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Highly Stereoselective Synthesis of α-D-Mannopyranosyl Phosphosugars

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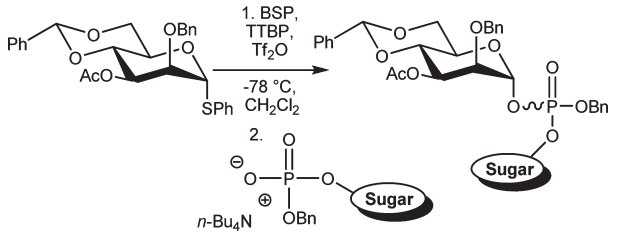
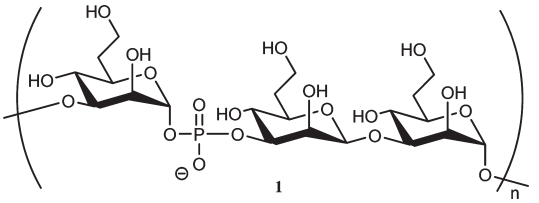


α-Mannopyranosyl phosphosugars are obtained in 61−90% yields from 4,6-*O*-benzylidene-protected mannosyl thioglycosides bearing ester functionality in the 3-*O*-position by coupling reactions with ammonium salts of phosphosugars on activation with 1-benzenesulfinyl piperidine, 2,4,6-tri-*tert*-butylpyrimidine, and trifluoromethanesulfonic anhydride. Due to the presence of the disarming ester group, only the formation of the α-isomer was observed.

Glycosyl phosphosugars are fragments of several glycoproteins. They are also structural blocks of poly(glycosyl phosphates) which are present in the cell walls and capsules of numerous bacteria. Such capsular polysaccharides are composed of mono- or polysaccharide units linked with phosphate diester bridges through hemiacetal and alcohol hydroxyl group neighboring units.[1] Recently, Vinogradov et al. determined the structure of the capsular polysaccharide from *Campylobacter jejuni* RM1221, the causative agent of human gastroenteritis.[2] This polysaccharide **1** has a regular structure made up of a linear main chain of trisaccharide repeating units, comprising two α- and one β-6-deoxy-D-manno-*hepto*-pyranose residues punctuated by a phosphodiester linkage. Continuing our interest in the stereocontrolled synthesis of mannoheptoside containing oligosaccharides and their deoxy congenors,[3] we have now directed our attention to the synthesis of this repeating unit and report here on the accomplishment of an essential first step—the highly stereocontrolled preparation of α-mannopyranosyl phosphosugars.



1

Glycosyl phosphosugars have previously been synthesized by the phosphate diester and phosphite triester methods,[4] as well as by the *H*-phosphonate approach.[5] More recently, Boons et al. employed glycosyl phosphoramidites in an approach to the synthesis of the proteophosphoglycans from *Leishmania*.[6] In our laboratory, we prepared β-mannopyrannosyl phosphoisoprenoids diastereoselectively by coupling of a 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-protected mannopyranosyl sulfoxide with tetrabutylammonium phosphates.[7] The stereoselectivity observed in these reactions was highly solvent-dependent, being highly β-selective in toluene, but giving α,β-mixtures in dichloromethane. In view of the importance of the acetals of the 4,6-*O*-benzylidene type in our general strategy toward the *Campylobacter jejuni* RM1221 repeating unit, it was necessary to adapt this chemistry so as to render it highly α-selective, ideally, in the optimal solvent for this chemistry—dichloromethane. To this end, we opted to investigate the influence of an ester in the 3-*O*-position, which we had previously shown to result in very high α-selectivity in the synthesis of a range of typical glycosides despite the presence of the 4,6-*O*-benzylidene group.[8,9]

The ammonium salts of phosphosugars required as glycosyl acceptors were prepared using the phosphoramidite strategy.[10] Thus, a range of phosphosugars **6** were obtained from known carbohydrates **2** (D-glucose, L-rhamnose, or D-mannose derived) by coupling with benzyl 2-cyanoethyl (CE) *N*,*N*-diisopropylphosphoramidite **3**[7] in the presence of benzimidazolium trifluoromethanesulfonate **4**[11] as

(1) (a) von Figura, K.; Hasilik, A. *Annu. Rev. Biochem.* **1986**, *55*, 167–194. (b) Raetz, C. R. H.; Whitfield, C. *Annu. Rev. Biochem.* **2002**, *71*, 635–700. (c) Nikolaev, A. V.; Botvinko, I. V.; Ross, A. J. *Carbohydr. Res.* **2007**, *342*, 297–334.

(2) Gilbert, M.; Mandrell, R. E.; Parker, C. T.; Li, J.; Vinogradov, E. *ChemBioChem* **2007**, *8*, 625–631.

(3) (a) Crich, D.; Banerjee, A. *Org. Lett.* **2005**, *7*, 1395–1398. (b) Crich, D.; Banerjee, A. *J. Am. Chem. Soc.* **2006**, *128*, 8078–8086. Crich, D.; Li, M. *J. Org. Chem.* **2008**, *73*, 7003–7010.

(4) (a) Cawley, T. N.; Letters, R. *Carbohydr. Res.* **1971**, *19*, 373–382. (b) Thiem, J.; Franzkowiak, M. *J. Carbohydr. Chem.* **1989**, *8*, 1–28.

(5) Nikolaev, A. V.; Ivanova, I. A.; Shibaev, V. N.; Kochetkov, N. K. *Carbohydr. Res.* **1990**, *204*, 65–78.

(6) (a) Elsayed, G. A.; Boons, G.-J. *Synlett* **2003**, *9*, 1373–1375. (b) Majumdar, D.; Elsayed, G. A.; Buskas, T.; Boons, G.-J. *J. Org. Chem.* **2005**, *70*, 1691–1697.

(7) (a) Crich, D.; Dudkin, V. *Org. Lett.* **2000**, *2*, 3941–3943. (b) Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2002**, *124*, 2263–2266.

(8) (a) Crich, D.; Cai, W.; Dai, Z. *J. Org. Chem.* **2000**, *65*, 1291–1297. (b) Crich, D.; Yao, Q. *J. Am. Chem. Soc.* **2004**, *126*, 8232–8236.

(9) For a discussion of this effect, see: Crich, D.; Hu, T.; Cai, F. *J. Org. Chem.* **2008**, *73*, 8942–8953.

(10) (a) Matteucci, M. D.; Caruthers, M. H. *J. Am. Chem. Soc.* **1981**, *103*, 3185–3191. (b) For a review, see: Beaucage, S.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223–2311.

(11) (a) Hayakawa, Y.; Kataoka, M.; Noyori, R. *J. Org. Chem.* **1996**, *61*, 7996–7997. (b) Sakakura, A.; Hayakawa, Y. *Tetrahedron* **2000**, *56*, 4427–4435.

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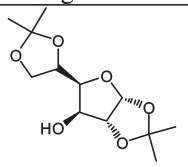
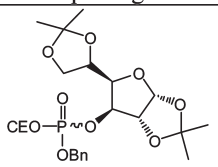
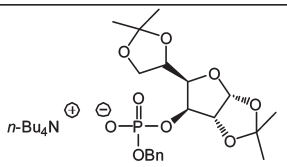
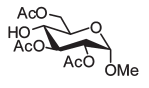
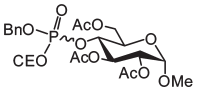
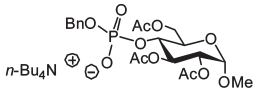
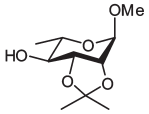
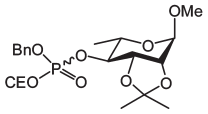
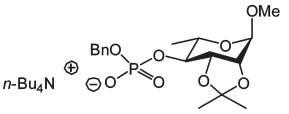
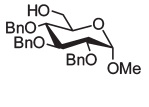
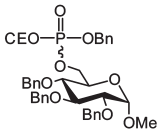
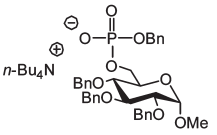
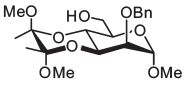
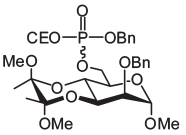
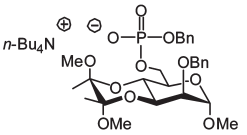
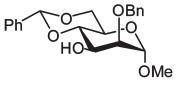
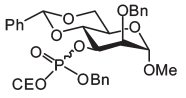
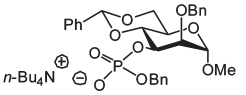
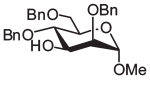
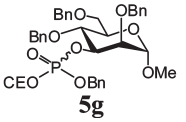
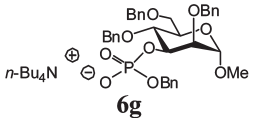
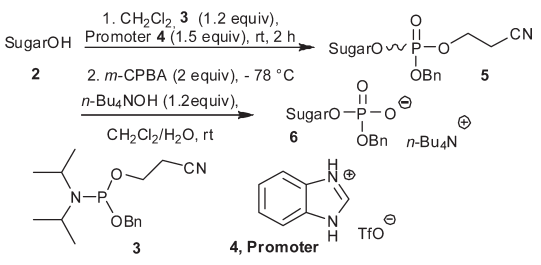
TABLE 1. Preparation of Acceptors 6

Entry	SugarOH **2**	Phosphosugars **5**	% Yield	Ammonium Salt **6**	% Yield
1	 **2a**	 **5a**	98	 **6a**	100
2	 **2b**	 **5b**	84	 **6b**	94
3	 **2c**	 **5c**	81	 **6c**	99
4	 **2d**	 **5d**	82	 **6d**	99
5	 **2e**	 **5e**	86	 **6e**	95
6	 **2f**	 **5f**	82	 **6f**	100
7	 **2g**	 **5g**	81	 **6g**	100

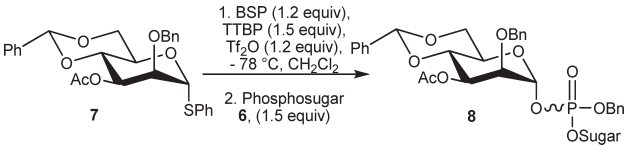
SCHEME 1. Preparation of Tetrabutylammonium Phosphosugars 6



promoter followed in situ by oxidation with *m*-CPBA (Scheme 1). The diesters **5** were isolated in high yields after this two-step sequence as approximately 1/1 mixtures of diastereoisomers (Table 1). This mixture was confirmed by the ^{31}P NMR, which showed two signals. The 2-cyanoethyl protecting group was removed by treatment of **5** with tetrabutylammonium hydroxide in a CH_2Cl_2/water mixture (Scheme 1) to give the ammonium phosphosugars **6** quantitatively (Table 1),

(12) The ammonium salts **6** were not stable to silica gel chromatography.

SCHEME 2. Preparation of α-Mannopyranosyl Phosphosugars 8



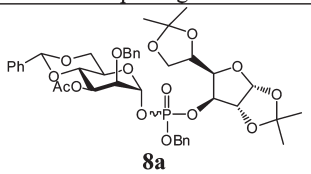
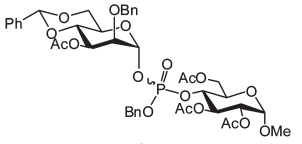
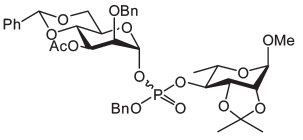
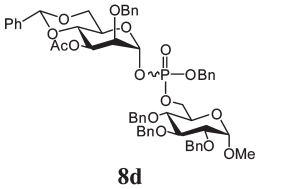
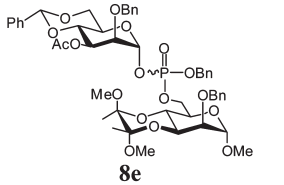
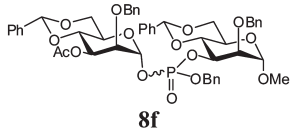
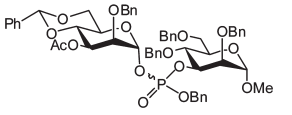
and these were employed in the subsequent glycosylation reactions without purification.[12]

The α-thiomannoside **7** was prepared from the phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thia-α-D-mannopyranoside[13] by acetylation in 98% yield (Scheme 2).

Glycosylation of the acceptors **6** with donor **7**, carried out by means of the 1-benzenesulfinyl piperidine (BSP)/Tf_2O preactivation protocol[14] in the presence of the hindered base 2,4,6-tri-*tert*-butylpyrimidine (TTBP),[15] was achieved in dichloromethane at −78 °C (Scheme 2). These reactions were rapid and, in line with our expectations, gave

(13) Crich, D.; Li, W.; Li, H. *J. Am. Chem. Soc.* **2004**, *126*, 15081–15086.
(14) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020.
(15) Crich, D.; Smith, M.; Yao, Q.; Picione, J. *Synthesis* **2001**, 323–326.

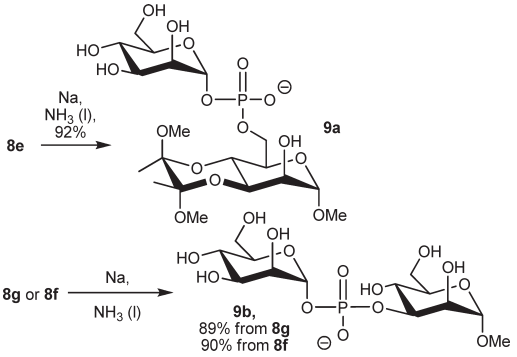
TABLE 2. Glycosylation Reactions of 7

Entry	Ammonium salt **6**	α-Mannopyranosyl Phosphosugars **8**	% Yield
1	**6a**	 **8a**	73
2	**6b**	 **8b**	74
3	**6c**	 **8c**	90
4	**6d**	 **8d**	61
5	**6e**	 **8e**	66
6	**6f**	 **8f**	90
7	**6g**	 **8g**	67

the α-anomers **8** with very high anomeric selectivity and good to high yield following silica gel chromatography[16] (Table 2). No significant selectivity was observed at the level of the stereogenic phosphorus center, and compounds **8** were therefore characterized as approximately 1/1 mixtures of diastereoisomers.

Perhaps not surprisingly in view of the occasional use of glycosyl phosphates as glycosyl donors,[17] we observed that compounds **8** were generally not stable in methanolic solution when they gave the corresponding methyl glycosides.[18]

SCHEME 3. Deprotection with Sodium in Liquid Ammonia



Finally, we turned our attention to deprotection. After several dead ends,[19] we had recourse to treatment of compounds **8** with sodium in liquid ammonia when cleavage of all benzyl esters and ethers, benzylidene acetals, and acetate esters was achieved cleanly in excellent yields (Scheme 3).

In summary, we have shown that α-mannopyranosyl phosphosugars can be obtained in good yield and excellent selectivity through the use of a donor carrying a stereodirecting, but nonparticipating, 3-*O*-carboxylate ester. This chemistry is currently being applied to the syntheses of capsular polysaccharide repeating units from *Campylobacter jejuni* RM1221, on which we will report in due course.

Experimental Section

General Procedure for the Formation of Phosphosugars 5. To a solution of sugars **2** (1.0 equiv) and benzimidazolium trifluoromethanesulfonate **4** (1.5 equiv) in dry CH_2Cl_2 (10 mL/mmol of **2**) was added at room temperature **3** (1.2 equiv). The reaction mixture was stirred 2 h at room temperature before it was cooled to −78 °C and *m*-CPBA (2 equiv) was added. The reaction mixture was stirred 1 h at this temperature, warmed to room temperature, and stirred overnight at room temperature before it was quenched with saturated aqueous $NaHCO_3$ and then extracted several times with CH_2Cl_2. The combined organic layer was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (EtOAc/heptane, 1:1) to afford phosphosugars **5**.

General Procedure for Cyanoethyl Ester Cleavage. To a solution of phosphosugars **5** (1.0 equiv) in a mixture of CH_2Cl_2/H_2O (2:1, 9 mL/mmol of **5**) was added at room temperature tetrabutylammonium hydroxide (1 M in water, 1.2 equiv). The reaction mixture was stirred 2 h at room temperature before it was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The obtained ammonium salt was used for the next step without purification.

General Procedure for Glycosylation. To a cooled (−78 °C) solution of **7** (1.0 equiv), BSP (1.2 equiv), and TTBP (1.5 equiv) in dry CH_2Cl_2 (20 mL/mmol of **7**) was added Tf_2O (1.2 equiv). The resulting orange reaction mixture was stirred 30 min

(16) The selectivity was determined by ^1H and ^{31}P NMR analyses of crude reaction mixtures and confirmed by the determination of the $^1J_{CH}$ coupling constants of pure diastereoisomers. Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297.

(17) (a) Hashimoto, S. I.; Honda, T.; Ikegami, S. *J. Chem. Soc., Chem Commun.* **1989**, *11*, 685–687. (b) Plante, O. J.; Palmacci, E. R.; Andrade, R. B.; Seeberger, P. H. *J. Am. Chem. Soc.* **2001**, *123*, 9545–9554. (c) For a review, see: Zhu, X.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 1900–1934.

(18) For this reason, ESIMS analyses of compounds **8** were conducted with solutions in acetonitrile.

(19) With a view to stabilizing the glycosyl phosphate linkage before removal of the benzylidene acetal, compounds **8** were first treated with NaI in acetone to afford quantitatively the corresponding stable sodium phosphates. Unfortunately, hydrogenolyses of these compounds with a variety of catalysts (Pd/C or $Pd(OH)_2/C$) were either incomplete or resulted in complex mixtures.

at −78 °C, and then a solution of ammonium phosphosugars **6** (1.5 equiv) in dry CH_2Cl_2 (1 mL/mmol) was added. The reaction mixture was stirred 20 min at −78 °C, warmed slowly to room temperature, and stirred 1 h at room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$ and then extracted twice with CH_2Cl_2. The combined organic layer was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (30% EtOAc in heptane) to afford the α-D-mannopyranosyl phosphosugars **8**.

General Procedure for Deprotection with Sodium and Liquid Ammonia. The protected α-D-mannopyranosyl phosphosugars **8** were dissolved in dry THF (0.1 mol/L) and placed in a three-necked flask equipped with a bubbler, an ammonia inlet, and a coldfinger condenser. The system was cooled to −78 °C, flushed with argon, and the condenser was filled with dry ice/acetone before ammonia gas was passed through the system. To the ensuing stirred solution was added Na as small spheres until the solution retained a dark blue color, after which stirring was continued for 0.5 h before the reaction was quenched with saturated aqueous NH_4Cl, warmed to room temperature, and concentrated under reduced pressure. The residue was purified on Sephadex LH20 (eluent absolute EtOH) to afford the α-D-mannopyranosyl phosphosugars **9**.

Benzyl 2-Cyanoethyl(methyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranosid-3-yl)phosphate (5g): Mixture of diastereoisomers; colorless oil, 81%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.26−2.50 (m, 4H), 3.36 (s, 6H), 3.71−3.86 (m, 6H), 3.88−4.14 (m, 8H), 4.51−4.60 (m, 4H), 4.68−4.80 (m, 12H), 4.98−5.12 (m, 4H), 7.20−7.40 (m, 40H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.1, 19.2, 19.3, 54.9, 61.6, 61.7, 61.8, 68.9, 69.6, 69.7, 69.8, 71.5, 73.1, 73.5, 74.0, 74.4, 74.7, 77.2, 78.8, 78.9, 79.0, 98.8, 166.2, 166.3, 127.5, 127.6, 127.7, 127.9, 128.3, 128.6, 135.4, 135.5, 137.9, 138.0, 138.2; ^{31}P NMR (202 MHz, $CDCl_3$) δ −2.40, −2.16; ESI-HRMS calcd for $C_{38}H_{42}NO_9PNa$ $[M + Na]^+$ 710.2495, found 710.2495.

Tetrabutylammonium Benzyl(methyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranosid-3-yl)phosphate (6g): Colorless oil, 100%; $[\alpha]^{26}_D$ + 26.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (t, J = 7.2 Hz, 12H), 1.31−1.44 (m, 8H), 1.48−1.62 (m, 8H), 3.21−3.28 (m, 8H), 3.31 (s, 3H), 3.66−3.84 (m, 3H), 3.94 (t, J = 9.5 Hz, 1H), 4.34 (s, 1H), 4.56−4.69 (m, 5H), 4.80 (d, J = 12.6 Hz, 1H), 4.97 (dd, J = 5.1 Hz, J = 12.7 Hz, 1H), 5.02 (d, J = 12.6 Hz, 1H), 5.03 (d, J = 12.4 Hz, 1H), 5.19 (d, J = 11.7 Hz, 1H), 7.16−7.35 (m, 18H), 7.42 (d, J = 6.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 19.8, 24.0, 54.6, 58.7, 68.6, 70.0, 71.9, 73.2, 73.5, 74.4, 75.0, 75.6, 77.2, 100.1, 126.5, 126.9, 127.2, 127.6, 127.8, 127.9, 128.2, 138.7, 139.4, 139.7, 140.2; ^{31}P NMR (202 MHz, $CDCl_3$) δ − 0.73; ESI-HRMS calcd for $C_{35}H_{38}O_9P$ $[M - n\text{-}Bu_4N^+]^-$ 633.2253, found 633.2263.

Benzyl (3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranos-1-yl) (methyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranosid-3-yl)phosphate (8g): Mixture of diastereoisomers; colorless oil, 67%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.03 (s, 3H), 2.04 (s, 3H), 3.36 (s, 6H), 3.70−3.87 (m, 10H), 4.41 (d, J = 11.7 Hz, 1H), 4.46−4.62 (m, 7H), 4.68−4.88 (m, 12H), 4.97−5.15 (m, 4H), 5.26−5.31 (m, 2H), 5.54 (s, 1H), 5.55 (s, 1H), 5.79−5.84 (m, 2H), 7.21−7.48 (m, 60H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.3, 55.3, 55.4, 66.1, 66.3, 68.6, 69.4, 69.8, 69.9, 70.0, 70.3, 72.0, 72.1, 73.4, 73.7, 73.8, 74.2, 74.3, 74.3, 74.4, 74.5, 74.6, 75.1, 75.4, 75.8, 75.9, 76.5, 76.6, 76.7, 79.6, 79.8, 96.7 ($^1J_{CH}$ = 178 Hz), 96.8 ($^1J_{CH}$ = 176 Hz), 99.3 ($^1J_{CH}$ = 171 Hz), 99.4 ($^1J_{CH}$ = 171 Hz), 102.2, 102.3, 126.6, 126.7, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.4, 128.5, 128.6, 129.0, 135.4, 135.5, 137.1, 137.2, 137.9, 138.0, 138.1, 138.2, 169.7, 169.9; ^{31}P NMR (202 MHz, $CDCl_3$) δ −3.65, −3.47; ESI-HRMS (solvent MeCN) calcd for $C_{57}H_{61}O_{15}PNa$ $[M + Na]^+$ 1039.3646, found 1039.3638.

Ammonium (methyl α-D-mannopyranosid-3-yl) (α-D-mannopyranos-1-yl)phosphate (9b): White powder, 89% from **8g**; $[\alpha]^{26}_D$ + 55 (*c* 0.15, CH_3OH); mp 180 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 3.38 (s, 3H), 3.50−3.56 (m, 1H), 3.61 (t, J = 9.7 Hz, 1H), 3.70 (dd, J = 6.3 Hz, J = 11.5 Hz, 1H), 3.74−3.85 (m, 5H), 3.86−3.93 (m, 2H), 4.03−4.05 (m, 1H), 4.32 (td, J = 2.0 Hz, J = 8.0 Hz, 1H), 4.68 (s, 1H), 5.51 (d, J = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 55.4, 62.4, 62.8, 67.4, 68.4, 71.2, 71.9, 72.4 (d, J = 9.2 Hz), 74.4, 75.8, 77.8 (d, J = 4.8 Hz), 98.0 (d, J = 5.5 Hz), 102.5; ^{31}P NMR (202 MHz, CD_3OD) δ −1.34; ESI-HRMS calcd for $C_{13}H_{24}O_{14}P$ $[M^-]^-$ 435.0904, found 435.0918.

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Supporting Information Available: Preparation of **7**, characterization of **5a**−**f**, **6a**−**f**, **7**, **8a**−**f**, and **9a**, ^{1}H, ^{13}C, and ^{31}P NMR spectra of **7**, **5a**−**g**, **6a**−**g**, **8a**−**g**, and **9a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.