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Synthesis of Glycosyl Phosphates from 1,2-Orthoesters and Application to in Situ Glycosylation Reactions

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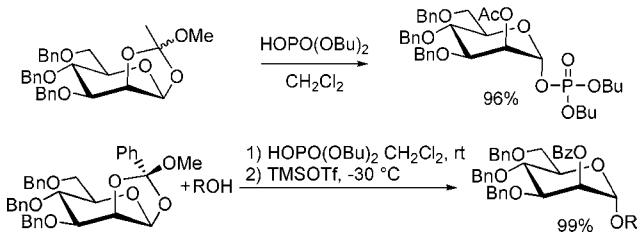
ABSTRACT



A series of glycosyl phosphates were prepared in high yield by treatment of the corresponding 1,2-orthoesters with dibutyl phosphate. Glycosyl phosphates are efficient glycosylating agents even when used in crude form or when generated in situ. The immunodominant epitope trirhamnoside of group B *Streptococcus* was prepared to demonstrate the synthetic utility of the method.

The chemical synthesis of oligosaccharides is of utmost importance to procure tools for glycobiology in sufficient amounts.[1] A plethora of glycosylating agents carrying a variety of anomeric leaving groups have been developed to construct glycosidic linkages.[2] Glycosyl chlorides, bromides, iodides, trichloroacetimidates, fluorides, *n*-pentenyl glycosides, anhydro sugars, as well as anomeric aryl sulfoxides, and thioglycosides have been applied to the construction of complex oligosaccharides and glycosylated natural products.[3]

Glycosyl phosphate triesters are effective glycosylating agents for the chemical synthesis of carbohydrates.[4] Difficulties associated with the synthesis of anomeric phosphates from either anomeric lactols[5] or other glycosylating agents such as glycosyl trichloroacetimidates, halides, or *n*-pentenyl- and thioglycosides[6] diminished the synthetic utility of this class of glycosylating agents. Introduction of a one-pot protocol[7] gave ready access to glycosyl phosphate building blocks that now serve as key monomers for the synthesis of complex oligosaccharides in solution and by automated solid-phase synthesis.[8] The conversion of glycals to glucosyl and galactosyl phosphates is efficient but requires the use of dimethyldioxirane (DMDO). DMDO has to be prepared freshly, can be dangerous to handle, and makes reaction scaleup difficult.[9] The need to access a host of glycosyl

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(1) (a) Garegg, P. *J. Med. Res. Rev*. **1996**, *16*, 345. (b) Seeberger, P. H. *Chem. Commun*. **2003**, 1115.

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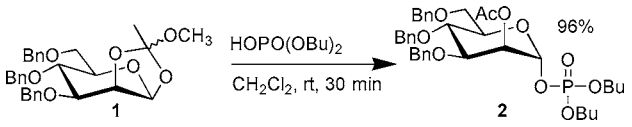
phosphate monomers for use in automated oligosaccharide synthesis prompted the pursuit of an efficient synthetic means to generate glycosyl phosphates.

General applicability and ready scaleup of such a transformation are essential. 1,2-Glycosyl orthoesters are valuable synthetic intermediates in the preparation of carbohydrate building blocks.[10] Like 1,2-glycals, 1,2-glycosyl orthoesters possess only three hydroxyl groups to be differentiated. Treatment of 1,2-glycosyl orthoesters with excess alcohol nucleophiles under Lewis acid activation generates 2-*O*-acyl glycosides with 1,2-trans configuration.[11] In the 1970s, peracetylated 1,2-orthoesters served as precursors to glycosyl phosphate monoesters and nucleotide 5′-diphospho sugars, albeit in highly variable yields.[12]

Here, we describe the stereoselective conversion of 1,2-orthoesters to glycosyl 1-phosphate triesters by employing phosphate diesters as both a nucleophile and an acidic activator.

Tribenzylmannosyl 1,2-orthoacetate **1** served as a test substrate en route to find the optimal reaction conditions. Slow addition of a solution of **1** in dichloromethane to a solution of dibutyl phosphate in the presence of molecular sieves (MS) yielded the desired glycosyl phosphate **2**. To minimize the excess of dibutyl phosphate and to simplify the workup procedures, different bases to quench the acidic dibutyl phosphate were tested. Just 3 equiv of dibutyl phosphate is sufficient to convert **1** to mannosyl phosphate **2** in 30 min at room temperature (Scheme 1). Activated 4 Å

Scheme 1. Conversion of 1,2-Orthoester **1** to Mannosyl Phosphate **2**[a]

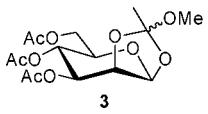
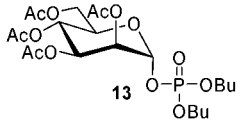
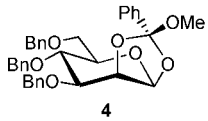
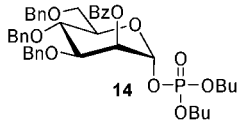
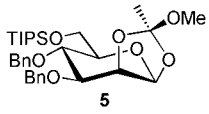
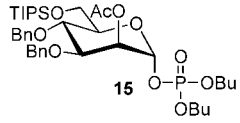
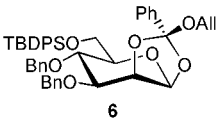
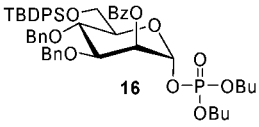
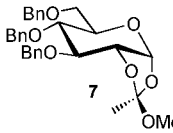
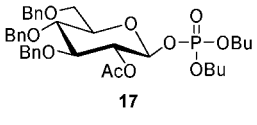
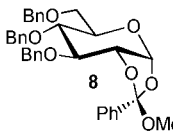
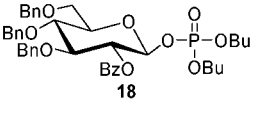
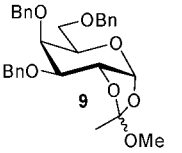
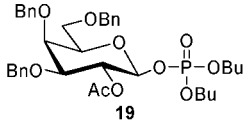
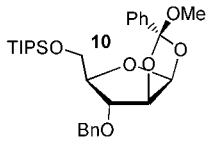
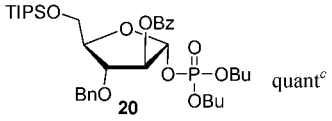
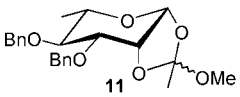
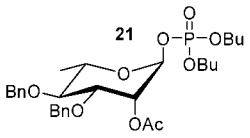
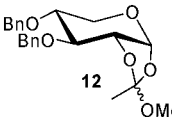
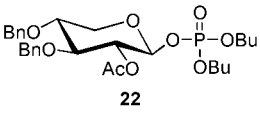


[a] Conditions: 1 equiv of **1** and 3 equiv of dibutyl phosphate.

MS function as drying agent,[13] and excess dibutyl phosphate was quenched by the addition of triethylamine. Filtration through a pad of Et_3N-deactivated silica gel, followed by column chromatography, afforded **2** in 96% yield.

The protocol can be further simplified by adding dibutyl phosphate dropwise to a solution of 1,2-orthobenzoate **4** in CH_2Cl_2. The corresponding mannosyl phosphate **14** was isolated in 97% yield. This addition sequence can also be employed when handling orthoacetates such as **1**, although longer reaction times are required to achieve full conversion.

Table 1. Preparation of Various Glycosyl Phosphates from 1,2-Orthoesters[a]

1,2-orthoester	product	yield
 **3**	 **13**	86[c]
 **4**	 **14**	97 86[b]
 **5**	 **15**	95[c]
 **6**	 **16**	89
 **7**	 **17**	83 89[c]
 **8**	 **18**	quant
 **9**	 **19**	92 90[c]
 **10**	 **20**	quant[c]
 **11**	 **21**	92
 **12**	 **22**	90

[a] 3 equiv of dibutyl phosphate was added by syringe to a CH_2Cl_2 solution of sugar at room temperature. [b] Multigram scale. [c] Addition of the sugar as solution to dibutyl phosphate (3 equiv) in CH_2Cl_2 at room temperature.

With a suitable protocol in hand, the synthetic scope of this transformation was explored using a range of mannose **3**−**6**, glucose **7** and **8**, galactose **9**, arabifuranose **10**, rhamnose **11**, and xylose **12** 1,2-orthoesters (Table 1).[14] The correspond-

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(10) Lemieux, R. U.; Morgan, A. R. *Can. J. Chem.* **1965**, *43*, 2199.
(11) (a) Ogawa, T.; Beppu, K.; Nakabayashi, S. *Carbohydr. Res.* **1981**, *93*, C6. (b) Leroux, J.; Perlin, A. S. *Carbohydr. Res.* **1981**, *94*, 108.
(12) (a) Volkova, L. V.; Danilov, L. L.; Évstigneeva, R. P. *Carbohydr. Res.* **1974**, *32*, 165. (b) Tsai, J.-H.; Behrman, E. J. *Carbohydr. Res.* **1978**, *64*, 297. (c) Salam, M. A.; Behrman, E. J. *Carbohydr. Res.* **1981**, *90*, 83; *Carbohydr. Res.* **1982**, *101*, 339. Behrman, E. J. *Carbohydr. Res.* **1982**, *102*, 139.
(13) AW 300 molecular sieves were also examined, but their acidity resulted in significant formation (5−20%) of the corresponding *O*-methyl-glycoside due to the rearrangement of the 1,2-orthoester.

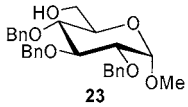
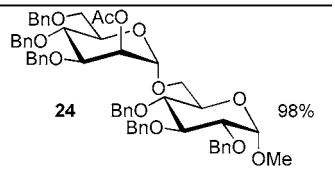
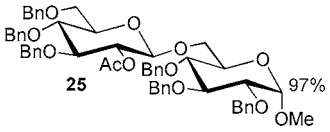
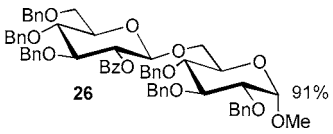
ing glycosyl phosphates were obtained in high yield. 1,2-Orthobenzoates (**4**, **6**, **8**, and **10**) reacted faster with dibutyl phosphate (30 min) than 1,2-orthoacetates (**1**, **3**, **5**, **7**, **9**, **11**, and **12**) that required reaction times of 2−3 h. These differences in reactivity can be rationalized when considering that the phenyl moiety at the orthoester helps to stabilize the carboxonium intermediate during orthoester ring opening.

Because the procurement of large amounts of monosaccharide building blocks is required to supply the starting materials for automated oligosaccharide synthesis, process scalability is important. The proof-of-principle reactions were carried out on a 0.2−0.5 mmol scale, but the process can be readily scaled up, as demonstrated for the preparation of several grams of **14**.

The workup and purification procedure called for filtration through a plug of silica followed by column chromatography. NMR spectra of the crude glycosyl phosphates obtained after simple silica gel filtration indicated very high purity of the desired products. Therefore, we began to examine the efficiency of crude glycosyl phosphates as glycosylating agents.

The crude mannosyl and glucosyl phosphates **2**, **17**, and **18** derived from the corresponding orthoesters (1.2 equiv) were coupled to methyl glucoside **23** (1.0 equiv) at −30 °C by TMSOTf activation (Table 2). The desired 1,2-trans-

Table 2. Glycosidations with Crude Glycosyl Phosphates[a]

donor	acceptor	product
2	 **23**	 **24** 98%
17	**23**	 **25** 97%
18	**23**	 **26** 91%

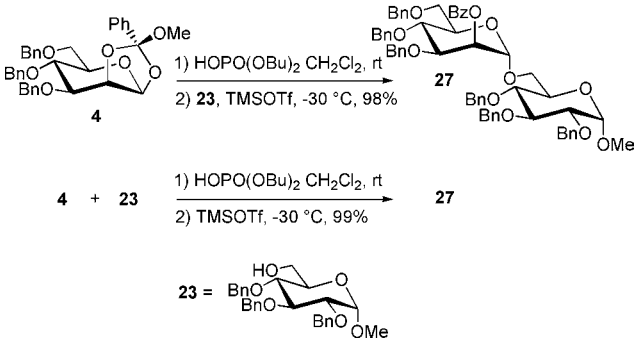
[a] Glycosidations were carried out with 1.2 equiv of donor, 1.0 equiv of acceptor, and 1.2 equiv of TMSOTf at −30 °C in CH_2Cl_2, within 1 h.

linked disaccharides **24**−**26** were obtained in excellent yield and complete stereoselectivity.[4] The formation of disaccharide orthoester byproducts was not observed.

The carbohydrate impurities generated during glycosyl phosphate synthesis did not interfere with the glycosylations. Thus, a one-pot coupling following the in situ generation of glycosyl phosphates should be possible because excess acidic dibutyl phosphate was not expected to negatively affect the glycosylation.

To evaluate the in situ route, mannosyl phosphate **14** was generated from orthoester **4** (1.2 equiv) as described above. Then, a solution of glucoside **23** (1.0 equiv) in CH_2Cl_2 was added. After cooling the reaction mixture to −30 °C, 3 equiv of TMSOTf was required to activate the glycosyl phosphate. Partial quenching of TMSOTf by some remaining phosphoric acid necessitated this increased amount of activator. Disaccharide **27** was isolated in 98% yield (Scheme 2). The

Scheme 2. Glycosidations Using in Situ Generated Glycosyl Phosphates[a]



[a] Conditions: 1.2 equiv of **4**, 1 equiv of **23**, 3.6 equiv of dibutyl phosphate, and 3.6 equiv of TMSOTf.

formation of *O*-methyl glycoside was not observed, probably because the molecular sieves that are present in the reaction mixture act as a methanol scavenger. A further simplification of the reaction protocol was achieved when orthoester **4** was treated with dibutyl phosphate in the presence of the coupling partner, nucleophile **23**. The in situ generated glycosyl phosphate was activated by simple addition of TMSOTf to furnish disaccharide **27** in excellent yield.

1,2-Glycosyl orthoesters had been used previously as glycosylating agents.[15] Direct coupling generally resulted in poor yield, and excess orthoester was required to improve coupling yields. Only a limited range of substrates such as 1,2-pentenol and -thio orthoesters were effective in the assembly of complex carbohydrates.[16] On the basis of the new procedure reported here, 1,2-orthoesters may be viewed as latent glycosylating agents similar to 1,2-glycals whereby the in situ conversion to glycosyl phosphates is employed for efficient couplings.

The synthetic utility of the new approach was further demonstrated by the rapid assembly of a group B *Streptococcus* trirhamnoside epitope (Scheme 3).[17] Rhamnose orthoester **11** served as the only building block for both glycosylating agent **21** and nucleophile **28** via chain elonga-

(14) See Supporting Information for the syntheses of **1** and **3**−**12**.

(15) (a) Kochetkov, N. K.; Khorlin, A. J.; Bochkov, A. F. *Tetrahedron* **1967**, *23*, 693. (b) Kochetkov, N. K.; Bochkov, A. F.; Sokolovskaya, T. A.; Snyatkova, V. J. *Carbohydr. Res.* **1971**, *16*, 17. (c) Wang, W.; Kong, F. *J Org. Chem.* **1998**, *63*, 5744.

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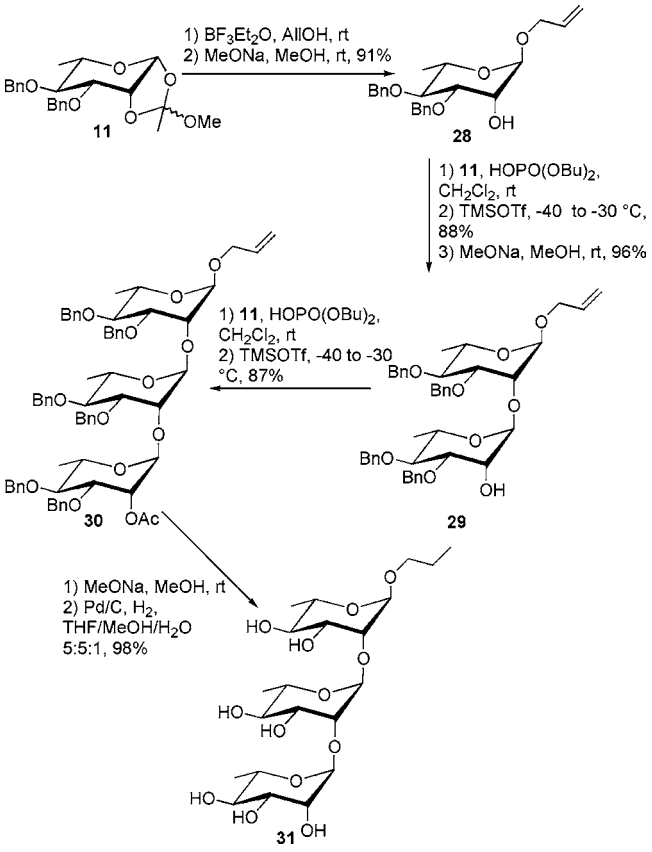
Scheme 3. Synthesis of Rhamnose Trisaccharide **31**



tion with in situ generation of glycosyl phosphates. Allyl rhamnoside **28** was obtained in 91% yield by selective opening of **11** under acidic conditions with an excess of allyl alcohol and subsequent cleavage of the acetate. Treatment of orthoester **11** with dibutyl phosphate in the presence of **28**, followed by the activation with TMSOTf, gave dirhamnoside in 88% yield. Removal of the acetate furnished **29** that served as a nucleophile in the coupling with the in situ generated rhamnosyl phosphate to yield 87% of trirhamnoside **30**. Removal of all protective groups furnished trirhamnoside **31** in 98% yield.

In summary, we developed a highly effective protocol to convert 1,2-glycosyl orthoesters to C2 acyl glycosyl phosphates. The synthetic scope was established by preparing a series of mannosyl, glucosyl, galactosyl, rhamnosyl, xylosyl, and arabinofuranosyl phosphates in high yield. Pure glycosyl phosphates are obtained following column chromatography. In situ generation of glycosyl phosphates from 1,2-orthoesters allows for simple and efficient glycoside formation as shown for a trirhamnoside antigen. Straightforward access to glycosyl phosphate building blocks will facilitate the synthesis of complex carbohydrates in solution and by automated solid-phase synthesis.

Acknowledgment. This research was supported by ETH Zürich and the Swiss National Science Foundation (SNF Grant 200121-101593).

Supporting Information Available: Detailed experimental procedures and compound characterization data, including ^{1}H, ^{13}C, and ^{31}P NMR spectral data for all described compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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