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Highly Stereoselective Synthesis of α -D-Mannopyranosyl Phosphosugars

David Crich and Sébastien Picard

Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, CNRS, 1 avenue de la Terrasse, 91198 Gif-sur-Yvette, France, and Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, Michigan 48202, USA.

Abstract

α -Mannopyranosyl phosphosugars are obtained in 61 to 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 groups 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, comprised of 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 congeners,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.

dcrich@icsn.cnrs-gif.fr .

Supporting Information Available: Preparation of **7**, characterisation of **5a-f**, **6a-f**, **7**, **8a-f** and **9a**, ¹H, ¹³C and ³¹P 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>.

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 a approach to the synthesis of the proteophosphoglycans from *Leishmania*.⁶ In our laboratory we prepared β -mannopyranosyl 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 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 ³¹P NMR which showed two signals. The 2-cyanoethyl protecting group was removed by treatment of **5** with tetrabutylammonium hydroxide in a CH₂Cl₂/water mixture (Scheme 1) to give the ammonium phosphosugars **6** quantitatively (Table 1), 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 -mannopyranoside¹³ by acetylation in 98% yield (Scheme 2).

Glycosylation the acceptors **6** with donor **7**, carried out by means of the 1-benzenesulfinyl piperidine (BSP)/Tf₂O preactivation protocol¹⁴ 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 the α -anomers **8** with very high anomeric selectivity and good to high yield following silica gel chromatography¹⁶ (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 donors¹⁷ we observed that compounds **8** were generally not stable in methanolic solution when they gave the corresponding methyl glycosides.¹⁸

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, benzyldiene 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 non-participating 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 CH₂Cl₂ (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 NaHCO₃ and then extracted several times with CH₂Cl₂. The combined organic layer was washed with brine, dried (MgSO₄) 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 CH₂Cl₂:H₂O (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 CH₂Cl₂ and washed with saturated aqueous NaHCO₃. The organic layer was washed with brine, dried (MgSO₄) 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 CH₂Cl₂ (20 mL/mmol of **7**) was added Tf₂O (1.2 equiv). The resulting orange reaction mixture was stirred 30 min at –78 °C and then a solution of ammonium phosphosugars **6** (1.5 equiv) in dry CH₂Cl₂ (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 NaHCO₃ and then extracted twice with CH₂Cl₂. The combined organic layer was washed with brine, dried (MgSO₄) 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** was dissolved in dry THF (0.1 mol/L) and placed in a three-necked flask equipped with a bubbler, an ammonia inlet and a cold finger 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. Colourless oil, 81%. ^1H 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. ESIHRMS Calcd for $\text{C}_{38}\text{H}_{42}\text{NO}_9\text{PNa}$ $[\text{M}+\text{Na}]^+$: 710.2495, found 710.2495.

Tetrabutylammonium benzyl (methyl 2,4,6-tri-O-benzyl- α -D-mannopyranosid-3-yl) phosphate (**6g**)

Colourless oil, 100%. $[\alpha]_D^{26} + 26.8$ (c, 1.0, CHCl_3). ^1H 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. ESIHRMS Calcd for $\text{C}_{35}\text{H}_{38}\text{O}_9\text{P} [\text{M}-n\text{-Bu}_4\text{N}^+]^-$: 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. Colourless oil, 67%. ^1H 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 ($^1J_{\text{CH}} = 178$ Hz), 96.8 ($^1J_{\text{CH}} = 176$ Hz), 99.3 ($^1J_{\text{CH}} = 171$ Hz), 99.4 ($^1J_{\text{CH}} = 171$ 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. ESIHRMS (Solvent MeCN) Calcd for $\text{C}_{57}\text{H}_{61}\text{O}_{15}\text{PNa} [\text{M} + \text{Na}]^+$: 1039.3646, found 1039.3638.

Ammonium (methyl α -D-mannopyranosid-3-yl) (α -D-mannopyranos-1-yl) phosphate (**9b**)

White powder, 89% from **8g**. $[\alpha]_D^{26} + 55$ (c, 0.15, CH_3OH); mp: 180 °C; ^1H 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. ESIHRMS Calcd for $\text{C}_{13}\text{H}_{24}\text{O}_{14}\text{P} [\text{M}]^-$: 435.0904, found 435.0918.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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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 $\text{Pd}(\text{OH})_2/\text{C}$) were either incomplete or resulted in complex mixtures.

Scheme 1.

Preparation of tetrabutylammonium phosphosugars 6

Scheme 2.
Preparation of α -Mannopyranosyl phosphosugars 8

Scheme 3.
Deprotection with sodium in liquid ammonia

Table 1

100

86

% Yield

Ammonium Salt 6

% Yield

% Yield	Ammonium Salt 6	% Yield
94	6b	84

% Yield	Ammonium Salt 6	% Yield
99		81

% Yield

Ammonium Salt 6

% Yield

99

82

% Yield

Ammonium Salt 6

% Yield

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95

98

% Yield

Ammonium Salt 6

% Yield

81

100

69

Table 2

Glycosylation reactions of 7

Entry	Ammonium salt 6	α -Mannopyranosyl Phosphosugars 8
1	6a	8a
2	6b	8b
3	6c	8c

Entry Ammonium salt 6

 α -Mannopyranosyl Phosphosugars 8

4

6d

8d

Entry	Ammonium salt	α -Mannopyranosyl Phosphosugars
5	6e	<p>8e</p>
6	6f	<p>8f</p>

Entry Ammonium salt 6

 α -Mannopyranosyl Phosphosugars 8

7

6g

8g