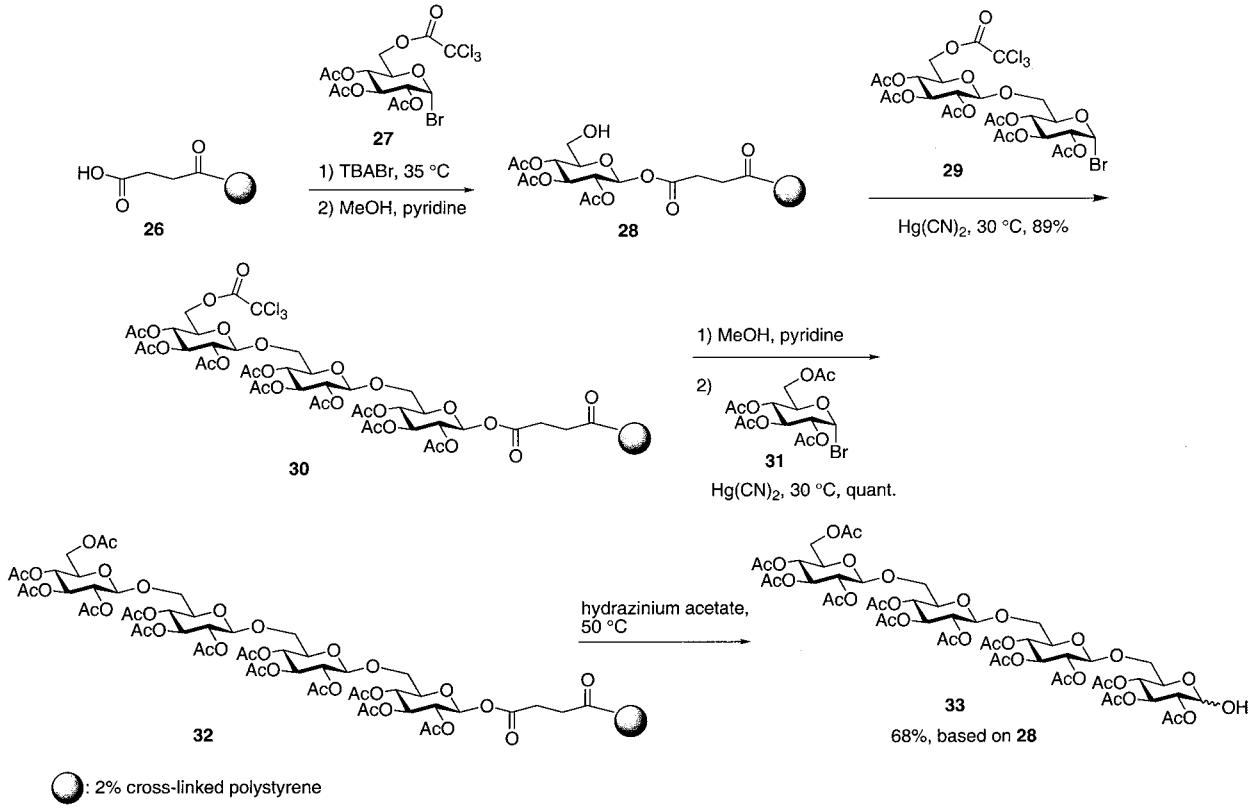
An ester linker for oligosaccharide synthesis was explored by Gagnaire. The C6 hydroxyl group of glucosamine **21** was immobilized by reaction with acid chloride on functionalized "popcorn" polystyrene. Protecting group manipulations and repeated glycosylation with excess glucosamine chloride **23** under Helferich conditions furnished β-linked disaccharide **24** in 85% yield.[21] Cleavage of the ester linker with sodium methoxide and reacetylation rendered 51% of disaccharide **25** (based on **22)** (Scheme 5). The

Scheme 5



same group had previously reported the synthesis of a β-(1→6) linked glucosamine dimer.[22] A major drawback of "popcorn" polystyrene was the reduced overall yield due to its partial solubility and thus considerable loss of material during the syntheses.

Scheme 6



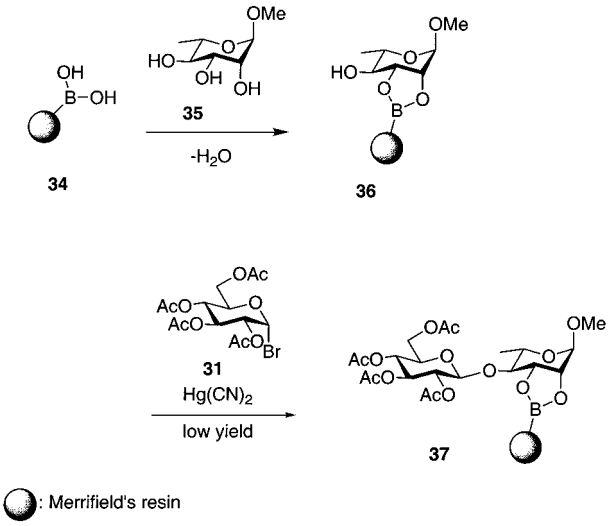
The synthesis of gentiotetraose, a β-(1→6) linked tetramer of glucose, was accomplished by using a benzoyl propionate linker[23] and temporary trichloroacetate protecting groups (Scheme 6).[24] After cleavage from the support, the desired tetramer **33** was obtained in 68% yield contaminated with traces of di- and trisaccharides. This concept was also applied to the synthesis of a β-(1→3) linked glucose dimer.[25]

Glycosylations yielded preferentially the α-linkage (4.4:1 α:β) when a nonparticipating benzyl group was used in place of the 2-*O*-acetyl group.[26] It should be noted that the stereochemical outcome of these solid-phase reactions was essentially identical to findings in solution studies carried out in parallel.

Fréchet described an unconventional mode for attaching the first monosaccharide to the solid support (Scheme 7).[27] A resin-bound cyclic boronic acid ester was selectively introduced to connect *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 resulted in liberation from the polymer. Unfortunately, couplings involving monosaccharide **36** as acceptor proceeded in poor yields.[11b]

An altogether different path for the immobilization of the first monosaccharide on polymeric supports was followed by Guthrie. The polymer support was created by copolymerization of styrene with a sugar monomer equipped with a polymerizable *O*-protecting group.[28] This linear, noncrosslinked, soluble polymer allowed for glycosylation reactions in homogeneous solution but was readily precipitated to facilitate purification. For the first time the glycosyl donor was attached to the support and reacted with an excess of solution-based acceptor. Carbohydrate monomer

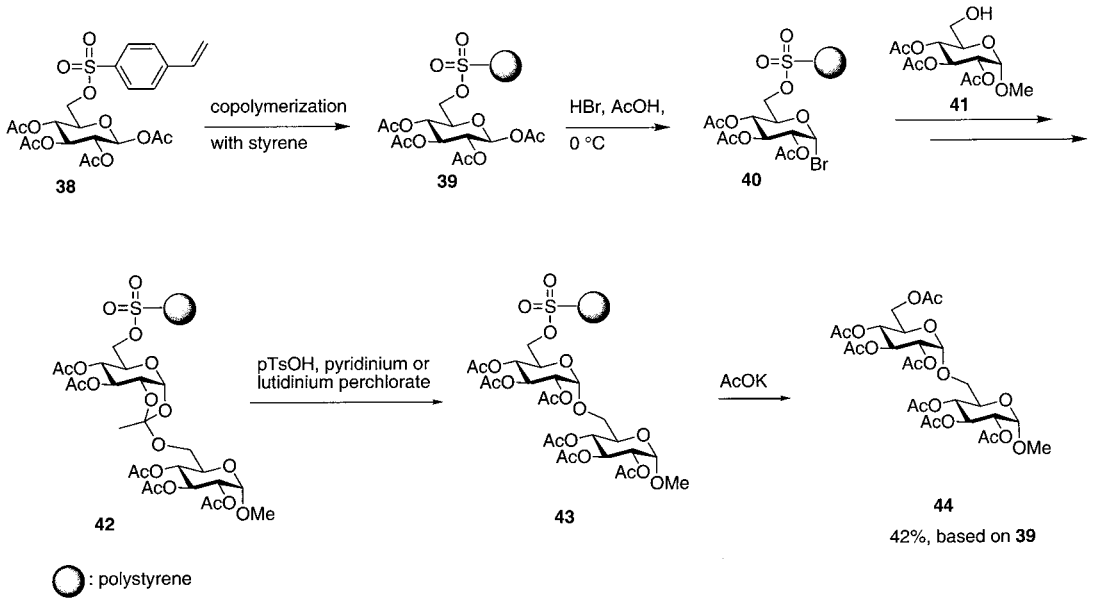
Scheme 7



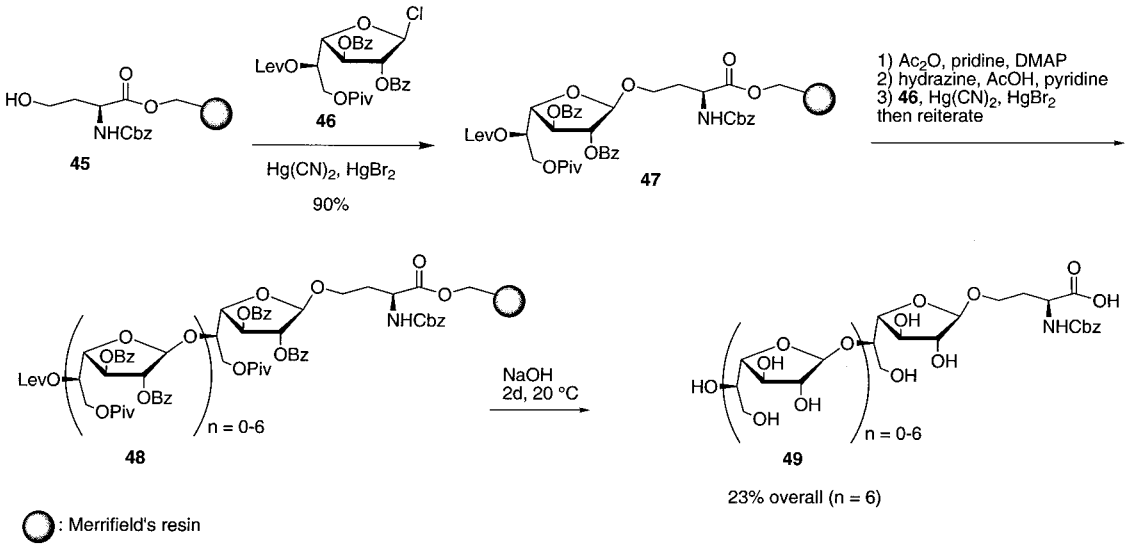
38 was copolymerized with styrene to yield soluble polystyrene **39**, containing approximately 0.1 mol % of monosaccharide. Disaccharide formation was effected via ortho ester[29] **42**, which was obtained from glycosyl bromide **40** (Scheme 8). Refluxing of the resin with potassium acetate yielded gentiobiose octaacetate **44** in 42% yield based on **39**.[30]

These early attempts explored many of the fundamental issues associated with solid-phase oligosaccharide synthesis, including different strategies (donor- vs acceptor-bound synthesis), various solid supports (soluble and insoluble), different linkers, and a variety of glycosylating agents. Most of the recent advances in the field have been based on the concepts developed almost 30 years ago. Ultimately,

Scheme 8



Scheme 9



it was the lack of reliable and efficient glycosylating agents suitable for use on solid support combined with the unavailability of on-resin analytical methods which hampered progress in this field and eventually led to a 20 year hiatus.

Major advances in solution-phase oligosaccharide synthesis including the development of more powerful glycosylating agents of improved selectivity, greater diversity of available protecting groups, and new analytical techniques opened the window of opportunity briefly glanced through by the pioneers.

The only notable advance during the period of dormancy in the 1980s was reported by van Boom et al. Linear β-(1→5)linked galactofuranosyl homopolymers, found to be immunologically active in *Aspergillus* and *Penicillium* species,[31] were chosen as targets for a repetitive oligosaccharide synthesis.[32] Galactofuranosyl chloride **46** was coupled to L-homoserine-functionalized Merrifield's resin **45** under Helferich conditions to furnish resin-bound monosaccharide **47** (Scheme 9). Chain elongation was achieved by selective removal of the C5 levulinoyl protecting group with hydrazine and subsequent β-stereospecific glycosylation with donor **46**. A capping step was introduced after each coupling in order to facilitate the purification of the final products. Acetylation of any unreacted hydroxyl groups ensured minimal contamination with deletion sequences. Deprotection, glycosylation, and capping were reiterated before base hydrolysis released heptamer **49** in 23% overall yield (89% average yield over 13 steps). These synthetic structures were the basis for studies correlating oligosaccharide length and immunogenicity. Completely deprotected oligomers **49** ($n = 0-6$) were used in rabbits as synthetic vaccines to demonstrate an increase of immunogenicity with increased chain length of the oligosaccharide.

While the chemical synthesis of oligosaccharides on insoluble supports with the exception of the above-mentioned example came to a complete halt during the 1980s, enzymatic methods for oligosaccharide synthesis on both insoluble[33] and soluble[34] supports

generated continuous interest following the initial disclosure by Zehavi in 1983. Complications associated with selectivity of glycosidic bond formation, regioselectivity, and the need for protecting groups did not apply with enzymatic methods. In particular, α-sialylic acid linkages[35] that are difficult to create by chemical methods were effectively accessed using enzymatic methods on solid support. These methods have been previously reviewed[36] and will not be covered in this article.

The complete regio- and stereoselectivity of glycosyl transferases makes them valuable catalysts for special linkages in polymer-supported synthesis. The current shortcoming of a rather limited set of available enzymes is expected to be overcome in the future. Still, the need to synthesize a variety of natural and unnatural oligosaccharides persists and chemical solid-phase oligosaccharide synthesis promises to meet these demands.

III. Synthetic Strategies

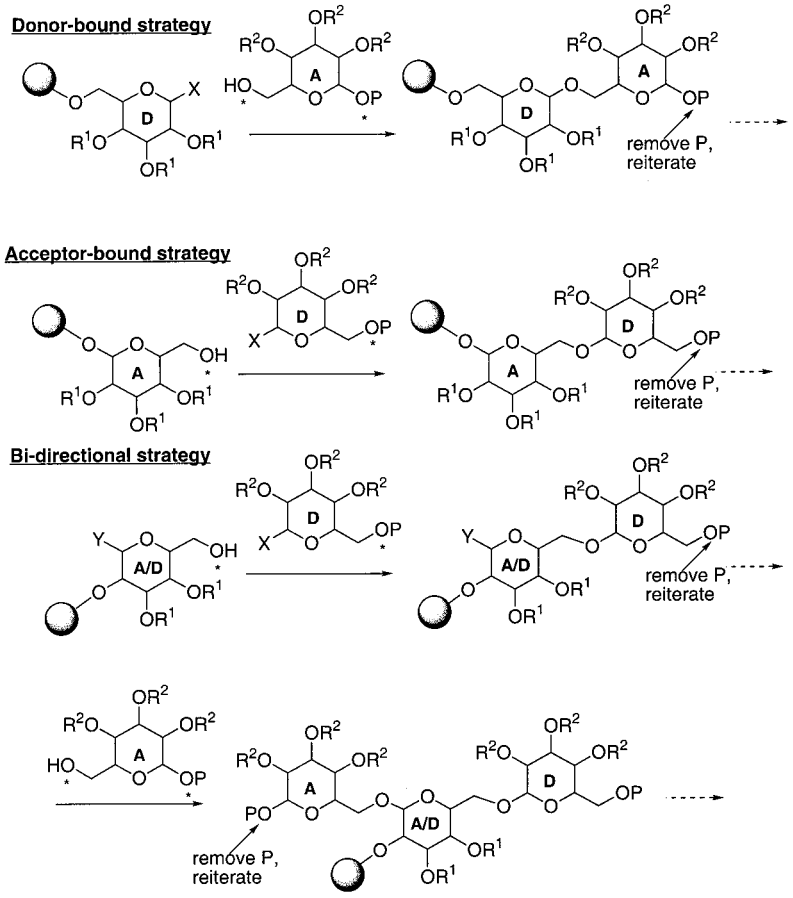
Renewed interest in polymer-supported oligosaccharide synthesis in the 1990s was fueled by significant advances in the solution-phase synthesis of these molecules. Versatile protecting-group strategies[37] and increasingly powerful and selective glycosylating agents[38] provided the basis for access to complex oligosaccharides by polymer-supported synthesis.

The creation of a new glycosidic linkage by the union of a glycosyl donor and a glycosyl acceptor is the central feature of any oligosaccharide synthesis. Thus, two general synthetic strategies present themselves. When the 'nonreducing' end of the first carbohydrate moiety is attached to the polymeric support via an anchoring group, a glycosyl donor is immobilized (donor-bound strategy, Scheme 10). Alternatively, the glycosyl acceptor is immobilized by fixing the anomeric position to the support (acceptor-bound strategy). Both alternatives had been explored early on (vide supra) and are the basis for all current syntheses of oligosaccharides on solid support. Below we will briefly outline these general strategies. Variations of either of these two main strategies lead to bidirectional synthesis plans explored more recently.

A. Donor-Bound Glycosylation Strategy

Danishefsky and co-workers employed the donor-bound strategy for the solid-phase synthesis of oligosaccharides by the glycal assembly method.[39] The first glycal monosaccharide **50** was attached to the polymeric support via a 6-*O*-diphenyl arylsilane linker that may be readily cleaved by treatment with tetrabutylammonium fluoride (TBAF). Treatment with dimethyldioxirane (DMDO)[40] converted the glycal double bond into the corresponding 1,2-anhydrosugar,[41] thus fashioning support-bound glycosyl donor **51**. The desired β-glycoside **53** was selectively prepared by coupling solution-based glycal acceptor **52** via activation with zinc chloride.[42] Repetition of this procedure was used to assemble β-(1→6)-linked

Scheme 10[a]



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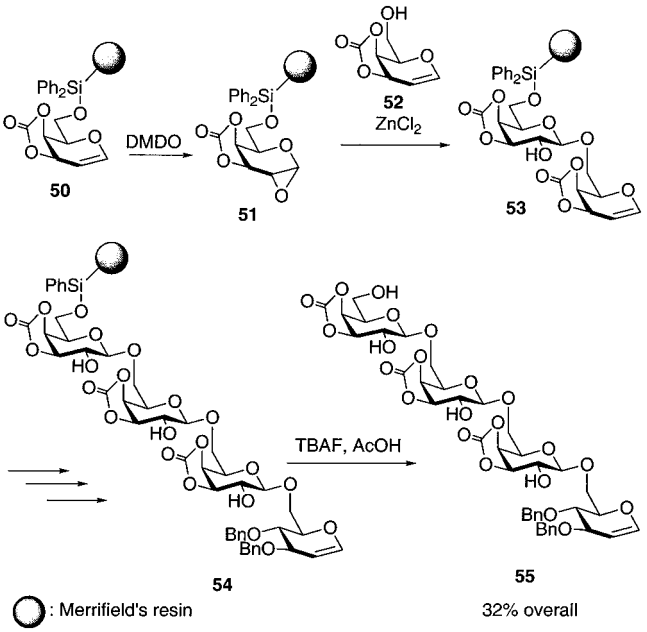
Scheme 11



tetrasaccharide **55** in 32% overall yield (Scheme 11).

A principle drawback of the donor-bound strategy has to be considered. Most side reactions during glycosylations involve the glycosyl donor and thus result in termination of chain elongation. A reduction of the overall yield in the donor-bound strategy is the consequence. These inherent challenges not withstanding, an impressive array of complex oligosaccharide structures have been synthesized by Danishefsky and co-workers using the glycal assembly method under the donor-bound paradigm (vide infra).[43]

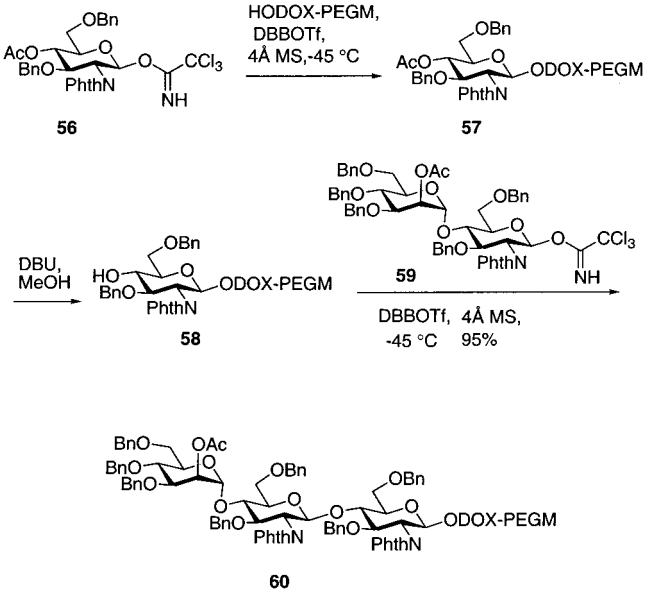
B. Acceptor-Bound Glycosylation Strategy

Immobilization of the acceptor on the solid support allows for an excess of side-reaction-prone glycosyl donor to be added in order to maximize coupling yields. Nonproductive side products are washed away after each coupling. It was this reasoning that has generated immense interest in the acceptor-bound approach to solid-phase oligosaccharide synthesis. We demonstrate this concept on the example of trisaccharide **60** prepared by Krepinsky and co-workers on a soluble polymeric support (Scheme 12).[44]

C. Bidirectional Strategy

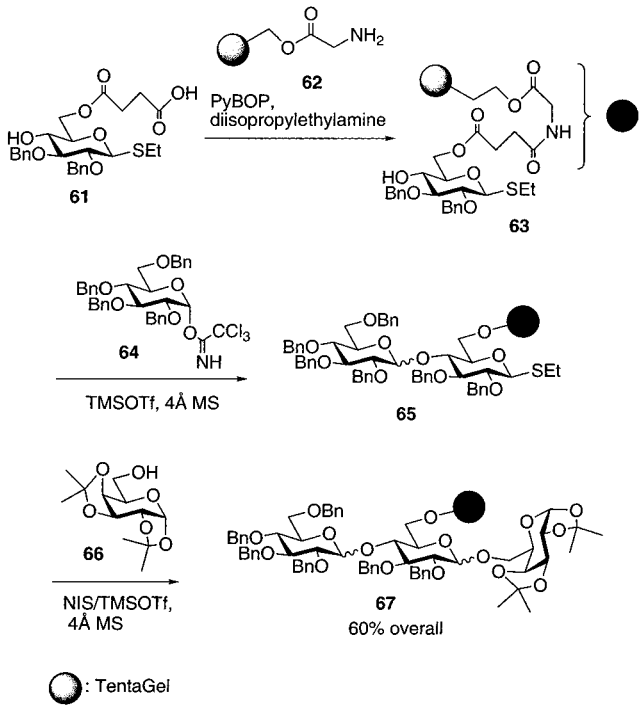
An ideal scenario would allow for the elongation of the growing oligosaccharide in both directions. Such a 'bidirectional' approach that constitutes a hybrid between the donor- and the acceptor-bound strategies has recently been disclosed.[45] The glycosyl donor is attached to the polymer matrix somewhere in the 'nonreducing' region. A suitably differentiated acceptor site and an anomeric donor function for chain elongation in two directions are available. The latent donor moiety, present on the reducing end of the saccharide that serves initially as a glycosyl acceptor, has to be completely inert toward the coupling conditions used for elongation of the acceptor-bound branch. Two sets of orthogonal glycosyl

Scheme 12



donors[46] are required. This approach lends itself particularly to the preparation of branched structures as illustrated by the synthesis of trisaccharide **67** (Scheme 13).

Scheme 13

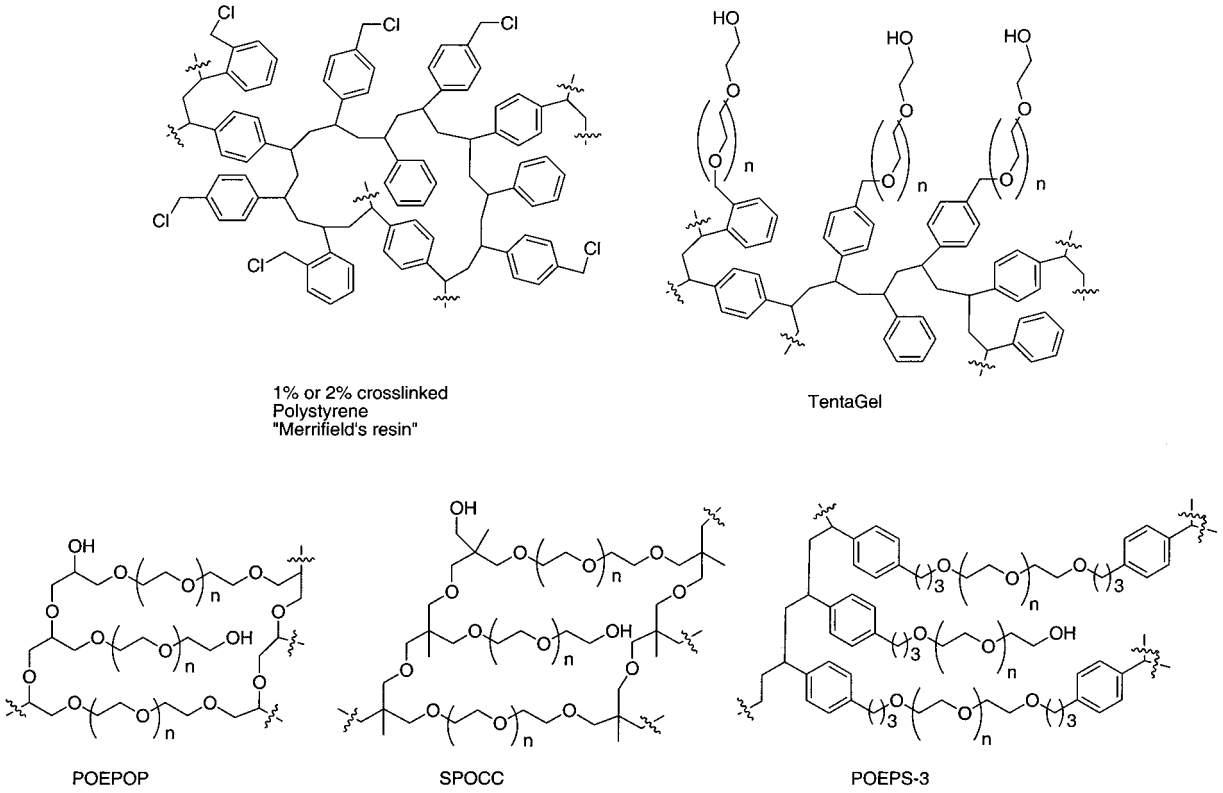


IV. Polymer Supports

A. Insoluble Supports

The choice of support matrix has an immense impact on the overall synthetic strategy, the choice of reagents, and the reaction conditions. Price and availability play an important role in the selection of a particular carrier as well. Most solid-phase oligosaccharide syntheses have relied on Merrifield's resin (polystyrene (PS), cross-linked with 1% divin-

Scheme 14[a]



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ylbenzene) due to its high loading capacity, compatibility with a broad range of reaction conditions, durability, and low price. Access to all reactive sites on Merrifield's resin requires swelling of the polymer in solvents such as dichloromethane, THF, DMF, and dioxane. This relatively narrow range of solvents compatible with cross-linked polystyrene limits the synthetic versatility of this popular resin. Still, most syntheses reported to date have been carried out on Merrifield's resin.

The compatibility of the polystyrene resin with more polar solvents has been improved by grafting poly(ethylene glycol) (PEG) chains onto the polystyrene backbone. Resins such as TentaGel[47] exhibit more desirable swelling properties (even in water) at the expense of lower loading capacities (0.2−0.3 mmol/g) and higher price. In recent years, higher loading PS−PEG polymers (e.g., ArgoGel, 0.4−0.6 mmol/g) have been introduced for solid-phase organic synthesis but have not yet found widespread use in the preparation of carbohydrates.

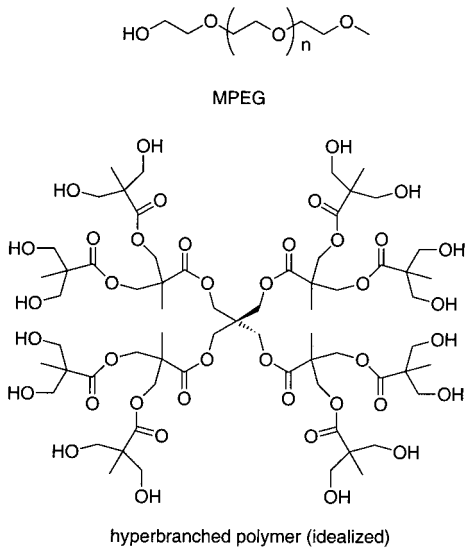
Meldal et al. presented novel types of PEG-based resins that enhance both loading capacities and swelling properties in a wide range of solvents, commonly used in enzymatic and chemical reactions. These resins consist of primary and secondary (POEPOP)[48] or exclusively of primary (SPOCC)[49] ether linkages and were prepared by cationic copolymerization of PEG and an oxirane or oxetane, respectively (Scheme 14). Another new resin, POEPS-3,[50] is a copolymer of 4-vinylbenzyl-substituted PEG chains. This matrix combines features of the TentaGel-type resins and the polyether resins. Compared to conventional PS and TentaGel-type supports, these new resins exhibited improved swelling properties and were amenable to on-bead analytical tools such as HR-MAS NMR spectroscopy (vide infra).[51] Initial studies also showed the compatibility of the SPOCC and POEPOP resins with the glycosylation of resin-bound peptides employing glycosyltrichloroacetimidate donors.[48,49] The influence of the resin on the rate of reaction has recently been studied, but no superior resin has emerged.[52] Also, the degree of swelling has a crucial impact on the chemical microenvironment of the resin and thus for its reactivity.[53] Further studies involving these designer supports will be required to demonstrate their utility in a wide range of coupling reactions.

Nonswelling controlled pore glass (CPG) supports, commonly used for automated DNA synthesis, have been evaluated for their performance in oligosaccharide synthesis with trichloroacetimidate donors. In contrast to polystyrene, only the surface of CPG is functionalized, thus resulting in lower loading but also possibly easier access of reagents. Since glass does not require swelling, a wide range of solvents may be applied. Mechanical instability due to fracture complicates the handling of CPG beads. The major limitation in a carbohydrate context is certainly the incompatibility with silyl ether protecting groups commonly employed for temporary hydroxyl protection. Depending on the type of CPG, loadings from 30 to 35 μmol/g for amino-functionalized CPG[54] to 0.3 mmol/g for mercaptopropyl-functionalized CPG[55] have been reported.

B. Soluble Supports

Insoluble supports often require extensive reaction development to render procedures developed for solution-phase synthesis amenable to the solid-phase paradigm. Soluble polymer supports combine advantages of the solution-phase regime with the easy workup of solid-phase synthesis. While all chemical transformations are carried out in homogeneous solution, the polymer is precipitated out after each step to ensure the removal of any excess reagents by simple filtration. A potential drawback is the loss of material during the precipitation step after each coupling, which lowers the overall yield in the assembly of large structures. Further restrictions are imposed by the limited temperature range under which the soluble polymer can be efficiently used (only above −45 °C due to potential precipitation of the support). Nevertheless, poly(ethylene glycol)-based soluble polymers (MPEG) are commonly used supports for oligosaccharide synthesis (Scheme 15).[56]

Scheme 15



Most recently, a polydisperse soluble hyperbranched polyester[57] was described for the synthesis of disaccharides (vide infra).[58] This support was prepared from pentaerythritol and 2,2-bis(hydroxymethyl)-propionic acid in one step, exhibited high solubility in most aprotic solvents, but was precipitated quantitatively by methanol or separated by size-exclusion chromatography. An interesting feature of this support is its rapid degradation by treatment with aqueous base. The final products of a synthesis can be procured by hydrolysis of the support and extraction of the products into an organic solvent.

V. Linker Systems

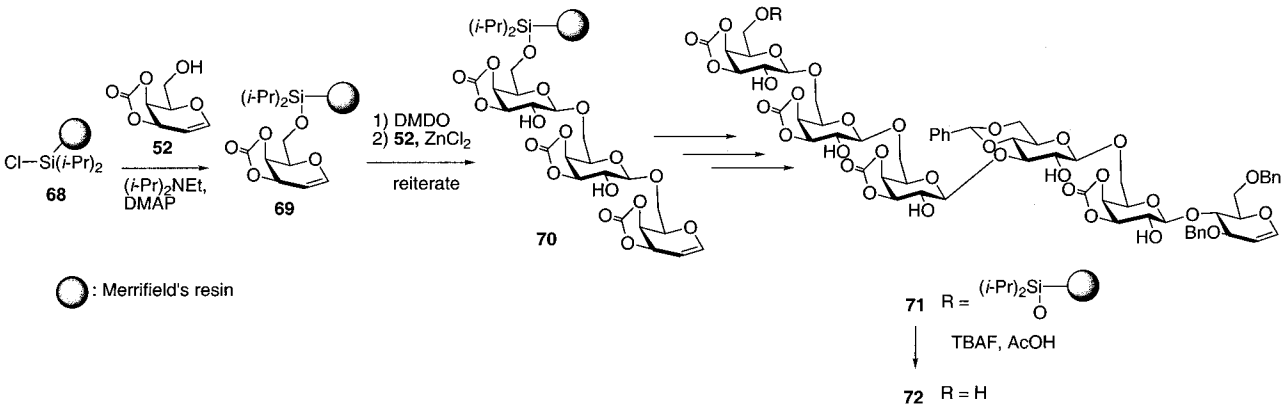
The linker[59] chosen to attach the first monosaccharide to the solid support is of crucial importance. The chemical nature of this anchor determines all other protecting-group and coupling manipulations that may be carried out during the entire synthesis. The linker may be viewed as a protecting group that is attached to the polymeric carrier. Therefore, any protecting group used in carbohydrate synthesis may in principle serve as a linker. Keeping orthogonality with commonly used temporary protecting groups in mind, the selection of the linker is an important strategic decision.

A. Silyl Ether Linkers

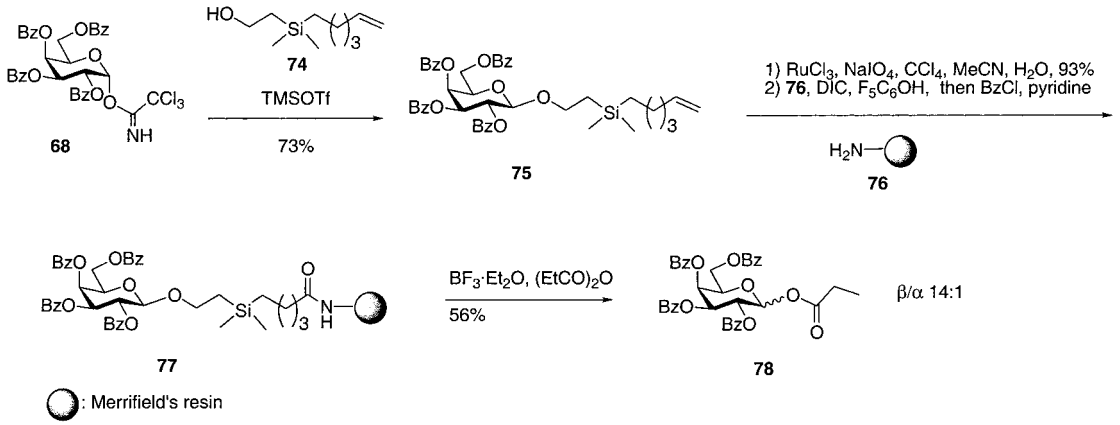
Silyl ethers are commonly used as temporary protecting groups for hydroxyl groups in oligosaccharide synthesis. Although a silane-based linker precludes the use of further silyl ethers as temporary means of protection, its selective and high-yielding cleavage made this class of reagents attractive anchors. Since temporary protection maneuvers were minimized and the need for temporary silyl ether groups was not as pressing during the synthesis, a silane anchor was successfully used in Danishefsky's donor-bound strategy employing glycal-derived donors (Scheme 16). The initially applied diphenyl aryl silane proved too labile during subsequent reactions and was replaced by the more robust diisopropyl arylsilane in more recent syntheses.

The compatibility of the silane anchoring concept with a range of different glycosylation agents such as thioglycosides, anomeric fluorides, trichloroacetimidates, and sulfoxides using various acidic promoters was recently demonstrated (vide infra).[60] Magnussen described an acid-labile silane anchor group for the anomeric position which permits the introduction of different aglycons during the cleavage step. While promising, it should be noted that this

Scheme 16



Scheme 17



approach has not been yet used in the synthesis of an oligosaccharide (Scheme 17).[61]

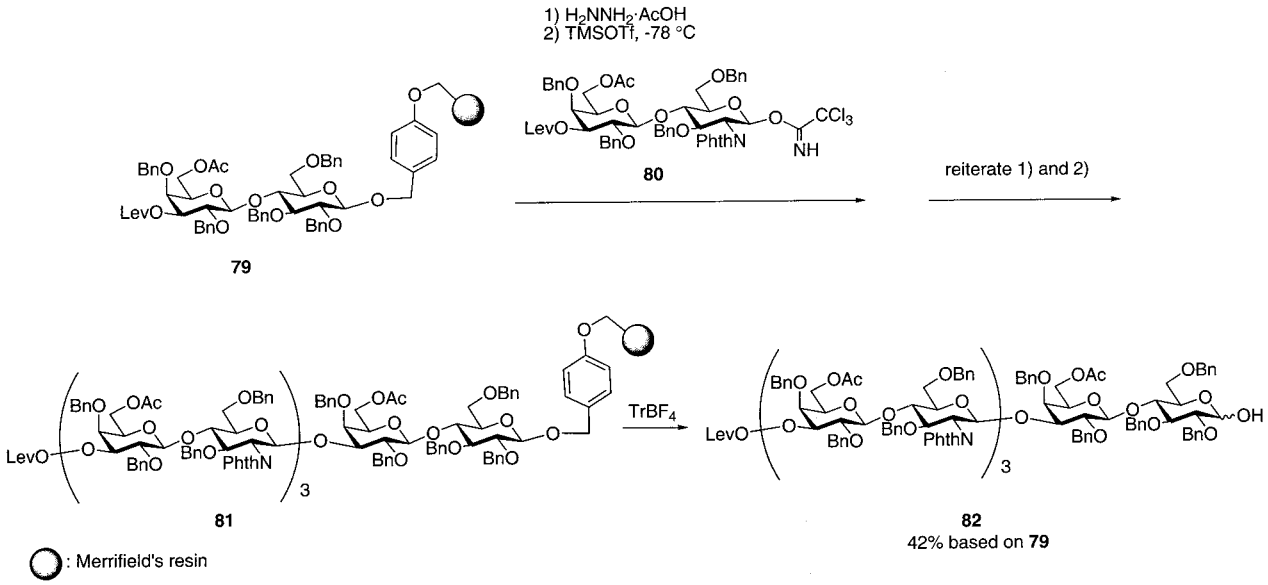
B. Acid- and Base-Labile Linkers

Acid-labile linkers are commonly employed for solid-phase peptide synthesis. Several of the linkers proven in peptide chemistry have been applied to oligosaccharide assembly on solid support. Amino-functionalized Rink resin was used by Silva et al. in the preparation of a disaccharide library (vide infra).[62] Hanessian described benzylidene acetal-type linkages of carbohydrates to Wang aldehyde resin as temporary protecting groups for the preparation of differentially protected monosaccharides. These linkages were readily cleaved with trifluoroacetic acid (TFA).[63] The acid-labile Wang resin linker was reported by Ogawa for the synthesis of a polylactosamine (Scheme 18).[64] This linker withstood the mildly acidic conditions employed for the activation of glycosyl trichloroacetimidates by catalytic amounts of TMSOTf. The oligosaccharide product **82** was cleaved from the resin by treatment with triphenylmethylborontetrafluoride ($TrBF_4$).

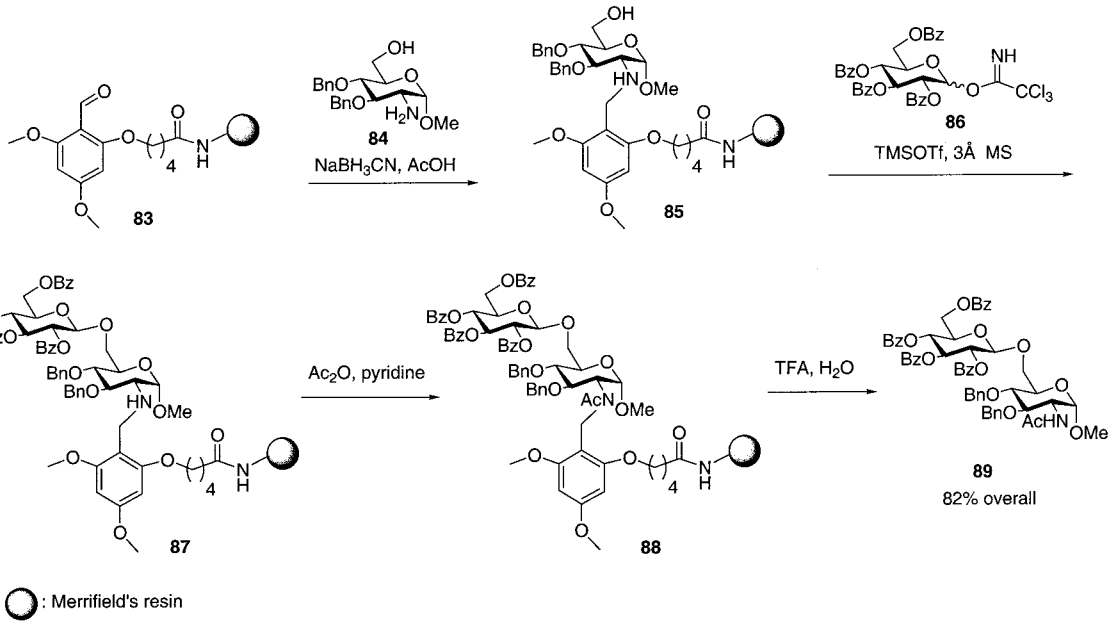
Wang resin has also been functionalized with a bis(dihydropyrane) linker, carrying a dihydropyrane moiety on either side to connect the carbohydrate to the resin. While cleavage from the resin using pyridinium *p*-toluenesulfonate (PPTS) at elevated temperature was achieved in quantitative yield, the initial loading of the resin with a carbohydrate moiety did not exceed 70%.[65] Very recently, the tris(alkoxy)benzylamine (BAL) safety-catch linker, originally developed for peptide synthesis,[66] was used to anchor an amino sugar to a support.[67] Acylation followed by treatment with TFA effected cleavage of the linker as demonstrated by the synthesis of a disaccharide (Scheme 19). Resin-bound glucosamine **85** served as acceptor in the reaction with perbenzoylated glucosyl trichloroacetimidate **86** to fashion **89** in 82% overall yield.

A base-labile succinoyl linker, commonly used in automated DNA synthesis, was early on employed in oligosaccharide syntheses on soluble supports.[68] It has also been used to improve the synthesis of polylactosamine oligosaccharides,[64] and it has been applied to the preparation of disaccharides on amino-functionalized TentaGel and PS supports (Scheme 20). Treatment with aqueous ammonia effected release of **94** from the resin.[54] A 9-hydroxymethylfluorene-2-succinic acid-based linker that was cleaved by

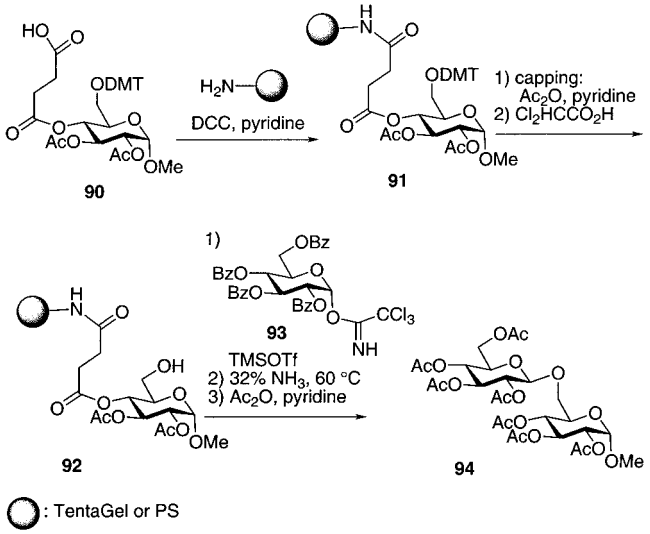
Scheme 18



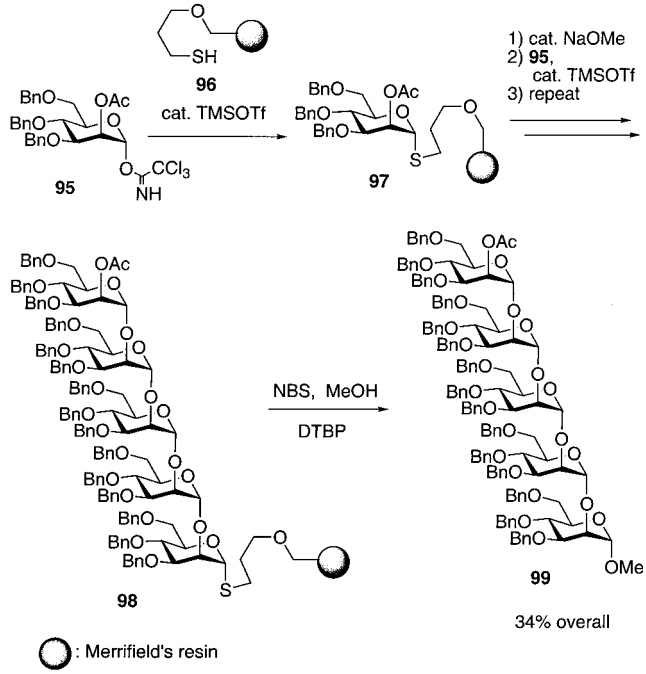
Scheme 19



Scheme 20



Scheme 21



treatment with 20% triethylamine was applied to the preparation of disaccharides on soluble support.[69] Meldal et al. showed that peptides bound to POEPOP resin via a hydroxymethyl benzoyl (HMBA) linker could be efficiently glycosylated. Cleavage by sodium methoxide was also demonstrated.[70]

C. Thioglycoside Linkers

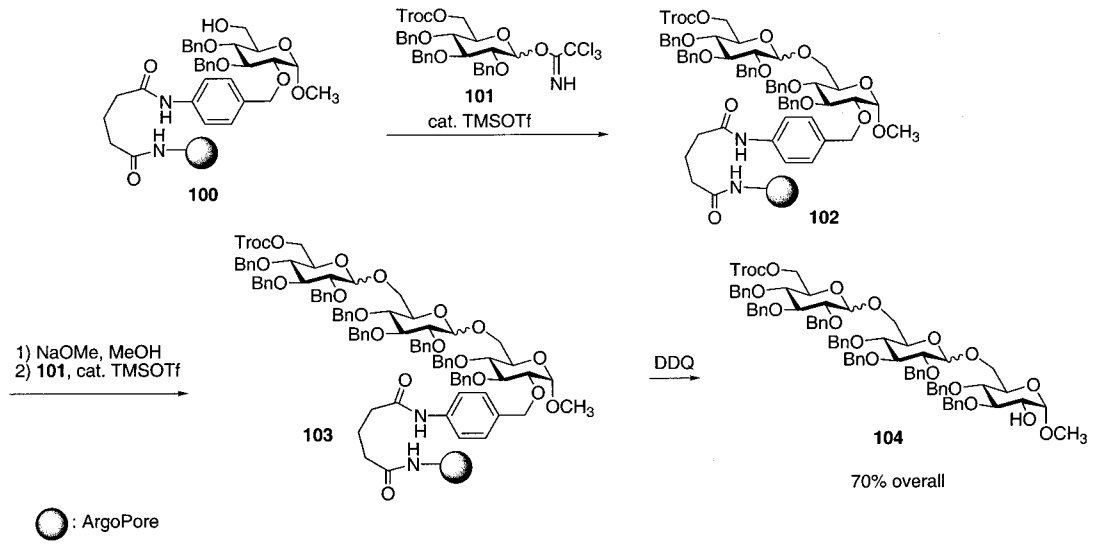
Thioglycosides are an attractive mode of anomeric attachment since they are stable to a wide range of activation conditions but may be readily activated by thiophiles. This concept was originally explored by Anderson.[18] More recently, several groups revitalized these anchors successfully. Schmidt chose this approach to prepare α-mannosidic oligomers on a Merrifield resin with 0.15−0.3 mmol/g loading.[71] Activation of the thioglycoside by NBS in the presence of di-*tert*-butyl pyridine (DTBP) and methanol resulted in cleavage of the oligosaccharide product **99** as a methyl glycoside (Scheme 21).

A *p*-hydroxythiophenyl glycoside was applied to Kahne's synthesis of oligosaccharides using glycosyl sulfoxides. This linkage was hydrolyzed at the end of the synthesis by treatment with trifluoroacetate[72] and was successfully employed in the preparation of a diverse library of oligosaccharides (vide infra).[73]

D. Linkers Cleaved by Oxidation

Oxidation can be a smooth and selective means for protecting-group removal. An oxidatively removable linker related to the *p*-methoxybenzyl group was designed by Fukase (Scheme 22).[74] To overcome the inherent acid lability associated with the PMB group, an acyl moiety was introduced to provide an acid-stable protecting group.[75] In its resin-bound form, this protecting group can be cleaved under oxidative

Scheme 22



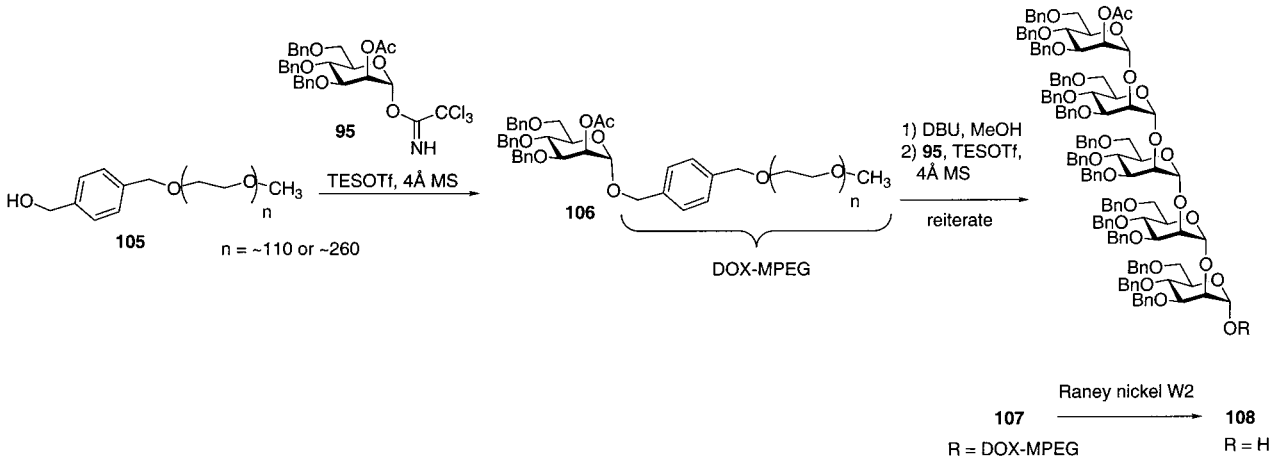
conditions by the action of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). To facilitate purification and analysis of the product, this linker was attached to hydroxyl groups other than the anomeric position. Connection of the first carbohydrate residue to ArgoPore resin was followed by acylation to cap all nonreacted sites. Using this functionalized resin, the highest yields were obtained when 6-*O*-Troc-protected glucosyl trichloroacetimidate **101** was coupled with TMSOTf as promoter. The desired trisaccharide **104** was prepared in 70% overall yield although selectivity was low ($\alpha:\beta = 2:1$). Better selectivity ($\alpha:\beta = 9:1$) was achieved when a 6-*O*-*tert*-butyldiphenylsilyl (TBDPS)-protected phenylthioglucoside in combination with a hypervalent iodine activator was used.

E. Linkers Cleaved by Hydrogenation

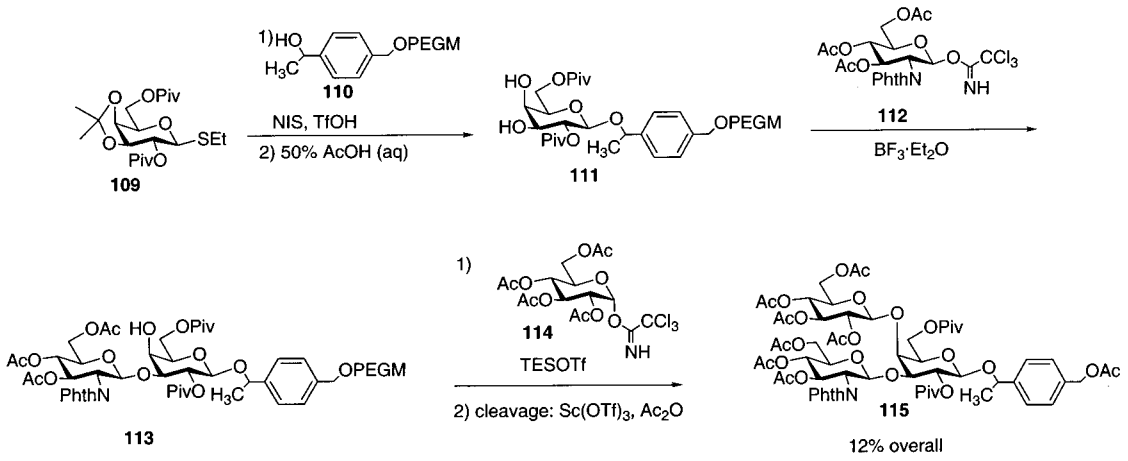
The linker system most often applied with soluble PEG supports is an α,α′-dioxyxylyl diether (DOX), which was introduced by Krepinsky for the synthesis of α-(1→2) pentamannoside **108** (Scheme 23).[76] This anchoring group is stable to treatment with Lewis acids, but the free reducing sugar can be obtained by hydrogenolysis. Alternatively, treatment with scandium(III) triflate yields the protected oligosaccharide as an acetoxyxylyl glycoside. In these cases cleavage at the PEG−DOX linkage is presumably accomplished by complexation of Sc^{3+} with the PEG chain.[77]

Recently, the synthesis of the repeating unit of group B *Streptococcus* type 1 capsular polysaccharide on a soluble polymer was studied in detail.[78] In particular, the problem of side reactions due to acyl transfer[79] from the acceptor to the donor was examined which appeared to be a serious problem under various glycosylation conditions.[80] Use of a sterically demanding C2 pivaloyl ester and modification of the initially applied DOX linker on MPEG polymer increased the yield of the desired product and allowed for the synthesis of trisaccharide **115** (Scheme 24). Galactose thiodonor **109** was coupled to the MDOX−MPEG polymer **110**. After cleavage of the isopropylidene protecting groups, glucosamine building block **112** was coupled to yield a mixture of regioisomers (2.2:1 in favor of the less hindered C3 position). This mixture was glycosylated to furnish trisaccharide **115** in 12% yield after cleavage from the support. In their recent comparison of lacto-*N*-tetraose syntheses, Whitfield et al. highlight the superiority of the

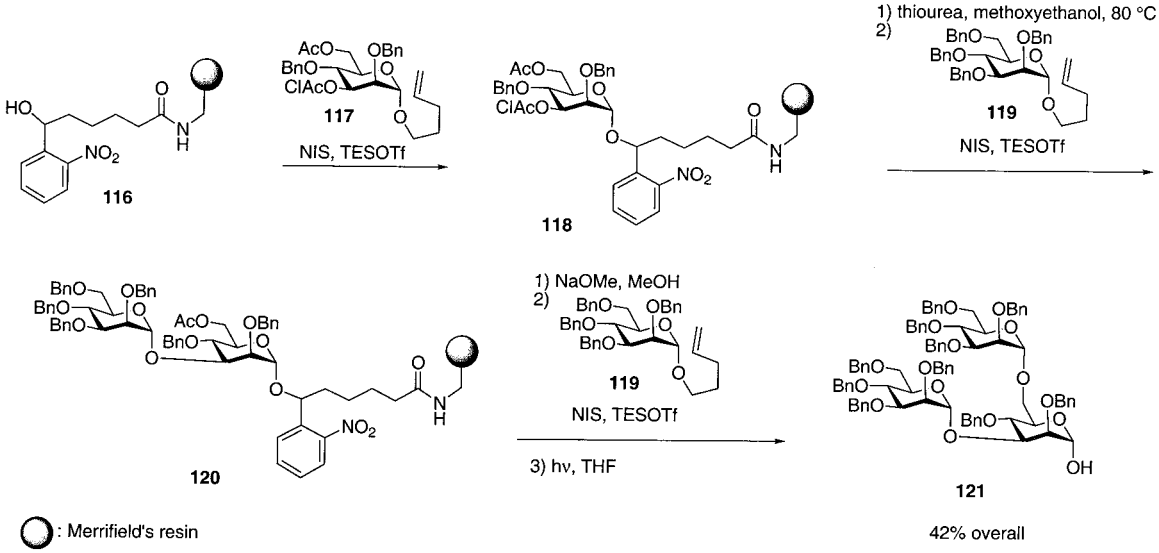
Scheme 23



Scheme 24



Scheme 25



polymer-bound approach using DOX-functionalized MPEG over classical solution synthesis in terms of yield and ease of purification.[81]

Chan et al. described a linker system for the use with low molecular weight MPEG supports (8−20 ethylene glycol units).[82] A *p*-carboxamide benzyl glycoside closely related to the DOX linker was applied and cleaved by hydrogenolysis to afford the free reducing sugar.

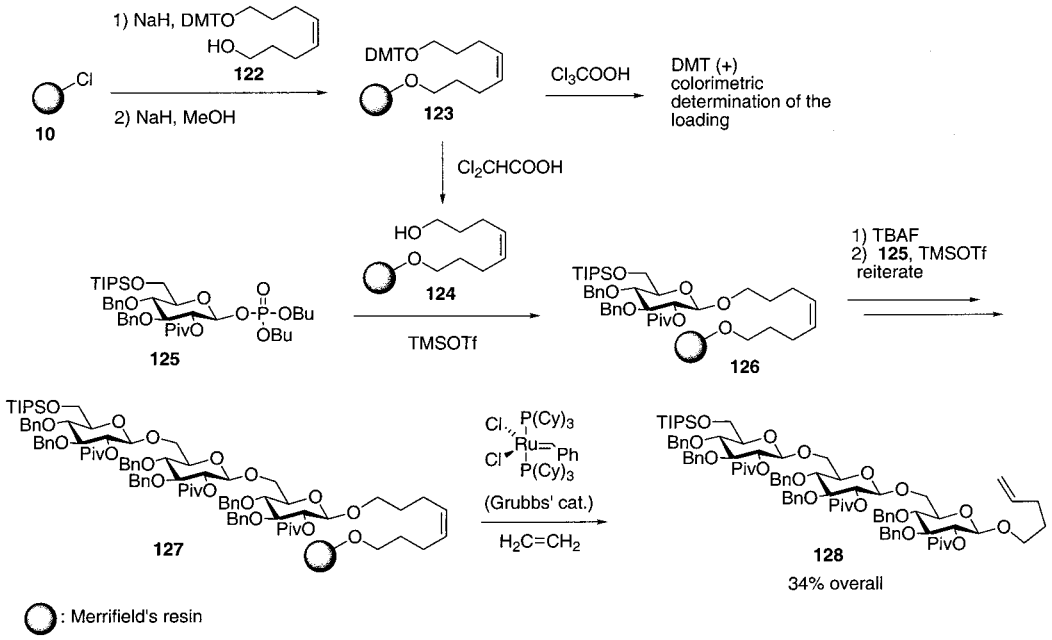
F. Photocleavable Linkers

Almost all linker systems for polymer-supported oligosaccharide synthesis interfere with some standard conditions for carbohydrate protecting-group manipulations, thus reducing the diversity and complexity of the oligosaccharides that may be prepared. In response to this shortcoming several groups revitalized the idea of employing a photocleavable linker[15] for chemical oligosaccharide synthesis. Photolabile *o*-nitrobenzylic linkers were used by Nicolaou and co-workers for the construction of branched carbohydrate structures (vide infra)[83,84] and by Meldal et al. in the direct glycosylation of a POEPOP-bound pentapeptide.[70] Since photolytic cleavage of primary *o*-nitrobenzyl linkers is usually very slow and incomplete, Fraser-Reid designed a new system based on a secondary *o*-nitrobenzyl ether linkage.[85] This linker was used in the synthesis of a branched trimannan oligosaccharide (Scheme 25). Differentially protected mannosyl *n*-pentenyl glycoside (NPG) **117** was coupled to the resin via linker **116**. Selective removal of the C6 chloroacetyl group and subsequent mannosylation of the C6 and C3 positions afforded trimer **121** as the free reducing sugar in 42% overall yield after photolytic cleavage from the resin.

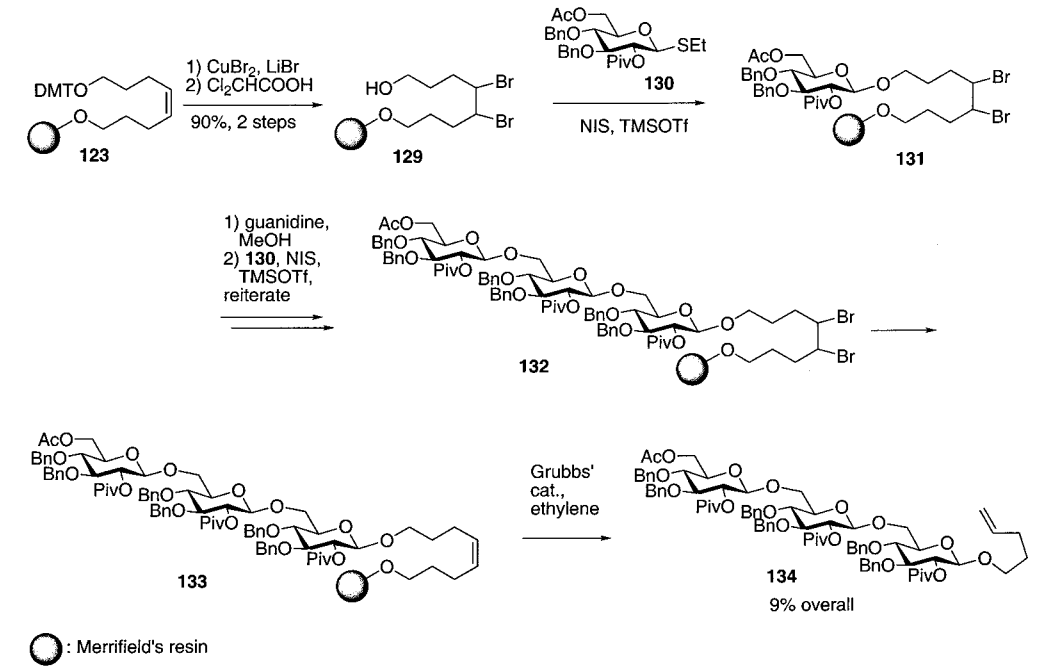
G. Linkers Cleaved by Olefin Metathesis

Ideally, the linker moiety is not only completely stable during the synthesis and effectively cleaved as the final step, but in addition can be removed in a fashion that renders the resulting oligosaccharide suitable for conjugation or further glycosylations in fragment couplings. These demands were met by a new linker concept recently developed in our laboratory (Scheme 26).[86] The first carbohydrate moiety was connected via a glycosidic bond to octenediol-functionalized Merrifield's resin **124**. The loading was readily determined by colorimetric methods after cleavage of the DMT protecting group of **123**. Resins with a loadings of up to 0.65 mmol/g were obtained

Scheme 26



Scheme 27

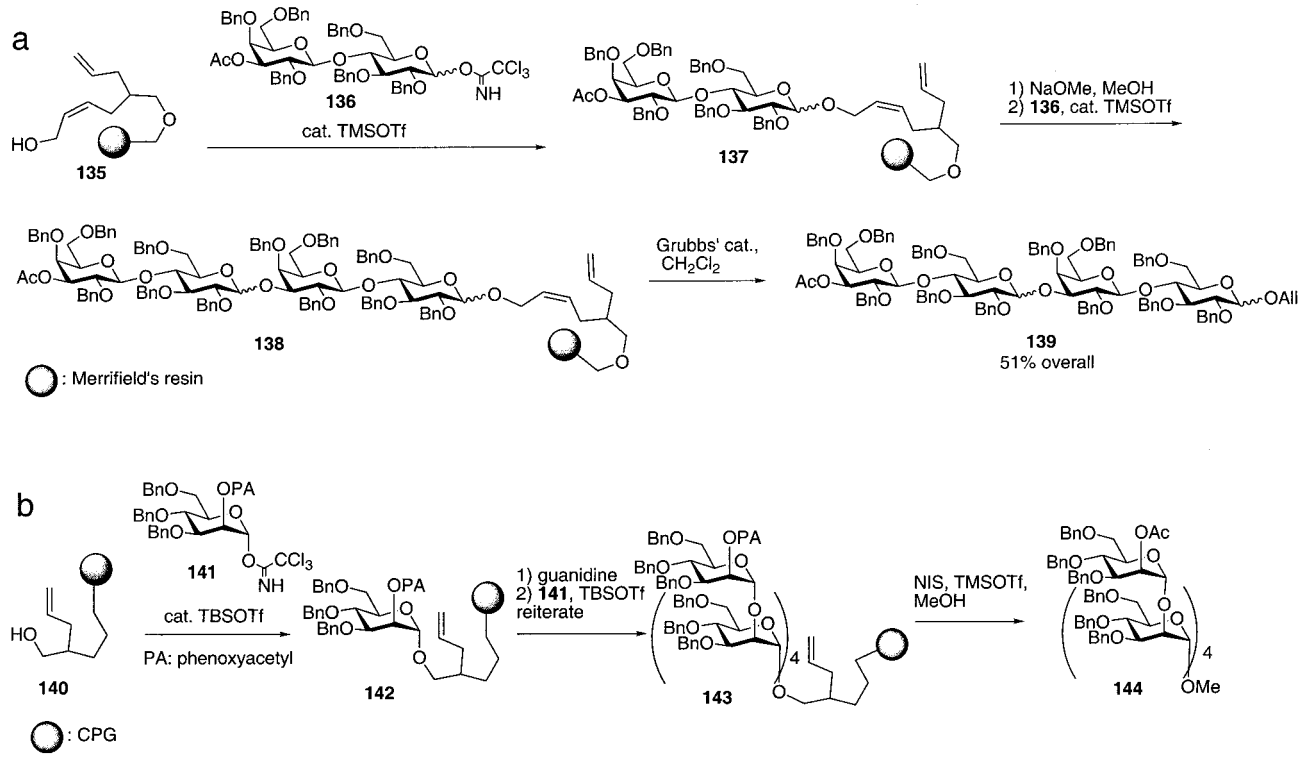


and employed in oligosaccharide synthesis. The octenediol linker was quantitatively cleaved by olefin cross metathesis using Grubbs' catalyst under an atmosphere of ethylene to afford fully protected oligosaccharides in the form of *n*-pentenyl glycosides. These fragments can serve as glycosyl donors using the conditions developed by Fraser-Reid.[87] The versatility of this linker was emphasized by ozonolytic cleavage to yield a terminal aldehyde ready for neoglycoconjugate formation via reductive amination.[88] Furthermore, *n*-pentenyl glycosides can serve as precursors for a vast array of anomeric functionalities and linking moieties.[89] The octenediol linker was applied to the preparation of β−(1→6) linked triglucoside **128** in 34% overall yield, using dibutyl phosphate glycosyl donors.[90,91]

To render the octenediol linker compatible with glycosylating agents that require electrophiles as activators, the double bond was converted into the corresponding dibromide.[92] This dibromide proved compatible with thiodonors requiring electrophilic promoters such as NIS. After formation of trisaccharide **132**, the double bond was reinstalled by elimination. Cleavage from the resin with Grubbs' catalyst and ethylene afforded trisaccharide **134** in 9% overall yield (Scheme 27).[93]

Ring-closing metathesis (RCM) with Grubbs' catalyst was applied to cleave δ,ϵ-double bond containing linker **135** introduced by Schmidt et al. (Scheme 28a).[94] A similar linker bearing a *n*-pentenyl functionality was used for the preparation of α-pentamannoside **144** using trichloroacetimidate donors on

Scheme 28



CPG. Cleavage from the support was accomplished by NIS/TMSOTf, although no yield was reported (Scheme 28b).[95]

VI. Protecting Groups

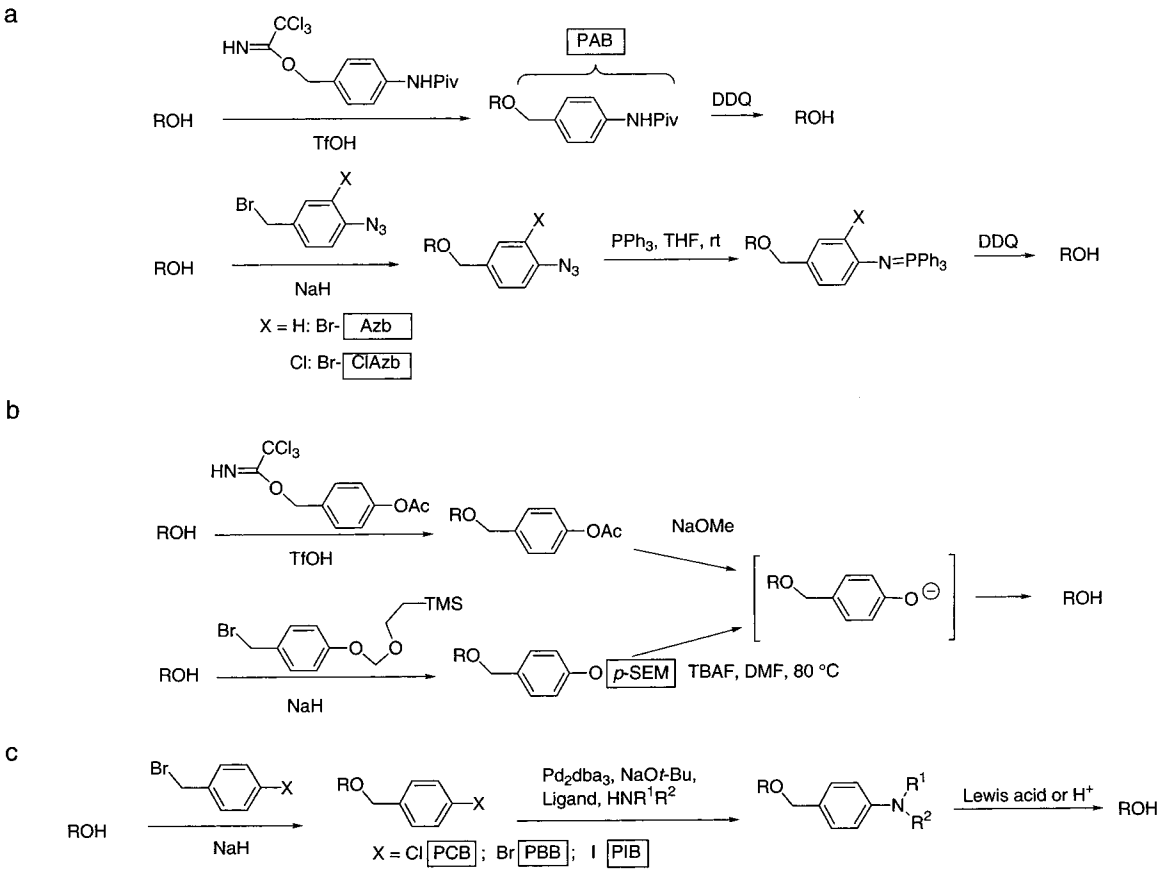
The multitude of hydroxyl groups present in carbohydrates requires efficient orthogonal protecting groups that allow for the selective manipulation of a particular functional group of interest. Permanent protection must be applied to hydroxyl groups not to be operated on during a given oligosaccharide synthesis. Temporary protection has to be used to mask hydroxyl groups that constitute connection sites at a later stage of the synthesis. For branched oligosaccharides and particularly for combinatorial carbohydrate-based libraries, careful synthetic planning with respect to the protecting-group ensemble is required.

A. Benzyl Ethers

With few exceptions, most polymer-supported oligosaccharide syntheses have relied on benzyl ethers as permanent protecting groups that remained inert during the assembly and were removed by catalytic hydrogenation after cleavage from the support. MPEG−DOX and similar soluble supports allow for the benzyl groups to be removed together with the DOX linkage. The heterogeneous nature of the Pd-catalyzed hydrogenolysis prevents benzyl groups from being used as temporary protecting groups on an insoluble solid support. A protecting-group system that combines the exceptional stability of benzyl groups with the possibility of selective cleavage holds great potential. A few laboratories have recently described approaches to meet these demands. Fukase described a concept that relies on masked *p*-aminobenzyl groups (Scheme 29a).[75] The *p*-pivaloylaminobenzyl (PAB) was found to be acid stable but was readily removed by oxidation with DDQ. More selective deprotections under mild conditions were effected with the *p*-azidobenzyl (Azb) and the *p*-azido-*m*-chlorobenzyl (ClAzb) protecting group after reduction of the azide to an iminophosphorane. The latter was shown to be stable to oxidative or acidic conditions but was selectively cleaved after conversion to the iminophosphorane and proved useful in solid-phase oligosaccharide synthesis (vide infra). A related concept employing acetate or silyl-protected *p*-hydroxybenzyl ethers was later introduced by Hindsgaul (Scheme 29b).[96] Although the removal of these benzyl ethers did not require catalytic hydrogenation, the treatment with base or fluoride followed by oxidative cleavage rendered them incompatible with ester, silyl, or PMB protecting groups.

Very recently, halogen-substituted benzyl ethers have been introduced as protecting groups that are as stable as benzyl ethers but were readily converted into acid-labile protecting groups. A Pd-catalyzed amination reaction[97] was used to create an arylamine that was readily cleaved by protic or Lewis acids (Scheme 29c).[98] By exploiting the different reactivities of *p*-iodobenzyl (PIB), *p*-bromobenzyl (PBB), and *p*-chlorobenzyl (PCB) groups this concept adds additional degrees of orthogonality. All of these novel benzyl protecting groups have been demonstrated in solution-phase oligosaccharide synthesis and are expected to prove useful in the synthesis of complex and branched carbohydrate structures on solid support.

Scheme 29



B. Base-Labile Protecting Groups

Acetyl, benzoyl, and pivaloyl esters have been extensively used in polymer-supported oligosaccharide synthesis as participating C2 protecting groups on glycosyl donors to ensure β-selective glycosylation reactions.

Most prominent among the commonly applied esters is the acetyl group. The standard method for cleavage of the acetyl group on solid support is treatment with excess sodium methoxide,[99] yielding quantitatively the free hydroxyl group in short reaction times. When used on soluble polymeric supports, DBU[44] or Hünig's base[82] have also been reported. Alternative cleavage procedures on solid support include guanidine[100] and hydrochloric acid.[93]

The more labile chloroacetyl esters have been selectively removed in the presence of acetyl groups by action of thiourea in methoxyethanol.[85] In some cases the less labile pivaloyl ester has been shown to give superior results to acetates or *p*-chlorobenzoates since fewer side reactions such as acyl transfer and ortho ester formation occur.[78] Repeated treatment with excess guanidine removed the phenoxyacetyl group in a CPG-based oligosaccharide synthesis.[55] Levulinoyl esters have been cleaved off with hydrazinium acetate in excellent yields.[64] C6 dinitrobenzoyl esters, employed to enhance the α-selectivity in glycosylation reactions, have been removed with sodium methoxide. The same reaction conditions were successfully used for the cleavage of a C6 trichloroethoxycarbonyl (Troc) group.[74] The 9-fluorene methyloxycarbonyl (Fmoc) group was quantitatively removed by treatment with 20% triethylamine.[83]

Although many new participating protecting groups for amino sugars have been introduced for solution-phase oligosaccharide synthesis,[101] virtually all glucosamine donors employed in polymer-supported oligosaccharide synthesis rely on the phthaloyl protecting group. The phthaloyl group served well in most cases but may cause severe problems arising from partial decomposition when exposed to strongly basic conditions. Other *N*-protecting groups such as a carbamate (Troc),[102] the *N*-trifluoroacetyl group (cleaved by LiOH in MeOH-THF),[103] or a dithiosuccinoyl (Dts) or azido group were used very rarely.[70] In the solid-phase glycal assembly method, aminosugar donors were installed by iodosulfonamidation of glycals,[104] thus incorporating *N*-arylsulfonamide-protected aminosugars.

C. Acid-Labile Protecting Groups

Acid-labile protecting groups have been used less frequently than base-labile protecting groups since many glycosylation reactions involve acidic conditions that may result in loss of temporary protecting groups during coupling. The dimethoxytrityl (DMT) group was readily released by action of 2% dichloroacetic acid in CH_2Cl_2,[105] while stronger acidic conditions (4% TFA in CH_2Cl_2) were used to cleave off trityl groups.[71] Trityl trifluoroacetate released in this reaction was used to determine the deprotection yield.[106] Quantitation of DMT release by a colorimet-

ric assay was applied to determine resin loading with an octenediol linker.[86]

4,6-*O*-Benzylidene acetals have been removed from soluble support-bound carbohydrates by 60% acetic acid.[76] Ethoxyethyl ether (EE) was released from a resin-bound sugar upon treatment with catalytic amounts of *p*-toluenesulfonic acid,[107] while a temporary tetrahydropyranyl (THP) protecting group was cleaved by glacial acetic acid in THF.[45]

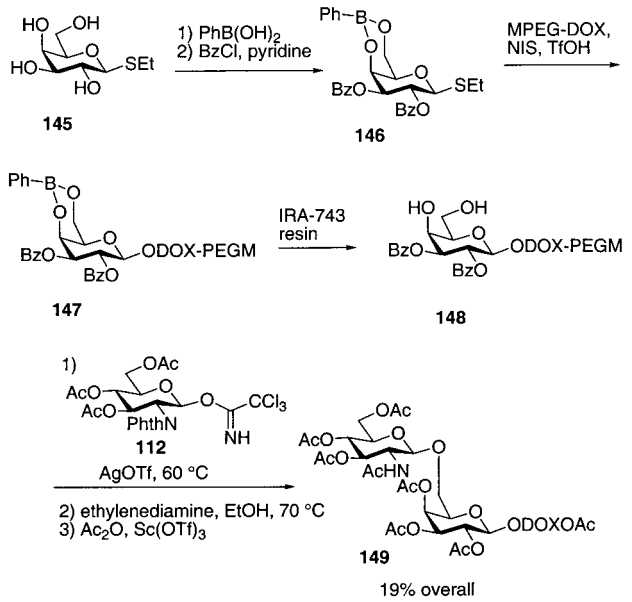
D. Silyl Ether Protecting Groups

The efficiency of silyl ether removal under conditions that do not affect other protecting groups has made these groups attractive means of temporary protection. The *tert*-butyl dimethylsilyl (TBDMS) ether group has been applied as temporary protecting group in various polymer-supported oligosaccharide syntheses. Removal of TBDMS from the C6 position with HF/pyridine complex[58] or with HCl in methanol[108] on soluble polymers has been reported. Quantitative cleavage of TBDMS from a secondary position of a support-bound carbohydrate upon treatment with tetrabutylammonium fluoride (TBAF) was also disclosed.[86] These conditions were also successfully used to remove a primary triisopropylsilyl (TIPS) ether protecting group.[86] The *tert*-butyl diphenylsilyl (TBDPS)[82] and triethylsilyl ether (TES)[100] groups were quantitatively removed by HF/pyridine.

E. Other Protecting Groups

The prevalence of highly branched oligosaccharide structures occurring in nature emphasizes the need for a host of orthogonal protecting groups for solid-phase oligosaccharide assembly. Thus, other groups with unique reactivities have been applied to hydroxyl group protection. Kunz et al. demonstrated the compatibility of a temporary allyl ether protecting group with the solid support paradigm. A secondary allyl-protected hydroxyl group was liberated by catalytic action of Ir{(COD)[PCH_3Ph_{22}]}PF_6.[109]

Scheme 30



A boronate diester similar to the boronate linker group proposed by Fréchet[11b] was applied as 4,6-*O*-protecting group for MPEG−DOX-supported carbohydrates and was readily removed by hydrolysis with IRA-743 Resin (Scheme 30).[110]

Recently, the 4-azido-3-chlorobenzyl (ClAzb) group has been explored for temporary protection of hydroxyl groups on solid support. The ClAzb group is stable during oligosaccharide synthesis but can be turned into a protecting group prone to cleavage under acidic or oxidative conditions by reduction of the azido function with triphenylphosphine (vide supra).[75,111] This protecting group was applied to the α- and β-selective preparation of tetrasaccharides on macroporous ArgoPore polystyrene.[112]

VII. Glycosylating Agents Used for Polymer-Supported Oligosaccharide Synthesis

The lack of powerful glycosylation reactions compatible with the solid-phase regime was a source of frustration that eventually resulted in waning interest in the pursuit of this mode of carbohydrate assembly. Not surprisingly, application of new glycosylating agents developed for solution-phase chemistry to soluble polymers ignited the interest in polymer-supported synthesis.

A. Glycosyl Trichloroacetimidates

Krepinsky reported the successful use of trichloroacetimidate glycosyl donors for the synthesis of a disaccharide on a soluble PEG support in 1991.[113] Glycosyl trichloroacetimidates[114] had been extremely successful in solution-phase oligosaccharide synthesis in terms of reactivity and selectivity. These donors can be activated under very mild conditions by catalytic amounts of TMSOTf and other triflates including dibutylboron triflate (DBBOTf). The latter agent was introduced to prevent acceptor silylation sometimes encountered with silyl triflates.[44]

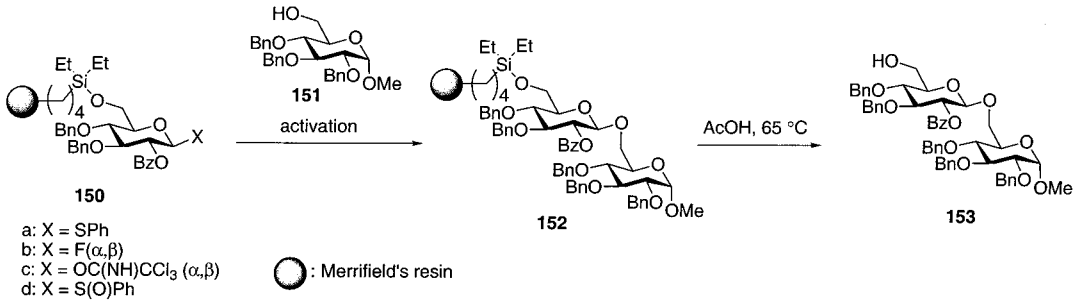
B. Glycosyl Sulfoxides

Anomeric glycosyl sulfoxides are highly reactive glycosylating agents upon activation with Lewis acidic promoters. Triflic anhydride was used most commonly to induce facile reactions at −78 °C and fashion even difficult linkages with hindered acceptors. Their use as glycosyl donors in solid-phase oligosaccharide synthesis has been initiated by Kahne and resulted in the preparation of oligosaccharides as well as an encoded combinatorial library of di- and trisaccharides (vide infra).[115]

C. 1,2-Anhydrosugars

1,2-anhydrosugars are readily derived from glycal precursors and have been activated to fashion a variety of glycosidic linkages. The glycal assembly method has been the basis for extensive synthetic studies under the donor-bound paradigm. Support-bound glycals were readily converted to the corresponding anhydrosugars by epoxidation with dimethyl dioxirane (DMDO).[42] Although questioned in the early 1990s,[116] the glycal assembly method has been

Scheme 31



very successfully used in the solid-phase synthesis of oligosaccharides and glycopeptides.

D. Thioglycosides

Thioglycosides,[117] readily prepared from anomeric acetates or 1,2-anhydrosugars,[118] have been frequently used as glycosyl donors. Thioglycosides can be prepared on large scale, stored over prolonged periods of time even at room temperature and can be selectively activated with a range of thiophilic promoters such as dimethylthiosulfonium triflate (DMTST), methyl triflate, or NIS/triflic acid. Drawbacks of thioglycoside donors on solid support are the high toxicity of the activators. Nevertheless, thioglycosides have found widespread use in solid-phase syntheses of oligosaccharides and it has been possible to achieve high α- or β-selectivity in glycosylation reactions.[110]

E. Glycosyl Fluorides

Anomeric halides, the staple of carbohydrate chemistry for over seventy years, have only rarely been used on polymer supports since the introduction of the glycosyl donors listed above. Activation by heavy metal salts makes these reagents difficult to use on solid supports. However, fucosyl fluorides are commonly employed for the installation of α-fucosidic linkages due to the excellent yield and diastereoselectivity of the reaction. α/β-Selectivities up to 80/20 could be obtained in glucosylation reactions of hindered 4-hydroxyl glucosamine acceptors using perbenzylated methyl thioglucoside or glucosyl fluoride in CH_2Cl_2/diethyl ether on a soluble PEG support.[119]

Many of these above-mentioned glycosyl donors usually employed to glycosylate resin-bound acceptors, have recently been compared in a glycosylation study under the donor-bound paradigm.[60] Glucosyl phenylsulfide and sulfoxide donors were found to be the most effective providing quantitative yields and high purity of the products (Scheme 31).

F. *n*-Pentenyl Glycosides

Fraser-Reid and his group studied the use of *n*-pentenyl glycosides (NPG) as glycosylating agents for solution-phase synthesis extensively.[87] The use of NPGs which are activated by electrophilic reagents such as NIS/TESOTf has also been extended to solid-phase oligosaccharide synthesis.[120] High average coupling yields exceeding 90% and excellent α- or β-selectivity were achieved with the acceptor-bound strategy as demonstrated by gel-phase ^{13}C NMR.

G. Glycosyl Phosphates

Glycosyl phosphates[90] have been recently applied to the solid-phase synthesis of oligosaccharides in our laboratory.[86] A straightforward new route to the preparation of these donors from glycal precursors was the key to providing sufficient quantities of differentially protected building blocks. Glycosyl phosphates are extremely reactive glycosylation agents that were activated at low temperatures to form sterically demanding linkages such as β-(1→4)-linked glucosides in very high yields within minutes.

VIII. "On-Bead" Analytical Tools

The synthetic organic chemist commonly relies upon a host of analytical techniques including thin-layer chromatography and NMR spectroscopy that allow him to rapidly assess the progress of the reaction in question. The development of reaction conditions for solid-phase synthesis has been hampered by the lack of tools to monitor reactions as they unfold on a polymeric matrix. Reaction development on the solid-phase matrix required a part of the sample to be separated followed by cleavage of the product from the resin and analysis by traditional solution-phase methods. This practice was time-consuming, expensive, and wasteful particularly in the context of multistep syntheses. To meet the need for nondestructive on-bead methods for the characterization of the oligosaccharides and intermediates, NMR and IR spectroscopy have been adapted for use on polymeric supports.[121] These methods have had an immense impact on the development of new methods for solid-phase oligosaccharide synthesis by allowing direct reaction monitoring.

A. High-Resolution Magic Angle Spinning NMR Spectroscopy

Detailed structural information can generally be derived from NMR spectra. Determination of the diastereoselectivity of a coupling reaction is of highest importance in the assembly of growing oligosaccharides on the solid support. Conventional NMR spectra acquired on a gel of the resin in a regular NMR tube exhibit very broad signals. While gel-phase ^{13}C NMR spectroscopy[122] has been used in the development of solid-phase oligosaccharide synthesis[120] on better swelling PEG−polystyrene composites, it was not

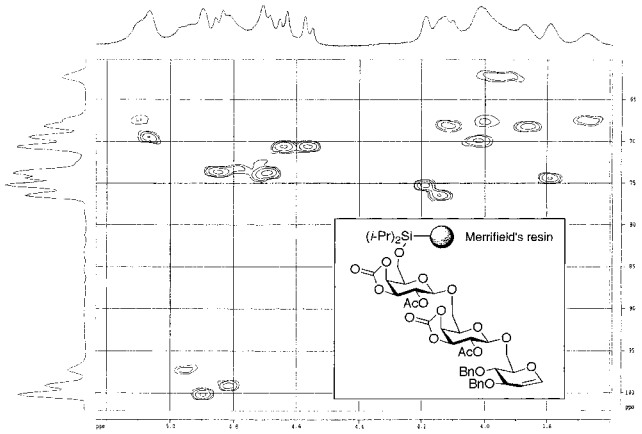
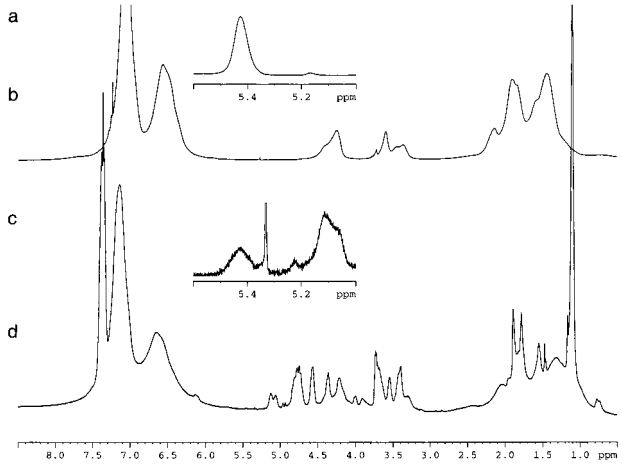


Figure 1. Reprinted with permission from ref 124. Copyright 1998 VCH Weinheim.



a: 123; b: 129; c: 132; d: resin-bound disaccharide

Figure 2.

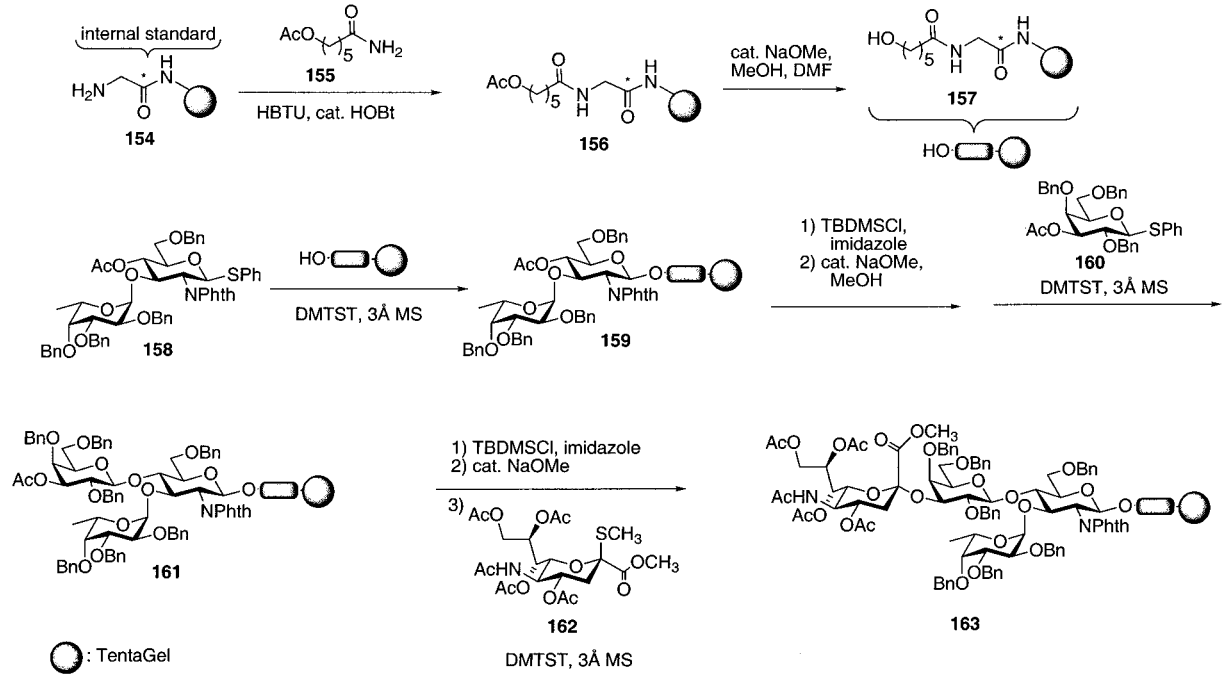
successful when applied to Merrifield's resin. High-resolution magic angle spinning NMR (HR-MAS) techniques[123] proved extremely useful for the analysis of support-bound oligosaccharides.[124] Well-resolved ^{1}H and ^{13}C NMR spectra as well as 2D-heteronuclear correlating HMQC spectra were obtained using HR-MAS for the characterization of a polymer-bound trisaccharide glycal (Figure 1).[124]

The power of the HR-MAS method for on-resin analysis has been further underscored in the development of new linkers. Without this method, only indirect analytical data after removal from the resin was available. Direct assessment of the resin-bound linker greatly facilitated the introduction of 4,5-dibromo octane-1,8-diol linker **129** (vide supra). The disappearance and reappearance of the olefinic protons as well as the growing oligosaccharide chain was clearly visible in the ^{1}H spectrum (Figure 2).[93]

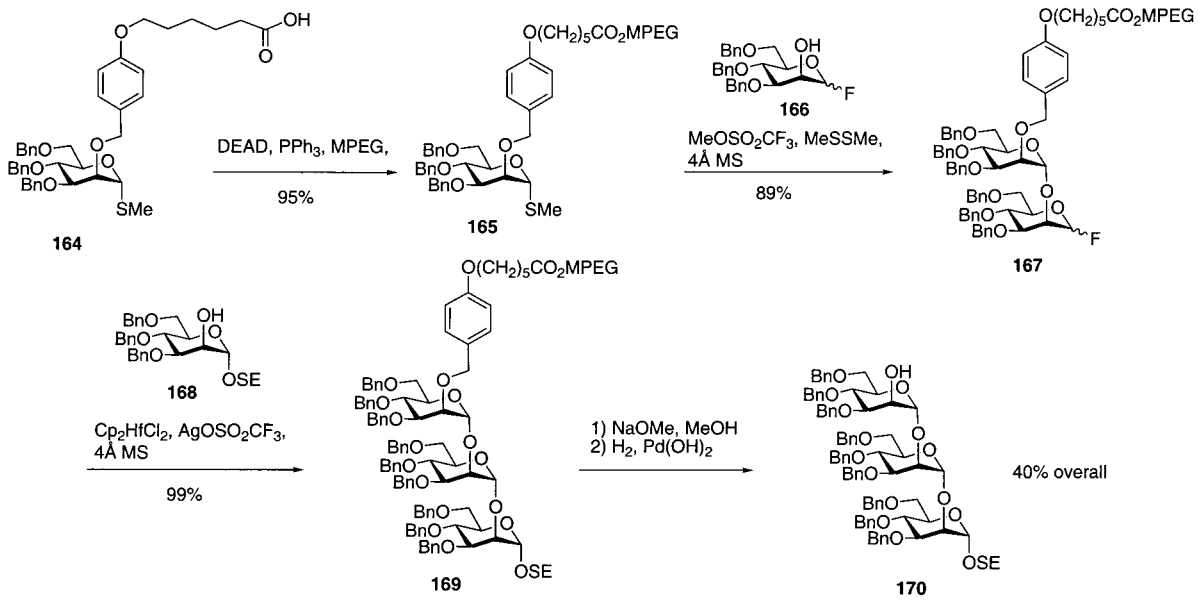
B. Gated-Decoupling ^{13}C NMR Spectroscopy

Although HR-MAS is an extremely powerful tool for the development of solid-phase oligosaccharide synthesis, it mandates a rather large expenditure for a special probe head. A conventional high-field NMR-spectrometer was used for the quantitative monitoring of solid-phase oligosaccharide synthesis by gated-decoupling ^{13}C NMR spectroscopy.[125] ^{13}C-Enriched protecting groups were employed to improve the signal-to-noise ratio when monitoring the carbon signals during the synthesis of a sialyl Lewis^{x} tetrasaccharide on a TentaGel support. Quantitative monitoring was achieved by comparison of the protecting-group signal with the signal of a ^{13}C-enriched glycine that had been incorporated into the linker as internal standard.[126] Coupling efficiency at each of the four coupling and deprotection steps was monitored by comparison of the internal standard with a ^{13}C-labeled protecting group incorporated via a thioglycoside donor (Scheme 32). While this analysis allows for the very effective and nondestructive assessment

Scheme 32



Scheme 33



of the reaction yield and necessitates no special probe head, it requires the use of ^{13}C-enriched protecting groups. Furthermore, no information about the anomeric composition of the generated oligosaccharide was obtained.

C. FT-IR Microspectroscopy

Fourier transform infrared (FT-IR) spectroscopy has been an important analytical tool to characterize compounds prepared by solution-phase synthesis. This concept has also been successfully applied to solid-phase organic chemistry. A single bead is often enough to acquire a FT-IR spectrum that allows for qualitative and quantitative interpretation.[121,127] FT-IR microspectrometry is a fast, nondestructive method that is extremely useful when reactions involving groups with characteristic absorption bands, e.g., esters and terminal double bonds, are monitored. Since spectra can be acquired in less than a minute, this method has proven very useful in the development of glycosylation, deprotection,[86,93] and cleavage conditions.[94] The reduction of TentaGel-bound 2-deoxy-2-azido glucuronic acid derivatives to the corresponding amines has been monitored by diffuse reflectance infrared Fourier transform spectroscopy[128] (DRIFTS).[129] An intriguing feature of the latter method is its potential for automation and thus for library screening. A general shortcoming, however, of FT-IR-based methods for oligosaccharide synthesis is the lack of information regarding the selectivity of glycosidic linkage formation that may be obtained by HR-MAS.

The analytical methods discussed in this section are expected to be of major importance in future studies aimed at the development of new methods and the preparation of complex structures on the solid support. Most of the molecules prepared to date and discussed below were composed without the benefit of on-resin analytical tools.

IX. Special Procedures for Polymer-Supported Oligosaccharide Synthesis

Besides the methods that were developed for the preparation of oligosaccharides on solid support, a number of innovative approaches that address very specific challenges of carbohydrate chemistry under the solid-phase paradigm have been reported. Although some methods have originally been designed for oligosaccharide synthesis on soluble polymers, they may eventually also be applied to solid-phase synthesis and are summarized in this section.

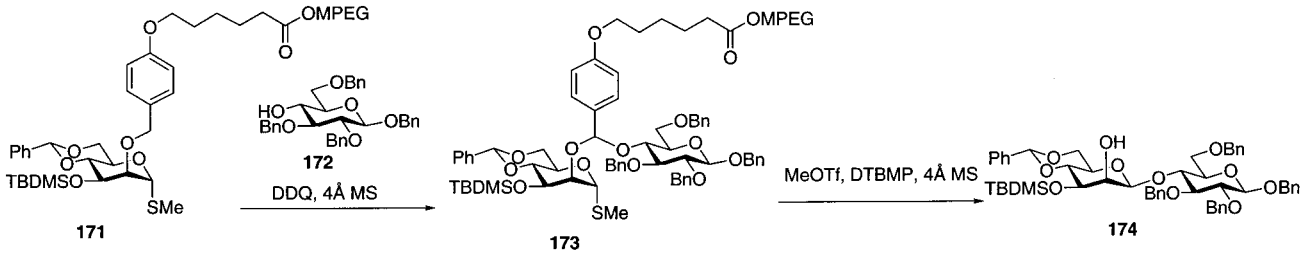
A. Orthogonal Glycosylations

The concept of "orthogonal glycosylation"[46b] is based on at least two sets of glycosyl donors. Each donor may be selectively activated under conditions which do not affect the second donor. Originally tested on soluble polymers by Ogawa and Ito, this approach had considerable impact on the design of oligosaccharide synthesis and combinatorial approaches.[130] Glycosyl fluorides and thioglycosides were chosen as orthogonal glycosyl donors for the polymer-supported synthesis of trimannan **170** (Scheme 33).[46a] Methyl thiomannoside **164** was attached to MPEG via an ester linkage and coupled with mannosyl fluoride **166** by activation with in situ generated DMTST to stereoselectively afford support-bound disaccharide **167**. Union with mannoside **168** followed by cleavage from the support afforded **170** in 40% overall yield.[131] To facilitate purification of the product by reverse-phase chromatography, monomer **168** was equipped with a hydrophobic trimethylsilylethyl (SE) tag.

B. "Gatekeeper Approach"

Ito and Ogawa also introduced the "gatekeeper approach" for the creation of β-mannosidic linkages on a soluble polymeric support.[132] The polymeric backbone "gatekeeper" ensured the selective formation of the desired linkage by intramolecular aglycon

Scheme 34



delivery to the glycosyl donor. The majority of the byproducts formed during this glycosylation reaction were retained on the polymeric support, while the β-mannoside was released into solution, thus greatly facilitating purification. Polymer-bound α-methylthiomannoside **171** which was tethered via a *p*-methoxyphenylacetal linker was reacted with a variety of acceptors to yield about 50% of the desired β-mannosides (Scheme 34). This method is an encouraging example for generation of 'difficult' glycosidic linkages on solid support, although yields and reaction times leave room for improvement.

C. Synthesis of Deoxyglycosides

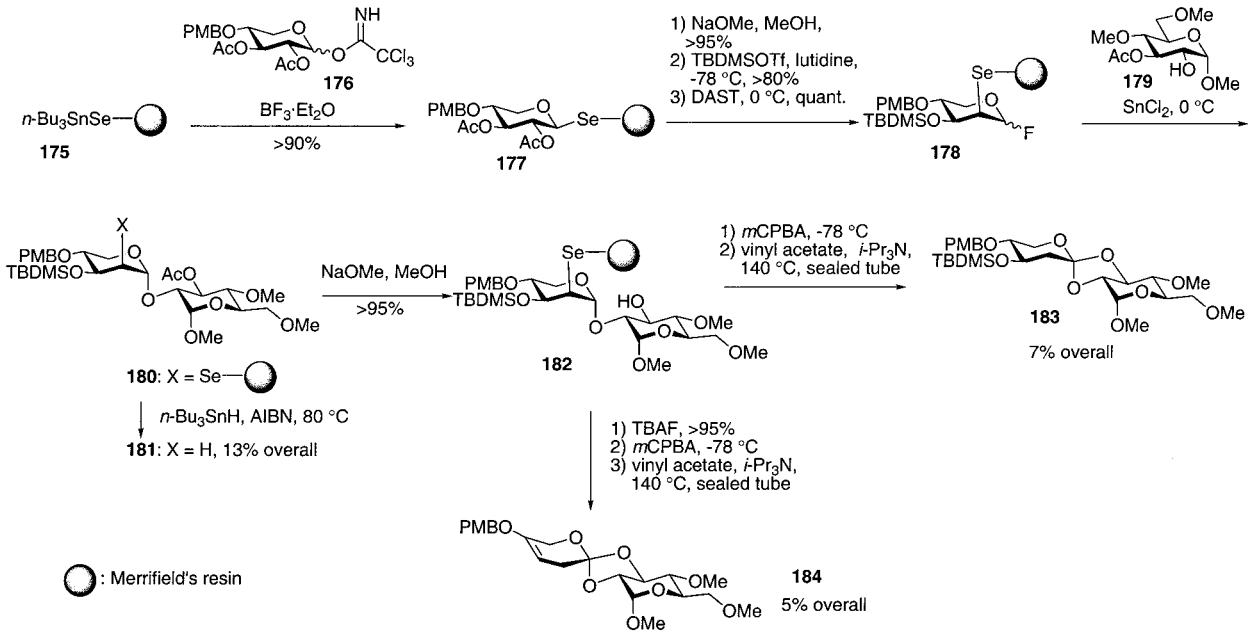
Trichloroacetimidate donors were also employed in the synthesis of 6-deoxysaccharides. A unique linker system that yielded 6-deoxy 6-iodo sugars upon cleavage with NaI in acetone was applied. These 6-deoxy 6-iodo sugars were readily reduced to the desired 6-deoxydisaccharides by action of tributyltin hydride and azo bisisobutyronitrile (AIBN).[100] Kirschning reported a solid-phase bound hypervalent iodonium reagent which was used in the synthesis of α-2-deoxy-2-iodomannosyl acetates from glycals.[133] These acetates are valuable intermediates for the synthesis of 2-deoxyglycosides.[134]

Nicolaou and co-workers developed a flexible method to install 2-deoxy glycosides, 2-deoxyortho esters, and 2,3-unsaturated 2-deoxyortho esters for the synthesis of the complex natural antibiotic everninomicin 13,-384−1.[135] Recently, this synthesis was successfully applied to the solid phase (Scheme 35).[136] Trichloroacetimidate donor **176** was β-glycosylated to polystyrene-based selenium resin **175**.[137] The stereochemical information introduced by virtue of the neighboring group was next exploited in a diethylaminosulfur trifluoride (DAST)-mediated stereospecific 1,2-seleno migration to yield resin-bound fluoride donor **178**. Glycosylation of various primary and secondary acceptors (e.g., **179**) was carried out in the presence of $SnCl_2$ to afford immobilized 2-deoxy-2-selenoglycosides (e.g., **180**). Reductive cleavage yielded free 2-deoxydisaccharide **181**. Deprotection of a vicinal hydroxyl group within the acceptor moiety gave access to 2-deoxyortho esters and 2,3-unsaturated 2-deoxyortho esters after oxidation/elimination or C3 hydroxyl deprotection followed by oxidation/elimination (Scheme 35).[138]

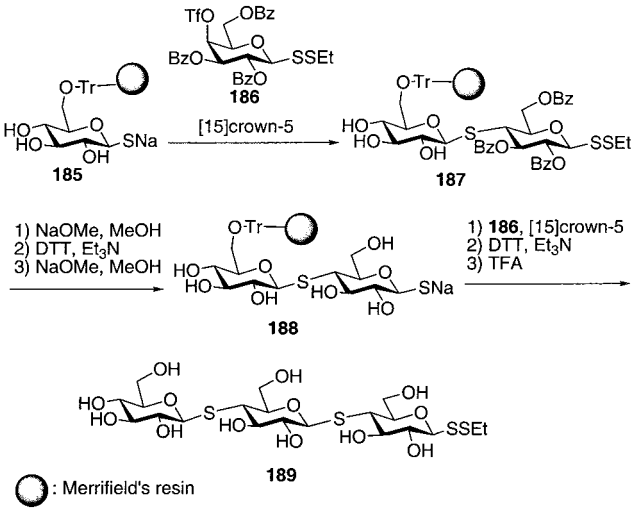
D. Synthesis of Thio-Oligosaccharides

Hummel and Hindsgaul recently described a method for the solid-phase synthesis of thio-oligosaccharides.[139] Since solution-phase methods to establish these glycosidase-stable linkages very often resulted in low yields, this procedure may prove to be advantageous for the preparation of these oligosaccharide analogues. Unprotected disulfides, available from either the anomeric thiol or an anomeric chloride

Scheme 35



Scheme 36



donor, were coupled to a tritylated polystyrene resin and reduced to the corresponding sulfides with dithiothreitol (DTT) (Scheme 36). Deprotonation, followed by complexation with a crown ether in the presence of an acceptor disulfide, furnished thiodisaccharide **187**. Further glycosylation, deprotection, and cleavage yielded 92% of thiotrisaccharide **189**.

The use of highly hindered iminophosphorane bases in the direct alkylation of anomeric thiols provides another method for the high-yielding solution-phase synthesis of thiosaccharides. Koh and co-workers proved the viability of their protocol under solid-phase conditions with the synthesis of disaccharide **195** on a Rink-functionalized PEG−polystyrene resin (Scheme 37). Cleavage from the support with 10% TFA afforded **195** in 48% yield based on resin-bound monomer **192**.[140]

E. Miscellaneous Procedures

Solid-phase extraction of oligosaccharides equipped with hydrophobic tags has been reported by several groups. Hindsgaul et al. tagged a galactose moiety with hydrophobic lauroyl in the synthesis of a combinatorial library of galactose neoconjugates.[141] Pozsgay described a lipophilic *p*-(dodecyloxy)benzyl ether protecting group that may also facilitate the purification of oligosaccharides synthesized on a polymer support.[142] Demonstrating the potential of this idea by means of lipophilic stearoyl ester protecting groups, Pozsgay very recently reported the blockwise synthesis of a complex 24-mer. Having equipped the reducing end tetrasaccharide unit with several stearoyl esters, block-coupling products were purified by simple C_{18} adsorbent technique up to the hexadecasaccharide stage.[143] Fluorous benzyl ether protecting groups[144] that were separated by extraction with fluorous solvents by phase separation are another potential tool for purification.[145]

X. Synthesis of Complex Oligosaccharides

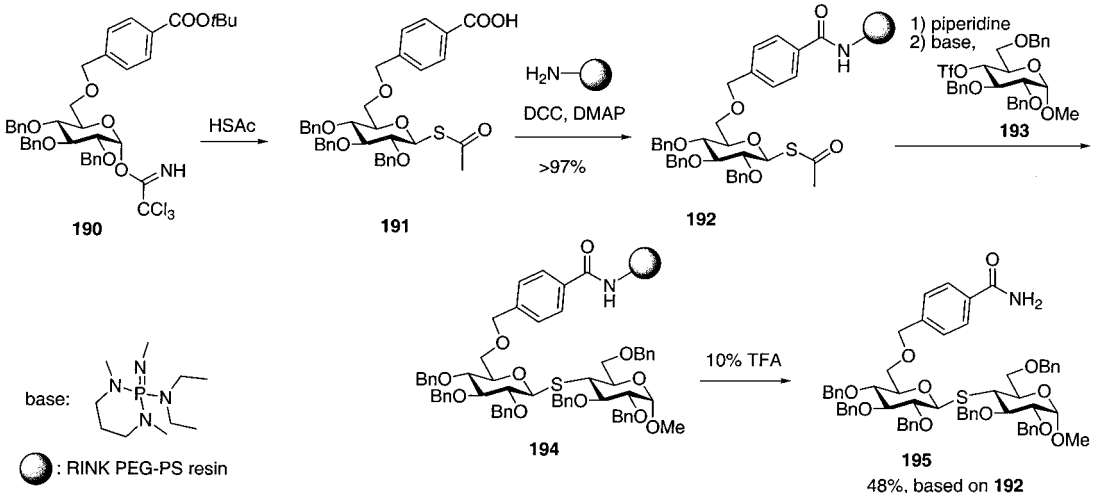
In this section some of the most successful approaches for the synthesis of complex oligosaccharides on solid support are summarized. The examples outlined below make use of the concepts described above. The advantages and disadvantages of the different strategies for the synthesis of complex oligosaccharides on solid support will be highlighted. The advances in this area are grouped according to the type of glycosyl donors that were used in each synthesis.

A. Glycal Assembly Approach

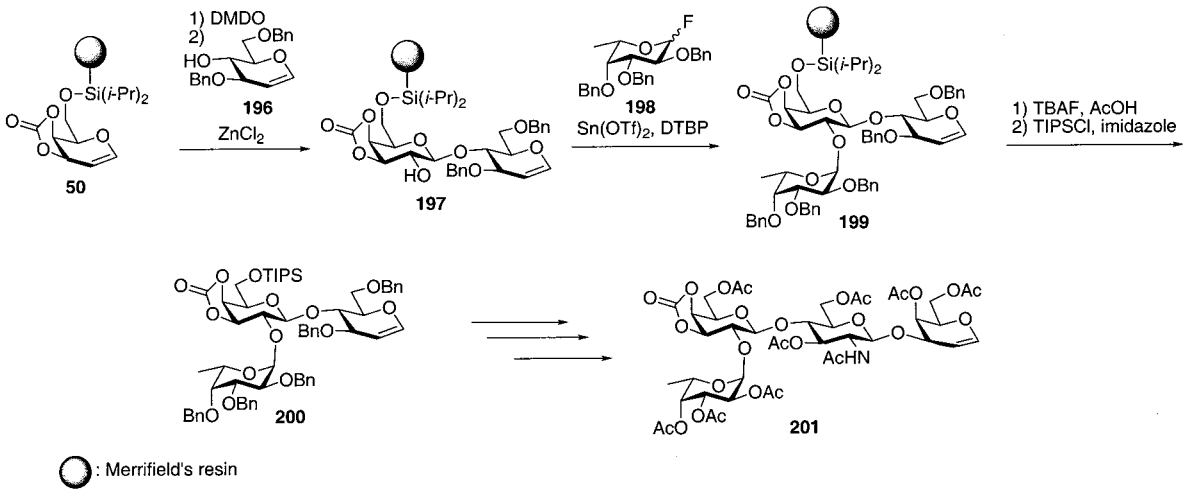
A host of complex oligosaccharides, glycoconjugates, and glycosylated natural products have been prepared by the glycal assembly method. Following the success in solution-phase synthesis, glycals were adapted to the solid-phase paradigm. Danishefsky and co-workers followed the donor-bound approach since glycals minimize protecting-group manipulations, serve as glycosyl acceptors, and may be readily converted into different glycosylating agents. The first glycal was linked via a diisopropylsilane to Merrifield's resin.[146] After the feasibility of the approach had been initially demonstrated on the example of a linear tetrasaccharide,[42] a linear hexasaccharide containing β-(1→3)-glucosidic and β-(1→6)-galactosidic linkages was prepared.[147]

Carbohydrate blood-group determinants are important for binding events including cell adhesion.[148]

Scheme 37



Scheme 38

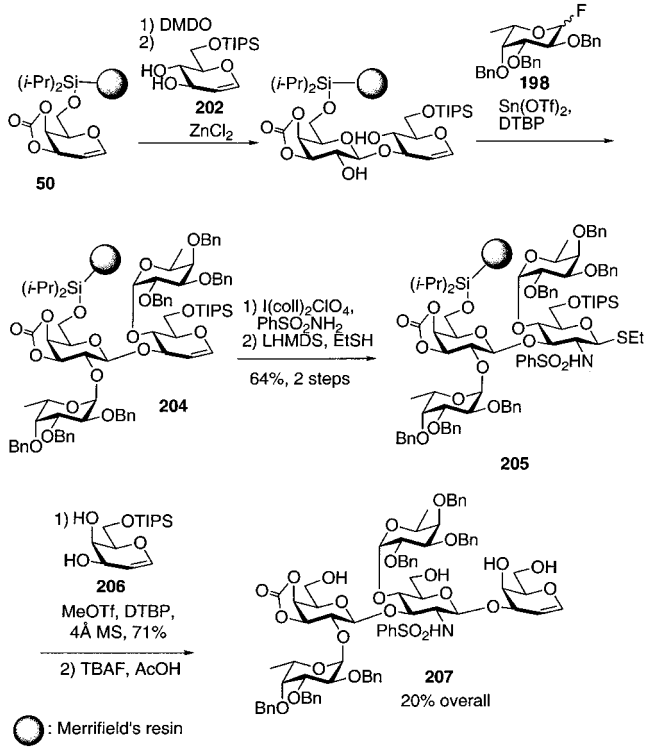


Since glycoconjugate antigens closely related to these blood-group structures have been found to be associated with various tumors, they are currently being evaluated for use in cancer immunotherapy.[149] Before the solid-phase methodology for the creation of the key β-*N*-acyl glucosamine linkage had been developed, a H-type 2 tetrasaccharide glycal **201** (Scheme 38) as well as a Lewis^b-hexasaccharide were in part synthesized on solid support.[150]

The Lewis^b (Le^b) blood-group antigen has been identified as a mediator for the binding of the pathogen *Helicobacter pylori* to human gastric epithelium.[151] Since bacterial attachment is essential for infection,[152] Le^b or its analogues may serve as therapeutic alternatives to broad spectrum antibiotics. Synthetic access to the Le^b antigen on the solid support was achieved after the iodosulfonamidation reaction, previously developed for solution-phase oligosaccharide synthesis,[153] was successfully accomplished to install a thioethyl glycosyl donor from a glycal precursor.[154] Branched tetrasaccharide **204** obtained by α-selective bisfucosylation with fucose donor **198**[155] was transformed into the thioethyl donor **205**. The coupling to galactal acceptor **206** was achieved in 71% to furnish the desired pentasaccharide in 20% overall yield after release from the support (Scheme 39).[154]

The reliable and selective installation of β-glucosidic linkages using the glycal assembly method on the solid support proved to be a challenge. The conformationally constrained galactal that had been initially explored was found to be relatively stable to mild Lewis acids such as zinc chloride and even allowed for galactosylation of hindered C4 hydroxyl acceptors. The lack of a constrained glucosyl epoxide rendered these donors highly reactive and prone to rapid decomposition upon treatment with Lewis acids. On the basis of solution-phase precedence,[156] the glucosyl epoxides could be converted reliably into thioethyl glycosyl donors[117] bearing a pivaloyl participating group at C2. It was demonstrated in model studies that donor **209** that was obtained in 91% yield from resin-bound glycal **208** was an excellent donor for the highly selective construction of β-(1→4), β-(1→3), and β-(1→6)-glucosidic linkages. Using this methodology, tetrasaccharide **212** containing exclusively β-(1→4)-glucosidic linkages was synthesized in 20% overall yield (84% yield per step) (Scheme 40).[157]

Scheme 39

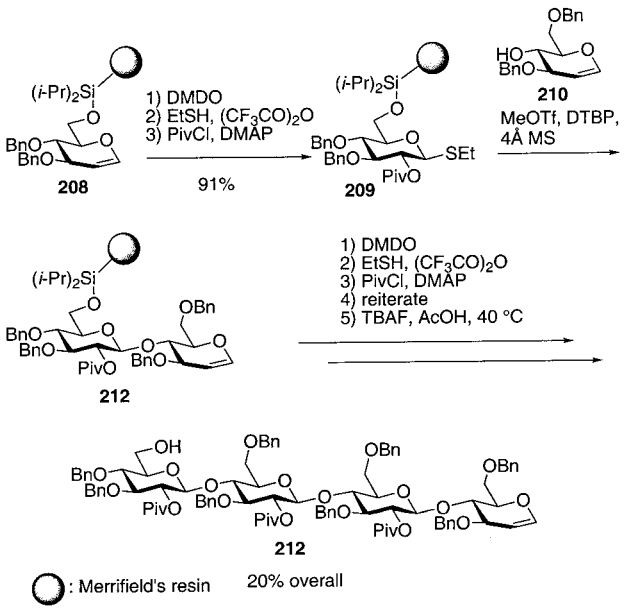


The glycal method was further extended to access *N*-linked glycopeptides by solid-phase synthesis (Scheme 41).[158]

B. Glycosyl Sulfoxides

Anomeric sulfoxides can be activated by triflic anhydride at low temperatures to function as very powerful glycosylating agents which readily react even with very hindered acceptors.[159] The high reactivity combined with excellent selectivity provided a set of useful building blocks for solid-phase oligosaccharide synthesis. Without a C2 participating group, anomeric sulfoxides gave high α-selectivity when coupled with secondary alcohols as demonstrated by the synthesis of fucosylated glucosamine

Scheme 40

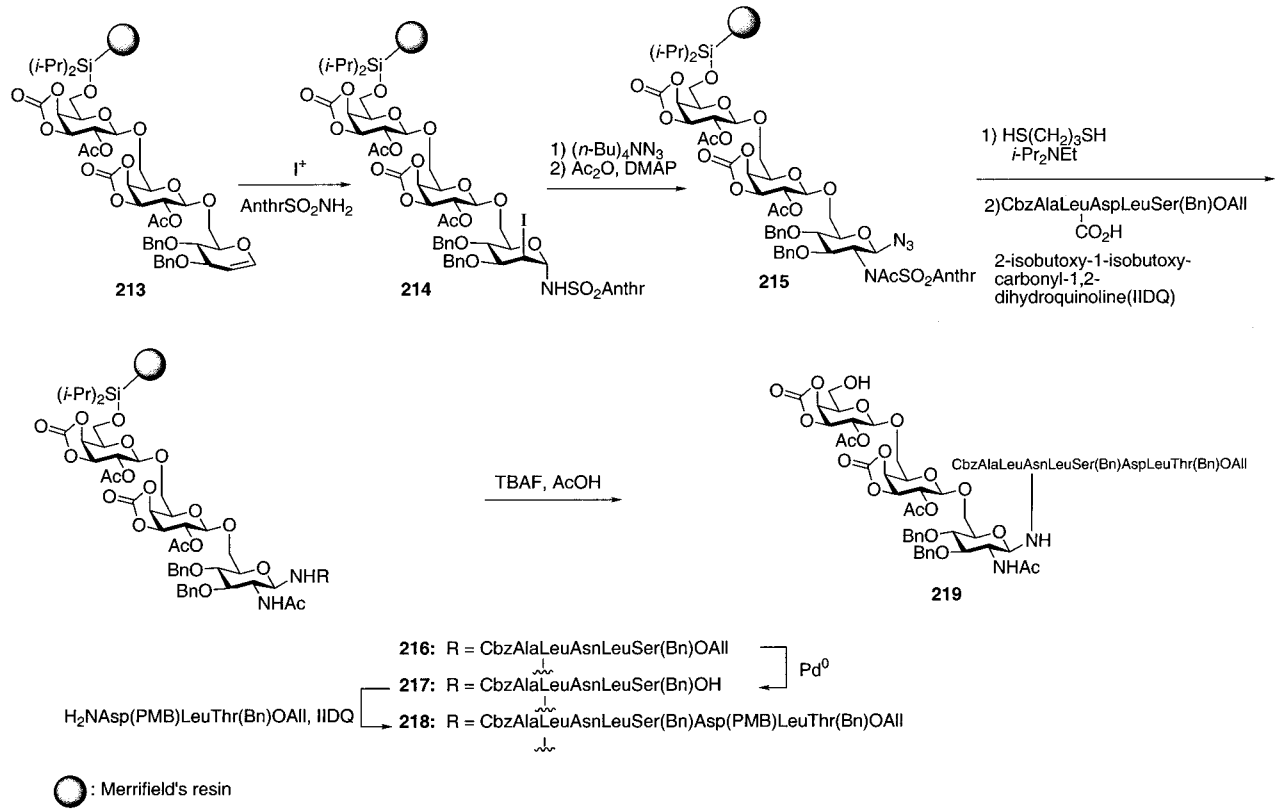


223. Coupling of fucosyl sulfoxide **221** proceeded in virtually complete α-selectivity and high yield (Scheme 42a).[73] The selective formation of β-glycosidic linkages was achieved by virtue of a C2 pivaloyl participating group to furnish β-(1→6) **228** linked trigalactoside (Scheme 42b). The only shortcoming of this synthesis was the thiophenyl linker that was used to attach the reducing end of the growing oligosaccharide to Merrifield's resin. While this linker was stable during the synthesis, model studies revealed that cleavage with mercuric trifluoroacetate resulted in yields of only 70−75%, thus lowering the overall yield of the solid-phase synthesis considerably. Assuming the same efficiency for the detachment from the support, an overall yield of 67% for the disaccharide and 52% for the trisaccharide corresponded to very good average coupling efficiency of 89−96% per step.

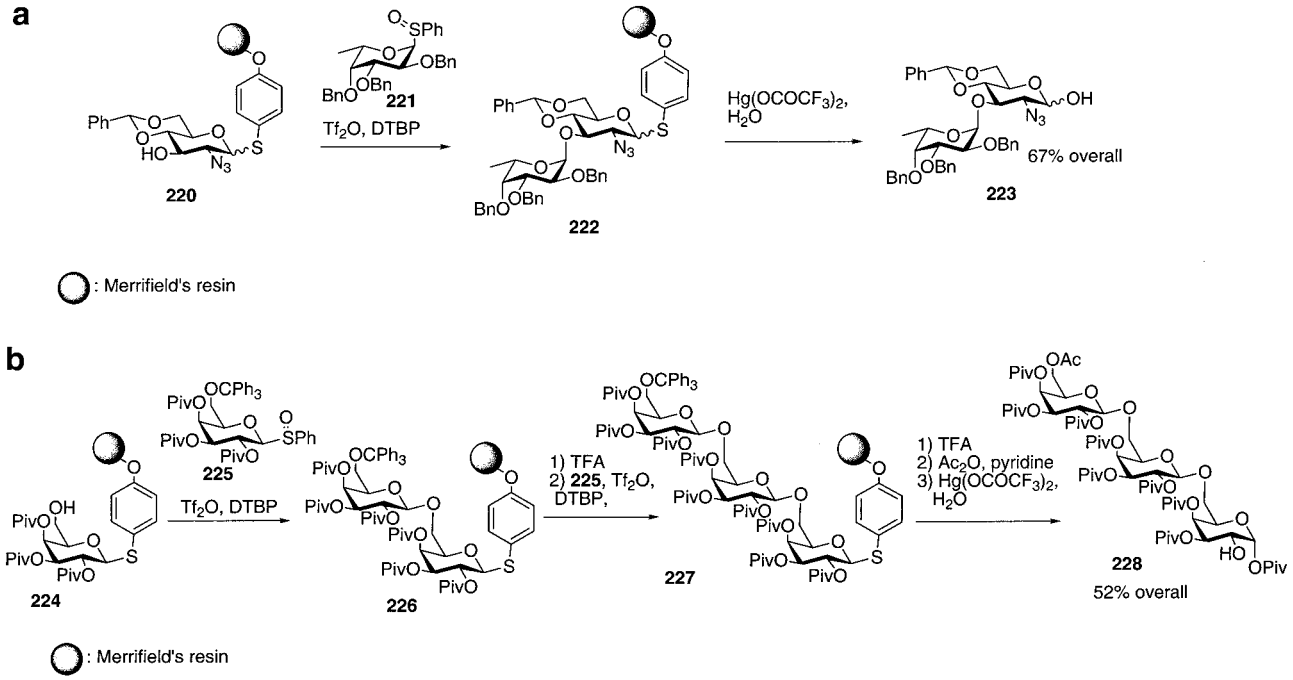
C. Glycosyl Trichloroacetimidates

Trichloroacetimidates have become the most commonly used glycosyl donors among the wide array of glycosylating agents. Versatility, high yields, and excellent selectivity in glycosylation reactions provide the basis for their outstanding success in solution-phase oligosaccharide synthesis.[114] These features rendered glycosyl trichloroacetimidates interesting candidates as building blocks for the assembly of oligosaccharides on solid support. After screening a range of linkers, Schmidt et al. initially relied on a thioether linkage and Merrifield's resin.[99] The synthesis of β-linked linear penta- and hexasaccharides[99,71] (vide supra) was recently followed by the solid-phase synthesis of a branched pentasaccharide common to most complex *N*-glycan structures (Scheme 43).[102] Mannosyl donor **229** was attached to thiol-functionalized Merrifield's resin via the reducing end. Treatment with sodium methoxide removed the C3 and C6 benzoyl protecting groups to furnish an acceptor ready for dimannosylation with trichloroacetimidate donor **95**. The polymer-bound trisaccharide **231** was selectively obtained in 38% overall yield, as determined after cleavage from the polymer matrix. Removal of the mannose C2 acetyl protecting groups and reaction with glucosamine donor **232** afforded branched pentasaccharide **233**. Cleavage of

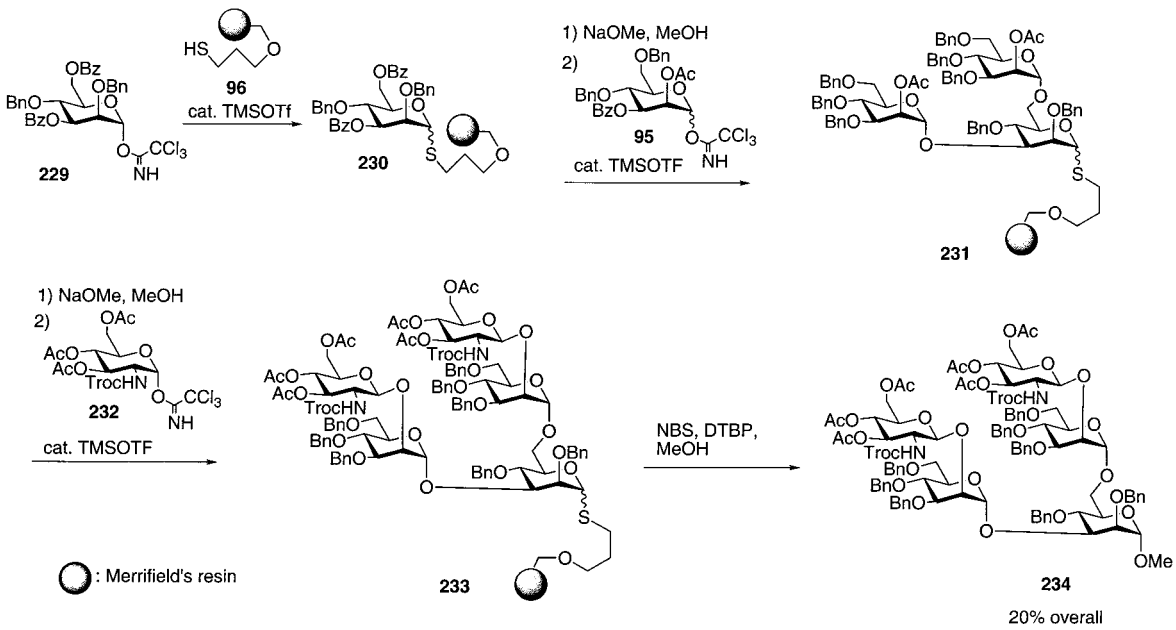
Scheme 41



Scheme 42



Scheme 43

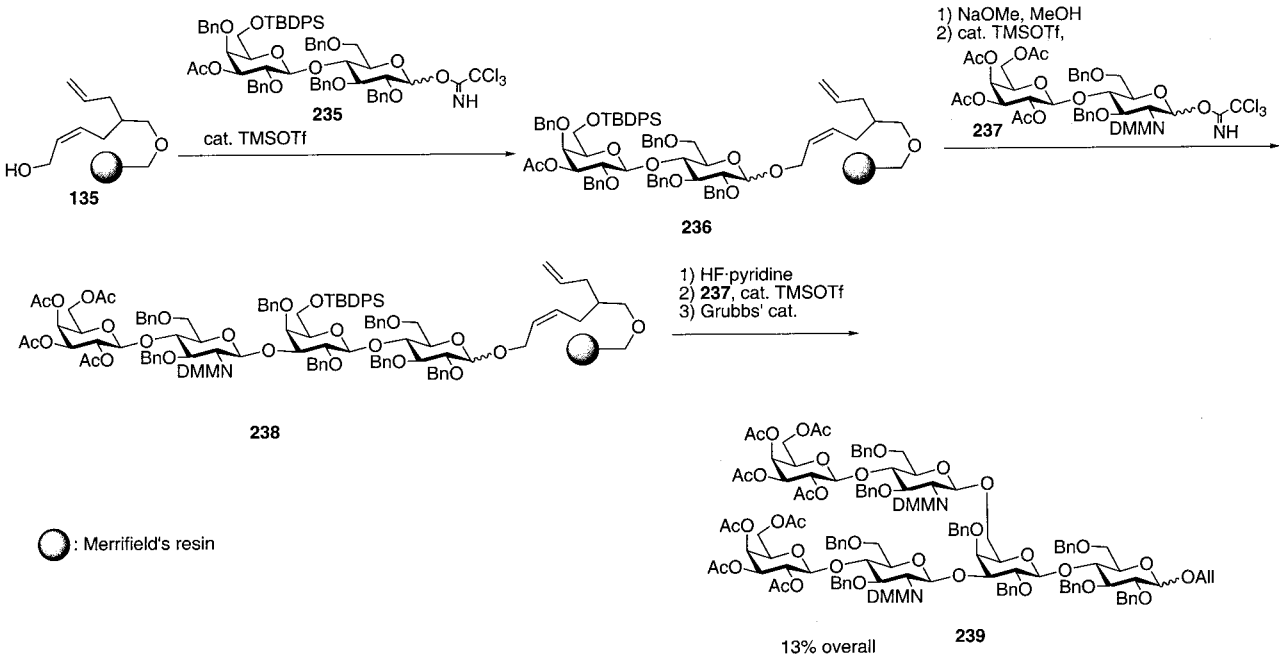


the thioether linker with *N*-bromosuccinimide (NBS) in the presence of methanol released pentasaccharide methyl glycoside **234** in 20% overall yield.

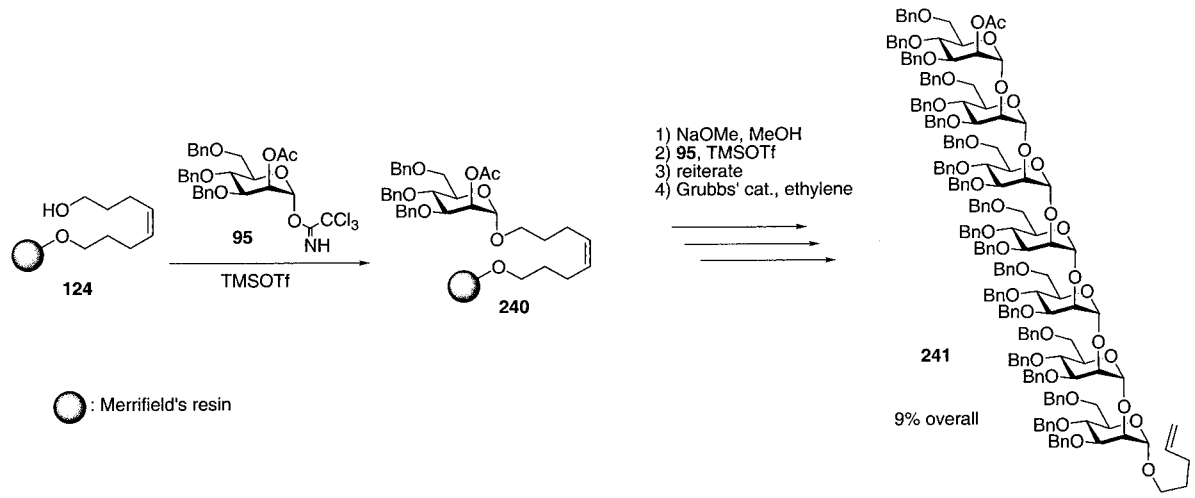
Very recently, Schmidt reported the synthesis of a branched lacto-*N*-hexaose-derived hexasaccharide employing his RCM-cleavable linker (vide supra).[160] Linker-modified Merrifield's resin **135** was double glycosylated using 3′,6′-differentiated lactose donor **235**. After deprotection of the 3′-position, the first lactosamine moiety was introduced by glycosylation with *N*-dimethylmaleoyl lactosamine trichloroacetate **237** under catalytic activation with TMSOTf. Although it did not affect the outcome of this synthesis, it is noteworthy that the TBDPS protecting group was not fully stable under the glycosylation conditions applied, as evidenced by traces of deprotected tetrasaccharide and hexasaccharide that were detected after cleavage of a resin sample. Complete deprotection of the 6′-position with HF−pyridine and subsequent glycosylation employing donor **237** yielded a support-bound hexasaccharide that was liberated by ring-closing metathesis and isolated as the allyl glycoside **239** in 71% stepwise or 13% overall yield (Scheme 44).

Use of trichloroacetimidate donors with octenediol linker **124** was particularly successful. Repetitive α-mannosylation employing trichloroacetimidate **95** furnished a linear heptasaccharide (Scheme 45).[86] The desired *n*-pentenyl heptamannoside **241** was obtained in 9% overall yield (84% per step), and the

Scheme 44



Scheme 45



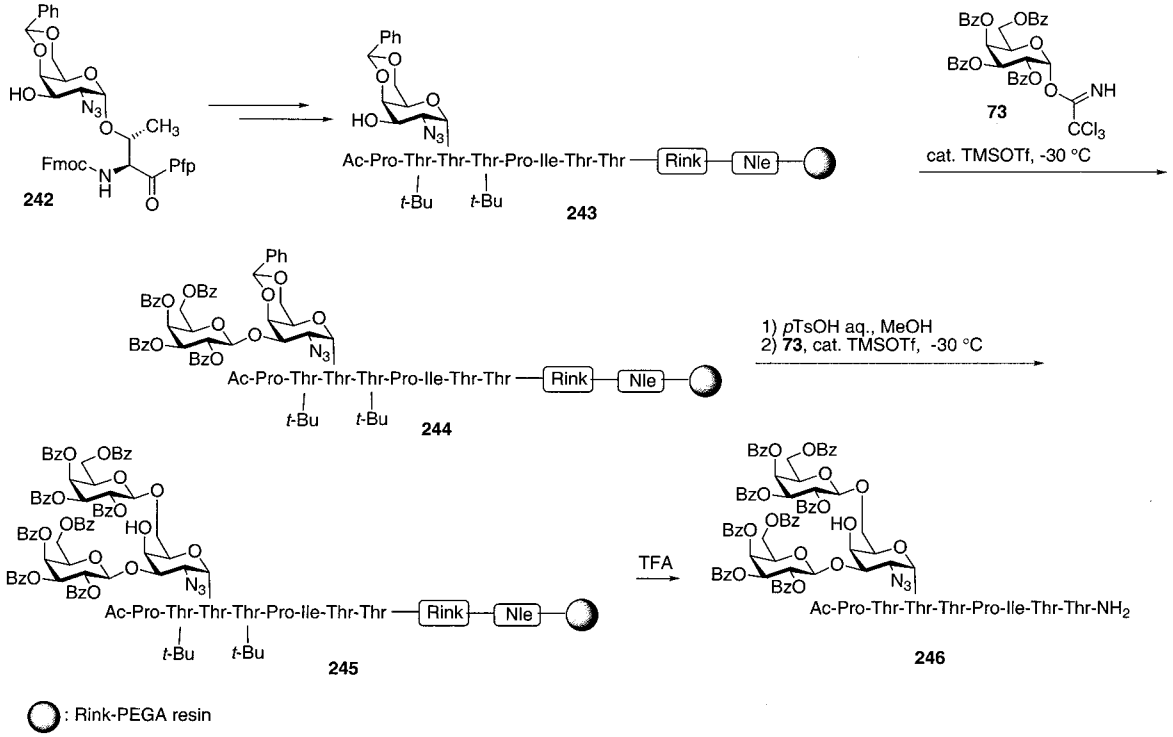
corresponding penta- and trimannosides were cleaved to yield 41% and 76%, respectively (91−95% per step).

Trichloroacetimidate donors also performed extremely well with other solid support materials. Iadonisi et al.[105,54] explored the performance of trichloroacetimidates in glycosylation reactions with acceptors bound to different polymeric supports. Coupling yields of up to 95% were reported using polystyrene or CPG. PEG-containing polymers were found to perform significantly poorer in these reactions. This group also explored glycosylations of CPG-bound oligonucleotides using trichloroacetimidates.[161]

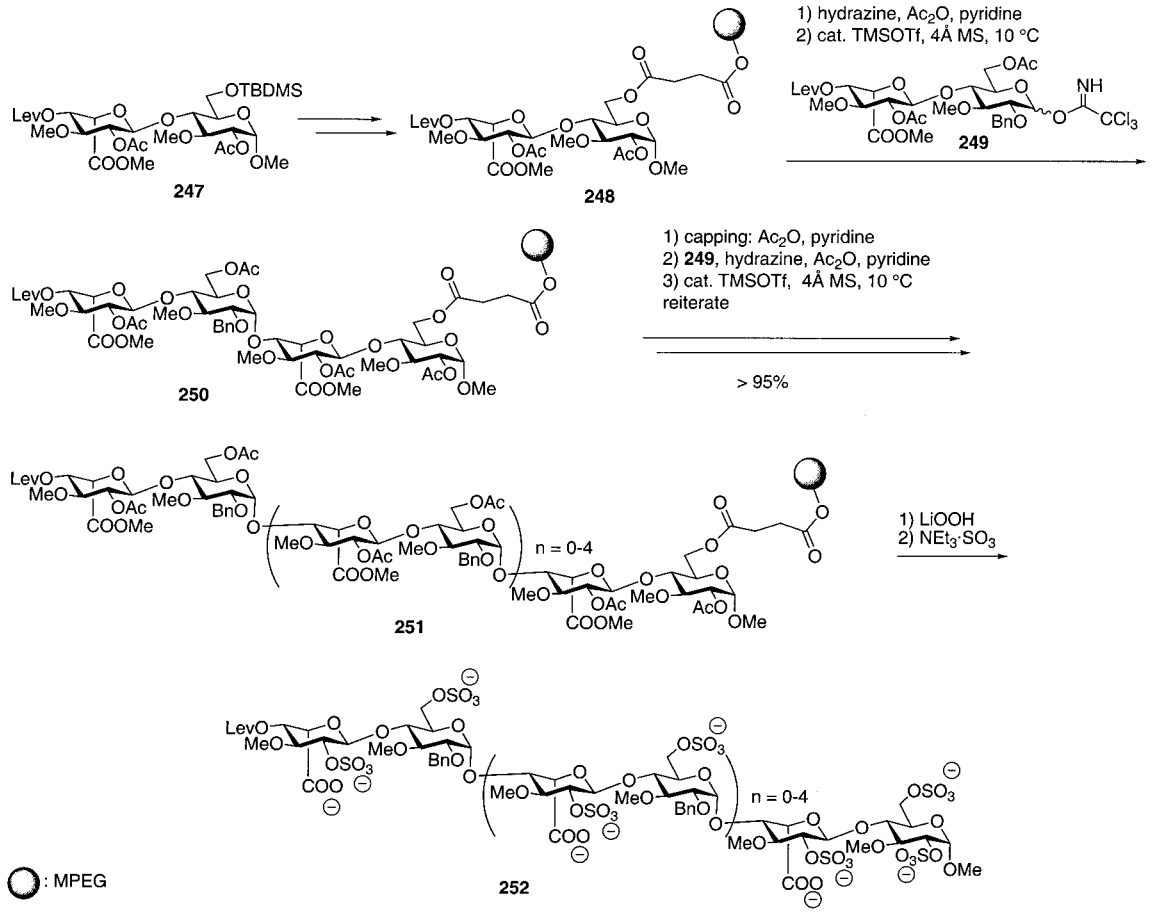
The solid-phase synthesis of glycopeptides[162] commonly relies on conventional peptide synthesis methods and the incorporation of glycosylated amino acid building blocks since direct glycosylation of oligopeptides on the polymer was often problematic.[163] Different trichloroacetimidate galactosamine and glucosamine donors were used to elaborate monoglycosylated threonine residues within an octapeptide chain on standard Rink-PEGA resin.[164] Building block **242** was used in the solid-phase synthesis of oligopeptide **243** followed by β-selective galactosylation to give **244** (Scheme 46). The same reaction was not successful on several other resins including Macrosorb, Tenta-Gel, and Polyhipe. Deprotection of the benzylidene and subsequent coupling to donor **73** furnished resin-bound glycopeptide **245** (64% of **246** after cleavage from the resin).

O-Methylated heparan sulfate-like oligomers of varying length were prepared on a soluble MPEG using trichloroacetimidate donors.[165] Resin-bound acceptor **248** was deprotected with hydrazinium acetate and glycosylated with excess donor **249** to yield exclusively α-linked tetrasaccharide **250**. Reaction temperature, excess of donor, and sometimes double glycosylations were found to be crucial for coupling efficiencies to exceed 95%. A capping step was introduced after each glycosylation to acetylate any unreacted acceptor sites. Reiteration of the deprotection, glycosylation, and capping cycle led to

Scheme 46



Scheme 47

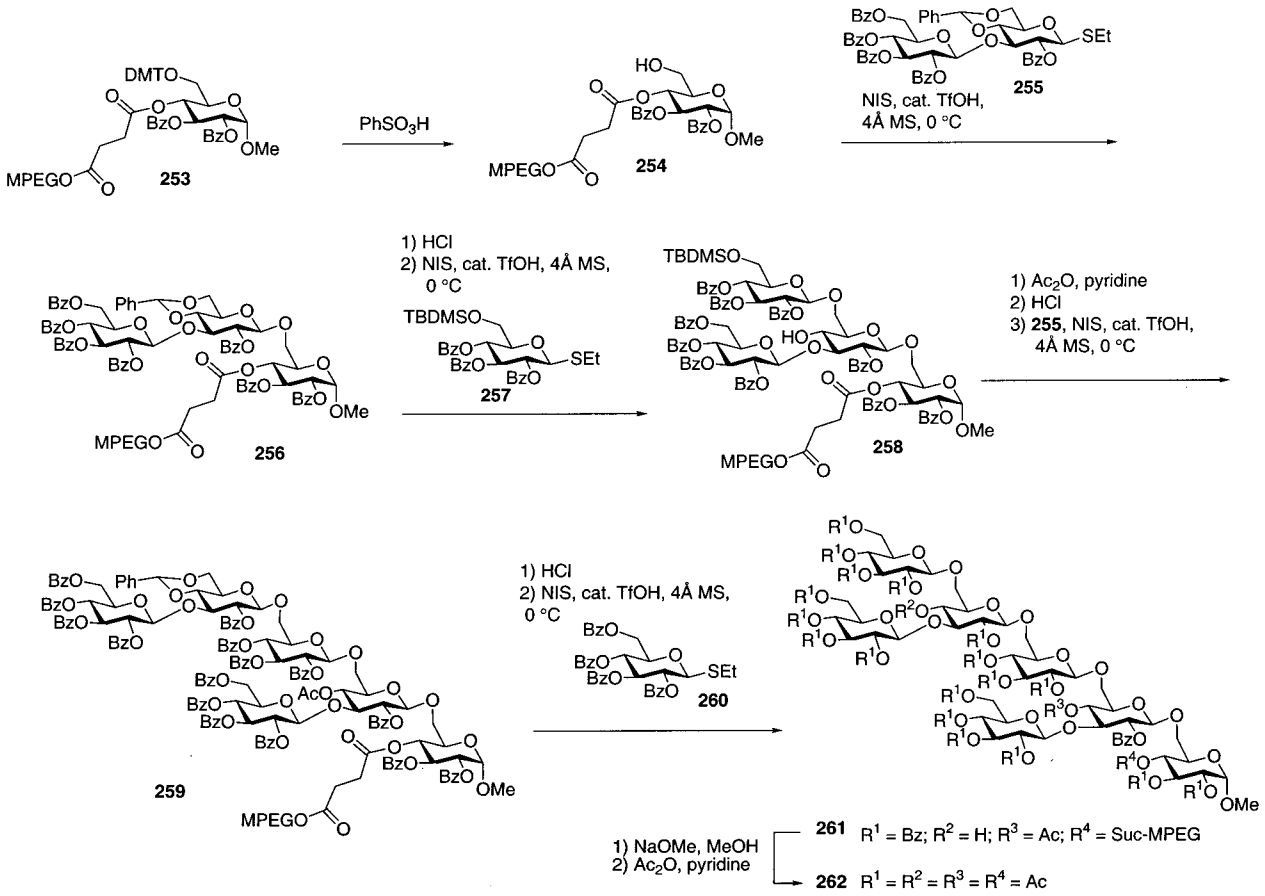


oligosaccharides up to dodecamer **251** ($n = 4$). Deprotection and cleavage from the support by saponification was followed by hydrogenolysis. After *O*-sulfation and purification, the heparan sulfate-like oligomers were obtained in good yields (Scheme 47).

D. Thioglycoside Donors

High-yielding synthetic procedures from a variety of precursors and activation of the resulting thioglycoside donors with different thiophilic reagents (e.g.,

Scheme 48



methyl triflate, DMTST, dimethylthiosulfonium tetrafluoroborate (DMTSB)) provides access to a range of glycosidic linkages in very good yield.[117] The stability of these donors, allowing for prolonged storage even at room-temperature, renders thioglycosides even more attractive.

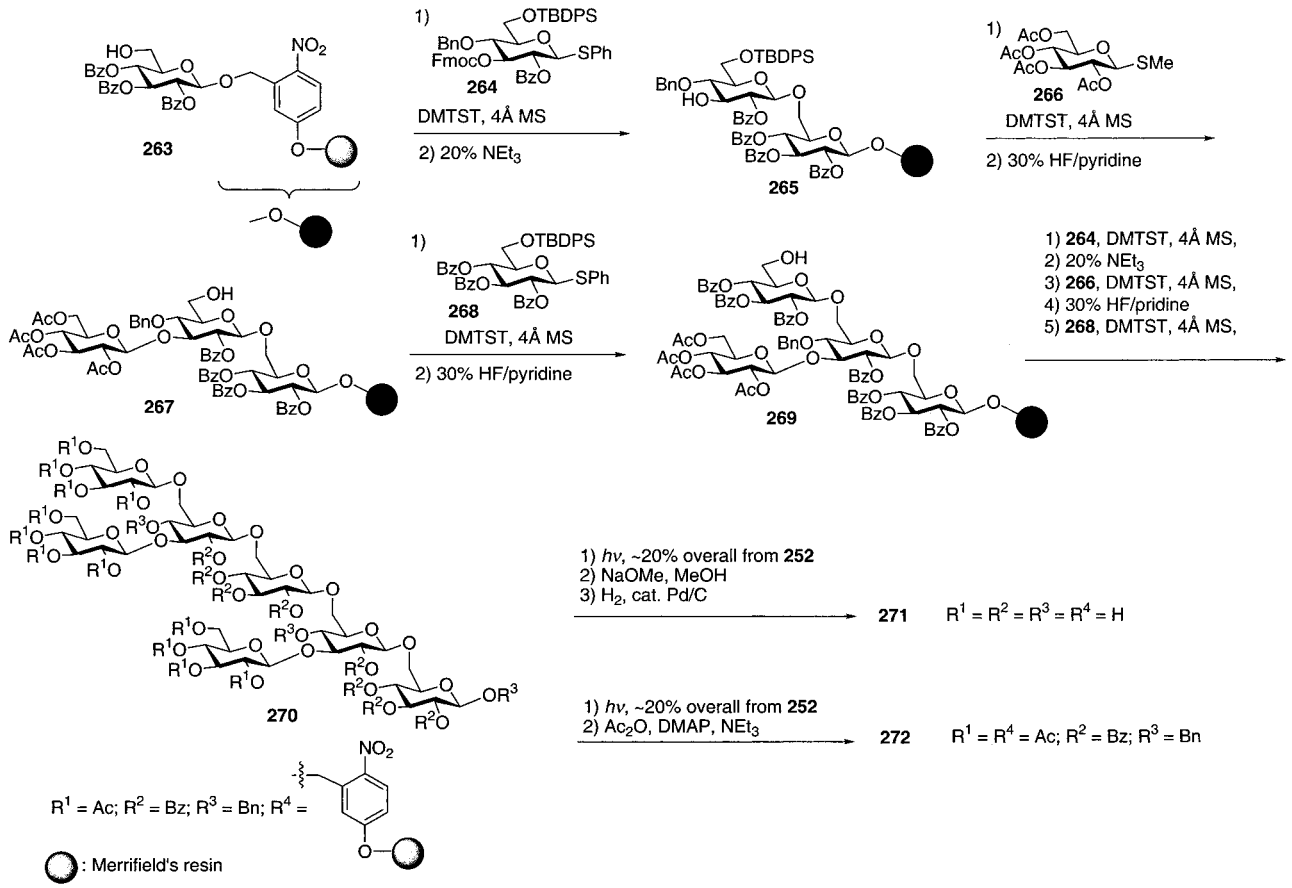
The application of ethyl thioglycosides to the synthesis of highly branched oligosaccharides on a polymer support was investigated by van Boom et al. in the synthesis of a heptaglucoside exhibiting phytoalexin elicitor activity.[108] This heptasaccharide containing β-(1→6)- and β-(1→3)-glucosidic linkages had previously been synthesized by conventional solution-phase methodology.[166] This synthesis was remarkable for two reasons. It constituted the first total synthesis of a large branched oligosaccharide structure on a polymeric support and made use of a regioselective glycosylation that reduced the need for elaborate protecting-group manipulations. Elaboration of the starting monomer **254** with glycosyl donor **255** yielded fully protected trisaccharide **256**. Subsequent deprotection and peracetylation steps furnished homogeneous heptasaccharide **262** in 18% overall yield after purification (Scheme 48). Full β-selectivity of all glycosidation reactions was achieved by virtue of C2 benzoyl groups.

The same phytoalexin elicitor (HPE) heptasaccharide was later synthesized by Nicolaou and coworkers on a polystyrene support equipped with a photolabile *o*-nitrobenzyl linker utilizing thiomethyl and thiophenyl glycosides.[83] This synthesis was achieved by subsequent coupling of monomers using the key 3,6-differentially protected glucose **264**. The first monosaccharide was attached to the linker which in turn was coupled to phenolic polystyrene. Acceptor sites were temporarily TBDPS or Fmoc protected, and glycosylations employed phenylthiodonors **264**, **266**, and **268**. Photolytic cleavage and acetylation of the lactol provided **271** in fully protected form as a mixture of the anomeric acetates in 20% overall yield. Photolytic cleavage followed by deacylation and hydrogenation in solution procured the fully deprotected HPE **272** (Scheme 49).

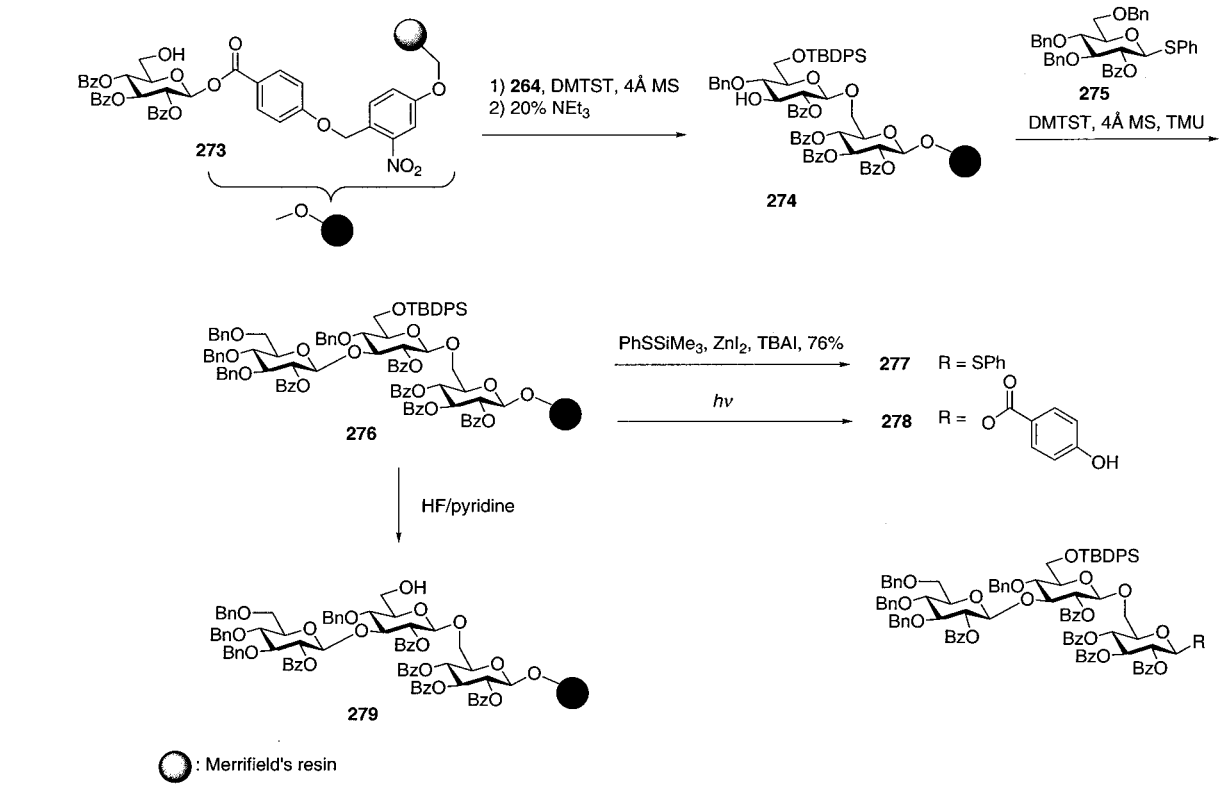
Incorporation of a 4-oxybenzoic acid spacer between the photolabile linker and the anomeric position of the first monosaccharide allowed for the generation of fully protected oligosaccharide fragments that in turn served as glycosylating agents (Scheme 50).[84] Trisaccharide **276** was either cleaved with $PhSSiMe_3$/ZnI_2/$n$$Bu_4NI$ to yield 76% of trisaccharide phenyl thioglycoside **277** or photolytically released from the support to afford 63% of the fully protected trisaccharide **278**. Fragment couplings using **277** in successive condensations furnished dodecamer **281** in 10% yield from **273** (Scheme 51).

Sialic acid plays a major role in the recognition of various tumor-associated oligosaccharides[167] and in antigens involved in the inflammatory response.[168] Still, the generation of α-sialic acid linkage remains difficult. For polymer-supported synthesis of oligosaccharides to become a general process, the effcient coupling of sialic acid building blocks will be required. Ogawa described an approach[169] by which sialic acid thiodonor **283** was attached to an MPEG support via a succinoyl linker following the donor-bound strategy. To direct the stereochemical outcome of glycosyla-

Scheme 49



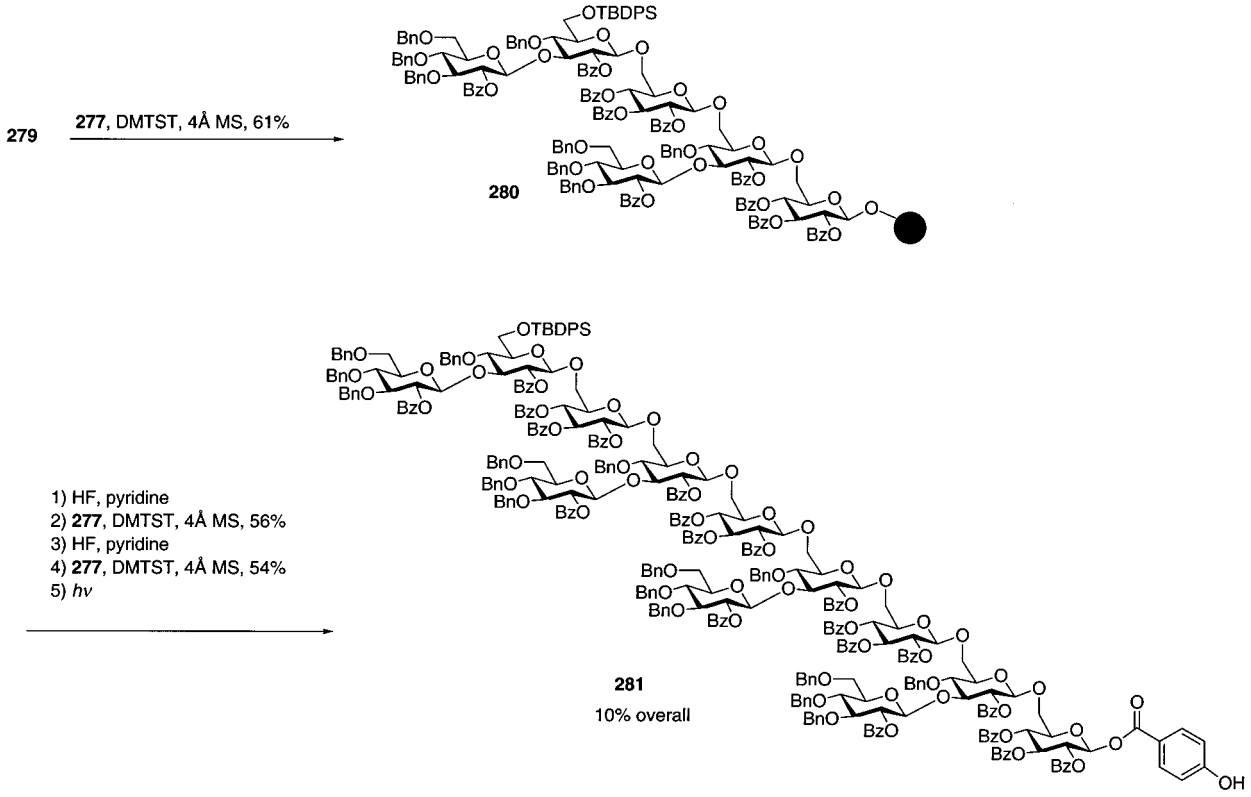
Scheme 50



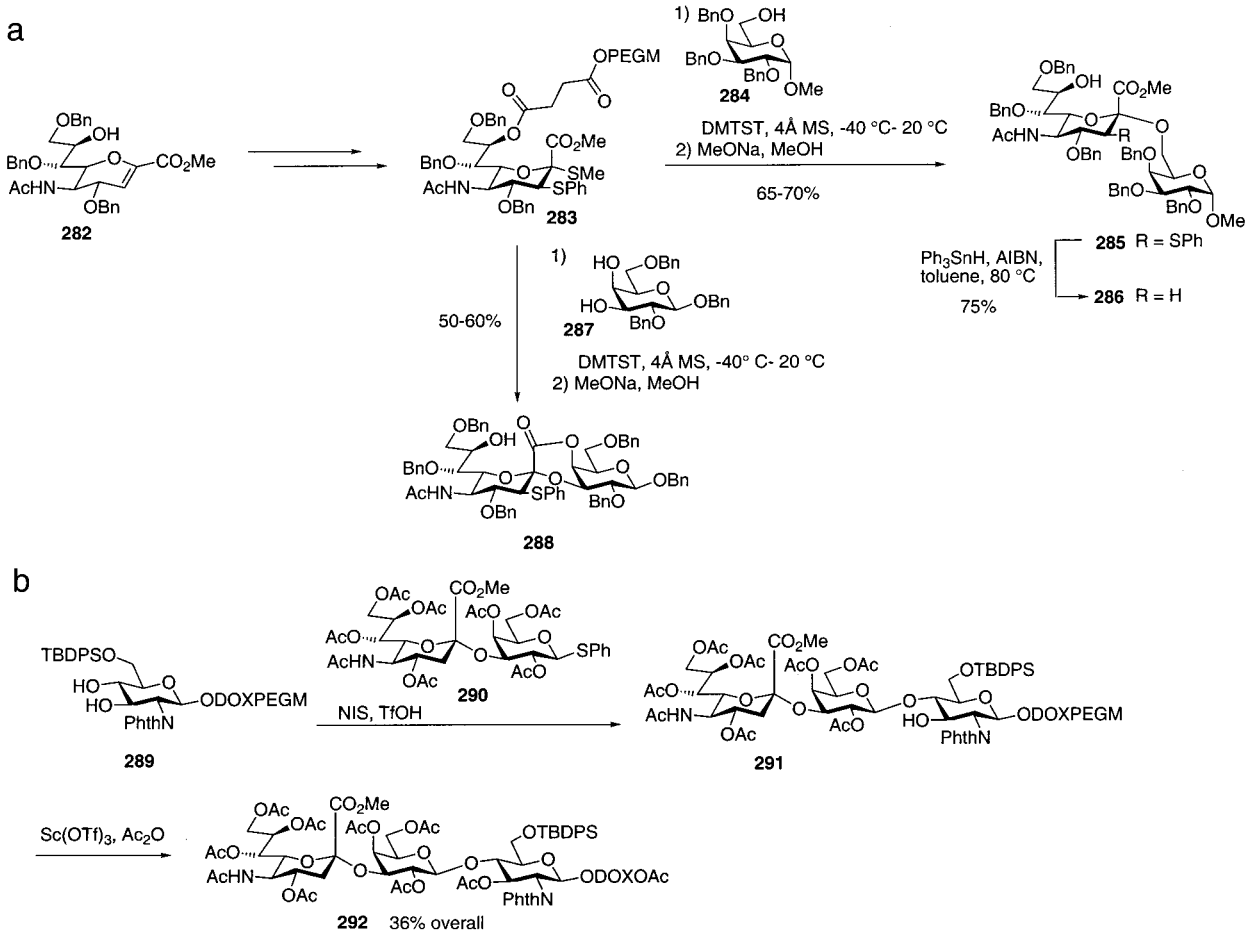
tions,[170] an equatorial C3 phenylsulfide moiety was installed. This donor served in the synthesis of disaccharides **286** and **288** (Scheme 52a).

For use under the acceptor-bound paradigm, Whitfield et al. reported the application of phenylthio sialylgalactoside **290** which was chemoenzymatically

Scheme 51

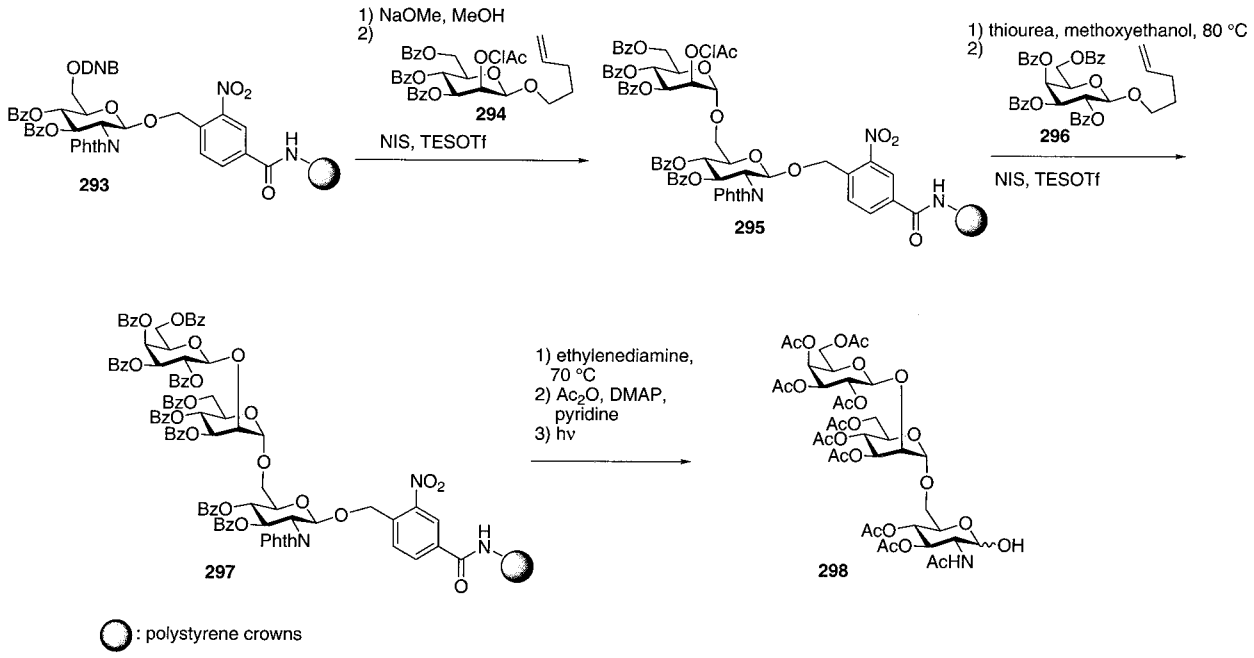


Scheme 52



synthesized in a highly efficient manner.[171] Synthesis of trisaccharide **292** on a soluble polymer and subsequent cleavage was achieved in 36% overall yield (Scheme 52b).

Scheme 53



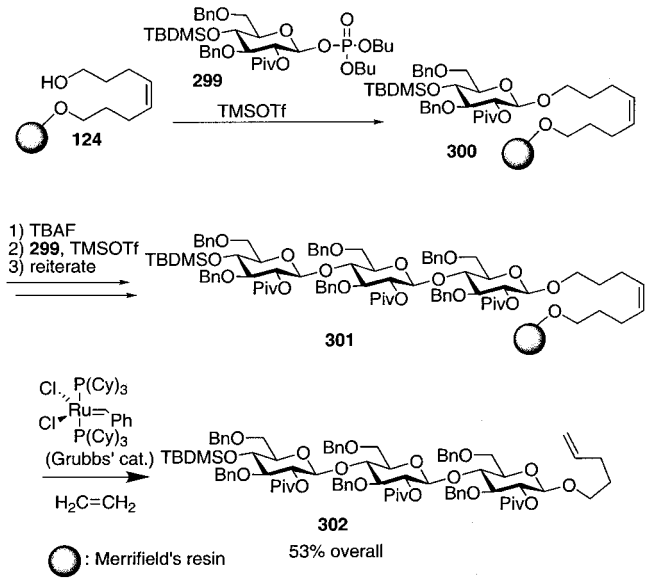
E. *n*-Pentenyl Glycosides

Fraser-Reid and his group extensively studied the use of NPGs as glycosylating agents for solution-phase synthesis.[87] Chiron's polystyrene-grafted "crowns" were applied to the construction of a trisaccharide utilizing a photocleavable *o*-nitrobenzyl linker and *n*-pentenyl-glycoside donors (Scheme 53). Polystyrene crowns were chosen with future applications in parallel synthesis in mind. After attachment of the first aminoglucosyl moiety to the linker via the anomeric position, the C6 DNB group was removed. Coupling with mannose donor **294**, deprotection of the C2 chloroacetyl group, and galactosylation with **296** furnished trisaccharide **297**. Global deprotection followed by peracetylation and photolytic cleavage from the support provided **298**, although no yield was reported.[120]

F. Glycosyl Phosphates

Glycosyl phosphates have been very recently explored as glycosylating agents in the context of solid-phase oligosaccharide synthesis. Following solution-phase studies which showed that excellent coupling yields[90] and remarkable selectivities[91] could be obtained in extremely short coupling times, the compatibility of this methodology with solid-phase synthesis was explored. Using octenediol linker **124**, β-(1→4)-linked trisaccharide **302** was readily assembled in 53% overall yield (7 steps). Couplings with hindered acceptors underscore the potential of glycosyl phosphates in solid-phase oligosaccharide synthesis (Scheme 54).[86]

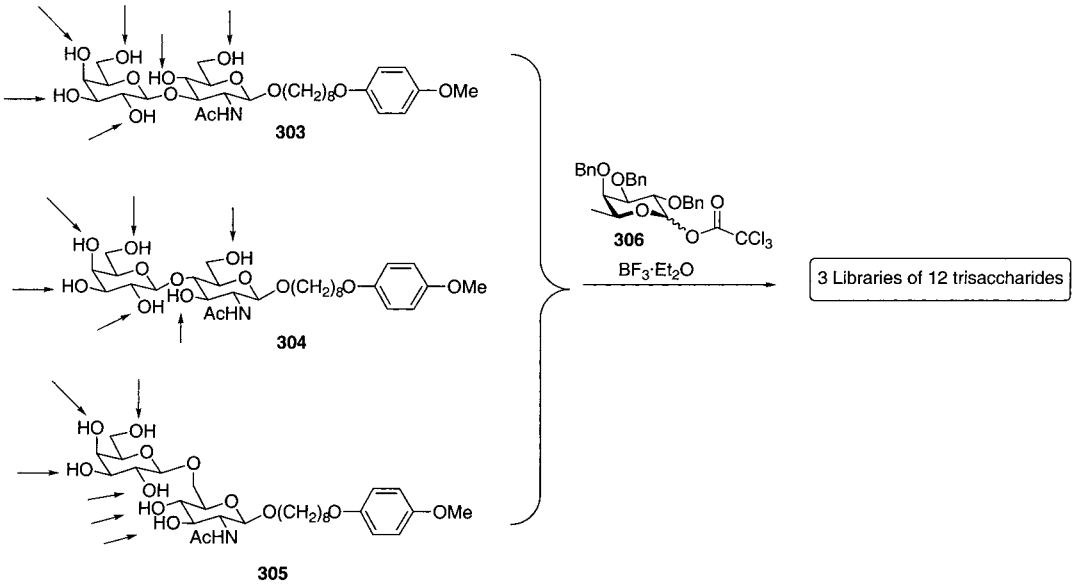
Scheme 54



XI. Synthesis of Combinatorial Carbohydrate Libraries

Many recognition processes of biological importance involve a diverse array of oligosaccharides and glycoconjugates presented on the cell surface. The many differently oriented alcohol and amine groups exposed even on a monosaccharide allow for the connection of a variety of groups to be presented to a receptor of interest. Given the nature of combinatorial or parallel synthesis, solid-phase oligosaccharide assembly holds great potential for the generation of carbohydrate libraries. The need for identifying natural and unnatural ligands for carbohydrate receptors has generated intense efforts in several areas. Efficient protocols for the combinatorial synthesis, screening, and hit identification of carbohydrate-derived libraries on the solid support as well

Scheme 55



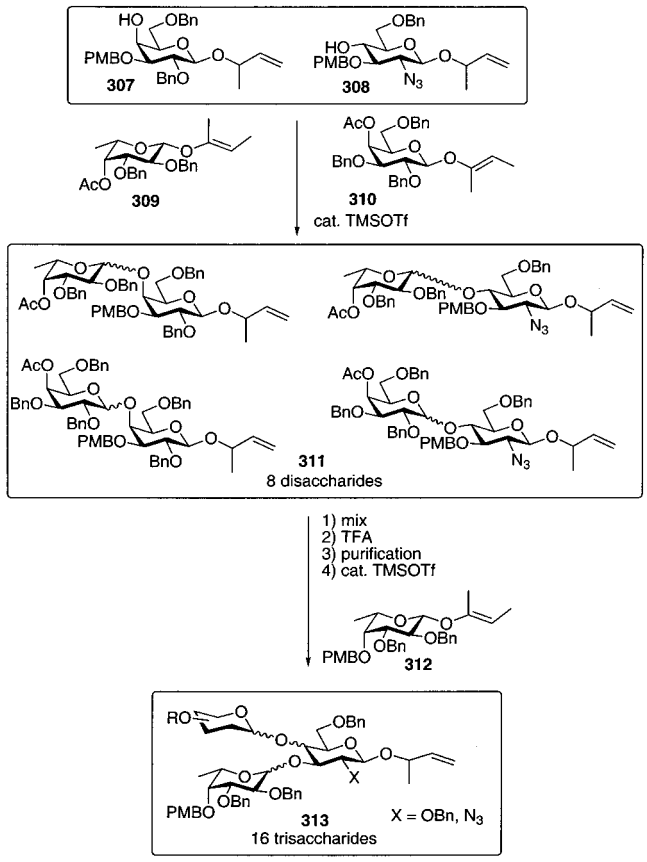
as in solution have been pursued. Progress includes new methods for the identification of binding oligosaccharide structures out of mixtures with the help of NMR techniques[172] or affinity chromatography[173] and the sequencing of complex oligisaccharide chains by means of mass spectrometry.[174] In this section we summarize the synthetic efforts made in this field, including oligosaccharide libraries, libraries of acylated di- and trisaccharides, and libraries based on carbohydrate scaffolds. Some special methods for the construction of carbohydrate-related libraries and carbohydrate conjugate libraries will also be briefly discussed. Previous reviews have covered some of the earlier work.[175]

A. Combinatorial Oligosaccharide Libraries

One of the first attempts at the preparation of oligosaccharide libraries was reported by Hindsgaul et al.[176] The "random glycosylation" of unprotected disaccharides involved the nonselective coupling of a fucosyl donor to create three sublibraries of α-fucosylated disaccharides in one step. Nearly statistical mixtures of all possible trisaccharides were obtained after chromatography (Scheme 55). The ease of library synthesis was an intriguing feature of this strategy that avoided lengthy protecting-group manipulations to differentiate diverse acceptor sites. Screening and hit identification, on the other hand, were severely complicated by mixtures of molecules with identical molecular weight.

Linear[177] and branched trisaccharide model libraries[178] were prepared by the "latent-active glycosylation".[179] This concept made use of the convenient access to both glycosyl donors and acceptors from common allyl glycoside precursors. Trisaccharide libraries of α/β-mixtures at every glycosidic linkage were prepared by the split-and-mix method (Scheme 56). Central acceptor building blocks were equipped with a C3 PMB group, and glycosylations were performed under conditions that reliably provided anomeric mixtures. A mixture of 16 fully protected trisaccharides was obtained by coupling **312** with the

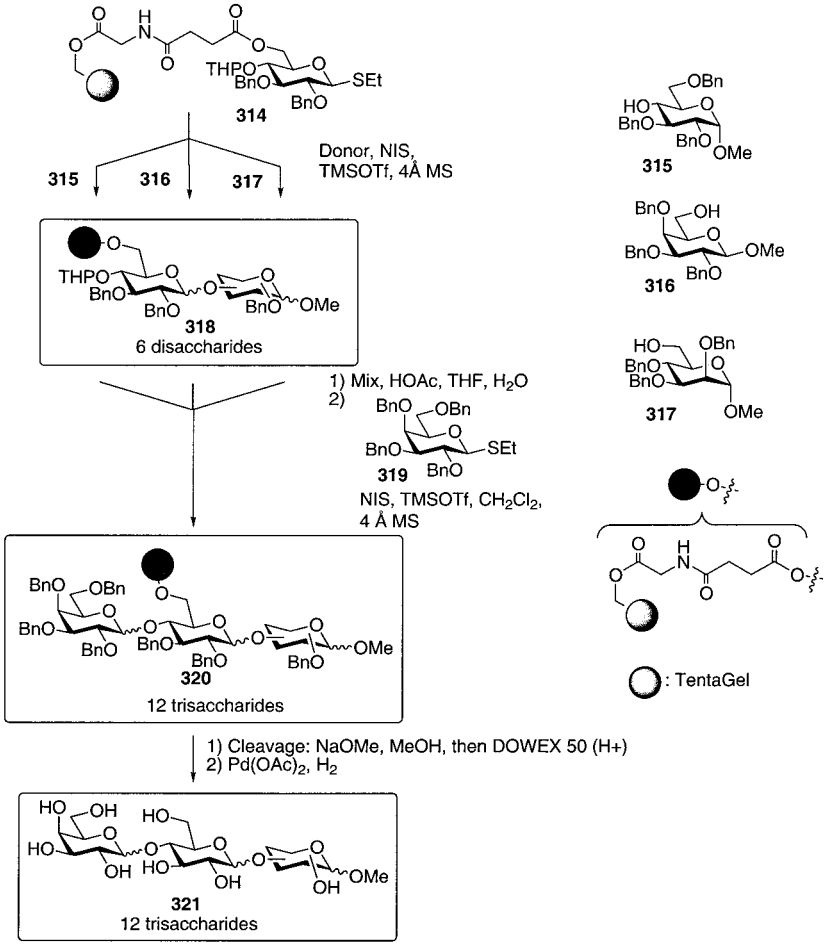
Scheme 56



disaccharides of library **311**. These disaccharides had in turn been prepared from the corresponding vinyl glycoside donors and allyl glycoside acceptors.

Boons also introduced a 'two-directional' glycosylation strategy for the solid-phase preparation of a 12-membered trisaccharide library.[45] After the C6 hydroxyl of the first thioglycoside was immobilized on TentaGel, a set of three different monomers **315**–**317** was coupled to furnish disaccharides **318**. Deprotection of the C4′ position and glycosylation with thiogalactoside **319** under conditions that yield ano-

Scheme 57



meric mixtures furnished 12 trisaccharides (Scheme 57).

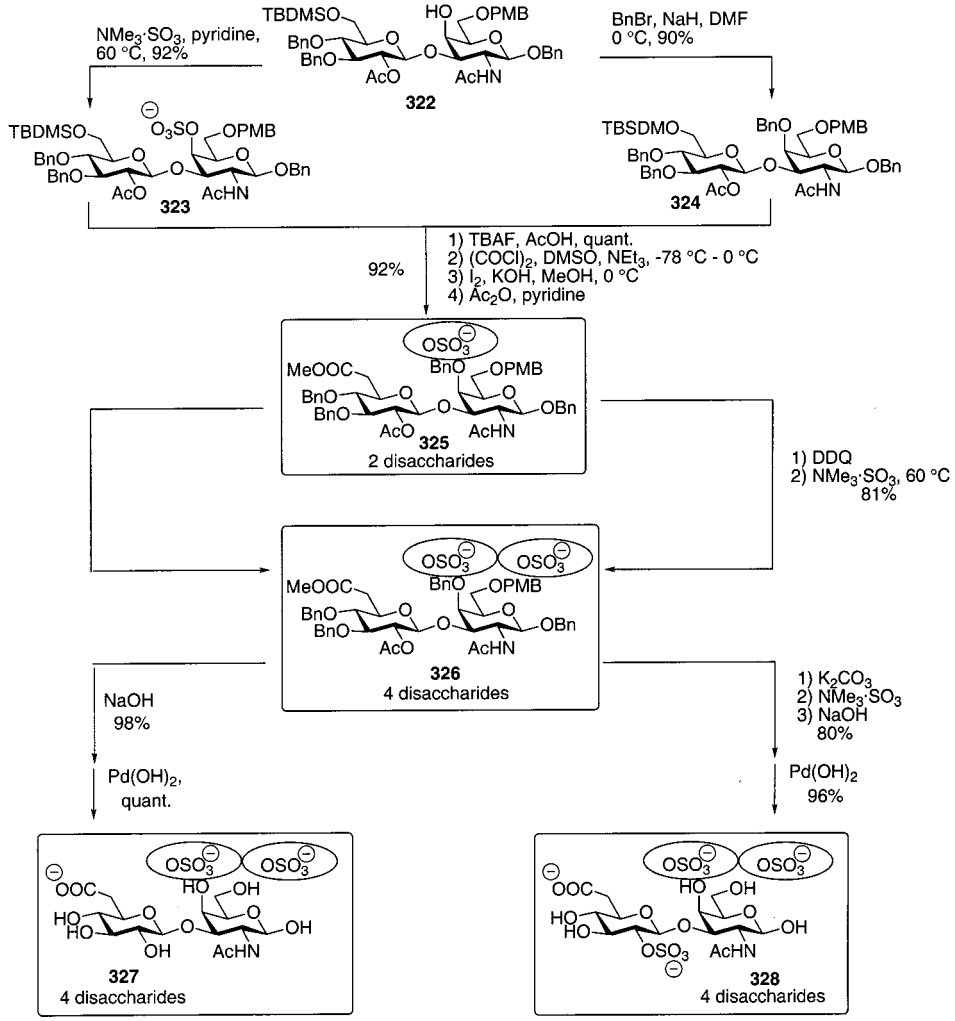
Lubineau and Bonnaffé used a split-and-mix approach to generate a small library of chondroitin sulfate disaccharides containing all eight possible sulfoforms (Scheme 58).[180] Starting from the differentially protected key disaccharide **322**, the C3-position of galactosamine was sulfated and benzylated in parallel reactions. After pooling all compounds, deprotection and oxidation of the C6 position of glucose furnished glucuronic acid derivatives **325**. Splitting and partial sulfation yielded library **326**. After splitting the library in half again, one part was saponified and deprotected to give the chondroitin sulfate sublibrary **327**. The other part was selectively deacetylated and *O*-sulfated to furnish sublibrary **328** after saponification and hydrogenation. Apart from the fact that this was the first approach to make all three natural chondroitin sulfation sites accessible for chemical sulfation, the use of the sulfate group as a means of protection during the synthesis of this library was remarkable.

Solution-phase split-and-mix approaches that are in principle capable of generating diverse libraries containing large numbers of compounds generally suffer from difficulties regarding hit identification and isolation. These problems are particularly severe in the case of oligosaccharide libraries that contain many compounds of identical mass that only differ in the stereochemistry of the anomeric center.

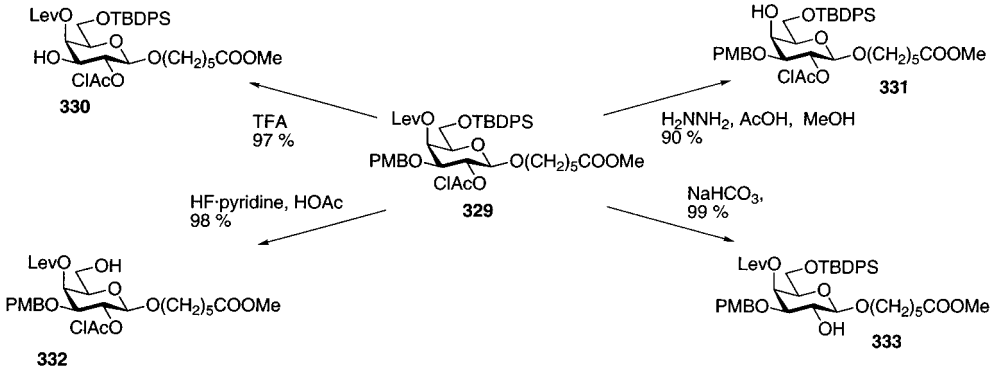
To realize a higher complexity and diversity of separable oligosaccharide libraries, parallel synthesis that includes more synthetic steps and creates defined glycosidic linkages is preferable. This approach requires a highly orthogonal protecting-group strategy. Relying on a central, fully differentially protected galactoside building block, a route to a virtual library of almost 40 000 branched pentasaccharides was presented.[181] Forty-five defined oligosaccharides were prepared as a proof-of-principle. Each of the four protecting groups could be selectively removed, and the free hydroxyl group was glycosylated with seven different donors (Scheme 59).

An important milestone in the development of combinatorial carbohydrate libraries was Kahne's synthesis of a 1300 member library of acylated amino di- and trisaccharides following a split-and-mix protocol.[73] Glycosyl sulfoxides were used as donors for solid-phase glycosylations on TentaGel resin (Scheme 60). Six different C2 azidosugars served as the first carbohydrate moiety, and 12 mono- and disaccharides constituted the second building block resulting in up to 72 different di- and trisaccharides. Reductive conversion of the azides into amines allowed for further differentiation using 18 acyl groups. On-bead screening against a bacterial lectin from *Bauhinia purpurea* was performed using a colorimetric assay to detect structures that bound the target. Compounds on selected beads were identified using Still's tagging technique.[182] Two carbohydrates that exhib-

Scheme 58



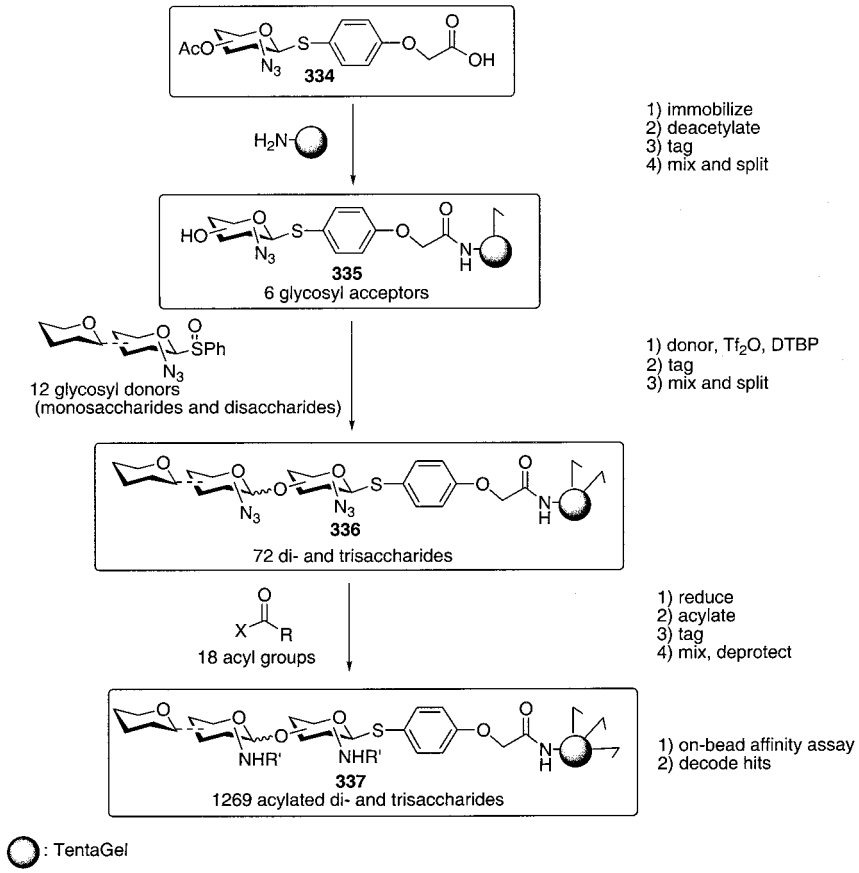
Scheme 59



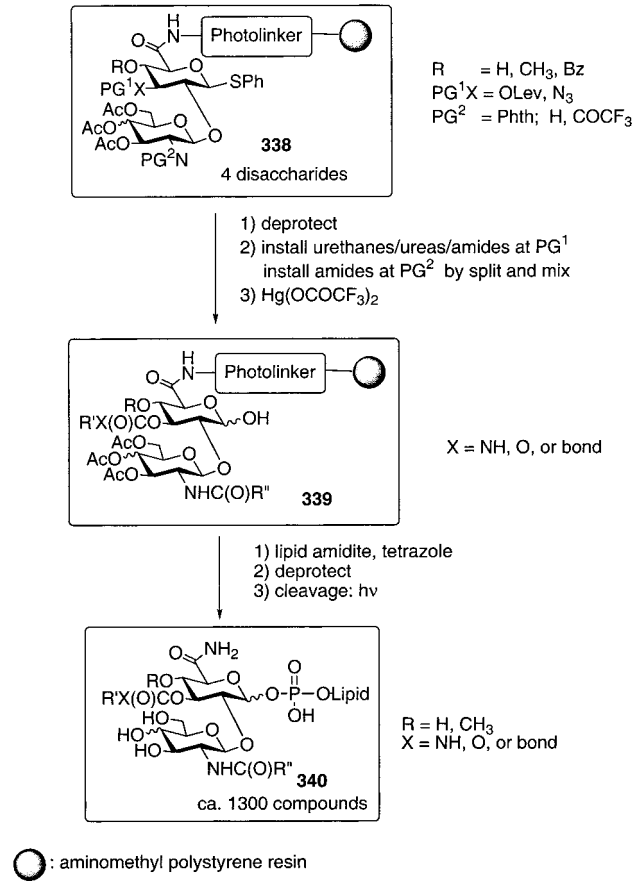
ited a higher affinity than the known natural ligand were identified by this assay.

Applying the idea of combinatorial acylation to an oligosaccharide library, the synthesis of a series of simplified moenomycin A analogues using solid-phase methodology was recently reported (Scheme 61).[183] The synthesis was carried out using the acceptor-bound strategy with the core aminoglucuronic moiety attached via a C6 amide bond to a photocleavable linker. This method allowed coupling of the fully differentiated library to the sensitive phospholipid in the last step prior to deprotection and cleavage from the resin. Four disaccharides were installed on the solid support bearing two differentially protected amine functionalities. Introduction of different *N*-acyl-, urethane-, and urea derivatives, followed by lactol formation at the reducing sugar and coupling to a range of different phospholipids, afforded a library of 1300 compounds. A directed split-and-mix synthesis was carried out using the IRORI technology.[184] Screening of the library for antibiotic activity against sensitive and resistant strains identified several potent antibiotic disaccharides. Previously, the synthesis of a closely related acylated disaccharide library lacking the degree of diversity in the carbohydrate backbone had been described.[103]

Scheme 60



Scheme 61

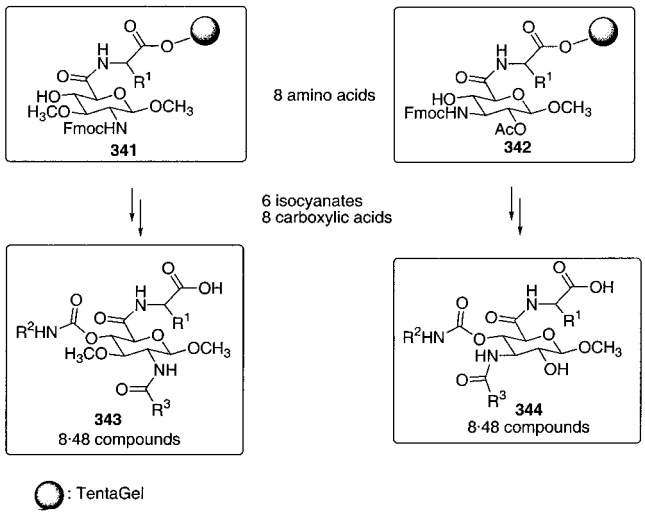


B. Carbohydrates as Scaffolds for Combinatorial Libraries

The diverse arrangement of hydroxyl groups around a monosaccharide core makes these structures ideal scaffolds for the preparation of diverse libraries. Hirschmann, Nicolaou, and Smith described potent β-D-glucose-derived mimetics of a cyclic hexapeptide somatostatin (SRIF) agonist.[185] After establishing that appropriately substituted carbohydrates can effectively serve as peptidomimetics,[186] much effort was devoted to the construction of libraries of differently functionalized monosaccharides. Since monosaccharides are enantiomerically pure, often possess a rigid conformation, and exhibit a high degree of functionalization, they provide a set of characteristics very suitable for combinatorial chemistry platforms. Hirschmann et al. reported binding studies of several derivatives using D- and L-glucose and L-mannose scaffolds, which revealed that potent ligands for various receptors can be generated by this method. Altering the sugar backbone or the substituents resulted in changes in the specificity of the compounds.[187]

The synthesis of a library based on 2-deoxy-2-aminoglucuronic acid and 3-deoxy-3-aminoglucuronic acid scaffolds bearing three differentiated sites for the introduction of diversity on a trityl-functionalized TentaGel was described by Sofia (Scheme 62).[188] A peptidic linker to the solid-phase was introduced as the first combinatorial element. Further diversity was created by carbamate formation at the free hydroxyl using a set of six different isocyanates,

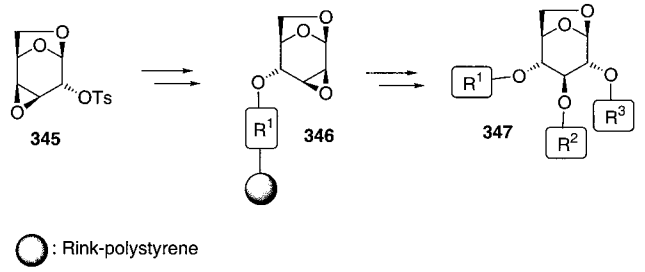
Scheme 62



followed by deprotection and acylation of the amino group with eight different carboxylic acids. The 16 48-member libraries **343** and **344** prepared in a directed sorting parallel synthesis using the IRORI radio frequency tagging system[184] were released by treatment with acid.

Another approach based on a differently substituted central carbohydrate building block for combinatorial solid-phase synthesis was presented by Brill (Scheme 63).[189] Immobilization of anhydrosugar **345**

Scheme 63



on amino-functionalized Rink−polystyrene resin provided scaffold **346** exposing three elements of diversity that were accessed by epoxide-opening reactions to create levoglucosan library **347**.

Carbohydrate scaffolds found further use in the solution-phase synthesis of a combinatorial neamine library with increased diversity due to substitutions at the C6′ amino functionality.[190] Another disaccharide based on tunicamycin bearing an amino and a hydroxyl group as sites of library diversification was recently described as a scaffold for combinatorial library formation.[191]

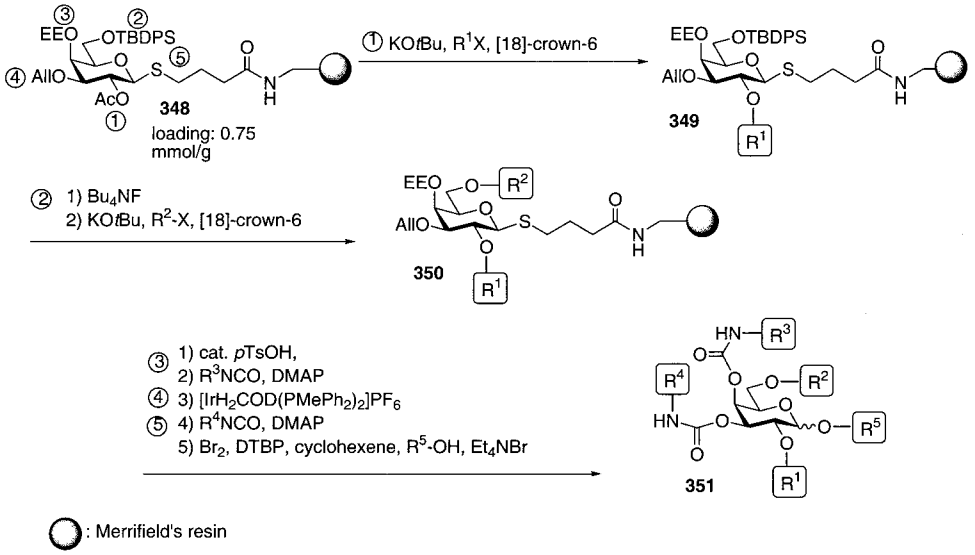
Recently, Kunz reported an improved orthogonal protection scheme for a central galactose scaffold in solid-phase combinatorial synthesis.[109] In addition to the previously described four degrees of orthogonality,[107] cleavage from the solid support by action of bromine furnished a galactosyl bromide. The galactosyl bromide served in turn as a glycosyl donor by activation with tetraethylammonium bromide (Scheme 64).

C. Special Methods for the Construction of Carbohydrate-Related Libraries

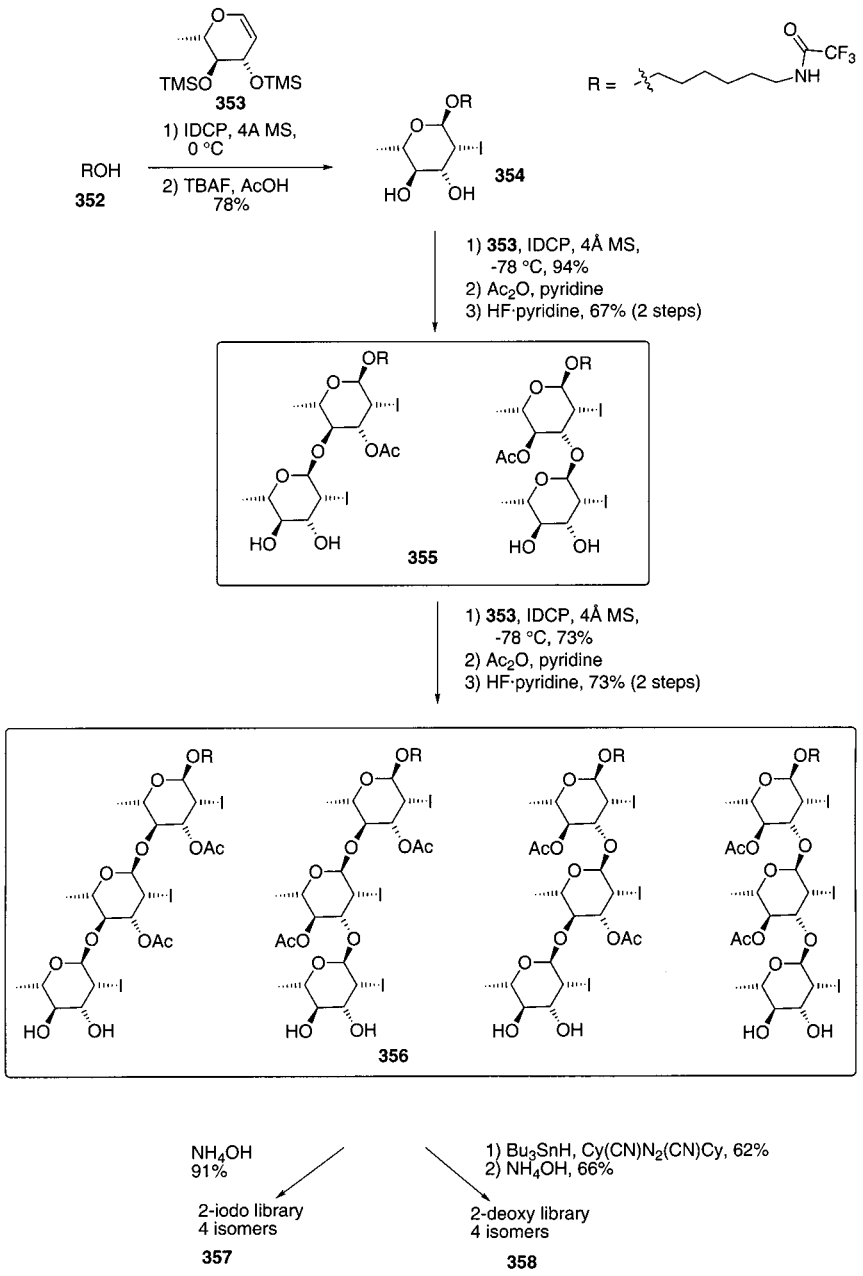
Enzymatic methods have also been successfully employed in the synthesis of oligosaccharide libraries. Acylated sialyl-Lewis^a- and sialyl-Lewis^x-analogue libraries bearing different acyl groups as well as natural and unnatural α-linked pyranosides in lieu of fucose were prepared.[192] Fucosyl transferase III was used for the glycosylation of a sialyl lactosamine residue and was found to be widely tolerant to an array of fucose analogues. More of the important enzymatic work in this field has been reviewed previously.[193]

Combining features of the "random glycosylation" and the split-and-mix strategies, Ichikawa's group synthesized a library of 2,6-dideoxy trisaccharides (Scheme 65).[194] Glycal **353** was coupled to acceptor **352** and desilylated. Random glycosylation with glycal donor **353** afforded library **355**. After capping the unreacted hydroxyl groups as acetates, desilylation, and further glycosylation, the resulting library **356** was split. *O*-Deacetylation or dehalogenation

Scheme 64



Scheme 65



followed by deacetylation furnished 2-iodo- and 2-deoxy sublibraries **357** and **358**.

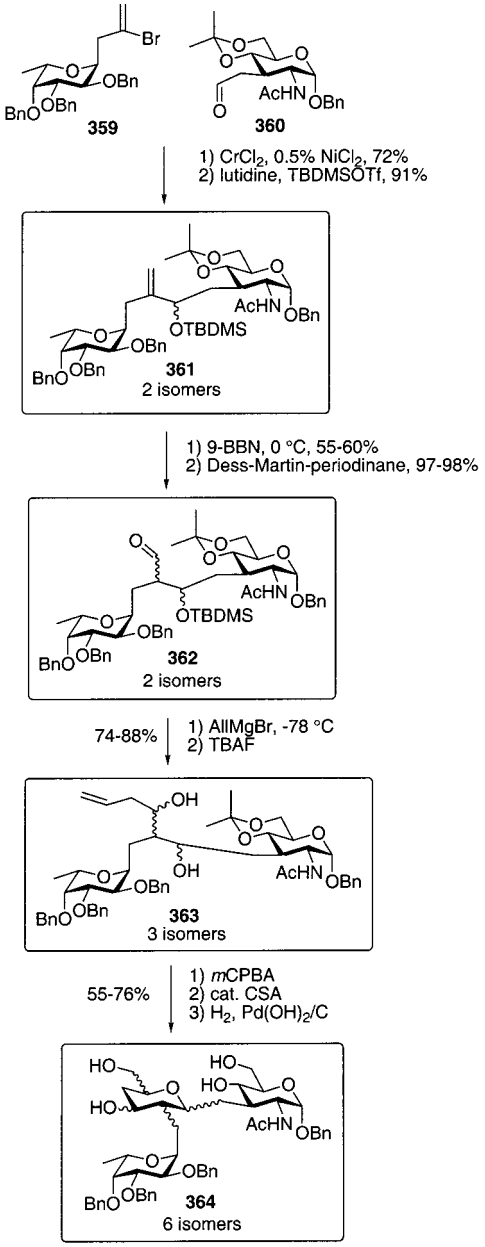
A larger library of 2-deoxy mono- and disaccharides as well as 2-deoxyortho esters and 2,3-unsaturated 2-deoxyortho esters was prepared recently in a parallel synthesis both in solution and on solid support by Nicolaou et al. using methodology described in section IX.C.[136]

Armstrong's approach to the synthesis of *C*-di-[195] or trisaccharide libraries relied on the de novo synthesis of a sugar.[196] The divergent synthesis of analogues of the H-type 1 blood-group determinant involved the reductive coupling of **359** and **360** followed by stereoselective hydroboration and Dess–Martin oxidation to afford the aldehydes **362** (Scheme 66). Addition of allylmagnesium bromide, cyclization, and deprotection resulted in the formation of six diastereomeric *C*-trisaccharides in three sublibraries. The flexibility of this route was demonstrated by the synthesis of some trisaccharides exhibiting different hydroxyl patterns in the central carbohydrate residue. A recursive stereochemical deconvolution strategy[197] was proposed to rapidly identify hits if stereochemically biased reactions for the de novo synthesis are available.

Fessner developed a de novo enzymatic strategy for the preparation of a *C*-disaccharide library (Scheme 67).[198] Syntheses of diverse monosaccharide derivatives on a solid support were described by Kobayashi.[199] Furthermore, a number of combinatorial libraries of oligosaccharide mimics based on the coupling of carbohydrate-derived amino acids on a rigid scaffold[200] or combinatorial oligomerization[201] have been reported.

The Ugi four-component condensation[202] was used to build up a scaffold bearing one,[203] up to three,[204] or four[205] carbohydrate moieties in a further approach to the combinatorial synthesis of glycoconjugates.

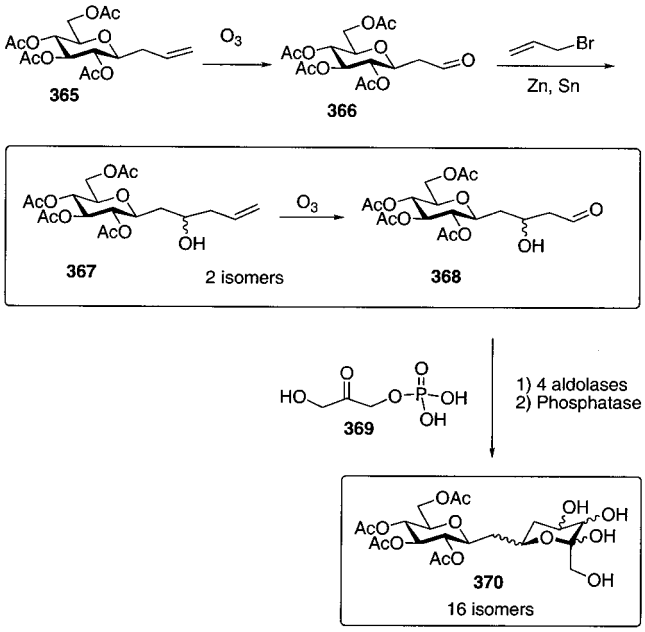
Scheme 66



Employing a set of appropriately functionalized sugar monomers, a library of 20 neoglycoconjugates was prepared in solution.[205] Since this one-pot reaction can be performed under standardized conditions, it holds potential for the automated preparation of glycopeptides. Wessel et al. describe the synthesis of a small libary of carbohydrate-anellated dihydroquinolines using a three-component one-pot reaction.[206]

Bols recently prepared the first combinatorial library of glycosidase inhibitors based on the iminosugar 1-azafagomine coupled to a variable peptide chain.[207] Another recent example of a library based on an unchanged carbohydrate monomer derivatized with variable aglycons was the synthesis of a 1-thio-β-D-galactopyranoside library synthesized in order to find potent ligands for galactose recognizing plant lectins, demonstrating C-18 solid-phase extraction as a facile purification method.[141]

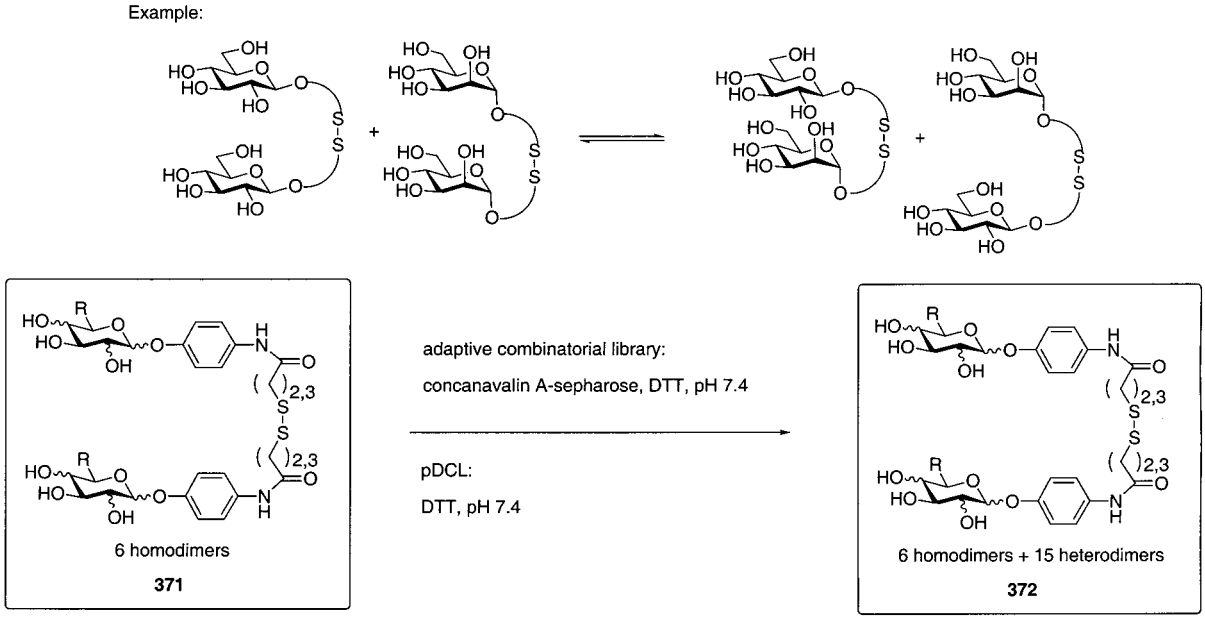
Scheme 67



Lerchen reported an approach to lectin-mediated drug targeting using fucose-modified epitopes on neoglycoconjugates for recognition by colon cancer cells.[208] A small library of fucose epitopes was synthesized and coupled to the fluorescent cytostatic agent batracyclin in the form of bovine serum albumin (BSA) conjugates. Screening for cellular uptake and cytotoxicity revealed one neoglycoconjugate with a high degree of specificity and cytotoxicity for a tumor cell line.

Dynamic combinatorial chemistry is an emerging and exciting concept for the synthesis of target-addressing, self-adjusting libraries.[209] Lehn very recently reported the first application of this concept to the synthesis of a library of disulfide-spacer tethered oligosaccharide mimics.[210] Exploiting the rapid interconversion between thiols and disulfides that can be switched on or off simply by changing the pH of the solution, two approaches were followed to find concanavalin A binding structures, mimicking the natural trimannoside ligand. A set of six different homodimers **371**, including mannose, galactose, glucose, arabinose, and xylyl headgroups and different spacer lengths (Scheme 68), was chosen as a starting mixture in a model experiment. Action of dithiothreitol at pH 7.4 initiated scrambling of the monomers, which was carried out in the presence of sepharose-bound lectin concanavalin A (adaptive combinatorial library/self-adjusting virtual combinatorial library) and without lectin (preequilibrated dynamic combinatorial library, pDCL). In the latter case a nearly statistical mixture of 21 homo- and heterodimers was obtained. Addition of immobilized lectin after the equilibrating scrambling process followed by filtration revealed that only mannose-containing structures were bound to the lectin with a marked predominance of the mannose homodimer (1.5:1). Interestingly, the adaptive protocol lead to an increased (2.1:1) selectivity in favor of the mannose homodimer, indicating that the presence of the lectin acts as a thermodynamical trap in the combinatorial

Scheme 68



process. These concepts may greatly facilitate the search for lectin-binding oligosaccharide mimics in the future. The preequilibrated protocol may be particularly useful in cases where the lectin is incompatible with the equilibrating conditions.

These recent examples of combinatorial methods for the identification of biologically active carbohydrate structures show a shift in the focus of library design. Variations around known core structures have replaced large random libraries. Further progress in solid-phase oligosaccharide chemistry combined with different approaches to combinatorial and parallel synthesis including dynamic combinatorial approaches are expected to facilitate the rapid identification of oligosaccharide ligands which interact with a host of receptors. Selection of non-carbohydrate ligands or carbohydrate hybrids may prove to be a source for interesting molecular tools and potentially new drugs.

XII. Toward Automation of Oligosaccharide Synthesis

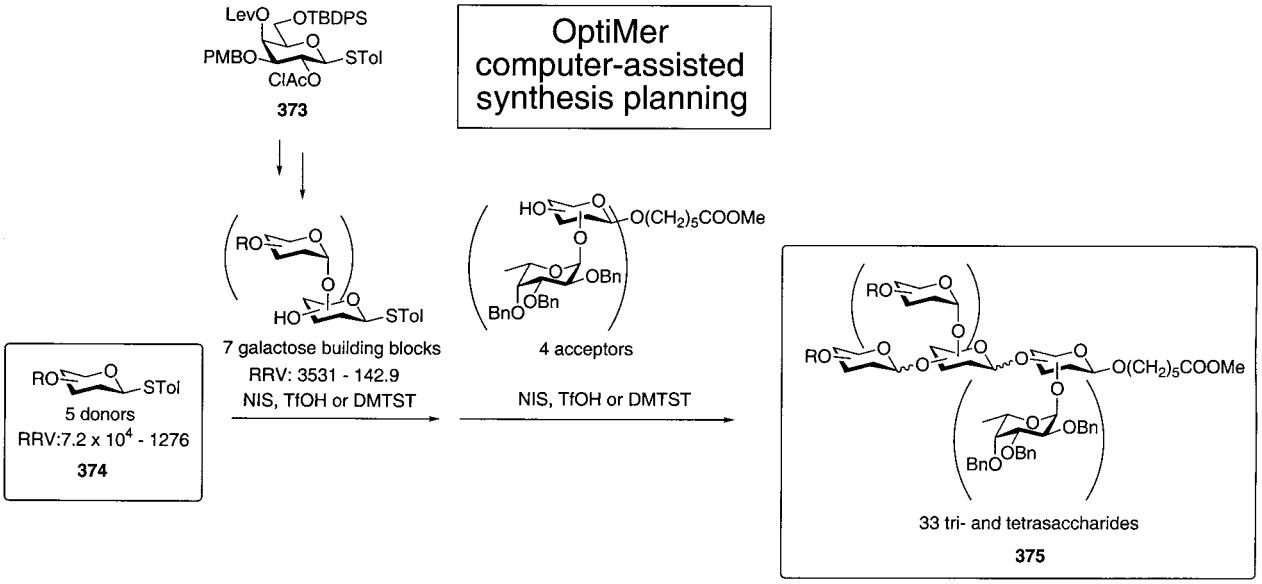
An ultimate goal of research in modern oligosaccharide synthesis is the development of an automated oligosaccharide synthesizer. Such a machine would ideally render complex carbohydrate structures available to any laboratory, enabling researchers to focus on exploring the many biological functions of these still poorly understood biooligomers. Recent years have seen some promising steps toward this end.

A. Solution-Phase Approaches

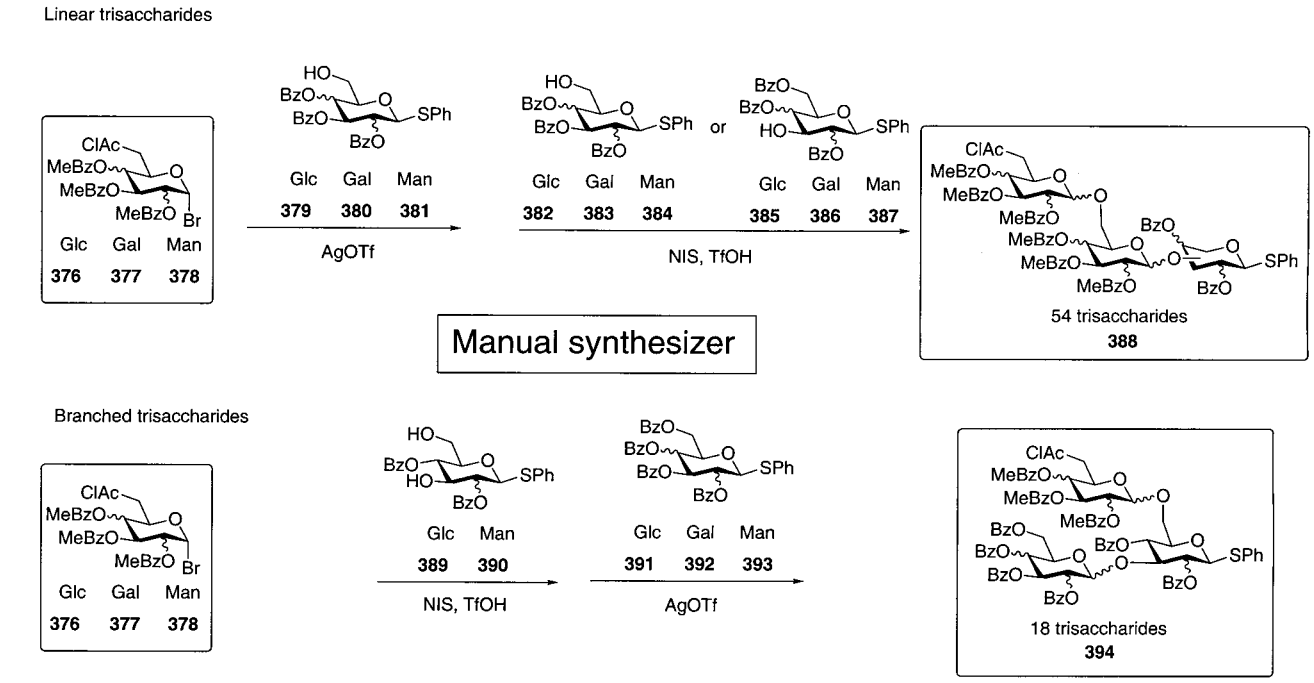
One-pot sequential glycosylation[87,211] depending on decreasing donor reactivity from the nonreducing to the reducing end of the growing oligosaccharide chain is an attractive synthetic method as it reduces the number of chromatographic steps at the end of the synthesis. In an extensive study Wong and coworkers recently evaluated and mapped 50 diverse and differently protected *p*-methylphenyl thioglycosides. The data of their relative anomeric reactivities (relative reactivity value, RRV) was obtained in competing glycosylation reactions and laid the basis for the development of a computer-assisted automated synthesis planning program ("OptiMer"), following the one-pot sequential glycosylation paradigm.[212] This software proposes an optimal one-pot donor sequence for a given linear (or branched if branched disaccharide donors are employed) oligosaccharide in terms of theoretical yield which ideally requires identical and high ratios of donor/acceptor reactivity on each glycosylation step. So far, this approach has only been applied to thiodonors using NIS/triflic acid or DMTST as promoting systems and it does not take into account losses in donor reactivity after glycosylation nor eventual side reactions. Nevertheless, additional studies will further increase the scope of this method and make it a valuable tool for oligosaccharide synthesis planning. A small library of 33 tri- and tetrasaccharides was synthesized,[213] based on differentially protected central galactose building block **373** previously employed in the construction of a pentasaccharide library.[181] Using the OptiMer synthesis planning methodology, a set of five different, highly reactive *p*-methylphenyl thioglycosides was chosen as nonreducing end sugars, seven differently deprotected and glycosylated derivatives of **373** exhibiting a medium RRV constitued the second carbohydrate moiety, while the reducing end was formed by three glucosamine and one disaccharide acceptor (Scheme 69).

A further step further toward full automation of oligosaccharide synthesis was recently demonstrated by Takahashi et al. With the prospect of studying DNA-cleaving selectivity of combinatorially glycosylated endiynes, these researchers described the rapid synthesis of linear and branched trisaccharide libraries employing one-pot sequential glycosylation protocols on a manual Quest 210 synthesizer.[214] Parallel synthesis of 54 linear trisaccharides was accomplished using three glycosyl bromides that carried a C6 ClAc protecting group for future conjugation with the enediyne moiety. These glycosyl bromides were

Scheme 69



Scheme 70



coupled under silver triflate activation with three phenyl thioglycosides to yield disaccharides that were reacted in turn with six acceptor glycosides (Scheme 70). A library of 18 branched trisaccharides was obtained by regioselectively coupling the glycosyl bromides to glucose and mannose acceptors **389** and **390**, each bearing two free hydroxyl groups. Subsequent glycosylation with perbenzoylated phenyl thioglycosides **391**−**393** furnished library **394**. All members of these libraries were isolated in good to excellent yield (64−99%), underscoring the efficiency of this approach.

B. Automation of Solid-Phase Oligosaccharide Synthesis

Oligosaccharide synthesis on solid supports offers the opportunity for automation even more than solution-phase methodologies. With a versatile linker technology and powerful glycosyl donors available, the first fully automated syntheses of oligosaccharides have been carried out in our laboratory using an appropriately modified peptide synthesizer.[215] Choosing the synthesis of linear heptamannoside **241** (Scheme 45) as a first model target, careful optimization of the reaction conditions employing mannosyl trichloroacetimidate **95** as glycosyl donor furnished the resin-bound heptamer within 19 h in a fully automated fashion without manual intervention in ~40% overall yield (HPLC). This yield was considerably higher than that achieved in the manual protocol (9%).[86]

Automated synthesis of complex branched oligosaccharide structures is a very important reality test for any synthesizer technology. Employing glycosyl phos-

phate donors, a hexasaccharide fragment of the phytoalexin elicitor β-glucan structure was prepared on the synthesizer in a fully automated process.

XIII. Conclusion and Outlook

Advances in glycobiology depend heavily upon the ability of synthetic organic chemists to provide defined compounds for biochemical and biophysical studies. Driven by the need to create more efficient methods for the synthesis of complex oligosaccharides, the past 10 years have seen a major push toward a reliable procedure for the solid-phase synthesis of oligosaccharides. Building upon strategies first outlined in the 1970s, the advent of new glycosylation procedures provided fertile ground for rapid developments. New linker systems and different solid support materials have been evaluated as have a variety of protecting groups. The development of on-resin analytical techniques such as HR-MAS NMR greatly facilitated the introduction of innovative solid-phase synthetic methods. Chemical and enzymatic methods were explored on soluble and insoluble polymeric supports, and methodological advances were illustrated by the preparation of large oligosaccharides. As the coupling yields have improved to 95% and sometimes above, larger structures have come within reach. In addition to single target structures, carbohydrate-based combinatorial libraries have generated much interest. These efforts are crucial for providing molecular tools to elucidate biologically important interactions.

The developments in the areas of new protecting groups, improved linker systems, and more powerful and versatile coupling agents have now brought about a situation in which automation is about to become a reality. Still, different glycosyl donors may be needed for the effective formation of a variety of linkages. It can now be anticipated that studying reactivities and optimizing conditions, necessary to obtain consistently high coupling yields for each new linkage, will eventually lead to a stage where automation of synthesis planning and execution will allow even the nonspecialist to create important molecular tools for biochemical, biophysical, and medical applications.

XIV. List of Abbreviations

AIBN	azo bisisobutyronitrile
All	allyl
Anthr	9-anthryl
Azb	*p*-azidobenzyl
BAL	backbone amide linker
9-BBN	9-borabicyclo[3.3.1]nonane
Bz	benzoyl
cat.	catalytic, catalyst
Cbz	benzyloxycarbonyl
ClAzb	*p*-azido-*m*-chlorobenzyl
CPG	controlled pore glass
DAST	diethylaminosulfur trifluoride
DBBOTf	dibutylboron triflate
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexyl carbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIC	2-dimethylaminoisopropyl chloride hydrochloride
DMAP	4-(dimethylamino)pyridine
DMDO	dimethyl dioxirane
DMM	dimethyl maleoyl
DMT	dimethoxytrityl
DMTST	dimethylthiosulfonium triflate
DMTSB	dimethylthiosulfonium tetrafluoroborate
DNB	dinitrobenzoyl
DOX	dioxyxylyl
DRIFTS	diffuse reflectance infrared Fourier transform spectroscopy
DTBP	2,6-di-*tert*-butyl pyridine
Dts	dithiosuccinoyl
DTT	dithiothreitol
EE	ethoxyethyl ether
Fmoc	9-fluorene methyloxycarbonyl
HBTU	*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HMBA	hydroxymethylbenzoyl
$h\nu$	irradiation (λ = wavelength)
HR-MAS	high-resolution magic angle spinning
IDCP	iodonium di(*sym*-collidine)perchlorate
IIDQ	2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline
Lev	levulinoyl
LHMDS	lithium hexamethyldisilazane
m-CPBA	*m*-chloroperoxybenzoic acid
MDOX	α-monomethyl dioxyxylyl
MPEG	monomethyl poly(ethylene glycol)
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
Nle	norleucine
NPG	*n*-pentenyl glycoside
PA	phenoxyacetyl
PAB	*p*-pivaloylamino benzyl
PEG	poly(ethylene glycol)
PBB	*p*-bromobenzyl
PCB	*p*-chlorobenzyl
pDCL	preequilibrated combinatorial library
Phth	phthaloyl
PIB	*p*-iodobenzyl
Piv	pivaloyl
PMB	*p*-methoxy benzyl
POEPOP	polyoxyethylene−polyoxypropylene
POEPS-3	polyoxyethylene−polystyrene
PS	polystyrene
PyBOP	benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate
quant.	quantitative
RCM	ring-closing metathesis
refl.	reflux
RRV	relative reactivity value
SE	trimethylsilylethyl
SEM	trimethylsilylethyleneoxymethyleneoxy
SPOCC	polyoxyethylene−polyoxetane
Suc	succinoyl
TBABr	tetrabutylammonium bromide
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TES	triethylsilyl
TFA	trifluoroacetic acid
THP	tetrahydropyranyl
TIPS	triisopropylsilyl
TMSOTf	trimethylsilyl trifluoromethane sulfonate
TMU	tetramethylurea
Tol	*p*-methoxyphenyl
Tr	triphenylmethyl (trityl)
Troc	trichloroethoxycarbonyl

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