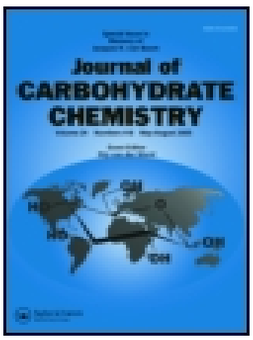

Journal of Carbohydrate Chemistry

ISSN: 0732-8303 (Print) 1532-2327 (Online) Journal homepage: http://www.tandfonline.com/loi/lcar20

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To cite this article: Gabriela Thiele & Thomas Norberg (1998) Synthesis of Carbohydrate Haptens to be Used for Generation of Catalytic Antibodies, Journal of Carbohydrate Chemistry, 17:1, 143-152, DOI: 10.1080/07328309808005774

To link to this article: http://dx.doi.org/10.1080/07328309808005774

Published online: 16 Aug 2006.

J. CARBOHYDRATE CHEMISTRY, 17(1), 143-152 (1998)

SYNTHESIS OF CARBOHYDRATE HAPTENS TO BE USED FOR GENERATION OF CATALYTIC ANTIBODIES

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Dedicated to Professor Hans Paulsen on the occasion of his 75th birthday

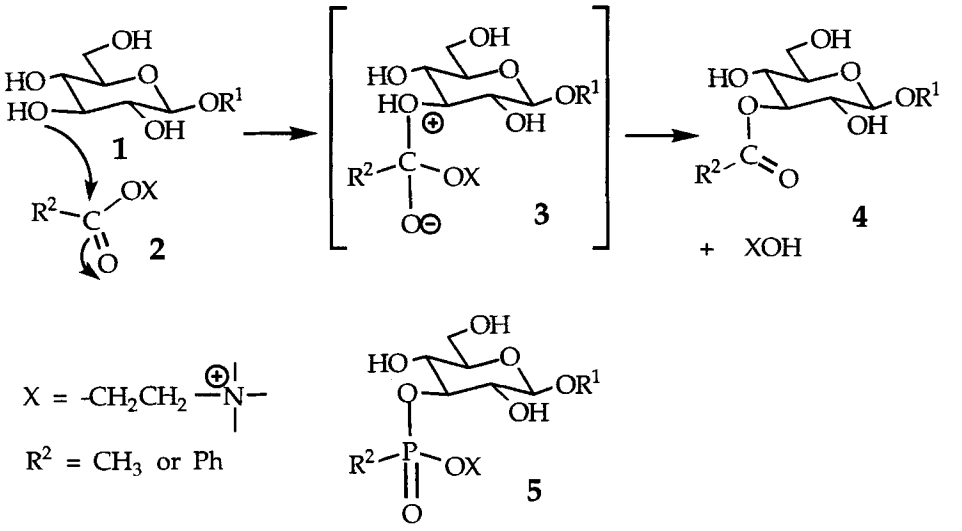
Received March 3, 1997 - Final Form September 26, 1997

ABSTRACT

The glycoside 2-(2-azidoethoxy)ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside was prepared and then reacted, first with methyl phosphonyl dichloride, then with dimethylaminoethanol, to give the methylphosphonyl diester **9**. *N*-Methylation of **9** with methyl iodide and subsequent catalytic hydrogenation gave the methylphosphonyl diester **11**. The corresponding phenylphosphonyl diester **14** was also prepared in a similar way. The phosphonyl diesters **11** and **14** are analogs of the transition states for 3-*O*-acetylation and 3-*O*-benzoylation of β-D-glucopyranose derivatives with acetyl- and benzoylcholine, respectively. They will be used for generation of catalytic antibodies.

INTRODUCTION

In 1986 Schultz[3] and Lerner,[4] independently, showed that monoclonal antibodies could be generated that catalyzed ester hydrolysis. The generation of the antibodies involved immunization with an ester hydrolysis transition state analog. Since then a large number of reports, summarized in several reviews,[5-9] have appeared on use of transition state analogs in generation of antibodies that catalyze a variety of organic reactions. Although many reports have dealt with various aspects of the initially studied reaction, ester hydrolysis (even selective hydrolysis



Scheme 1

of diesters has been reported[10]), only a few[11] have dealt with the reverse reaction, ester formation (acylation). It would be of great interest, e.g. for carbohydrate chemists, if antibodies could be produced that catalyze selective acylation reactions. We now report synthesis of the transition state analogs **11** and **14**, to be used for generation of such catalytic antibodies.

RESULTS AND DISCUSSION

A reaction of a β-D-glucopyranose derivative **1** with an acyl ester **2** to give the 3-*O*-acyl derivative **4** occurs via a transition state closely resembling the tetrahedral intermediate **3** (Scheme 1). The phosphonate derivative **5** should be a reasonably stable transition state analog here, and is therefore a good candidate for synthesis (in a form suitable for protein conjugation) and use for generation of catalytic antibodies. The nature of the X group in **5**, derived from the acylating ester **2**, is important. It should confer good leaving group properties to the XO group in **2**, *i.e.* it should make the forward reaction thermodynamically favorable. It should also be polar enough to make the acylating ester water soluble, since this is the solvent that has to be used with antibodies. Initial attempts to use phenyl groups substituted with polar, electronegative groups as X groups failed, because the corresponding phosphonate esters were difficult to prepare. However, if X was ethyldimethylammonium, phosphonate esters could be prepared in resonable

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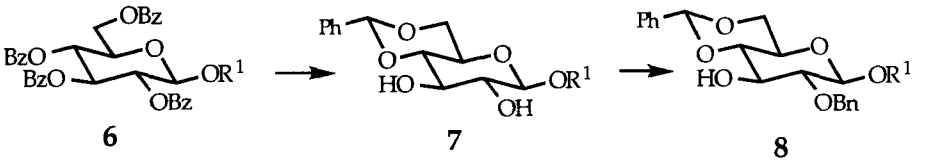
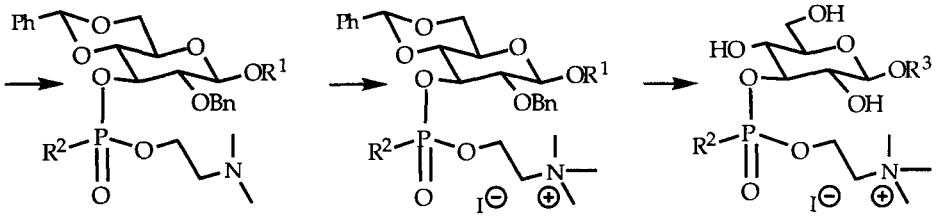
yields. Subsequent *N*-methylation resulted in an ethyltrimethylammonium group, which reasonably fulfills both criteria above. Two transition state analogs with this group were prepared, the methylphosphonate diester **11** (corresponding to an acetylation of **1** with acetylcholine), and the phenylphosphonate diester **14** (corresponding to a benzoylation of **1** with benzoylcholine). The following synthetic steps were carried out:



6 **7** **8**



9: $R^2 = CH_3$ **10:** $R^2 = CH_3$ **11:** $R^2 = CH3$

12: $R^2 = Ph$ **13:** $R^2 = Ph$ **14:** $R^2 = Ph$

Reaction of 2-(2-azidoethoxy)ethanol[12] with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide,[13] using silver triflate promotion[14] gave the 2-(2-azidoethoxy)ethyl glycoside **6** in 76 % crystalline yield. The 2-(2-azidoethoxy)ethyl "linker" group is introduced to make the final product suitable for attachment to an immunogenic protein. Debenzoylation of **6** with sodium methoxide in methanol and treatment of the product with benzaldehyde and anhydrous zinc chloride gave the 4,6-benzylidene derivative **7** in 68 % crystalline yield. Partial benzylation of **7**, using phase transfer conditions[15] gave, as expected, the 2-*O*-benzyl derivative **8** as the major product (63 % crystalline yield). The 2-position of the benzyl group was evident from the ^1H NMR spectrum of the acetylated derivative (downfield shift of H-3). Treatment[16] of **8** with an excess of methylphosphonyl dichloride in pyridine gave a reactive methylphosphonate monoester derivative, which was reacted *in situ* with dimethylaminoethanol to give the methylphosphonate diester **9** (73 %). *N*-methylation of **9** with methyl iodide gave **10** (83 % yield), catalytic hydrogenation of which gave the final methylphosphonate diester **11** (72 % yield). An analogous reaction sequence to the above, starting with the treatment of **8** with an excess of phenylphosphonyl dichloride, produced, via **12** (54%) and **13** (89 %), the

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phenylphosphonate diester **14** (82 %). All the phosphonate derivatives above were obtained as 1:1 mixtures of diastereomers, which could not easily be separated by chromatography. The physical constants given in the experimental part are therefore for the mixtures, with the exception of the NMR data, where assignments of signals to individual diastereomers were possible.

Conjugation of **11** and **14** to protein, immunization, and screening for catalytic activity of the obtained antibodies will be reported in a coming paper.

EXPERIMENTAL

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General methods. Melting points were determined on a Mettler FP 62 apparatus. Optical rotations were measured at 23 °C (*c* 1.0, chloroform) unless otherwise stated, using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at 298 °K with Bruker DRX 400 (400.13 MHz for ^{1}H and 11.625 MHz for ^{13}C) or Bruker DRX 600 (600.13 MHz for ^{1}H and 150.91 MHz for ^{13}C), instruments. Chemical shifts are given in ppm downfield from internal TMS (external when D_2O was the solvent). Assignments were corroborated by appropriate 2-D experiments. In the NMR data of diastereomeric H-phosphonate mixtures, the signals from one of the isomers are differentiated with an asterisk (*). HRMS spectra were recorded in the positive ion mode with a JEOL SX/102A mass spectrometer, using the FAB ion source and 3-nitrobenzyl alcohol as matrix. For thin layer chromatography, glass plates precoated with silica gel 60 F_{254} (E. Merck, Darmstadt, Germany) were used, with detection by UV light, charring with 10% sulfuric acid in ethanol, or ninhydrin spray. Column chromatography was performed using silica gel 60 (0.035-0.070 mm, Matrex LC 60A, Grace, Helsingborg, Sweden). Molecular sieves (4Å, rods, from Union Carbide) were dried and immediately ground before use at 300 °C /1 torr overnight. Water-washed organic solutions were dried over anhydrous sodium sulfate prior to concentration. Commercial methylphosphonyl dichloride and phenylphosphonyl dichloride (Fluka) were used without purification. Dimethylaminoethanol (Acros) was distilled under reduced pressure and kept over molecular sieves prior to use.

2-(2-Azidoethoxy)ethyl 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranoside (6). 2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[13] (5 g, 7.6 mmol) and 2-(2-azidoethoxy)ethanol[12] (2 g, 15.3 mmol) were dissolved in anhydrous dichloromethane (30 mL) and molecular sieves (4Å) were added. The mixture was cooled to -25 °C and a solution of silver triflate (3 g, 11.6 mmol) in anhydrous toluene (10 mL) was added. The reaction mixture was further stirred for 3 h at a temp between -15 and -25 °C. After additon of an excess of pyridine, the mixture was warmed to rt and then filtered. The filtrate was diluted with dichloromethane,

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then washed with, successively, aqueous sodium thiosulfate, 2 M aqueous sulfuric acid, aqueous sodium bicarbonate, and water, dried and concentrated. The residue was purified by column chromatography (toluene/ethyl acetate 4:1 v/v). Crystallization of the main fraction (R_f = 0.55) from methanol with activated carbon gave **6** (4.1 g, 76 %) as colourless needles: mp 96-97 °C; $[\alpha]_D$ + 18°; ^{1}H NMR ($CDCl_3$) δ 3.09 (dt, 2H, H-4'), 3.46 (dd, 2H, $J_{3',4'}$ = 5.1 Hz, H-3'), 3.60 (m, 2H, H-2'), 3.81 (ddd, 1H, $J_{1'a,2'}$ = 3.8 Hz, H-1'a), 4.00 (ddd, 1H, $J_{1'a,1'b}$ = 11.5 Hz, $J_{1'b,2'}$ = 4.2 Hz, H-1'b), 4.19 (ddd, 1H, H-5), 4.51 (dd, 1H, $J_{6a,5}$ = 5.1 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.66 (dd, 1H, $J_{6b,5}$ = 3.1 Hz, H-6b), 4.98 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.54 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2), 5.69 (dd, 1H, $J_{4,3}$ = $J_{4,5}$ = 9.8 Hz, H-4), 5.93 (dd, 1H, H-3), 7.25-8.04 (m, 20 H, 4 Ph); ^{13}C NMR ($CDCl_3$) δ 50.7 (C-4'), 63.2 (C-6), 69.6 (C-1'), 69.9 (C-4), 70.2 (C-3'), 70.5 (C-2'), 72.1 (C-2), 72.4 (C-5), 73.0 (C-3), 101.5 (C-1), 128.4-133.6 (4 Ph), 165.2, 165.5, 166.1, 166.5 (4 *C*=*O*). HRMS Calcd for $C_{38}H_{36}N_3O_{11}$: 710.2350. Found: 710.2446 $(M+H)^+$.

2-(2-Azidoethoxy)ethyl 4,6-*O*-Benzylidene-β-D-glucopyranoside (7). A solution of **6** (5 g, 2.8 mmol) in methanol (30 mL) containing sodium methoxide (0.1M in methanol, 1.0 mL), was stirred at rt until TLC detected no further change, then it was neutralised with Dowex 50 (H^+), filtered and concentrated. The residue was suspended in water (15 mL), the solution was washed twice with ethyl acetate and then lyophilized. The obtained material was dissolved in benzaldehyde (10 mL), anhydrous zinc chloride (1 g, 7.3 mmol) was added and the solution was stirred at rt for 24 h. The reaction mixture was poured into ice-water with vigorous stirring. After 20 min, the aqueous phase was washed with petroleum ether (2x) and then extracted with dichloromethane (3 x 50 mL). The combined dichloromethane layers were washed with water, dried (sodium sulfate) and concentrated. Crystallization from toluene (~8 mL) gave **7** (1.85 g, 68% from **6**) as white needles: mp 72-73 °C; $[\alpha]_D$ - 44°; ^{1}H NMR ($CDCl_3$) δ 3.38 (t, 2H, $J_{4',3'}$ = 5.1 Hz, H-4'), 3.44 (ddd, 1H, $J_{5,4}$ = 9.6 Hz, $J_{5,6ax}$ = 9.7 Hz, H-5), 3.52 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 3.55 (dd, 1H, $J_{4,3}$ = 9.2 Hz, H-4), 3.67 (t, 2H, H-3'), 3.71 (m, 2H, H-2'), 3.77 (dd, 1H, H-6ax), 3.78, 3.81 (m, 2H, H-1'a, H-3), 4.04 (ddd, 1H, $J_{1'a,1'b}$ = 11.1 Hz, $J_{1'b,2'}$ = 3.2 Hz, H-1'b), 4.33 (dd, 1H, $J_{6eq,6ax}$ = 10.4 Hz, $J_{6eq,5}$ = 4.9 Hz, H-6 eq), 4.45 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 5.52 (s, 1H, Ph-C*H*), 7.34-7.50 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ 50.7 (C-4'), 66.6 (C-5), 68.7 (C-6), 69.3 (C-1'), 70.1 (C-3'), 70.3 (C-2'), 73.2 (C-3), 74.6 (C-2), 80.6 (C-4), 102.0 (Ph-CH), 103.7 (C-1), 126.4, 128.4, 129.3, 137.1 (Ph). HRMS Calcd for $C_{17}H_{24}N_3O_7$: 382.1614. Found: 382.1685 $(M+H)^+$.

2 - (2-Azidoethoxy)ethyl 2-*O*-Benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (8). Tetrabutylammonium hydrogen sulfate (360 mg, 1 mmol), benzyl bromide (1.55 g, 9.1 mmol), and compound **7** (2 g, 4.2 mmol) were dissolved in dichloromethane (30 mL). Aqueous sodium hydroxide (8 mL of a 5% solution) was added and the mixture was boiled under reflux for 8 h. After cooling to rt, the layers were separated. The organic layer was washed with water, dried, and concentrated.

Purification of the residue by column chromatography (petroleum ether/ethyl acetate 2:1 v/v) gave a major product (R_f = 0.39) **8** (1.55 g, 63%), which crystallized as white needles from ether/petroleum ether: mp 80-81 °C; $[\alpha]_D$ - 27°; ^{1}H NMR ($CDCl_3$) δ 2.28 (b, 1H, OH), 3.33 (dt, 2H, H-4'), 3.38 (dd, 1H, $J_{2,3}$ = 7.8 Hz, H-2), 3.43 (ddd, 1H, $J_{5,6eq}$ = 4.9 Hz, $J_{5,6ax}$ = 9.8 Hz, H-5), 3.54 (dd, 1H, $J_{4,3}$ = 9.3 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.65 (dd, 2H, $J_{3',4'}$ = 5.1 Hz, H-3'), 3.71 (m, 2H, H-2'), 3.78 (dd, 1H, H-6ax), 3.83 (m, 1H, H-1'a), 3.85 (m, 1H, H-3), 4.04 (ddd, 1H, $J_{1'a,1'b}$ = 11.3 Hz, $J_{1'b,2'}$ = 3.5 Hz, H-1'b), 4.34 (dd, 1H, $J_{6ax,6eq}$ = 10.4 Hz, H-6eq), 4.58 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.77 (d, 1H, $J_{Ha,Hb}$ = 11.4 Hz, C*Ha*H-Ph), 4.99 (d, 1H, CH*Hb*-Ph), 5.52 (s, 1H, Ph-C*H*), 7.14-7.52 (m, 10H, 2Ph); ^{13}C NMR ($CDCl_3$) δ 50.8 (C-4'), 66.2 (C-5), 68.8 (C-6), 69.4 (C-1'), 70.1 (C-3'), 70.5 (C-2'), 73.2 (C-3), 74.7 (*CH_2*-Ph), 80.5 (C-4), 81.8 (C-2), 101.9 (Ph-CH), 104.1 (C-1), 126.4, 128.1, 128.4, 128.6, 129.2, 129.8, 137.2, 137.5 (2Ph). HRMS Calcd for $C_{24}H_{30}N_3O_7$: 472.2084. Found: 472.2144 $(M+H)^+$.

2-(2-Azidoethoxy)ethyl 2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-[(2-*N*,*N*-dimethylaminoethyl)methylphosphonyl]-β-D-glucopyranoside (9). Molecular sieves (4Å, 1 g) were added to a solution of **8** (500 mg, 1.1 mmol) in anhydrous pyridine (10 mL). The mixture was flushed with dry nitrogen and cooled to 0 °C. Cold methylphosphonic dichloride (1 g, 7.5 mmol) was added and the mixture was stirred for 30 min under dry nitrogen at rt until all starting material had disappeared (TLC, (toluene/ethyl acetate 2:1 and ethyl acetate/methanol 3:1). Then dry 2-dimethylaminoethanol (5 g, 56 mmol) was added and the stirring was continued for 40 h at 45 °C under dry nitrogen. Then the mixture was kept in a refrigerator for 1 h. The solids were removed by filtration and washed with dichloromethane (3 x 10 mL). The filtrate was washed with water, dried, concentrated and co-evaporated with toluene (2 x 20 mL). Purification of the residue by column chromatography (ethyl acetate/triethylamine/MeOH 40:10:3 v/v) gave the main product (R_f = 0.62) **9** (481 mg, 73%) as a yellow syrup, $[\alpha]_D$ - 26°; ^{1}H NMR ($CDCl_3$) δ 1.33, 1.38 (2 d, 6H, $J_{Me,P}$ = 18.2 Hz, Me-P, Me-P*), 2.03, 2.08 (2s, 12H, NMe_2, NMe_2*), 2.19, 2.24 (2 dd, 4H, $J_{CH2\text{-}O,CH2\text{-}N}$ = 5.5 Hz, P-O-CH_2-C*H_2*, P-O-CH_2-C*H_2**), 3.24 (m, 4H, H-4', H-4'*), 3.45 (m, 2H, H-5, H-5*), 3.52 (m, 2H, H-2, H-2*), 3.55, 3.57 (2dd, 4H, $J_{3',4'}$ = 5.0 Hz, H-3', H-3'*), 3.62, 3.64 (dd, m, 2H, H-4, H-4*), 3.66 (m, 4H, H-2', H-2'*), 3.72, (ddd, 1H, P-O-C*Ha*H), 3.77, 3.78 (m, 3H, P-O-C*Ha*H*, H-6ax, H-6ax*), 3.79 (m, 2H, H-1'a, H-1'a*), 3.94, 3.97 (2ddd, 2H, P-O-CH*Hb*, P-O-CH*Hb**), 4.02 (m, 2H, H-1'b, H-1'b*), 4.33, 4.34 (2dd, 2H, $J_{6eq,6ax}$ = 10.5 Hz, $J_{6eq,5}$ = 4.9 Hz, H-6eq, H-6eq*), 4.60 (dd, 1H, H-3), 4.62 (d, 2H, $J_{1,2}$ = 7.6 Hz, H-1, H-1*), 4.66 (m, 3H, C*H*aH-Ph, H-3*, C*H*aH-Ph*), 5.05, 5.07 (2d, 2H, $J_{Ha,Hb}$ = 11.4 Hz, CH*Hb*-Ph, CH*Hb*-Ph*), 5.47, 5.50 (2s, 2H, Ph-C*H*, Ph-C*H**), 7.24-7.55 (m, 20H, 2Ph, 2Ph*); ^{13}C NMR ($CDCl_3$) δ 11.6, 12.6 (MeP, MeP*), 45.1, 45.2 (NMe_2, NMe_2*), 50.6 (C-4', C-4'*), 58.8 (P-O-CH_2, P-O-CH_2*), 62.1 (P-O-CH_2-CH_2, P-O-CH_2-CH_2*), 65.8, 65.9 (C-5, C-5*), 68.7, 68.8 (C-6, C-6*), 69.5 (C-1', C-1'*), 70.0 (C-3', C-3'*), 70.4 (C-2', C-2'*), 74.3, 74.4 (CH_2Ph,

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CH_2Ph*), 76.1, 76.4 (C-3, C-3*), 79.6, 79.7 (C-4, C-4*), 80.8, 80.9 (C-2, C-2*), 101.9, 102.1 (Ph-CH, Ph-CH*), 104.1, 104.2 (C-1, C-1*), 126.3-138.1 (2Ph, 2Ph*). HRMS Calcd for $C_{29}H_{42}N_4O_9P$: 621.2689. Found: 621.2703 $(M+H)^+$.

2-(2-Azidoethoxy)ethyl 2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-[(2-*N,N,N*-trimethylaminoethyl)methylphosphonyl]-β-D-glucopyranoside iodide (10). A solution of **9** (100 mg, 0.16 mmol) in ethanol (10 mL) was treated with methyl iodide (30 mg, 0.21 mmol) for 4-5 h, until all starting material had disappeared (TLC; $CHCl_3$/MeOH 10:1 v/v). Then the solution was gently concentrated to give **10** as a syrup (104 mg, 83 %), $[\alpha]_D$ -17°; ^1H NMR ($CDCl_3$) δ 1.33, 1.42 (2d, 6H, $J_{Me,P}$ = 18.0 Hz, Me-P, Me-P*), 3.04, 3.11 (2s, 18H, NMe_3, NMe_3*), 3.15, 3.23 (2ddd, 2H, P-O-CH_2-C*Ha*H, P-O-CH_2-C*Ha*H*), 3.24, 3.26 (dd, dt, 4H, $J_{4,3} = J_{4,3}$ = 9.6 Hz, H-4', H-4'*), 3.40 (ddd, 1H, $J_{Ha,Hb}$ = 14.4 Hz, $J_{Hb,\ CH2O}$ = 7.1 Hz, P-O-CH_2-CH*Hb*), 3.46, 3.47 (m, dd, 2H, P-O-CH_2-CH*Hb**, H-2), 3.52, 3.55, 3.57 (m, 3H, H-5, H-2*, H-5*), 3.58, 3.59 (2dd, 4H, $J_{3',4'}$ = 5.0 Hz, H-3', H-3'*), 3.66 (m, 5H, H-2', H-2'*, H-4), 3.72 (dd, 1H, H-4*), 3.76, 3.78, 3.79, 3.81 (m, 4H, H-6ax, H-1'a, H-6ax*, H-1'a*), 3.97 (ddd, 1H, P-O-C*Ha*H), 4.02 (2ddd, 2H, H-1'b, H-1'b*), 4.13 (m, 1H, P-O-CH*Hb*), 4.21, 4.24 (m, 2H, P-O-C*Ha*H*, P-O-CH*Hb**), 4.33, 4.36 (2dd, 2H, $J_{6eq,6ax}$ = 10.7 Hz, $J_{6eq,5}$ = 5.0 Hz, H-6eq, H-6eq*), 4.59 (2d, 2H, $J_{Ha,Hb}$ = 11.4 Hz, C*Ha*H-Ph, C*Ha*H-Ph*), 4.60, 4.66 (2dd, 2H, $J_{3,4} = J_{3,2}$ = 9.6 Hz, H-3, H-3*), 4.68, 4.73 (2d, 2H, $J_{1,2}$ = 7.6 Hz, H-1, H-1*), 5.08, 5.17 (2d, 2H, CH*Hb*-Ph, CH*Hb*-Ph*), 5.51, 5.58 (2s, 2H, Ph-C*H*, Ph-C*H**), 7.26-7.55 (m, 20H, 2Ph, 2Ph*); ^{13}C NMR ($CDCl_3$) δ 12.2, 13.2 (MeP, MeP*), 51.5 (C-4', C-4'*), 55.3 (NMe_3, NMe_3*), 58.8, 59.1 (P-O-CH_2, P-O-CH_2*), 66.5, 66.7 (C-5, C-5*), 66.8, 66.9 (P-O-CH_2-CH_2, P-O-CH_2-CH_2*), 69.5, 69.6 (C-6, C-6*), 70.3 (C-1', C-1'*), 70.8 (C-3', C-3'*), 7 1.2 (C-2', C-2'*), 75.1, 75.3 (*C*H_2Ph, *C*H_2Ph*), 77.5, 77.6 (C-3, C-3*), 80.0, 80.3 (C-4, C-4*), 81.5, 81.6 (C-2, C-2*), 102.6, 103.0 (Ph-CH, Ph-CH*), 104.8, 105.0 (C-1, C-1*), 127.3-139.2 (2Ph, 2Ph*). HRMS Calcd for $C_{30}H_{44}N_4O_9P$: 635.2846. Found: 635.2861 $(M)^+$.

2-(2-Aminoethoxy)ethyl 3-*O*-[(2-*N,N,N*-trimethylaminomethyl)methylphosphonyl]-β-D-glucopyranoside iodide (11). A solution of **10** (350 mg, 0.46 mmol) in ethanol-water (1:1, 8 mL) was hydrogenated at 5 MPa/rt overnight in the presence of Pd/C (10 %, 200 mg) and conc hydrochloric acid (0.1 mL). The catalyst was filtered off and washed carefully with ethanol-water (1:1, 35 mL), the combined filtrates were concentrated to 10 mL, and hydrogenated again with fresh portions of Pd/C (200 mg) and hydrochloric acid (0.1 mL) until finished (monitoring by TLC, ethyl acetate-methanol-acetic acid-water, 4:6:3:2 and MALDI-TOF MS). The catalyst was filtered off and washed carefully with ethanol-water (1:1, 35 mL), the combined filtrates were first concentrated to 10 mL, then lyophilized. The residue was purified by gel filtration (Bio-Gel P-2, elution with 95:5:0.2 water/1-butanol/acetic acid). Lyophilization of appropriate fractions (TLC monitoring with ninhydrin or sulfuric acid visualization) gave amorphous **11** (185 mg, 72 %), $[\alpha]_D$ - 8 (*c* 0.5, water). The product was stored at - 20 °C in the presence of ≈ 10 μL of acetic acid. ^1H NMR

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(D_2O) δ 1.64, 1.65 (2d, 6H, $J_{Me,P}$ = 18.0 Hz, Me-P, Me-P*), 3.12 (s, 18H, NMe_3, NMe_3*), 3.14 (m, 4H, H-4', H-4'*), 3.41 (m, 4H, H-2, H-2*, H-5, H-5*), 3.51, 3.52 (2dd, 2H, H-4, $J_{4,5}$ = 9.7 Hz, H-4*), 3.62 (2dd, 4H, $J_{1',2'}$ = 4.6 Hz, H-2', H-2'*), 3.61, 3.66, 3.67 (m, 6H, H-6, H-6*, P-O-C*Ha*H, P-O-C*Ha*H*), 3.70 (dd, 4H, $J_{3',4'}$ = 5.1 Hz, H-3', H-3'*), 3.78 (2dd, 2H, P-O-CH_2-C*Ha*H, P-O-CH_2-C*Ha*H*), 3.82, 3.84 (2dd, 2H, P-O-CH*Hb*, P-O-CH*Hb**), 3.97 (m, 2H, P-O-CH_2-CH*Hb*, P-O-CH_2-CH*Hb**), 4.18 (2dd, 2H, $J_{3,4}$ = $J_{3,2}$ = 9.1 Hz, H-3, H-3*), 4.44, 4.45 (2d, 2H, $J_{1,2}$ = 8.0 Hz, H-1, H-1*), 4.48 (m, 4H, H-1', H-1'*); ^{13}C NMR (D_2O) δ 10.9, 11.9 (MeP, MeP*), 39.9 (C-4', C-4'*), 54.7 (NMe_2, NMe_2*), 59.9 (C-1', C-1'*), 61.2 (P-O-CH_2, P-O-CH_2*), 66.5 (C-2', C-2'*), 67.2 (C-3', C-3'*), 69.2, 69.3 (C-4, C-4*), 69.9 (P-O-CH_2-CH_2, P-O-CH_2-CH_2*), 70.5 (C-6, C-6*), 72.9, 73.0 (C-2, C-2*), 76.3, 76.4 (C-5, C-5*), 82.7, 82.8 (C-3, C-3*), 102.9, 103.0 (C-1, C-1*). HRMS Calcd for $C_{16}H_{36}N_2O_9P$: 431.2158. Found: 431.2177 $(M)^+$.

2-(2-Azidoethoxy)ethyl 2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-[(2-*N*,*N*-dimethylaminoethyl)phenylphosphonyl]-β-D-glucopyranoside (12). Molecular sieves (4Å, 1 g) were added to a solution of **8** (200 mg, 0.42 mmol) in anhydrous pyridine (10 mL). The reaction vessel was flushed with dry nitrogen and cooled to 0 °C, then cold phenylphosphonic dichloride (170 mg, 0.87 mmol) was added and the mixture was stirred at rt for 5 min and then at 40 °C while adding a small amount of the dichloride every 30 min, until most of the starting material had disappeared (no more than 3.5 - 4 h, TLC toluene/ethyl acetate 2:1 and ethyl acetate/methanol 3:1, v/v). Then dry 2-dimethylaminoethanol (1.5 g, 17 mmol) was added and the stirring was continued for 40 h at 50 °C (the entire reaction sequence was performed under dry nitrogen). Then the mixture was kept in a refrigerator for 1 h. The solids were removed by filtration and washed with dichloromethane (3 x 10 mL). The filtrate was washed with water, dried, concentrated and co-concentrated with toluene (2 x 20 mL). Purification of the residue by column chromatography (ethyl acetate/ triethylamine 10:1 v/v) gave the main product (R_f = 0.55) **12** (155 mg, 54%) as a colourless syrup, $[\alpha]_D$ -31°; ^{1}H NMR ($CDCl_3$) δ 2.04, 2.08 (2s, 12H, NMe_2, NMe_2*), 2.29, 2.34 (2dd, dd, 4H, $J_{Ha,Hb}$ = 11.2 Hz, $J_{CH2\text{-}O,CH2\text{-}N}$ = 5.8 Hz, P-O-CH_2-CH_2, P-O-CH_2-CH_2*), 3.21, 3.28 (2dt, 4H, H-4', H-4'*), 3.43, 3.44 (m, 2H, H-5, H-2), 3.46 (ddd, 1H, $J_{5,6ax}$ = $J_{5,4}$ = 9.7 Hz, $J_{5,6eq}$ = 5.0 Hz, H-5*), 3.52, 3.57 (2dd, 4H, $J_{3',4'}$ = 5.1 Hz, H-3', H-3'*), 3.56 (m, 1H, H-2*), 3.58, 3.64 (2m, 4H, H-2', H-2'*), 3.61, 3.69 (2m, 2H, H-4, H-4*), 3.71, 3.74, 3.76, 3.78 (m, 4H, H-6ax, H-1a', H-1a'*, H-6ax*), 3.95, 3.96 (m, 2H, P-O-C*Ha*H, P-O-C*Ha*H*), 3.98, 3.99 (m, 2H, H-1'b, H-1'b*), 4.02, 4.03 (m, 2H, P-O-CH*Hb*, P-O-CH*Hb**), 4.27 (d, 1H, $J_{Ha,Hb}$ = 11.4 Hz, C*Ha*H-Ph), 4.31, 4.35 (2dd, 2H, $J_{6eq,6ax}$ = 10.5 Hz, H-6eq, H-6eq*), 4.59, 4.63 (2d, 2H, $J_{1,2}$ = 7.6 Hz, H-1, H-1*), 4.73 (2d, 2H, C*Ha*H-Ph*, CH*Hb*-Ph), 4.78, 4.84 (2dd, 2H, $J_{3,2}$ = $J_{3,4}$ = 9.3 Hz, H-3, H-3*), 5.02 (d, 1H, CH*Hb*-Ph*), 5.30, 5.51 (2s, 2H, Ph-C*H*, Ph-C*H**), 7.26-7.65 (m, 30H, 3Ph, 3Ph*); ^{13}C NMR ($CDCl_3$) δ 46.1, 46.3 (NMe_2, NMe_2*), 51.4, 51.5 (C-4', C-4'*), 59.5 (P-O-CH_2-CH_2, P-O-CH_2-CH_2*), 64.3 (P-O-CH_2, P-O-CH_2*), 66.7 (C-5, C-5*), 69.5, 69.6 (C-6, C-

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6*), 70.3 (C-1', C-1'*), 70.7, 70.8 (C-3', C-3'*), 71.1, 71.2 (C-2', C-2'*), 74.7, 75.0 (CH_2Ph, CH_2Ph*), 78.0, 78.1 (C-3, C-3*), 80.3 (C-4, C-4*), 81.5, 81.6 (C-2, C-2*), 102.4, 102.8 (Ph-CH, Ph-CH*), 104.9, 105.1 (C-1, C-1*), 127.2-139.3 (3Ph, 3Ph*). HRMS Calcd for $C_{34}H_{44}N_4O_9P$: 683.2846. Found: 683.2851 $(M+H)^+$.

2-(2-Azidoethoxy)ethyl 2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-[(2-*N,N,N*-trimethylaminomethyl)phenylphosphonyl]-β-D-glucopyranoside iodide (13). A solution of **12** (100 mg, 0.15 mmol) in ethanol (10 mL) was treated with methyl iodide (30 mg, 0.21 mmol) for 4-5 h, until all starting material had disappeared(TLC, $CHCl_3$/MeOH 10:1 v/v). Then the solution was gently concentrated to give **13** as a syrup (108 mg, 89 %), $[\alpha]_D$ - 22°; ^1H NMR ($CDCl_3$) δ 2.97, 3.04 (2s, 18H, NMe_3, NMe_3*), 3.21, 3.23 (dt, t, 4H, H-4', H-4'*), 3.35, 3.37 (m, 2H, P-O-CH_2-C*Ha*H, P-O-CH_2-C*Ha*H*), 3.39 (dd, 1H, H-2), 3.49 (ddd, 1H, $J_{5,6ax}$ = 9.6 Hz, H-5), 3.52 (dd, 2H, $J_{3',4'}$ = 5.2 Hz, H-3'), 3.53, 3.55 (m, 2H, P-O-CH_2-CH*Hb*, H-5*), 3.57 (dd, 2H, H-3'*), 3.58, 3.59, 3.60, 3.61 (m, 5H, H-2*, H-2', P-O-CH_2-CH*Hb**, H-4), 3.65, 3.70 (m, 3H, H-2'*, H-6ax), 3.73 (ddd, 1H, H-1'a), 3.78, 3.79, 3.81 (m, 3H, H-6ax*, H-4*, H-1'a*), 3.97, 4.02 (2ddd, 2H, $J_{1'a,1'b}$ = 11.5 Hz, $J_{1'b,2'}$ = 4.0 Hz, H-1'b, H-1'b*), 4.11 (d, 1H, C*Ha*H-Ph), 4.11, 4.20, 4.23 (m, 4H, P-O-C*Ha*H, P-O-C*Ha*H*, P-O-CH*Hb*, P-O-CH*Hb**), 4.30, 4.33 (2dd, 2H, $J_{6eq,6ax}$ = 10.7 Hz, $J_{5,6eq}$ = 5.0 Hz, H-6eq, H-6eq*), 4.58 (d, 1H, $J_{Ha,Hb}$ = 11.5 Hz, C*Ha*H-Ph*), 4.64 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.72 (d, 1H, CH*Hb*-Ph), 4.73 (d, 1H, H-1*), 4.77, 4.82 (2dd, 2H, $J_{3,2}$ = $J_{3,4}$ = 9.2 Hz, H-3, H-3*), 5.18 (d, 1H, $J_{Ha,Hb}$ = 11.5 Hz, CH*Hb*-Ph*), 5.28, 5.59 (2s, 2H, Ph-C*H*, Ph-C*H**), 7.0-7.55 (m, 30H, 3Ph, 3Ph*); ^{13}C NMR ($CDCl_3$) δ 51.4, 51.5 (C-4', C-4'*), 55.3 (NMe_3, NMe_3*), 59.8, 60.0 (P-O-CH_2, P-O-CH_2*), 66.6 (C-5, C-5*, P-O-CH_2-CH_2, P-O-CH_2-CH_2*), 69.4, 69.5 (C-6, C-6*), 70.3 (C-1', C-1'*), 70.7, 70.8 (C-3', C-3'*), 71.2 (C-2', C-2'*), 74.7, 74.9 (CH_2Ph, CH_2Ph*), 78.4, 78.5 (C-3, C-3*), 80.0, 80.1 (C-4, C-4*), 81.2, 81.5 (C-2, C-2*), 102.3, 103.0 (Ph-CH, Ph-CH*), 104.7, 104.9 (C-1, C-1*), 127.2-139.4 (3Ph, 3Ph*). HRMS Calcd for $C_{35}H_{46}N_4O_9P$: 697.3002 Found: 697.3026 $(M)^+$.

2-(2-Aminoethoxy)ethyl 3-*O*-[(2-*N,N,N*-trimethylaminomethyl)phenylphosphonyl]-β-D-glucopyranoside iodide (14). Compound **13** (270 mg, 0.33 mmol) was hydrogenated and purified essentially as described for **11** to give **14** (170 mg, 82 %, >80 % purity), $[\alpha]_D$ - 6° (c 0.5, water); ^1H NMR (D_2O) δ 3.09 (s, 18 H, NMe_3, NMe_3*), 3.08, 3.11 (2 m, 4 H, H-4', H-4'*), 3.34 (dd, 1H, H-2), 3.36, 3.40 (m, dd, 2H, H-5, H-5*), 3.47 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 3.48 (dd, 1H, H-2*), 3.60 (m, 1H, H-4*), 4.27, 4.29 (2 dd, 2H, $J_{3,4}$ = $J_{3,2}$ = 9.1 Hz, H-3, H-3*), 4.41, 4.47 (2d, 2H, $J_{1,2}$ = 8.0 Hz, H-1, H-1*), 4.52 (m, 4H, H-1', H-1'*); ^{13}C NMR (D_2O) δ 39.9 (C-4', C-4'*), 54.8 (NMe_3, NMe_3*), 60.7 (C-1', C-1'*), 61.2, 69.9 (P-O-CH_2-CH_2, P-O-CH_2-CH_2*, P-O-CH_2-CH_2, P-O-CH_2-CH_2*), 66.5 (C-2', C-2'*), 67.1, 67.2 (C-3', C-3'*), 69.2 (C-4', C-4'*), 70.5 (C-6, C-6*), 72.8, 72.9 (C-2, C-2*), 76.3 (C-5, C-5*), 83.4 (C-3, C-3*), 102.9 (C-1, C-1*), 125.7, 126.9, 129.7, 129.9, 132.2, 132.3, 134.7 (aromatic C). HRMS Calcd for $C_{21}H_{38}N_2O_9P$: 493.2315. Found: 493.2332 $(M)^+$.

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ACKNOWLEDGEMENTS

We are indebted to the Deutsche Forschungsgemeinschaft for a postdoctoral grant (Th 627/1-1), and to the Swedish National Defense Research Establishment (FOA) for financial support. We also thank Dr. Corine Sandström at the Chemistry Department's NMR laboratory for invaluable support and expert help with NMR interpretations.

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