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The stepwise synthesis of oligo(glycosyl phosphates) via glycosyl hydrogenphosphonates. The chemical synthesis of oligomeric fragments from *Hansenula capsulata* Y-1842 exophosphomannan and from *Escherichia coli* K51 capsular antigen

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ABSTRACT

A stepwise approach has been used in the syntheses of pentamannosyl tetraphosphate HO-[-6Man(α)-PO_4-]$_4$-6Man(α)-OMe and tetra(*N*-acetylglucosaminyl) triphosphate HO-[-3GlcNAc(α)-PO_4]$_3$-3GlcNAc(β)-$OC_6H_4NO_2$, which are fragments of the yeast and bacteria extracellular phosphoglycans. Elongation of the chain was performed with the use of suitably protected glycosyl hydrogenphosphonate derivatives for successive introduction of glycosyl phosphate residues. Partially protected monosaccharide derivatives and oligomeric blocks served as hydroxylic components.

INTRODUCTION

In recent papers, a highly efficient method for the synthesis of glycosyl phospho sugars via glycosyl hydrogenphosphonates has been described[1–3]. A series of glycosyl phospho sugars that are fragments of yeast phosphoglycans[4–6], of several glycoproteins[5], and of poly(glycosyl phosphates) from bacterial cell walls and capsules[1,5,6] was synthesized by this approach. Till now, the chemical synthesis of longer fragments of poly(glycosyl phosphates) containing several phosphate diester units has not been achieved. The polycondensation of a partially protected mannosyl hydrogenphosphonate derivative, investigated as a possible approach to oligo(glycosyl phosphates), was found to give an unusual cyclic (1 → 6)-linked di(α-D-mannopyranosyl phosphate) as the main product[7,8]. In addition, linear oligo(mannosyl phosphates) with dp of ~ 3–7 were obtained in an overall yield of 16%.

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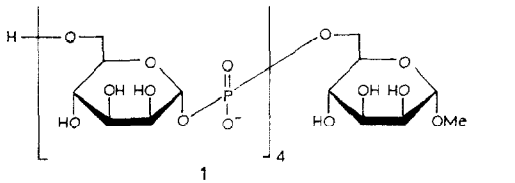

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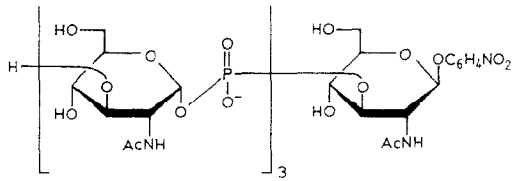

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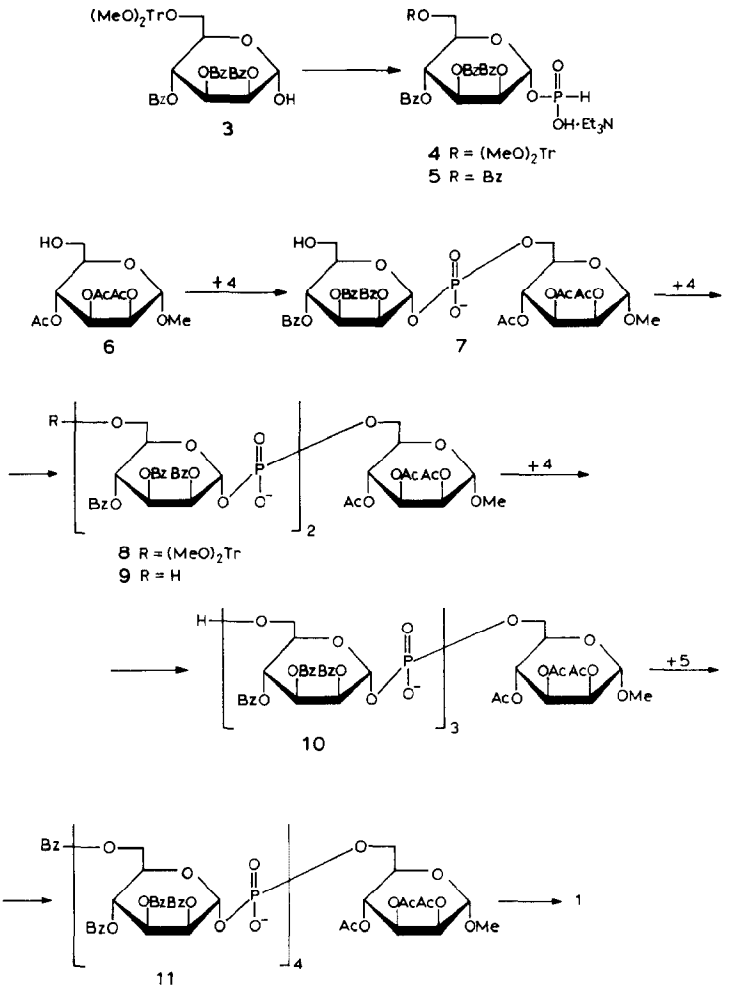
We now report the application of a stepwise approach to chain elongation for the synthesis of linear oligo(glycosyl phosphates). The construction of the phosphate diester units was achieved by the use of the glycosyl hydrogenphosphonate method.

RESULTS AND DISCUSSION

This paper reports the chemical synthesis of the oligo(glycosyl phosphates) **1** and **2**, which are fragments of the extracellular phosphomannan from *Hansenula capsulata* Y-1842 (ref 9) and from the capsular antigen of *Escherichia coli* K51 (ref 10), respectively. The elongation cycle included coupling of a glycosyl hydrogenphosphonate derivative with a hydroxylic acceptor followed by oxidation of the resulting hydrogenphosphonate diester to the phosphate and removal of a temporary protecting group. The oxidation of the hydrogenphosphonate group to the phosphate in each step is an essential feature of this approach. The relatively high stability of the glycosyl phosphate diesters allows a selective deprotection and chromatographic isolation of the products, whereas the same operations for the glycosyl hydrogenphosphonate diesters lead to significant degradation[4].

The synthesis of the pentamannosyl tetraphosphate **1**.—To synthesize the (1 → 6)-linked oligomer **1**, the mannosyl hydrogenphosphonate derivative **4**, containing a temporary dimethoxytrityl protecting group at O-6, was used for the consecutive introduction of the mannosyl phosphate residues (Scheme 1). It was obtained from the 1-OH-derivative **3** (ref 8) and tri-imidazolylphosphine as described previously[4,8], the final product **4** being isolated by chromatography on SiO_2 (94%). The ^1H NMR data for **4** [H-1, δ 5.93 (dd, $^3J_{H,P}$ 8.9 Hz); HP, δ 7.15 (d, $^1J_{H,P}$ 638 Hz)] were typical of glycosyl hydrogenphosphonate derivatives[1–8]. The monohydroxylic 6-OH-derivative **6** (ref 15) was used as the first acceptor, terminating the chain at the reducing end.



Scheme 1.

Interaction of **4** and **6** was accomplished in pyridine in the presence of trimethylacetyl chloride as a condensing reagent. The intermediate hydrogenphosphonate diester was oxidized in situ with iodine in aqueous 95% pyridine. Subsequent detritylation with 1% CF_3CO_2H in dichloromethane (0°C, 1 min) led to the phosphate diester **7** in an overall yield of 90%.

The next stage, the condensation of the hydrogenphosphonate **4** and the phosphate diester block **7**, was critical for the evaluation of the selected strategy of the stepwise oligo(glycosyl phosphate) synthesis. The product of the reaction and subsequent oxidation, performed under the conditions described above, was isolated in 78% yield after chromatography on SiO_2 and was identified as **8**. The NMR spectra confirmed the presence of two phosphate diester groups [^{31}P, δ −2.68, −2.92 (1:1)] and three mannopyranose residues (^1H, $\delta_{H\text{-}1}$ 4.68, $\delta_{H\text{-}1'}$ and

$\delta_{H-1''} \sim 5.82$). Thus, a possible side reaction, the interaction of the hydrogenphosphonate **4** with the phosphate group in the disaccharide acceptor **7**, was found to be unimportant. The further elongation of the chain by the sequence of reactions described was shown to be possible. The monohydroxylic trisaccharide block **9** was obtained from **8** by mild acidic detritylation in 97% yield.

The synthesis of the linear tetramannosyl triphosphate derivative **10** involved the condensation of **4** and **9** followed by oxidation and detritylation under the standard conditions (71%). The final step of the chain elongation was performed with the use of the mannosyl hydrogenphosphonate derivative **5** which had been described previously[3]. The O-protected derivative **11** was obtained from **5** and **10** by the standard procedures of condensation and oxidation in a yield of 72%. The deacylation of **11** with 0.1 M NaOMe in methanol–dioxane led to the pentamannosyl tetraphosphate **1** (88%), which was isolated by ion-exchange chromatography on Fractogel TSK DEAE (HCO_3^- form). The overall yield of the pentamer **1** amounted to 31%, calculated on the first acceptor **6**.

The structures of the oligomers **7–11** and **1** were confirmed by the NMR data. The ^{31}P NMR data (see Experimental) are characteristic of phosphate diesters of this type[1–8]. For the protected di- and tri-phosphates **8** and **10**, the spectra consisted of two signals, which can be explained by the non-equivalence of the phosphate groups in these compounds. The presence of the (1 → 6)-phosphodiester bonds in the pentamer **1** was confirmed by the signals of C-1, C-5, and C-6 of the corresponding mannopyranose units in the ^{13}C NMR spectra (Table I), which were shifted (as a result of the α- and β-effects of phosphorylation) and coupled with P (or broadened). The α configuration of the mannosyl phosphate fragments followed from the positions of the C-3′–C-3‴″ and C-5′–C-5‴″ resonances, which were close to the chemical shifts of C-3 and C-5 of α-D-mannopyranosyl phosphate[11]. The chain length of **1** was confirmed by the ratio of the total integral intensities of C-1′–C-1‴″ and C-6–C-6″ to the intensities of C-1 and C-6‴″, respectively. In both cases, these ratios were found to be 4:1. The ratios of the intensities of C-5, C-5′–C-5‴, and C-5‴″ were close to 1:3:1.

The synthesis of the tetraglycosyl triphosphate **2**.—The two glycosyl hydrogenphosphonate derivatives **19** and **20** were synthesized as potential key synthons for the synthesis of (1 → 3)-linked oligomer **2** (Scheme 2). Dimethoxytrityl and methoxybenzyl groups were used for temporary protection of the hydroxylic group at C-3. Both derivatives were obtained from the common precursor, 4,6-diol **12** (ref 12). The latter was debenzylated (H_2/Pd–C) followed by benzoylation and selective *O*-deacylation by acidic methanolysis[13] to give the tribenzoate **14**. Its tritylation with $(MeO)_2TrClO_4$ in the presence of 2,4,6-collidine or benzylation with *p*-methoxybenzyl trichloroacetimidate[14] in the presence of CF_3SO_3H led to the 3-dimethoxytrityl and 3-methoxybenzyl ethers **15** (70%) and **16** (56%), respectively. Each compound was *O*-1-debenzoylated with dimethylamine in acetonitrile[1,3,8] to give the α-OH-derivatives **17** (62%) and **18** (67%), which were further converted into hydrogenphosphonates **19** and **20** by reaction with tri-im-

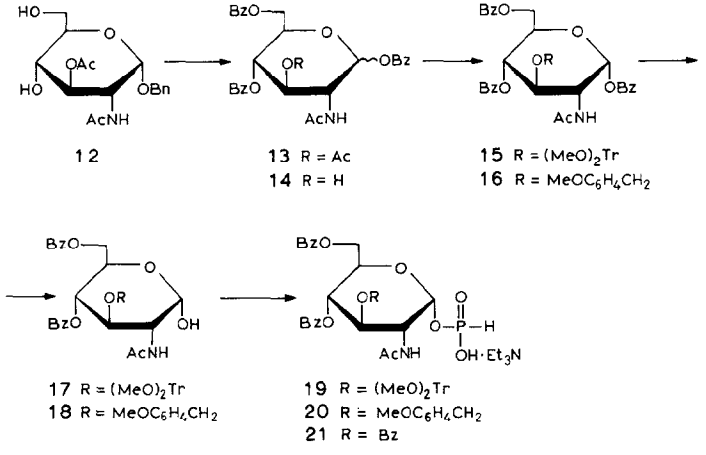
TABLE I

^{13}C NMR data (D_2O, δ in ppm, J in Hz) for oligo(glycosyl phosphates) **1** and **2** ($J_{C,P}$ values in brackets)

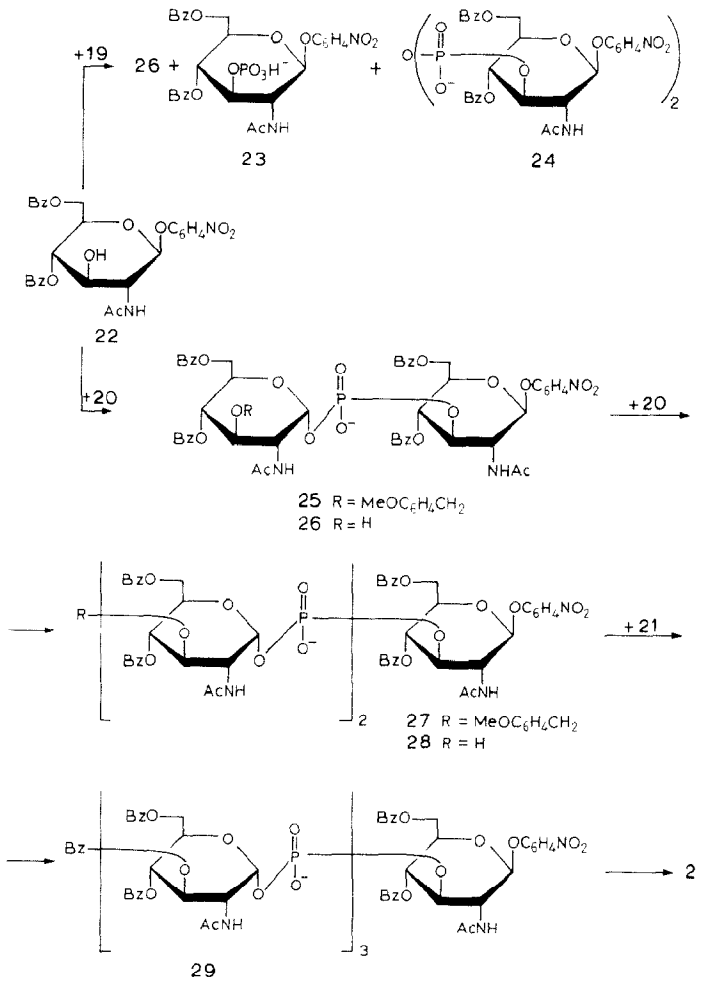
Atom	1	Atom	2
C-1	102.3	C-1	99.8
C-2	71.2	C-2	56.1br
C-3	71.8	C-3	79.3d (5.3)
C-4	67.8	C-4	70.6
C-5	72.8d (7.5)	C-5	77.4
C-6	66.3d (4.9)	C-6	61.8
C-1′–C-1‴′	97.7br	C-1′,C-1″	95.7d (6.0)
C-2′–C-2‴′	71.9d (6.4)	C-2′,C-2″	54.5d (6.8)
C-3′–C-3‴′	71.3	C-3′,C-3″	77.4d (5.3),77.8d (5.3)
C-4′–C-4‴′	67.3	C-4′,C-4″	70.3
C-5′–C-5‴′	74.0d (6.5)	C-5′,C-5″	74.3
C-6′–C-6‴′	66.0br	C-6′,C-6″	61.8
C-1‴″	97.7br	C-1‴′	95.7d (6.0)
C-2‴″	71.9d (6.4)	C-2‴′	55.2d (7.1)
C-3‴″	71.3	C-3‴′	72.1
C-4‴″	67.8	C-4‴′	70.9
C-5‴″	75.2	C-5‴′	74.1
C-6‴″	62.2	C-6‴′	61.8

idazolylphosphine followed by hydrolysis at pH 8 (refs 4 and 8). The 3-OH-derivative **22**, which was described earlier[5], served as a first acceptor during the chain elongation.

The condensation of the dimethoxytrityl derivative **19** and **22** was carried out in pyridine in the presence of Me_3CCOCl followed by oxidation and detritylation as described for the synthesis of the (1 → 6)-linked phosphate diester **7** (Scheme 3). In



Scheme 2.



Scheme 3.

contrast with the previous synthesis, the expected phosphate diester **26** was obtained in an overall yield of only 15%; the phosphate monoester **23** (9%) and the pyrophosphate diester **24** (11%) were also isolated. To investigate the results, the reaction of **19** and **22** was monitored by ^{31}P NMR spectroscopy. After mixing the reactants and the condensing reagent, only the two signals of the diastereomers of the expected hydrogenphosphonate diester were observed at δ 7.33 and 9.06 ($^{1}J_{P,H}$ 729 Hz). The subsequent oxidation of these products with iodine in aqueous pyridine presumably caused a partial cleavage of the glycosyl hydrogenphosphonate linkage and led to the side products **23** (δ 1.53) and **24** (δ −7.80) in addition to the corresponding phosphate diester (δ −1.05). The ^{1}H NMR data confirmed the structures of the phosphate esters **23**, **24**, and **26** (Table II).

On the other hand, the condensation of the methoxybenzyl derivative **20** and

TABLE II

^{1}H NMR data ($CDCl_3$, δ in ppm, J in Hz) for phosphate esters **23–26**

Atom	**23** [a]	**24** [a]	**25** [a,b]	**26** [a]
H-1	5.17d	5.28d	5.23d	5.41d
	($J_{1,2}$ 8.1)	($J_{1,2}$ 8.0)	($J_{1,2}$ 8.0)	($J_{1,2}$ 8.0)
H-2	4.56dt	4.44m	4.51m	4.39dt
	($J_{2,3}$ 10.2)			($J_{2,NH}$ 8.0)
H-3	4.87dt	5.01dt	4.86q	4.83q
	($J_{3,P}$ 9.5)	($J_{2,3}$ 10.3, $J_{3,P}$ 9.7)	($J_{2,3} = J_{3,P} \approx$ 9.3)	($J_{2,3} = J_{3,P}$ = 9.3)
H-4	5.28t	5.44t	5.40t	5.37t
	($J_{3,4} = J_{4,5}$ = 9.5)	($J_{3,4} \approx J_{4,5}$ = 9.7)	($J_{3,4} = J_{4,5}$ = 9.3)	($J_{3,4} = J_{4,5}$ = 9.3)
H-5	4.20m	4.23ddd	4.17ddd	4.15m
	($J_{5,6a}$ 7.5)	($J_{5,6a}$ 6.9)	($J_{5,6a}$ 7.0)	($J_{5,6a}$ 7.0)
H-6a	4.31dd	4.41dd	4.44dd	4.38dd
	($J_{6a,6b}$ 11.5)	($J_{6a,6b}$ 11.9)	($J_{6a,6b}$ 11.7)	($J_{6a,6b}$ 11.7)
H-6b	4.42dd	4.53dd	4.59dd	4.51dd
	($J_{5,6b}$ 2.0)	($J_{5,6b}$ 2.7)	($J_{5,6b}$ 2.7)	($J_{5,6b}$ 3.0)
H-1′			5.43dd	5.45dd
			($J_{1',2'}$ 3.3, $J_{1',P}$ 8.4)	($J_{1',2'}$ 3.2, $J_{1',P}$ 8.3)
H-2′			4.43m	4.10m
H-3′			3.93dd	3.82t
			($J_{2',3'}$ 10.1)	($J_{2',3'}$ 9.8)
H-4′			5.56t	5.40t
			($J_{3',4'} = J_{4',5'}$ = 9.4)	($J_{3',4'} = J_{4',5'}$ = 9.8)
H-5′			4.33dt	4.25dt
			($J_{5',6a'} = J_{5',6b'}$ = 2.7)	($J_{5',6a'} = J_{5',6b'}$ = 2.6)
H-6a′			4.07dd	4.12dd
			($J_{6a',6b'}$ 11.1)	($J_{6a',6b'}$ 12.0)
H-6b′			4.67dd	4.63dd
NH	5.67d	7.67d	6.60–8.20	6.60–8.20
	($J_{2,NH}$ 8.1)	($J_{2,NH}$ 8.0)		
CH_3CO	2.08s	2.06s	2.18s, 2.28s	2.13s, 2.17s

[a] Additional signals of Et_3NH^+ (δ 1.13–1.33t and 2.84–3.03q) and of C_6H_4 and C_6H_5 (6.60–8.20) were present. [b] Additional signals of the *p*-methoxybenzyl group were present at δ 3.68 (s, 3 H, CH_3O) and 4.54 and 4.73 (2 d, 2 H, J 10.5 Hz, CH_2).

acceptor **22** followed by oxidation under the standard conditions proceeded smoothly and gave the phosphate diester **25** in 74% yield. The monohydroxylic block **26** was readily obtained from **25** after debenzylation with ammonium cerium(IV) nitrate in aqueous acetonitrile (67%).

The synthesis of the triglycosyl diphosphate **28** was achieved by condensation of **20** and **26** followed by oxidation and selective deprotection as described for **25** and **26**. The oligomers **27** and **28** were obtained in yields of 59 and 73%, respectively. The final step of the chain elongation was performed with the use of the benzoylated hydrogenphosphonate **21** which had been synthesized earlier[5]. The protected tetramer **29** was obtained from **21** and **28** by the standard procedure of condensation and oxidation in a yield of 40%. The debenzoylation was accom-

plished with 0.05 M NaOMe in methanol–1,4-dioxane (1°C) to give the tetraglycosyl triphosphate **2** (70%).

The structures of the oligomers **25–29** and **2** were confirmed by the NMR data as described for oligo(mannosyl phosphates) (see above). Thus, the ^{31}P NMR spectra of the protected di- and tri-phosphates **28** and **29** consisted of two and three signals, respectively (δ_P −1.64, −2.83 for **28**; and −0.58, −2.22, and −2.75 for **29**), which indicated, presumably, the non-equivalence of the phosphate groups in the oligomers. The presence of (1 → 3)-phosphodiester linkages in **2** was confirmed by the signals of C-1′–C-1‴, C-2–C-2‴, C-3–C-3‴, H-1′–H-1‴, and H-3–H-3‴, shifted and coupled with P, in the ^{13}C and ^1H NMR spectra (Table I and Experimental). The α configuration of the glycosyl phosphate fragments followed from the proton coupling constants $J_{1',2'}$, $J_{1'',2''}$, and $J_{1''',2'''}$ of 3.2–3.6 Hz. The chain length of **2** was confirmed by the presence of four different signals for C-3–C-3‴ in the ^{13}C NMR spectrum and by the ratios of the total integral intensities of the C-6–C-6‴, C-1′–C-1‴, and C-1 signals, which were close to 4:3:1. The analogous intensity ratios for H-3–H-3‴, H-1′–H-1‴, and H-1 in the ^1H NMR spectrum were found to be 3:3:1.

These results illustrate the high efficiency of the suggested stepwise approach for the synthesis of oligo(glycosyl phosphates) via glycosyl hydrogenphosphonates. The possibility has been shown of using oligomeric blocks containing phosphate diester groups as hydroxylic components. It seems quite possible to use this approach for the preparation of oligomers with longer chains.

EXPERIMENTAL

General materials and methods were described in a recent paper[3]. TLC was performed on Kieselgel 60 F_{254} (Merck), using *A*, 4:1 $CHCl_3$–MeOH; *B*, 85:20:2 $CHCl_3$–MeOH–H_2O; *C*, 7:3 2-propanol–H_2O, *D*, 4:1 benzene–acetone; *E*, 5:1 benzene–acetone; *F*, 4:1 CH_2Cl_2–MeOH; *G*, 17:3 CH_2Cl_2–MeOH; *H*, 9:1 CH_2Cl_2–MeOH; *I*, 34:15:1 CH_2Cl_2–MeOH–H_2O; *J*, 5:2 2-propanol–H_2O; with detection under UV light or by charring with 10% H_2SO_4 in MeOH. Column chromatography was performed on Silicagel L 40/100 (Chemapol, C.S.F.R.) and Silpearl (Sklarny Kavalier, 25–40 μm, C.S.F.R.), using *K*, 97:2:1 → 92:7:1 CH_2Cl_2–MeOH–Et_3N; *L*, 96:3:1 → 83:16:1 CH_2Cl_2–MeOH–Et_3N; *M*, 94:5:1:0.5 → 83:15:1:1.5 CH_2Cl_2–MeOH–Et_3N–H_2O; *N*, 90:9:1 → 69:30:1 CH_2Cl_2–MeOH–H_2O. Ion-exchange chromatography was accomplished on a column (18 × 1 cm) of Fractogel TSK DEAE-650 (S) (HCO_3^- form) (Merck) by elution at 1 mL · min^{-1} with a linear gradient of aq NH_4HCO_3 (0 → 0.33 M).

p,p′-*Dimethoxytriphenylmethyl perchlorate.*—A solution of *p,p′*-dimethoxytriphenylmethyl chloride (10 g, 29.5 mmol) in pyridine (100 mL) and water (10 mL) was kept for 1 h at 20°C, then concentrated, and toluene was evaporated from the residue. Column chromatography in 99:1 benzene–Et_3N gave *p,p′*-dimethoxytriphenylmethanol (9.2 g, 97%) as a solid. The latter was dissolved in

Ac_2O (85 mL), and aq 57% $HClO_4$ (6.7 mL) was added dropwise with stirring at 0°C. The solution was poured into anhyd ether (250 mL); a precipitate which formed was separated, washed with ether (5 × 50 mL), and dried in vacuo to yield *p,p'*-dimethoxytriphenylmethyl perchlorate (11 g, 95%) as a purple amorphous powder.

*Triethylammonium 2,3,4-tri-*O*-benzoyl-6-*O*-p,p'-dimethoxytrityl-α-*D*-manno-pyranosyl hydrogenphosphonate* (**4**).—To a stirred solution of imidazole (228 mg, 3.36 mmol) in MeCN (6 mL) at 0°C was added PCl_3 (0.089 mL, 1.01 mmol) followed by Et_3N (0.49 mL, 3.53 mmol). Stirring was continued for 15 min, and a solution of **3** (ref 8) (196 mg, 0.247 mmol) in MeCN (6 mL) was added dropwise during 30 min at 0°C. The mixture was stirred for 5 min at 20°C, then quenched with M Et_3NHHCO_3 (TEAB) (pH 8, 1.4 mL), and the clear solution was stirred for 15 min and then concentrated. 4:1 Pyridine–Et_3N was evaporated from the residue, a solution of which in $CHCl_3$ (50 mL) was washed with 0.5 M TEAB (4 × 30 mL), dried by filtration through cotton, and concentrated. Column chromatography (solvent *K*) of the residue gave **4** (223 mg, 94%), as a solid, $[\alpha]_D^{26}$ −83.7° (*c* 1, $CHCl_3$, R_f 0.43 (solvent *A*). ^1H NMR data ($CDCl_3$): δ 1.30 (t, 9 H, 3 CH_3CH$_2$), 2.98 (q, 6 H, 3 CH$_3$CH_2), 3.17 (dd, 1 H, $J_{5,6a}$ 3.5, $J_{6a,6b}$ 10.2 Hz, H-6a), 3.45 (dd, 1 H, $J_{5,6b}$ 2.0 Hz, H-6b), 3.66, 3.68 (2 s, 6 H, 2 CH_3O), 4.54 (ddd, 1 H, H-5), 5.78 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 5.87 (dd, 1 H, H-3), 5.93 (dd, 1 H, $J_{1,2}$ 2.0, $J_{1,P}$ 9.0 Hz, H-1), 6.22 (t, 1 H, $J_{3,4} = J_{4,5}$ = 10.0 Hz, H-4), 6.62 and 6.67 (2 d, 4 H, *o*-protons of C_6H_4), 7.15 (d, 1 H, $J_{H,P}$ 638.3 Hz, HP), and 7.05–8.15 (m, 24 H, C_6H_5, *m*-protons of C_6H_4).

*Triethylammonium 2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl hydrogenphosphonate* (**5**).—This compound was obtained as described earlier[3] followed by isolation using column chromatography (solvent *K*).

*Methyl 2,3,4-tri-*O*-acetyl-α-*D*-mannopyranoside 6-(2,3,4-tri-*O*-benzoyl-α-*D*-mannopyranosyl phosphate), triethylammonium salt* (**7**).—A mixture of **4** (108 mg, 0.113 mmol) and **6** (ref 15) (40 mg, 0.125 mmol) was dried by evaporation of pyridine (3 × 1 mL) therefrom. The residue was dissolved in the same solvent (1 mL), trimethylacetyl chloride (0.033 mL, 0.30 mmol) was added, the mixture was stirred for 10 min at 20°C, and a freshly prepared solution of iodine (59 mg, 0.23 mmol) in 19:1 pyridine–H_2O (2 mL) was added. After 10 min, $CHCl_3$ was added, and the organic layer was successively washed with cold M aq $Na_2S_2O_3$ and cold M TEAB, dried by filtration through cotton, and concentrated. The residue was dissolved in CH_2Cl_2 (6 mL), and 2% CF_3CO_2H in CH_2Cl_2 (6 mL) was added at 0°C. After 1 min, the solution was washed successively with ice-cold satd aq $NaHCO_3$ and M TEAB, dried by filtration through cotton, and concentrated. Column chromatography of the residue (solvent *K*) gave **7** (99 mg, 90%) as a solid; $[\alpha]_D^{28}$ −50° (*c* 1, $CHCl_3$); R_f 0.38 (solvent *A*). NMR data ($CDCl_3$): ^1H, δ 1.39 (t, 9 H, 3 CH_3CH$_2$), 1.99, 2.06, 2.12 (3 s, 9 H, 3 CH_3CO), 3.12 (q, 6 H, 3 CH$_3$CH_2), 3.39 (s, 3 H, CH_3O), 3.76 (dd, 1 H, $J_{5',6a'}$ 5.2, $J_{6a',6b'}$ 12.7 Hz, H-6a'), 3.82 (dd, 1 H, $J_{5',6b'}$ 2.2 Hz, H-6b'), 3.96–4.21 (m, 3 H, H-5,6a,6b), 4.51 (ddd, 1 H, H-5'), 4.65 (d, 1 H, $J_{1,2}$

1.7 Hz, H-1), 5.20 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 5.27 (t, 1 H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 5.34 (dd, 1 H, H-3), 5.77 (t, 1 H, $J_{3',4'} = J_{4',5'} = 10.3$ Hz, H-4'), 5.79 (m, 1 H, H-2'), 5.80 (dd, 1 H, $J_{1',2'}$ 2.2, $J_{1',P}$ 8.9 Hz, H-1'), 6.02 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-3'), 7.15–8.10 (m, 15 H, 3 C_6H_5); ^{31}P, δ −2.45.

Methyl 2,3,4-tri-O-acetyl-α-D-mannopyranoside 6-[2,3,4-tri-O-benzoyl-α-D-mannopyranosyl phosphate 6-(2,3,4-tri-O-benzoyl-6-O-p,p'-dimethoxytrityl-α-D-mannopyranosyl phosphate)], bis-triethylammonium salt (**8**).—This compound was obtained by condensation of **4** (48 mg, 0.05 mmol) and **7** (35 mg, 0.036 mmol) in the presence of trimethylacetyl chloride (0.016 mL, 0.125 mmol) followed by oxidation with iodine, as described above. Column chromatography (solvent *K*) gave **8** (54 mg, 78%) as a solid; $[\alpha]_D^{28}$ −57.4° (*c* 1, $CHCl_3$); R_f 0.42 (solvent *A*). NMR data ($CDCl_3$): ^1H, δ 1.22 (t, 18 H, 6 CH_3CH_2), 1.95, 2.04, 2.06 (3 s, 9 H, 3 CH_3CO), 2.93 (q, 12 H, 6 CH_3CH_2), 3.05 (dd, 1 H, $J_{5'',6a''}$ 2.4, $J_{6a'',6b''}$ 10.5 Hz, H-6a''), 3.34 (s, 3 H, CH_3O), 3.39 (dd, 1 H, $J_{5'',6b''}$ 1.5 Hz, H-6b''), 3.63 (s, 6 H, 2 CH_3OAr), 4.02–4.18 (m, 3 H, H-5,6a,6b), 4.20–4.33 (m, 2 H, H-6a',6b'), 4.47 (ddd, 1 H, H-5''), 4.65–4.72 (m, 1 H, H-5'), 4.68 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.19 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 5.24 (t, 1 H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.34 (dd, 1 H, H-3), 5.76–5.86 (m, 5 H, H-1',1'',2',2'',3''), 5.84 (t, 1 H, $J_{3',4'} = J_{4',5'} = 9.9$ Hz, H-4'), 5.99 (dd, 1 H, $J_{2',3'}$ 2.9, H-3'), 6.31 (t, 1 H, $J_{3'',4''} = J_{4'',5''} = 10.3$ Hz, H-4''), 6.58 and 6.62 (2 d, 4 H, *o*-protons of C_6H_4), 7.10–8.20 (m, 39 H, C_6H_5, *m*-protons of C_6H_4); ^{31}P, δ −2.68, −2.92 (1 : 1).

Methyl 2,3,4-tri-O-acetyl-α-D-mannopyranoside 6-[2,3,4-tri-O-benzoyl-α-D-mannopyranosyl phosphate 6-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl phosphate)], bis-triethylammonium salt (**9**).—Compound **8** (54 mg) was treated with 1% CF_3CO_2H in CH_2Cl_2 during 1 min at 0°C, as described for the synthesis of **7**. Column chromatography (solvent *L*) gave **9** (44 mg, 97%) as a solid; R_f 0.20 (solvent *A*). NMR data ($CDCl_3$): ^{13}C, δ 9.6 (CH_3CH_2), 20.65, 20.75 (CH_3CO), 45.7 (CH_3CH_2), 55.0 (CH_3O), 61.3 (C-6''), 64.7, 65.05 (2 d, $J_{C,P} \sim 4.0$ Hz, C-6,6'), 66.4 (C-4'), 67.4 (C-4 + C-4''), 69.5 (C-3), 69.55 (d, $J_{C,P}$ 7.7 Hz, C-5), 69.6 (C-2), 70.1 and 70.2 (C-3',3''), 70.5, 70.7, and 70.8 (3 d, $J_{C,P} \sim 7.3$ Hz, C-2',2'',5'), 72.1 (C-5''), 93.6 and 93.7 (2 d, $J_{C,P} \sim 4.2$ Hz, C-1',1''), 97.95 (C-1), 128.1–129.7 and 132.9–133.2 (C_6H_5), 165.1, 165.4, 165.7 (PhCOO), 169.8, and 170.2 (MeCOO); ^{31}P, δ −2.59.

Methyl 2,3,4-tri-O-acetyl-α-D-mannopyranoside 6-{2,3,4-tri-O-benzoyl-α-D-mannopyranosyl phosphate 6-[2,3,4-tri-O-benzoyl-α-D-mannopyranosyl phosphate 6-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl phosphate)]}, tris-triethylammonium salt (**10**).—This compound was obtained by condensation of **4** (148 mg, 0.154 mmol) and **9** (168 mg, 0.103 mmol) in the presence of trimethylacetyl chloride (0.049 mL, 0.385 mmol) followed by oxidation with iodine (76 mg, 0.3 mmol) and treatment with 1% CF_3CO_2H in CH_2Cl_2 (1 min, 0°C), as described for the synthesis of **7**. Column chromatography (solvent *M*) gave **10** (167 mg, 71%) as a solid; $[\alpha]_D^{26}$ −69.3° (*c* 1, $CHCl_3$); R_f 0.07 (solvent *A*), 0.35 (solvent *B*). ^{31}P NMR data ($CDCl_3$): δ −2.54, −3.05 (2 : 1).

Methyl 2,3,4-tri-O-acetyl-α-D-mannopyranoside 6-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl phosphate 6-{2,3,4-tri-O-benzoyl-α-D-mannopyranosyl phosphate 6-[2,3,4-tri-O-benzoyl-α-D-mannopyranosyl phosphate 6-(2,3,4-tri-O-benzoyl-α-D-mannopyranosyl phosphate)]})tetrakis-triethylammonium salt (**11**).—This compound was obtained by condensation of **5** (79 mg, 0.104 mmol) and **10** (167 mg, 0.073 mmol) in the presence of trimethylacetyl chloride (0.033 mL, 0.26 mmol) followed by oxidation with iodine (76 mg, 0.3 mmol), as described above. Column chromatography (solvent *M*) gave **11** (159 mg, 72%) as a solid; $[\alpha]_D^{30}$ −55.9° (*c* 1, $CHCl_3$), R_f 0.43 (solvent *B*). NMR data ($CDCl_3$): ^{13}C, δ 8.9 (CH_3CH_2), 22.5 (CH_3CO), 45.6 (CH_3CH_2), 55.0 (CH_3O), 62.3 (C-6‴), 64.9 (br, C-6–C-6″), 66.7 and 67.1 (C-4–C-4‴), 69.1 (C-3), 69.5 (s + d, $J_{C\text{-}5,P}$ ~ 7 Hz, C-2 + C-5), 70.0–70.15 [m, (C-2′–C-2‴) + (C-3′–C3‴) + (C-5′–C-5‴)], 93.7 (br, C-1′–C-1‴), 97.9 (C-1), 128.1–129.8, 132.75–133.0 (C_6H_5), 165.1, 165.2, 165.8 (PhCOO), 169.8 and 170.2 (MeCOO); ^{31}P, δ −2.97.

Methyl α-D-mannopyranoside 6-(α-D-mannopyranosyl phosphate 6-{α-D-mannopyranosyl phosphate 6-[α-D-mannopyranosyl phosphate 6-(α-D-mannopyranosyl phosphate)]}), tetra-ammonium salt (**1**).—A solution of **11** (155 mg) in MeOH (10 mL), 1,4-dioxane (17 mL), and 1 M NaOMe in MeOH (3 mL) was kept for 1 h at 20°C. The mixture was deionized with Dowex 50W-X4 (H^+) resin, filtered, immediately neutralized with Et_3N, and concentrated to dryness. Ion-exchange chromatography of the residue (see general part of Experimental) gave **1** (55 mg, 88%) as a solid; $[\alpha]_D^{25}$ +34° (*c* 1, H_2O); R_f 0.18 (solvent *C*). NMR data (D_2O): ^1H, δ 3.32 (s, 3 H, CH_3O), 3.93 (dd, the lines are broadened, 4 H, $J_{2'-2''',3'-3'''}$ ~ 3.0 Hz, H-2′–H-2‴), 4.05 (m, 8 H, H-6–H-6″), 4.70 (H-1, under HOD signal), and 5.35 (dd, the lines are broadened, 4 H, $J_{1'-1''',2'-2'''}$ ~ 1.5, $J_{1'-1''',P-P'''}$ ~ 7.6 Hz, H-1′–H-1‴); ^{31}P, δ −1.12; ^{13}C, see Table I.

2-Acetamido-3-O-acetyl-1,4,6-tri-O-benzoyl-2-deoxy-α,β-D-glucopyranose (**13**).—A solution of **12** (ref 12) (4.2 g, 12 mmol) in MeOH (100 mL) was hydrogenolysed over 10% Pd–C for 8 h at 20°C. The mixture was filtered and concentrated to dryness, and the residue was dissolved in pyridine (50 mL) and treated with benzoyl chloride (4.5 mL, 38 mmol) at 20°C. After 16 h, the mixture was diluted with $CHCl_3$ and washed successively with satd aq $NaHCO_3$ and water, dried, and concentrated. Column chromatography (9:1 benzene–acetone) of the residue gave **13** (4.98 g, 72%) as a solid; $[\alpha]_D^{27}$ +91° (*c* 1, $CHCl_3$); R_f 0.4 (solvent *D*). ^1H NMR data ($CDCl_3$): δ 1.90, 1.95, 1.98, and 1.99 (4 s, CH_3CO), 4.21 (ddd, $J_{5,6a}$ 4.5, $J_{5,6b}$ 3.0, $J_{4,5}$ 9.9 Hz, H-5β), 4.36–4.58 (m, H-5α, H-6aα,β, H-6bα), 4.60 (dd, $J_{6a,6b}$ 12.5 Hz, H-6bβ), 4.66 (ddd, $J_{2,3}$ 10.7 Hz, H-2β), 4.75 (ddd, $J_{2,3}$ 9.1 Hz, H-2α), 5.45 (dd, H-3β), 5.62 (t, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4β), 5.63 (t, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4α), 5.74 (dd, H-3α), 5.86 (d, $J_{NH,2}$ 10.5 Hz, NH), 6.03 (d, $J_{1,2}$ 9.0 Hz, H-1β), 6.56 (d, $J_{1,2}$ 3.6 Hz, H-1α), 7.15–7.73 and 7.92–8.20 (m, C_6H_5); α:β = 1.7:1.

2-Acetamido-1,4,6-tri-O-benzoyl-2-deoxy-α,β-D-glucopyranose (**14**).—Acetyl chloride (4 mL) was added dropwise to MeOH (100 mL) at 0°C and **13** (5.1 g) was dissolved in the mixture, which was kept for 12 h at 1°C and 7 h at 20°C. The

mixture was neutralized with Et_3N and concentrated. Column chromatography (1:1 hexane–acetone) gave **14** (2.78 g, 60%) as a solid; $[\alpha]_D^{26}$ +113° (*c* 1, $CHCl_3$); R_f 0.26 (solvent *E*). ^1H NMR data ($CDCl_3$): δ 1.89 and 1.93 (2 s, CH_3CO), 4.14 (dd, $J_{2,3}$ 10.3 Hz, H-3β), 4.26 (dd, $J_{2,3}$ 10.8 Hz, H-3α), 4.33–4.53 (m, H-2β, H-5α,β, H-6aα,β, H-6bα,β), 4.56 (ddd, $J_{2,NH}$ 8.0 Hz, H-2α), 5.42 (t, $J_{3,4} = J_{4,5} = 8.9$ Hz, H-4β), 5.48 (t, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4α), 6.11 (d, $J_{1,2}$ 8.6 Hz, H-1β), 6.25 (d, NH), 6.53 (d, $J_{1,2}$ 3.6 Hz, H-1α), 7.14–7.67 and 7.90–8.17 (m, C_6H_5); α:β = 2.5:1. *Anal.* Calcd for $C_{29}H_{27}NO_9$: C, 65.28; H, 5.10; N, 2.63. Found: C, 65.28; H, 5.34; N, 2.93

*2-Acetamido-1,4,6-tri-*O*-benzoyl-2-deoxy-3-*O*-*p,p'*-dimethoxytrityl-α-*D*-gluco-pyranose* (**15**).—2,4,6-Collidine (0.165 mL, 1.25 mmol) was added to a solution of **14** (300 mg, 0.56 mmol) and dimethoxytriphenylmethyl perchlorate (450 mg, 1.12 mmol) in CH_2Cl_2 (6 mL). The mixture was kept for 24 h at 20°C, and dimethoxytriphenylmethyl perchlorate (225 mg, 0.56 mmol) and 2,4,6-collidine (0.082 mL, 0.62 mmol) were added. After 72 h, the reaction was quenched with 3:1 pyridine–MeOH (2 mL). The mixture was diluted with $CHCl_3$ and washed successively with satd aq $NaHCO_3$ and water, dried, and concentrated. Column chromatography (19:1 benzene–acetone) of the residue gave **15** (330 mg, 70%) as a solid; $[\alpha]_D^{28}$ +80° (*c* 1, $CHCl_3$); R_f 0.47 (solvent D). ^1H NMR data ($CDCl_3$): δ 1.61 (s, 3 H, CH_3CO), 3.65 and 3.73 (2 s, 6 H, 2 CH_3O), 3.97 (ddd, 1 H, $J_{5,6a}$ 4.5, $J_{5,6b}$ 3.3, $J_{4,5}$ 9.7 Hz, H-5), 4.15 (t, 1 H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 4.23 (dd, 1 H, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.36 (dd, 1 H, H-6b), 4.77 (dt, 1 H, $J_{2,NH}$ 9.7 Hz, H-2), 5.76 (t, 1 H, H-4), 6.34 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 6.70d, 6.83d, 7.02–7.78m, and 8.00d (29 H, C_6H_4, C_6H_5, NH); minor signals of the β-anomer (~ 10%) were also present.

*2-Acetamido-1,4,6-tri-*O*-benzoyl-2-deoxy-3-*O*-p-methoxybenzyl α-*D*-glucopyranose* (**16**).—To a stirred solution of **14** (900 mg, 1.68 mmol) and *p*-methoxybenzyl trichloroacetimidate (950 mg, 3.36 mmol) (ref. 14) in 2:1 ether–1,4-dioxane (9 mL) was added a solution of trifluoromethanesulfonic acid (0.006 mL, 0.067 mmol) in ether (3 mL) under Ar. After 15 min, the mixture was neutralized with Et_3N, diluted with $CHCl_3$, washed successively with satd aq $NaHCO_3$ and water, dried, and concentrated. Column chromatography (9:1 benzene–acetone) of the residue gave **16** (621 mg, 56%) as a solid; $[\alpha]_D^{25}$ +121° (c 1, $CHCl_3$); R_f 0.37 (solvent *D*). ^1H NMR data ($CDCl_3$): δ 1.82 (s, 3 H, CH_3CO), 3.76 (s, 3 H, CH_3O), 4. 17 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-3), 4.35 (m, 2 H, H-5,6a), 4.51 and 4.66 (2 d, 2 H, *J* 11 Hz, CH_2Ar), 4.52 (dd, 1 H, $J_{5,6b}$ 2.4, $J_{6a,6b}$ 11 Hz, H-6b), 4.58 (ddd, 1 H, $J_{2,NH}$ 8.0 Hz, H-2), 5.16 (d, 1 H, NH), 5.69 (t, 1 H, $J_{3,4} = J_{4,5} = 9.1$ Hz, H-4), 6.52 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 6.83d, 7.20d, 7.33–7.70m, and 7.95–8.12m (19 H, C_6H_4, C_6H_5). *Anal.* Calcd for $C_{37}H_{35}NO_{10}$: C, 67.98; H, 5.39; N, 2.14. Found: C, 67.88; H, 5.62; N, 2.87.

*2-Acetamido-4,6-di-*O*-benzoyl-2-deoxy-3-*O*-p,p'-dimethoxytrityl-α-*D*-gluco-pyranose* (**17**).—Dimethylamine (1.1 mL, 16.73 mmol) was added to a solution of **15** (2 g, 2.39 mmol) in MeCN (20 mL) at −20°C, and the mixture was kept at 20°C. Additional dimethylamine (0.55 mL × 2) was added after 24 h and 40 h; the

reaction was monitored by TLC (solvent *D*). After 48 h, the mixture was concentrated to dryness and MeCN was evaporated from the residue. Column chromatography (7:3 toluene–acetone) gave **17** (1.08 g, 62%) as a solid; $[\alpha]_D^{28}$ +56° (*c* 1, $CHCl_3$); R_f 0.15 (solvent *D*). NMR data ($CDCl_3$): ^1H, δ 1.65 (s, 3 H, CH_3CO), 3.66 and 3.76 (2 s, 6 H, 2 CH_3O), 4.00 (dd, 1 H, $J_{2,3}$ 9.4 Hz, H-3), 4.12 (ddd, 1 H, $J_{5,6a}$ 3.2, $J_{5,6b}$ 4.5 Hz, H-5), 4.22 (dd, 1 H, $J_{6a,6b}$ 11.8 Hz, H-6a), 4.41 (dd, 1 H, H-6b), 4.42 (dt, 1 H, $J_{2,NH}$ 9.4 Hz, H-2), 4.97 (d, 1 H, NH), 5.18 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 5.50 (t, 1 H, $J_{3,4} = J_{4,5} = 8.6$ Hz, H-4), 6.65d, 6.76d, 7.00–7.57m, 7.66d, and 7.96d (23 H, C_6H_4, C_6H_5); ^{13}C, δ 23.4 (CH_3CO), 53.6 (C-2), 55.0, 55.2 (CH_3O), 63.5 (C-6), 68.7 (C-4), 71.0 (C-5), 72.0 (C-3), 88.4 (Ar_3C), 91.5 (C-1, $J_{C\text{-}1,H\text{-}1}$ 171 Hz), 113.0–113.4, 126.9–132.9, 145.8, 158.4, and 158.7 (C_6H_4, C_6H_5), 165.3, 166.4 (COO), and 170.1 (CON).

*2-Acetamido-4,6-di-*O*-benzoyl-2-deoxy-3-*O*-p-methoxybenzyl-α-*D*-glucopyranose* (**18**).—Dimethylamine (0.35 mL, 5.25 mmol) was added to a solution of **16** (490 mg, 0.75 mmol) in MeCN (6 mL) at −20°C, and the mixture was kept at 20°C. After 48 h, the mixture was concentrated to dryness and MeCN was evaporated from the residue. Column chromatography (7:3 benzene–acetone) of the residue followed by crystallization ($CHCl_3$–hexane) gave **18** (275 mg, 67%); mp 194–96°C; $[\alpha]_D^{25}$ +63° (*c* 1, $CHCl_3$); R_f 0.1 (solvent *D*). ^1H NMR data ($CDCl_3$): δ 1.9 (s, 3 H, CH_3CO), 3.75 (s, 3 H, CH_3O), 4.05 (dd, 1 H, $J_{2,3}$ 11.1 Hz, H-3), 4.23 (ddd, 1 H, $J_{2,NH}$ 8.8 Hz, H-2), 4.31 (dd, 1 H, $J_{5,6a}$ 4.3, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.44 (ddd, 1 H, $J_{5,6b}$ 2.9 Hz, H-5), 4.45 and 4.57 (2 d, 2 H, *J* 11.6 Hz, CH_2Ar), 4.58 (dd, 1 H, H-6b), 5.32 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.56 (t, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.57 (d, 1 H, NH), 6.75d, 7.10d, 7.32–7.65m, 8.00d, and 8.07d (14 H, C_6H_4, C_6H_5). *Anal.* Calcd for $C_{30}H_{31}NO_9$: C, 65.56; H, 5.68; N, 2.55. Found: C, 65.59; H, 5.39; N, 2.93.

*Triethylammonium 2-acetamido-4,6-di-*O*-benzoyl-2-deoxy-3-*O*-p,p′-dimethoxytrityl-α-*D*-glucopyranosyl hydrogenphosphonate* (**19**).—This compound was obtained from **17** (430 mg, 0.6 mmol) as described for **4**. Column chromatography (solvent *L*) gave **19** (440 mg, 83%) as a solid; $[\alpha]_D^{22}$ +61° (*c* 1, $CHCl_3$); R_f 0.25 (solvent *F*). NMR data ($CDCl_3$): ^1H, δ 1.21 (t, 9 H, 3 CH_3CH_2), 1.59 (s, 3 H, CH_3CO), 2.92 (q, 6 H, 3 CH_3CH_2), 3.65 and 3.71 (2 s, 6 H, 2 CH_3O), 3.95 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-3), 4.14 (dd, 1 H, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.19 (dt, 1 H, $J_{5,6a} = J_{5,6b} = 3.3$ Hz, H-5), 4.40 (dd, 1 H, H-6b), 4.52 (dt, 1 H, $J_{2,NH}$ 10.0 Hz, H-2), 5.25 (d, 1 H, NH), 5.51 (dd, 1 H, $J_{1,2}$ 3.5, $J_{1,P}$ 8.3 Hz, H-1), 5.63 (t, 1 H, $J_{3,4} = J_{4,5} = 9.1$ Hz, H-4), 6.74 (d, 1 H, $J_{H,P}$ 635 Hz, HP), 6.61d, 6.72d, 6.95–7.55m, 7.63d, and 8.00d (23 H, C_6H_4, C_6H_5); ^{13}C, δ 8.5 (CH_3CH_2), 23.3 (CH_3CO), 45.5 (CH_3CH_2), 53.3 (d, $J_{C,P}$ 6.1 Hz, C-2), 55.0 and 55.1 (CH_3O), 63.0 (C-6), 69.4 (C-4), 71.4 (C-5), 71.7 (C-3), 88.3 (Ar_3C), 93.0 (d, $J_{C,P}$ 6.1 Hz, C-1), 112.9–113.3, 126.9–132.9, 145.8, 158.3, and 158.6 (C_6H_4, C_6H_5), 165.1, 166.2 (COO), and 169.9 (CON); ^{31}P, δ 1.51 (in $CDCl_3$), 1.19 (in C_5H_5N).

*Triethylammonium 2-acetamido-4,6-di-*O*-benzoyl-2-deoxy-3-*O*-p-methoxybenzyl-α-*D*-glucopyranosyl hydrogenphosphonate* (**20**).—This compound was obtained from

18 (220 mg, 0.4 mmol) as described for **4**. Column chromatography (solvent *L*) gave **20** (275 mg, 96%) as a solid; $[\alpha]_D^{31}$ +55° (*c* 1, $CHCl_3$); R_f 0.2 (solvent *F*). NMR data ($CDCl_3$): ^1H, δ 1.29 (t, 9 H, 3 CH_3CH_2), 1.91 (s, 3 H, CH_3CO), 3.01 (q, 6 H, 3 CH_3CH_2), 3.71 (s, 3 H, CH_3O), 4.03 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-3), 4.27 (dd, 1 H, $J_{5,6a}$ 3.7, $J_{6a,6b}$ 11.8 Hz, H-6a), 4.41 (ddt, 1 H, $J_{2,NH}$ 9.9, $J_{2,P}$ 1.5 Hz, H-2), 4.48, 4.54 (2 d, 2 H, *J* 11.0 Hz, CH_2Ar), 4.50 (m, 1 H, H-5), 4.56 (dd, 1 H, $J_{5,6b}$ 2.5 Hz, H-6b), 5.60 (t, 1 H, $J_{3,4} = J_{4,5} = 9.2$ Hz, H-4), 5.61 (dd, 1 H, $J_{1,2}$ 3.2, $J_{1,P}$ 8.1 Hz, H-1), 6.33 (d, 1 H, NH), 7.01 (d, 1 H, $J_{H,P}$ 638 Hz, HP), 6.69d, 7.05d, 7.35–7.60m, and 7.95–8.06m (14 H, C_6H_4, C_6H_5); ^{13}C, δ 8.6 (CH_3CH_2), 23.2 (CH_3CO), 45.6 (CH_3CH_2), 52.6 (d, $J_{C,P}$ 6.0 Hz, C-2), 55.1 (CH_3O), 62.8 (C-6), 69.2 (C-5), 71.0 (C-4), 73.6 (CH_2Ar), 77.2 (C-3), 93.4 (d, $J_{C,P}$ 5.3 Hz, C-1), 113.7, 120.8, 128.3–133.3, and 159.0 (C_6H_4, C_6H_5), 165.0 and 166.2 (COO), and 170.4 (CON); ^{31}P, δ 0.98.

Interaction of **19** *and* **22**.—A condensation of **19** (220 mg, 0.225 mmol) and **22** (83 mg, 0.15 mmol) (ref 5) in pyridine in the presence of trimethylacetyl chloride (0.07 mL, 0.56 mmol), followed by oxidation with iodine (112 mg, 0.45 mmol), and treatment with 1% CF_3CO_2H in CH_2Cl_2 (1 min, 0°C) were performed as described for the synthesis of **7**. Column chromatography (solvent *L*) gave *p*-nitrophenyl 2-acetamido-4,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside 3-phosphate (**23**) (10 mg, 9%, amorphous); $[\alpha]_D^{31}$ −4.3° (*c* 1, $CHCl_3$); R_f 0.6 (solvent *G*). NMR data ($CDCl_3$): ^1H, see Table II; ^{31}P, δ 0.05, 3.12 (5:1, mono-anion and di-anion respectively). Eluted second was P^1,P^2-bis(*p*-nitrophenyl 2-acetamido-4,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside) 3,3′-pyrophosphate (**24**) (24 mg, 11%, amorphous); $[\alpha]_D^{28}$ −12° (*c* 1, $CHCl_3$); R_f 0.5 (solvent *G*). NMR data ($CDCl_3$): ^1H, see Table II; ^{31}P, δ −7.53. Also obtained was the phosphate diester **26** (26 mg, 15%); physical properties, see below.

p-*Nitrophenyl 2-acetamido-4,6-di*-O-*benzoyl-2-deoxy-β*-D-*glucopyranoside 3-(2-acetamido-4,6-di*-O-*benzoyl-2-deoxy-3*-O-p-*methoxybenzyl-α*-D-*glucopyranosyl phosphate), triethylammonium salt* (**25**).—A mixture of **20** (78 mg, 0.11 mmol) and **22** (55 mg, 0.1 mmol) was dried by evaporation of pyridine (3 × 1 mL) therefrom. The residue was dissolved in the same solvent (0.5 mL), trimethylacetyl chloride (0.031 mL, 0.25 mmol) was added, the mixture was stirred for 10 min at 20°C, and a freshly prepared solution of iodine (50 mg, 0.2 mmol) in 19:1 pyridine–H_2O (1 mL) was added. After 15 min, $CHCl_3$ was added, and the organic layer was washed successively with M aq $Na_2S_2O_3$ (2 × 25 mL) and 0.5 M TEAB (2 × 25 mL), dried by filtration through cotton, and concentrated. Column chromatography of the residue (solvent *L*) gave **25** (93 mg, 74%) as a solid; $[\alpha]_D^{19}$ +29° (*c* 1, $CHCl_3$); R_f 0.25 (solvent *H*). NMR data ($CDCl_3$): ^1H and ^{13}C, see Tables II and III, respectively; ^{31}P, δ −3.62.

p-*Nitrophenyl 2-acetamido-4,6-di*-O-*benzoyl-2-deoxy-β*-D-*glucopyranoside 3-(2-acetamido-4,6-di*-O-*benzoyl-2-deoxy-α*-D-*glucopyranosyl phosphate), triethylammonium salt* (**26**).—(*a*) To a stirred solution of **25** (40 mg, 0.03 mmol) in 9:1 MeCN–H_2O (1 mL) was added ammonium cerium(IV) nitrate (33 mg, 0.06 mmol). The mixture was stirred for 1 h at 20°C, then diluted with $CHCl_3$, washed

TABLE III

^{13}C NMR data ($CDCl_3$, δ in ppm, J in Hz) for O-protected oligo(glycosyl phosphates) **25**, **28**, and **29** ($J_{C,P}$ values in brackets)

Atom	**25** [a,b]	**28** [a]	**29** [a]
C-1	99.1	97.8	97.8
C-2	55.5	57.4	57.7d (5.0)
C-3	76.0d (4.9)	74.4d (4.9)	74.1br
C-4	70.7d (4.9)	71.4br	71.6br
C-5	72.7	72.5	72.6
C-6	63.3	63.4	63.6
C-1′	95.1d (4.9)	94.6d (4.5) [c]	94.7d (5.0) [c]
C-2′	53.2d (7.3)	54.3d (4.8)	54.1d (6.0) [d]
C-3′	77.3	73.6d (4.2)	74.1br
C-4′	71.0	70.3d (4.5)	70.5d (4.8)
C-5′	69.0	68.8	68.7 [e]
C-6′	62.2	62.2	62.4 [f]
C-1″		94.4d (4.5) [c]	94.3d (5.0) [c]
C-2″		55.1d (4.8)	54.9d (6.0) [d]
C-3″		71.0	74.1br
C-4″		72.0	70.5d (4.8)
C-5″		69.3	68.8 [e]
C-6″		62.2	62.6 [f]
C-1‴			94.9d (5.0)
C-2‴			52.1br
C-3‴			71.7
C-4‴			69.3
C-5‴			69.7
C-6‴			61.9
CH_3CO	23.3,24.1	23.1,23.6(×2)	23.0,23.6(×2),23.7

[a] Additional signals of Et_3NH^+ (δ 8.4–8.7 and 45.3–45.5), $NO_2C_6H_4$ (116.9, 125.5, 142.8, and 161.9), C_6H_5 (128.4–133.3), COO (165.0–168.0), and CON (171.7–172.6) were present. [b] Additional signals of the *p*-methoxybenzyl group were present at δ 55.1 (CH_3), 74.1 (CH_2), 113.6, 120.8, 130.0, and 159.0 (C_6H_4). [c,d,e,f] Assignments may be interchanged.

successively with satd aq $NaHCO_3$ and 0.5 M TEAB, dried, and concentrated. Column chromatography (solvent *L*) of the residue gave **26** (23 mg, 67%), as a solid, $[\alpha]_D^{26}$ +42° (*c* 1, $CHCl_3$); R_f 0.25 (solvent *G*). NMR data ($CDCl_3$): ^1H, see Table II; ^{31}P, δ −3.21.

(*b*) Condensation of **20** (100 mg, 0.14 mmol) and **22** (77 mg, 0.14 mmol) in the presence of trimethylacetyl chloride, followed by oxidation with iodine, and working up were performed as described for the synthesis of **25**. The residue was dissolved in aq MeCN and treated with ammonium cerium(IV) nitrate (152 mg, 0.28 mmol), as described under (*a*). Column chromatography gave **26** (80 mg) in 50% overall yield.

p-Nitrophenyl 2-acetamido-4,6-di-O-benzoyl-2-deoxy-β-D-glucopyranoside 3-[2-acetamido-4,6-di-O-benzoyl-2-deoxy-α-D-glucopyranosyl phosphate 3-(2-acetamido-

4,6-di-O-benzoyl-2-deoxy-3-O-p-methoxybenzyl-α-D-glucopyranosyl phosphate)], *bis-triethylammonium salt* (**27**).—This compound was obtained by the condensation of **20** (50 mg, 0.069 mmol) and **26** (53 mg, 0.046 mmol) in pyridine in the presence of trimethylacetyl chloride (0.021 mL, 0.172 mmol) followed by oxidation with iodine, as described for the synthesis of **25**. Column chromatography (solvent *N*) gave **27** (50 mg, 59%) as a solid; $[\alpha]_D^{28}$ +52° (*c* 1, $CHCl_3$). NMR data ($CDCl_3$): ^1H, δ 2.17, 2.20, and 2.27 (3 s, 9 H, 3 CH_3CO), 3.68 (s, 3 H, CH_3O), 4.12 (t, 1 H, $J_{2'',3''} = J_{3'',4''}$ = m9.7 Hz, H-3''), 4.57 and 4.76 (2 d, 2 H, *J* 11.1 Hz, CH_2Ar), 4.92 (q, 1 H, $J_{2,3} = J_{3,4} = J_{3,P}$ = 9.7 Hz, H-3), 5.43 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 5.52 (dd, 1 H, $J_{1',2'}$ 3.2, $J_{1',P}$ 9.9 Hz, H-1'), and 5.60 (dd, 1 H, $J_{1'',2''}$ 3.0, $J_{1'',P}$ 7.6 Hz, H-1''); ^{31}P, δ −3.43.

p-*Nitrophenyl 2-acetamido-4,6-di-O-benzoyl-2-deoxy-β-D-glucopyranoside 3-[2-acetamido-4,6-di-O-benzoyl-2-deoxy-α-D-glucopyranosyl phosphate 3-(2-acetamido-4,6-di-O-benzoyl-2-deoxy-α-D-glucopyranosyl phosphate)]*, *bis-triethylammonium salt*. (**28**).—(*a*) This compound was obtained from **27** (50 mg, 0.027 mmol) by treatment with ammonium cerium(IV) nitrate (30 mg, 0.054 mmol) in 9:1 MeCN–H_2O (1 mL) for 2 h at 20°C, as described for the synthesis of **26** (*a*). Column chromatography (solvent *N*) gave **28** (34 mg, 73%) as a solid; $[\alpha]_D^{29}$ +62° (*c* 1, $CHCl_3$); R_f 0.4 (solvent *I*). NMR data ($CDCl_3$: ^{13}C, see Table III; ^{31}P, δ −1.64 and −2.83 (1:1).

(*b*) Compound **28** was obtained by the condensation of **20** (108 mg, 0.15 mmol) and **26** (115 mg, 0.1 mmol) in the presence of trimethylacetyl chloride, followed by oxidation and debenzylation, as described for the synthesis of **26** (*b*). Column chromatography (solvent *N*) gave **28** (80 mg) in 46% overall yield.

p-*Nitrophenyl 2-acetamido-4,6-di-O-benzoyl-2-deoxy-β-D-glucopyranoside 3-{2-acetamido-4,6-di-O-benzoyl-2-deoxy-α-D-glucopyranosyl phosphate 3-[2-acetamido-4,6-di-O-benzoyl-2-deoxy-α-D-glucopyranosyl phosphate 3-(2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-α-D-glucopyranosyl phosphate)]}*, *tris-triethylammonium salt* (**29**).—This compound was obtained by the condensation of **21** (70 mg, 0.1 mmol) and **28** (110 mg, 0.063 mmol) in pyridine in the presence of trimethylacetyl chloride, followed by oxidation with iodine, as described for the synthesis of **25**. Column chromatography (solvent *N*) gave **29** (60 mg, 40%) as a solid; $[\alpha]_D^{28}$ +63° (*c* 1, $CHCl_3$); R_f 0.3 (solvent *I*). NMR data ($CDCl_3$): ^{13}C, see Table III; ^{31}P, δ −0.58, −2.22, and −2.75 (1:1:1).

p-*Nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside 3-{2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate 3-[2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate 3-(2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate)]}*, *triammonium salt* (**2**).—The oligomer **29** (50 mg) was treated with 0.05 M NaOMe in 1:1 MeOH–1,4-dioxane (2 mL) for 24 h at 1°C and isolated as described for the synthesis of **1**, to give **2** (17 mg, 70%) as a solid; $[\alpha]_D^{28}$ +65° (*c* 1, H_2O); R_f 0.5 (solvent *J*). NMR data (D_2O): ^1H, δ 2.08 and 2.14 (2 s, 6 H, 2 CH_3CO), 2.16 (s, 6 H, 2 CH_3CO), 3.57 (t, 1 H, $J_{3''',4'''} = J_{4''',5'''}$ = 9.5 Hz, H-4'''), 3.67–4.20 (m, 16 H, H-2–H-2''', H-3''', H-4–H-4'', H-5–H-5''', H-6–H-6'''), 4.34 (q, the lines are broadened, 3 H, $J_{H,P} = J_{H,H}$ ~ 9 Hz, H-3–H-3''), 5.44 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.53 (dd, $J_{H,H}$ 3.2, $J_{H,P}$ 7.3

Hz), 5.56 (dd, $J_{H,H}$ 3.2, $J_{H,P}$ 7.5 Hz), 5.58 (dd, $J_{H,H}$ 3.6, $J_{H,P}$ 7.9 Hz) [3 H, H-1′–H-1‴], 7.23 and 8.29 (2 d, 4 H, C_6H_4); ^{13}C, see Table I; ^{31}P, δ −0.69.

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