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Large-scale synthesis of D-mannose 6-phosphate and other hexose 6-phosphates

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ABSTRACT

The syntheses of D-mannose 6-phosphate (**4**), several D-mannopyranoside 6-phosphates, and methyl α-D-glucopyranoside 6-phosphate are described. Phosphorylation of methyl 2,3,4-tri-*O*-(trimethylsilyl)-α-D-mannopyranoside (**2**) with phosphorus oxychloride followed by careful hydrolysis gave methyl α-D-mannopyranoside 6-phosphate (**10**, 81%). Direct phosphorylation of 1,2,3,4,6-penta-*O*-(trimethylsilyl)-α-D-mannopyranoside with phosphorus oxychloride followed by hydrolysis gave **4** (50% yield based on D-mannose). The first method was further used in the synthesis of methyl, butyl, and hexadecyl α-D-mannopyranoside 6-phosphate disodium salts, and in the synthesis of methyl α-D-glucopyranoside 6-phosphate disodium salt.

Compound **2** was obtained in 67% yield, from methyl 2,3,4,6-tetra-*O*-(trimethylsilyl)-α-D-mannopyranoside, by selective hydrolysis with a saturated solution of potassium carbonate in methanol.

Butyl and hexadecyl α-D-mannopyranosides were prepared by glycosidation of the respective alcohols with tetra-*O*-benzoyl-α-D-mannopyranosyl bromide in silver triflate-promoted reactions.

INTRODUCTION

It has been shown that D-mannose 6-phosphate is a potent inhibitor of inflammation in the central nervous system[1]. This effect may be due to inhibition of the interaction between the D-mannose 6-phosphate receptors (MRs) and lysosomal enzymes, as these interactions are partly responsible for intracellular transport and binding of the lysosomal enzymes to the cell surface[1–7].

To investigate this hypothesis in more detail, large amounts of D-mannose 6-phosphate were required, however, no practical procedure for large-scale synthesis of this compound appears to be described in the literature. The direct phosphorylation of D-mannose in concentrated sulfuric acid, as described for D-glucose[8], has not been reported. We did not observe sufficient selectivity for

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substitution at the primary hydroxyl group by reaction with phosphorus oxychloride in trimethyl orthophosphate, despite analogy with reactions proposed for selective phosphorylation of arabino- or ribo-furanose derivatives[9,10].

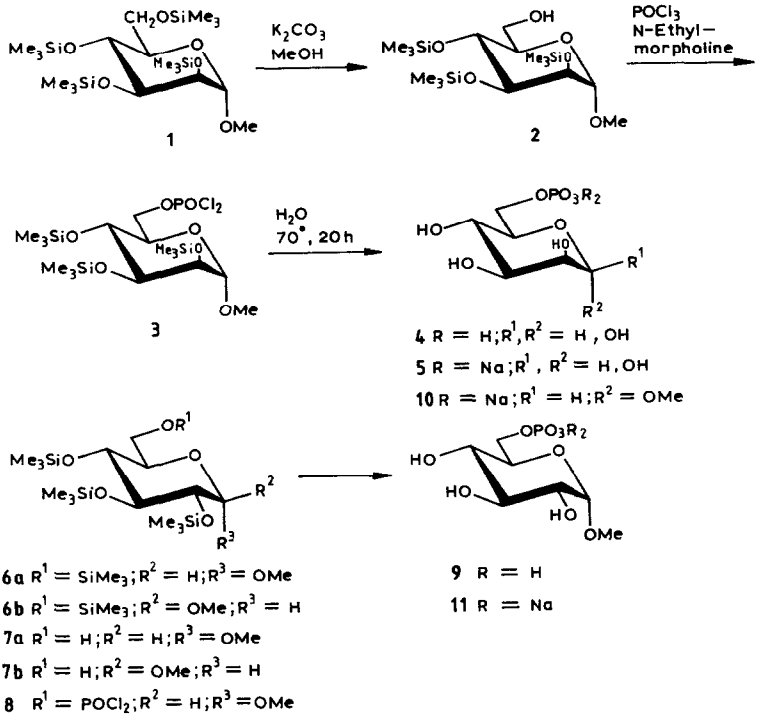
The most straightforward procedure published for the preparation of D-glucose 6-phosphate involves temporary protection of the methyl glucoside with chlorotriphenylmethane followed by acetylation, detritylation[11,12], and phosphorylation. The method is, however, quite cumbersome for large-scale synthesis. This work describes the use of silyl derivatives for large-scale preparation of D-mannose 6-phosphate.

The synthesis of long-chain alkyl glycopyranosides of D-mannose 6-phosphate are also described since they, via the long fatty chain, may be assumed to have affinity to the cell membrane and hence increase the concentration of D-mannose 6-phosphate in the vicinity of the membrane-bound receptor.

RESULTS AND DISCUSSION

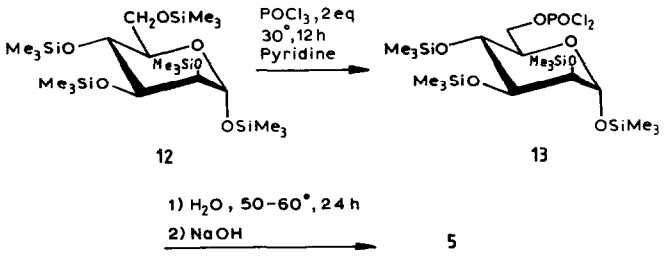
Methyl α-D-mannopyranoside was converted into its tetra-*O*-trimethylsilyl derivative **1** in quantitative yield. The crude product was selectively hydrolyzed with potassium carbonate in methanol[13,14] affording the 6-hydroxy compound **2** in 67% yield isolated by crystallization at low temperature from pentane. Phosphorylation with phosphorus oxychloride catalyzed by *N*-ethylmorpholine gave, after work up, the crystalline, rather hygroscopic and unstable dichlorophosphate **3**, which was hydrolyzed to give methyl α-D-mannopyranoside 6-phosphate. This was purified on a weakly basic ion-exchange resin and transformed into methyl α-D-mannopyranoside 6-phosphate disodium salt (**10**, 81%). Analogously, methyl α- and β-glucopyranoside were per(trimethylsilyl)ated and, upon selective hydrolysis of **6a** and **6b**, crystallization from aqueous acetonitrile afforded **7a** and **7b** in 60 and 28% yield, respectively. Conversion of **7a** to the dichlorophosphate **8** and hydrolysis as described for **10** yielded methyl α-D-glucopyranoside 6-phosphate disodium salt (**11**).

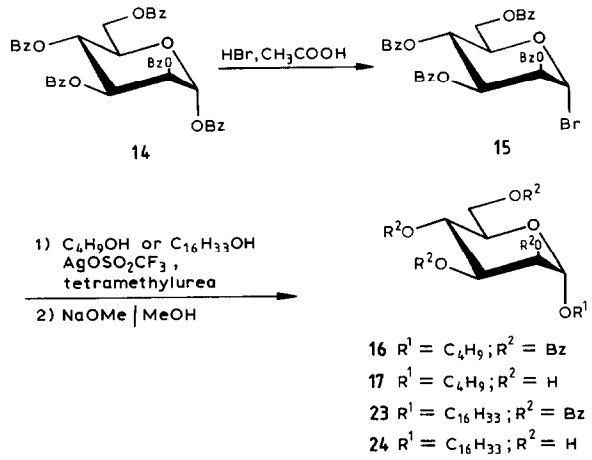
Attempts to hydrolyze **3** to give D-mannose 6-phosphate (**4**) gave decomposition products before the hydrolysis was complete. To avoid the decomposition products and unreacted methyl glycoside in the synthesis of **4**, direct phosphorylation of 1,2,3,4,6-penta-*O*-(trimethylsilyl)-α-D-mannose (**12**) was attempted[15]. Treatment of **12** with an excess of phosphorus oxychloride and pyridine in toluene, and heating the mixture to 30° overnight yielded the unstable dichlorophosphate **13**, which was hydrolyzed by addition of water and heating for 24 h at 50–55°. Hydrolysis of the silyl ether groups was complete within 1 h, but during hydrolysis, an intermediate cyclic phosphate was formed, which was converted into D-mannose 6-phosphate on heating. The crude product **4** was purified on a weakly basic ion-exchange resin and transformed into D-mannose 6-phosphate disodium salt (**5**, 50% overall yield). Compound **5** was characterized by its ^{1}H and ^{13}C NMR spectroscopic data, which were in agreement with those of an authentic sample. Thus, a simple three-step



procedure for the large-scale synthesis (up to 100 g) of pure D-mannose 6-phosphate has been developed.

Butyl α-D-mannopyranoside 6-phosphate (**21**) was synthesized as a model compound for hexadecyl α-D-mannopyranoside 6-phosphate. Thus, 1-butanol was glycosylated with tetra-*O*-benzoyl-α-D-mannopyranosyl bromide[16] (**15**) in a silver triflate-promoted reaction. After deprotection, the butyl glycoside **17** was quantitatively per(trimethylsilyl)ated and selectively hydrolyzed using potassium carbonate in methanol (35% yield). Phosphorylation of the selectively protected butyl glycoside **19**, as described for **2**, afforded the unstable dichlorophosphate **20**, which was

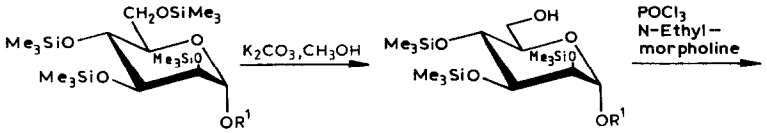




hydrolyzed and converted into the disodium salt **22** in 20% overall yield. The same procedure was used for the synthesis of hexadecyl α-D-mannopyranoside 6-phosphate disodium salt (**29**). Attempts to glycosylate hexadecanol with tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**15**), in a silver triflate-promoted reaction, led to quantitative formation of hexadecyl acetate. The transesterification was also observed in the glycosylation of butanol with tetra-*O*-acetyl-α-D-mannopyranosyl bromide, albeit to a lesser extent; in the latter reaction a small amount of butyl 3,4,6-tri-*O*-acetyl-α-D-mannopyranoside could also be isolated.

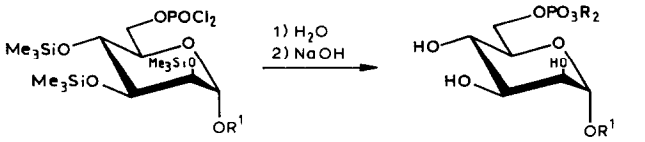
Banoub and Bundle[17,18] observed the same phenomenon in a stannic tetrachloride-catalyzed glycosylation. They suggested that the acetyl migration could take place via the orthoester. However, this cannot be the only mechanism responsible for the transesterification during glycosylation of hexadecanol as, in our hands, the acetylated hexadecanol was formed quantitatively, and the ratio of bromide-to-hexadecanol was initially 1:1. The mechanism suggested by Banoub and Bundle requires at least 2 equivalents of the glycoside acceptor. Instead, acid-catalyzed transesterification may be the mechanism responsible for the acetyl migration, with the acetyl group at the 2-position being most reactive.

When benzoyl groups were used for protection in the glycosylation reaction with 1-butanol or hexadecanol in the presence of tetramethylurea, acyl migration was effectively eliminated and glycosides were isolated in 81 and 70% yields, respectively. After deprotection, the hexadecyl glycoside **24** was quantitatively per(trimethylsilyl)ated and selectively hydrolyzed in 56% yield, using potassium carbonate in methanol. Phosphorylation, as described for compound **2**, afforded the unstable dichlorophosphate **27**, which was hydrolyzed by the addition of water and stirring at room temperature, and the hexadecyl α-D-mannopyranoside 6-phosphate (**28**) crystallized in 56% overall yield. The compound was insoluble in water, dichloromethane, methanol, butanol, dimethyl sulfoxide, chloroform, toluene and



18 $R^1 = C_4H_9$
25 $R^1 = C_{16}H_{33}$

19 $R^1 = C_4H_9$
26 $R^1 = C_{16}H_{33}$



20 $R^1 = C_4H_9$
27 $R^1 = C_{16}H_{33}$

21 $R^1 = C_4H_9$; $R^2 = H$
22 $R^1 = C_4H_9$; $R^2 = Na$
28 $R^1 = C_{16}H_{33}$; $R^2 = H$
29 $R^1 = C_{16}H_{33}$; $R^2 = Na$

other solvents, but dissolved in pyridine to form a pyridinium salt. This salt was soluble in chloroform. ^1H NMR showed distinct signals from the aliphatic chain, while the signals from the carbohydrate part completely lacked their fine structure indicating the formation of micelles. The spectrum was partly resolved by addition of Me_2SO-d_6. In the ^{13}C NMR spectra of **29**, a coupling between C-5 and phosphorus (7 Hz) was observed. This coupling, as well as the coupling between phosphorus and H-6 and H-6′ (12 Hz), were also observed in all other phosphorylated compounds.

In conclusion, the trimethylsilyl group was found to be an ideal protecting group in the phosphorylation reaction, as the per(trimethylsilyl)ation is quantitative, the group is stable under the reaction conditions, and is easily removed by acid hydrolysis. The phosphorylation of alkyl glycosides works as a general procedure. Selective hydrolysis of the per(trimethylsilyl)ated butyl and hexadecyl glycosides may be further optimized, and the yields thereby improved. It was found important to have two different phosphorylation procedures. One involves the phosphorylation of alkyl glycosides, where the per(trimethylsilyl)ated glycoside is selectively hydrolyzed at the primary position and then quantitatively phosphorylated. This procedure affords pure alkyl glycoside 6-phosphates, but these cannot be cleanly hydrolyzed to the free monosaccharide 6-phosphates. The other procedure involves the direct phosphorylation of per(trimethylsilyl)ated monosaccharides in a nonquantitative phosphorylation reaction, followed by ready purification by adsorption on an ion-exchange resin. This procedure yields pure monosaccharide 6-phosphates and can only be used for synthesis of phosphorylated alkyl glycosides when these are water soluble, allowing ion-exchange purification.

EXPERIMENTAL

General methods.—Analytical grade solvents were dried over molecular sieves and pyridine was distilled. Chlorotrimethylsilane, *N*-ethylmorpholine and phosphorus oxychloride were purchased from Fluka, and an authentic sample of D-mannose 6-phosphate, ion-exchange resin, and methyl glycosides were from Sigma. IR spectra were recorded on a Perkin–Elmer 157 spectrophotometer, optical rotations were recorded on a Perkin–Elmer 141 polarimeter, TLC was performed on Merck coated aluminum foil plates F_{254} by eluting with a mixture of CH_2Cl_2 and EtOAc, or hexane and EtOAc. Vacuum liquid chromatography (VLC)[19] was performed on Silica Gel 60 H (Merck). ^1H NMR spectra were recorded on Bruker AM500 and AMX600 instruments. ^{13}C NMR spectra were recorded at 300 K in $CDCl_3$ relative to $CDCl_3$ (δ 77.24) or in D_2O with external reference 1,4-dioxane (δ 67.4). ^1H NMR spectra were recorded at 300 K in $CDCl_3$ relative to Me_4Si (δ 0.000), or in D_2O relative to acetone (δ 2.225). Assignment of ^{13}C NMR spectra was achieved by heteronuclear correlated 2D-spectroscopy.

*Methyl 2,3,4,6-tetra-*O*-(trimethylsilyl)-α-*D*-mannopyranoside* (**1**).—Chlorotrimethylsilane (300 g, 2762 mmol) was added dropwise over a period of 1 h to a suspension of mechanically stirred methyl α-D-mannopyranoside (100 g, 515 mmol) in pyridine (600 mL) keeping the temperature between 50 and 60°. The mixture was stirred for 1 h and diluted with CH_2Cl_2 (600 mL). The mixture was left overnight and the pyridine hydrochloride was filtered off and washed with CH_2Cl_2. The mixture was concentrated at 35° in vacuo and the residue diluted with toluene (200 mL), and filtered. The toluene was removed at 35° and 0.1 Pa to yield **1** (233 g). The product was pure according to ^1H NMR and TLC; $[\alpha]_{25}^{D} + 52.6°$ (*c* 7, $CHCl_3$). ^1H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{19}H_{46}O_6Si_4$: C, 47.26; H, 9.60. Found: C, 47.29; H, 9.58.

*Methyl 2,3,4-tri-*O*-(trimethylsilyl)-α-*D*-mannopyranoside* (**2**).—Method *A*: Compound **1** (233 g, 482 mmol) was dissolved in MeOH (300 mL) and satd anhyd K_2CO_3 in MeOH (10 mL) was added with stirring at 20°. The progress of the reaction was monitored by TLC (1:19 EtOAc – CH_2Cl_2). After 2 h, TLC showed optimal product distribution, and the solution was cooled to −78° yielding **2** as crystals (100 g), mp 65° (after drying). Additional K_2CO_3 solution (10 mL) was added to the filtrate and the reaction was monitored by TLC. After 2 h at 20°, toluene (200 mL) was added and the organic solvents were removed in vacuo at 20°. The residue was diluted with dry CH_2Cl_2 (200 mL) and passed through a short column of silica gel (50 g) by elution with 1:9 EtOAc–CH_2Cl_2 (350 mL). The solvents were removed in vacuo by repeated concentrations from hexane (100 mL). The combined products were redissolved in hexane (400 mL) and filtered. Cooling to −78° for 2 h, filtration at low temperature and washing with cold hexane (−78°, 250 mL) afforded **2** (133 g, 67%).

Method *B*: The foregoing reaction was allowed to proceed for 4.5 h and toluene (100 mL) was added. The solvents were removed in vacuo at 20° and the syrup

TABLE I

^{1}H NMR chemical shift data (δ, ppm) for compounds **1–28**

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-6′
1 [e]	4.50	3.78	3.75	3.83	3.47	3.83	3.74
2 [e]	4.55	3.81	3.78	3.89	3.56	3.81	3.73 [a]
3 [e]	4.54	3.83	3.78	3.87	3.78	4.62	4.43
4α [f]	5.16	3.92	3.83	3.72	3.91	4.12	4.11
4β [f]	4.88	3.90	3.64	3.62	3.48	4.17	4.06
5α [f]	5.18	3.905	3.839	3.780	3.868	4.013	3.958
5β [f]	4.93	3.937	3.655	3.727	3.439	4.086	4.002
6a [e]	4.65	3.52	3.80	3.48	3.55	3.81	3.72
6b [e]	4.10	3.32	3.47	3.48	3.21	3.85	3.73
7a [e]	4.64	3.52	3.83	3.52	3.62	3.84	3.73 [b]
7b [e]	4.28	3.32	3.48	3.50	3.30	3.86	3.71 [c]
10 [f]	4.79	3.92	3.78	3.86	3.65	3.96	4.05
11 [f]	4.81	3.69	3.62	3.68	3.61	4.07	3.93
12 [e]	4.93	3.67	3.84	3.86	3.61	3.79	3.74
16 [e]	5.13	5.77	5.97	6.14	4.53	4.73	4.53
17 [e]	4.85	3.95	3.87	3.97	3.54	3.99	3.79
18 [e]	4.60	3.79	3.78	3.83	3.51	3.83	3.74
19 [e]	4.61	3.79	3.81	3.89	3.59	3.72	3.68
21 [f]	4.89	3.80	3.63 [d]	3.58 [d]	3.66 [d]	4.11	4.05
22 [f]	4.89	3.90	3.79	3.87	3.65	3.93	4.07
23 [e]	5.17	5.74	5.97	6.14	4.47	4.73	4.53
24 [g]	4.67	3.81	3.74	3.64	3.56	3.86	3.72
25 [e]	4.51	3.81	3.74	3.83	3.50	3.78	3.72
26 [e]	4.51	3.81	3.77	3.89	3.58	3.79	3.71
28 [h]	4.49	3.59 [d]	3.44 [d]	3.55 [d]	3.23 [d]	4.04 [d]	3.78 [d]

[a] HO-6 resonates at 2.05 ppm as a triplet. [b] HO-6 resonates at 2.00 ppm as a singlet. [c] HO-6 resonates at 1.98 ppm as a triplet. [d] Assignments may be reversed. Solvents used were as follows: [e] $CDCl_3$, [f] D_2O, [g] CD_3OD, [h] $CDCl_3$–Me_2SO-d_6.

redissolved in a mixture of EtOAc in CH_2Cl_2 (1:9, 150 mL). The solution was filtered through silica gel (40 g) and the gel washed with the same solvent (150 mL). The organic solvents were removed and the product was crystallized from hexane at −78° for 2 h and filtered to yield **2** (100 g, 50%); mp 72–73°; $[\alpha]_{25}^{D}$ + 58.3° (*c* 3, $CHCl_3$). ^{1}H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{16}H_{38}O_6Si_3$: C, 46.78; H, 9.33. Found: C, 46.83; H, 9.29.

*Methyl 6-O-dichlorophosphinyl-2,3,4-tri-O-(trimethylsilyl)-α-*D*-mannopyranoside* (**3**).—Compound **2** (133 g, 325 mmol) and *N*-ethylmorpholine (45.56 mL, 360 mmol) were dissolved in dry CH_2Cl_2 (700 mL). Phosphorus oxychloride (30.2 mL, 330 mmol) was added during 5 min with stirring on a water bath and the reaction was monitored by TLC (CH_2Cl_2). After 5 h the reaction was complete but the mixture was left overnight. Dichloromethane (350 mL) was removed by evaporation and the mixture was diluted with hexane (700 mL). After stirring at 0° (10 min) the precipitate was removed by filtration. The filtrate was cooled to −5° and extracted with a mixture of HCl (37%, 3 mL), water (100 mL) and ice (200 mL), and then twice with water (200 mL). After drying ($MgSO_4$), the organic solvents

TABLE II

^{1}H NMR coupling constants [a] (J, Hz) for compounds **1–28**

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
1 [e]	2.0	2.6	9.0	9.0	2.0	6.0	11.5
2 [e]	2.0	2.5	9.0	9.0	2.9	5.5	11.5 [b]
3 [e]	0.5	3.0	9.0	9.0	2.0	2.5	10.0
4α [f]	2.0	3.0	9.0	9.0	m	m	m
4β [f]	1.2	3.3	9.0	9.0	m	m	m
5α [f]	2.1	3.5	9.0	9.0	2.5	5.5	m
5β [f]	0.5	3.5	9.5	9.0	3.0	4.5	m
6a [e]	3.5	9.0	8.5	9.5	2.2	5.2	11.4
6b [e]	7.6	8.2	m	8.6	1.9	5.1	11.4
7a [e]	3.5	9.0	8.5	9.5	2.5	4.6	11.6
7b [e]	7.5	m	m	m	5.8	9.4	11.7 [c]
10 [f]	1.8	3.0	9.8	9.8	m	m	m
11 [f]	3.5	9.5	9.5	9.5	1.8	7.8	11.7 [d]
12 [e]	1.8	2.8	9.0	9.0	1.9	5.2	11.8
16 [e]	1.8	3.1	10.0	10.0	1.9	4.5	11.8
17 [e]	1.5	2.5	9.2	9.2	2.0	5.0	11.8
18 [e]	1.5	2.5	9.0	9.0	2.1	5.0	11.8
19 [e]	1.5	2.5	9.0	9.0	2.0	5.1	11.8
21 [f]	2.0	3.0	9.0	9.0	m	m	m
22 [f]	1.8	3.0	9.8	9.8	2.0	4.0	11.8 [d]
23 [e]	1.8	3.0	10.0	10.0	2.1	4.5	11.8
24 [g]	1.5	2.8	9.8	9.8	2.1	4.5	11.8
25 [e]	1.8	2.8	9.8	9.8	2.1	5.0	11.8
26 [e]	1.8	2.8	9.8	9.8	2.1	5.0	11.8

[a] Observed first-order values 0.3 Hz. [b] $J_{OH,H-6}$ and $J_{OH,H-6'} = 6.2$. [c] $J_{OH,H-6}$ and $J_{OH,H-6'} = 6.4$. [d] $J_{P,H-6} = 5.0$, $J_{P,H-6'} = 7.8$. Solvents used were as follows: [e] $CDCl_3$, [f] D_2O, [g] CD_3OD.

were removed by concentration in vacuo. The product crystallized to give **3** (148 g, 85%); mp 50–55°; an analytical sample could be obtained by recrystallization from pentane at −78°, mp 57.5–59.5°. The product was very hygroscopic and was used directly in the next step.

D-*Mannose 6-phosphate* (**4**).—Crude **13** (83.2 g) was treated with 150 mL of water in a rotary evaporator during 30 min at 30°. The solution was heated for 24 h at 50–60° and the water was removed in vacuo. Concentrations were repeated 3 times with water (150 mL). ^{1}H and ^{13}C NMR data are presented in Tables I–III.

D-*Mannose 6-phosphate disodium salt* (**5**).—Crude **4**, obtained from **13** (83.2 g), was dissolved in water and adsorbed onto a weakly basic ion-exchange resin (300 g Amberlyst A-21), and impurities were eluted with water. The resin-containing compound **4** was suspended in water and pH was adjusted to 9.0 with M NaOH. Compound **5** was eluted with water and lyophilized, yielding 33.9 g (80%). The compound was characterized by ^{1}H and ^{13}C NMR spectroscopy (Tables I–III) and comparison of its spectral data with an authentic commercial sample from Sigma (No. M 6876). The product was pure according to the NMR spectral data, and its

TABLE III

^{13}C NMR chemical shift data (δ, ppm) for compounds **1–28**

Compound	C-1	C-2	C-3	C-4	C-5	C-6	O-CH_3
1 [b]	99.66	73.98	72.27	72.07	75.34	62.25	54.51
2 [b]	102.02	72.46	73.53	68.13	73.76	62.27	54.64
4α [c]	95.00	71.60	71.00	67.20	72.60	63.65	
4β [c]	94.80	73.60	72.02	67.00	76.70	63.65	
5α [c]	94.99	71.47	70.69	67.12	72.67 $^4J_{CP}$ 7 Hz	63.76	
5β [c]	94.61	72.05	73.43	66.88	76.31 $^4J_{CP}$ 7 Hz	63.76	
6a [b]	100.02	73.85	74.86	71.99	71.56	61.94	54.89
6b [b]	104.05	76.32	78.88	71.64	76.43	62.23	56.33
7a [b]	100.02	73.92	75.20	72.10	71.60	61.90	54.80
7b [b]	104.47	75.96	78.27	71.58	76.00	62.18	56.90
10 [c]	101.93	71.03 [a]	67.22 [a]	70.82 [a]	72.86	63.45	55.69
11 [c]	100.29	72.21 [a]	69.62 [a]	72.11 [a]	73.44	63.19	55.94
12 [b]	95.59	75.21	72.19	68.42	74.60	62.49	
16 [b]	97.65	70.17 [a]	68.83 [a]	67.06	70.68	62.99	
17 [b]	100.02	71.06 [a]	71.59 [a]	66.18	72.20	60.89	
18 [b]	100.56	72.83 [a]	73.68 [a]	66.96	74.78	62.70	
19 [b]	100.86	72.51 [a]	73.77 [a]	67.30	73.82	62.30	
21 [c]	98.47	67.25 [a]	66.72 [a]	62.99 [a]	67.94 [a]	62.00	
22 [c]	100.69	72.89 [a]	68.57 [a]	71.01 [a]	72.95 [a]	66.76	
23 [b]	97.64	70.17	68.82	67.09	70.67	62.99	
24 [d]	99.63	70.76	66.72	70.37	72.64	61.01	
25 [b]	100.54	73.68	68.49	72.84	74.79	62.68	
26 [b]	100.84	73.77	68.17	72.51	72.83	62.29	
28 [e]	99.84	70.85 [a]	66.52 [a]	70.31 [a]	71.99	65.38	

[a] Assignments may be reversed. Solvents used were as follows: [b] $CDCl_3$, [c] D_2O, [d] CD_3OD, [e] $CDCl_3$–Me_2SO-d_6.

optical rotation, $[\alpha]_{25}^{D}$ + 7.39 (*c* 3.72, H_2O) as compared with $[\alpha]_{25}^{D}$ + 5.84 (*c* 3.43, H_2O) for the commercial sample from Sigma.

Methyl 2,3,4-tri-O-(trimethylsilyl)-α-D-glucopyranoside (**7a**).—Methyl 2,3,4,6-tetra-*O*-(trimethylsilyl)-α-D-glucopyranoside (**6a**, 24.3 g) was prepared, as already described for compound **1** (100% yield); $[\alpha]_{25}^{D}$ + 84.4° (*c* 4, $CHCl_3$). ^1H and ^{13}C NMR data are given in Tables I–III.

Anal. Calcd for $C_{16}H_{46}O_6Si_4$: C, 47.26; H, 9.60. Found: C, 47.33; H, 9.65.

The trimethylsilyl group was removed from the 6-position of **6a** (24 g, 50 mmol) as already described for compound **2** with K_2CO_3 in MeOH, and after 1 h toluene was added and the solvent was removed in vacuo. The product was purified by VLC (20:1 CH_2Cl_2–EtOAc), and crystallized from MeCN and water to yield **7a** (12.5 g, 61%); mp 69.0–69.5; $[\alpha]_{25}^{D}$ + 92.6° (*c* 3, $CHCl_3$). ^1H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{16}H_{38}O_6Si_3$: C, 46.78; H, 9.33. Found: C, 46.69; H, 9.26.

Methyl 2,3,4-tri-O-(trimethylsilyl)-β-D-glucopyranoside (**7b**).—Methyl 2,3,4,6-tetra-*O*-(trimethylsilyl)-β-D-glucopyranoside (**6b**, 12.2g) was prepared, as already

described for compound **1** (100% yield); $[\alpha]_{25}^{D}$ −2.5° (*c* 3, $CHCl_3$). ^1H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{19}H_{46}O_6Si_4$: C, 47.26; H, 9.60. Found: C, 47.42; H, 9.76.

The silyl group was removed from the 6-position as already described for compound **2** and **7a** with K_2CO_3 in MeOH and, after 1.5 h, toluene (10 mL) was added and the solvent was removed in vacuo. The product was purified by VLC (20:2 CH_2Cl_2–EtOAc) and crystallized from MeCN and water to yield **7b** (3 g, 29%); mp 69.0–69.5; $[\alpha]_{25}^{D}$ −9.6° (*c* 3, $CHCl_3$). ^1H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{16}H_{38}O_6Si_3$: C, 46.78; H, 9.33. Found: C, 46.78; H, 9.26.

Methyl α-D-mannopyranoside 6-phosphate disodium salt (**10**).—Compound **3** (604 mg, 1.25 mmol) was treated with 20 mL of water on a rotary evaporator for 30 min at 20°, and the water was removed in vacuo. Concentrations were repeated 3 times with water (20 mL). The resulting colorless syrup was dissolved in water and adsorbed onto a weakly basic ion-exchange resin (6 g, Amberlyst A-21), and impurities were eluted with water. The resin containing compound **4** was suspended in water and pH was adjusted to 9.0 with M NaOH. Compound **10** was eluted with water and lyophilized, yielding 296 mg (81%, hygroscopic product). The compound was pure according to ^1H and ^{13}C NMR and the data are presented in Tables I–III.

Methyl α-D-glucopyranoside 6-phosphate disodium salt (**11**).—Compound **7a** (1.0 g, 2.43 mmol) was phosphorylated with $POCl_3$, as described for compound **2**, to give compound **8** (704 mg, 1.34 mmol, 55%), which was hydrolyzed and purified by adsorption on a weakly basic ion-exchange resin, as for **10**, affording the hygroscopic compound **11** (349 mg, 82%). The product was pure according to ^1H and ^{13}C NMR spectroscopy. ^1H and ^{13}C NMR data are presented in Tables I–III.

1,2,3,4,6-Penta-O-(trimethylsilyl)-α-D-mannopyranose (**12**).—α-D-Mannose (50 g, 279 mmol) was added to a solution of chlorotrimethylsilane (247.8 mL, 1953 mmol) in pyridine (170.6 mL, 2100 mmol) in small portions with stirring during 2 h, keeping the temperature at ~ 0°. The stirring was continued for another hour at 0°. The mixture was diluted with CH_2Cl_2 (200 mL) and left overnight. The reaction mixture was diluted with toluene (200 mL), concentrated at 30° in vacuo and the residue was diluted with toluene (200 mL) and filtered. The toluene was removed at 30° and 0.1 Pa and the residue was again diluted with toluene and filtered. The toluene was removed at 30° and 0.1 Pa to yield **12** (117 g, 78%). The colorless syrup was pure according to ^1H NMR and TLC, with $[\alpha]_{20}^{D}$ + 51.0° (*c* 1, $CHCl_3$). ^1H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{21}H_{52}O_6Si_5$: C, 46.62; H, 9.69. Found: C, 46.69; H, 9.65.

6-O-Dichlorophosphinyl-1,2,3,4-tetra-O-(trimethylsilyl)-α-D-mannopyranose (**13**).—Compound **12** (117 g, 216 mmol) was dissolved in toluene (250 mL) and pyridine (38.3 mL, 474 mmol) and $POCl_3$ (40.2 mL, 431 mmol) were added. The solution was heated to 30° and stirred at this temperature overnight. The mixture was concentrated, diluted with toluene (250 mL), filtered and concentrated at 35° and

0.1 Pa. The residue was dissolved in pentane (200 mL), cooled to −5° and extracted first with 100 mL of a mixture of HCl (37%, 3 mL), brine (100 mL) and ice (200 mL), and then twice with brine. After drying ($MgSO_4$) and filtration the pentane was removed in vacuo to yield **13** (83.2 g, 64%). The colorless syrup was used directly to prepare compound **4**.

Butyl 2,3,4,6-tetra-O-benzoyl-α-D-mannopyranoside (**16**).—Tetra-*O*-benzoyl-α-D-mannopyranosyl bromide **15** (1.6 g, 2.37 mmol), 1-butanol (0.23 mL, 2.5 mmol) and tetramethylurea (0.30 mL, 2.5 mmol) were dissolved in CH_2Cl_2 (10 mL) and stirred under Ar over molecular sieves (3A, 0.5 g) at −18° for 2 h. Silver trifluoromethanesulfonate (0.64 g, 2.5 mmol) was added quickly, and the mixture was stirred at −18° overnight. The mixture was allowed to reach room temperature and filtered through Celite. The filtrate was poured onto ice and washed with cold solutions of $NaHCO_3$, $Na_2S_2O_3$ (10%), water, H_2SO_4 (M), water and $NaHCO_3$. The CH_2Cl_2 layer was dried ($MgSO_4$) and concentrated. TLC (3:2 hexane–EtOAc) showed 2 products, and the syrup was subjected to VLC (3:1 hexane–EtOAc) to yield **16** (1.26 g, 81%), as a syrup. $[\alpha]_{20}^{D}$ − 16.7° (*c* 0.6, CH_2Cl_2). ^1H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{38}H_{36}O_{10}$: C, 69.93; H, 5.56. Found: C, 69.88; H, 5.43.

Butyl α-D-mannopyranoside (**17**)—Compound **16** (1.26 g, 1.9 mmol) was treated with NaOMe in MeOH (0.1 M, 15 mL) for 1 h at room temperature and neutralized with an ion-exchange resin (Amberlite IR C-50 (H^+), 1.0 g). Filtering, washing with MeOH and concentration in vacuo yielded **17** (0.46 g, 100%), $[\alpha]_{20}^{D}$ − 128.4° (*c* 1.6, MeOH). ^1H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{10}H_{20}O_6$: C, 50.84; H, 8.66. Found: C, 50.82; H, 8.66.

Butyl 2,3,4,6-tetra-O-(trimethylsilyl)-α-D-mannopyranoside (**18**).—The same procedure, as described for compound **1**, was applied to compound **17**, to give **18** as a colorless syrup (3.0 g, 91%); $[\alpha]_{20}^{D}$ + 35.6° (*c* 7.3, CH_2Cl_2). ^1H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{22}H_{52}O_6Si_4$: C, 50.33; H, 9.98. Found: C, 50.50; H, 9.54.

Butyl 2,3,4-tri-O-(trimethylsilyl)-α-D-mannopyranoside (**19**).—Compound **18** (1.0 g, 1.91 mmol) was dissolved in MeOH (20 mL) and a saturated solution of anhyd K_2CO_3 in MeOH (40 μL) was added with stirring at 20°. The reaction was left for 3 h, and the MeOH was evaporated quickly. The residue was dissolved in MeOH (1 mL) and another 80 μL of the K_2CO_3 solution was added. The reaction progressed slowly and, after 8 h, the optimal product distribution had not been reached. The mixture was heated to 40° for 2 h and the reaction was stopped by adding toluene and evaporating the MeOH. The mixture was subjected to VLC (CH_2Cl_2) to yield **19** (300 mg, 35%) as a syrup, $[\alpha]_{20}^{D}$ + 49.0° (*c* 2, CH_2Cl_2). ^1H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{19}H_{44}O_6Si_3$: C, 50.40; H, 9.79. Found: C, 50.43; H, 9.77.

Butyl 6-O-dichlorophosphinyl-2,3,4-tri-O-(trimethylsilyl)-α-D-mannopyranoside (**20**).—The same procedure, as described for compound **3**, was applied to com-

pound **19**, to give **20** as as syrup (221 mg, 58%). The product was used directly in the next step.

Butyl α-D-mannopyranoside 6-phosphate (**21**)—Compound **20** (221 mg, 0.39 mmol) was treated with 780 μL of water on a rotary evaporator at 20° for 3 h, and then the water was removed in vacuo. ^{1}H NMR (Table I and II) showed that the trimethylsilyl groups had been hydrolyzed. ^{1}H and ^{13}C NMR data are presented in Tables I–III.

Butyl α-D-mannopyranoside 6-phosphate disodium salt (**22**).—The pH of the above solution was adjusted to 7 with NaOH (0.5 M) and was lyophilized to yield **22** (134 mg, 96%), $[\alpha]_{20}^{D}$ + 24.0° (*c* 1, MeOH). ^{1}H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{10}H_{19}Na_2O_9P$: C, 33.34; H, 5.31. Found: C, 33.55; H, 5.19.

Hexadecyl 2,3,4,6-tetra-O-benzoyl-α-D-mannopyranoside (**23**).—Tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (**15**) (6.4 g, 9.5 mmol) and tetramethylurea (1.26 mL, 10.4 mmol) were dissolved in toluene (20mL) and stirred under Ar over molecular sieves (3A, 1 g) for 1 h at room temperature. Hexadecan-1-ol (2.53 g, 10.4 mmol) and silver trifluoromethanesulfonate (2.68 g, 10.4 mmol) were successively added to the solution. The reaction was monitored by TLC (1:1 pentane–EtOAc) and after 5 h it was complete. The mixture was filtered through Celite and the filtrate poured onto ice and washed with cold solutions of $NaHCO_3$, $Na_2S_2O_3$ (10%), water, H_2SO_4 (2%), water and $NaHCO_3$. The organic phase was dried ($MgSO_4$) and concentrated. The resulting syrup was subjected to VLC (10:1 pentane–ETOAc) to yield **23** as a syrup (5.5 g, 70%); $[\alpha]_{20}^{D}$ + 44.5° (*c* 2, CH_2Cl_2). ^{1}H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{50}H_{60}O_{10}$: C, 73.15; H, 7.37. Found: C, 73.19; H, 7.56.

Hexadecyl α-D-mannopyranoside (**24**).—Compound **23** (4.04 g, 4.92 mmol) was treated with NaOMe in MeOH (0.1 M, 15 mL) at room temperature. After 0.5 h the product had crystallized. The crystals were collected and dissolved in MeOH, and the solution was neutralized. The product was recrystallized from 1:1 MeOH–toluene to yield **24** (1.99 g, 100%); mp 195–196°; $[\alpha]_{20}^{D}$ + 31.0° (*c* 1, MeOH). ^{1}H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{22}H_{44}O_6$: C, 65.31; H, 10.96. Found: C, 65.25; H, 10.93.

Hexadecyl 2,3,4,6-tetra-O-(trimethylsilyl)-α-D-mannopyranoside (**25**)—The same procedure, as described for compound **1**, was applied to compound **23**, to give **25** as a syrup (2.96 g, 91%); $[\alpha]_{20}^{D}$ + 35.3° (*c* 4.3, CH_2Cl_2). ^{1}H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{34}H_{76}O_6Si_4$: C, 58.90; H, 11.05. Found: C, 59.12; H, 11.11.

Hexadecyl 2,3,4-tri-O-(trimethylsilyl)-α-D-mannopyranoside (**26**).—Compound **25** (1.0 g, 1.44 mmol) was dissolved in a mixture of MeOH (1 mL) and CH_2Cl_2 (20 μl) and satd anhyd K_2CO_3 in MeOH (30 μL) was added with stirring. TLC (1:19 EtOAc–CH_2Cl_2) showed optimal product distribution after 20 min, toluene (10 mL) was added and the solution was concentrated. The mixture was subjected to

column chromatography (CH_2Cl_2) to yield **26** (0.49 g, 55%); $[\alpha]_{20}^{D}$ + 36.5° (*c* 1.7, CH_2Cl_2). ^1H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{31}H_{68}O_6Si_3$: C, 59.95; H, 11.03. Found: C, 59.89; H, 11.14.

Hexadecyl 6-O-dichlorophosphinyl-2,3,4-tri-O-(trimethylsilyl)-α-D-mannopyranoside (**27**).—The same procedure, as described for compound **3**, was applied to compound **26**, except that the extractions with aqueous phases were omitted. The product was used directly in the next step.

Hexadecyl α-D-mannopyranoside 6-phosphate disodium salt (**28**).—Compound **27** was treated with water on a rotary evaporator at 20°, whereupon the product crystallized. The crystals were collected and suspended in water and NaOH (2 M) was added to pH 7. A small sample was recrystallized from pyridine–water. Yield: 0.375 g (100%), mp > 250°, $[\alpha]_{20}^{D}$ + 62.3° (*c* 1.3, pyridine). ^1H and ^{13}C NMR data are presented in Tables I–III.

Anal. Calcd for $C_{22}H_{43}Na_2O_{10}P \cdot 3\,H_2O$: C, 45.01; H, 7.78. Found: C, 45.47; H, 8.23.

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