One-Step, Stereocontrolled Synthesis of Glycosyl 1-Phosphates, Uridine-5′-diphosphogalactose, and Uridine-5′-diphosphoglucose from Unprotected Glycosyl Donors

Stephen Hanessian,* Pu-Ping Lu, and Hideki Ishida

Contribution from the Department of Chemistry, Université de Montréal, P.O. Box 6128, Station Centre-ville, Montreal, P.Q., Canada H3C 3J7

Received August 4, 1998

Abstract: The reaction of 2-(1,2-*trans*-glycopyranosyloxy)-3-methoxypyridines (MOP glycosides) with phosphoric acid leads to the corresponding 1,2-*cis*-1-phosphates in good yield and excellent stereoselectivity. 1-Phosphate esters of α-D-glucopyranose, α-D-galactopyranose, and 2-azido-2-deoxy-α-D-galactopyranose were thus prepared without recourse to protective groups. In the L-fucose series, the major product was the α-L-fucosyl 1-phosphate. An alternative method that relies on neighboring group participation allowed the preparation of a protected β-L-fucosyl 1-phosphate. Reaction of unprotected β-D-glucopyranosyloxy and β-D-galactopyranosyloxy MOP donors with uridine diphosphoric acid gave UDP-Glc and UDP-Gal with preponderance of the desired α-anomeric configuration.

Glycosyl 1-phosphates and glycosyl 5′-nucleotide esters play a vital role in life processes that involve carbohydrates in particular.[1] Thus, glycosyl 1-phosphates are key intermediates in the metabolism of sugars and their transformation to nucleotides. These in turn are nature's reagents in the enzyme-mediated transfer of a sugar unit (as a glycosyl donor) to an acceptor molecule in the general synthesis of simple and complex glycosides, including oligosaccharides, glycoproteins, and related macromolecules.[2] Unlike protein biosynthesis, the assembly of oligomeric sugars is done through the action of specific glycosyltransferases in the presence of nucleotide 5′-phosphates, hence their paramount importance.

Glycosyl 1-Phosphates. The biosynthesis of glycosyl 1-phosphates involves glycosylkinases and ATP. Whitesides and coworkers[2] have reported on practical methods for the enzyme-mediated synthesis of a number of glycosyl 1-phosphates using crude enzyme extracts. For example, α-D-galactosyl 1-phosphate (α-D-Gal-1-P) barium salt and α-D-galactosamine 1-phosphate (α-GalN-1-P) barium salt could be obtained in a purity of 85−80% and yields of 74−50%, respectively. The 1-phosphates of D-glucose, D-galactose, D-galactosamine, and L-fucose are commercially available at a cost of dollars per milligram depending on the sugar.

The chemical synthesis of glycosyl 1-phosphates[3] has relied on multistep procedures in which an *O*-acetylated glycosyl halide is condensed with a salt of phosphoric acid or its ester, followed by de-esterification. For example, treatment of tetra-*O*-acetyl-α-D-glycopyranosyl bromide with trisilver phosphate or silver diphenyl phosphate in refluxing benzene gives, after a tedious purification protocol, α-D-glucosyl 1-phosphate (α-D-Glc-1-P) as the barium salt.[4] When silver dibenzyl phosphate is used, the product is the β-phosphate barium salt.[5] Although a number of aldosyl 1-phosphates could be synthesized by these and related procedures,[6] the synthesis of β-L-fucosyl 1-phosphate (β-L-Fuc-1-P) has required particular attention. Acetate, benzoate, and *O*-benzyl protective groups have been choice derivatives in the reaction of the corresponding *O*-protected fucosyl halides with phosphate nucleophiles. Problems associated with reproducibility and overall efficiency have been independently addressed by Hindsgaul,[6] Ohrlein,[7] and van Boom.[8] Despite improvements, the synthesis of β-L-Fuc-1-P still involves four to five chemical steps from L-fucose.[9] In fact, the natures of *O*-protecting groups and the anomeric leaving group play key roles in the stereoselectivity of phosphorylation.[10,11] The benzoate esters have been reported[10] to provide less of the unwanted anomeric phosphates and to confer some stability compared to acetates for example. Unlike other aldosyl 1-phosphates derivatives, those derived from β-L-fucose must be stored at low temperature and they have a propensity to anomerize to the α-phosphate triesters at room temperature.[6−10]

(1) For pertinent references, see: Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683. Kennedy, J. F.; White, C. A. In *Bioactive Carbohydrates*; Ellis Horwood Ltd: West Sussex, U.K. 1983. Sharon, N. In *Complex Carbohydrates*; Addison-Wesley Publishing Co.: Reading, MA, 1975.

(2) (a) Heidlas, J. E.; Lees, W. J.; Whitesides, G. M. *J. Org. Chem.* **1992**, *57*, 152. (b) For a pertinent review, see: Heidlas, J. E.; Williams, K. W.; Whitesides, G. M. *Acc. Chem. Res.* **1992**, *25*, 307.

(3) Cori, C. F.; Colowick, S. P.; Cori, G. T. *J. Biol. Chem.* **1937**, *121*, 465. Putman, E. W. *Methods Carbohydr. Chem.* **1963**, *2*, 261.

(4) Wolfrom, M. L.; Smith, C. S.; Pletcher, D. E.; Brown, A. E. *J. Am. Chem. Soc.* **1942**, *64*, 23. Zervas, L. *Naturwiss* **1939**, *27*, 317; see also ref 5.

(5) See, for example: Sabesan, S.; Neira, S. *Carbohydr. Res.* **1992**, *223*, 169. Roy, R.; Tropper, F. D.; Grand-Maitre, C. *Can. J. Chem.* **1991**, *69*, 1462. Inage, M.; Chaki, H.; Kusumoto, S.; Shiba, T. *Chem. Lett.* **1982**, 1281.

(6) Gokhale, U. B.; Hindsgaul, O.; Palcic, M. M. *Can. J. Chem.* **1990**, *68*, 1063.

(7) Baisch, G.; Ohrlein, R. *Bioorg. Med. Chem.* **1997**, *5*, 383.

(8) Heskamp, B. M.; Broxterman, H. J. G.; Van der Warl, G. A.; Van Boom, J. M. *J. Carbohydr. Chem.* **1996**, *15*, 611. Veeneman, G. H.; Broxterman, H. J. G.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1991**, *32*, 6175.

(9) (a) Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260. See also: (b) Nunez, H. A.; O'Connor, J. V. (c) Rosevear, P. R.; Barker, R. *Can. J. Chem.* **1980**, *59*, 2086. (d) Prihar, H. S.; Behrman, E. J. *Biochemistry* **1973**, *12*, 997. (e) Leaback, D. H.; Heath, E. C.; Roseman, S. *Biochemistry* **1969**, *8*, 1351.

(10) Adelhorst, K.; Whitesides, G. M. *Carbohydr. Res.* **1993**, *242*, 69.

(11) Schmidt, R. R.; Wegmann, R.; Jung, K.-H. *Liebigs Ann. Chem.* **1991**, 121.

10.1021/ja982783i CCC: $15.00
Published on Web 12/09/1998

Glycosyl 5′-Nucleotide Esters: UDP-Gal and UDP-Glc. The biosynthesis of UDP-Gal can take place via more than one pathway.[12] In the most common protocol, the enzyme UDP-galactopyrophosphorylase catalyses the synthesis of UDP-Gal from α-D-Gal-1-P and uridine triphosphate (UTP). Another pathway involves the transfer of a uridyl unit from UDP-Glc to α-D-Gal-1-P with release of Glc-1-P.[13] In some instances, specific isomerases can transform one sugar residue to another at the UDP-sugar stage as in the biosynthesis of UDP-GalNAc from UDP-GlcNAc and the transformation of UDP-Glc to UDP-Gal.[14] UDP-Glc and UDP-Gal have been isolated from bean seedlings[15] and from yeast[16] by extraction and fractionation techniques. The most practical nonchemical synthesis of UDP-Gal involves the use of UDP-Glc as UMP donor in the presence of α-D-Gal-1-P and Gal-1-P uridyltransferase.[2] Thus, UDP-Gal could be isolated as the disodium salt in 43% yield on a 2.5-g scale.

The chemical syntheses of glycosyl 5′-nucleotides have relied mostly on the condensation of glycosyl 1-phosphates with appropriate 5′-nucleotide derivatives.[17] Thus, the ready availability of glycosyl 1-phosphates can be appreciated in this context, since most nucleotide 5′-phosphates or diphosphates are easily accessible.[18]

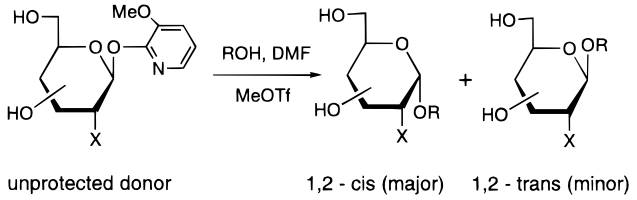
It is only recently that the synthesis of UDP-Glc by a method that does not involve the condensation of α-D-Glc-1-P with UMP was disclosed.[19] Thus, 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide was condensed with uridine 5′-diphosphoric acid to give, after deprotection, an anomeric mixture of the corresponding UDP-glucose. The same technique was applicable to the synthesis of UDP-Ara and UDP-Fuc in yields ranging from 10 to 30% and rather modest selectivities (α/β, 1:1 to 3:1). Related anomeric phosphorylations with nucleotide 5′-monophosphates have also been reported utilizing *O*-protected sugar derivatives.[20]

In this paper, we describe the stereocontrolled synthesis of α-1-phosphate esters of D-glucose, D-galactose, L-fucose, and 2-amino-2-deoxygalactose, adopting a method that involves a novel one-step synthesis from unprotected glycosyl donors. The same method is also applicable to the synthesis of UDP-Gal and UDP-Glc. A synthesis of β-L-Fuc-1-P is also described.

Results and Discussion

We have previously reported on a practical synthesis of *O*-glycosides utilizing 2-(β-D-glycosyloxy)-3-methoxypyridine (MOP) donors (Scheme 1).[21] One of the major attributes of the method is the feasibility of stereocontrolled glycoside synthesis *without the need for protective groups*, reminiscent of enzyme-mediated reactions. In general, 1,2-*trans*-MOP glycosides with

Scheme 1



hydroxy or azido groups at C-2 in the D-gluco, D-galacto, 2-azido-2-deoxy-D-galacto series led to the corresponding 1,2-*cis*-*O*-glycosides as preponderant isomers, provided the acceptor (alcohol) component was used in excess (5 equiv or more). Utilizing partially *O*-esterified MOP glycosides as acceptors resulted in the synthesis of di- or oligosaccharides with a latent cleavable MOP group at the new reducing end. De-*O*-esterification and iteration of the process provided a protocol for the assembly of small quantities of certain oligosaccharides.[21]

The utility of MOP and the related 2-pyridylthiocarbonate leaving groups in *O*-protected sugar glycosides as donors in the synthesis of 1,2-*cis*-oligosaccharides has also been reported.[22,23] Utilizing participating ester groups, in these glycosyl donors led to the corresponding 1,2-*trans*-glycosides in excellent yields. In the case of *O*-protected glycosyl donors, only a slight excess of acceptor alcohol was used.

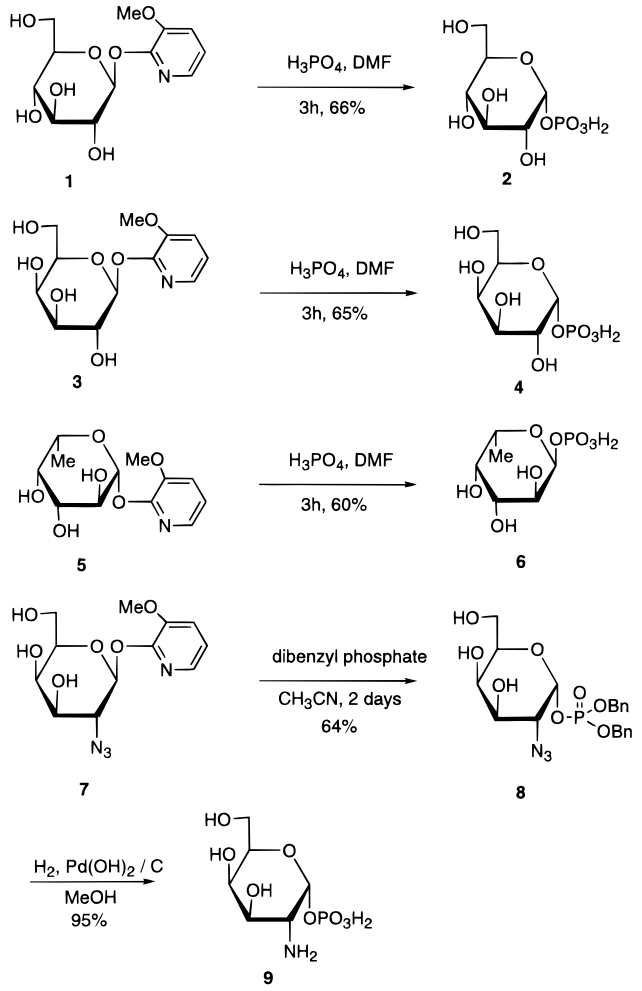
In the present study, we sought to develop a one-step stereocontrolled synthesis of four biosynthetically relevant glycosyl 1-phosphates with a desired anomeric configuration. Treatment of the readily available D-glucopyranosyloxy MOP donor (**1**)[21] with a 6 M excess of phosphoric acid in DMF as solvent led to α-D-Glc-1-P (**2**), which was isolated as the dicyclohexylammonium salt in 66% yield, identical with material reported by Putman and Hassid[24] (Scheme 2). Application of this simple procedure to glycosyl MOP donors derived from D-galactopyranose (**3**) and L-fucopyranose (**5**) gave the corresponding crystalline α-D- and α-L-glycopyranosyl 1-phosphates in yields ranging from 60 to 66%. In the case of 2-azido-2-deoxy-α-D-galactopyranosyl MOP (**7**), phosphorylation was found to proceed more stereoselectively when dibenzyl phosphate was utilized instead of the free acid. The reaction time was considerably longer than in the above-described cases, requiring 2−3 days for completion. The desired α-1-dibenzyl phosphate triester (**8**) was isolated in 64% yield as a homogeneous syrup and characterized by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopies and by chemical correlation. Thus, catalytic hydrogenation of **8** in the presence of $Pd(OH)_2$ on charcoal under 40 psi of hydrogen pressure, followed by lyophilization, gave the expected α-D-galactosamine 1-phosphate[3] (**9**) as a white powder in 95% yield.

As in the case of oligosaccharide synthesis with unprotected 1,2-*trans*-glycosyloxy MOP donors,[21] the major isolable products were the 1,2-*cis*-phosphates. We assume that the reaction proceeds via an S_N2-like mechanism in which a protonated 3-methoxy-2-pyridyloxy moiety is a leaving group. Whether an intermolecular or an intramolecular reaction is involved in the case of phosphoric acid is not known at this time (Scheme 3). Anomerization of the MOP group was not detected under the conditions of the reaction, which corroborates the "inversion" mechanism.

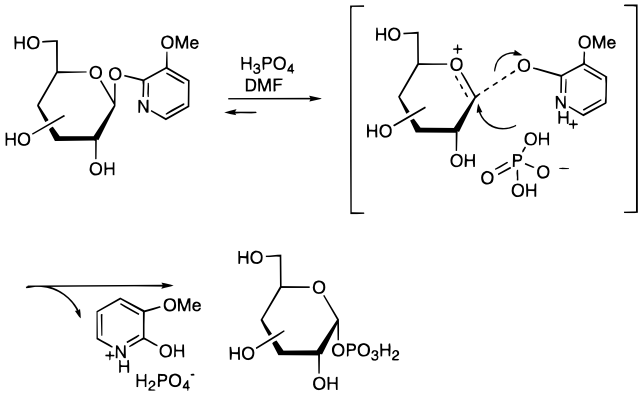
The longer reaction time with 2-azido-2-deoxy-β-D-galactopyranosyloxy MOP (**7**) is not surprising in view of the presence

(12) Caputto, R.; Leloir, L. F.; Cardini, C. E.; Paladini, A. C. *J. Biol. Chem.* **1980**, *184*, 333.
(13) Kalckar, H. M. *Adv. Enzymol.* **1958**, *20*, 111. Abraham, H. D.; Howell, R. R. *J. Biol. Chem.* **1969**, *244*, 545.
(14) Maley, F.; Tarentino, A. L.; McGarrahan, J. F.; Del Giacco, R. *Biochem. J.* **1968**, *107*, 637. Glaser, L. *J. Biol. Chem.* **1959**, *234*, 2801.
(15) Ginsburg, V.; Stumpf, P. K.; Hassid, W. Z. *J. Biol. Chem.* **1956**, *223*, 977.
(16) Leloir, L. F.; Cabib, E. *Methods Enzymol.* **1963**, *6*, 777.
(17) See, for example: Moffatt, J. G. *Methods Enzymol.* **1966**, *8*, 136.
(18) Hall, R. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1954**, *76*, 5056.
(19) Arlt, M.; Hindsgaul, O. *J. Org. Chem.* **1995**, *60*, 14.
(20) See, for example: Schmidt, R. R.; Braun, H.; Jung, K.-H. *Tetrahedron Lett.* **1992**, *33*, 1585. Martin, T. J.; Braun, H.; Schmidt, R. R. *Bioorg. Med. Chem.* **1994**, *2*, 1203. Chappell, M. D.; Halcomb, R. L. *Tetrahedron* **1997**, *53*, 11109.
(21) Hanessian, S. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Dekker: New York, 1996; p 381. Lou, B.; Reddy, G. V.; Wang, H., Hanessian, S. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Dekker: New York, 1996; p 389.
(22) Lou, B.; Huynh, H. K.; Hanessian, S. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Dekker: New York, 1996; p 413.
(23) Lou, B.; Huynh, H. K.; Hanessian, S. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Dekker: New York, 1996; p 431.
(24) Putman, E. W.; Hassid, Z. *J. Am. Chem. Soc.* **1957**, *79*, 5057.

Scheme 2



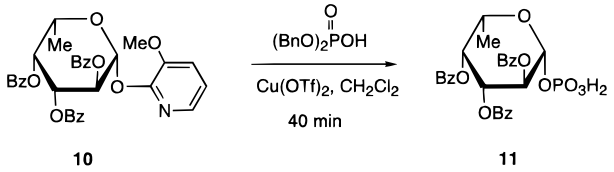
Scheme 3



of the electron-withdrawing azido group at C-2 which makes the pyridyl moiety less prone to protonation.[21] The same behavior was observed in the formation of *O*-glycosides, and the use of triflic acid as an activator was necessary.[21]

As noted above, the direct phosphorylation of a β-L-fucopyranosyl MOP donor led exclusively to α-L-fucosyl 1-phosphate (**6**) (Scheme 2). Unfortunately, many attempts to obtain the nucleotide precursor β-L-fucosyl 1-phosphate from an unprotected MOP donor always led to the anomerically more stable α-phosphate as the major product. We therefore explored the utility of 2,3,4-tri-*O*-benzoyl-β-L-fucopyranosyl MOP (**10**) as a glycosyl donor. In the presence of 0.8 equiv of copper triflate, added portionwise, and a slight excess of dibenzylphosphoric acid, we obtained the desired β-fucosyl phosphate ester **11** in 51% yield as a spectroscopically pure compound (Scheme 4). Minor quantities of the unwanted α-phosphate (<10%) and starting donor (<10%) could be easily separated by chromatography. The portionwise addition of the catalyst appeared to be an important factor in securing high 1,2-*trans* selectivity. As previously mentioned, and following the initial observations of Whitesides,[10] the synthesis of **11** has been accomplished from the anomeric bromide,[25] thioether,[8] and trichloroacetimidate derivatives.[11]

Scheme 4



On the other hand, a recent careful study of the reaction of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide with dibenzylphosphoric acid in the presence of 3-Å molecular sieves and silver carbonate by Baisch and Ohrlein[7] has produced a protocol to prepare the *O*-acetylated β-L-fucosyl 1-phosphate triester on a 800-g scale. This contrasts previous difficulties in working with acetate esters[10] that have proved to be unstable and problematic in isolation and storage.

The MOP technology for anomeric activation has proved its versatility in the above cited examples of direct anomeric phosphorylations with excellent stereocontrol as dictated by the presence or absence of protective groups in the glycosyl donor.

In view of the successful stereoselective phorphorylations of glycosyl MOP derivatives to afford the corresponding anomeric 1-phosphates shown in Schemes 2 and 4, we investigated the analogous 5′-uridyldiphosphorylation reaction. Thus, condensation of the commercially available uridine 5′-diphosphate (UDP) with a glycosyloxy MOP donor in an appropriate solvent was expected to lead to the corresponding UDP-glycose in one step and without recourse to protective groups. Since UDP-Glc and UDP-Gal are biologically relevant nucleotides, we focused on their chemistry in this phase of our work.

In the case of phosphorylations, the acidity and nucleophilicity of phosphoric acid were sufficient to activate the MOP group in an S_N2-like reaction to afford the α-glycosyl 1-phosphates from β-MOP glycosides. It was therefore of interest to see if UDP free acid would be capable of mimicking the reactivity observed in the phosphorylations. Issues related to the generation of UDP free acid[27] and its stability under the conditions of the reaction in DMF as solvent were of concern.

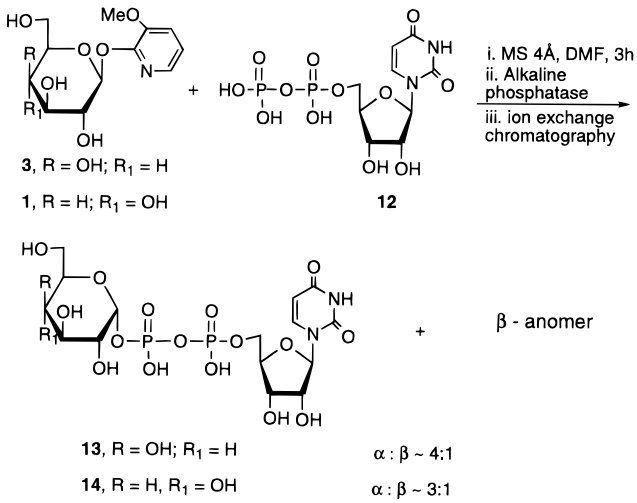
In the event, UDP free acid, prepared from the commercially available trisodium salt **12** by treatment with a cation-exchange resin in DMF, was treated with β-D-galactopyranosyloxy MOP (**3**) in the presence of powdered 4-Å molecular sieves (Scheme 5). After a reaction time of 3 h, the donor was completely consumed with formation of the desired nucleotide contaminated with residual UDP, which was easily separated off by treatment with alkaline phosphatase and its conversion to uridine. Purification of the mixture by ion exchange chromatography gave UDP-Gal (**13**) as a white powder in 60% yield. Spectroscopic analysis (^{1}H, ^{13}C, and ^{31}P NMR) indicated an α/β ratio

(25) Ichikawa, Y.; Sim, M. M.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 2943.

(26) Paulsen, H.; Richter, A.; Sinwell, V.; Stenzel W. *Carbohydr. Res.* **1974**, *64*, 339.

(27) Simon, E. S.; Grabowski, S.; Whitesides, G. M. *J. Org. Chem.* **1990**, *55*, 1834.

Scheme 5



of 4:1 in favor of the desired nucleotide anomer. UDP-Glc (**14**)-was obtained in a similar manner in ∼55% yield and an α/β ratio of 3:1. As reported by Hindsgaul,[19] the presence of the β-isomer does not affect the catalytic activity of glycosyltransferases in enzymatic oligosaccharide synthesis.

In conclusion, we have demonstrated that unprotected 2-(β-glycosyloxy)-3-methoxypyridyl donors are versatile intermediates for the one-step stereocontrolled synthesis of α-1-phosphates of D-glucose, D-galactose, L-fucose, and 2-amino-2-deoxy-D-galactose (via the corresponding 2-azido precursor). The same donors are also capable of transferring glucopyranosyl and galactopyranosyl units to UDP-free acid to afford the corresponding uridine 5′-diphospho sugars in one step. The synthesis of β-L-fucosyl 1-phosphate was achieved through the intermediacy of the *O*-benzoate ester.

The reactions are reminiscent of enzyme-catalyzed syntheses, in that no protection of the hydroxy groups in the glycosyl donor is needed. Future work will focus on extension to nucleotides of other biologically relevant sugars and to further improve anomeric stereocontrol.

Experimental Section

General Methods. Ion-exchange resin Dowex 1×2-200 (chloride form) and 50W×8-100 (H^+ form) were purchased from Aldrich. Uridine 5′-diphosphate (UDP) sodium salt was from Sigma. Ion-exchange resin chromatography was performed on a Bio-Rad Econo system. Alkaline phosphatase (from calf intestine) was purchased from Boehringer Mannheim. ^{1}H NMR spectra were recorded on 400- or 300-MHz spectrometers with DOH (δ = 4.81 ppm) or $CHCl_3$ (δ = 7.26 ppm) as an internal reference.^{13}C NMR and ^{31}P NMR spectra were recorded at 100.6 and 162.0 MHz, respectively. Mass spectra were determined by fast atom bombardment (FAB) or electrospray ionization (ESI) techniques. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter at ambient temperature. Melting points measured are uncorrected.

General Procedure for Preparation of Glycosyl 1-Phosphates: To a solution of crystalline phosphoric acid (250 mg, 2.5 mmol) in anhydrous DMF (1 mL) was added the glycopyranosyloxy MOP[21] (0.35 mmol). The reaction mixture was stirred at room temperature for 3 h, then neutralized with saturated barium hydroxide. The precipitated barium phosphate was removed by centrifugation and washed with water. The supernatant and washings were combined and concentrated. The residue was dissolved in a small amount of water. Ethanol was added to precipitate the glycosyl 1-phosphate barium salt. The precipitate was collected by centrifugation, and the crude precipitate was reprecipitated with water−ethanol. A solution of glycosyl 1-phosphate barium salt in water (2 mL) was acidified with Amberlite IR-120 (H^+), filtered, neutralized with 1 M cyclohexylamine in ethanol, and then concentrated. Precipitation of the residue with water−ethanol gave the glycosyl 1-phosphate bis(cyclohexylammonium) salt in 60−66% yield.

α-D-Glucopyranosyl 1-Phosphate Bis(cyclohexylammonium) Salt (2).[24] According to the general procedure described above, **2** was obtained in 66% yield: mp 164−168 °C; $[\alpha]_D$ +64 (*c* 2.0, H_2O); reported,[24] mp 163−169 °C; $[\alpha]_D$ +64.0 (H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 5.45 (dd, J = 7.3, 3.4 Hz, 1H, H-1), 3.93 (ddd, J = 9.8, 5.6, 2.1 Hz, 1H, H-5), 3.86 (dd, J = 12.2, 2.1 Hz, 1H, H-6b), 3.77 (dd, J = 9.8, 9.8 Hz, 1H, H-3), 3.72 (dd, J = 12.2, 5.6 Hz, 1H, H-6a), 3.46 (ddd, J = 9.8, 3.4, 1.0 Hz, 1H, H-2), 3.38 (dd, 1H, J = 9.8, 9.8 Hz, 1H, H-4), 3.14 (m, 2H), 1.01−2.00 (m, 20H); ^{13}C NMR (D_2O) δ 94.26, 73.85, 72.90, 72.67, 70.46, 61.42, 51.02, 31.03, 24.98, 24.49; ^{31}P NMR (D_2O) δ 2.84.

α-D-Galactopyranosyl 1-Phosphate Bis(cyclohexylammonium) Salt (4).[24] By following the general procedure described above, **4** was obtained in 65% yield: mp 147−152 °C; $[\alpha]_D$ +76 (*c* 1.0, H_2O); reported,[24] mp 147−153 °C; $[\alpha]_D$ +78.5 (H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 5.45 (dd, J = 7.2, 3.5 Hz, 1H, H-1), 4.13 (dd, J = 6.2, 6.2 Hz, 1H, H-5), 3.95 (d, J = 3.4 Hz, 1H, H-4), 3.86 (dd, J = 10.2, 3.2 Hz, 1H, H-3), 3.66−3.79 (m, 3H, H-2, H-6a and H-6b), 3.16 (m, 2H), 1.04−1.99 (m, 20H); ^{13}C NMR (D_2O) δ 94.84, 71.91, 70.20, 70.09, 69.53, 61.98, 51.05, 31.04, 24.99, 24.50; ^{31}P NMR (D_2O) δ 2.06.

α-L-Fucopyranosyl 1-Phosphate Bis(cyclohexylammonium) Salt (6).[9e] By following the general procedure described above, **6** was obtained in 60% yield: mp 168−176 °C; $[\alpha]_D$ −76 (*c* 1.0, H_2O); reported,[9e] $[\alpha]_D$ −77.8 (H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 5.44 (dd, J = 6.8, 3.4 Hz, 1H, H-1), 4.28 (q, J = 6.6 Hz, 1H, H-5), 3.93 (dd, J = 10.2, 3.2 Hz, 1H, H-3), 3.82 (d, J = 3.2 Hz, H-4), 3.71 (ddd, J = 10.2, 3.4, 1.0 Hz, 1H, H-2), 3.16 (m, 2H), 1.21 (d, J = 6.6 Hz, 1H, H-6), 1.16−2.01 (m, 20H); ^{13}C NMR (D_2O) δ 94.53, 72.78, 70.52, 69.56, 67.60, 51.06, 31.04, 24.99, 24.50, 16.18; ^{31}P NMR (D_2O) δ 2.62.

2-Azido-2-deoxy-α-D-galactopyranosyl 1-Dibenzyl Phosphate (8). To a solution of dibenzyl phosphate (555 mg, 4.96 mmol) in anhydrous acetonitrile (30 mL) at 0 °C was added a solution of **7** (155 mg, 0.496 mmol) in CH_3CN (6 mL) dropwise. The reaction mixture was warmed to room temperature and stirred for 2−3 days. After the reaction was completed, the mixture was neutralized with pyridine and concentrated to dryness. Column chromatography of the residue on silica gel using EtOAc−MeOH−Et_3N (90:10:1) as an eluant afforded the desired product **8** as a syrup (148 mg, 64%): $[\alpha]_D$ +34 (*c* 1.5, MeOH); ^{1}H NMR (CD_3OD, 300 MHz) δ 7.32−7.39 (m, 10H, Ph), 5.79 (dd, J = 6.0, 3.2 Hz, 1H, H-1), 5.13 and 5.11 (2 AB, J = 11.0 Hz, 4H, CH_2Ph), 3.94 (m, 1H, H-5), 3.93 (d, J = 2.9 Hz, 1H, H-4), 3.87 (dd, J = 10.4, 2.9 Hz, 1H, H-3), 3.76 (ddd, J = 10.4, 3.2, 3.2 Hz, H-2), 3.67 (d, J = 6.6 Hz, 2H, H-6); ^{13}C NMR (CD_3OD) δ 131.36, 131.22, 131.00, 130.83, 130.51, 100.48, 76.68, 72.75, 72.70, 71.39, 71.30, 64.01; ^{31}P NMR (D_2O) δ −1.61. The product was unstable upon storage.

2-Amino-2-deoxy-α-D-galactopyranosyl 1-Phosphate (α-GalN-1-P, 9).[2a] To a solution of the preceding compound **8** (60 mg, 0.13 mmol) in MeOH (2 mL) was added $Pd(OH)_2$ (150 mg, 20 wt % on charcoal). The reaction mixture was stirred under 40 psi H_2 for 2 h, filtered, and concentrated. Lyophilization of the residue afforded the title compound **9** as a white powder (31 mg, 95%): $[\alpha]_D$ +137.7 (*c* 0.44, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 5.75 (dd, $J_{1,2}$ = 3.3 Hz, $J_{1,P}$ = 6.7 Hz, 1H, H-1), 4.20 (br t, $J_{1,6}$ = 6.5 Hz, 1H, H-5), 4.14 (dd, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 3.0 Hz, 1H, H-3), 4.08 (br d, $J_{3,4}$ = 3.0 Hz, 1H, H-4), 3.80 (dd, J_{gem} = 11.8 Hz, $J_{5,6a}$ = 7.2 Hz, 1H, H-6a), 3.74 (dd, J_{gem} = 11.8 Hz, $J_{5,6b}$ = 5.4 Hz, 1H, H-6b), 3.56 (dt, $J_{2,3}$ = 10.9 Hz, $J_{1,2}$ = $J_{2,p}$ = 3.2 Hz, 1H, H-2); ^{13}C NMR (D_2O) δ 92.38 (d, C1, J = 5.0 Hz), 72.42 (C5), 68.76 (C4), 67.07 (C3), 61.63 (C6), 51.85 (d, J 8.1 Hz, C2); ^{31}P NMR (D_2O) δ 0.50.

2-(2,3,4-Tri-*O*-benzoyl-β-L-fucopyranosyloxy)-3-methoxypyridine (10). A mixture of 2-(β-L-fucopyranosyloxy)-3-methoxypyridine[21] (68 mg, 0.25 mmol), benzoic anhydride (1.02 g, 4.50 mmol), DMAP (138 mg, 1.13 mmol), and pyridine (2 mL) was stirred at room temperature for 2 h. Methanol (1 mL) was added, and the mixture was stirred for 30 min, then diluted with CH_2Cl_2 (10 mL), washed with saturated $NaHCO_3$, H_2O, and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography using hexanes−

EtOAc−Et_3N (100:50:1) as an eluant to provide the title compound (132 mg, 90%) as a white foam: $[\alpha]_D$ −191.6 (*c* 0.80, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15, 7.88, 7.82 (ddd, J = 8.0, 1.5, 1.0 Hz, each 2H, benzoyl C2-H, C6-H), 7.75 (dd, J = 4.9, 1.5 Hz, 1H, MOP H-4), 7.61 (dddd, J = 8.0, 7.8, 1.2, 1.0 Hz, 1H, Ar-H), 7.49 (dddd, J = 8.0, 7.8, 1.2, 1.0 Hz, 2H, Ar-H), 7.48−7.41 (m, 2H, Ar-H), 7.29, 7.26 (ddd, J = 8.0, 7.8, 0.8 Hz, each 2H, Ar-H), 7.01 (dd, J = 7.8, 1.5 Hz, 1H, MOP H-6), 6.90 (dd, J = 7.8, 4.9 Hz, 1H, MOP H-5), 6.53 (d, $J_{1,2}$ = 8.3 Hz, 1H, H-1), 6.12 (dd, $J_{2,3}$ = 10.4 Hz, 1H, H-2), 5.81 (dd, $J_{3,4}$ 3.5 Hz = $J_{4,5}$ 1.0 Hz, 1H, H-4), 5.71 (dd, 1H, H-3), 4.35 (dq, $J_{5,6}$ = 6.4 Hz, 1H, H-5), 3.70 (s, 3H, OCH_3), 1.38 (d, 3H, H-6); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 166.0, 165.6, 165.1 (C═O), 152.0 (MOP C2), 144.2, 136.7, 133.3, 133.1, 132.9, 130.01, 129.99, 129.97, 129.95, 129.72, 129.70, 129.68, 129.66, 129.65, 129.60, 129.59, 129.57, 129.43, 129.18, 128.87, 128.47, 128.45, 128.43, 128.21, 128.19, 128.17, 128.16, 128.14, 128.13, 128.11, 128.10, 128.08, 119.13, 118.82 (aromatic C, CH), 94.55 (C-1), 72.4, 71.0, 70.5, 69.4 (C2, C3, C-4, C5), 55.7 (OCH_3), 16.3 (C6); FAB MS (*m*/*z*) 584 (M + H) $^+$; exact FAB MS calcd for $C_{33}H_{30}NO_9$ (M + H)$^+$ 584.1920, found 584.1921.

Dibenzyl 2,3,4-Tri-*O*-benzoyl-β-L-fucopyranosyl 1-Phosphate (11). To a mixture of **10** (58.4 mg, 0.10 mmol), dibenzyl phosphate (55.7 mg, 0.20 mmol), crushed 4-Å molecular sieves (584 mg), and anhydrous CH_2Cl_2 (10 mL) was added copper triflate (29 mg, 0.08 mmol) in five portions within 40 min under argon. The reaction was monitored by TLC and neutralized with pyridine. The suspension was filtered through a Celite pad, and the filtrate was concentrated. The residue was subjected to silica gel chromatography using CH_2Cl_2−acetone−Et_3N (98:2:1) as an eluant to give the title compound (38 mg, 51%);[8,25] the α-anomer (7.4 mg, 10%), and unreacted starting material (6 mg, 10%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12, 7.96, 7.80 (dd, J = 8.0, 1.2 Hz, each 2H, benzoyl C2-H, C6-H), 7.68−6.99 (m, 19H, Ar-H), 5.89 (dd, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 8.0 Hz, 1H, H-2), 5.76 (dd, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 1.0 Hz, 1H, H-4), 5.67 (dd, $J_{1,P}$ = 7.2 Hz, 1H, H-1), 5.57 (dd, 1H, H-3), 5.14, 5.12 (ABM, J = 11.8 Hz, 7.4 Hz, each 1H, $PhCH_2$), 4.86, 4.77 (ABM, J = 11.6, 6.4 Hz, each 1H, $PhCH_2$), 4.22 (dq, $J_{5,6}$ = 6.4 Hz, 1H, H-5), 1.35 (d, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 165.7, 165.4, 165.2 (C═O), 135.5, 135.4, 135.04, 134.96, 133.45, 133.35, 133.21, 129.89, 129.73, 129.68, 129.03, 128.80, 128.63, 128.53, 128.39, 128.26, 128.21, 127.83, 127.35 (aromatic C, CH), 96.94 (d, $J_{1,P}$ = 4.7 Hz, C-1), 71.7, 70.8, 70.5 (C3, C4, C5), 69.6 (d, $J_{2,P}$ = 9.3 Hz, C-2), 69.5 (d, $J_{C,P}$ = 5.6 Hz, $PhCH_2$), 69.2 (d, $J_{C,P}$ = 5.6 Hz, $PhCH_2$), 16.1 (C6); ^{31}P NMR ($CDCl_3$) δ −2.41; FAB MS (*m*/*z*) 737 (M + H)$^+$; exact FAB MS calcd for $C_{41}H_{38}O_{11}P$ (M + H)$^+$ 737.2156, found 737.2154.

Uridine 5′-Diphospho-D-galactose (UDP-Gal, 13). To a suspension of UDP trisodium salt dihydrate (500 mg, 1 mmol) in DMF (16 mL) at 4 °C, was added Amberlite IR-120 (H^+ form) resin until the UDP was completely dissolved. The resin was filtered and washed with DMF. The combined filtrate and washing were concentrated in vacuo, the residue was dissolved in anhydrous DMF (8 mL), and the solution was concentrated. This process was repeated three times. The residue was then dissolved in anhydrous DMF (4 mL), and crushed 4-Å molecular sieves (500 mg) were added to the solution. The resulting mixture was stirred at room temperature for 2 h, cooled to 0 °C, and treated with a solution of **3** (152 mg, 0.5 mmol) in DMF (2 mL) dropwise. The reaction mixture was warmed to room temperature within 10 min and stirred for 3 h until **3** was completely consumed. The reaction mixture was cooled to 0 °C, diluted with cold water (30 mL) and 0.5 M NH_4HCO_3 (5 mL), and passed through a bed of Celite. The filtrate and washings were combined and lyophilized. The residue was dissolved in deionized water (5 mL), then treated with alkaline phosphatase (Boehringer Mannheim, 500 μL, 1 unit/μL), and the mixture was kept at room temperature until the unreacted UDP was no longer detected by TLC. The mixture was diluted with water (20 mL) and loaded onto a Dowex-1×2-200 column (bicarbonate form, 2.5 × 12 cm). The column was first eluted with water (400 mL) to remove the neutral compounds, then eluted with a linear gradient of 0.05 M ammonium bicarbonate (900 mL) and 0.5 M NH_4HCO_3 (200 mL). Fractions which contained UDP-Gal were pooled and concentrated to 10 mL at 25 °C. The concentrated solution was neutralized to pH 7 with Dowex 50W×8 resin. After removal of the resin by filtration, and lyophilization, the desired UDP-Gal was obtained as a white powder[27] (∼60%, α/β ratio 4:1). A portion was converted to the sodium salt by passage over Dowex-50W×8 (Na^+ form), elution with water, and lyophilization: ^{1}H NMR (D_2O, 400 MHz) δ 7.94 (d, J = 8.1 Hz, 1H, H-6”), 5.96 (d, J = 8.1 Hz, 1H, H-5”), 5.94 (d, J = 4.2 Hz, 1H, H-1′), 5.62 (dd, J = 7.0, 3.0 Hz, H-1 of α-Gal), 4.94 (dd, J = 7.4, 7.2 Hz, H-1 of β-Gal), 4.20 (d, J = 2.5 Hz, H-4), 3.62 (dd, J = 9.6, 7.3 Hz, H-2 of β-Gal); ^{13}C NMR (D_2O) δ 166.95, 152.54, 142.33, 103.37, 96.55 (d, J = 6.9 Hz, C1), 89.07, 83.91, 76.56, 74.48, 72.61, 70.36, 70.02, 69.12 (d, J = 8.5 Hz, C2), 65.64 (d, J = 5.8 Hz, C5′), 61.91, 61.71; ^{31}P NMR (D_2O) δ −10.49 and −12.03; ESI MS *m*/*z* 633 (M + Na)$^+$, 611 (M + H)$^+$. The ammonium salt of **13** was not suitable for MS analysis.

Uridine 5′-Diphospho-D-glucose (UDP-Glc, 14). UDP-Glc was prepared from UDP free acid and GlcOMOP using the same procedure as described above for the preparation of UDP-Gal except that the time of reaction was extended to 6 h. UDP-Glc was obtained as a white powder (∼50%, α/β ratio 3:1): ^{1}H NMR (D_2O, 400 MHz) δ 7.92 (d, J = 8.0 Hz, 1H, H-6”), 5.99 (d, 1H, J = 3.5 Hz, 1H, H-1′), 5.94 (d, J = 8.0 Hz, 1H, H-5”), 5.60 (dd, J = 7.3, 3.3 Hz, H-1 of α-Glc), 5.01 (dd, J = 8.0, 7.9 Hz, H-1 of β-Gal), 3.78 (dd, J = 9.8, 9.8 Hz, H-3), 3.53 (ddd, J = 9.8, 3.3, 3.0 Hz, H-2 of α-Glc), 3.47 (dd, J = 9.8, 9.8 Hz, H-4), 3.39 (ddd, J = 9.8, 8.0, 3.0 Hz, H-2 of β-Gal); ^{13}C NMR (D_2O) δ 166.94, 152.52, 142.28, 103.39, 103.33, 98.55 (C1 of β-Glc), 96.27 (d, J = 6.7 Hz, C1 of α-Glc), 89.02, 88.92, 83.94, 83.85, 77.17, 74.44, 74.25, 73.49, 70.40, 70.30, 70.10, 69.82, 65.62, 65.56, 61.46, 60.92; ^{31}P NMR (D_2O) δ −10.62 and −12.26; ESI MS *m*/*z* 633 (M + Na)$^+$, 611 (M + H)$^+$.

Acknowledgment. We thank NSERCC for generous financial support through the Medicinal Chemistry Chair Program.

Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR spectra for all compounds (24 pages, print/PDF). See any current masthead page for ordering information and Internet access instructions.

JA982783I