Oligosaccharide Synthesis in Solution and on Solid Support with Glycosyl Phosphates

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The synthesis of glycosyl 1-phosphate triesters and their use in solution-phase and solid-phase oligosaccharide chemistry is reviewed. The preparation of anomeric phosphates from lactol and glycal starting materials and their application in the synthesis of nucleotide diphosphate sugars is described. Glycosyl phosphates have been employed successfully in the construction of *O*- and *C*-glycosides and give highly *trans*-selective coupling products even in the absence of C-2 participating groups. Solid-phase techniques utilizing glycosyl phosphate triesters have enabled the assembly of biologically important complex carbohydrates. Most recently, glycosyl phosphates have been the basis for the development of an automated oligosaccharide synthesizer.

Introduction

The role of carbohydrates and glycoconjugates in biological signaling pathways is now well appreciated.[1,2] The details of this information transfer process, however, remain to be unraveled and have sparked much interest in oligosaccharide chemistry and biochemistry. Cell−cell recognition events mediated by cell−surface glycoconjugates underlie many disease manifestations, such as cancer and viral infections.[3,4] Specific carbohydrates have been identified as markers for certain types of tumors and as binding sites for viral and bacterial pathogens. Oligosaccharides are involved in the blood coagulation cascade, wound healing, and nerve growth.[5,6]

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Emma R. Palmacci (left) was born in Melrose, MA in 1976. She attended Worcester Polytechnic Institute and worked under the direction of Prof. James P. Dittami on novel photocyclization reactions. After graduating from WPI in 1998 with a B.S. in chemistry, she moved to MIT to begin her doctorate studies. She is currently a member of the Seeberger laboratory and is working on the automated solid-phase synthesis of interesting oligosaccharides, such as glycosaminoglycans.

Peter H. Seeberger (center) received his B.S. in 1989 from the Universität Erlangen-Nürnberg, where he studied chemistry as a Bavarian government fellow. In 1990 he moved as a Fulbright scholar to the University of Colorado, where he earned his Ph.D. in biochemistry under the guidance of Marvin H. Caruthers in 1995. After a postdoctoral fellowship with Samuel J. Danishefsky at the Sloan-Kettering Institute for Cancer Research in New York City, he began his independent career at the Massachusetts Institute of Technology in January 1998. Since 1999 he has been the Firmenich Assistant Professor of Chemistry. His research interests focus on the interface of chemistry and biology and in particular on the role of complex carbohydrates and glycoconjugates in information transfer in biological systems. His group has developed new methods for the automated solid-phase synthesis of complex carbohydrates and glycosaminoglycans that serve as molecular tools. Other interests include synthetic methodology, total synthesis, immunology, and biochemical and biophysical studies of carbohydrates. He has received a Research Corporation "Research Innovation" Award (1999), an Edgerly Science Partnership Fund Award (1999), a Mizutani Foundation for Glycoscience Award (1999), was named a Liebig Fellow (1997) and a GlaxoSmithKline Chemistry Scholar (2002), and received the Technology Review Top 100 Young Innovator Award (1999) for the development of an automated oligosaccharide synthesizer.

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The limited supply of pure, structurally defined complex carbohydrates and glyconjugates continues to impede the field of molecular glycobiology. These structures are found only in small amounts in nature and are often difficult to isolate in pure form. The procurement of sufficient quantities of defined oligosaccharides required for detailed biophysical and biochemical studies therefore relies on efficient synthetic methods. While much progress has been made in oligosaccharide synthesis, the construction of complex carbohydrates remains challenging and is carried out in a small number of specialized laboratories.

The preparation of synthetic oligosaccharide sequences presents a significant challenge to the organic chemist. The desired structures possess a myriad of hydroxy groups, are often highly branched, and require the stereospecific formation of glycosidic linkages. Sophisticated and versatile protecting group schemes are necessary to allow for the regio- and stereospecific synthesis of biologically interesting molecules. In order to construct a glycosidic bond, an acceptor hydroxy group is exposed and serves as a nucleophile in the reaction with an activated donor. Repetition of this deprotection-glycosylation procedure affords the oligosaccharide sequence of the desired length. Unfortunately, a purification step is necessary after each manipulation, thus rendering the synthesis of larger structures extremely time-consuming.

Advances in the development of versatile and powerful glycosylating agents have provided access to complex molecules of interest.[7,8] Most notably, glycosyl trichloroacetimidates, thioglycosides, glycosyl sulfoxides, fluorides, and *n*-pentenyl glycosides have found application in the synthesis of complex oligosaccharides.[8] Limitations of these glycosyl donors, such as lengthy syntheses and long reaction times, fuel current efforts directed at the development of new glycosylating agents. Similarly, continued investigations into new protecting groups for oligosaccharide synthesis have yielded novel means for hydroxy group differentiation.[9] One-pot procedures have been developed to alleviate the need for multiple protecting group manipulations and the purification and isolation of intermediates.[10−12]

While solution-phase methods are the most frequently utilized for the construction of oligosaccharides, solid-phase chemistry has seen a recent surge in interest and has allowed complex molecules to be synthesized in a rapid and efficient fashion.[13] Reaction sequences performed on solid-phase matrixes may benefit from the use of excesses of reagents and the elimination of intermediate purification and isolation steps. Any impurities not bound to the support can be removed simply by washing the resin, and the final product is obtained after cleavage from the support and a single purification step. Peptides and nucleic acids are routinely synthesized on solid supports in an automated fashion.[14,15] While the synthesis of oligosaccharides has been aided by the evolution of solid-phase methods, an automated solid-phase oligosaccharide synthesizer would ultimately have a huge impact on the field of glycobiology. Recent advancements in carbohydrate synthesis have brought a generally available automated oligosaccharide machine within reach.[12,16]

Nature employs glycosyltransferases to catalyze the formation of new glycosidic linkages. These enzymes utilize nucleotide diphosphosugars (NDPs) as substrates.[17,18] The preparation of NDPs is most commonly accomplished through the coupling of glycosyl 1-phosphates and nucleoside 5′-monophosphates. The variety of procedures available for the synthesis of anomeric phosphates was previously in stark contrast to the limited uses of glycosyl phosphates as glycosylating agents. Recent advancements, however, have resulted in increased interest in phosphorus-based glycosyl donors.

Here we discuss the chemical synthesis of glycosyl 1-phosphates and their application in oligosaccharide construction. The focus of this review is on chemical methods and not enzymatic approaches, which have been the subject of excellent recent reviews.[19] The synthesis of anomeric phosphates from lactol and glycal starting materials and the use of glycosyl 1-phosphates in the synthesis of NDPs is summarized. Next, the utilization of glycosyl phosphate triesters in the construction of *O*- and *C*-glycosides is discussed. Finally, solid-phase techniques employing glycosyl phosphates for the automated synthesis of complex structures are evaluated.

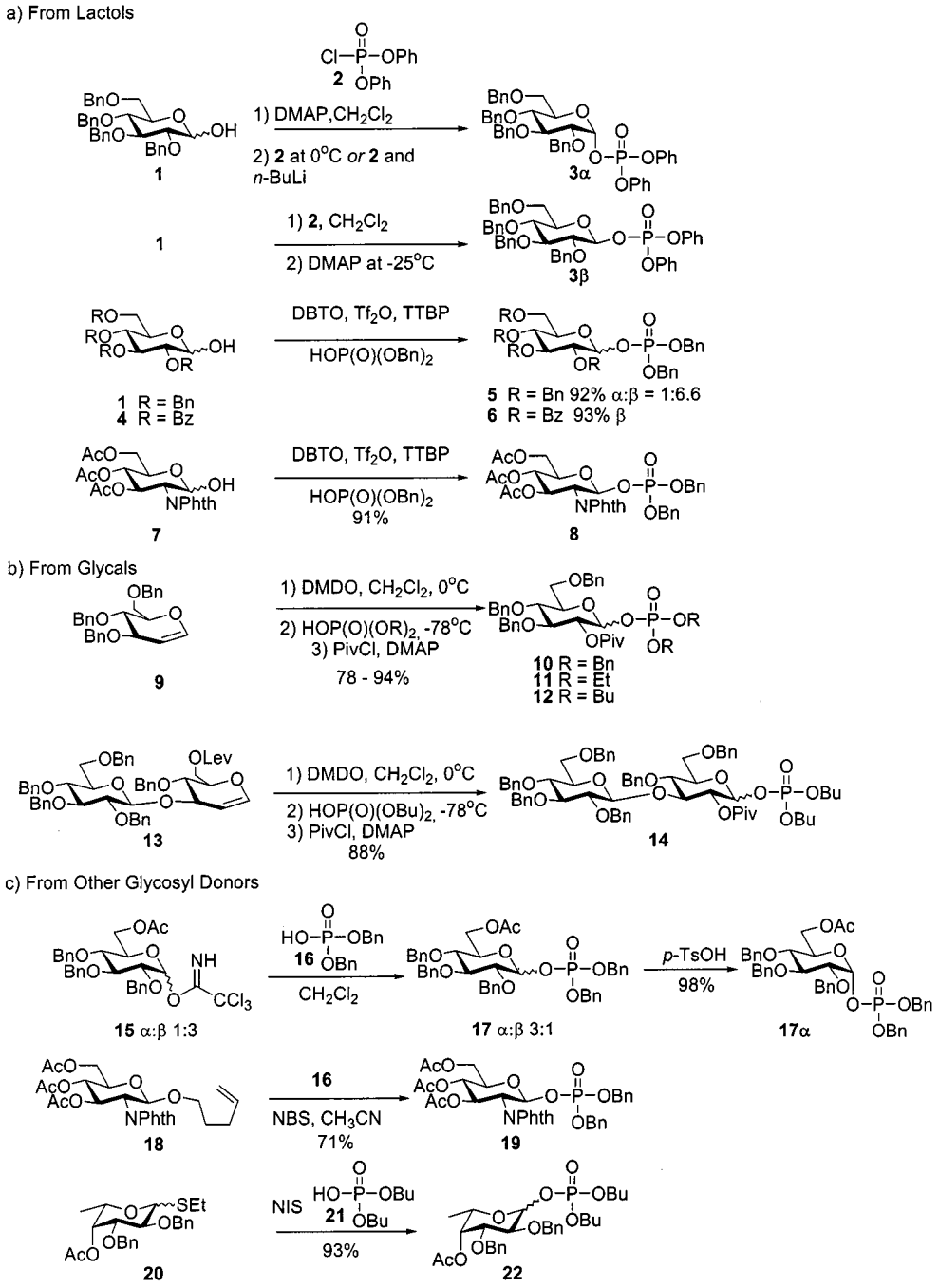
Synthesis of Glycosyl Phosphate Triesters

The generation of anomeric phosphate triesters for use as glycosyl donors and for the synthesis of NDPs has been accomplished from a variety of intermediates. Anomeric lactols and 1,2-anhydro sugars are commonly employed, while derivatization of other glycosyl donors, such as imidates and *n*-pentenyl glycosides, has also seen use in generating glycosyl phosphates. These methods allow for the use of phosphate diesters containing various alkyl and aryl substituents.

Conversion of anomeric lactols into glycosyl phosphates has been accomplished by treatment with an appropriate base and the desired chlorophosphate. Either anomer can be prepared selectively by using diphenyl chlorophosphate (**2**) and DMAP under either thermodynamic or kinetic conditions,[20] as demonstrated in the efficient synthesis of **3α** and **3β**. (Scheme 1a) Similarly, the free reducing sugar **1** has been treated with *n*-butyllithium and **2** to afford glycosyl phosphate **3α**.[21]

In addition, lactol derivatives have been converted into anomeric phosphates by dehydrative glycosylation[22] (Scheme 1a). Selectively protected glucose, galactose, and mannose monosaccharides were treated with trifluoromethanesulfonic (triflic) anhydride and dibenzothiophene oxide (DBTO) to generate a proposed anomeric oxosulfonium species. This reactive intermediate readily underwent substitution with dialkyl phosphate to afford glycosyl donors **5**, **6**, and **8** in good yields.

Glycals are attractive starting materials for the synthesis of glycosyl donors, since they possess only three hydroxy groups that need to be differentiated. Recent exploitation of glycal-derived 1,2-anhydrosugars[23] resulted in the direct



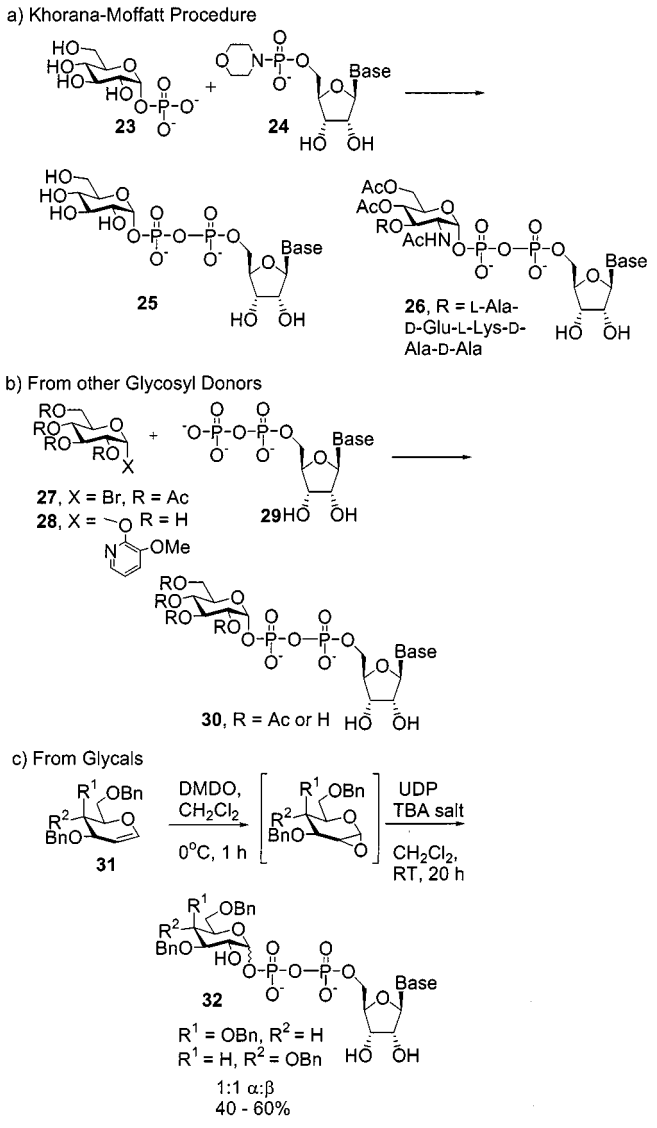
Scheme 1. Synthesis of glycosyl phosphates

construction of glycosyl phosphates in a one-pot procedure.[24] (Scheme 1b) Generation of the anhydrosugar with dimethyldioxirane followed by epoxide opening at low temperature and in situ acylation afforded donors **10**–**12** and **14**. This method worked well for both monosaccharide and disaccharide glycal moieties. Both α- and β-enriched phosphates could be generated with this method, depending on the solvent employed for the opening of the 1,2-anhydrosugar. Coordinating solvents such as THF predominantly afforded α-phosphates, while dichloromethane resulted in β-selective epoxide opening.

The derivatization of glycosyl donor species into anomeric phosphates was initially utilized to construct enzyme substrates for glycosyltransferases. These methods have more recently served as a useful means for the synthesis of glycosyl phosphate donors. Trichloroacetimidate **15** was converted into phosphate **17** in high yield by treatment with dibenzyl phosphate[25] (Scheme 1c). Conversion of the α/β mixture of anomeric phosphates into the more stable α-phosphate was accomplished with *p*-toluenesulfonic acid. Glucosamine *n*-pentenyl glycoside **18** and fucosyl thioglycoside **20** were converted into the anomeric phosphates **19** and

22 by activation in the presence of the phosphate diester[26,27] (Scheme 1c). While the conversion of one glycosyl donor species into a second is not ideal, glycosyl phosphates have successfully been shown to glycosylate hindered or unreactive hydroxy groups, thus making the two-step synthesis of glycosyl phosphates worthwhile in the completion of large structures. Other anomeric groups such as anomeric nitrates,[28] vinyl glycosides,[29] and oxazolines have also been converted into glycosyl phosphates.[30,31]

The development of chemical methods for the synthesis of NDPs has allowed for their use in important biochemical studies and in the enzymatic assembly of oligosaccharides. Until recently, the most widely used synthetic technique was the condensation of an unprotected glycosyl 1-phosphate **23** with a nucleoside 5′-phosphomorpholidate **24**, known as the Khorana−Moffatt procedure[32] (Scheme 2a). Variants of this procedure utilizing fully protected derivatives have been developed in order to overcome solubility problems, as demonstrated in the construction of a UDP-*N*-acetylmuramyl pentapeptide **26** precursor[33] (Scheme 2a).



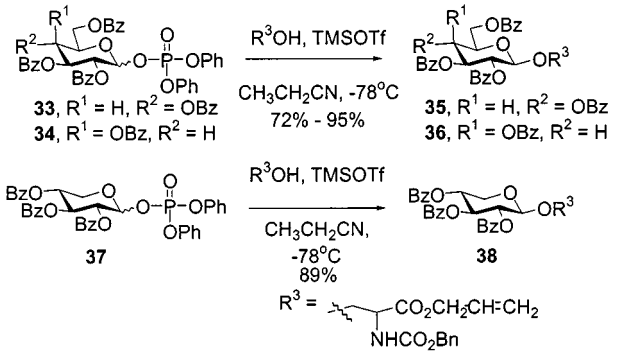
Scheme 2. Synthesis of NDPs

A second route to NDP sugars relies on the direct coupling of a glycosyl donor to a nucleoside diphosphate (Scheme 2b). Since the initial success in coupling anomeric bromide **27** with uridine 5′-diphosphoric acid **29**,[34] other glycosylating agents have also been employed in this context. Anomeric 3′-methoxypyridines such as **28** were used as unprotected glycosyl donors in the synthesis of glycosyl 1-phosphates and in the UDP-Gal and UDP-Glu derivatives.[35]

By utilizing nucleophilic opening of 1,2-anhydrosugars,[24] Klaffke and co-workers have developed an expedient route to NDPs.[36] In a one-step procedure, D-glucal starting material **31** was converted into **32** by epoxide opening with the tetrabutylammonium salt of UDP (Scheme 2c). Removal of the benzyl protecting groups by hydrogenolysis afforded the desired sugar nucleotide diphosphosugar; L-configured glycals were also employed to give the corresponding NDPs.

Use of Glycosyl Phosphates in the Synthesis of *O*-Glycosides

Ikegami and co-workers were the first to investigate the use of glycosyl phosphates as glycosyl donors. Perbenzoylated glycosyl diphenyl phosphates **33**, **34**, and **37** were synthesized and coupled to various acceptors including secondary and hindered alcohols[37] (Scheme 3). Glycosylations were carried out in propionitrile with a stoichiometric amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as an activator and furnished β-linked products in high yields (72−95%) after only 10 min at −78 °C.

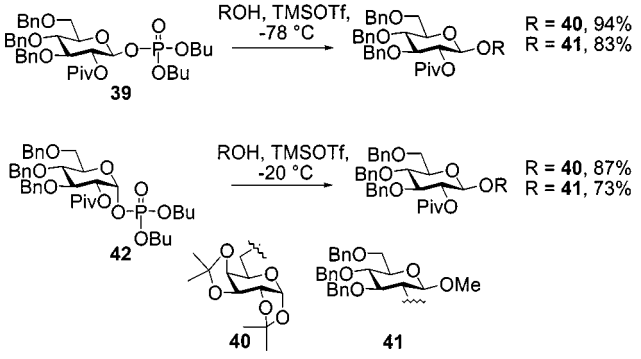


Scheme 3. Use of glycosyl phosphates in β-glycoside construction

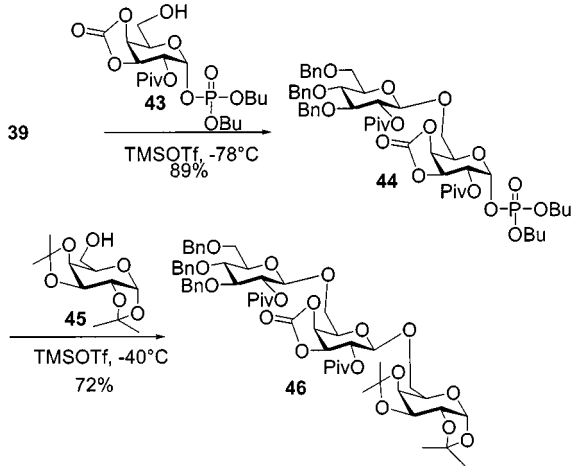
Following this initial disclosure, several other illustrations of successful glycosylations with glycosyl phosphates were reported. Waldmann et al. employed lithium perchlorate ($LiClO_4$) to activate peracetylated glycosyl phosphates for glycoside construction.[38] The neutral conditions allowed for the glycosylation of acid-sensitive groups in moderate yields.

During our investigations of glycosyl phosphates, we discovered that α-configured phosphates such as **42** were more stable than their β-linked counterparts (**39**) and that higher temperatures were required to effect glycosylation of the former[24] (Scheme 4). We recently reported an anomeric glycosylation strategy that took advantage of this reactivity

difference between α- and β-glycosyl phosphates in the synthesis of a trisaccharide[39] (Scheme 5). The central building block 6-OH-α-galactosyl phosphate **43** displayed both acceptor and donor capabilities. Glycosylation of **43** with β-glucosyl phosphate **39** at −78 °C furnished β-(1→6)-linked disaccharide **44**, bearing an anomeric α-phosphate. After chromatography, disaccharide donor **44** was coupled with acceptor **45** at −40 °C to afford trisaccharide **46** in good yield (64%) and in only two steps.

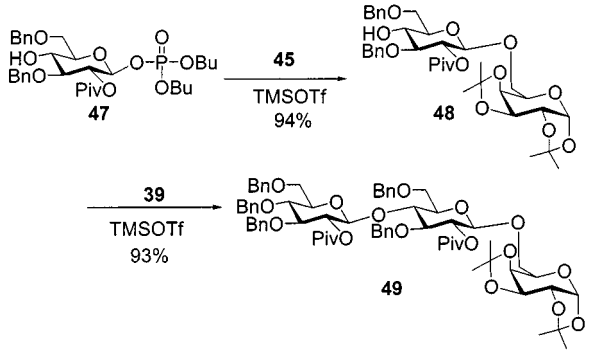


Scheme 4. Reactivity difference between α- and β-glycosyl phosphates



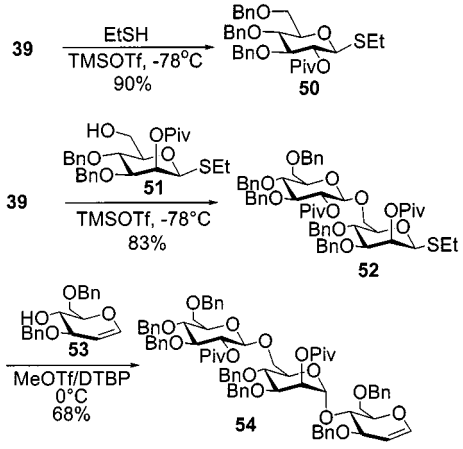
Scheme 5. Anomer-controlled glycosylation with α- and β-glycosyl phosphates

We have described other examples of one-pot trisaccharide construction using regioselective glycosylations. Treatment of donor **47**, containing a secondary hydroxy acceptor, with **45** at −78 °C afforded β-(1→6)-linked disaccharide **48** in excellent yield (94%) within 10 min (Scheme 6). After chromatography, **48** was glycosylated with glucosyl phosphate **39** to provide trisaccharide **49** in 87% overall yield. When the two glycosylations were carried out in a sequential, one-pot procedure without intermediate purification, trisaccharide **49** was isolated in 72% overall yield.



Scheme 6. Regioselective glycosylations

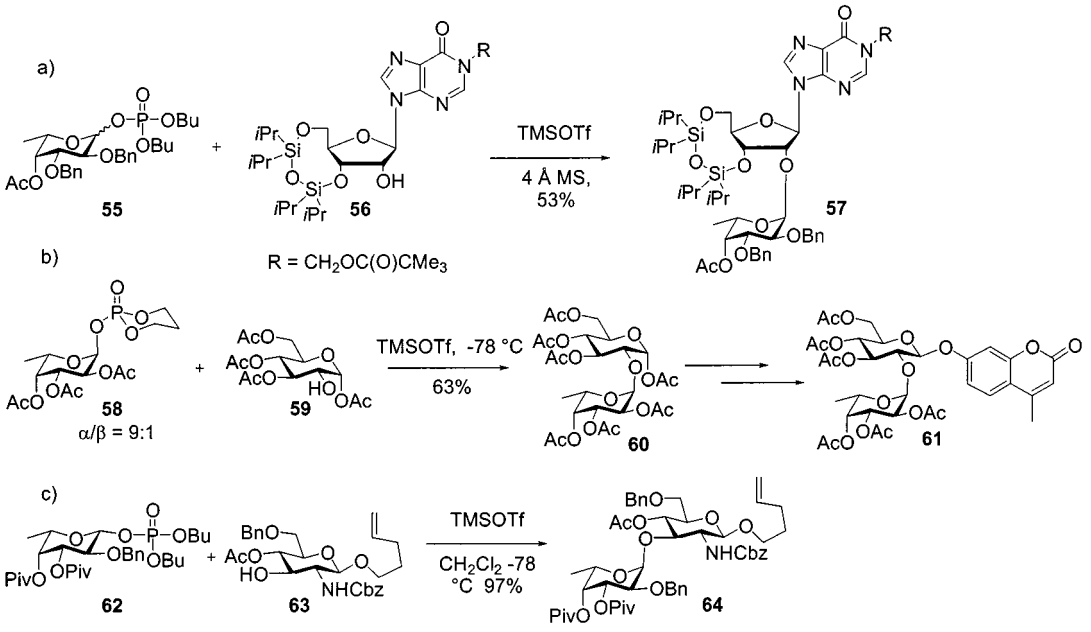
To expand the scope of phosphate-based glycosylations further, we introduced a glycosylation strategy that employed both glycosyl phosphates and thioglycoside donors (Scheme 7). Since thioglycoside **50** was stable under phosphate activation conditions, it was anticipated that other thioglycosides would also be stable.[39] Activation of **39** in the presence of **51** with TMSOTf at −78 °C afforded disaccharide **52** in good yield. Coupling of **52** with glycal acceptor **53** was accomplished with methyl triflate and di-*tert*-butylpyridine to afford trisaccharide **54**. The orthogonality of glycosyl phosphates and thioglycosides provides a convenient two-step method for preparing trisaccharides.



Scheme 7. Orthogonal glycosylation with glycosyl phosphates and thioglycosides

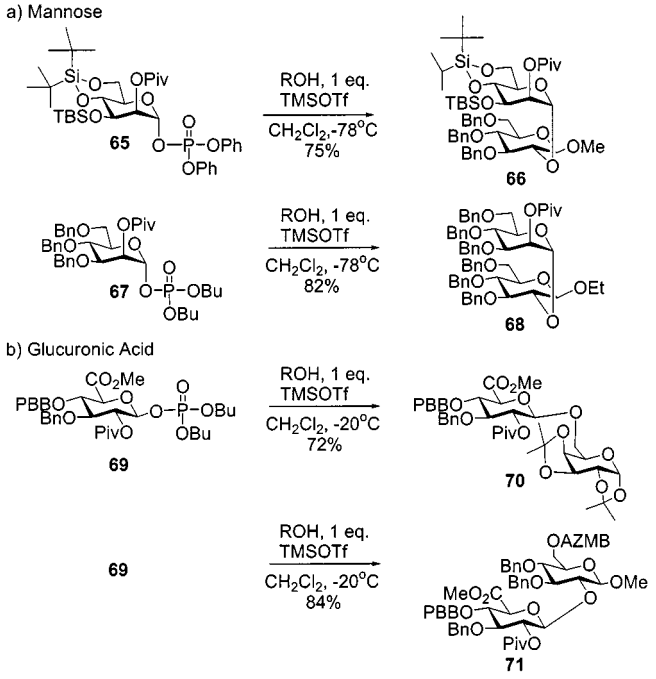
Fucosyl, mannosyl, and glucuronic acid glycosyl phosphates have also been explored as glycosyl donors in the synthesis of biologically important oligosaccharides; α-fucosidic linkages, examples of difficult to achieve 1,2-*cis* glycosidic linkages, have been constructed by employing fucosyl phosphate donors in the synthesis of a Shimofuridin analogue **57**[27] (Scheme 8a) and fucosidase substrate **61**[40] (Scheme 8b). Incorporation of an ester functionality on the C-3 and C-4 hydroxy groups in donor **62** allowed for completely α-selective fucosylation in excellent yield (97%).[39]

Mannosyl donors incorporating a C-2 participating group, such as **65** and **67**, have been used in the preparation of α-mannose-containing disaccharides **66** and **68**[41]



Scheme 8. Glycosylations with fucosyl phosphates

(Scheme 9a) and for the high-yielding construction of an α-mannosidic linkage in the total synthesis of bleomycin A.[42] Essential to the synthesis of heparin-like glycosaminoglycans was the development of an efficient uronic acid donor. Glucuronic acid phosphate **69** was synthesized from the corresponding glycal[43] and served as a highly effective donor upon treatment with primary or secondary hydroxy groups[41] (Scheme 9b).

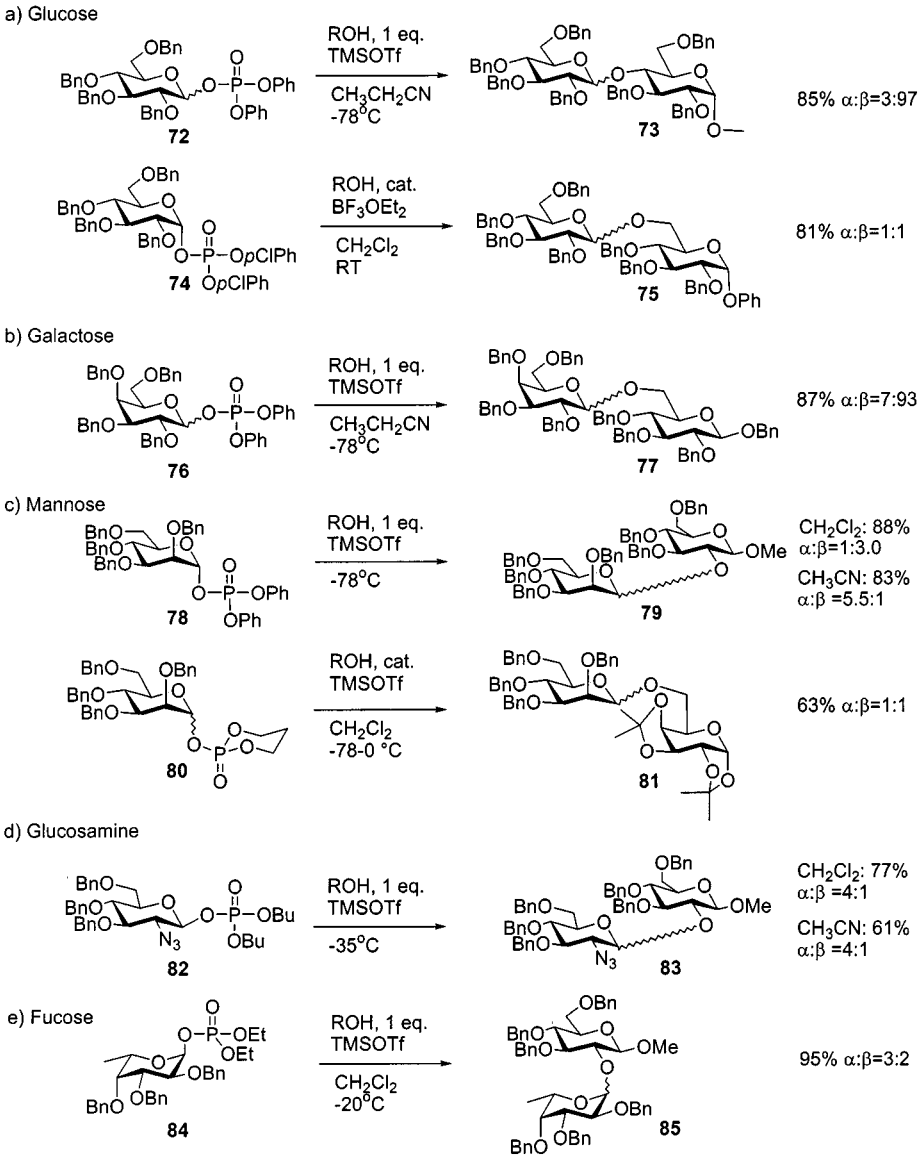


Scheme 9. Formation of α-mannoside and β-glucuronic acid glycosides

The ability to obtain glycosidic linkages in a stereospecific fashion is of utmost importance in oligosaccharide chemistry. The *trans* linkages are incorporated with the aid of C-2 participating groups, but frequent orthoester formation and C-2 ester base lability can be problematic to a synthesis. Conversely, there is no direct method for the construction of 1,2-*cis*-glycosides and these structures usually require lengthy syntheses. Ultimately, the formation of these linkages through easily controlled parameters, such as choice of solvent and donor species, would greatly benefit the area of oligosaccharide chemistry. The application of glycosyl phosphates in the synthesis of 1,2-*trans*-glycosidic linkages in the absence of participating groups and in the construction of 1,2-*cis*-glycosides have been reported.

Ikegami et al. employed 2-*O*-benzylated glycosyl donors **72** and **76** in the synthesis of β-glycosides[37] (Scheme 10a). Both donors showed remarkable β-selectivity in coupling reactions with various glycosyl acceptors. Similarly, β-selectivity was observed by Singh et al. in reactions between a cyclic glucosyl propane-1,3-diyl phosphate and primary acceptors when activated by a stoichiometric amount of TMSOTf.[44] Singh et al. also reported the use of propane-1,3-diyl mannose phosphate **80** in the synthesis of mannosidic linkages (Scheme 10c). Unlike in the glucose series, α-mannosides were formed exclusively upon activation of cyclic mannosyl phosphate **80** with excess amounts of TMSOTf.[45] Interestingly, when a catalytic amount of activator was employed, an increase in β-mannoside formation was observed (α/β = 1:1).

Ubiquitous in nature, β-mannosides are major constituents of lipophosphoglycans and, along with α-mannosides, essential components of *N*-linked glycoproteins. We reported a route to both α- and β-enriched mannosides with perbenzylated donor **78**, depending on the choice of solvent[41] (Scheme 10c). Coupling between **78** and a secondary acceptor preferentially afforded the α-linked product when the reaction was carried out in CH_3CN (β/α = 1:5.5). Inter-



Scheme 10. Glycosyl phosphates with C-2 nonparticipating groups

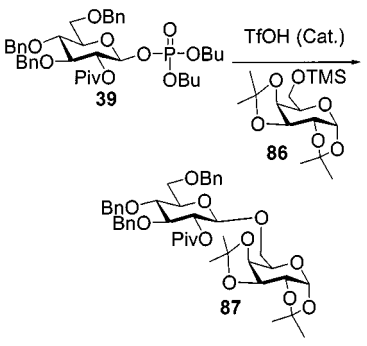
estingly, a reversal of the anomeric selectivity (β/α = 3:1) was observed when CH_2Cl_2 was used. Cyclic protecting groups influence the selectivity observed in β-mannosylation reactions;[46] however, attempts to use a 4,6-*O*-benzylidene-protected donor proved unsuccessful, due to partial hydrolysis of the cyclic acetal functionality under the acidic reaction conditions.

After the initial results with mannosyl phosphates, 2-azido-2-deoxy glycosyl phosphate **82** was coupled to secondary acceptors in participating and nonparticipating solvents[41] (Scheme 10d). Interestingly, there was no obvious solvent effect on the resulting stereochemistry. Use of both dichloromethane and acetonitrile resulted in β-selective glycosylation in the synthesis of **83**. Notably, the 2-azido-2-deoxy sugars were less reactive than their 2-*O*-counterparts, requiring higher reaction temperatures and affording lower yields.

Perbenzylated fucosyl phosphate **84** was effective in the glycosylation of a C-2 hydroxy acceptor, although a mixture of anomers was obtained (95%, α/β = 3:2) (Scheme 10e). Diaryl phosphate **74** was activated with a catalytic amount of $BF_3{\cdot}OEt_2$ at ambient temperature to give **75** in moderate yield, although no selectivity was observed in the resulting linkages[47] (Scheme 10a). Similarly, when glycosyl phosphates with C-2 nonparticipating groups were activated with $LiClO_4$, low α/β selectivities were observed.[48] It should be noted that the most effective glycosylations with phosphate donors were promoted with TMSOTf, and that this method of activation has resulted in many examples in the generation of the new glycosidic linkage with modest to excellent stereospecificity.

Although no detailed mechanistic evidence for the activation of glycosyl phosphates has yet been reported, new results may provide an insight into the reaction pathway.

There is an apparent requirement for a stoichiometric amount of TMSOTf for successful phosphate couplings. The driving force in phosphate activation is likely to be the formation and release of a stoichiometric amount of silyl phosphate as a by-product. This hypothesis is supported by results involving treatment of phosphate donor **39** with trimethylsilyl-protected acceptor **86** (Scheme 11).[49,50] Varying amounts of triflic acid were added at low temperatures (−78 °C) to effect glycosylation. Remarkably, as little as 0.01 equiv. of triflic acid afforded coupled product **87** in excellent yield (91%), suggesting that the in situ generation of catalytic amounts of TMSOTf is necessary for an efficient glycosylation reaction.



Scheme 11. Catalytic glycosylations with glycosyl phosphates

Use of Glycosyl Phosphates in the Synthesis of *C*-Glycosides

C-Glycoside natural products exhibit medicinally interesting properties, including antifungal and antitumorigenic responses.[51] The most common method for *C*-glycoside construction exploits the electrophilicity of the anomeric carbon atom by coupling nucleophiles to a glycosyl donor. Various leaving groups, including anomeric acetates, trichloroacetimidates, thioglycosides, halides, and methyl glycosides, have been used as glycosyl donors in the electrophilic approach to the preparation of *C*-glycosides.[51,52] Glycosyl phosphates have recently been utilized in the synthesis of *C*-aryl and *C*-alkyl glycosides.

An indirect route to the production of *C*-aryl glycosidic linkages involves the initial creation of an *O*-glycoside, followed by a Fries-like *O*-to-*C* rearrangement (Scheme 12a). Mannosyl and glucosyl phosphate donors (**72** and **78**) were activated to generate electrophilic anomeric species, which were coupled to an aromatic phenol to afford *O*-glycosides. The initial *O*-glycoside was then converted into the *C*-aryl bond under Lewis acid conditions; *C*-mannoside and *C*-glucoside derivatives of naphthol, 3,4,5-trimethoxyphenol, and resorcinol were synthesized in high yields[53] (Scheme 12b), and *O*-glycosides of glucosyl phosphate **72** could be isolated, while the *O*-to-*C* conversion with **78** was rapid and did not allow for the observation of intermediate *O*-linked products. Glycosyl phosphate donors **72** and **78** were also applied in the generation of *C*-alkyl glycosides with the use of silicon-derived *C*-nucleophiles (Scheme 12c).

Unlike in the above examples, in which the oxonium species is generated, glycosyl phosphates **98** and **72** have been activated by samarium diiodide to generate anomeric anions.[54] The resulting anomeric nucleophiles were coupled with various electrophilic species (**99**, **101**) to fashion *C*-glycosides such as **100** and **102** (Scheme 12d).

Use of Glycosyl Phosphates on Solid Supports

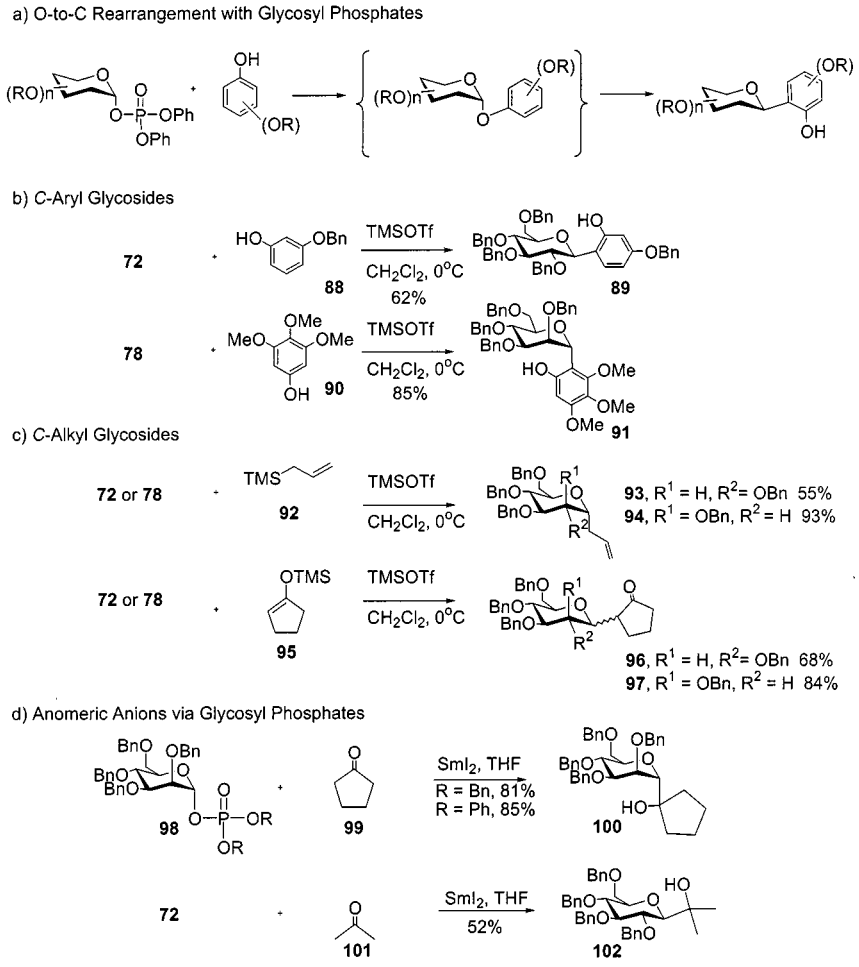
Glycal-derived glycosyl phosphates have proven to be readily accessible and effective glycosyl donors, as detailed above. They require only liquid, nontoxic activators, and the addition of molecular sieves is generally unnecessary. Therefore, glycosyl phosphates lend themselves particularly well to the synthesis of oligosaccharides on solid supports.

We have recently reported a high-yielding solid-phase syntheses of oligosaccharides through the employment of a novel octenediol linker (Scheme 13);[55] β-(1→6)-linked triglucoside **108** was obtained in 35% yield over 7 steps, with 3 equiv. of glycosyl phosphate donor **103** being used for each coupling step. The glycosylations were carried out at −50 °C for 1 h, followed by removal of the 6-*O*-silyl protecting group with tetrabutylammonium fluoride (TBAF). The trisaccharide was finally released from the support by cross-metathesis with Grubbs' catalyst under ethylene to yield *n*-pentenyl glycoside **108**.

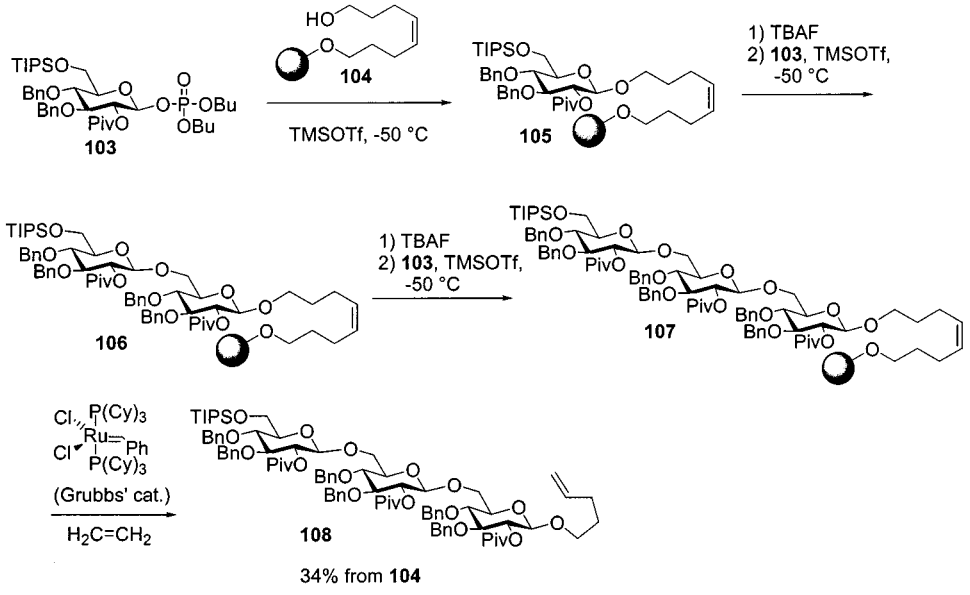
By a similar reaction sequence, the sterically challenging β-(1→4)-glucosidic linkage was effectively incorporated on the support with the 4-*O*-TBS-protected glucosyl phosphate donor **109**. Repetitive glycosylation and deprotection furnished trisaccharide **112** in 53% overall yield after cleavage from the resin (Scheme 14).

Following the success of glycosyl phosphates in the manual solid-support synthesis of oligosaccharides, their application to the automated solid-phase synthesis of oligosaccharides was evaluated.[16] The fully protected phytoalexin elicitor (PE) β-glucan **113** was selected as a complex target structure,[56] as it had previously been synthesized both in solution[57,58] and on a solid support[59] and so would serve well as a benchmark for the automation of glycosyl phosphates. For the synthesis of the branched β-(1→3)/β-(1→6) structure, two different glycosyl phosphate donors, **114** and **115** (Scheme 15), were synthesized. Levulinoyl esters were employed as temporary 6-*O*-protecting groups and the 2-*O*-pivaloyl group was used to ensure complete β-selectivity in the glycosylation reaction. Each cycle incorporated double glycosylations and double deprotections to ensure high-yielding steps. This automated cycle resulted in an excellent yield and high purity of a model β-(1→6) trisaccharide **116** (Scheme 16).

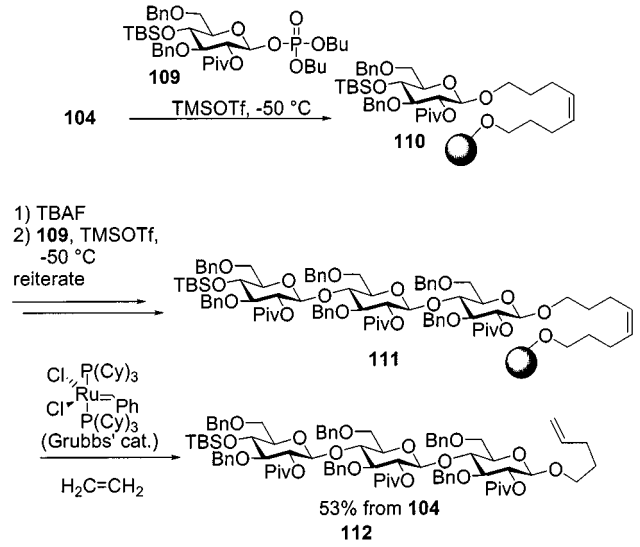
The automated cycle was then applied to the synthesis of more complex PE oligosaccharides using alternating phosphate building blocks (Scheme 17). Branched hexasaccharide **117** was constructed in 10 h in over 80% yield as judged



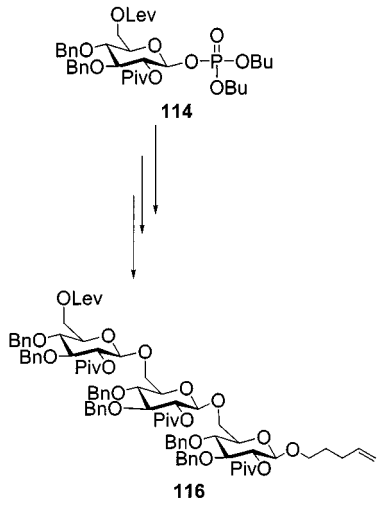
Scheme 12. Synthesis of *C*-glycosides from glycosyl phosphates



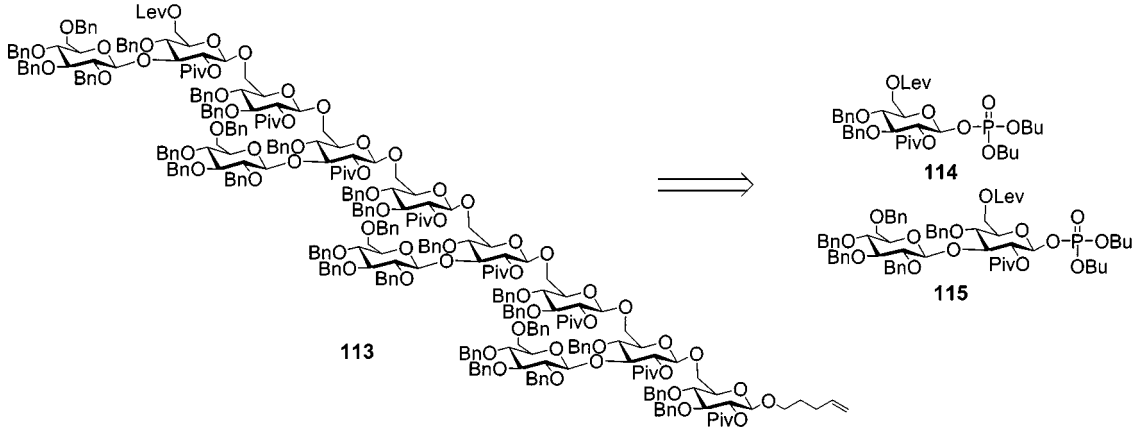
Scheme 13. Solid-phase synthesis with glycosyl phosphates



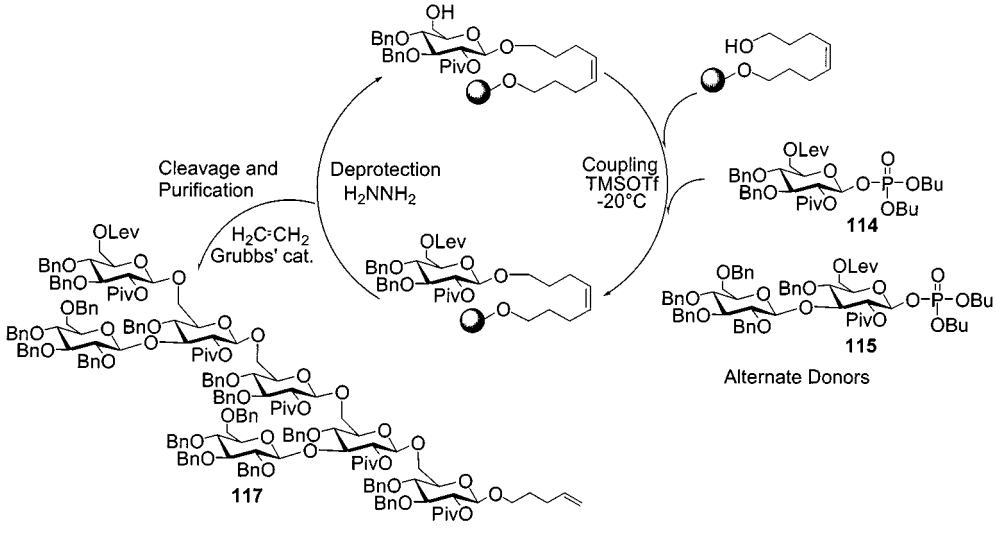
Scheme 14. Solid-phase synthesis with glycosyl phosphates



Scheme 16. Synthesis of model trisaccharide **116**



Scheme 15. Phytoalexin elicitor dodecasaccharide



Scheme 17. Automated synthesis of elicitor hexasaccharide

by HPLC analysis. In addition, dodecasaccharide **113**[59] was prepared in a fully automated fashion in 17 h and in 50% yield by use of the same cycle. Notably, solution-phase synthesis of only two phosphate building blocks was necessary, greatly reducing the manual labor usually required to assemble a structure of this size. This expedient generation of materials by automation represents a major improvement over conventional methods for polysaccharide synthesis. Currently, we are utilizing glycosyl phosphates in the automated synthesis of other biologically important oligosaccharides such as the Lewis blood group determinants and heparin-like glycosaminoglycans.

Conclusion

Glycosyl phosphates are a class of powerful glycosyl donors that compare favorably to other types of glycosylating agents with respect to their ability to react with hindered glycosyl acceptors in high yield. High anomeric selectivity can be achieved in these glycosylations, as demonstrated in the syntheses of **73** and **77**. Optimal glycosylation conditions require no additive other than stoichiometric amounts of TMSOTf, and high-yielding couplings have been carried out on solid supports and on an automated machine. Acid-sensitive compounds can be glycosylated under very mild neutral conditions in concentrated solutions of lithium perchlorate in organic solvents, or with a silylated acceptor and a catalytic amount of TfOH.

Various glycosyl phosphate derivatives offer the potential to fine-tune the donor reactivity, which can be used beneficially to simplify the synthesis of complex carbohydrates. Since glycosyl phosphates have become available through a straightforward one-pot synthesis from glycals, minimizing protecting group manipulations, they lend themselves as viable alternatives to the established set of glycosyl donors. This class of donors, in concert with other glycosylating agents, should serve well in the assembly of complex oligosaccharides.

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